Structure activity relationships of 1,8-disubstituted thioxanthenones.

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STRUCTURE ACTIVITY RELATIONSHIPS

OF

1,8-DISUBSTITUTED THIOXANTHENONES

by

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A Thesis
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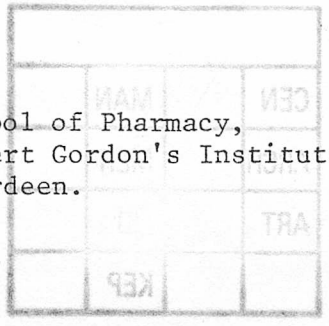

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May, 1978.

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CONTENTS

Summary

Lucanthone, a thioxanthenone bearing a diethylaminoethylamino substituent, is orally active against *Schistosoma mansoni* infections in man. The structure-activity relationships of lucanthone and related compounds have been extensively investigated and the need for a 1-dialkylaminoalkylamino substituent and 4-methyl group established. The symmetrical nature of the thioxanthenone ring presents an opportunity for producing 1,8-disubstituted compounds which may possess enhanced biological activity.

To investigate this situation a series of 1,8-di(dialkylamino-alkylamino)-4-methylthioxanthen-9-ones and 1,8-dipiperazinyl-4-methylthioxanthen-9-ones have been prepared from 1,8-dichloro-4-methylthioxanthen-9-one. The latter compound has been synthesised from 2-chloro-6-nitrotoluene and from 2-amino-4-chlorotoluene.

In the course of this work it was established that selective dehalogenation of 1,8-dichloro-4-methylthioxanthen-9-one occurred and this resulted in the isolation of a series of 1-substituted alkylamino-alkylamino- and piperazinyl-5-methylthioxanthen-9-ones. The structures of these compounds were elucidated by their n.m.r. and mass spectral properties. In addition an unambiguous synthesis of the parent 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one from 1-chloro-5-methylthioxanthen-9-one confirmed the site of dehalogenation at C-8.

The biological activity of these compounds has been examined by means of the heat denaturation profile of native DNA and by assessment of their schistosomicidal activity in experimental *S. mansoni* infections in mice. The results obtained show that 1,8-di(diethylaminoethylamino)- and 1,8-di(dimethylaminoethylamino)-substitution leads to an increased

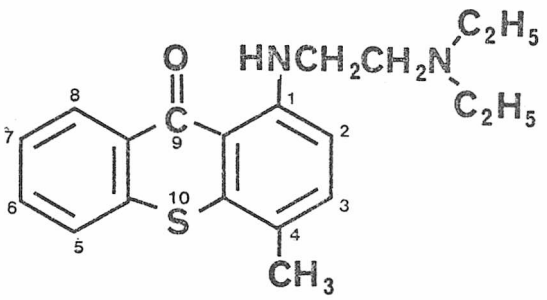
DNA interaction compared with lucanthone. A mechanism of interaction with DNA consistent with a proposed intercalation model is discussed for the 1,8- and 1-substituted methylthioxanthenones.

Enhanced schistosomicidal activity was found for 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one against S. mansoni infection in mice compared with lucanthone. The occurrence of activity with 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one has demonstrated that the presence of a methyl group para to the basic side chain in lucanthone and related compounds is not an absolute requirement for schistosomicidal activity.

INTRODUCTION

INTRODUCTION

The thioxanthenones became of interest in chemotherapy when the compound 1-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one, called Miracil D (1) was introduced for the treatment of schistosomiasis during the Second World War. This compound, now known by its official name lucanthone (1), has been extensively employed in the treatment of human schistosomiasis and has attracted interest in experimental cancer chemotherapy.

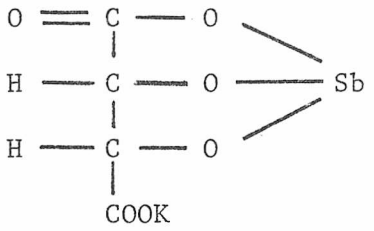


(1)[a]

Schistosomiasis is the term used to describe a number of disabling diseases in humans caused by trematode parasites which affect over 200 million people throughout the world;[1] the three main causative organisms being Schistosoma haematobium, S. mansoni and S. japonicum. These organisms have a complex life-cycle with alternating parasitic and free-living stages and involving two hosts: primates and the genera of snails, Bulinus, Biomphalaria and Oncomelonia.[2]

Prior to 1938 the main treatment for the disease had been inorganic trivalent antimonials, such as tartar emetic (2), introduced by Christopherson in 1918.[3]

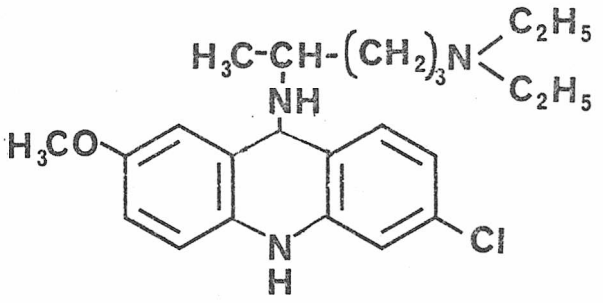
(a) The Chemical Abstracts system of ring numbering is employed in this thesis.



(2)

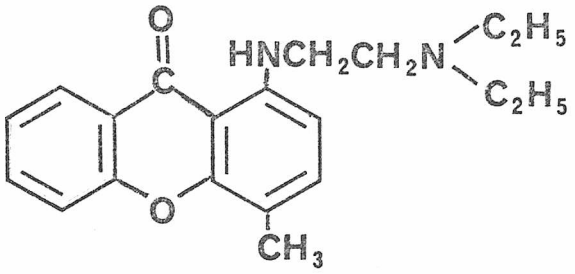
These compounds, whilst being effective, were extremely toxic and therefore unsuitable for mass chemotherapy.

Synthetic studies on acridine analogues by Mauss and Mietzch[4] in 1932, and subsequent investigation of antimalarial activity by Kikuth,[5] led to the introduction of mepacrine (3) which showed considerable activity against the malaria parasite.



(3)

Related xanthenones were also tested[6] but were not reported to have significant antimalarial activity. However, work begun by research workers at Bayer, Germany in 1932 resulted in the development of a technique for infecting small mammals with S. mansoni, and their employment in the screening of a large number of chemical compounds for schistosomicidal activity.[7] When the compounds synthesised by Mauss were tested in this newly established screening test slight activity was noted for the xanthenones. One compound, however, 1-[[2-(diethylamino)-ethyl]amino]-4-methylxanthen-9-one (4), subsequently called Miracil A (4) showed pronounced activity against S. mansoni infections in mice.[5]



(4)

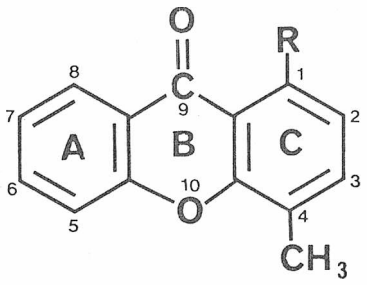
The discovery of schistosomicidal activity in the xanthenone series was a chemotherapeutic achievement, as these were the first orally effective, non-metallic, organic compounds found to possess activity against schistosomes.

A series of Miracil A analogues was prepared and investigated by Gönnert and Kölling[8] to find other active members of the series and certain structure-activity relationships emerged as summarised in Tables 1 and 2.

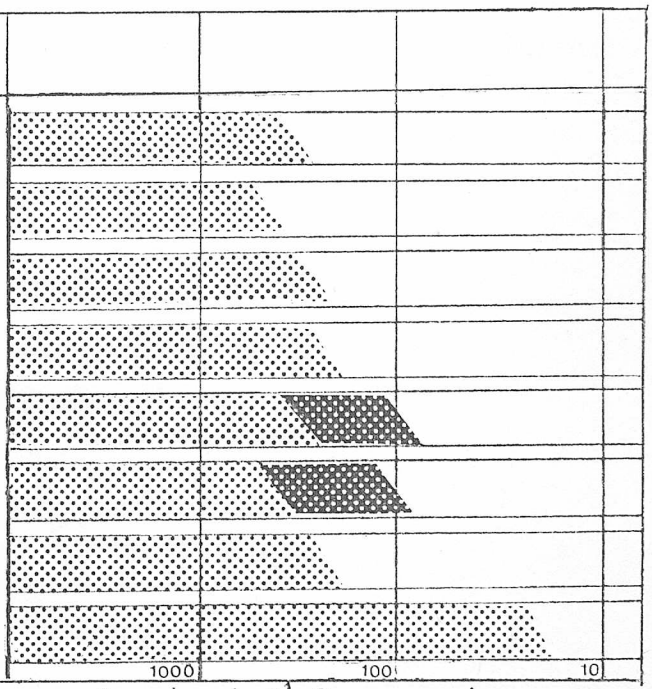
The results in Table 1 show that compounds containing only one nitrogen were inactive against schistosomes (5,6,7) and the diamino compound (8) without terminal alkyl substitution also had no schistosomicidal effect. Replacement of one ethyl group on the terminal nitrogen by hydrogen resulted in a compound (9) which had a slight increase in activity against S. mansoni and a reduced toxicity.

Table 1. Xanthenone derivatives; variations in the side chain

(Gönnert and Kölling[8])



No.	R
(5)	$-NH_2$
(6)	$-NHC_2H_5$
(7)	$-N(C_2H_5)_2$
(8)	$-NHCH_2CH_2NH_2$
(9)	$-NHCH_2CH_2NHC_2H_5$
(4) (Miracil A)	$-NHCH_2CH_2N(C_2H_5)_2$
(10)	$-NHCH_2CH(OH)CH_2N(C_2H_5)_2$
(11)	$-NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$



Dose mg kg^{-1} S. mansoni infection in mice.

In this and subsequent diagrams the doses are expressed in mg kg^{-1}. The clear area on the right covers the ineffective dose range, the black area indicates the "schistosomicidal range" and the stippled area the toxic range for each drug in S. mansoni infection in mice. (This type of presentation permits the demonstration of the graded effectiveness of individual doses together with the toxicity of the compound in question. In addition the gradual transition from toxic to tolerated and from effective to ineffective doses is illustrated).

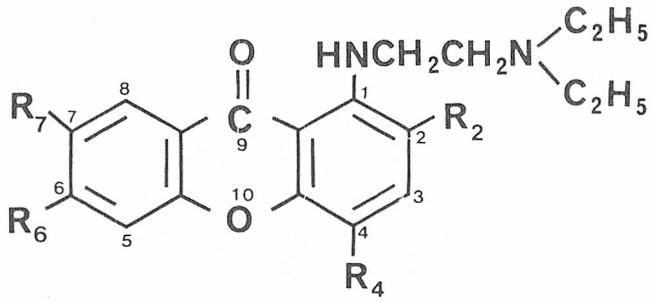
The data in Table 2 show that a methyl group at position 4 of the xanthenone ring was necessary for schistosomicidal activity (4, 14, 15, 16) as well as a basic side chain at position 1 in the molecule. Introduction of a methyl group at position 2 (4,17) caused total loss of activity. Gönnert and Kölling[8] reported that additional substituents

in position 5, 6, 7 or 8 proved to be advantageous and resulted in some increase in activity (14, 15, 16) although no results were quoted for compounds with substitution at positions 5 and 8. The introduction of a chlorine atom in position 6 (14) or a methoxy group in position 6 or 7 of the xanthenone molecule (15,16) was particularly interesting since the 6-chloro derivative of Miracil A (4) proved to be the most effective compound in mice and was later designated Miracil B (14).

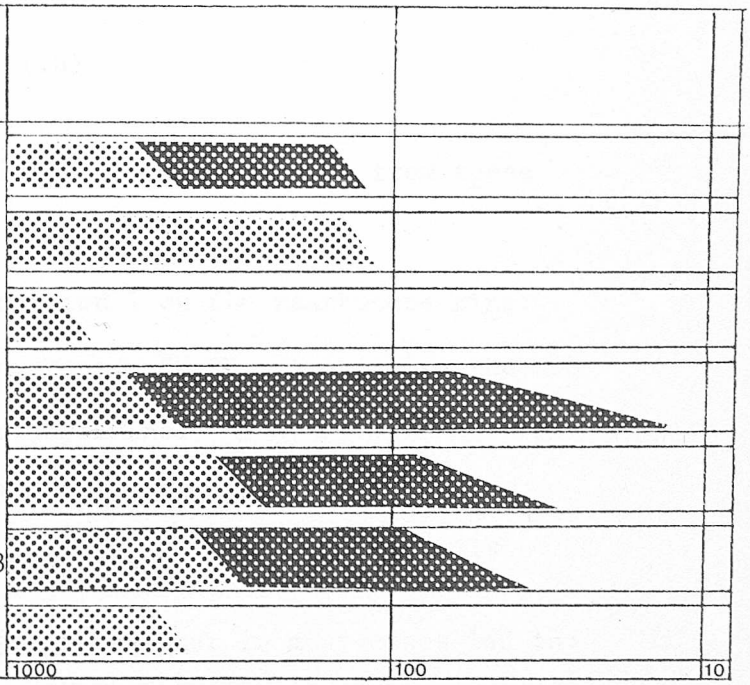
Transposition of the two substituents at positions 1 and 4, or the introduction of a higher alkyl group, methoxy group or halogen atom instead

Table 2. Xanthenone derivatives; variations in ring substituents

(Gönnert and Kölling[8])



No.	R_2	R_4	R_6	R_7
(4) (Miracil A)	-H	$-CH_3$	-H	-H
(12)	-H	$-C_2H_5$	-H	-H
(13)	-H	-Cℓ	-H	-H
(14) (Miracil B)	-H	$-CH_3$	-Cℓ	-H
(15)	-H	$-CH_3$	$-OCH_3$	-H
(16)	-H	$-CH_3$	-H	$-OCH_3$
(17)	$-CH_3$	$-CH_3$	-H	-H



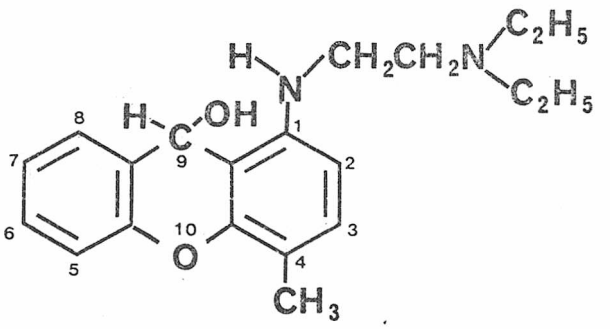
Dose mg kg^{-1}. S. mansoni infection in mice

(Explanation of diagram and conditions page 4)

of the methyl group at position 4 of the molecule, were reported to lead to loss of activity (12, 13).[8]

Besides the introduction of substituents into the ring system, particular attention was paid to variation of the basic side chain at position 1. An optimal efficiency was obtained when the two amino groups were separated by an alkyl chain of two or three carbon atoms. Moreover, a terminal mono-or diethylamino group proved to be especially favourable (4, 9).

Other changes in the molecule involved the carbonyl group at position 9. Reduction to the xanthydrol yielded Miracil C (18) which showed increased activity in mice infections of S. mansoni.



(18)

The key features of schistosomicidal activity emerging from these studies were:-

1. A diamino side chain at position 1 on the xanthenone ring.
2. An optimal side chain of distance $-CH_2CH_2-$ separating the two nitrogen atoms, i.e. aminoethylamino.
3. A terminal mono-or diethyl alkyl group on the side chain.
4. A methyl group at position 4, para to the side chain.[2,8]

Replacement of the ring oxygen by sulphur, in most cases led to improved activity, and on the basis of evaluation in mice it was

established that the thioxanthen-9-one analogue (1) was more active than Miracil A (4).[8]

This compound, designated Miracil D (1), now known by its approved name of lucanthone (1), was found to be active orally against human S. mansoni infections.[9]

Table 3 shows the schistosomicidal activity of the Miracils by different routes in mice. All four compounds could be administered favourably by mouth, an advantage in the mass administration of schistosomicides to large populations of humans in underdeveloped countries.

Table 3. Evaluation of oral, subcutaneous and intravenous routes of the schistosomicidal activity of the Miracils in mice.

(Archer and Yarinsky[2])

	Miracil A (4)		Miracil B (14)		Miracil C (18)		Miracil D (lucanthone)(1)	
Route	Dose	Rating	Dose	Rating	Dose	Rating	Dose	Rating
P.O.	165	C	65	C	65	C	125	C
	125	A	35	A	50	Tr	65	A
	100	Tr	16.5	Tr	33	0	35	Tr
	85	0	8.5	0			16.5	0
S.C.	165	C	125	C	65	A	125	C
	125	Tr	65	A	35	Tr	65	A
	85	0	35	Tr	16.5	0	35	0
			16.5	0				
I.V.	12.5	0	25	0			65	A
							35	0

P.O. = orally in suspension
S.C. = subcutaneous injection
I.V. = intravenous injection
0 = inactive, eggs excreted continuously for 3 weeks
Tr = some dead worms and brief cessation of egg excretion
A = no excretion of eggs for 3 weeks but relapse and live worms at autopsy
C = curative - no relapse, no live worms at autopsy

African strain of S. mansoni used, dosages given (mg kg^{-1}) for 6 consecutive days.

When these compounds were also studied in monkeys infected with the same strain of S. mansoni vastly different results were obtained, as shown in Table 4.

Table 4. Schistosomicidal activity of the Miracils on S. mansoni infections in monkeys.

(Archer and Yarinsky[2])

	Miracil A (4)		Miracil B (14)		Miracil C (18)		Miracil D (lucanthone) (1)	
Route	Dose	Rating	Dose	Rating	Dose	Rating	Dose	Rating
ORAL	20x2	0	100x2	0	25x2	0	20x1	C
							10x2	C

Dose mg kg^{-1}.

The sulphur analogue of Miracil B (14) was more active than Miracil D (1) in mice but was ineffective in the monkey. In mouse infections involving S. mansoni, Miracil B (14) was more effective than the other compounds, but in the monkey, lucanthone (1) showed higher activity, while Miracil B (14) produced results only when given in doses which caused vomiting. In large doses lucanthone (1) caused vomiting and diarrhoea, but no severe or fatal results were observed.

Few comparisons have been made between the different Miracils in man. Blair et al.[9] treated each of three patients, infected with S. mansoni, with one of the three Miracils A, B or C (4, 14, 18). The daily dose was initially 100 mg and continued for 6 days in the case of Miracil A (4) and Miracil C (18) and for 5 days in the case of Miracil B (14); it was then increased to 200 mg for a similar period. None of the patients showed any toxic signs whatsoever, the drugs had no apparent effect on the schistosome worms and egg production proceeded unhindered.

Subsequently these doses have been shown to be too small.[10]

Newsome and Halawani[11] treated eight patients with Miracil A (4). Four had severe infections of S. mansoni and were cured by three courses of treatment each consisting of 1 g twice a day for three days. Lucanthone (1) produced similar results and both compounds caused mild gastro-intestinal side effects.

Kikuth and Gönnert[7] have also reported that in experimental mice the therapeutic indices of the Miracils were very much affected by the age of the worm, as shown in Table 5.

Table 5. Therapeutic indices[(a)] of Miracil compounds in mouse S. mansoni infections

(Kikuth and Gönnert[7])

Age of schistosomes	Miracil A (4)	Miracil B (14)	Miracil C (18)	Miracil D (lucanthone) (1)
Sexually mature worms (48 days or more after infection in mice)	1:17	1:15	1:35	1:4
Immature worms (33 days after infection in mice)	1:1	1:4	1:1	1:1

(a) 'Chemotherapeutic index' defined by Ehrlich as the ratio of minimum curative dose: maximum tolerated dose.

However, in the case of lucanthone (1) age was not found to be so critical to schistosomicidal activity as in the other compounds.

The Miracil compounds were most active against sexually mature worms, and doses which killed mature schistosomes were ineffective against worms 4 and 15 days old.

Gönnert[12] has studied the effects of lucanthone (1) in mice. During

and after treatment the worms leave the mesenteric vessels and are carried to the liver. Coupled male and female worms separate and, depending on the strain of mice used, the schistosomes die one or two weeks after treatment has started. The first signs of degeneration of the worms are vacuolation of the cuticle and invasion of the parasite tissues by eosinophils and macrophages from the blood of the mouse. Of the worms which are not killed the affected males recover more quickly than the females, although these could recommence egg laying within a couple of months.

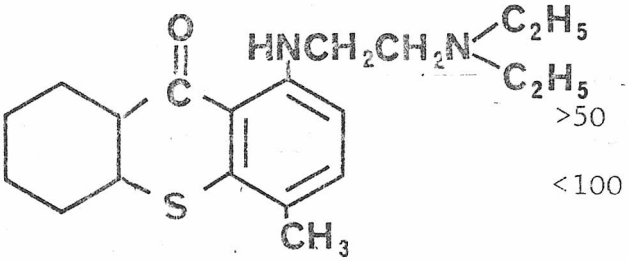
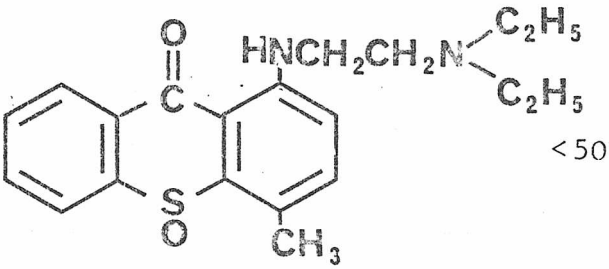
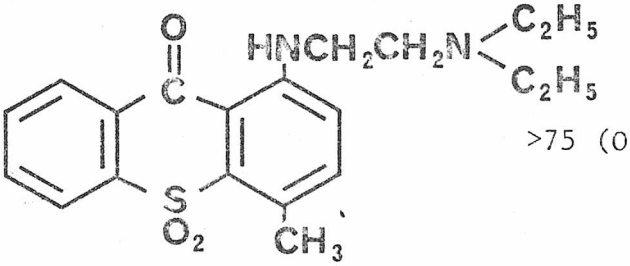
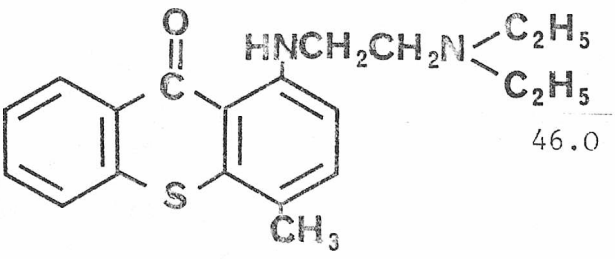
The Egyptian strain of S. mansoni has been found to be more resistant to lucanthone (1) than the Liberian strain.[13] The death rates were also affected by the strains of mice used and it was noted that heavy infections were proportionately more difficult to cure than lighter ones.

Lucanthone (1) has been found to be effective against S. mansoni infections in rabbits, Rhesus monkeys and golden hamsters, but when exhibited in the same doses to animals harbouring S. japonicum it had no effect on the worms and the discharge of eggs in the faeces was uninterrupted.[14]

Following the work on the Miracils by Gönnert and his colleagues[7,8,12,13] an extensive series of thioxanthenones have been prepared by Archer and Suter,[15] using synthetic methods similar to those employed by Mauss,[6] and these have been tested by Berberian and Freele.[16] The strain of S. mansoni used originated in Puerto Rico and the intermediate snail host was Biomphalaria glabrata. Female Swiss mice were infected by intraperitoneal injection of 250 cercariae per mouse. Male hamsters (Cricetus auratus) were each injected with 200 cercariae. Medication was begun 46 days after injection and continued for 5 days. The results of these drug evaluations are recorded in Tables 6, 11, 13, 14 and 16.

Table 6. Effect of modification of the thioxanthenone ring on schistosomicidal activity in S. mansoni infections

(Archer and Yarinsky[2])

No.	Compound	ED_{50} mg kg^{-1}*	% Reduction in live worms
		Mice	Hamster
(19)		>50 (28%) (P.O.) <100 (98%)	>50 (14%) (P.O.) <100 (89%)
(20)		<50 (94%) (P.O.)	>25 (26%) (P.O.) <50 (73%)
(21)		>75 (0%) (P.O.)	>12.5 (0%) (P.O.
(1) (lucanthone)		46.0 (P.O.)	9.8 (P.O.)

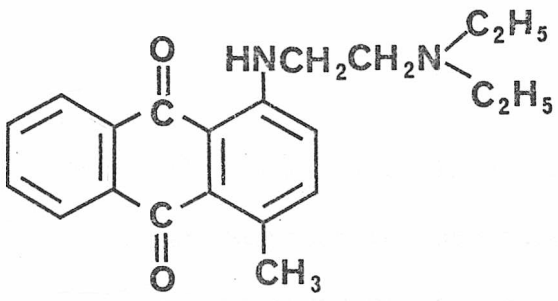
P.O. = given by oral suspension

* The ED_{50} is the dose which kills 50% of the schistosomes. In cases where ED_{50} values were not obtained, doses are given with the percentage mortality of adult worms shown in parenthesis.

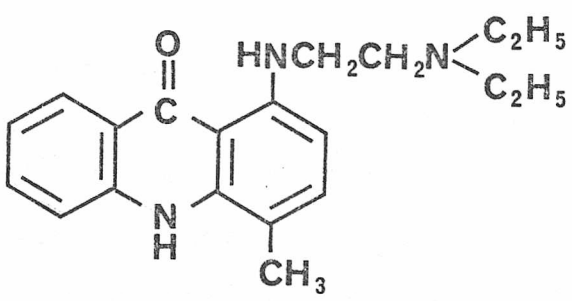
Oxidation of the sulphur atom of the thioxanthenone to form the sulphoxide (20) resulted in no loss in activity in the mouse but reduced schistosomicidal activity in the hamster compared with lucanthone (1) (Table 6); however, the sulphone (21) showed no activity at dose levels above the ED_{50} for lucanthone (1) in both species.

Archer and Yarinsky[2] have shown that the lucanthone analogue (19) with a fully saturated "A" ring retained schistosomicidal activity against S. mansoni in mice and hamsters but they also report that in no case studied was a compound found which was more schistosomicidal in the hamster than lucanthone (1) against S. mansoni. In the 76 compounds studied by Archer and colleagues a methyl group at the 4 position and a basic side chain in the 1 position of the thioxanthenone ring were found to be essential for schistosomicidal activity.

Certain compounds retaining this arrangement, with, however, variants in the nucleus, proved to be inactive; the anthraquinone (22) and the acridone (23) analogues of lucanthone (1) were ineffective in laboratory animals.[17]



(22)



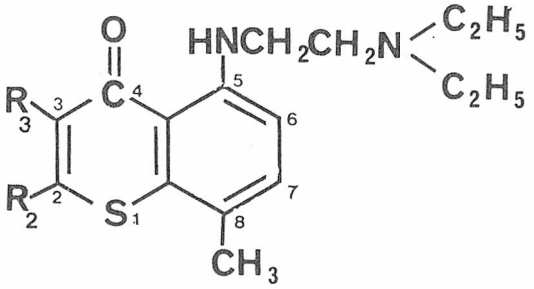
(23)

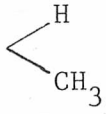
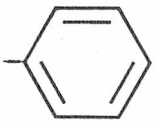
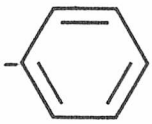
Gönnert and Kölling[8] have reported that the elimination of the unsaturated carbocyclic ring "A" completely to yield a thiochrome led

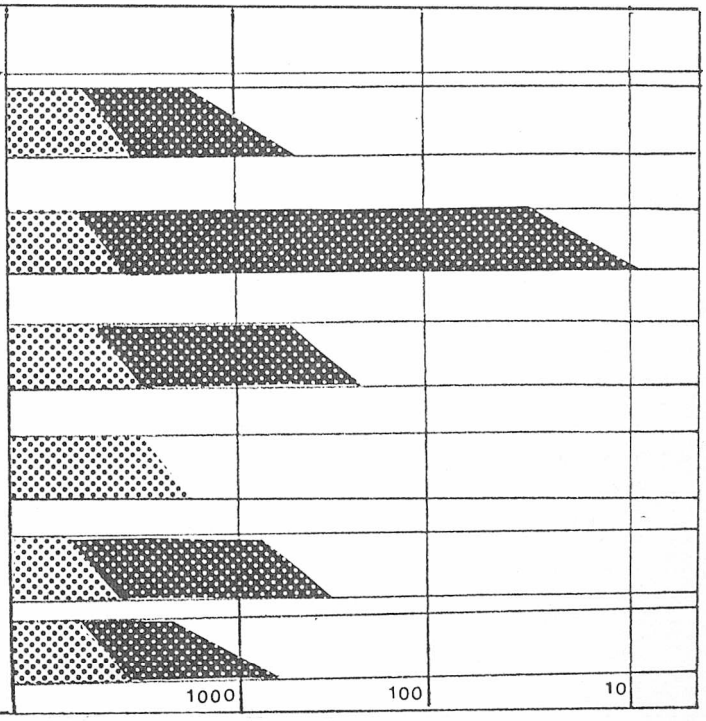
to little loss of activity (Table 7).

Table 7. Schistosomicidal activity of thiochrome derivatives

(Gönnert and Kölling[8])



No.	R_2	R_3
(24)		$-H_2$
(25)	$-CH_3$	$-H$
(26)		$-H$
(27)	$-H$	
(28)	$-CH_2-CH_2-CH_2-$	
(19)	$-CH_2-CH_2-CH_2-CH_2-$	



Dose mg kg^{-1}. S. mansoni infection in mice

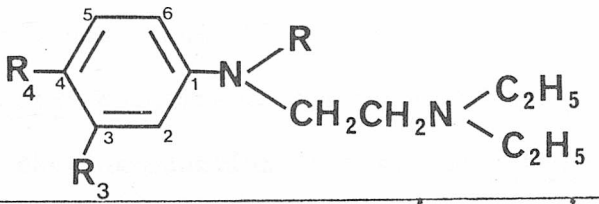
(Explanation of diagram and conditions page 4)

While the thiochrome class yielded no compound with increased effectiveness compared to lucanthone (1) a continuation of studies on thiochromes has proved of interest. A basic side chain in position 5 and a methyl group in the 8 position are prerequisites for schistosomicidal activity. The presence of a third substituent in the 2 position produced a series of compounds which retained schistosomicidal activity, the most

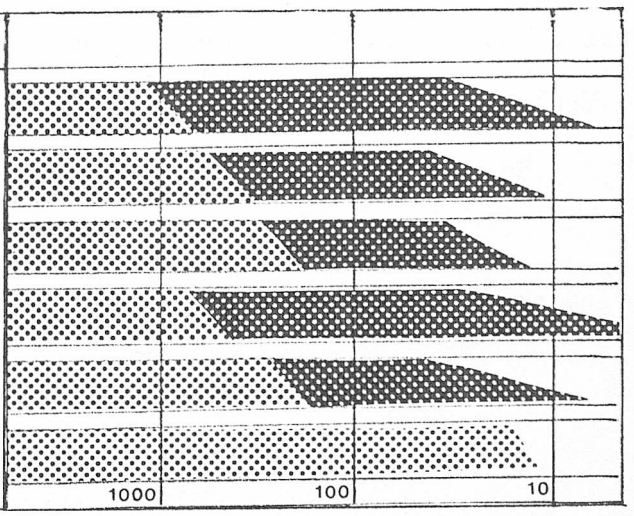
potent of which was compound (25). Substitution of a phenyl group at position 2 (26) gave activity against schistosomes but not at position 3 (27). Compounds (28) and (19) substituted at positions 2 and 3 by a carbon bridge retained schistosomicidal activity. Further, removal of ring "A" and "B" of the thioxanthenone molecule led to derivatives of 4-aminotoluene and 4-aminoxylene. In the anilinic series shown in Table 8 the principle of having a 1-N-alkylaminoalkylamino side chain and 4-methyl group on an aromatic ring still proved to be effective.

Table 8. Schistosomicidal activity of aniline derivatives.

(Gönnert and Kölling[8])



No.	R_4	R_3	R
(29) (Mirasan)	$-CH_3$	$-C\ell$	$-H$
(30)	$-CH_3$	$-Br$	$-H$
(31)	$-CH_3$	$-NO_2$	$-H$
(32)	$-CH_3$	$-C\ell$	$-CH_3$
(33	$-CH_3$	$-C\ell$	$-n-C_3H_7$
(34)	$-OCH_3$	$-C\ell$	$-H$



Dose mg kg^{-1}. S. mansoni infection in mice.

(Explanation of diagram and conditions page 4)

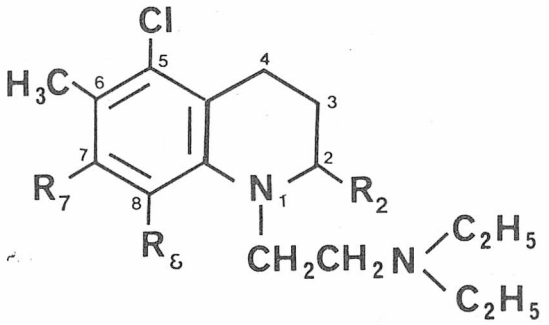
Mauss, Kölling and Gönnert[18] found that substitution at the 3 position of the benzene ring was necessary for activity. This substitution could be a halogen atom, a nitro- or a cyano- group. Substitution with chlorine gave a compound, Mirasan (29), which has found some clinical use.

Replacement of the methyl group in position 4 by another substituent resulted in completely ineffective compounds (29,34). The introduction of a second aliphatic group onto the proximal nitrogen atom of Mirasan (29) created compounds with retained schistosomicidal activity in mice (32, 33). Compound (32)was more active than Mirasan (29) in mice but has not been reported as being of use clinically. Numerous other substitutions in the ring and modifications of the side chain were permissible. The 2,5-dimethyl and 2,6-dichloro derivatives had reduced activity. The Mirasans were more active than the Miracils in mice but were inactive in monkeys.

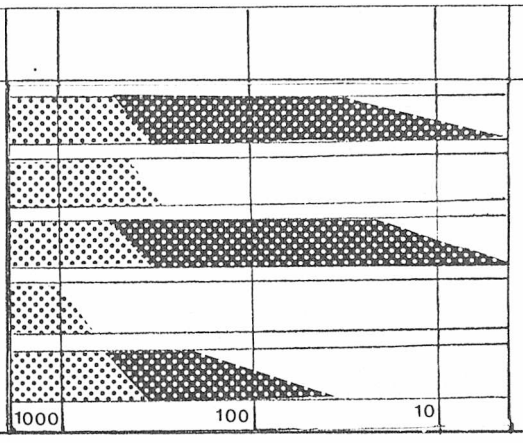
These workers have also reported on a group of tetrahydroquinolines and quinaldines (Table 9) derived from Mirasan (29).[18] Compound (35) with an ortho chlorine to the methyl group as in Mirasan (29) was active, however, the introduction of a second ortho chlorine at position 7 led to loss of schistosomal activity (36). Substitution of a methyl group at position 8 (37) led to no loss of activity compared with compound (35) but the introduction of a second methyl group at R_2 led to loss of activity against S. mansoni in mice (38). Compound (39) with substitution in the 2 and 8 positions showed decreased schistosomicidal activity compared with (35).

Table 9. Schistosomicidal activity of tetrahydroquinoline derivatives

(Gönnert and Kölling[8])



No.	R_2	R_7	R_8
(35)	-H	-H	-H
(36)	-H	-Cℓ	-H
(37)	-H	-H	$-CH_3$
(38)	$-CH_3$	-H	$-CH_3$
(39)	$-CH_3$	-H	-Cℓ



Dose mg kg^{-1}. S. mansoni infection in mice

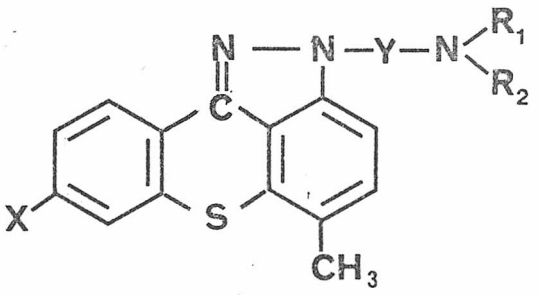
(Explanation of diagram and conditions page 4)

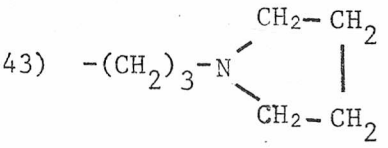
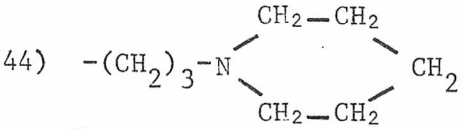
Elslager at al.[19] have recently reported on a series of benzothiopyranoindazoles (Table 10) in which the carbonyl of the thioxanthenone is replaced by an imino group thus giving an increase in the complexity of the ring structure. These were prepared by the condensation of an alkylaminoalkylhydrazine with a 1-chloro-4-methylthioxanthen-9-one forming a bridge between the C_1 and C_9 positions of the thioxanthenone.

Table 10. Oral schistosomicidal activity of benzothiopyrano (4,3,2-cd) indazoles in S. mansoni infections in mice

(Archer and Yarinsky[2])

Compound

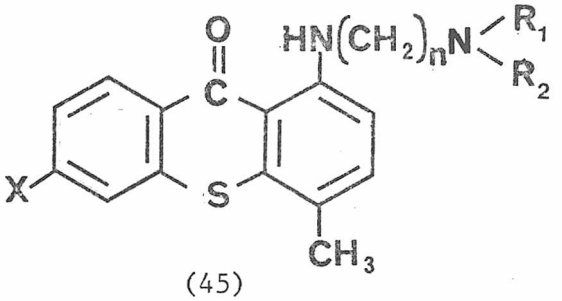


No.	Y-NR_1R_2	X	Regimen mg $kg^{-1}d^{-1}$ x days	% Reduction in live worms
(40)	$-(CH_2)_2N(C_2H_5)_2$	-H	33x14 (D)	32
(41)	$-(CH_2)_2N(C_2H_5)_2$	-Cℓ	389x14 (D)	88
(42)	$-(CH_2)_2N(CH_3)CH(CH_3)_2$	-Cℓ	214x14 (D)	75
(43)		-Cℓ	55x14 (P.O.)	38
(44)		-Cℓ	181x14 (D) 200x5 (P.O.)	66 61

D = given in diet P.O. = given orally in suspension d = day

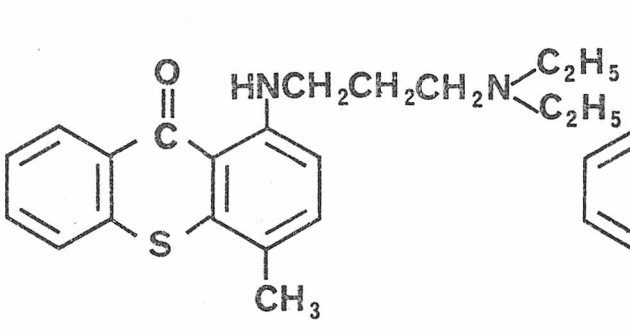
The highest activity in mice infections was shown by compound (41) which shows structure-activity requirements similar to those found in thioxanthenones, but the dose was much larger in this case.

Archer et al.[17] prepared a number of active compounds of general formula (45). In one series of substituted thioxanthenones of varied

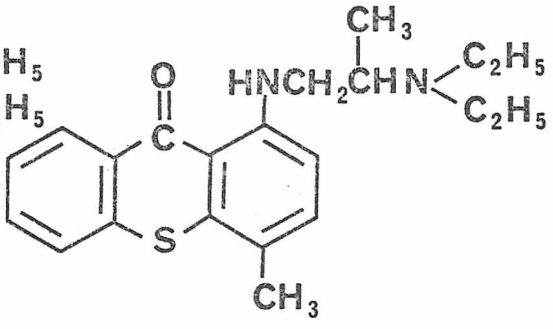


(45)

side chain length the activity was found to drop markedly when the side chain exceeded two carbons separating the nitrogen atoms. Thus 1-(3-diethylaminopropylamino)-4-methylthioxanthen-9-one (46) was inactive but the isomeric 1-(2-diethylaminopropylamino)-4-methylthioxanthen-9-one (47) was about equal to lucanthone (1) in effectiveness against S. mansoni in mice.



(46)



(47)

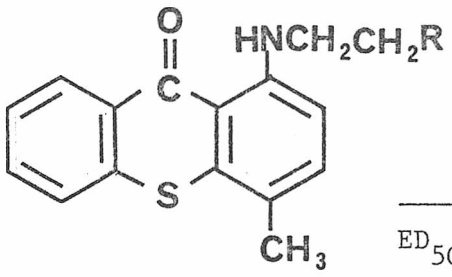
The dimethyl, dipropyl, dibutyl, dipentyl, and dihexyl homologues of lucanthone (1) were also found to be less active than the diethyl parent compound. Table 11 illustrates other variations at the 1 position of the thioxanthenone nucleus tested.[17]

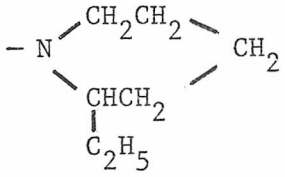
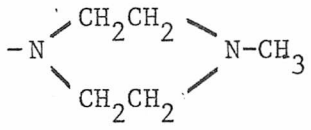
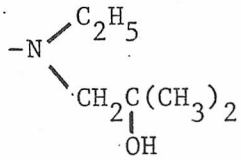
Replacement of the terminal ethyl groups of lucanthone (1) by n-butyl groups (51) led to a reduction of activity in mice; however, Archer claims that this was compensated for by the reduced toxicity, resulting in a favourable therapeutic ratio.[20] Replacement of the terminal ethyl groups by the N-methylpiperazinyl group (52) led to loss of schistosomicidal activity at the dose tested in mice and hamsters, while the ethylpiperidine

Table 11. Effect of variation at the 1-position of the thioxanthenone nucleus on schistosomicidal activity

(Archer and Yarinsky[2])

Compound



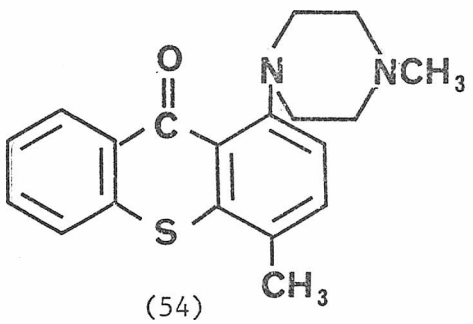
No.	R	ED_{50} mg kg^{-1}* Mice	% Reduction in live worms (S. mansoni) Hamsters
(48)	$-NHC_2H_5$	>50(20%)(P.O.)	>50(0%)(P.O.) >12.5(0%)(I.M.)
(49)		42.5(P.O.)	22.5(P.O.) >62(0%)(I.M.)
(50)	$-NHCH_2C(CH_3)_2$ (OH)	51.0(P.O.)	>50(42%)(P.O.) ≤100(65%)(I.M.)
(51)	$-N(C_4H_9)_2$	155.0(P.O.)	84.0(P.O.)
(52)		>25(0%)(P.O.) >25(0%)(I.M.)	>6.25(0%)(P.O.) >6.25(0%)(I.M.)
(53)		79.0(P.O.)	52.0(P.O.)
(1) (lucanthone)	$-N(C_2H_5)_2$	46.0(P.O.) >150(I.M.)	9.8(P.O.) >50(I.M.)

P.O. = given by oral suspension. I.M. = intramuscular injection

* Explanation page 11.

compound (49) showed similar oral activity to lucanthone (1) in mice but reduced activity in hamsters. Unlike its xanthenone analogue (9), compound (48), with only one terminal ethyl group had reduced activity to the diethyl analogues (4, 1) in mice.

While the terminal piperazinyl compound (52) had little activity Kushner[21] has reported that the replacement of the entire side chain by a piperazine ring at position 1 of the thioxanthenone ring led to an active series of compounds. The compound 1-[1-(4-methyl-1-piperazinyl)]-4-methylthioxanthen-9-one (54) was active against S. mansoni infections in mice, Table 12.



(54)

Table 12. Activity of 1-[1-(4-methylpiperazinyl)]-4-methylthioxanthen-9-one (54) against S. mansoni in mice.

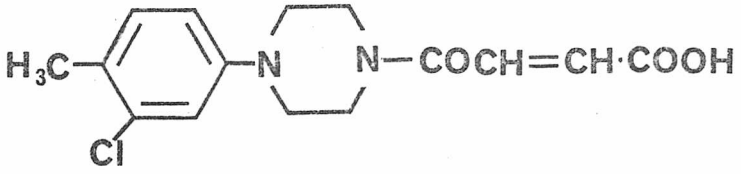
(Kushner[21])

Regimen mg kg^{-1} x days	% Reduction in live worms
10 Ip. b.i.d. x 12	22
100 P.O. b.i.d. x 6	100
150 P.O.- 1 dose	78
300 P.O.- 1 dose	83

Ip. = intraperitoneal

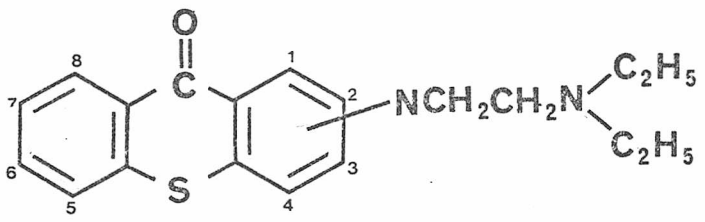
b.i.d. = twice a day

This compound (54) was developed from an N-substituted piperazine derivative (55) of Mirasan which had previously been found to be very active against S. mansoni in mice.[22]



(55)

Mann and Turnbull[23] found that compounds in which the basic side chain was in the 2, 3 or 4 positions were inactive but these compounds were also without the methyl group at position 4 (56). The introduction



(56)

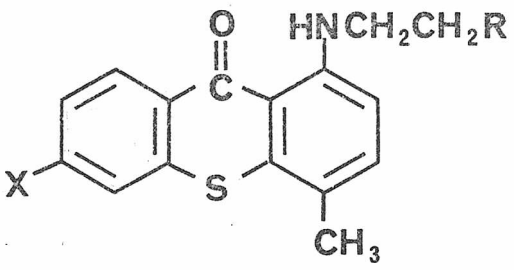
of additional substituents to the thioxanthenone ring in positions 5, 6 and 7 has been shown to be advantageous and results in some increase in activity, whereas additional substitution at positions 2 and 3 caused loss of activity against S. mansoni in mice.[8]

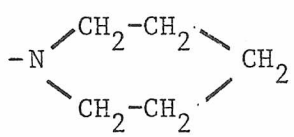
A number of active compounds have been prepared in which variations in the dialkylaminoalkylamino basic side chain were associated with chloro-substitution in the 6-position, (Table 13).[15] In contrast to Kikuth's observations[5] these compounds were shown to have a higher therapeutic index than lucanthone (1) in hamsters and mice but these have not proved of value in the clinical field.[24]

Table 13. Schistosomicidal activity of 6-chlorothioxanthenone derivatives in mice and hamsters

(Archer and Yarinsky[2])

Compound



No.	R	X	ED_{50} mg kg^{-1}* Mice	% Reduction in live worms hamsters
(1) (lucanthone)	$-N(C_2H_5)_2$	-H	46.0(P.O.) >150(I.M.)	9.8(P.O.) >50(I.M.)
(57)	$-N(C_2H_5)_2$	-Cℓ	18.5(P.O.)	>3.1(37%)(P.O.) <6.25(58%)
(58)	$-NHC_2H_5$	-Cℓ	<25(88%)(P.O.) >25(0%)(I.M.)	>100(0%)(P.O.)
(59)		-Cℓ	25.0(P.O.)	>25(0%)(P.O.)

P.O. = orally in suspension. I.M. = intramuscular injection

* Explanation page 11. S. mansoni infection

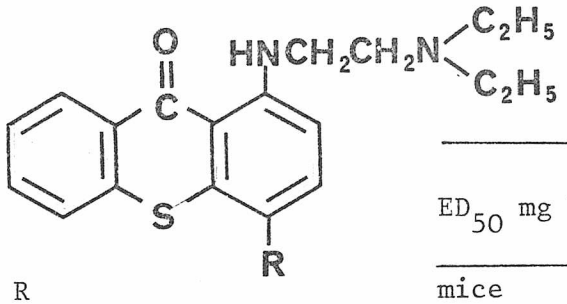
Standen[24] suggests that the variation in results between the two laboratories may be accounted for by the difference in strain and hence the sensitivity of the parasite used, the one used by Archer[15] being a Puerto Rican strain of S. mansoni and that used by Kikuth[5] at Bayer, being Liberian.

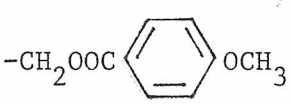
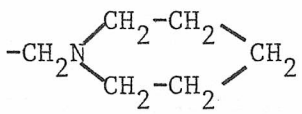
Extensive modifications to the 4 position of lucanthone (1) have been reported by Archer and Yarinsky[2] in an attempt to further define the structure-activity relationships (Table 14).

Table 14. Effect of variation of the substituent at the 4-position of the thioxanthenone molecule in S. mansoni infections

(Archer and Yarinsky[2])

Compound



No.	R	ED_{50} mg kg^{-1}* mice	% Reduction in live worms hamsters
(1) (lucanthone)	$-CH_3$	46.0(P.O.) >150(I.M.)	9.8(P.O.) >50(I.M.)
(60)	$-CH_2NH_2$	>100(0%)(P.O.)	>50(toxic)(P.O.)
(61)	-CHO	96.0(P.O.)	6.25(P.O.) >6.25(0%)(I.M.)
(62)	-COOH	>100(0%)(P.O.)	>3.1(0%)(P.O.)
(63)	-H	>400(0%)(P.O.) >25(0%)(I.M.)	>200(0%)(P.O.)
(64)	$-CH_2OOCCH_3$	13.9(P.O.) 10.9(I.M.)	0.75(P.O.) 1.34(I.M.)
(65)	$-CH_2OCH_3$	48.0(P.O.) >25(0%)(I.M.)	c.a. 80(P.O.)
(66) (hycanthone)	$-CH_2OH$	14.2(P.O.) 13.0(I.M.)	0.82(P.O.) 0.93(I.M.)
(67)	$-CH_2OOCC_6H_5$	>25(37%)(P.O.) <400(100%)(P.O.)	<25(99%)(P.O.)
(68)		>25(11%) <400(100%)(P.O.) >50(0%)(I.M.)	1.5(P.O.) <25(69%)(I.M.)
(69)		>200(0%)(P.O.)	>3.1(0%) <200(88%)(P.O.) >25(0%)(I.M.)
(70)	$-CH_2OOCH$	>25(9%)(P.O.) >7.5(5%)(I.M.)	<3.1(85%)(P.O.) <25(92%)(I.M.)
(71)	$-NO_2$	>100(0%)(P.O.)	-
(72)	$-CHOHCH_3$	>200(0%)(P.O.)	>100(0%)(P.O.)

P.O. = orally in suspension. I.M. = intramuscular injection

* Explanation see page 11.

Removal of the methyl group (63) caused total loss of activity, and replacement by other simple substituents such as methylamine (60), carboxylic acid (62) and nitro (71) groups also led to loss of activity in mice.

Considerable species variation was seen in the effect of substitution. Compound (60) was toxic to the hamster while compound (68) showed high intramuscular activity in the hamster and low activity in the mouse. The piperidine compound (69) was inactive in the mouse yet showed some oral activity in the hamster. The aldehyde (61) was more active than lucanthone (1) orally in the hamster but much less active in the mouse.

The esters (67) and (70) retain some activity but the hydroxymethyl derivative now known as hycanthone (66) and the acetate (64) were more active than lucanthone (1) against S. mansoni in the mouse and hamster.

Complete cures have been achieved in mice with doses of 150 mg $kg^{-1}d^{-1}$ x 5 days P.O. with lucanthone (1) and 100 mg $kg^{-1}d^{-1}$ x 5 days P.O. with hycanthone (66).[16] In hamsters complete irradication was not achieved with a dose of 50 mg $kg^{-1}d^{-1}$ x 5 days P.O. with lucanthone (1) but could be accomplished with 12.5 mg $kg^{-1}d^{-1}$ x 5 days P.O. with hycanthone (66). Intramuscular administration of lucanthone (1) showed no activity in either the mouse or the hamster yet a 50 mg kg^{-1} I.M. dose of hycanthone (66) effected a 90% reduction in live worms. In the Cebus monkey 10 mg $kg^{-1}d^{-1}$ x 5 days completely irradicated the worms in an S. mansoni infection; therefore hycanthone (66) showed a significant schistosomicidal activity in primates. This raised the question as to whether hycanthone (66) was an active metabolite of lucanthone (1) in primates. Newsome and Robinson[25] obtained eleven distinct chromatographic fractions from the urine of patients treated with lucanthone (1). None of these fractions, including lucanthone (1) itself, was active in vitro against schistosomes. Kikuth and Gönnert[7] had noted that lucanthone (1) was more effective in monkeys

than in mice and later after human trials had progressed it was found that the compound was more active in monkeys than man. Lucanthone (1) cured S. mansoni infections in a single dose of 20 mg kg^{-1} body weight in monkeys[10] but in clinical trials in man a total dose of 100-120 mg kg^{-1} was needed over three to five days.[7]

Struffe[26] noted that lucanthone (1) was metabolised more extensively in the monkey than in man and this observation suggested that a metabolic transformation product of lucanthone (1) was indeed the therapeutic active compound. He attempted to isolate an active metabolic product from the urine of a number of species of animal and man, treated with lucanthone (1). In general the urine samples were acidified and the components fractionated into acid, basic and neutral materials. Thus the major metabolic product in mouse urine was found to be the sulphone (21) and in monkey urine the sulphoxide (20). Neither of these metabolites was particularly active in S. mansoni infections in mice and hamsters.[2]

Rosi et al.[27] reported at this time on the discovery of hycanthone (66). The addition of lucanthone (1) to fermentation media of Aspergillus sclerotiorum led to its rapid oxidation to hycanthone (66) as well as to the corresponding aldehyde (61) and acid (62) (Fig. 1).

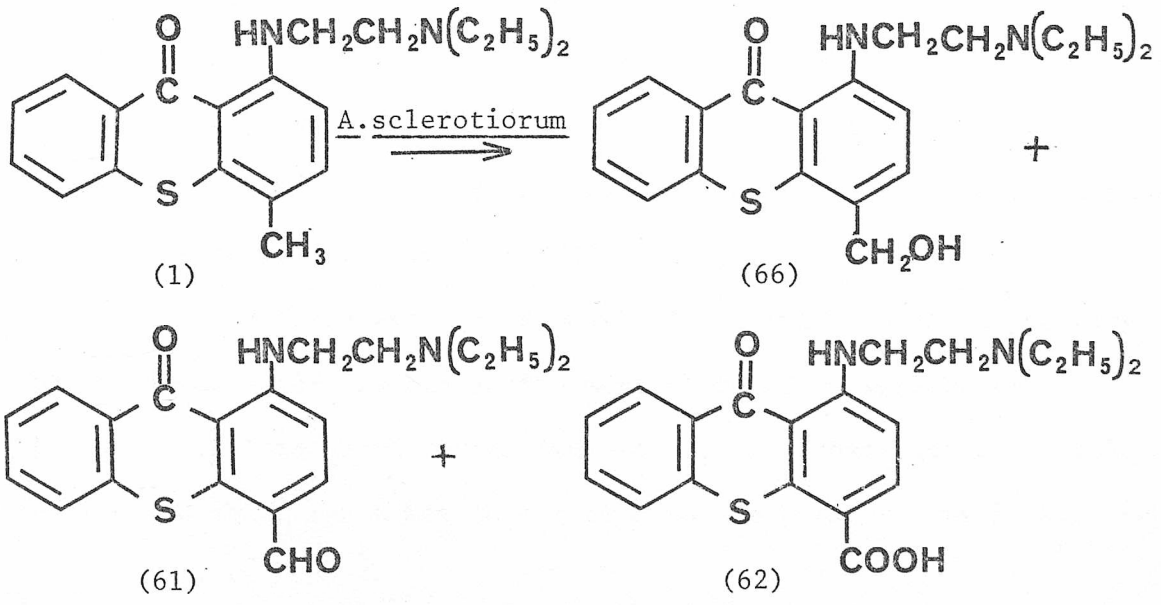
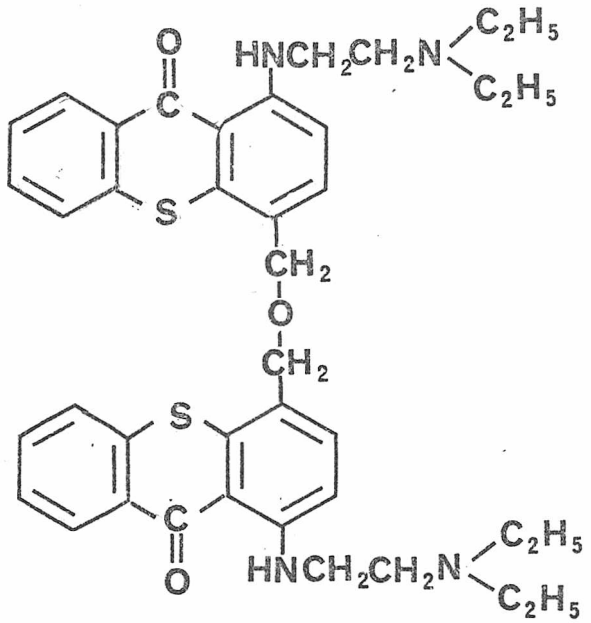


Fig. 1. Microbiological oxidation of lucanthone (1)

The hydroxylated derivative (66) was readily oxidised to the aldehyde (61) and the acid (62) thus establishing a possible metabolic relationship. It was observed that hycanthone (66) was acid sensitive and could be converted readily in dilute acid to the structure (73).



(73)

With this new information at hand these workers[27] repeated Struffe's experiments omitting the acid treatment. Thin-layer chromatograms revealed only trace amounts of hycanthone (66). However, when the urine was treated with β-glucuronidase, hydrolysis of the hycanthone glucuronide occurred and the metabolite was identified as hycanthone (66). This was found to be the next most abundant metabolite to lucanthone sulphoxide (20) in monkey urine. Hycanthone (66) is also metabolised by the monkey and the principle metabolites are the sulphoxide (74) and the desethylhycanthone (75) (Fig. 2).[28]

Archer and Yarinsky[2] have summarised the evidence for suggesting that hycanthone (66) is the active metabolite of lucanthone (1). Hycanthone (66) is about three times more active than lucanthone (1), in mice and about ten times more active in the hamster; it is also the

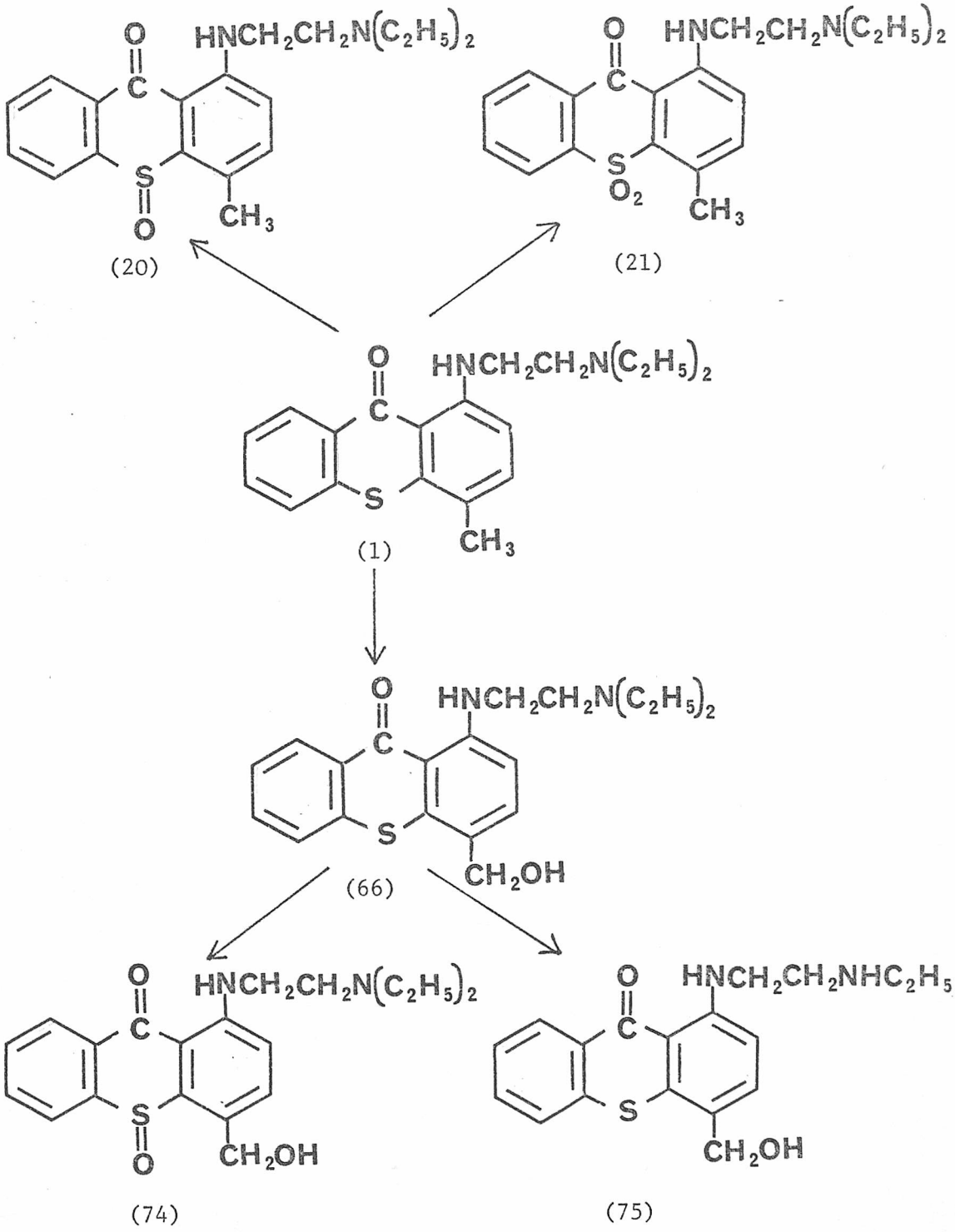


Fig. 2. Metabolic conversions of lucanthone (1) and hycanthone (66) in the monkey.

most active metabolite found in monkey urine. Lucanthone (1) is inactive when administered by intramuscular injection, in contrast however a 0.8 mg kg^{-1} (I.M.) dose of hycanthone (66) was as active as the same dose given orally for 5 days. The observed species differences can be correlated to the ability of the host to carry out the requisite enzymatic hydroxylation of lucanthone (1). Lastly, tritiated hycanthone can be detected in the adult S. mansoni after treatment of mice with intramuscularly administered tritium labelled lucanthone.

It is probable that previous investigations into discovering an active metabolite of lucanthone (1) in urine, had failed because of the acid sensitivity of hycanthone (66). The formation of a glucuronide necessitated an enzymatic rather than a chemical hydrolysis to liberate the free hycanthone (66).

Newsome and Robinson[25] and Elslager[19] have reported that hycanthone (66) has no activity in vitro although in both cases the test conditions were inadequate as even the untreated worms did not survive more than 30 days.

Interlaboratory comparison of the evaluation of hycanthone (66) has been rendered difficult because of the differences in strains of S. mansoni used for infections, together with differences in modes of administration and evaluation of activity as has been discussed by Farah and his colleagues[29] (Table 15).

Table 15. Efficacy of a single intramuscular injection of hycanthone (66) against S. mansoni strains of different origins.

(Farah et al.[29])

Source of schistosome strain	Origin of schistosome strain	Hycanthone efficacy	
		ED_{50} mg kg^{-1} (95% confidence limits*)	
		Mice	Hamsters
Sterling-Winthrop Research Institute	Puerto Rico	12(6.9-20)	1.46(0.88-2.44)
University of Michigan	St. Lucia	16(11-24)	1.64(1.15-2.38)
	Puerto Rico	34(25-49)	5.5(3.6-10.1)
Cheever	St. Lucia	20(14-30)	2.7(1.9-3.6)

* The Bliss Method for Quantal Response Data was employed for estimating the dose response curves.

Despite the differences in activity against different strains of S. mansoni hycanthone (66) is however an extremely effective compound against infections in mice and hamsters.

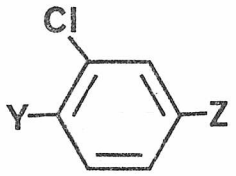
Following the increased schistosomicidal activity achieved by hydroxymethylation of the 4-position of lucanthone (1) a study was made of the effect of hydroxymethylation para to an alkylamino substituent in other active aromatic compounds.

Generally the introduction of the hydroxymethyl group led to enhanced activity over the methyl analogue (Table 16).

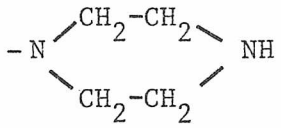
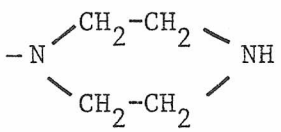
Table 16. Effect of a hydroxymethyl group in a variety of active compounds in S. mansoni infections

(Archer and Yarinsky[2])

Compound



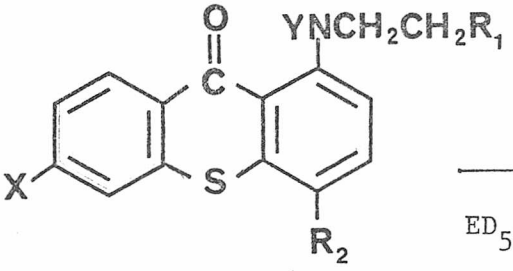
Mirasans

No.	Y	Z	ED_{50} ± s.e.* mg $kg^{-1}d^{-1}$ x 5 days mice	hamster
(29) (Mirasan)	$-CH_3$	$-NHCH_2CH_2N(C_2H_5)_2$	121.0±2.1	53.0±13.4
(76)	$-CH_2OH$	$-NHCH_2CH_2N(C_2H_5)_2$	15.0±2.8	7.0±0.9
(77)	$-CH_3$		5.2±0.7	>100.0
(78)	$-CH_2OH$		2.1±0.3	3.0±0.4

Continued

Table 16. Continued

Compound



Thioxanthenones

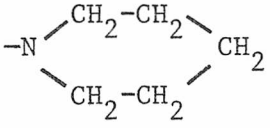
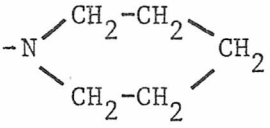
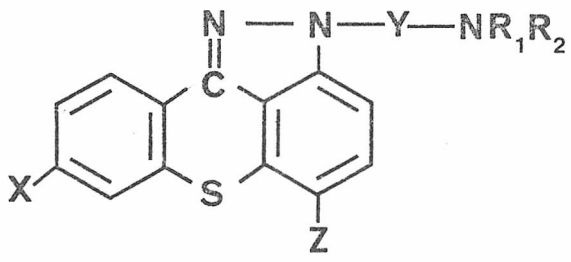
No.	X	Y	R_1	R_2	ED_{50} mg kg^{-1}* mice	% Reduction in live worms hamsters
(1) (lucanthone)	-H	-H	$-N(C_2H_5)_2$	$-CH_3$	46.0(P.O.) >150(I.M.)	9.8(P.O.) >50(I.M.)
(66) (hycanthone)	-H	-H	$-N(C_2H_5)_2$	$-CH_2OH$	14.2(P.O.) 13.0(I.M.)	0.82(P.O.) 0.93(I.M.)
(48)	-H	-H	$-NHC_2H_5$	$-CH_3$	>50(20%)(P.O.)	>50(0%)(P.O.) >12.5(0%)(I.M.)
(79)	-H	-H	$-NHC_2H_5$	$-CH_2OH$	19.5(P.O.)	2.45(P.O.)
(80)	-H	-H		$-CH_3$	>50<100(62%) (P.O.)	25.0(P.O.)
(81)	-H	-H		$-CH_2OH$	28.0(P.O.)	5.0(P.O.) <12.5(89%) (I.M.)
(82)	-H	$-CH_3$	$-N(C_2H_5)_2$	$-CH_3$	>25(45%)(P.O.) >25(0%)(I.M.)	>6.25(0%)(P.O.) >6.25(2%)(I.M.)
(83)	-H	$-CH_3$	$-N(C_2H_5)_2$	$-CH_2OH$	46(P.O.) >25(12%)(I.M.)	3.8(P.O.) >6.25(3%)(I.M.)
(57)	-Cℓ	-H	$-N(C_2H_5)_2$	$-CH_3$	18.5(P.O.)	>3.1(37%) <6.25(58%)(P.O.)
(84)	-Cℓ	-H	$-N(C_2H_5)_2$	$-CH_2OH$	13.0(P.O.)	-
(58)	-Cℓ	-H	$-NHC_2H_5$	$-CH_3$	<25(88%)(P.O.)	>100(0%)(P.O.)
(85)	-Cℓ	-H	$-NHC_2H_5$	$-CH_2OH$	<25(100%)(P.O.)	<6.25(90%)(P.O.)

Table 16. Continued

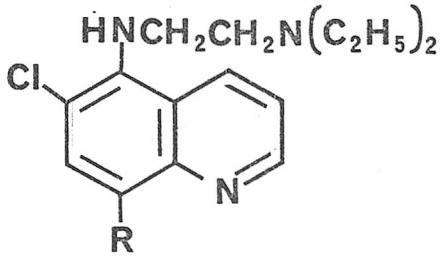
Compound



Chloroindazoles

No.	$Y-NR_1R_2$	X	Z	Regimen mg $kg^{-1}d^{-1}$ x days mice	% Reduction in live worms
(40)	$(CH_2)_2N(C_2H_5)_2$	-H	$-CH_3$	133 x 14 (D)	32
(86)	$(CH_2)_2N(C_2H_5)_2$	-H	$-CH_2OH$	143 x 14 (D)	72
(41)	$(CH_2)_2N(C_2H_5)_2$	-Cℓ	$-CH_3$	400 x 1 (P.O.)	60
(87)	$(CH_2)_2N(C_2H_5)_2$	-Cℓ	$-CH_2OH$	100 x 1 (P.O.)	72
((66)	hycanthone			100 x 5 (P.O.)	81)

Compound



Quinolines

No.	R	Route	ED_{50}* mg $kg^{-1}d^{-1}$ x 5 days (P.O.) mg $kg^{-1}d^{-1}$ x 1 day (I.M.) mice	hamsters
(88)	$-CH_3$	P.O.	23.0	>200
		I.M.	39.5	>200
(89)	$-CH_2OH$	P.O.	17.0	c.a. 70
		I.M.	20.00	>100

P.O. = given orally in suspension. D = given in diet
I.M. = intramuscular injection

* Explanation see page 11.

Some of the compounds tested (76,78) were very active in mice and hamsters but were not active in primates and so no further development has been made. Compound (82) may be slightly more active than its hydroxymethyl analogue (83) orally in mice, and was about as active intramuscularly in the hamster, but this was the only anomaly to the increased activity of the hydroxymethyl analogues.

None of the 6-chloro analogues were more effective than hycanthone (66) itself in hamsters either by oral or intramuscular routes. Archer and Yarinsky[2] conclude that in the thioxanthenone series hycanthone (66) embodies all the necessary structural features for high schistosomicidal activity. Hycanthone (66) has been shown to be well tolerated and highly effective parenterally in man.[30] However Hulbert, Bueding and Hartman[31] have summarized reports from a number of laboratories which indicate that hycanthone (66) is mutagenic, teratogenic, and that it induces prophage, mitotic crossing-over, cytogenic changes and malignant transformations. It has also been reported that hycanthone (66) is carcinogenic in mice infected with S. mansoni.

The principle toxic reactions of lucanthone (1) in human subjects have been gastrointestinal symptoms such as nausea, vomiting, anorexia and epigastric pain,[10,32] effects on the central nervous system,[10] and cardiovascular effects.[32] The nature of the salt of lucanthone (1) administered had a definite effect on toxicity; the use of resinates in place of the usual hydrochloride caused a significant diminution in the severity of the side effects.[33]

Gastrointestinal side effects were also fairly extensive with hycanthone (66)[34] and there have been reports of dizziness and headache. Of serious concern has been the recent discovery of hepatotoxicity leading to acute hepatitis and hepatocellular injury.[35]

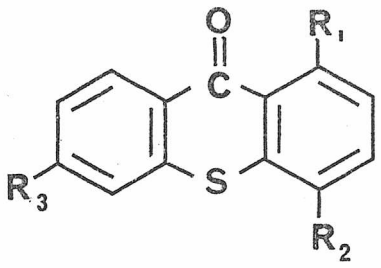
The first indications of possible mutagenic effects were demonstrated by Lüers in 1955[36] when a significant number of chromosome mutations were demonstrated in a wild strain of Drosophila melanogaster.

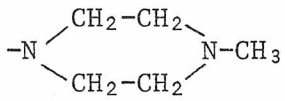
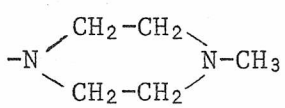
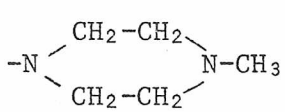
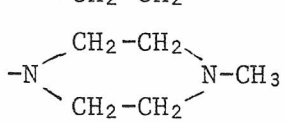
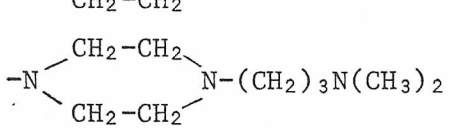
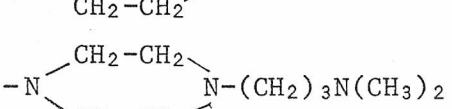
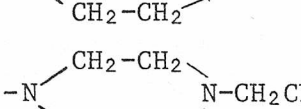
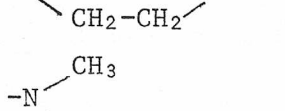
Hartman et al.[37] compared lucanthone (1) and hycanthone (66) in a test system consisting of T4 bacteriophage mutants during growth of Escherichia coli K-12; both compounds increased the reversion frequencies of two frame-shift mutations. Significant mutagenicity of hycanthone (66) has also been observed in a mammalian cell system involving forward mutations of the thymidine kinase locus of heteroxygous mutants of LS178Y mouse lymphoma cells in cell culture.[37]

Hirchberg et al.[38] have reported that lucanthone (1) exhibits carcinostatic activity against a number of transplantable mouse tumours at non-toxic dose levels. Blanz and French[39] tested a number of analogues including hycanthone (66) for carcinostatic activity, and reported that the presence of an intact thioxanthenone ring bearing a structurally compact substituent, e.g. methyl in the 4 position with an unsubstituted proximal nitrogen in the side chain at the 1 position, are the structural features necessary for optimum carcinostatic activity. In an investigation on the frame-shift mutations caused by hycanthone (66) and its congeners in bacteria (Salmonella typhimurium and Escherichia coli), the nature of the substituent in the 4 position of the thioxanthenone ring was found to be critical for mutagenicity.[40] Activity was found when this group was a hydroxymethyl or an aldehyde, but the analogues having a carboxylic acid or methyl group in this position were inactive. Strauss[41] reports that in a study of the relative mutagenicity of twenty thioxanthenones (Table 17) only compounds (66), (81), (85), (90), (92), (94), and (97)-(101) with a hydroxymethyl group, or (64) with an acetate group at the 4 position, were effective

Table 17. Relative mutagenic activities of some thioxanthenones in Salmonella

(Strauss[41])



No.	R_1	R_2	R_3	Approximate relative mutagenicity for Salmonella
(1) (lucanthone)	$-NH-(CH_2)_2-N(C_2H_5)_2$	$-CH_3$	-H	<.01
(66) (hycanthone)	$-NH-(CH_2)_2-N(C_2H_5)_2$	$-CH_2OH$	-H	1
(57)	$-NH-(CH_2)_2-N(C_2H_5)_2$	$-CH_3$	-Cℓ	<.01
(54)		$-CH_3$	-H	<.01
(90)		$-CH_2OH$	-H	.01
(91)		$-CH_3$	-Cℓ	<.01
(92)		$-CH_2OH$	-Cℓ	.01
(93)		$-CH_3$	-Cℓ	<.01
(94)		$-CH_2OH$	-Cℓ	.02
(95)		$-CH_3$	-H	<.01
(82)		$-CH_3$	-H	<.01

Continued

Table 17. Continued

No.	R_1	R_2	R_3	Approximate relative mutagenicity for Salmonella
(96)	$-NH-(CH_2)_2-N^+(C_2H_5)_2$ with O^-	$-CH_3$	$-H$	<.01
(97)	$-NH-(CH_2)_2-N^+(C_2H_5)_2$ with O^-	$-CH_2OH$	$-H$	.05
(64)	$-NH-(CH_2)_2-N(C_2H_5)_2$	$-CH_2-O-C(=O)-CH_3$	$-H$	2
(85)	$-NH-(CH_2)_2NHC_2H_5$	$-CH_2OH$	$-Cl$	3
(98)	$-NH-(CH_2)_2N(C_2H_5)-CH_2C(CH_3)(OH)-CH_3$	$-CH_2OH$	$-H$	.5
(99)	$-NH-(CH_2)_2-N(C_2H_5)(CH_2-CH_2OH)$	$-CH_2OH$	$-Cl$	2
(81)	$-NH-(CH_2)_2-N$ (morpholine)	$-CH_2OH$	$-H$	2
(100)	$-NH-(CH_2)_2-N$ (morpholine)	$-CH_2OH$	$-H$	2
(101)	$-NH-(CH_2)_2-N$ (morpholine)	$-CH_2OH$	$-H$	1

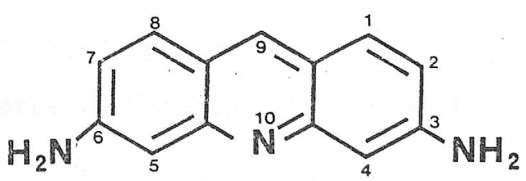
"Approximate relative mutagenicity" refers to the average effects on two test systems: TA 1537 and TA 1538.[35] Relative mutagenicity is a ratio of induced to spontaneous mutations in treated and control tests. <0.01 indicates no mutagenicity detected.

mutagens for Salmonella in vitro.

The lack of mutagenicity in Salmonella of lucanthone analogues (1), (54), (57), (82), (91), (95) and (96), retaining a methyl group at the 4 position, has been reported by a number of researchers;[37,42] of interest was the low or absent mutagenic activity in Salmonella of compounds (54), (82), (90), (92) and (95). These compounds also failed to mutate T4 bacteriophage. Modification of the basic side chain of lucanthone (1) to a piperazine or methylpiperazine (54) has been shown not to appreciably affect schistosomicidal activity[43] and these compounds show low or no mutagenic activity in Salmonella.

Hirschberg, Weinstein et al.[44,45,46] have demonstrated that both hycanthone (66) and lucanthone (1) completely inhibit the growth of B. subtilis and E. coli at concentrations of 3-6 x 10^{-5}M. In both organisms almost complete inhibition of ribonucleic acid (RNA) synthesis occurred and partial blockage of deoxyribonucleic acid (DNA) synthesis occurred in E. coli. They showed that inhibition of the DNA-dependent RNA synthesis by lucanthone (1) was due to complexing by the drug with DNA. Waring[47] has shown from measurements of the changes in the sedimentation coefficient of circular duplex DNA of bacteriophage ØX174RF, that hycanthone (66) intercalates between base pairs of DNA and this could explain why hycanthone (66) and lucanthone (1) are frame-shift mutagens in T4 bacteriophage.[37]

The concept of intercalation of compounds with nucleic acids was first put forward by Lerman in 1961[48] who described the complex between proflavine [3,6-diaminoacridine , (102)] and DNA.



(102)

He suggested that the interaction involves the insertion of the 3,6-diaminoacridine molecule between two layers of base pairs of the DNA double helix in such a way that the primary amino groups were held in ionic linkage by two phosphoric acid residues of the Watson and Crick spiral,[49] the flat skeleton of the acridine ring resting on the purine and pyrimidine molecules to which it was held by van der Waals forces.[50] Lerman based his concept of intercalation between proflavine (102) and DNA on the following evidence; fibres drawn from the complex gave much simpler X-ray diffration patterns than those given by pure DNA, suggesting that each DNA molecule was now more closely packed than pure DNA and hence had a smaller diameter;[48] small X-ray scattering results were consistent with a rod-like structure for the complex in dilute solution whose mass and radius of gyration around its axis decreased with increasing concentration of proflavine (102);[51] fluorescent intensities of flowing and stationary solutions were related in a way that was compatible with perpendicularity of the acridine molecules to the helical axis.[52] The interaction between proflavine (102) and DNA also gave a three-fold increase in viscosity. This was attributed to the extension, stiffening and straightening of the helix by the intercalated molecules.[48] The complex was found to have a lower sedimentation coefficient than free DNA, caused by a decrease in the average mass per unit length of the molecule, proflavine (102) having less than half the mass of the same volume of DNA. An alteration in the optical density profile of the aminoacridine, upon the addition of native DNA, occurred and a change in the heat-denaturation profile of DNA in the presence of the acridine was also noted.[50]

Weinstein and Hirschberg[45] have summarised the physicochemical evidence which supports the concept that lucanthone (1) and hycanthone

(66) are intercalating agents. Both produce a similar increase in viscosity of DNA like the aminoacridines. Flow-dichroism studies indicate that the lucanthone (1) chromophore is coplanar with the DNA bases as is the case with aminoacridines which are known to intercalate with DNA. The interaction of lucanthone (1) and the aminoacridines can both be prevented in media of high ionic strength by polycations such as spermine. Under these conditions the DNA is held in a more rigid conformation which impairs the relaxation necessary to permit intercalation.

The alteration in the ultraviolet absorption profile of lucanthone (1) upon the addition of DNA and in the heat denaturation of DNA, provides strong evidence for complex formation between lucanthone (1) and DNA.[44]

Weinstein et al.[46] showed that at neutral pH lucanthone (1) had absorption maxima at 330 and 442 nm and that both native and denatured DNA caused a shift in maxima to 337 and 450 nm respectively and decrease of absorption at the original maxima. Another approach has been the determination of the effect of lucanthone (1) and hycanthone (66) on the heat stability of calf thymus DNA. When aqueous solutions of DNA are slowly heated, there is no change in the secondary structure until a temperature is reached when denaturation occurs and the two strands of the helix uncoil (melt) over a temperature range of a few degrees. The progress of the melting can be followed by measuring the absorbance at 260 nm as denaturation produces a hyperchromic shift of about 40%. The idealized appearance of a plot of extinction, or more usually the ratio of absorbance at temperature T to absorbance at 25°, is shown in Fig. 3, and is called a "melting profile".

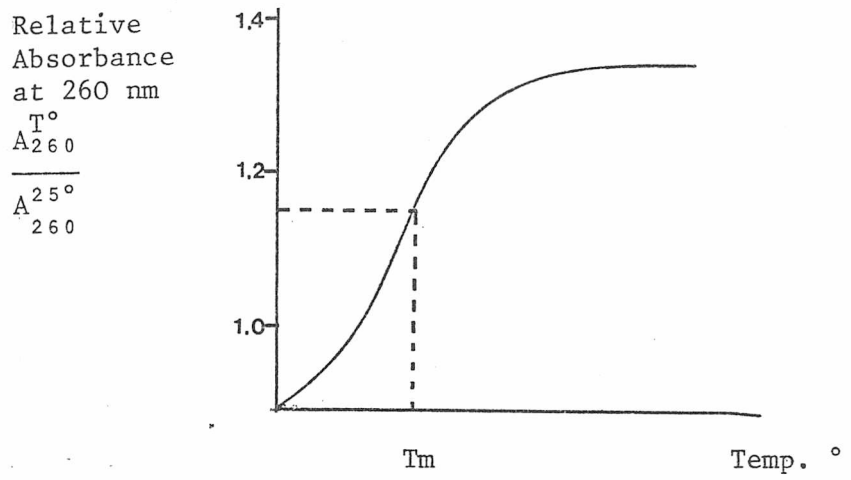


Fig. 3. Melting profile of native calf-thymus DNA.

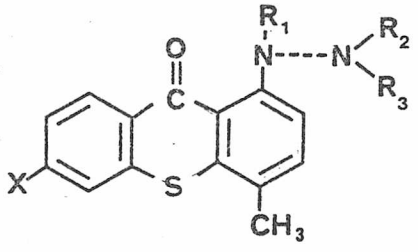
The melting temperature (Tm) is defined as the temperature at which the hyperchromic effect is 50% of its maximum value.[53] The value of Tm is a function of the ionic strength of the buffer and the nucleotide composition of DNA. For solutions of different DNA samples in a standard buffer Marmur[54] has shown that the guanine-cytosine ratio has a linear relationship to the value of Tm.

When assayed in buffer of low ionic strength, the heat denaturation profile of DNA reached 50% of total hyperchromicity (Tm) at 56°. This was raised to 71° in the presence of 6×10^{-6} mol ℓ^{-1} lucanthone (1) (ΔTm = 15°) and to 79° at twice that concentration (ΔTm = 23°). In high ionic strength saline-citrate this effect was not observed. Hycanthone (66) was found to be equivalent to lucanthone (1) and structure-activity studies (Table 18) have been carried out using the heat stability of DNA technique.[44,46,55]

Table 18. Comparison of relative growth-inhibitory activity for Bacillus subtilis and effect on the heat-denaturation profile of native DNA with lucanthone analogues.

(Hirschberg et al.[44])

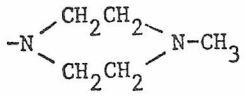
Compound



No.	R_1	X	N--N	R_2	R_3	B. subtilis Rating (a)	DNA (ΔTm) (b)
(1) (lucanthone)	-H	-H	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	6	11-14
(50)	-H	-H	$-CH_2CH_2-$	-H	$-CH_2C(CH_3)_2OH$	12	12
(53)	-H	-H	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2C(CH_3)_2OH$	12	11
(103)	-H	-H	$-CH_2CH_2CH_2-$	$-CH_3$	$-CH_3$	9	16
(46)	-H	-H	$-CH_2CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	16	10
(104)	-H	-H	$-CH_2CH_2CH_2-$	$-CH_2CH_2OH$	$-CH_2CH_2OH$	6	9
(105)	-H	-H	$-CH_2CHOHCH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	5	11
(106)	-H	-H	$-CH_2CH_2CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	18	11
(107)	-H	-H	$-CH(CH_3)CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	25	12
(82)	$-CH_3$	-H	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	1	3
(108)	$-CH_3$	-H	$-CH(CH_3)CH_2-$	$-CH_3$	$-CH_3$	1	5
(109)	-H	-Cℓ	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CHOHCH_3$	21	11
(110)	$-CH_3$	-Cℓ	$-CH_2CH_2-$	$-CH_3$	$-CH_3$	3	9
(57)	$-CH_3$	-Cℓ	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	4	10

Continued

Table 18. Continued

No.	R_1	X	N--N	R_2	R_3	B.subtilis Rating (a)	DNA (ΔTm) (b)
(111)	$-CH_3$	$-C\ell$	$-CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_2OH$	3	6
(112)	$-CH_3$	$-C\ell$	$-CH_2CH_2-$	$-n-C_4H_9$	$-n-C_4H_9$	18	4
(113)	$-CH_3$	$-C\ell$	$-CH_2CH_2CH_2-$	$-CH_2CH_3$	$-CH_2CH_3$	<2	
(114)[d]	-H;	$-CH_2CH_2C\ell$	-	-	-	<2	
(115)[d]	$-CH_3$;	$-CH_3$	-	-	-	3	3
(54)	-	-H		-		<2	7

(a) Rating = $\frac{25 \times 10^{-5}}{\times}$ where × is the molar concentration of compound needed to cause 50-100% inhibition of growth, without cell lysis, during the first 20 minutes after the addition of drug to the culture medium.

(b) ΔTm = Tm observed with DNA (20µg $m\ell^{-1}$) in the presence of drug (5×10^{-6}mol ℓ^{-1}) minus the Tm obtained with DNA in the absence of drug. Tm is the temperature at which 50% hyperchromicity occurs.

(c) This low rating indicates that 50% inhibition of growth was not observed at 12×10^{-5}mol ℓ^{-1}, the highest level tested because of solubility problems.

(d) This compound does not have a second nitrogen in the side chain.

In the lucanthone series the introduction of a methyl group onto the proximal nitrogen produced compounds (82) and (108) which exhibited a significant reduction in interaction with DNA. This degree of reduction of interaction was not observed in compounds (57) and (110) closely related to N-methyllucanthone (82) which also contained a 6-chloro substituent, although variation in the substituents on the terminal nitrogen did produce compounds (111) and (112) with relatively low ΔTm values.

Hirschberg et al.[44] suggest that alkyl substitution in the lucanthone series would be expected to alter the basicity or electron-donor capacity of the proximal nitrogen, which would account for the lowering of activities of the resulting derivatives, (62) and (100) (Table 18); but this does not explain the ability of the 6-chloro-N-methyl compounds (110) and (57) to interact with DNA.

These workers[44] further conclude from their results that the length of the side chain and the nature of the substituents on the terminal nitrogen have little effect on the ability of the drug to complex with DNA. The former observation is seen in compounds (1), (46) and (106) of increasing side chain length; however the effect of substituents on the terminal nitrogen is not clear from the results reported. The piperazine compound (54) whilst not containing a proximal hydrogen shows stabilisation of the DNA, unlike the N-methyllucanthone (82), but the effect is less than lucanthone (1).

In the B. subtilis system replacement of the hydrogen on the proximal nitrogen by a methyl group (82, 1) led to a significant loss of activity. Truncation of the side chain to a single nitrogen with the terminal dialkylamino group replaced by a chlorine atom (114, 1) led to diminished activity against B. subtilis. Increase in the length of the carbon chain between the two side chain nitrogens (1, 46, 106) resulted in increased

activity. Replacement of the terminal ethyl groups of (46) by methyl groups (103) or hydroxyethyl groups (104) led to decreased activity.

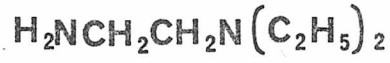
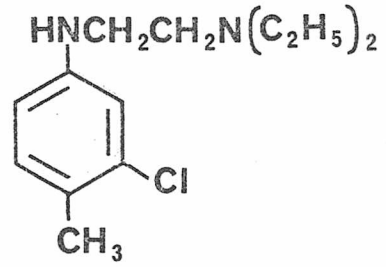
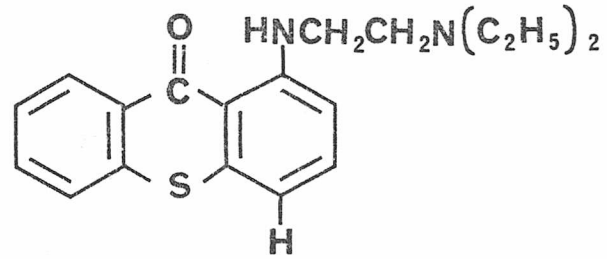
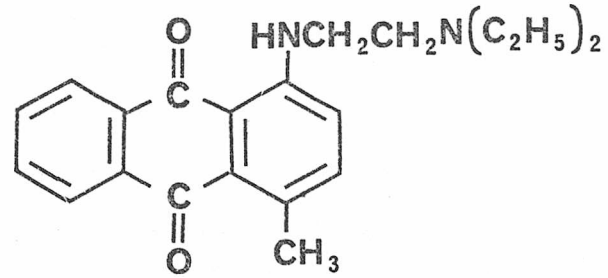
Methyl substitution on the proximal nitrogen of the side chain in the chlorolucanthone derivative (57) led to increased activity over the deschloro compound (82). Hirschberg et al.[44] suggest the 6-chloro substituent enhances the lipophilicity of the compounds and favours their passage across bacterial cell membranes. They also suggest that in contrast to the mepacrine-DNA model[57] it seems unlikely that the diamino side chain of lucanthone (1) forms interstrand bridges between the two phosphate groups of the DNA backbone, since the two side chain nitrogens in this instance are separated by two rather than four carbons and the basicity of the terminal nitrogen in the lucanthone series does not influence activity significantly.

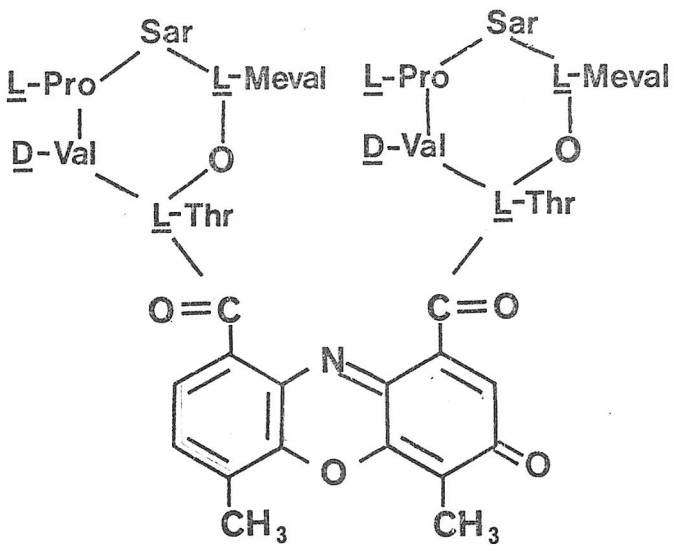
Diethylaminoethylamine (116) (Table 19), which represents the complete side chain unattached to a ring system, has a significant effect on the stability of DNA to heat denaturation like many other polyamines.

Replacement of the 4-methyl group by hydrogen (63) does not cause a significant decrease in the stabilizing effect on the DNA helix to heat denaturation. Related three ring structures such as the anthraquinone (22), actinomycin D (117), mepacrine (3) and proflavine (102) are at least as effective as lucanthone (1) at equimolar concentrations in raising the Tm of native DNA; chloroquine (118) is slightly less effective whereas the toluene derivative Mirasan (29) has no effect on the Tm of DNA.

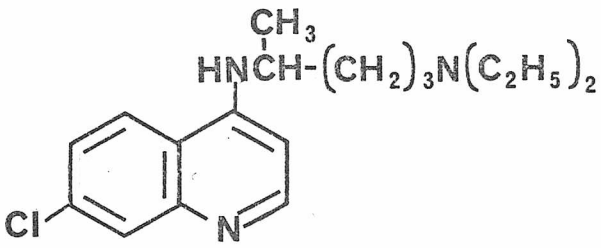
Table 19. Comparison of the effects of other compounds on the growth of B. subtilis, heat denaturation of DNA.

(Hirschberg et al.[44])

Compound No.		B. subtilis rating (a)(page 42)	DNA (ΔTm°)
(116)		<2	8
(29) (Mirasan)		<2	0
(63)		2	9
(22)		2	16
(117)	actinomycin D	100	>16
(3)	mepacrine	2	>16
(102)	proflavine	2	16
(118)	chloroquine	<2	10



(117)



(118)

In buffer of low ionic strength, but not in standard saline-citrate, the interaction with DNA of lucanthone (1), hycanthone (66) and their biologically active congeners caused an appreciable increase in the relative viscosity of the DNA. This increase was prevented by the addition of low concentrations of Mg^{++}, or high concentrations of Na^{+}. The structure-activity relationship for this effect correlated well with those for the increased heat stability of DNA.[44]

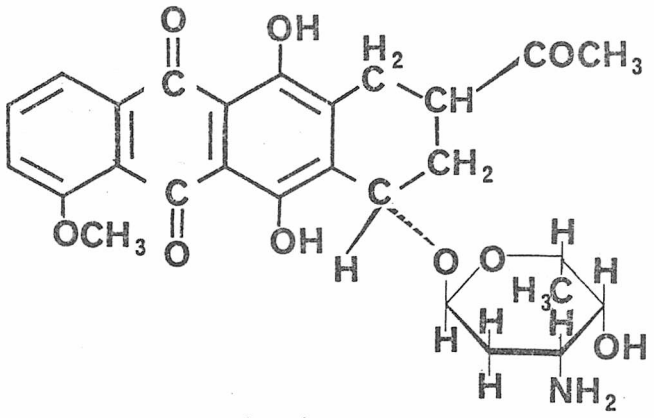
Hirschberg et al.[44,45,56] have suggested several molecular models for the interaction of thioxanthenones with DNA. The first of these involves the proximal nitrogen of the side chain of lucanthone (1)

interacting with a phosphate residue of the DNA backbone, analogous to proflavine (102) and mepacrine (3),[57] the three-ring system interacting with the base residues in DNA by intercalation. This interpretation is supported by previous data and also by the significant increase in the viscosity of calf thymus DNA with lucanthone (1), and its analogues.

Secondly, an alternative concept, equally compatible with the experimental results, suggests that in the formation of the DNA-lucanthone complex the oxygen atom of the carbonyl group at position 9 and the proximal nitrogen of the side chain are involved in hydrogen bonding with base residues in the DNA helix and the terminal nitrogen interacts with a phosphate residue of the DNA backbone.

The most recent formulation visualizes intercalation of the ring system between consecutive base pairs of DNA, with the terminal nitrogen extending outward to the periphery of the DNA helix to interact with the DNA backbone. This concept is more in line with the recent studies on the interaction of aminoacridines with DNA carried out by Pritchard et al.[59] whose findings were incompatible with the original Lerman model.[48]

There is also evidence from work on acridine and phenanthridines that two kinetic processes are occurring. Intercalation is a first order reaction which reaches equilibrium when one molecule of the compound has been bound per four or five nucleotides; further secondary and weaker binding can occur by a higher order process until one molecule of compound per nucleotide has been fixed. This secondary binding has been thought to consist of an adsorption of acridine or phenanthridine molecules on to those already bound by intercalation and it has been suggested that these additional molecules are "stacked" on the surface of the nucleic acid.[60] A similar process occurs in other compounds known to intercalate with DNA. Daunomycin (119) binds "strongly"

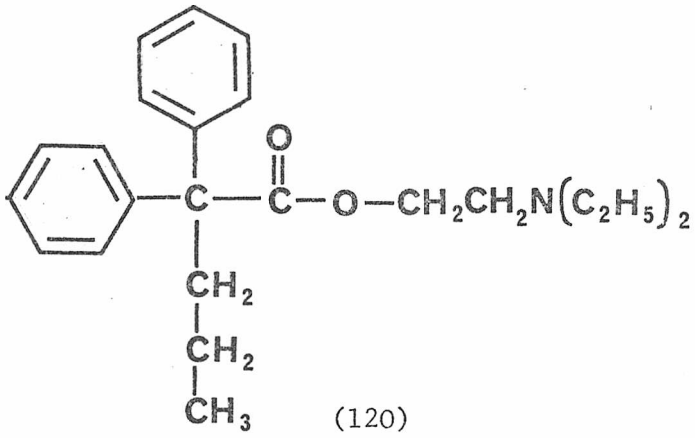


(119)

by intercalation between successive base pairs of the double helix and "weakly" by means of electrostatic interaction involving the DNA phosphate groups and the daunomycin amino group. The association constant for the strong binding type is reported to be of the same order of magnitude as those found for the acridine dyes and actinomycin D (117).[61] Zunino et al. argue that the double helix is not necessary for the formation of a complex, a complex may also be formed with denatured DNA. However, binding equilibrium experiments indicate that the double-helical structure is a necessary condition for the strong binding process. The absence of a specific increase in the viscosity of denatured DNA with daunomycin (119) suggests that this effect is a specific feature of a strong binding with native DNA.

While the antitumour and antibacterial action of the thioxanthenones could be attributed to the ability of these drugs to intercalate with the relevant DNAs, Archer and Yarinsky[2] propose that it is doubtful whether the schistosomicidal action of hycanthone (66) and its analogues is based on their ability to intercalate with schistosomal DNA. Hycanthone (66) and lucanthone (1) are about equally active in the tumour and bacterial systems but hycanthone (66) is far more active as a schistosomicidal agent. The antitumour activity of both can be abolished

by pretreatment with the metabolic inhibitor, SKF-525A (120), which selectively inhibits microsomal drug oxidations in the liver, by a non-competitive action.



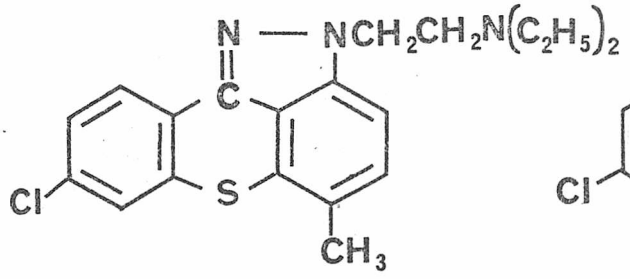
(120)

The schistosomicidal activity of hycanthone (66) in mice is not impaired by pretreatment with SKF-525A (120). These observations suggest that hycanthone (66) is itself biotransformed to the active antitumour agent.

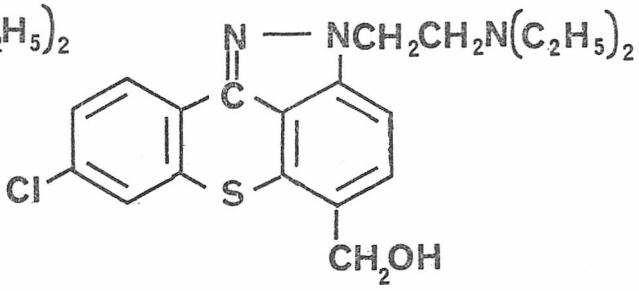
Hirschberg et al.[44] found that Mirasan (29) is not an anti-tumour drug nor does it complex with DNA, yet it is a highly effective schistosomicide in S. mansoni infections in mice and it too is biotransformed to its corresponding hydroxymethyl analogue (76); which is probably the active metabolite (76).

Bueding et al.[62] have reported on some hycanthone analogues with equivalent activity to hycanthone (66) but with reduced acute toxicity and hepatotoxicity. These compounds, benzothiopyranoindazoles, failed to induce malignant transformations in cells infected with Rauscher virus and had a reduced mutagenic activity in Salmonella, bacteriophage T4 and mouse lymphoblasts. No cytogenic effects were detected in rat bone marrow cells, unlike experiments carried out with hycanthone (66). The two compounds of principle interest have been designated 1A-3 (41)

and 1A-4 (87).



(41)
(122) N-oxide

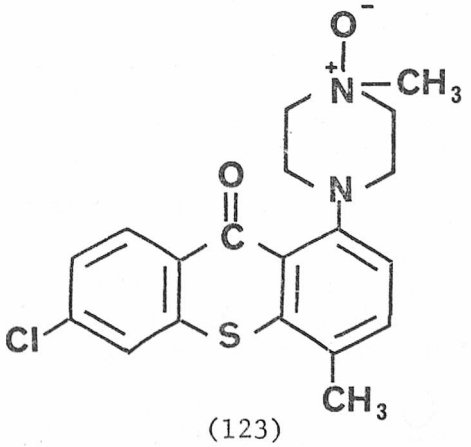


(87)
(121) N-oxide

Compound 1A-3 (41) had lower schistosomicidal activity than 1A-4 (87) but also had decreased acute toxicity giving an equivalent chemotherapeutic index. The chlorine atom at position 8 produced a marked decrease in acute toxicity of the indazole analogues in mice compared with the corresponding deschloro derivatives. N-Oxidation at the diethylaminoethyl group of active thioxanthenones and benzothiopyrano-indazoles consistently resulted in a marked reduction in mutagenicity for Salmonella strain TA 1538 (Table 20).

However, with the exception of hycanthone (66) schistosomicidal activity was either maintained or even increased.[31] The schistosomicidal activity of 1A-3 N oxide (122) is at least twice as high as that of hycanthone (66), while its mutagenicity is less than 1% of hycanthone (66).

An N-oxide of the methylchloromethylpiperazinyl analogue (123) failed to exhibit any demonstrable activity in the Salmonella system,[63] yet it had activity against schistosomes. Hence, mutagenicity can be

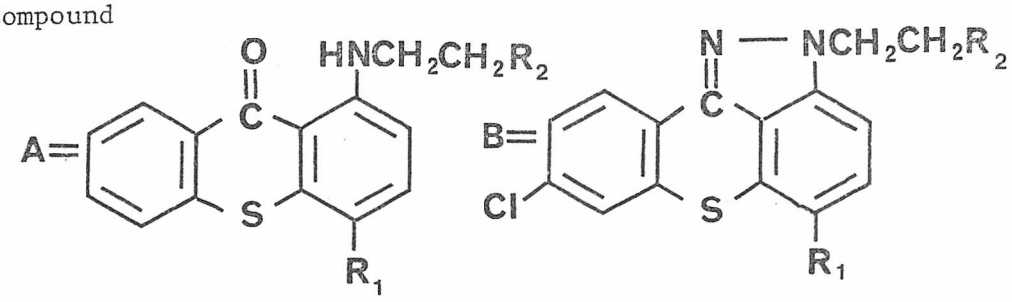


(123)

Table 20. Mutagenic and schistosomicidal activity in mice of hycanthone (66), hycanthone analogues and their N-oxides.

(Bueding et al.[62])

Compound



No.	Ring System	R_1	R_2	Mutagenic activity (colonies/ plate)(a)	Relative schistosomicidal activity (b)	
					I.M.	ORAL
(66) (hycanthone)	A	$-CH_2OH$	$-N:(C_2H_5)_2$	1262	1.0	0.5
(97) (hycanthone N-oxide)	A	$-CH_2OH$	$-\overset{+}{N}(\rightarrow O^-):(C_2H_5)_2$	56	0.3	0.25
(1) (lucanthone)	A	$-CH_3$	$-N:(C_2H_5)_2$	70	0.1	0.2
(96) (lucanthone N-oxide)	A	$-CH_3$	$-\overset{+}{N}(\rightarrow O^-):(C_2H_5)_2$	22	0.5	0.4
(87) (1A-4)	B	$-CH_2OH$	$-N:(C_2H_5)_2$	100	1.0	1.5
(121) (1A-4 N-oxide)	B	$-CH_2OH$	$-\overset{+}{N}(\rightarrow O^-):(C_2H_5)_2$	18	1.0	1.2
(41) (1A-3)	B	$-CH_3$	$-N:(C_2H_5)_2$	70	0.6	0.7
(122) (1A-3 N-oxide)	B	$-CH_3$	$-\overset{+}{N}(\rightarrow O^-):(C_2H_5)_2$	5	2.0	2.0

(a) Average numbers of drug induced mutations/plate above spontaneous mutation background (17 colonies/plates)
Values represent the averages of 4 or more plates, each containing 0.5 µmol drug.

(b) Relative activity = $\frac{\%\ \text{Reduction in live worms}}{(\text{Dose (m mol kg}^{-1}) \times 600)}$
based on the % reduction of live worms produced by a single dose of compound to mice infected with S. mansoni and autopsied 5 weeks after treatment.

dissociated from schistosomicidal activity both quantitatively and qualitatively.

The N-oxidation of the terminal amino grouping of the side chain of 1A-4 (87) yielded a compound (121) whose mutagenic activity is five times lower and whose acute toxicity is three times lower than those of 1A-4 (87)[42] while schistosomicidal potency in mice is similar to that of 1A-4 (87) and that of hycanthone (66), in a single intramuscular dose. The chemotherapeutic index of 1A-4 N-oxide (121) administered as a single intramuscular dose is more than twelve times higher than that of hycanthone (66).

Zilversmit[64] has reported on studies on lucanthone (1) and its N-methyl derivative (82). N-methyl substitution resulted in virtual deletion of the bacteriostatic and carcinostatic activity of lucanthone (1) and its ability to combine with DNA. Studies of electronic spectra, vibrational spectra and ionization constants indicate that N-methyl substitution precludes co-planarity of the amine side chain and the thioxanthenone ring system. The change in molecular configuration drastically reduced biological activities of the N-methyl derivative. Blanz and French[39] suggest that diminished hydrogen bonding capacity may be responsible for the reduced carcinostatic activity of the N-methyl derivatives. N-Methyllucanthone (82) is only a weak schistosomicidal agent but does have some activity despite the lack of co-planarity.[2]

These observations concerning the N-oxides and N-methyl derivatives of thioxanthenones confirm that the schistosomicidal effects of hycanthone (66) and lucanthone (1) are brought about by mechanisms different from those producing mutagenic and some acute toxic effects. Hence structural modifications to hycanthone (66) provide opportunities to reduce or eliminate certain acute toxic host effects while maintaining

or increasing schistosomicidal activity.

Of more than three hundred publications to date dealing with lucanthone (1) and hycanthone (66) very few have dealt with the mechanism of action of these drugs, which shows the fragmentary nature of the present knowledge of the subject. Biochemical and physiological experiments have shown that in schistosomes carbohydrates are an important source of energy, and protein metabolism is of central concern.[65] Bueding found a decrease in the glycolytic rate in schistosomes isolated from infected mice following treatment with lucanthone (1), but not when the drug was added *in vitro* to a suspension of *S. mansoni*. Oxygen uptake was unaffected in either instance and there was no apparent effect on nucleoprotein metabolism or proteolytic activity.[65]

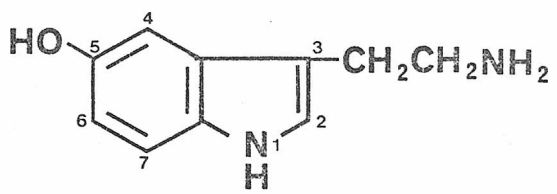
Early workers[7,9] noticed a loss of motility in *S. mansoni*, when lucanthone (1) was administered, the specific damage to the gonads and yolk cells, and the resultant interference with egg production. It was thought that lucanthone (1) specifically blocked the initiation of mitosis but permitted the mitotic process, once begun, to be completed.

Rogers and Bueding[66] described the biphasic alteration in glycogen levels and changes in the motor activity of schistosomes obtained from mice and hamsters following a single intramuscular injection of lucanthone (1). Depletion of glycogen stores, damage to the female reproductive system, and eventually a hepatic shift of the worms were observed. Hycanthone-resistant schistosomes were produced from those parasites which survived this treatment when they resumed production of viable eggs after a period of 6-12 months.

Hillman and Senft[67] have recently reported that hycanthone (66) is an inhibitor of acetylcholinesterase from *S. mansoni*, and is less

effective against acetylcholinesterase of mammalian origin. In contrast, physostigmine inhibits the mammalian enzyme more effectively than it does the helminth enzyme. These observations suggest that schistosome acetylcholinesterase differs from the mammalian enzyme with respect to the structure of the active centre, and that hycanthone (66) may have a selective affinity for schistosome cholinergic systems. This has been supported by research using a fluorescent dansylated choline derivative as a probe for locating acetylcholine receptors. Using this technique, the central ganglia in the head appear to contain a high density of acetylcholine receptors, especially in the male worms.[68]

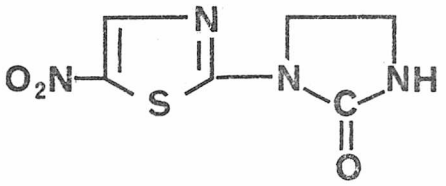
Bennet and Bueding[69] have reported that in spite of the high concentration of 5-hydroxytryptamine (124) in S. mansoni, synthesis of



(124)

this amine by the parasite could not be demonstrated. The worm has a high- and low-affinity uptake mechanism (below and above 2μmol ℓ^{-1} respectively). The high-affinity uptake mechanism provides the parasite with a means to obtain 5-hydroxytryptamine (124), even at the low concentrations of this amine prevailing in the physiological environment of the worm. The concentration of plasma 5-hydroxytryptamine (124) is highest in the hepatic veins, therefore the establishment of adult schistosomes in the mesenteric-portal veins, where 5-hydroxytryptamine (124) is relatively high could be ascribed to dependence of the worm

upon this amine. Robinson, Bueding and Fischer[70] have suggested that the schistosomicidal nitroheterocycle niridazole (125) may have 5-hydroxytryptamine antagonism.



(125)

A reduction of the glycogen stores of adult S. mansoni occurs in mice treated with niridazole (125);[71] this precedes the hepatic shift and can be attributed to the inhibiting action of the drug on the conversion of S. mansoni glycogen phosphorylase to the active form. Nitrogen basicity may be a critical factor determining schistosomicidal activity in niridazoles[70] and lucanthone analogues.[44] In the hypothesised mechanism of Hillman and Senft[67] this would be of importance in an acetylcholine blockade either by binding of the drug to acetylcholine sites or by binding of the drug to acetylcholinesterase, inhibiting the destruction of intrinsic acetycholine. These results would be consistent with a partial agonist model of the action of hycanthone (66) on acetylcholine receptors and would explain the stimulation of the schistosomes by low dosages of hycanthone (66) due to a direct effect.

Goodman and Gilman[72] have summarised the anthelminthic action of lucanthone (1) and hycanthone (66). They report that the drugs act primarily against the adult form of S. haematobium and S. mansoni and interfere with the laying of eggs, induce separation of paired worms, produce degenerative changes, and induce a shift of worms to the liver within a period of 3 to 7 days. Death of the adult worm then follows.

The mechanism of action is unknown, but may be associated with the stimulation of the schistosome's low-affinity 5-hydroxytryptamine (124) uptake into non-neuronal tissue. At the same time there is impairment of the ability of neuronal structures to store this excitatory neurotransmitter.[73] Other workers[67] have postulated that hycanthone (66) inhibits the destruction of intrinsic acetycholine by acetylcholinesterase.

The structural requirements for activity of thioxanthenones in different biological systems have been summarised in Table 22.

The formation of a symmetrical molecule in a number of drug types containing the essential structural requirements for biological activity can increase the activity of a compound. It was found, for instance, in the study of acetycholine antagonists that an essential requirement for activity in these compounds was a cationic group at one end of a hydrocarbon chain. Further, the formation of a symmetrical molecule containing two cationic groups markedly increased antagonism so that the polymethylene bis-methonium drugs (127) and (128) are potent antagonists, (Table 21).

Table 21. Symmetry in polymethylene bis-methonium drugs.

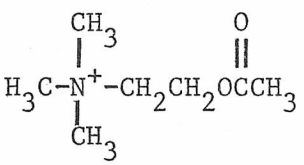
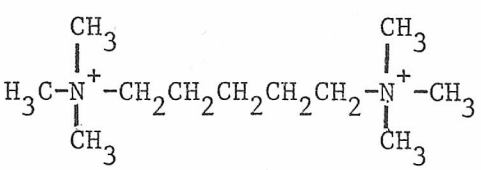
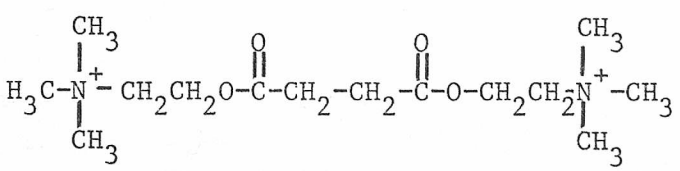
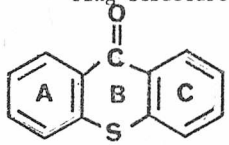
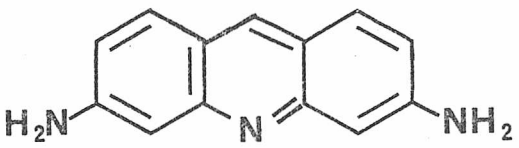
No.	
(126) Acetylcholine	
(127) Pentamethonium	
(128) Succinylcholine	

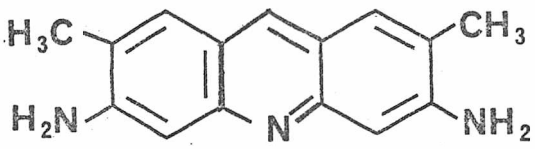
Table 22. Structure-Activity of Thioxanthenones and Benzothiopyranoindazoles in Schistosomiasis, Mutagenesis, Carcinostasis and Intercalation.

	Structure	Schistomiasis	Mutagenicity (Salmonella)	Carcinostasis	Intercalation	Growth-inhibition B. subtilis
1.	Intact thioxanthenone ring structure 	essential for activity in primates; loss of ring 'A' and 'B'; replacement of sulphur with oxygen; reduction of carbonyl or ring 'A'; and sulphoxide formation; permissible in mice.	active	active	essential for 'strong' binding	active, replacement of sulphur by carbonyl reduces activity.
2.	Position of N---N< chain	position 1 active; indazole bridge also active	position 1 active; indazole loses activity	position 1 active	position 1 active; indazole retains activity; side chain alone has some activity	position 1 active
3.	Length of side chain, distance between nitrogens -N....N<	optimal $-CH_2-CH_2-$		increase from two to four carbons increases activity	increase from two to four carbons has no effect on activity	increase from two to four carbons increases activity
4.	Terminal alkyl groups 	optimal mono- or diethyl				
5.	Proximal nitrogen of side chain	substitution reduces activity	N-methyl inactive, piperazine no detected activity	substitution leads to loss of activity	substitution leads to greatly reduced binding; piperazine active	substitution reduces activity
6.	6-Chloro substitution	increased activity against S. mansoni in mice, but not in primates	no change observable	inhibits activity	retains activity	
7.	Substitution at the 4 position	CH_3 or CH_2OH optimal	CH_2OH or CHO active; CH_3 and carboxy inactive	structurally 'compact' substituent eg CH_3 optimal	little effect replacement by H causes little loss of activity	replacement by H causes reduction in activity
8.	N-oxidation of the terminal nitrogen	maintained or increased activity	reduced activity	reduced activity		

Similarly, the acridine compounds, proflavine (102) and acridine yellow (129) both strongly bind to DNA by intercalation.



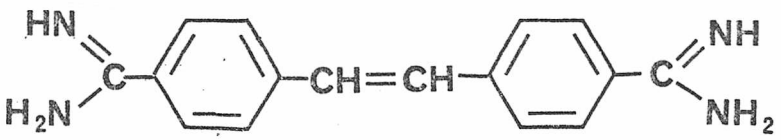
(102)



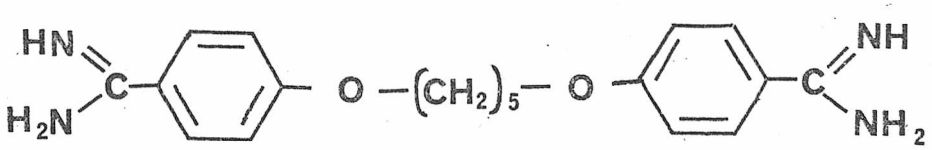
(129)

The diamino substitution enables a double linking of the acridine to two chains of the DNA helix.[74,75]

Aromatic diamidines such as stilbamidine (130) and pentamidine (131) inhibit the growth of protozoa, bacteria, fungi and neoplastic cells.[76]



(130)

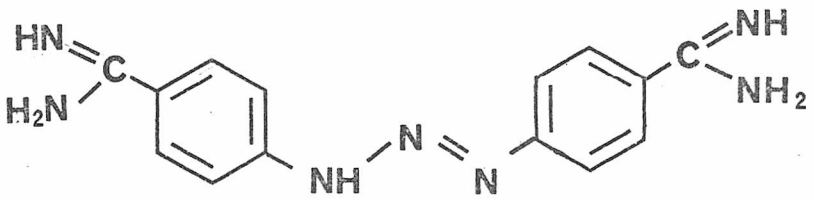


(131)

The biological activity of these compounds is known to be markedly affected by alterations in chain length and by modification of the guanyl

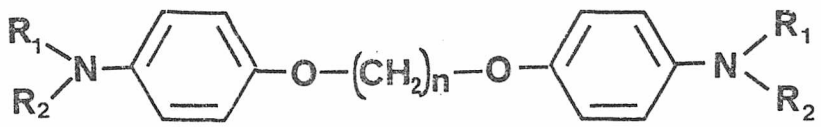
group. The growth inhibitory actions of aromatic diamidines has been demonstrated to be due to direct interaction with nucleic acid.[77]

Similarly the drug Berenil (132) has also been shown to form complexes with DNA.[78]



(132)

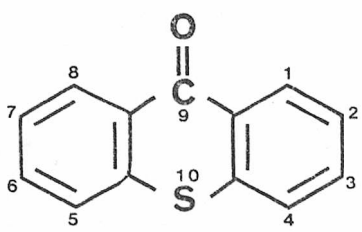
Raison and Standen[79] have described the activity of a series of symmetrical diaminophenoxyalkanes where the general formula (133) was subject to considerable variation in the chain and in the amino group.



(133)

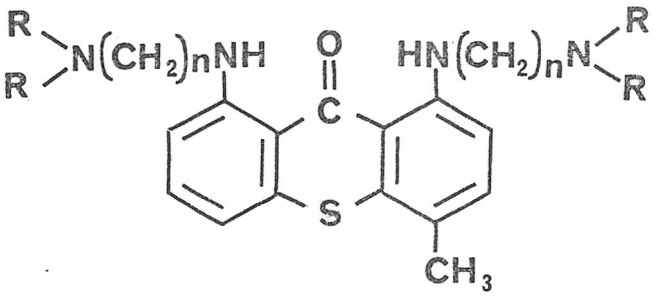
Schistosomicidal activity was found against S. haematobium and S. mansoni infections in mice, and n = 7 or 8 in the amino and dimethylamino series and n = 5,7 or 9 in the diethylamino series was found optimal for schistosomicidal activity.

The three ring system of thioxanthenone (134) is also symmetrical and the 1 position is equivalent to the 8 position.



(134)

The need for substitution of a dialkylaminoalkylamino side chain at position 1 has already been demonstrated for biological activity of these compounds; therefore the possibility exists that the 1,8-disubstituted compound (135) may possess enhanced activity.



(135)

CHAPTER 1

1,8-Dichloro-4-methylthioxanthen-9-one

CHAPTER 1

Index

1,8-Dichloro-4-methylthioxanthen-9-one (136) from 2-Chloro-6-nitrotoluene (137)

Introduction

The work described in this chapter had as its object the preparation of 1,8-dichloro-4-methylthioxanthen-9-one (136). This was synthesised by two routes analagous to the methods described by Archer and Suter.[15]

Lucanthone (1) was produced from 1-chloro-4-methylthioxanthen-9-one (138) by Mauss[16] by condensing it with diethylaminoethylamine (116) according to the procedure of Ullmann and Glenck,[80] the thioxanthen-9-one (138) being obtained mixed with the isomeric 4-chloro-1-methylthioxanthen-9-one (139) from the treatment of 2-mercaptobenzoic acid (140) with 4-chlorotoluene (141) in sulphuric acid, Fig. 4. 1-Chloro-4-methylthioxanthen-9-one (138) was sufficiently reactive to condense with an amine at elevated temperature, whereas the 4-chloro compound (139) was relatively unreactive; the 1-chloro position being more prone to nucleophilic attack than the 4-chloro position because of the electron withdrawing nature of the thioxanthenone carbonyl group, *peri* to the chlorine atom.

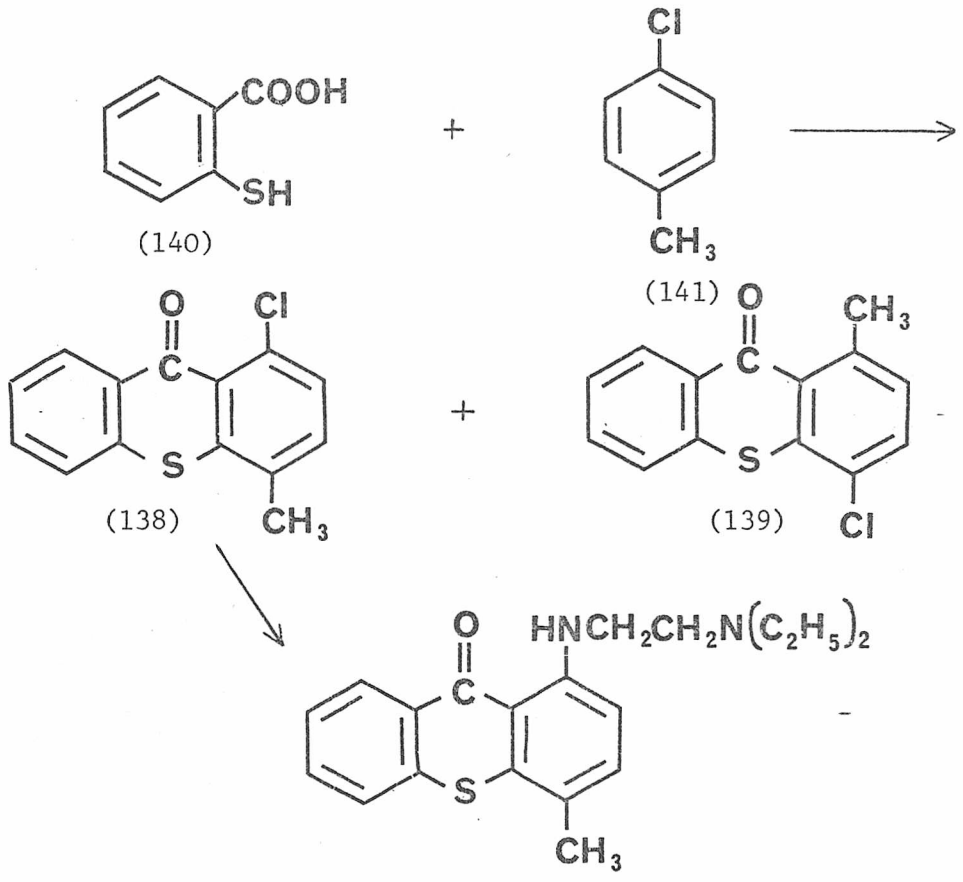
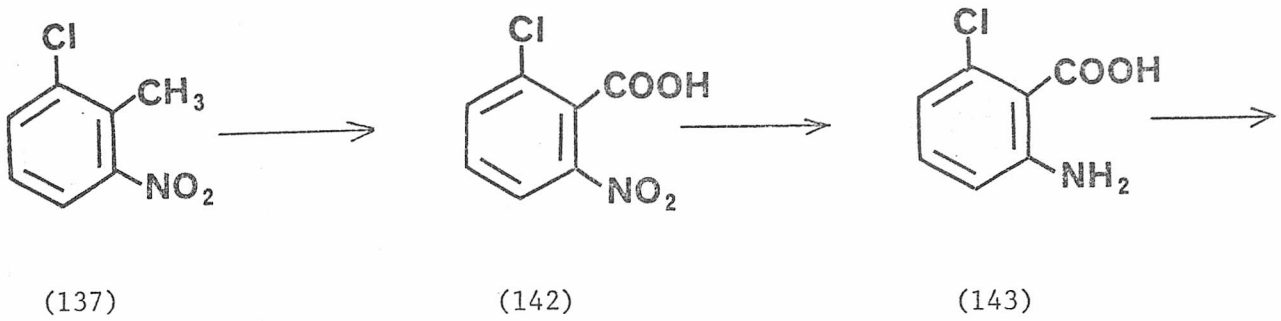
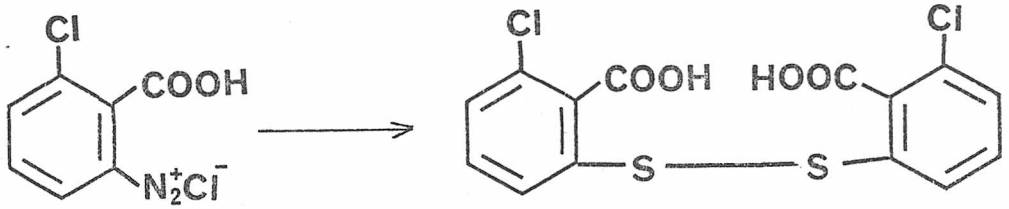


Fig. 4

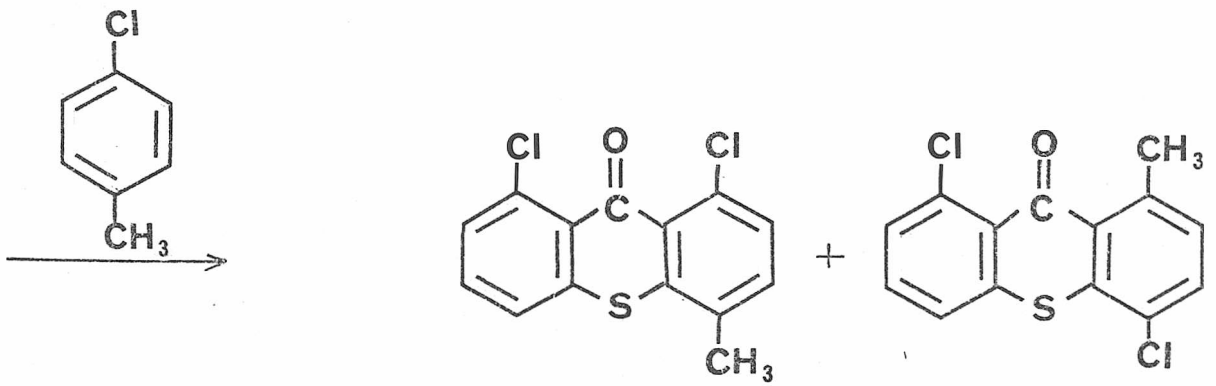
In the present study the preparation of 1,8-dichloro-4-methyl-thioxanthen-9-one (136) has been attempted using this method. The starting material chosen was 2-chloro-6-nitrotoluene (137) and the reaction scheme is outlined in Fig. 5.



(137) (142) (143)



(144) (145)



(141) (136) (146)

Fig. 5

2-Chloro-6-nitrotoluene (137) was oxidised, the nitro group reduced, and the resulting chloroanthranilic acid (143) diazotised and converted to the disulphide (145). The disulphide (145) was reacted with 4-chlorotoluene (141) to yield a mixture of the thioxanthenone isomers (136) and (146).

Oxidation of 2-Chloro-6-nitrotoluene (137)

Synthesis of 2-chloro-6-nitrobenzoic acid (142) has been attempted by two oxidative procedures. Oxidation with alkaline permanganate following the method of Vogel[81] yielded 32% 2-chloro-6-nitrobenzoic acid (142) which was characterised by its melting point and spectral properties. An alternative method described by Lehmstedt and Schrader[82] involved the bromination of the toluene (137) to give the bromo derivative (147) followed by its conversion to the ester (148). Hydrolysis of the ester (148) produced the alcohol (149) which was more susceptible to permanganate oxidation than the parent toluene (137) but the overall yield was only 20%, (Fig. 6).

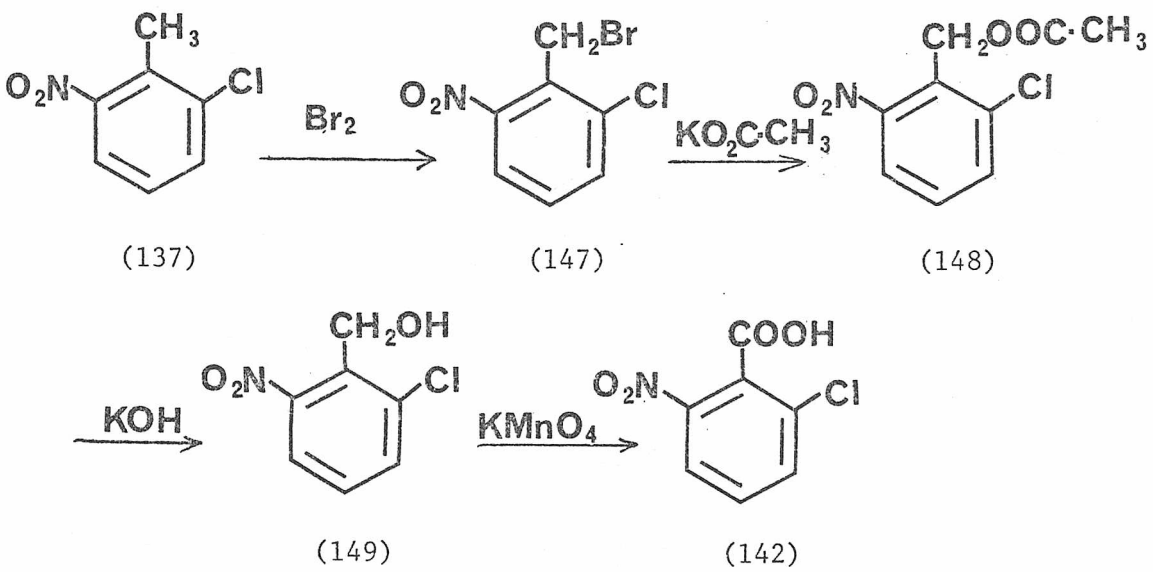


Fig. 6

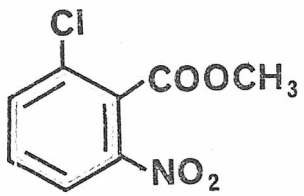
Attempts to attain the yield of 2-chloro-6-nitrobenzoic acid (142) quoted in the literature[82] (60%) were without success.

The initial bromination stage appeared to be the obstacle to higher yields of acid probably because of inadequate exposure of the reaction mixture to ultraviolet radiation. Replacement of photobromination by

N-bromosuccinimide (NBS), following the method of Chapman and Williams[83] produced no evidence of bromo derivative formation.

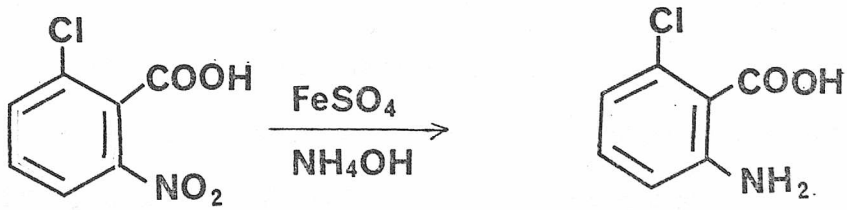
Reduction of 2-Chloro-6-nitrobenzoic acid (142)

The reduction of 2-chloro-6-nitrobenzoic acid (142) using hydrogen sulphide has been described by Cohen.[84] This method has the disadvantage of precipitating the ammonium salt of the benzoic acid when ammonia is added to the alcoholic solution of the compound before passage of hydrogen sulphide; however, the use of the methyl ester (150) circumvents this disadvantage[85] but yielded no detectable amine after introduction of the hydrogen sulphide.



(150)

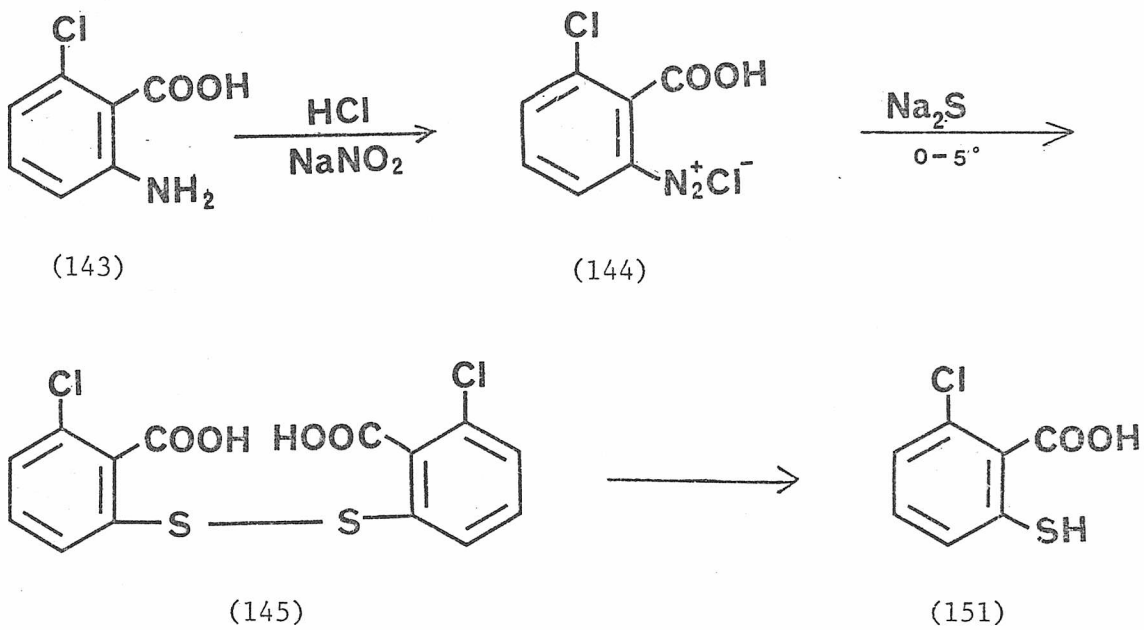
The reduction of 2-chloro-6-nitrobenzoic acid (142) to 2-amino-6-chlorobenzoic acid (143) was achieved using ferrous sulphate in ammonia.[85]



(142) (143)

Synthesis of 6-Chloro-2-mercaptobenzoic acid (151)

Synthesis of 6-chloro-2-mercaptobenzoic acid (151) was carried out using the method of Allen and MacKay,[86] Fig. 7. The anthranilic acid (143) was diazotised and the product reacted with sodium sulphide solution at 5° to form the disulphide (145).



(143) (144)

(145) (151)

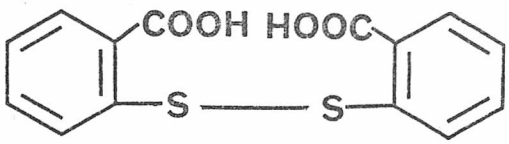
Fig. 7

Reduction of the disulphide using zinc in glacial acetic acid gave 6-chloro-2-mercaptobenzoic acid (151).[87]

Condensation of 6-Chloro-2-mercaptobenzoic acid (151) with 4-Chlorotoluene (141)

The first method used to synthesise 1-chlorothioxanthenones was reported by Smiles[88] who found that 2-mercaptobenzoic acid (140) condensed with certain hydrocarbons in sulphuric acid to furnish thioxanthenones. Ullmann[80] used 90% sulphuric acid to synthesise 1-chlorothioxanthenones from 4-chlorotoluene (141), but more concentrated acid has been found to be a more effective condensing agent.[15] Archer and Suter[15] studied the use of both the mercaptobenzoic acid (140) and

the disulphide (152) in the reaction with benzene and 4-chlorotoluene (141). The readily available crude disulphide (152) furnished the



(152)

thioxanthenone mixture in yields which were not improved by the use of pure mercaptobenzoic acid (140). This is because the mercaptobenzoic acid (140) is readily oxidised, almost quantitatively, to the disulphide (152) under the sulphuric acid conditions required for condensation.[15] Therefore, in the present synthesis of 1,8-dichloro-4-methylthioxanthen-9-one (136), it was not found necessary to reduce the disulphide (145) to the 2-chloro-6-mercaptobenzoic acid (151) before condensation with 4-chlorotoluene (141). Accordingly crude bis-(2-carboxy-3-chlorophenyl)-disulphide (145) was reacted with 4-chlorotoluene (141) in sulphuric acid to give a thioxanthenone mixture in 38% yield, Fig. 8.

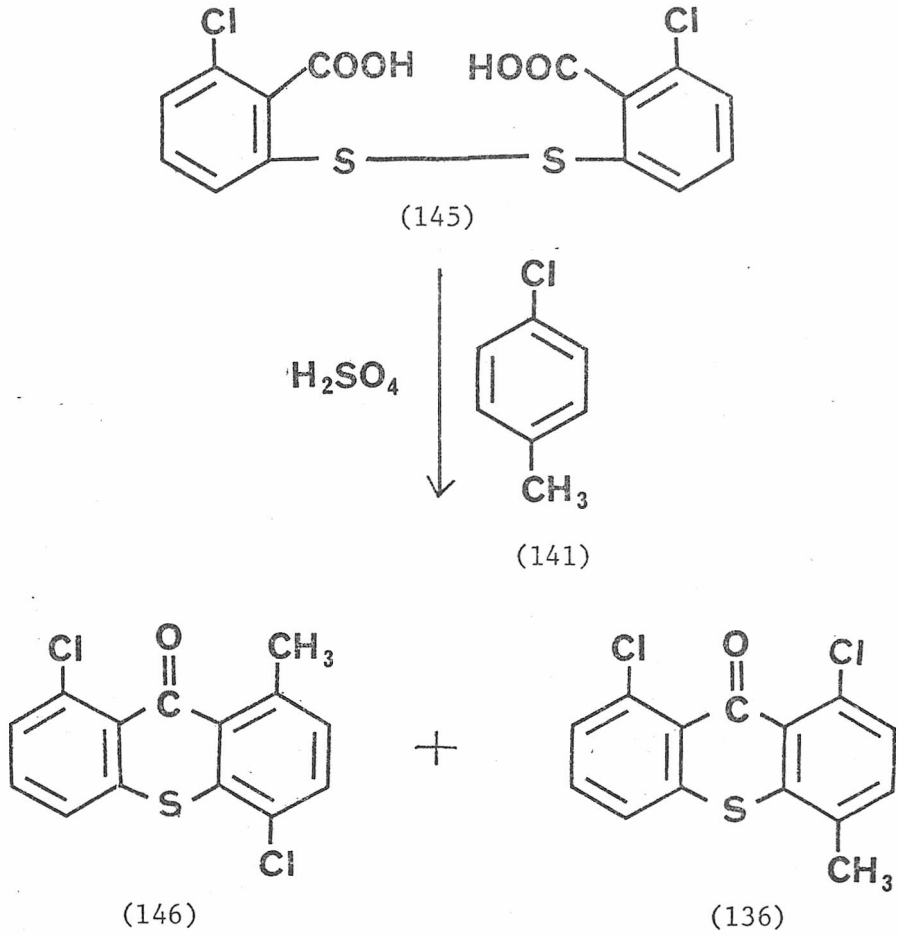


Fig. 8

The n.m.r. spectrum of this product indicated that two isomers were present; a multiplet at δ 6.9-7.2 accounted for the aromatic protons and two sharp singlets at δ 2.67 (3 protons) and δ 2.41 (3 protons) indicated the presence of an isomeric mixture. The singlet at δ 2.41 was assigned to a 4-methyl group as this methyl group was less deshielded than in the case of an 1-methyl group which lies in closer proximity to the 9-carbonyl group. Integration revealed a ratio of 41% 4,8-dichloro-1-methylthioxanthen-9-one (146) and 59% 1,8-dichloro-4-methylthioxanthen-9-one (136). This result was in accordance with the findings of other workers who reported that in

a number of different chlorothioxanthenone syntheses the 1-chloro isomer was found to be the more abundant,[115] the ortho position of 4-chlorotoluene (141) being more susceptible to electrophilic substitution than the meta position.

The two isomers were separated by preparative thin-layer chromatography and the n.m.r. spectrum of compound R_f 0.63 revealed a sharp singlet at δ 2.41 (3 protons) and a multiplet at δ 7.24-7.50 (5 protons). This would correspond to 1,8-dichloro-4-methylthioxanthen-9-one (136). The compound was fully characterised by its elemental analysis, accurate mass measurement and by its spectra which included absorptions in the i.r. and u.v. at 1672 cm^{-1} (carbonyl) and 204, 259 and 303 nm.

The n.m.r. spectrum of compound R_f 0.31 showed a sharp singlet at δ 2.67 (3 protons) and a multiplet at δ 7.06-7.58 (5 protons), corresponding to 4,8-dichloro-1-methylthioxanthen-9-one (146), and this structure was further characterised by its elemental analysis, accurate mass measurement and spectra. The i.r. and u.v. spectra showed peaks at 1672 cm^{-1} (carbonyl) and 204, 260, 300 nm.

A detailed analysis of the n.m.r. and mass spectra of these chloro-substituted compounds is reported on pages 73 and 78.

1,8-Dichloro-4-methylthioxanthen-9-one (136) from 2-Amino-4-chlorotoluene (153)

Introduction

An unambiguous synthesis of 1,8-dichloro-4-methylthioxanthen-9-one (136) was carried out involving the condensation of the potassium salt of 2,6-dichlorobenzoic acid (154) with 5-chloro-2-methylthiophenol (155), followed by cyclisation of the intermediate phenylthiobenzoic acid (156) in sulphuric acid,[15] (Fig. 9).

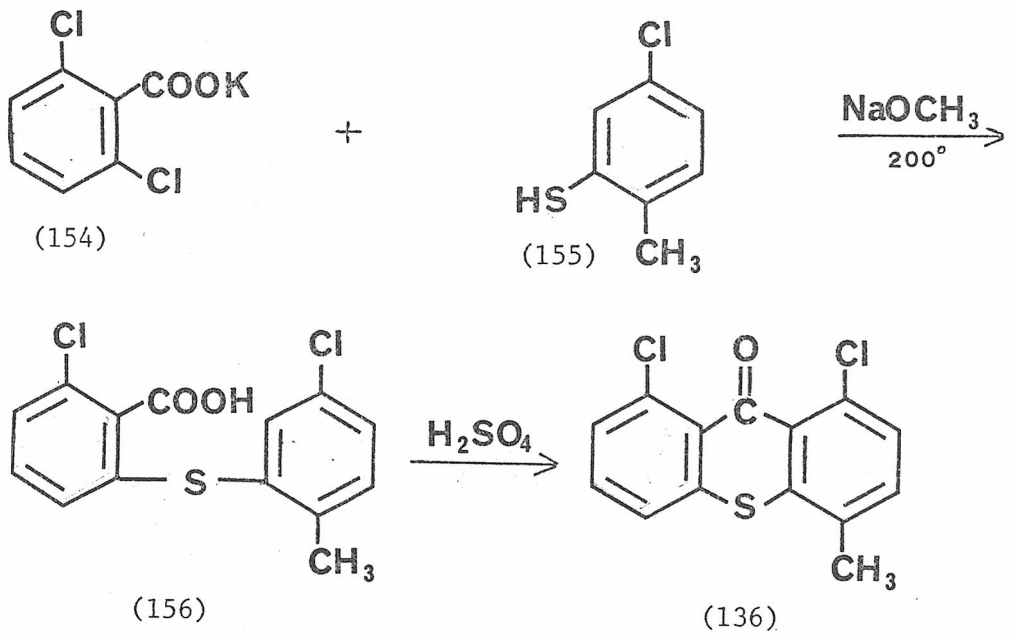
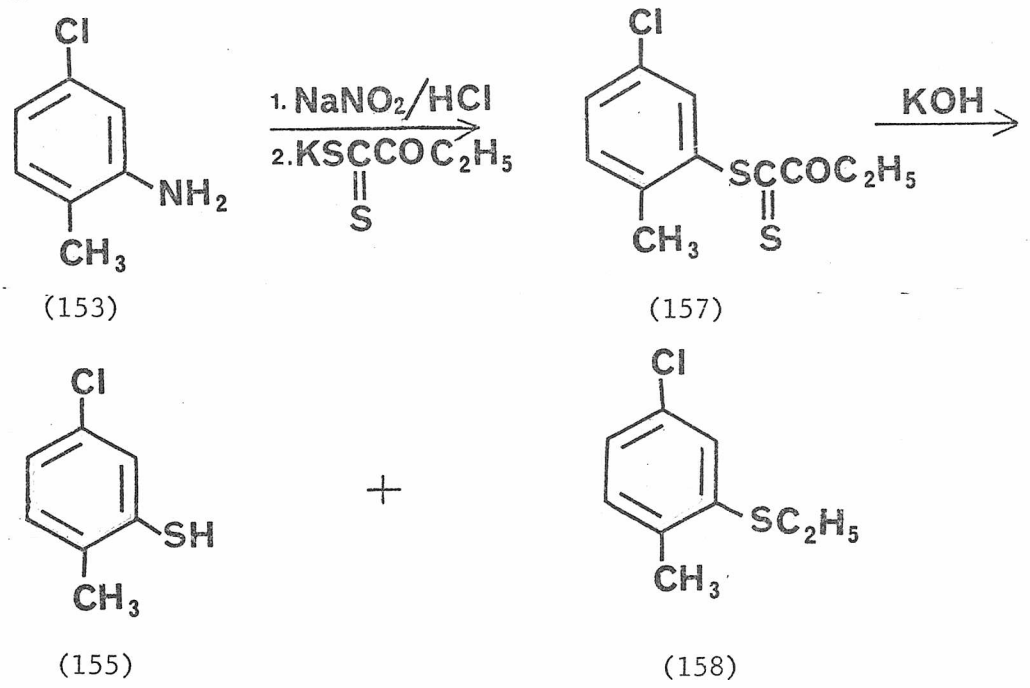


Fig. 9

Synthesis of 5-Chloro-2-methylthiophenol (155)

5-Chloro-2-methylthiophenol (155) has been synthesised by a number of routes which include treatment of 5-chloro-2-methylphenylmagnesium bromide with sulphur;[89,90] reduction of 5-chloro-2-methylbenzenesulphonic acid prepared from 2-amino-4-chlorotoluene;[15] and chlorosulphonation of 4-chlorotoluene.[91] Archer and Suter[15] found that the best method for preparing this compound consisted of converting 2-amino-

4-chlorotoluene (153) to the xanthate (157) and then hydrolysing this ester to give the thiol (155).[92] A neutral fraction was also obtained which was shown to be ethyl 5-chloro-2-methylphenyl sulphide (158), (Fig. 10).



(153) (157)

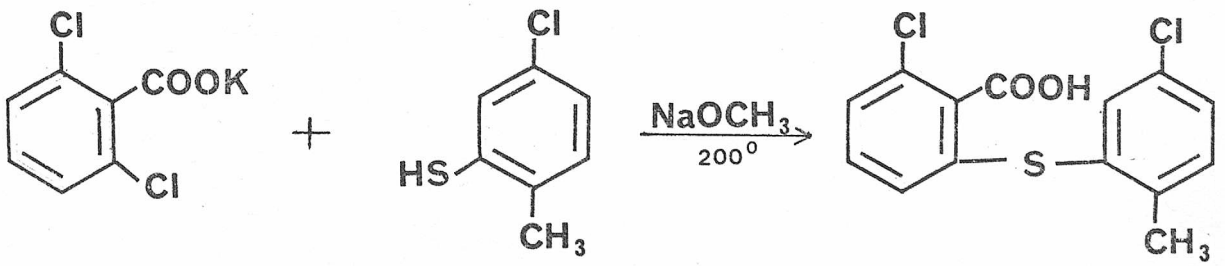
(155) (158)

Fig. 10

Using this route a yield of 78% of 5-chloro-2-methylthiophenol (155) was obtained and the structure was confirmed by its elemental analysis, mass spectrometry and spectral characteristics.

Synthesis of 6-Chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid (156)

Synthesis of the phenylthiobenzoic acid (156) involved condensation of 5-chloro-2-methylthiophenol (155) with the potassium salt of 2,6-dichlorobenzoic acid (154) at 200° giving a 53% yield of the required product.



(154) (155) (156)

Characterisation of this compound was confirmed by its elemental analysis and by its spectral properties which showed i.r. and u.v. absorptions at 2980-2880 cm^{-1} (O-H stretching), 1733 cm^{-1} (carbonyl), 211, 246 and 289 nm, respectively.

The n.m.r. spectrum showed a singlet at δ 2.38 (3 protons, methyl), a multiplet at δ 6.8-7.1 (6 protons, aromatic) and a singlet at δ 10.13 (1 proton, COOH, exchangeable with D_2O). Mass spectral data showed a molecular ion at m/e 312 and elemental analysis supported a molecular formula of $C_{14}H_{10}Cl_2O_2S$.

Cyclisation of 6-Chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid (156)

Archer and Suter[15] have reported that ring closures of phenylthiobenzoic acids were best carried out in sulphuric acid (100%) at steam bath temperatures and this technique was found to be satisfactory for the preparation of 1,8-dichloro-4-methylthioxanthen-9-one (136). The product of the reaction showed spectral characteristics identical with 1,8-dichloro-4-methylthioxanthen-9-one (136) prepared from 2-chloro-6-nitrotoluene (137), thus confirming the structure of the compound.

Nuclear Magnetic Resonance Spectroscopy of the Chlorothioxanthenones

N.m.r. data of the three chlorothioxanthenones (136), (138), (146) and 1-chloro-5-methylthioxanthen-9-one (159) (prepared in Chapter 2) are discussed in this section and summarised in Table 23.
(Chemical structures p. 98.)

Table 23. N.m.r. data of the chlorothioxanthenones

Compound	(136)	(146)	(138)	(159)
Ring CH_3	2.41(3H,s)	2.67(3H,s)	2.42(3H,s)	2.42(3H,s)
Aromatic protons	7.24-7.50(5H,m)	7.06-7.58(5H,m)	7.00-7.60(5H,m)	7.14-7.66(5H,m)
H-8			8.10-8.45(1H,m)	8.08-8.43(1H,dd, J=8Hz and 2Hz)

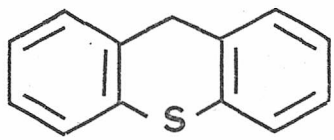
Values on δ scale.

Methyl protons

The compounds show methyl proton resonances in the range δ 2.41-2.42 with the exception of compound (146) where the resonance is at δ 2.67 due to considerable deshielding by the peri location of the carbonyl group in this thioxanthenone.

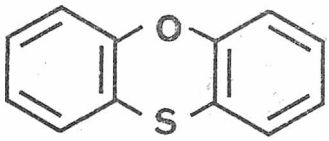
Aromatic protons

The aromatic protons of thioxanthene (160) resonate as a multiplet at δ 7.00-7.54 (8H), the sulphur atom being only weakly shielding.[93]



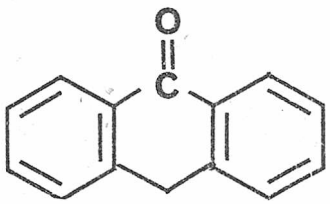
(160)

Similarly in phenoxathiin (161) the aromatic protons all possess very similar chemical shifts and are observed as a peak at δ 6.92 (8H).[93]



(161)

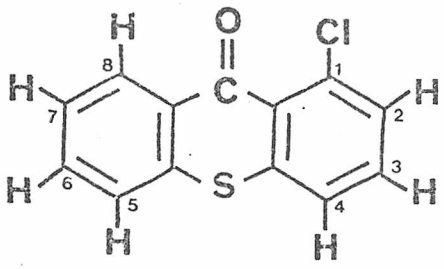
Introduction of a carbonyl group into the centre ring as in anthrone (162) results in a strong deshielding of the adjacent aromatic protons which resonate as a multiplet at δ 7.88 (2H) downfield from the other aromatic protons at δ 7.53 (6H, m).[93] This suggests that the multiplet,



(162)

integrating as one proton at δ 8.10-8.45 and δ 8.08-8.45 in compounds (138) and (159) respectively, arises from the resonance of proton H-8. The phenomenon is characteristic of protons attached to the β-carbon atom of an α,β—unsaturated carbonyl system.[93]

Assignment of this multiplet to the H-8 proton is further supported in the literature by the n.m.r. spectrum of compound (163),[94] where the resonance at δ 8.40 is assigned to the proton peri to the thioxanthenone carbonyl group.



(163)

n.m.r. ($CDCl_3$): δ 7.15-7.55 (6H, m, aromatic), 8.40 (1H, m, peri H).[94]

The multiplet nature of the absorption at δ 8.10-8.45 in compound (138) arises from an ortho-meta splitting, producing a doublet of doublets which is further split by the para hydrogen, H-5. In compound (159) where the H-8 proton is para to the methyl group, a doublet of doublet pattern is observed at δ 8.08-8.43. Compounds (136) and (146) bearing no single proton peri to the carbonyl group show no resonance in the region δ 8.0-8.5.

Chloro- and methyl- substitution on the thioxanthenones studied had little effect on the main aromatic resonance at δ 7.0-7.66 compared to thioxanthenone (160).[93] Thus the splitting patterns (10 ppm) in this region were not sufficiently resolved to allow a more detailed assignment of the aromatic protons.

The aromatic protons in compound (136) produced two major peaks which upon expansion of the scale to 5 ppm showed the fine structure presented in Fig. 11.

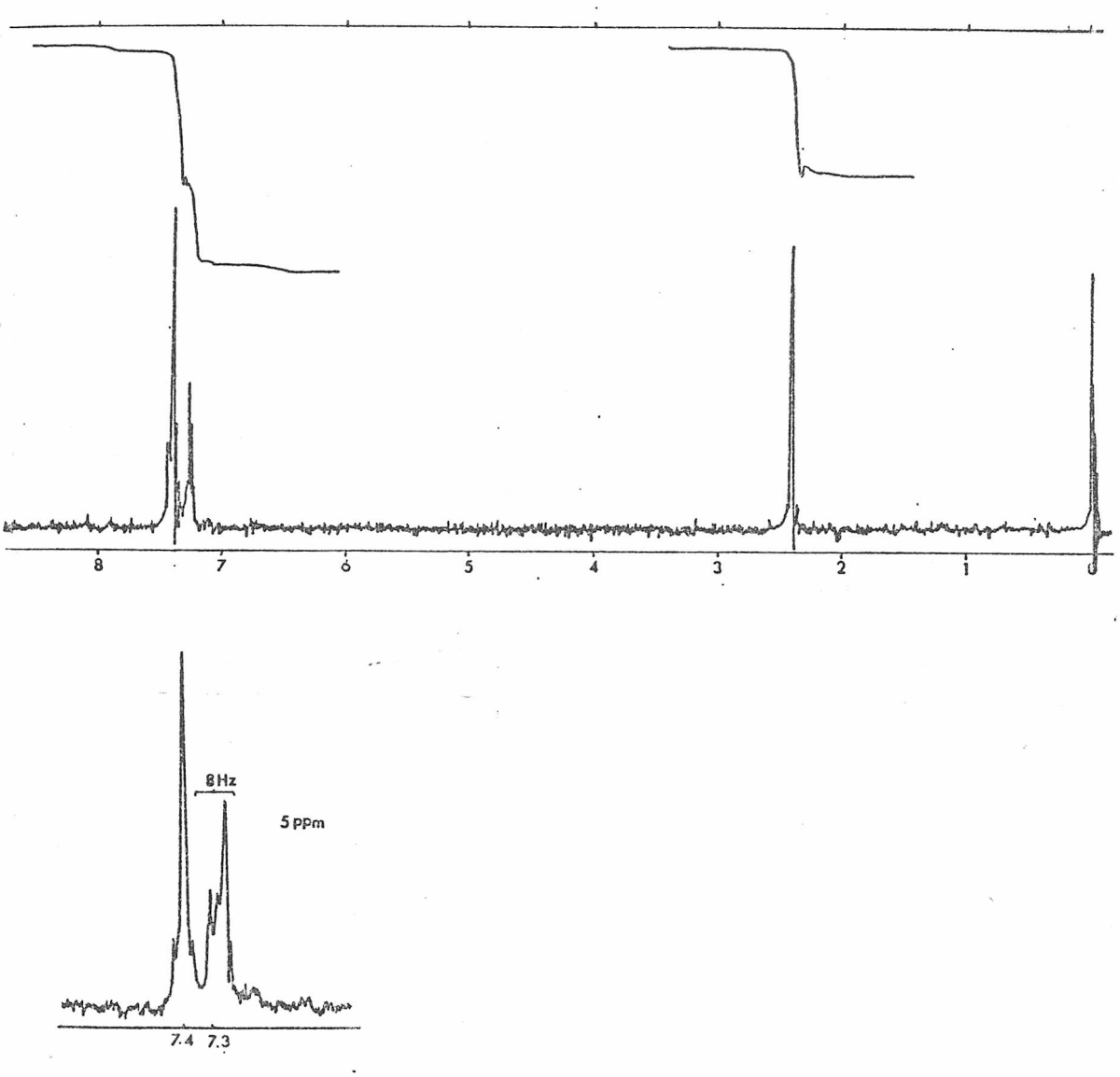


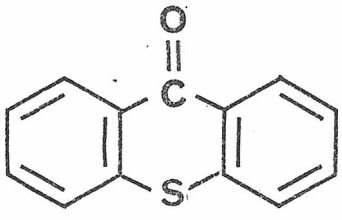
Fig. 11. N.M.R. spectrum of 1,8-dichloro-4-methylthioxanthen-9-one (136).

It would be expected that the proton ortho to the 4-C methyl would be slightly shielded and give rise to an ortho doublet, possibly the one centred at δ 7.3 (J = 8Hz). Attempts to confirm this suggestion by decoupling the H-2 and H-3 protons using the double resonance technique were unsuccessful.

Mass Spectrometry of Chlorothioxanthenones

Mass spectral data of the three chlorothioxanthen-9-ones (136), (138), (146) and 1-chloro-5-methylthioxanthen-9-one (159) are discussed in this section. (chemical structures p. 98).

The mass spectra of chlorothioxanthenones and lucanthone analogues have not been reported in the literature, however, the fragmentation of thioxanthen-9-one (134) has been described by Heiss and Zeller [95] (Fig. 12).



(134)

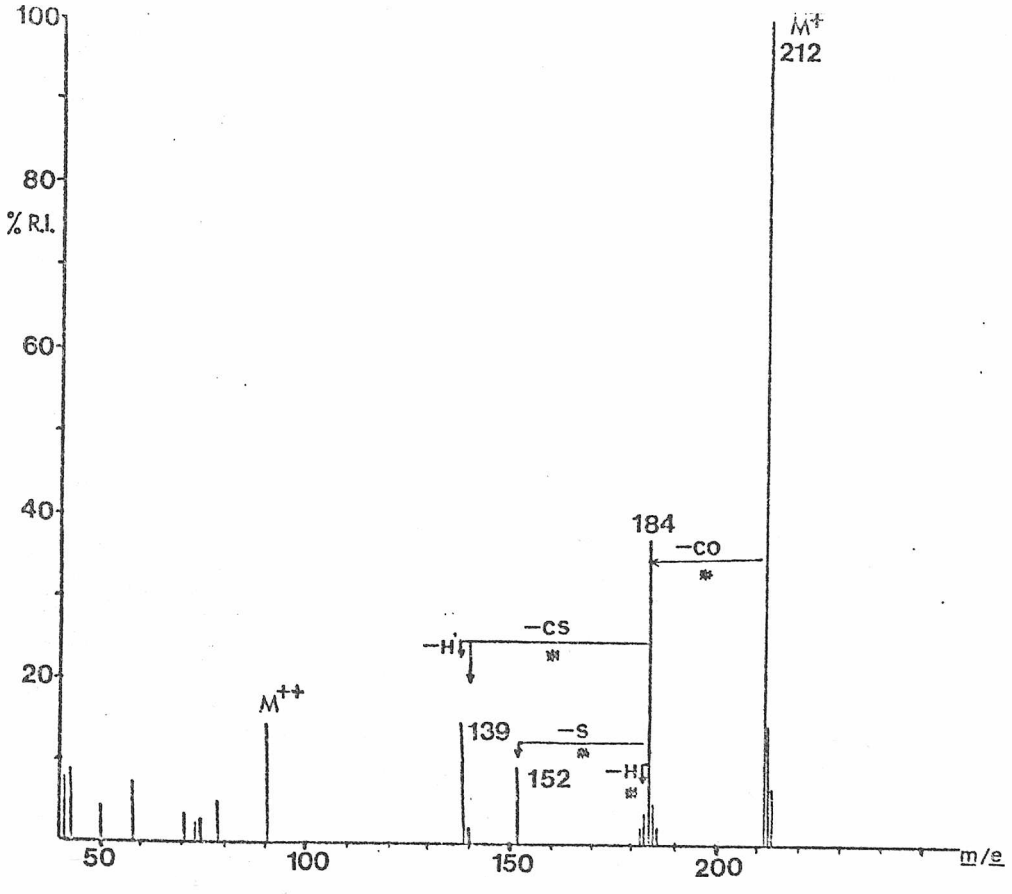
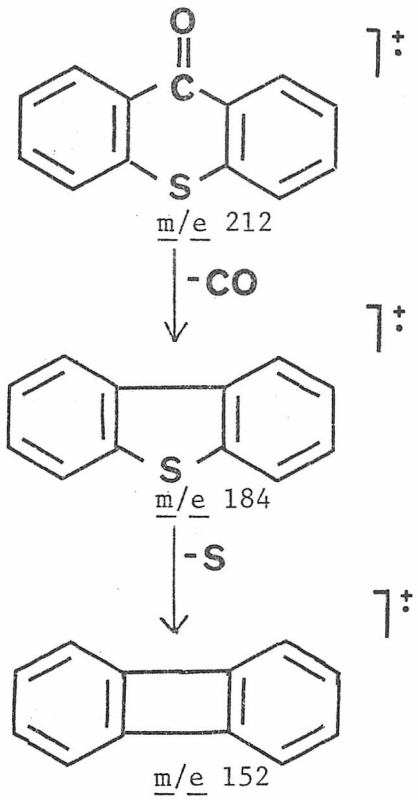


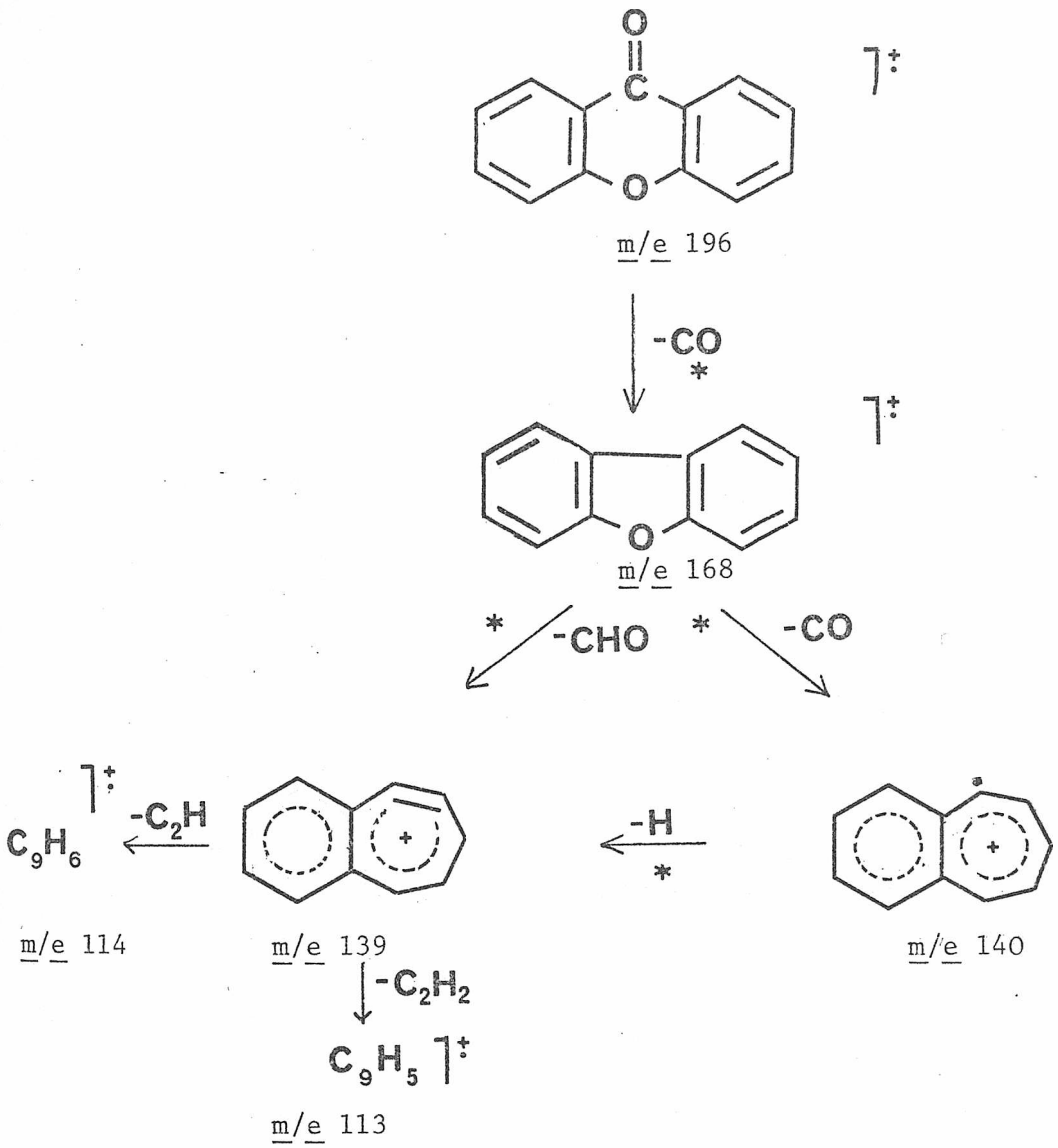
Fig. 12. Mass spectrum of thioxanthen-9-one (134)

Fragmentation occurs by loss of a carbonyl group to give the ion m/e 184, and the diphenylene sulphide cation further fragments by loss of sulphur (m/e 152) or CS + H (m/e 139). Schumann, Frese and Schönberg[96] report a fuller spectrum: m/e (%) 212(100), 184(21), 152(3), 149(8), 108(3), 106(4), 92(10), 89(15), and suggested the following degradation pattern (Scheme 1).



Scheme 1

This is similar to the fragmentation of xanthen-9-one (164) proposed by Arends et al.[97] (Scheme 2).



Scheme 2

the ions m/e 139 and 140 being common to both schemes.

Compounds (138) and (159) show similar fragmentation patterns, fragmentation occurring by the loss of the carbonyl group followed by loss of the chlorine atom. This latter fragmentation is supported by the change in isotope abundance pattern.

The spectrum of compound (138) is given in Fig. 13 and fragmentation pathway in Scheme 3.

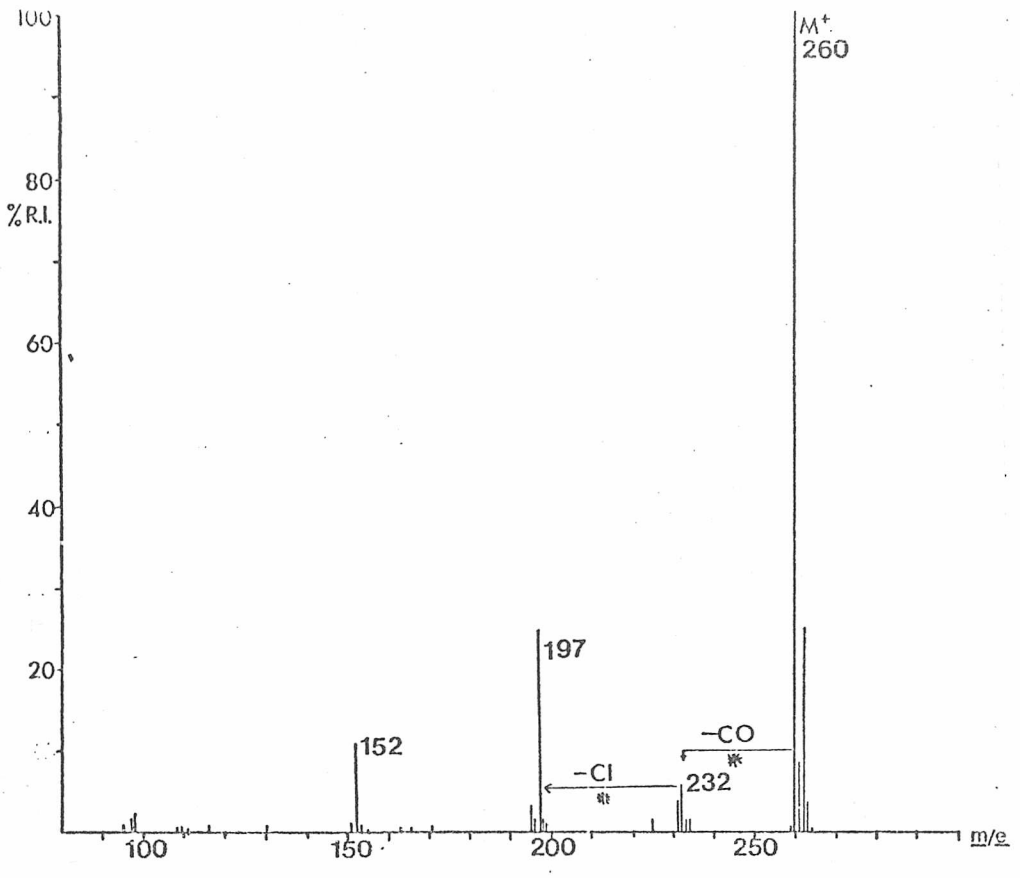
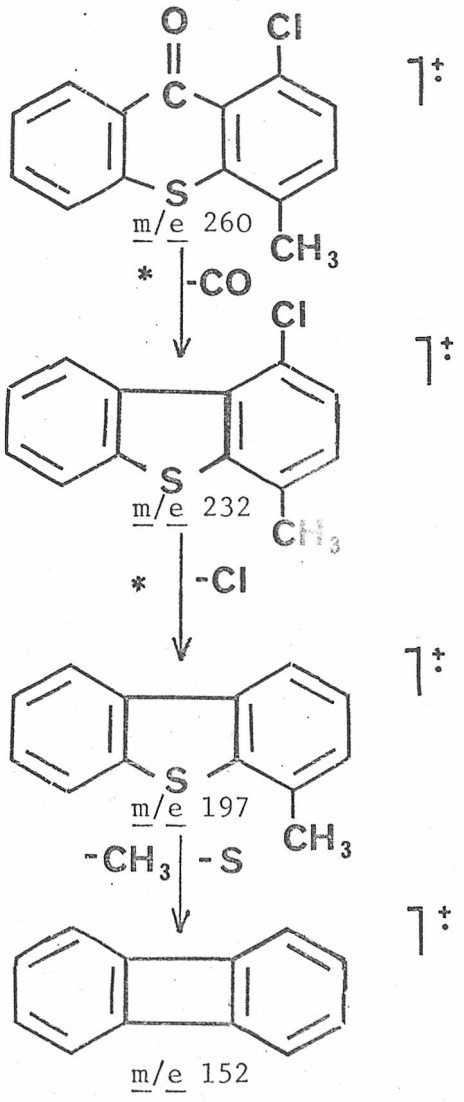


Fig. 13. Mass spectrum of 1-chloro-4-methylthioxanthen-9-one (138)



Scheme 3

In both compounds (138) and (159) the ion m/e 152 is the main fragmentation product through the loss of the sulphur and of the methyl group; the ions at m/e 139 and 140 are not observed.

A similar pattern of fragmentation is seen in 1,8-dichloro-4-methylthioxanthen-9-one (136); however, the isotope abundance pattern is more complex as a result of the presence of the two chlorine atoms.

Loss of a carbonyl group occurs primarily followed by the loss of the two chlorine atoms, (Fig. 14).

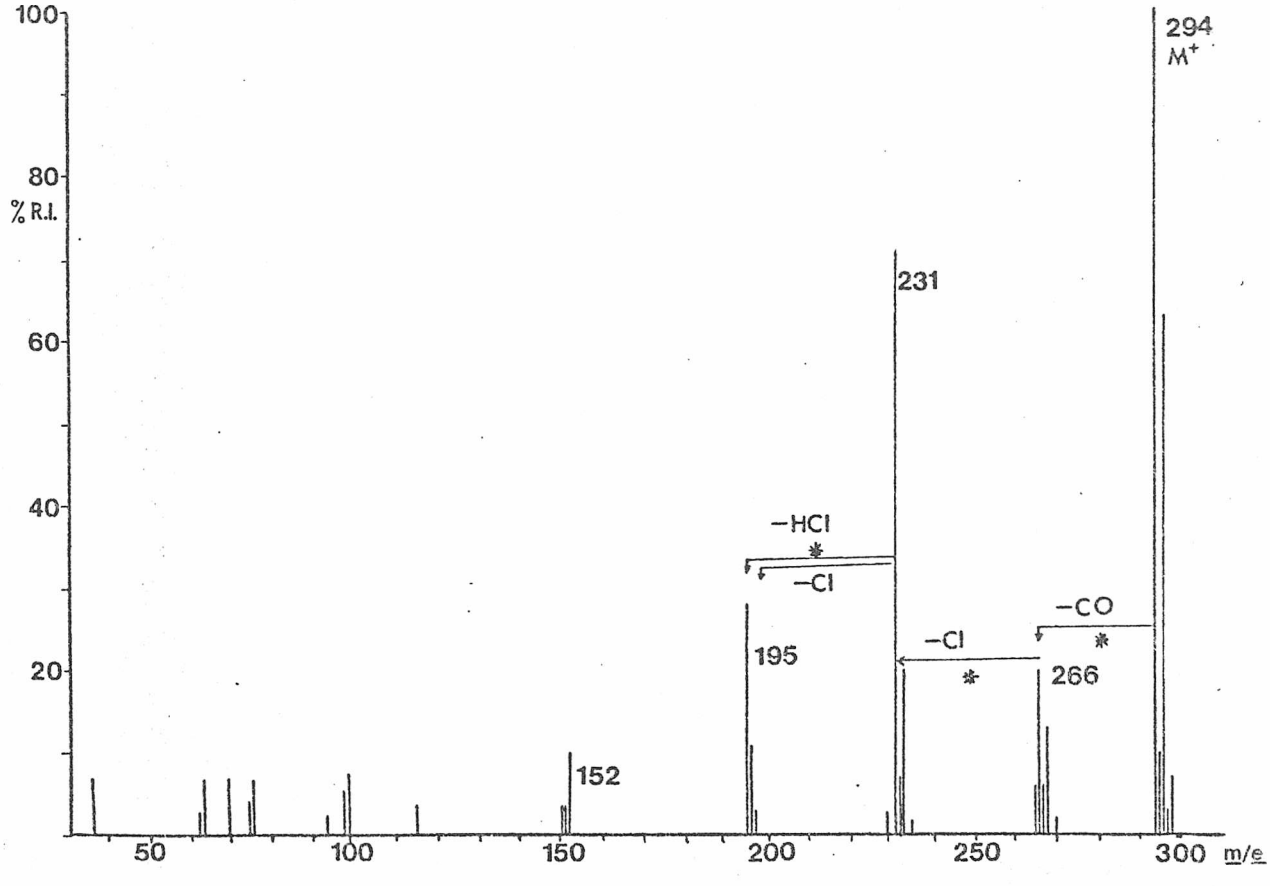


Fig. 14. Mass spectrum of 1,8-dichloro-4-methylthioxanthen-9-one (136).

In contrast to 1,8-dichloro-4-methylthioxanthen-9-one (136), 4,8-dichloro-1-methylthioxanthen-9-one (146) exhibits a complex fragmentation pattern with initial loss of 92 mass units indicating a cleavage of one aromatic ring to give an ion m/e 202 as the base peak, showing an isotope

abundance characteristic of only one chlorine [204:202 (28:100)]. Therefore the cleavage of the ring must have been accompanied by a loss of one chlorine atom. Accurate mass measurement of the ion m/e 202 gave a mass which corresponded to the molecular formula $C_7H_3Cl S_2O$ indicating sulphur capture during fragmentation. This suggests that the molecule fragments by a different pathway to the general scheme outlined for the other chlorothioxanthenones examined.

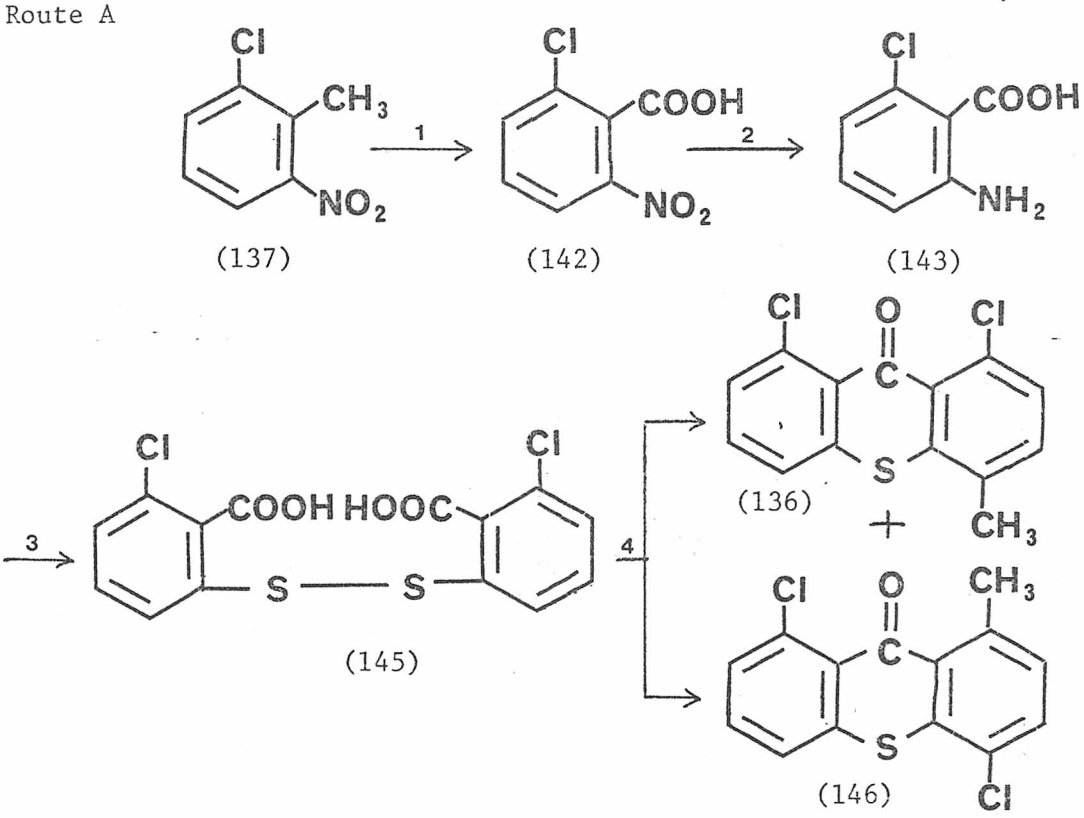
Comparison of the two Syntheses of 1,8-Dichloro-4-methylthioxanthen-9-one (136)

Maximum and minimium yields obtained by repetition of each stage of the synthesis of 1,8-dichloro-4-methylthioxanthen-9-one (136) from 2-chloro-6-nitrotoluene (137) and 2-amino-4-chlorotoluene (153) are recorded in Table 24.

The 2-amino-4-chlorotoluene route has the overall advantage of producing the 1,8-dichloro isomer (136) only, whereas the 2-chloro-6-nitrotoluene route produces an equivalent overall percentage yield of mixed thioxanthenone of which only 58% was the desired isomer. Therefore the higher yield and single isomer produced from the 2-amino-4-chlorotoluene (153), together with the relative ease of this reaction experimentally, has favoured the use of this route in subsequent production of quantities of 1,8-dichloro-4-methylthioxanthen-9-one (136) for further reactions.

Table 24. Comparison of yields of 1,8-dichloro-4-methylthioxanthen-9-one (136) by two independent routes.

Route A



Yields % w/w	Reaction stage 1*	2	3	4	Net
Minimum	30	80	80	40	7.7
Maximum	40	95	95	50	18.2

Minimum of 5 attempts at each stage

average = 12.9% (isomeric mixture)

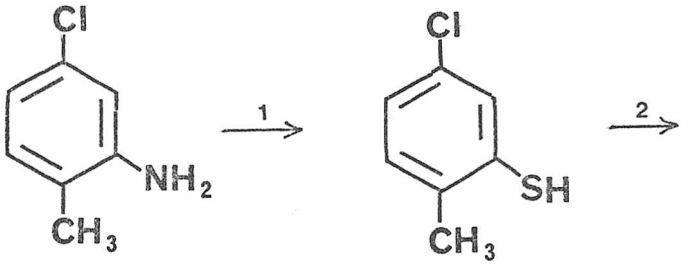
* 40 experiments

As only 58% of the final product was the 1,8-dichloro isomer (136) average yield of the 1,8-dichloro isomer is 7.5% overall.

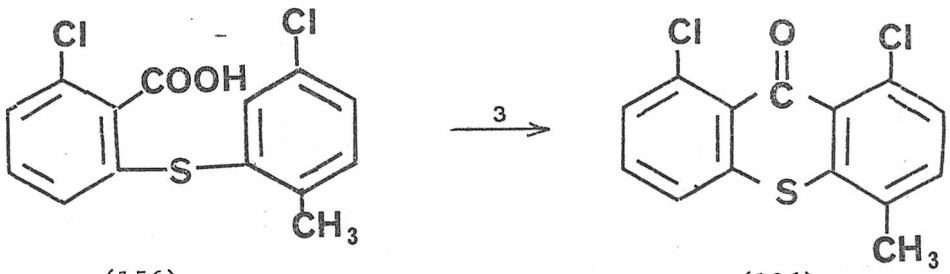
Continued

Table 24. Continued

Route B



(153) (155)



(156) (136)

Yields % w/w	Reaction stage 1	2	3	Net
Minimum	40	50	50	10
Maximum	54	70	55	21

average = 15%

EXPERIMENTAL

General Experimental Details.

Infrared Spectra

Infrared spectra were recorded on a Hilger Infrascan spectrophotometer. Unless otherwise stated the spectra were run as potassium bromide discs.

Ultraviolet Spectra

Ultraviolet spectra were recorded on a Unicam S.P. 800 spectrophotometer using 1 cm quartz cells, and refer to solutions in ethanol.

Nuclear Magnetic Resonance Spectra

All spectra were recorded using a Varian A60 spectrometer, except those of substituted methylthioxanthen-9-ones which were recorded on a Perkin-Elmer R12B 60 MHz spectrometer. Spectra were recorded in deuterochloroform containing tetramethylsilane ($\delta = 0$) as an internal standard, unless otherwise stated in the text.

Mass Spectra

Mass spectra were measured with an A.E.I. M.S. 30 low resolution mass spectrometer, at an electron beam energy of 70 eV.

Melting Points

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected, except those designated "Corr." which were determined on a precalibrated 'Kofler' hot bench apparatus.

Chromatography

(a) Thin-layer chromatography

Polygram SiℓN-HR coated plastic thin layer chromatography plates containing 0.2 mm, MN Silica Gel (Camlab), were used for all t.l.c. work.

(b) Column chromatography

Silica gel MFC 100 to 200 mesh, supplied by Hopkins and Williams was used for column chromatography.

(c) Preparative thin-layer chromatography

Silica gel/CT, supplied by Reeve Angel was used for preparing preparative plates, using silica (80 g) stirred in water (160 g) and spread onto five glass plates 20 x 20 x 0.75 cm, dried at room temperature for 6 hours, then at 70° overnight and activated at 110° for 30 minutes.

1-Chloro-4-methylthioxanthen-9-one (138)

A sample of 1-chloro-4-methylthioxanthen-9-one was supplied by the Wellcome Research Laboratories, Beckenham. This sample contained an impurity, the isomeric 4-chloro-1-methylthioxanthen-9-one which was removed by chromatogrpahic separation on a silica column eluted with chloroform. The material was recrystallised from alcohol, forming yellow needles, m.p. 142-145° (Lit.,[91] 143-145°); $\nu_{max.}^{KBr}$ 1640 (carbonyl), 1600, 1435, 1425, 1300, 810 (2 adj. free hydrogens), 740 (4 adj. free hydrogens) cm^{-1}; $\lambda_{max.}^{EtOH}$ 205 (log ε 4.12), 222 (4.10), 259 (4.50), 305 (3.70), 380 nm (3.64); δ 2.42 (3H, s, CH_3), 7.00-7.60 (5H, m, aromatic), 8.10-8.45 (1H, m, H-8); R_f 0.27 ($CC\ell_4$); m/e (%) 262(25), 260(100), 234(2), 232(6), 197(25), 152(11), 130(1), 98(3); m^* 207.0 (260 → 232), 167.3 (232 → 197).

The Synthesis of 1,8-Dichloro-4-methylthioxanthen-9-one (136) from 2-Chloro-6-nitrotoluene (137)

(i) Potassium permanganate (direct oxidation)

2-Chloro-6-nitrotoluene (10.0 g, 1 mol) was added to a hot solution of potassium hydroxide (3.6 g in 25 mℓ water) and refluxed gently for 10 minutes. Potassium permanganate (33 g, 3.6 mol) was dissolved in water (200 mℓ) at 65°, added to the solution and the mixture refluxed gently with stirring. After 3 hours most of the purple colour had disappeared and the solution was allowed to cool before adding 5% hydrochloric acid to acidify. The mixture was gently refluxed for ½ hour. After cooling, sodium metabisulphite was added portionwise until the precipitate of manganese dioxide was dissolved.

The organic fraction was extracted using ether (x 3) and separated

into a neutral fraction containing starting material and an acidic fraction using aqueous sodium carbonate solution (5%). After acidifying with dilute hydrochloric acid the acid fraction was extracted with ether, dried over anhydrous sodium sulphate, and the ether distilled off on a rotary evaporator under vacuum, yielding 2-chloro-6-nitrobenzoic acid (3.75 g, 32%). The acid was recrystallised from dilute ethanol; m.p. 155-157° (Lit.[82] 161°); $\nu_{max.}^{KBr}$ 3100-2560 (O-H stretching), 1710 (carbonyl, α-halo aryl acid), 1532, 1460, 1359(NO_2), 1270, 1155, 1110, 813, 765 and 750 cm^{-1}; $\lambda_{max.}^{EtOH}$ 212 (log ε 4.30), 260 nm (3.78), in alkali 218(4.22), 266 nm (3.72); δ 7.82 (1H, dd, J = 8Hz and 2Hz, H-5), 7.37 (1H, t, J = 8Hz, H-4), and 7.06 (1H, dd, J = 7Hz and 2Hz, H-3). No resonance associated with the carboxylic acid proton was detected; R_f 0.16 (chloroform-ethanol 4:1).

(ii) Bromination route

Oxidation of 2-chloro-6-nitrotoluene was carried out by the method of Lehmstedt and Schrader.[82] 2-Chloro-6-nitrotoluene (10.3 g) was refluxed with bromine (3.6 mℓ) for 2 hours at 165° under ultraviolet light (optimum wavelength 256 nm). The temperature was not allowed to exceed 170° to avoid charring. After cooling, a solution of potassium acetate (7 g) in ethanol (50 mℓ) was added to the intensely lachrymatory bromide and the solution gently refluxed for 2 hours to yield a dark brown alcoholic solution and white precipitate which was filtered off. Water was added to the alcoholic solution and the ester separated as a brown oil. The oil was washed with water (x 3) and then refluxed with potassium hydroxide (7.0 g) in water (70 mℓ) for 2 hours and the resulting alcohol oxidised by the addition of finely divided potassium permanganate (10 g) with gentle refluxing. After 30 minutes the excess permanganate was reduced

using dilute sulphuric acid (50 mℓ) and sodium metabisulphite was added until the solution became clear. The acid was extracted with chloroform (x 2) and dried over anhydrous sodium sulphate, giving a brown solid upon evaporation of the solvent (2.4 g) which, after recrystallisation from ethanol, gave 2-chloro-6-nitrobenzoic acid, m.p. 158-160° (Lit.[82] 161°). Identical u.v., i.v. spectra and R_f were obtained with the product of the direct permanganate oxidation of 2-chloro-6-nitrotoluene.

Methyl-2-chloro-6-nitrobenzoate (150)

2-Chloro-6-nitrobenzoic acid (8.5 g) was dissolved in methanol (17.5 g), methyl iodide (17.5 g) and freshly prepared silver oxide (14.3 g) were added and the mixture gently warmed for 1 hour following the method of Cohen and McCandlish.[98] The reaction proceeded spontaneously and was completed by refluxing for 30 minutes. Silver residue was filtered off and washed with hot methanol. The washings and reaction mixture evaporated down to yield an oil which was extracted with ether. Starting material was removed by washing the extract with aqueous sodium carbonate (5%) and the ethereal layer dried with anhydrous magnesium sulphate before being evaporated on a rotary evaporator under vacuum to give methyl 2-chloro-6-nitrobenzoate (94%), m.p. 88-90°. Upon recrystallisation from methanol, pale yellow crystals were produced, m.p. 89-91°, (Lit.[98] 80-82°; [99] 94-95°); $\nu_{max.}^{KBr}$ 1735 (carbonyl), 1527, 735 cm^{-1}; $\lambda_{max.}^{EtOH}$ 212 (log ε 4.38), 258 nm (3.80); δ 3.88 (3H, s, $COOCH_3$), 7.00-7.55 (2H, m, aromatic), and 7.82 (1H, dd, $\underline{J}$ = 8Hz and 2Hz, H-5). R_f 0.38 ($CHC\ell_3$).

2-Amino-6-chlorobenzoic acid (143)

Reduction of 2-chloro-6-nitrobenzoic acid was undertaken using the method of Newman and Childers[85] with modifications in the extraction. 2-Chloro-6-nitrobenzoic acid (10 g) was dissolved in concentrated ammonia (60 mℓ, 0.880) and added to a solution of ferrous sulphate heptahydrate (100 g) in water (300 mℓ) at 80°. The reaction mixture was allowed to stand for 10 minutes and then the hot solution was filtered and the cake extracted with hot ammonia solution and filtered. The amino acid was extracted with ethyl acetate after acidifying the solution to pH2 with dilute sulphuric acid. The extract was dried over anhydrous magnesium sulphate and removal of the solvent produced a yellow crystalline material, 2-amino-6-chlorobenzoic acid (87%), m.p. 144-146° (Lit.,[84] 146-147°); $\nu_{max.}^{KBr}$ 3470 and 3360 (NH_2), 3000-2500 (O-H stretching, carboxylic acid), 1660 (carbonyl), 1615, 1590, 1300, 1235, 915, 750 cm^{-1}; $\lambda_{max.}^{EtOH}$ 212 (log ε 4.12), 251 nm (3.59); δ (CD_3OD) 4.73 (3H, s, NH_3^+), 6.40 (1H, dd, $\underline{J}$ = 8Hz and 2Hz, H-5), 6.5-7.0 (2H, m, H-3 and H-4); R_f 0.32 (ether-chloroform, 1:1).

2-Chloro-6-mercaptobenzoic acid (151)

2-Chloro-6-mercaptobenzoic acid was prepared by the method of Allan and MacKay.[86] Sodium sulphide (7.8 g) was dissolved in boiling water (15 mℓ) and powdered sulphur (1.02 g) added. A solution of sodium hydroxide (6 mℓ, 20%) was added and the mixture cooled and placed in a freezing mixture of calcium chloride and ice (c. -5°). 2-Amino-6-chlorobenzoic acid (5.15 g) was dissolved in water and concentrated hydrochloric acid (1 mℓ) and cooled to 6° before adding to the sulphide solution. Sodium nitrite solution (2.1 g) was slowly added below the surface of the liquid by means of a pipette and the addition controlled so

that the reaction temperature did not exceed 5°. When the addition was complete the ice bath was removed and the reaction allowed to warm to room temperature and stand for 2 hours. Concentrated hydrochloric acid (0.9 mℓ) was added until the solution became acid to Congo red, precipitating bright orange crystals of bis-(2-carboxy-3-chlorophenyl)-disulphide.

The disulphide was reduced to the chloromercaptobenzoic acid by the method of Amoretti and Pagani.[87] A suspension of the disulphide with zinc powder (3 g) in glacial acetic acid (60 mℓ) was boiled gently with stirring for 10 hours. The reaction was cooled and the product, collected by filtration, was washed and resuspended in water. The suspension was acidified with concentrated hydrochloric acid and extracted with ether. The ethereal extract was dried with anhydrous sodium sulphate, evaporated to a solid, and recrystallised from warm ethanol to give 2-chloro-6-mercaptobenzoic acid (50%), m.p. 85-90° (Lit.,[86] 106-108°); $\nu_{max.}^{KBr}$ 3600-3400 (COOH), 2650, 2555 (S-H), 1707 (carbonyl), and 750 cm^{-1}; $\lambda_{max.}^{EtOH}$ 215 nm (log ε 4.41); δ 3.50 (1H, s, S-H), and 7.20 (3H, s, H-3, H-4 and H-5); R_f 0.49 (ether-chloroform 1:1).

1,8-Dichloro-4-methylthioxanthen-9-one (136) synthesis from Bis-(2-carboxy-3-chlorophenyl) disulphide (145)

Crude bis(2-carboxy-3-chlorophenyl) disulphide (2.0 g) prepared from 6-chloroanthranilic acid was treated with 100% sulphuric acid (25 mℓ). The mixture was cooled to 22° and 4-chlorotoluene (25 mℓ, Emanuel laboratory grade) added with vigorous stirring. Within one minute the temperature rose to 30°. The mixture was cooled in an ice-alcohol bath, but the temperature increased until it reached 35°. The cooling bath was removed and stirring continued until a total of 15 hours had elapsed.

The red solution was poured into water and the yellow precipitate collected on a filter. The crude thioxanthenone mixture was boiled with dilute ammonia for 15 minutes, filtered, washed with water, and dried at 70°, yielding 1.2 g (38%) of mixed thioxanthenone isomers, m.p. 112-115 °. Thin-layer chromatography demonstrated the presence of disulphide starting material R_f 0.45 and two other spots detectable by ultraviolet fluorescence. Separation of this crude product by preparative t.l.c. (silica gel; chloroform) gave a band at R_f 0.31 which corresponded to 4,8-dichloro-1-methylthioxanthen-9-one. This band was removed and extracted to give a white crystalline solid (<10 mg), m.p.174-176°; $\nu_{max.}^{KBr}$ 1672 (carbonyl), 1568, 1432, 795, 777 (3 adj. free H) cm^{-1}; $\lambda_{max.}^{EtOH}$ 204 (log ε 4.12), 260,(4.35), 300(3.51), 360 nm (3.20); δ 2.67 (3H, s, CH_3), 7.06-7.58 (5H, m, H-2,3,4,6, and 7). m/e (%) 298(0.3), 296(1.5), 294(2), 204(28), 202(100), 174(3), 158(3), 140(8), 139(18), 138(35), 110(9), 105(4). m^* 138.8 (294 → 202). Found M (mass spectrometry): 294.9676. $C_{14}H_8Cl_2OS$ requires 294.9673. Found (mass spectrometry): 201.9315. $C_7H_3ClS_2O$ requires 201.9313.

Removal and work up of the band at R_f 0.63 corresponding to 1,8-dichloro-4-methylthioxanthen-9-one led to the isolation of a white crystalline solid (30 mg), m.p. 168-169°. This compound showed spectral characteristics associated with a thioxanthenone ring system; $\nu_{max.}^{KBr}$ 1672, (carbonyl), 1575, 1565, 1432, 1292, 1267, 1240, 1200, 1105, 970, 860, (2 adj. free H), 797, 777 (3 adj. free H) cm^{-1}; $\lambda_{max.}^{EtOH}$ 204 (log ε 4.20), 259(4.41), 303(3.72), 370 nm (3.45); δ 2.41 (3H, s, CH_3), 7.24-7.50 (5H, m, H-2,3,5,6 and 7). m/e (%) 298(11), 297(5), 296(65), 295(10), 294(100), 270(2), 268(13), 267(6), 266(20), 265(6), 259(3), 233(22), 232(2), 231(71), 229(3), 197(3), 196(11), 195(28), 152(10), 151(4), 150(3.5), 115(4), 98(10), 75(7); m^* 240.7 (294 → 266), 200.6 (266 → 231), 166.3 (231 → 196). Found: M (mass

spectrometry) 294.9674. $C_{14}H_8Cl_2OS$ requires 294.9674. Found: C 56.82; H, 2.65; S, 11.03. $C_{14}H_8Cl_2OS$ requires C, 56.85; H, 2.71; S, 10.83%.

The Synthesis of 1,8-Dichloro-4-methylthioxanthen-9-one (136) from 2-Amino-4-chlorotoluene (153)

5-Chloro-2-methylthiophenol (155)

To a mixture of concentrated hydrochloric acid (75 mℓ) and ice (75 g), was added 2-amino-4-chlorotoluene (54.5 g). The suspension was kept at 0-5° and diazotised with sodium nitrite (28 g) dissolved in water (62 mℓ), previously cooled to 5°. Nitrite solution was added slowly, maintaining the reaction temperature below 5°. After all the nitrite had been added the solution was stirred for one hour and the insoluble residue filtered off. A solution of potassium ethylxanthate (72 g) in water (75 mℓ) at 60° was added dropwise to the clear diazonium solution with the aid of mechanical stirring. The temperature was kept at 55-60°. When all the solution had been added the mixture was kept at 70-80° for one hour.

The red xanthate layer was removed and the aqueous solution was extracted with ether, dried with anhydrous sodium sulphate, to yield an additional amount of xanthate in the form of a red oil. The combined oil layers corresponded to a 98% yield of xanthate.

The oil (93 g) was dissolved in ethanol (50 mℓ) and heated to boiling and then a solution of potassium hydroxide (87.5 g) in water (200 mℓ) was added. The solution was refluxed for 10 hours. The ethanol was removed *in vacuo* and water (500 mℓ) was added to the gummy product followed by ether extraction. The ether layer was dried and concentrated yielding the side-product ethyl 5-chloro-2-methylphenyl sulphide (7.2 g, 10%).

The alkaline aqueous layer was carefully acidified and the oil which separated was extracted with ether, dried and the ether distilled off to give 5-chloro-2-methylthiophenol (36.6 g, 59%). In subsequent experiments

the initial ether extraction was omitted and the gum was steam distilled. Ether extraction of the steam distillate yielded, after drying, 5-chloro-2-methylthiophenol (70-78%), b.p. 247-248° (760 mm), [Lit.,[15] 126-128° (30 mm)]; $\nu_{max.}^{KBr}$ 2940, 2585 (S-H), 1587, 1470, 1380, 1113, 1072, 1028, 835 and 812 cm^{-1}; $\lambda_{max.}^{EtOH}$ 213 (log ε 4.46), 242 (3.97), 284 nm (3.39); δ 2.23 (3H, s, CH_3), 3.30 (1H, s, S-H), 6.99 (1H, s, H-6) and 8.20 (2H, m, H-3 and H-4); R_f 0.51 (chloroform-ether, 1:1); m/e (%) 160(22), 159(9), 158(100), 157(18), 156(5.5), 144(4.5), 147(13), 145(22), 127(10), 126(5), 125(50), 124(8), 123(28), 121(7), 112(5.5), 111(16), 110(18), 109(20), 99(4), 89(18), 84(6), 77(8), 75(28), 74(22), 73(5.5), 69(3), 63(9). Found: C, 52.09; H, 4.45; Cℓ, 23.24; S, 18.83. $C_7H_7C\ell S$ requires C, 52.96; H, 4.41; Cℓ, 22.38; S, 20.18%.

6-Chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid (156)

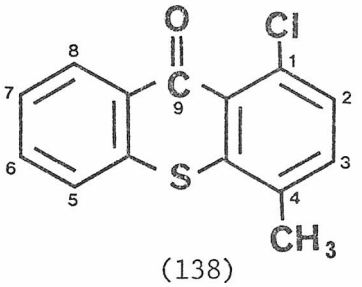
To a solution of sodium (2.54 g) in methanol (45 mℓ) was added a mixture of 5-chloro-2-methylthiophenol (44 g), potassium salt of 2,6-dichlorobenzoic acid (46.6 g) and copper-bronze powder (0.2 g). The mixture was warmed gently so that the methanol evaporated slowly with minimum loss of the volatile thiophenol.

The reaction mixture was stirred mechanically and heated to 140° in a Wood's metal bath to produce a molten mass. The temperature increased spontaneously to 200° with visible evolution of vapour and was held at 210-215° for 15-20 minutes. Careful temperature control of this reaction was found necessary as there was a tendency for overheating and charring in the molten mass. Loss of volatile thiophenol was minimised by the use of a reflux condenser instead of the open vessel technique originally used. A change in appearance of the molten mass from orange to green-yellow was observed. On cooling the solid was treated with sodium carbonate

solution (100 mℓ, 5%) and the suspension heated to boiling and filtered. The filtrate was acidified and the excess thiophenol recovered by steam distillation (4.9 g, 11%). After all the volatile material had been removed the residue was cooled, filtered, washed with water and ether extracted. The grey solid (45.9 g, 53%) remaining was air dried and recrystallised from glacial acetic acid to give 6-chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid, m.p. 77-78°; $\nu_{max.}^{KBr}$ 2980-2880 (O-H), 1733 (carbonyl), 1580, 1565, 1435, 1285, 805, and 778 cm^{-1}; $\lambda_{max.}^{EtOH}$ 211 (log ε 4.64), 246 (4.17), 289 nm (3.65); δ 2.38 (3H, s, CH_3), 6.8-7.1 (6H, m, aromatic) and 10.13 (1H, s, COOH, exchangeable with D_2O); R_f 0.63 (chloroform-ether, 1:1); m/e (%) 316(3), 314(18), 312(28), 160(7), 159(25), 158(40), 157(100), 156(28), 155(4), 125(5), 123(2.5), 122(5), 121(10), 112(1.5), 89(4), 78(2.5), 77(10), 69(3), 64(3), 52(3), 46(63); Found: C, 53.26; H, 3.62. $C_{14}H_{10}Cl_2O_2S$ requires C, 53.65; H, 3.20%.

1,8-Dichloro-4-methylthioxanthen-9-one (136) synthesis from 6-Chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid (156)

6-Chloro-2-[(5-chloro-2-methylphenyl)thio]benzoic acid (51.3 g) was cyclised with 100% concentrated sulphuric acid (513 g) at 95° for two hours. The solution became red and was poured cautiously into water. Ice was added to prevent excessive heating, the yellow flocculent precipitate produced was collected and the residue boiled with dilute ammonia solution (10%) for one hour. The oily material associated with the precipitate dissolved leaving a fine yellow suspension. The yellow solid was filtered off, washed with water and dilute alcohol and dried at 70° (26.6 g, 55%). A sample was recrystallised from acetic acid; m.p. 168-168.5°. This compound showed spectral characteristics and melting point identical to 1,8-dichloro-4-methylthioxanthen-9-one prepared from 2-chloro-6-nitrotoluene.



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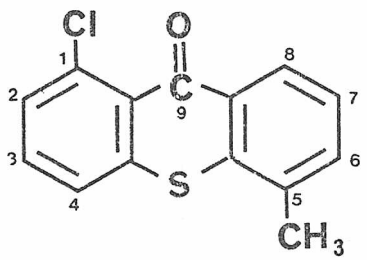

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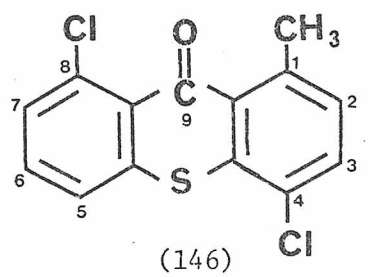

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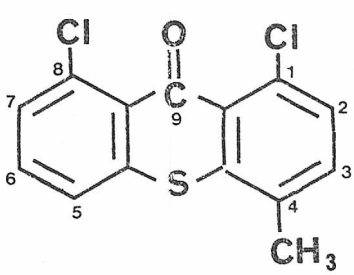


(136)

CHAPTER 2

1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen--9-ones and Related Compounds

CHAPTER 2

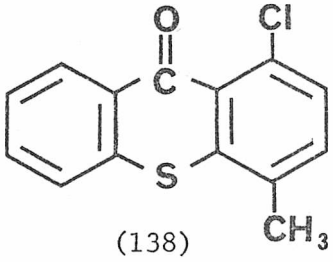
Index

1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and Related Compounds

Introduction

A number of methylthioxanthen-9-ones have been prepared substituted with dialkylaminoalkylamino and piperazinyl side chains in the 1 and 8 positions.

The original method for the preparation of lucanthone (1) involved a condensation of 1-chloro-4-methylthioxanthen-9-one (138) with diethylaminoethylamine (116) in the presence of copper catalyst in pyridine under pressure.[80]



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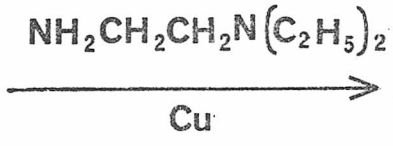
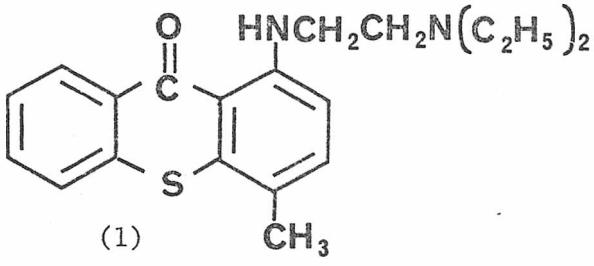


(116)

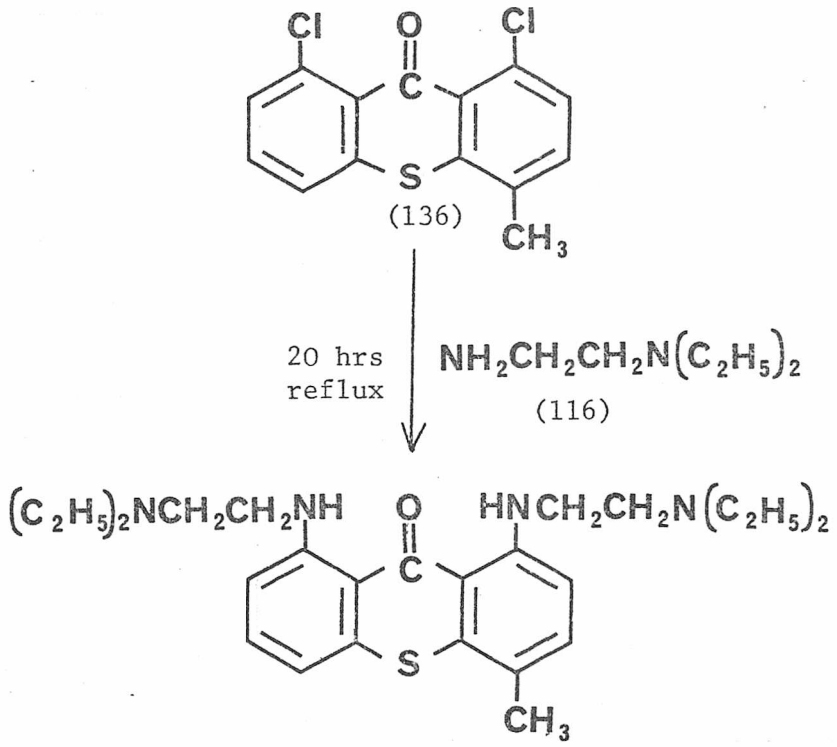


(1)

However, other workers have demonstrated that the copper[6] and the pyridine[15] can be eliminated and the reaction run at atmospheric pressure.[15]

Synthesis of 1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165)

1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) has been prepared from 1,8-dichloro-4-methylthioxanthen-9-one (136) by reaction with diethylaminoethylamine (116) in pyridine.

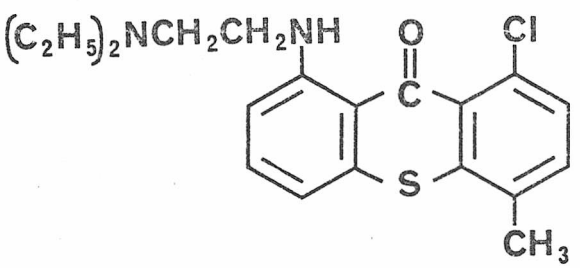


(165)

Preparative t.l.c. separation of the reaction mixture yielded two products. Material isolated from the band of R_f 0.13 was identified as 1,8-di[[2-diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) and was fully characterised by its elemental analysis, accurate mass measurement of the molecular ion and by its spectral properties which showed i.r. and u.v. absorption at 1612 cm^{-1} and 205.5, 258 nm respectively. The n.m.r. and mass spectra of the compound are discussed on pages 122 and 130 respectively. Material isolated from the band of R_f 0.55

showed molecular ion data which was consistent with a monosubstituted diethylaminoethylaminochloromethylthioxanthenone. The n.m.r. of this thioxanthenone (discussed on page 121), however suggested that the amino side chain of this compound was not para to the methyl group and that chloro substitution at position 1 had been retained.

The Ullmann condensation is facilitated when electron withdrawing groups are present in the ortho and para positions to the halogen atom on the aromatic ring; conversely electron donating groups in the ortho and para positions cause deactivation and hinder the reaction; thus the chlorine atom at position 1 of 1,8-dichloro-4-methylthioxanthen-9-one (136) will be the less reactive centre for nucleophilic substitution, being para to the methyl group. Position 8 therefore will be preferentially substituted yielding 1-chloro-8-[[2-(diethylamino)ethyl]-amino]-4-methylthioxanthen-9-one (166). The compound having R_f 0.55



(166)

has been established as having the structure (166) and has been fully characterised by its elemental analysis, accurate mass measurement of the molecular ion and by its spectral properties which included i.r. and u.v. absorption at 1630 cm^{-1}, 206 and 257 nm, respectively.

The yield of 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) was improved by the use of a fusion reaction which omitted the pyridine. Preparative t.l.c. separation of the products of this reaction gave 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one

(165), (55%) and a compound which showed similar t.l.c. properties to the monosubstituted product (166). Mass spectrometry, however, indicated that the compound did not contain a chlorine atom and this was further supported by the elemental analysis data. Retreatment of this compound with diethylaminoethylamine (116) yielded no 1,8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165).

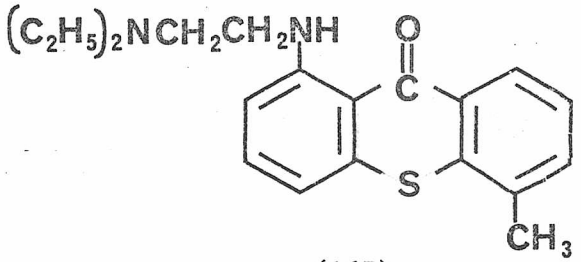
Bunnett and Zahler[101] report that the Ullmann condensation suffers some disadvantages, one being that reductive dehalogenation may sometimes become the major reaction. This is favoured by high temperatures and so the use of the lowest possible reaction temperature is recommended to minimise the dehalogenation.

Complete dehalogenation of 2,6-dichlorobenzoic acid by aniline occurs in the presence of copper, diphenylamine-2-carboxylic acid being formed; no reaction occurred however in the absence of catalyst.[82] Dehalogenation of 2,6-dichlorobenzoic acid to benzoic acid was also effected by N-dimethylaniline and copper under similar conditions.[82]

Loss of chlorine from the 8 position and its replacement by a proton would yield lucanthone (1); however, the spectral data of compound of R_f 0.52 and that of lucanthone (1) did not correspond. This therefore suggested that dehalogenation had taken place at the 1-position, i.e. *para* to the methyl group. This would be in accordance with the greater reactivity of the chlorine at position 8 compared to the chlorine at the 1 position, which is *para* to the electron donating methyl group as outlined previously.[101] This is supported by the n.m.r. spectrum (discussed on page 118) which shows a resonance corresponding to a proton *peri* to the carbonyl group in this thioxanthenone, and a methyl resonance corresponding to a methyl group not *para* to an amino side chain on an aromatic ring. Further the aromatic region of the n.m.r. spectrum integrates to six

protons and shows a shielded aromatic resonance corresponding to two protons ortho and para to an amino side chain.

This data supports the structure (167) which was confirmed by its elemental analysis, accurate mass measurement of the molecular ion and by an unambiguous synthesis carried out according to the route



(167)

outlined in Fig. 15.

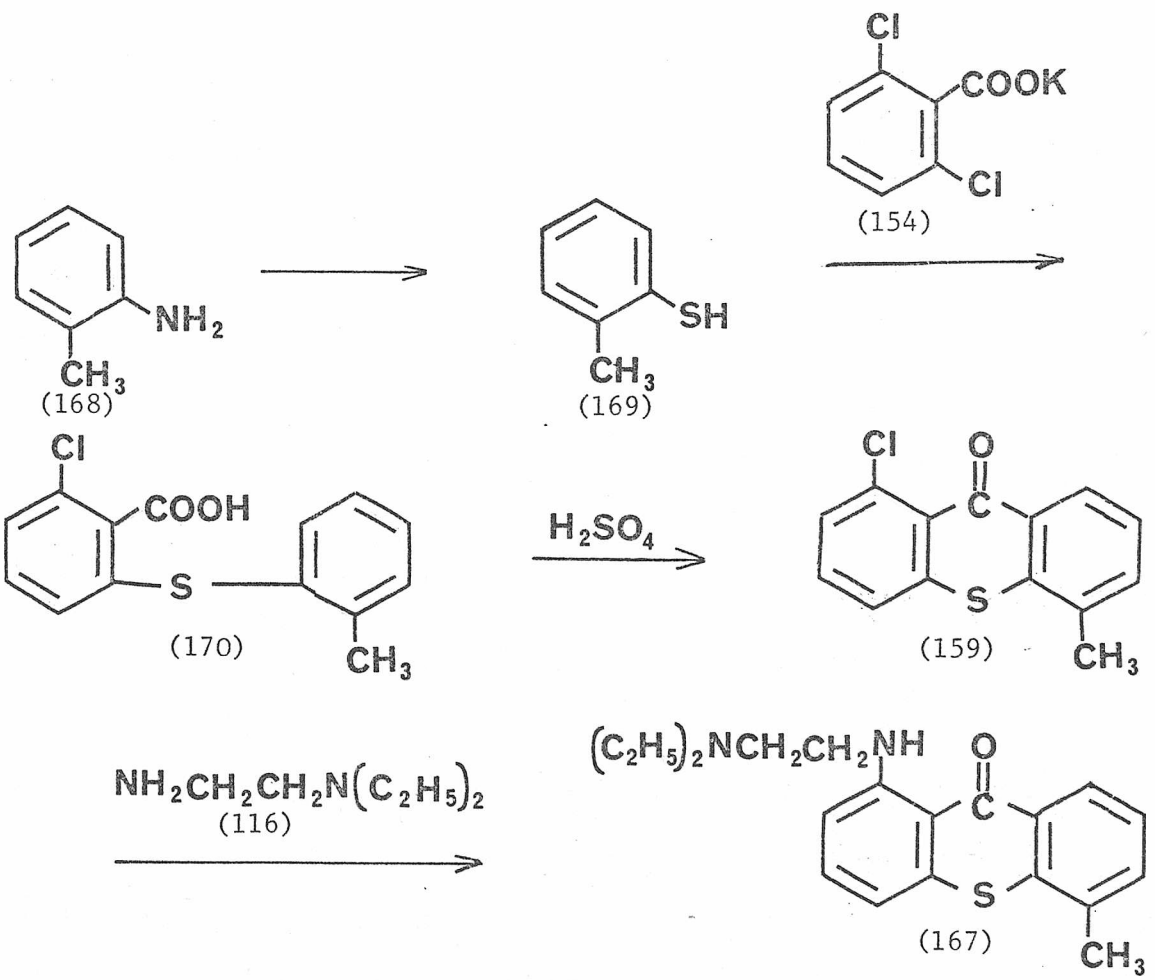


Fig. 15

Cyclisation of the phenylthiobenzoic acid (170) gave 1-chloro-5-methylthioxanthen-9-one (159) which was fully characterised by its elemental analysis and by its spectral properties which showed i.r. and u.v. absorptions at 1640 cm^{-1} and 205, 258 nm, respectively. The n.m.r. and mass spectra are discussed in detail on pages 73 and 80 respectively.

Condensation of 1-chloro-5-methylthioxanthen-9-one (159) with diethylaminoethylamine (116) yielded 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167) the n.m.r., mass spectra, melting point, mixed melting point and R_f values of which were identical to the product from the dehalogenation reaction.

The possible condensation and dehalogenation reactions of 1,8-dichloro-4-methylthioxanthen-9-one (136) in the modified Ullmann reaction are summarised in Fig. 16.

The mechanistic reasons for suggesting that condensation preferentially occurs at the 8 position have already been outlined and confirmed in the identification of 1-chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (166) as the major side product of the pyridine condensation. In addition, during monitoring of the modified Ullmann reaction by t.l.c., no spot corresponding to lucanthone (1) has been detected. This suggests therefore that the 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) arises from the 1,8-dichloro compound (136) via the 1-chloro compound (166) rather than the 8-chloro compound (171).

The dehalogenated product 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167) may arise by two routes. Firstly by dehalogenation of the 1-chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (166) and secondly by dehalogenation of the 1,8-dichloro compound (136) and subsequent reaction with diethylaminoethylamine (116).

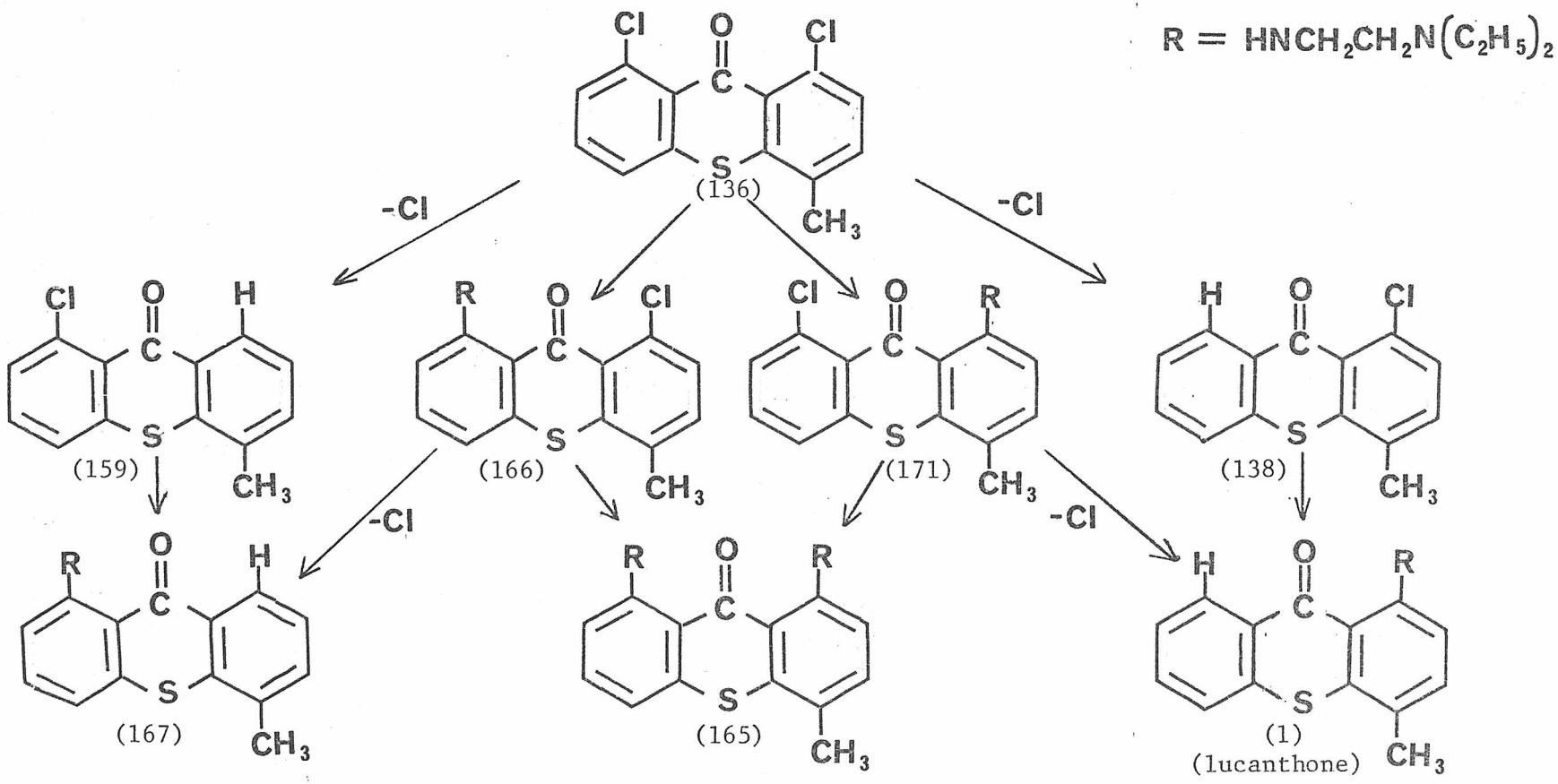


Fig. 16. Possible routes in the condensation of 1,8-dichloro-4-methylthioxanthen-9-one (136) with diethylaminoethylamino (116) in the presence of copper catalyst.

An investigation of the reaction of 1-chloro-8-[[2-(diethylamino)-ethyl]amino]-4-methylthioxanthen-9-one (166) with diethylaminoethyl-amine (116) under the modified Ullmann reaction conditions demonstrated that the reaction sequence shown in Fig. 17 can occur.

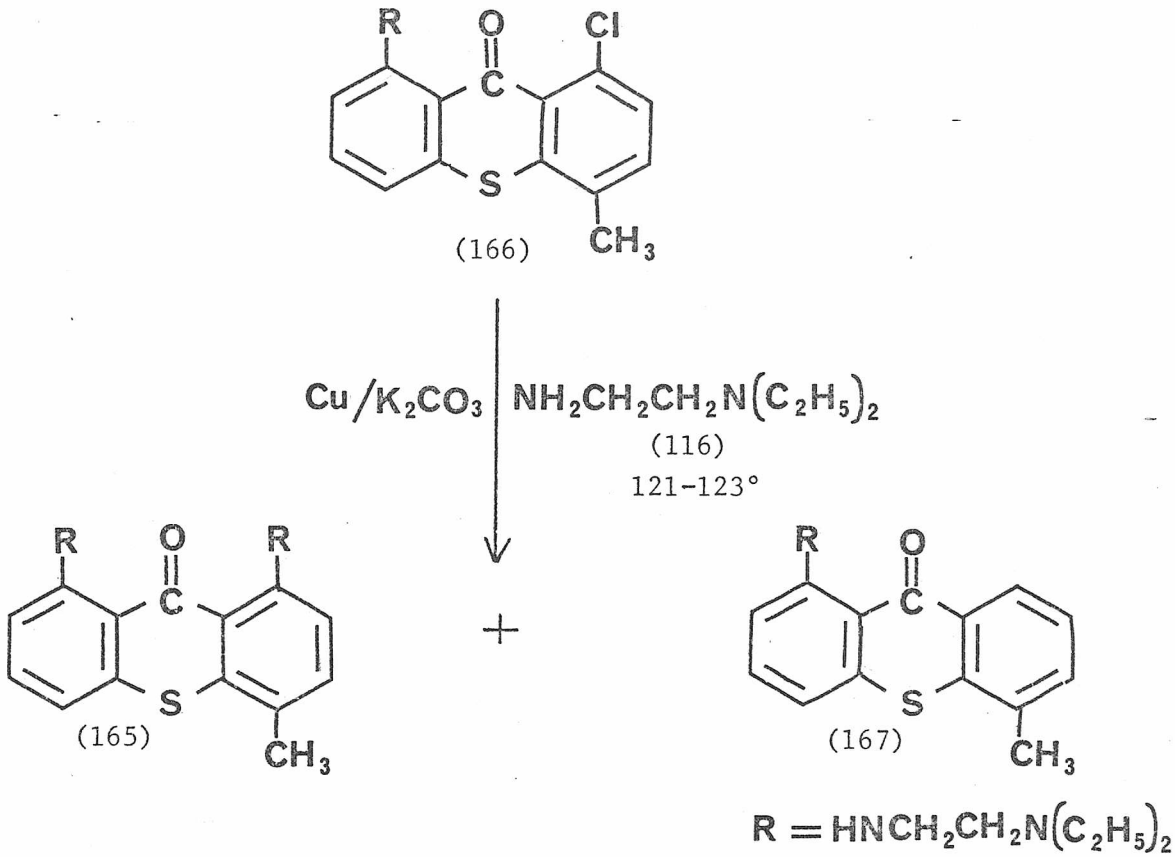


Fig. 17

High pressure liquid chromatographic (h.p.l.c.) examination of the products of the reaction between 1,8-dichloro-4-methylthioxanthen-9-one (136) and diethylaminoethylamine (116) using the modified Ullmann conditions demonstrated that after five hours gentle reflux all the starting material (136) had been converted to diethylaminoethylamino-thioxanthenones or to 1-chloro-5-methylthioxanthen-9-one (159). The absence of detectable 1-chloro-4-methylthioxanthen-9-one (138) by

h.p.l.c. also indicated the preferential dehalogenation of the 1 position of 1,8-dichloro-4-methylthioxanthen-9-one (136). As 1-chloro-5-methylthioxanthen-9-one (159) can be converted to 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167), both routes to the compound (167) have been demonstrated to be feasible under modified Ullmann conditions, (Fig. 16).

Synthesis of 1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and Related Compounds

In order to investigate the nature of the substitution at the 1 and 8 positions in the thioxanthenone molecule required for optimum biological activity a number of 1,8-di(dialkylaminoalkylamino)- and 1,8-dipiperazinyl-4-methylthioxanthen-9-ones (Table 25) have been synthesised (Fig. 18).

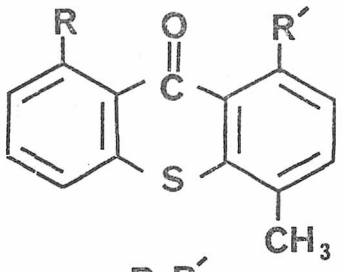
The aminoalkylaminothioxanthenones were fully characterized by their spectral characteristics and elemental analysis and in each case the corresponding dehalogenated compound has been isolated and characterised. Hydrochloride salts were found suitable for the *Schistosoma* screening experiments.

Elemental Analysis

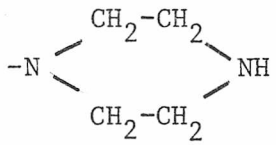
The results obtained from the elemental analysis of the hydrochloride salts of the dialkylaminoalkylamino and piperazinyl substituted methylthioxanthen-9-ones were found to be unsatisfactory (Table 26).

Archer and Suter[15] report that the salts of lucanthone-type compounds appeared to be hydrates which readily lost water on drying. Dihydrochlorides of some bases were obtained but only when there were at least three methylene groups separating the nitrogen atoms of the side chain.

Table 25.



R R´

$-HN(CH_2)_2NH_2$	$-HN(CH_2)_2N(CH_3)_2$	$-HN(CH_2)_2N(C_2H_5)_2$	$-HN(CH_2)_2N[CH(CH_3)_2]_2$
(172)	(173)	(165)	(174)
		$-HN(CH_2)_3(C_2H_5)_2$	
		(175)	
		$-HN(CH_2)_4N(C_2H_5)_2$	
		(176)	
			
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		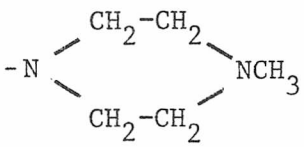	
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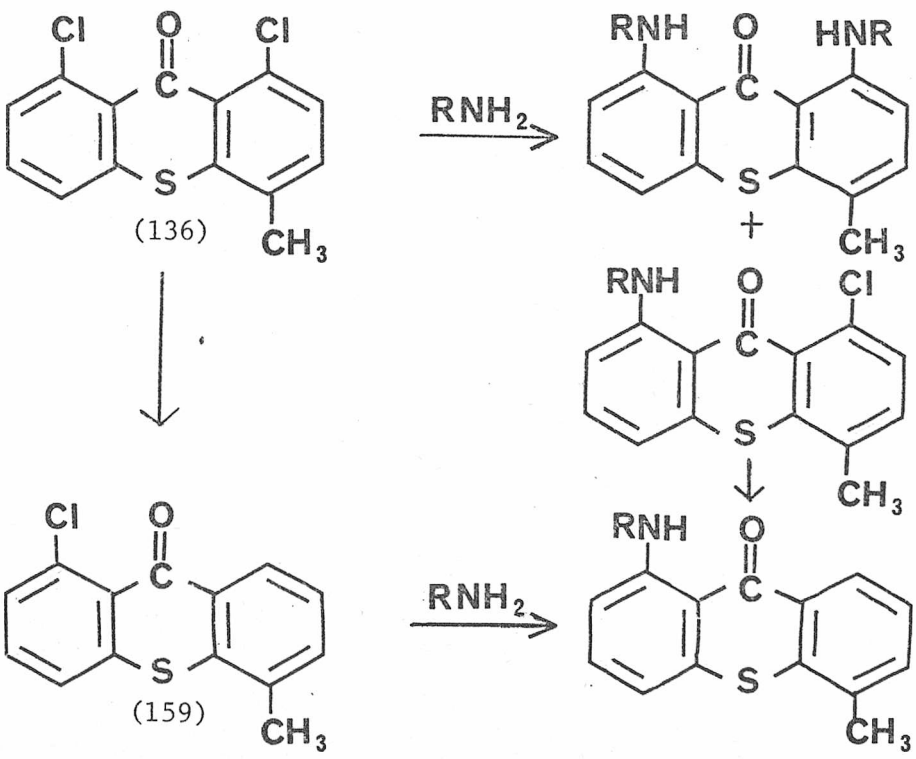


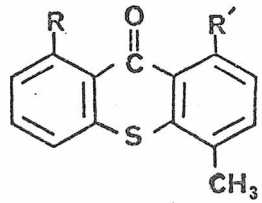
Fig. 18

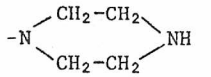
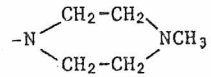
The infrared spectrum of lucanthone hydrochloride (1a) shows an N-H stretching vibration (3250 cm^{-1}) and an ammonium band of a tertiary amine (2650-2490 cm^{-1}); this is well separated from the C-H stretching frequencies at 3060-2870 cm^{-1} indicating that only the terminal tertiary amine of the side chain is protonated.[64] N-Methyl-lucanthone (82) readily produces a dihydrochloride in which the proximal nitrogen is also protonated; in the case of lucanthone (1) the dihydrochloride is only formed under conditions of low pH.[64]

Difficulty in preparing hydrochloride salts of lucanthone-type compounds has been reported by a number of workers.[15,103,104] Generally the elemental analyses of these compounds were unsatisfactory and Munro[104] reports that the monohydrochlorides contained more than, and the dihydrochlorides less than, the stoich iometric amount of acid. In the series of eleven compounds synthesised by Elslager et al.[103] it was found necessary to utilise five different recrystallisation solvents and dry conditions.

Laidlaw et al.[94] obtained good elemental analyses in a series of hycanthone analogues by using the thioxanthenone bases instead of the hydrochloride hydrates. Similarly in the present study the bases were found to give satisfactory analyses, after recrystallisation and drying under vacuum, (Tables 32 and 33, p. 148-149).

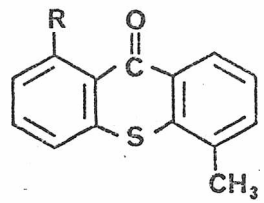
Table 26a. Elemental analyses of 1,8-di(dialkylaminoalkylamino)- and 1,8-dipiperazinyl-4-methylthioxanthen-9-one hydrochlorides

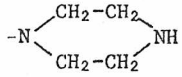
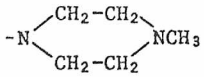


No.	Thioxanthenone RR'	Recrystallisation solvent	m.p.°	Formula	Analysis:	Carbon %	Hydrogen %	Nitrogen %	Sulphur %	Chlorine %
(172a)	$-NH(CH_2)_2NH_2$	EtOH-Ac	285-290(d)	$C_{13}H_{22}N_4OS$	Calcd.	41.22	5.73	10.69	6.11	27.10
				$.2H_2O.4HCl$	Found	39.10	4.98	9.36	8.29	20.88
(173a)	$-NH(CH_2)_2N(CH_3)_2$	EtOH-Ac	269-271(d)	$C_{22}H_{30}N_4OS$	Calcd.	60.76	7.13	12.89	7.36	8.17
				$.HCl$	Found	61.90	6.31	8.02	9.37	8.50
(165a)	$-NH(CH_2)_2N(C_2H_5)_2$	EtOH-Ac	174-177(d)	$C_{26}H_{38}N_4OS$	Calcd.	55.42	7.82	9.95	5.68	12.61
				$.2H_2O.2HCl$	Found	55.28	7.66	9.61	5.57	15.74
(175a)	$-NH(CH_2)_3N(C_2H_5)_2$	EtOH-Ac	176.5-177.5(d)	$C_{28}H_{42}N_4OS$	Calcd.	50.60	7.53	8.43	4.82	21.39
				$.2H_2O.4HCl$	Found	51.90	7.48	8.23	4.85	20.52
(174a)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	EtOH-Ac	183-185(d) (corr.)	$C_{30}H_{46}N_4OS$	Calcd.	49.45	7.97	7.62	-	19.50
				$.4H_2O.4HCl$	Found	53.70	8.78	8.44	-	22.04
(176a)	$-NH(CH_2)_4N(C_2H_5)_2$	EtOH-Ac	175-177(d)	$C_{30}H_{46}H_4OS$	Calcd.	49.45	7.96	7.69	4.40	19.50
				$.4H_2O.4HCl$	Found	48.30	7.15	7.85	4.70	16.60
(177a)		EtOH-Ac	178-179	$C_{22}H_{26}N_4OS$	Calcd.	48.98	6.68	10.39	-	13.17
				$.4H_2O.2HCl$	Found	47.92	6.24	9.33	-	15.83
(178a)		EtOH-Ac	217-220	$C_{24}H_{30}N_4OS$	Calcd.	45.00	6.56	8.75	-	22.19
				$.4H_2O.4HCl$	Found	45.64	6.09	8.76	-	18.72

Ac = acetone; (d) = melted with decomposition; (a) subscripts refer to hydrochloride salts

Table 26b. Elemental analyses of 1-dialkylaminoalkylamino- and 1-piperazinyl-5-methylthioxanthen-9-one hydrochlorides



No.	Thioxanthenone R	Recrystallisation solvent	m.p.°	Formula	Analysis:	Carbon %	Hydrogen %	Nitrogen %	Sulphur %	Chlorine %
(179a)	$-NH(CH_2)_2NH_2$	EtOH-Ac	143-146(d)	$C_{16}H_{16}N_2OS$	Calcd.	46.89	4.64	6.84	7.89	-
				$.H_2O.2HCl$	Found	45.53	4.67	6.20	10.75	-
(180a)	$-NH(CH_2)_2N(CH_3)_2$	EtOH-Ac	197-200(d)	$C_{18}H_{20}N_2OS$	Calcd.	61.98	6.03	8.03	9.18	10.18
				$.HCl$	Found	62.12	6.31	8.02	9.37	8.50
(167a)	$-NH(CH_2)_2N(C_2H_5)_2$	Ac	195-197.5	$C_{20}H_{24}N_2OS$	Calcd.	60.84	6.84	7.10	8.11	9.00
				$.H_2O.HCl$	Found	60.65	6.68	6.93	8.53	10.17
(181a)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	EtOH-Ac	214-216	$C_{22}H_{28}N_2OS$	Calcd.	62.49	7.34	13.25	-	8.40
				$.H_2O.HCl$	Found	59.25	6.40	15.14	-	9.78
(182a)	$-NH(CH_2)_3N(C_2H_5)_2$	Ac	184.5-186	$C_{21}H_{26}N_2OS$	Calcd.	56.63	6.74	6.29	7.19	15.96
				$.H_2O.2HCl$	Found	57.61	6.92	6.03	7.39	14.73
(183a)	$-NH(CH_2)_4N(C_2H_5)_2$	EtOH-Ac	183-185	$C_{22}H_{28}N_2OS$	Calcd.	55.35	7.13	5.87	-	14.88
				$.2H_2O.2HCl$	Found	53.70	8.78	8.44	-	22.04
(184a)		EtOH-Ac	238-240(d)	$C_{18}H_{18}N_2OS$	Calcd.	53.87	5.49	6.98	-	17.71
				$.H_2O.2HCl$	Found	53.70	5.31	6.96	-	17.56
(185a)		EtOH-Ac	236-238(d)	$C_{19}H_{20}N_2OS$	Calcd.	57.50	6.30	7.06	-	8.95
				$.2H_2O.HCl$	Found	58.15	6.12	7.05	-	10.63

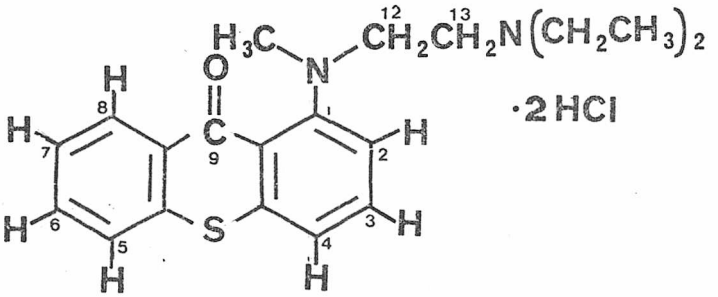
Nuclear Magnetic Resonance Spectroscopy of 1,8-Di(dialkylamino-alkylamino)-4-methylthioxanthen-9-ones and Related Compounds

The n.m.r. spectroscopy of lucanthone base (1) and its hydrochloride (1a) have been studied in detail together with compounds (165), (166) and (167) and the interpretation of these spectra have been used to substantiate the spectra of the other mono- and di-substituted methylthioxanthenones. The n.m.r. data for these compounds are presented in Table 27. (chemical Structures p. 159).

(a) Lucanthone (1)

The n.m.r. spectrum of lucanthone (1) does not appear in the literature, however, the spectra of related compounds (186) and hycanthone (66) have been reported.[94,27]

Laidlaw et al.[94] report the n.m.r. spectrum of compound (186):



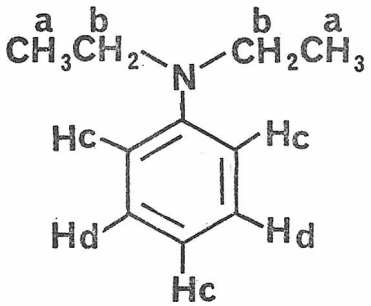
(186)

δ ($CDCl_3$) 7.65-9.00 (m, 7H, 2H-8H), 5.13 (s, 2H, exchangeable H of quarternary salt), 4.58 (m, 2H, NCH_2), 3.87 (s, 3H, NCH_3), 3.33-4.08 (m, 6H, $3CH_2$), 1.70 (t, 6H, $2CH_3$);

and for hycanthone (66).[94]

δ ($CDCl_3$) 10.1 (t, 1H, NH), 8.40 (m, 1H, peri H), 7.30 (m, 4H, aromatic H), 6.30 (d, 1H, aromatic H), 4.60 (s, 2H, CH_2O), 3.76 (s, 1H, OH), 2.33-3.50 (m, 8H, $4NCH_2$), 1.08 (t, 6H, $2CH_3$).

In the case of compound (186) no interpretation of the aromatic multiplet occurring at δ 7.65-9.00 is given, however in hycanthone (66) the multiplet at δ 8.40 is assigned to the 8-H proton peri to the carbonyl and the doublet at δ 6.30 is recorded as arising from an aromatic proton. This aromatic proton shows strong shielding and is likely to be the 2-H hydrogen ortho to the amino side chain. This effect is seen in N,N-diethylaniline (187) where the ortho and para protons resonate at a higher field than the meta protons.

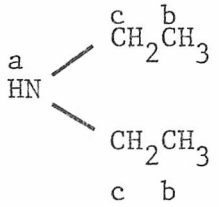


(187)

δ ($CC\ell_4$): a = δ 1.10(6H); b = 3.27(4H); c = c6.55(3H);
d = c7.05(2H).[93]

Rosi et al.[27] assign the δ 6.30 doublet (1H, aromatic) of hycanthone (66) to the 2-H proton.

The eight methylene group protons of hycanthone (66) are assigned to the multiplet at δ 2.33-3.50;[94] however in compound (186) there is a differentiation into a six-proton multiplet at δ 3.33-4.08 (m, 6H, $3CH_2$) and a two-proton multiplet at δ 4.58 (2H, NCH_2). The ethyl group protons would be expected to give rise to an A_3X_2 triplet-quartet pattern as seen in diethylamine (188):



(188)

δ ($CC\ell_4$): a = 0.90(1H, 5); b = 1.04(6H, t); c = 2.58(4H, q).

However, the methylene quartet overlaps the triplet arising from the 13-CH_2 methylene group, giving rise to a multiplet integrating to six protons. The 13-CH_2 triplet would be expected to occur at approximately δ 2.6 as in diethylamine (188). The δ 4.58 multiplet in compound (186) is assigned to the 12-CH_2 methylene protons resonating downfield from the other methylene hydrogens because of the anilinic group attached. This effect is also seen in N,N-diethylaniline (187), (δ 3.27,NCH_2); however the effect is greater due to compound (186) being a hydrochloride salt. Similarly the methyl groups in (186) show deshielding (δ 1.70, t, 6H), compared to hycanthone (66), (δ 1.08, t, 6H), because of this salt formation.[94]

The observed spectrum of lucanthone (1) is shown in Fig. 19.

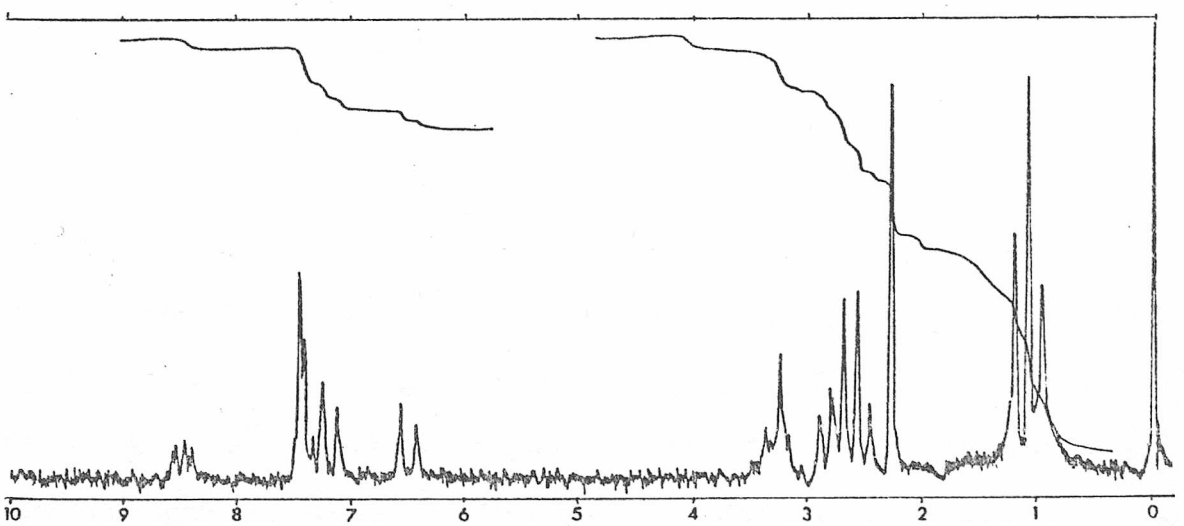


Fig. 19. N.m.r. spectrum of lucanthone (1)

The spectrum shows a singlet at δ 2.29(3H) arising from the methyl group at C-4. The ethyl group protons of the side chain give rise to a A_3X_2 triplet-quartet pattern at δ 1.08(6H) and 2.64 similar to that of diethylamine (188), (δ 1.10, 6H, t; δ 2.58, 4H, q);[93] however, the methylene proton quartet overlaps the triplet arising from the 13-CH_2 methylene group centred at δ 2.82, a little downfield from a similarly located methylene group in ethylenediamine (δ 2.60, 4H, s).[93] This gives rise to an overall multiplet integrating to six protons.

The 12-CH_2 protons resonate as a triplet at δ 3.24 in a similar position to the corresponding protons of diethylaniline (187), (δ 3.27).[93]

In the aromatic region a doublet is observed at δ 6.50 (1H, J = 8Hz) which can be assigned to the 2-H proton as in hycanthone (66), (δ 6.30, 1H, aromatic),[94] showing the shielding effect on the ortho proton observed in N,N-diethylaniline (187), (δ 6.55). A further doublet at δ 7.17 (1H, J = 7.5Hz) arises from the coupled 3-H proton and these two protons can be decoupled by double resonance.

The one proton multiplet centred at δ 8.4 arises from the H-8 proton peri to the thioxanthenone carbonyl group. This is substantiated in hycanthone (66) (δ 8.40, m, 1H, peri H)[27] and is also consistent with the results reported for the chlorothioxanthenones (138) and (159). Irradiation of this proton by double resonance caused a slight simplification of the multiplet at δ 7.1-7.6 indicating a coupling. Further analysis of the multiplet was attempted but the individual splittings for the three protons were not identified.

The spectrum of lucanthone hydrochloride (1a) (Fig. 20) shows some deshielding of the side chain protons. The 12-CH_2 triplet is centred at δ 3.90(2H) compared with δ 3.24 for the base, and the 13-CH_2 ethyl

methylene multiplet occurs at δ 3.06-3.55(6H) compared with δ 2.55-2.9 for its base.

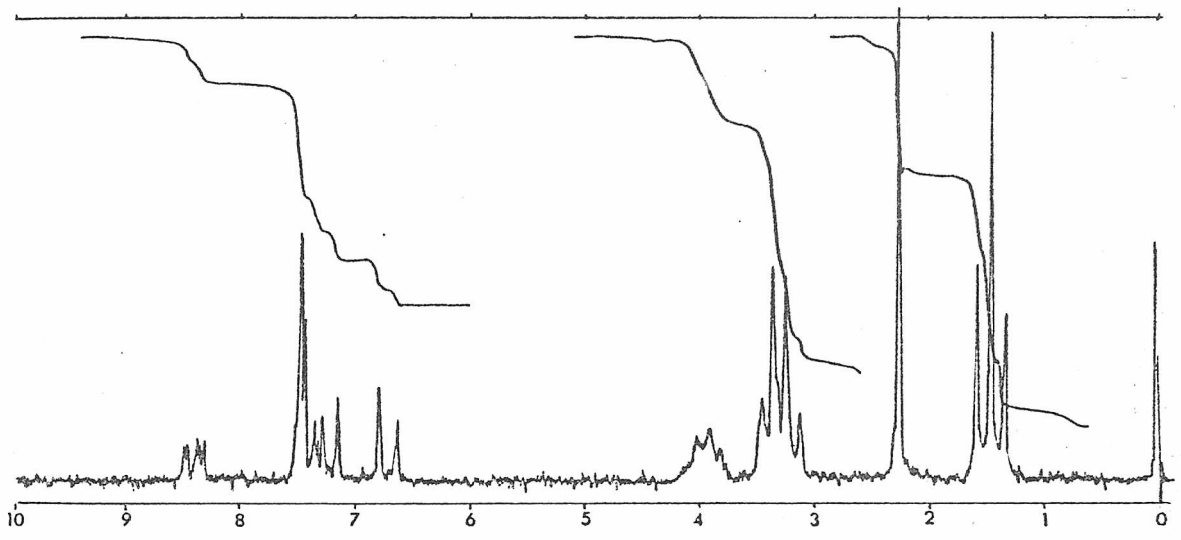


Fig. 20. N.m.r. spectrum of lucanthone hydrochloride (1a)

This deshielding effect is also seen in the hydrochloride salt of compound (186), (δ 4.58, m, 2H, N-CH_2; δ 3.33-4.08, m, 6H, $3CH_2$).[94]

In both lucanthone (1) and its hydrochloride (1a) the 12-CH_2 triplet shows some secondary splitting caused by the hydrogen attached to the proximal nitrogen. This hydrogen is bonded to the carbonyl group and appears as a triplet at δ 10.15(1H) in both compounds. It was exchangeable with D_2O and this simplified the 12-CH_2 triplet.

(b) 1-[[2-(Diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167)

1-[[2-(Diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167) exhibits an n.m.r. spectrum similar to lucanthone (1), (Fig. 21), except that the thioxanthenone ring methyl group resonates at δ2.40 (3H, s) and the aromatic 'triplet' at δ6.60 integrates to two protons; as expected for a compound with the amino side chain not *para* to the methyl group. The *ortho* and *para* hydrogens at C-2 and C-4 give rise to two *ortho*-*meta* triplets resonating at higher field to the other aromatic protons (δ 6.4-6.8, 2H, asym. t).

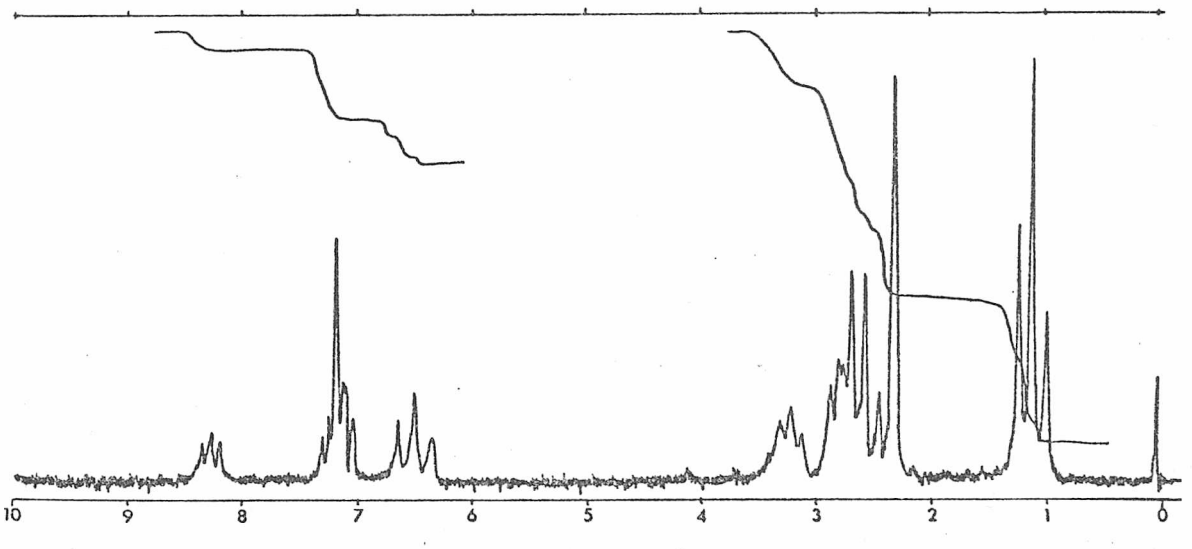


Fig. 21. N.m.r. spectrum of compound (167)

Double resonance of the aromatic region δ 7.1-7.5 of compound (167) caused the 'triplet' at δ 6.60 to collapse to two doublets (*J* = 2Hz) arising from the *meta* splitting of protons 2-H with 4-H, as shown in Fig. 22.

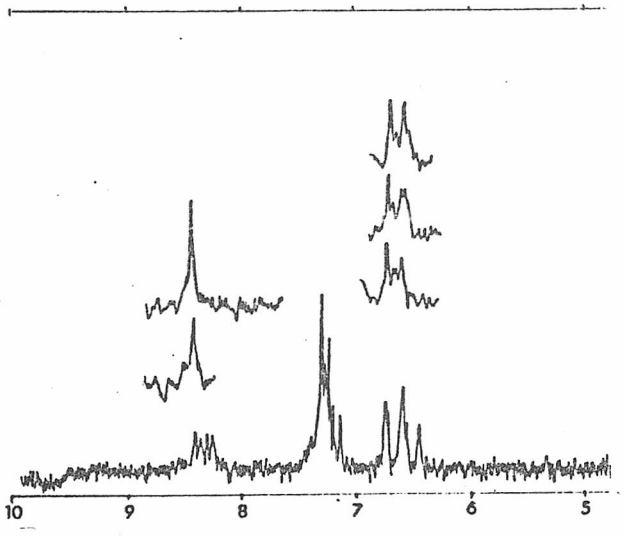


Fig. 22. Double resonance of the aromatic region of compound (167)

Similarly resonance of the aromatic protons at δ 7.1-7.5 causes the doublet of doublets at δ 8.4 to collapse to a sharp singlet (Fig. 22). Double resonance of the 'triplet' at δ 6.60 (6.8-6.4) causes a simplification of the aromatic multiplet at δ 7.1-7.5 by collapsing the triplet, arising from 3-H, to a singlet. Resonance of the 8-H doublet of doublets results in a simplification of the multiplet at δ 7.1-7.5 arising from the collapse of the 6-H doublet of doublets and the 7-H triplet.

(c) N-Methyllucanthone (82)

1-[Methyl[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (82) shows a spectrum (Fig. 23) similar to that of lucanthone (1) with the addition of a methyl resonance at δ 2.90 (3H, s). The position of

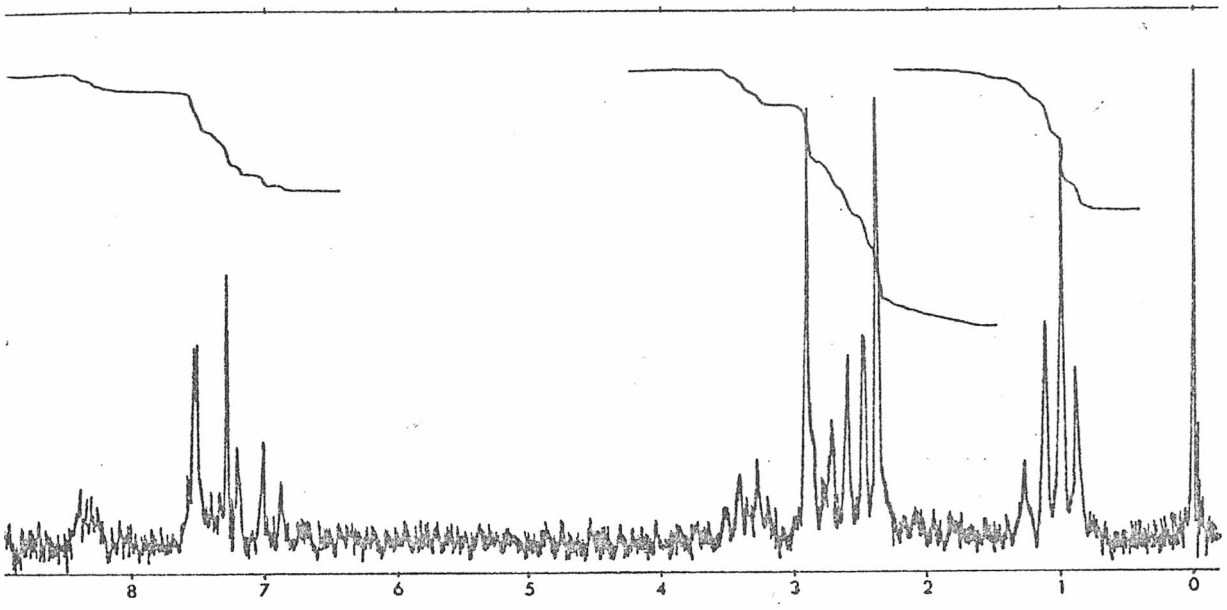


Fig. 23. N.m.r. spectrum of N-methyllucanthone (82)

the C-4 methyl resonance at δ 2.38 (3H, s) is similar to that found in compounds (166) and (167) where the side chain is not para to the methyl group. This is because the N-methyl group on the side chain reduces the interaction of the lone-pair of electrons of the proximal nitrogen with the π-electron cloud. This also reduces the shielding effect on the H-2 doublet and this occurs at δ 6.94 (1H, d, J=8Hz) compared to that of δ 6.50 (1H, d, J=8Hz) in lucanthone (1).

The absence of the hydrogen from the proximal nitrogen results in a slight sharpening of the 12-CH_2 triplet at δ 3.28 and the H-2 doublet at δ 6.94 as compared with lucanthone (1).

(d) 1-Chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (166)

The n.m.r. spectrum of 1-chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (166), (Fig. 24) shows a ring methyl group resonance at δ 2.42 (3H, s) suggesting that this group is not in the para position to an amino side chain as is lucanthone (1), (δ 2.29). This is further supported by the two proton aromatic resonance at δ 6.36 which can be assigned to the protons ortho and para to the amino side chain. The absence of resonance at δ 8.3-8.6 and the integration of five aromatic protons confirms that there is substitution at the 1 position and that dehalogenation has not occurred in this molecule.

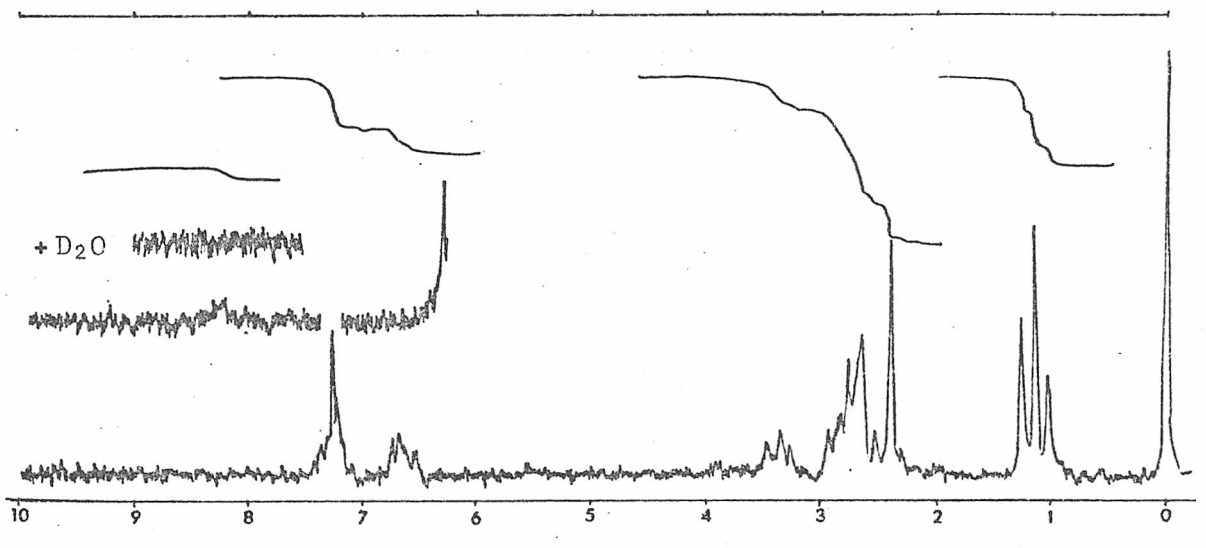


Fig. 24. N.m.r. spectrum of compound (166)

(e) 1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165)

The major differences in the spectrum of 1,8-di[[2-diethylamino)-ethyl]amino]-4-methylthioxanthen-9-one (165) and that of lucanthone (1) are the loss of the C-8 proton resonance and an increase in the number of protons resonating at δ 6.30-6.70 from one to three resulting from the 2-H, 5-H, and 7-H protons ortho and para to the amino groups. This gives rise to a 'triplet' centred at δ 6.6 (2-H doublet; 7-H, 5-H, ortho-meta doublet of doublets), (Fig. 25).

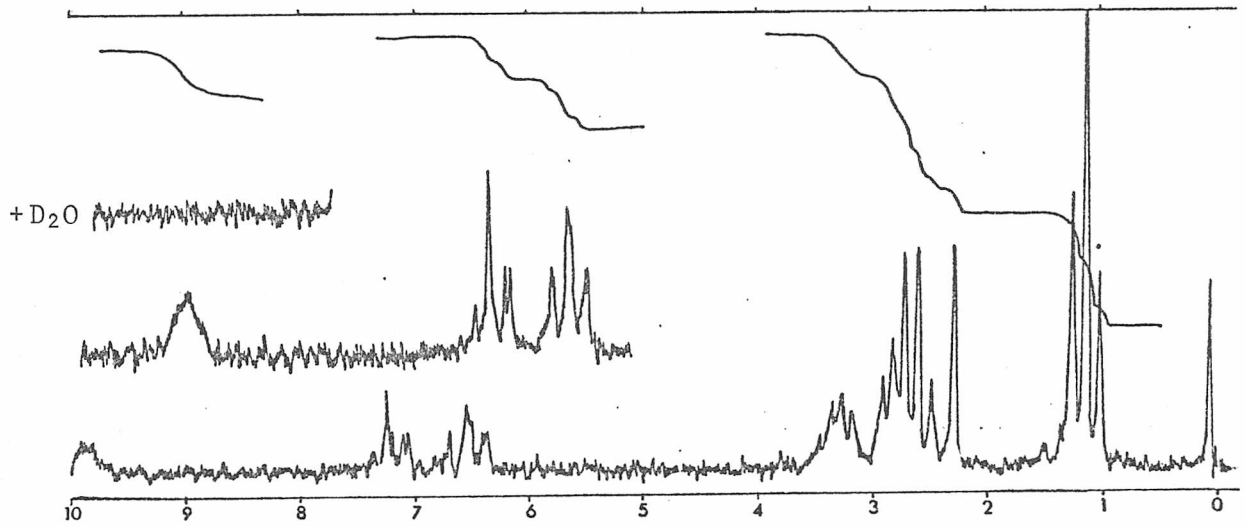


Fig. 25. N.m.r. spectrum of compound (165)

Protons 3-H and 6-H are dissimilar and give rise to a multiplet (2H) which consists of two groups of lines between δ 6.96 and 7.36. Proton 3-H ortho to the methyl group at C-4, being more shielded than 6-H, resonates upfield as an ortho doublet (J=8Hz, δ 7.10) overlapping slightly the diortho triplet (J=8Hz, δ 7.20) arising from the resonance of 6-H.

The resonance at δ 9.80 (2H, m) arises from the N-H groups attached to the ring; its position downfield suggests bonding with the carbonyl group. A D_2O shake caused the peak to diminish and resulted in a

sharpening of the split triplet at δ 3.2 suggesting that the 12-CH_2 and 12'-CH_2 proton triplets had been further split by the hydrogens of the proximal nitrogens.

The n.m.r. data of the diethylaminoethylaminomethylthioxanthen-9-ones is summarised in Table 27.

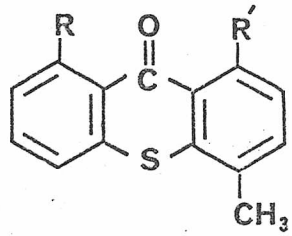
(f) Related substituted methylthioxanthenones

The n.m.r. data on the dialkylaminoalkylamino- and piperazinyl-methylthioxanthenones is recorded in Tables 39 and 40 (p. 155-156). Generally, except for minor differences in chemical shift the mono- and di-substituted methylthioxanthenone series show the same pattern of aromatic n.m.r. splitting as in compounds (167) and (165), previously reported in detail. The major differences in each spectrum studied arise from the variation in the length and nature of the aliphatic side chain and the terminal nitrogen substituents. However, these differences in the chemical shifts of the spectra were in accordance with the shifts found for the pure diamines. The main variations from the previously discussed spectra are given below:-

1. Aromatic resonances

The piperazinyl compounds were the only exception to the general pattern resulting from the aromatic substitution in the two series. In compounds (177), (178), (184), and (185) no upfield 'triplet' was observed at δ 6.6 as in the open chain compounds. This is due to the weaker shielding effect on the piperazinyl nitrogen proximal to the thioxanthenone ring compared with the corresponding anilinic compounds. The lone-pair of electrons of the proximal nitrogen are no longer able to contribute to the aromatic π-electron cloud to the same extent, consequently there is an overlap of

Table 27. N.m.r. data for diethylaminoethylaminomethylthioxanthen-9-ones



No.	R	R'
(1)	H	$HN(CH_2)_2N(C_2H_5)_2$
(1a)	H	$HN(CH_2)_2N(C_2H_5)_2.HCl$
(165)	$(C_2H_5)_2N(CH_2)_2NH$	$HN(CH_2)_2N(C_2H_5)_2$
(166)	$(C_2H_5)_2N(CH_2)_2NH$	Cl
(167)	$(C_2H_5)_2N(CH_2)_2NH$	H
(82)	H	$H_3CN(CH_2)_2N(C_2H_5)_2$

Compound	(1)	(1a)	(167)	(82)	(166)	(165)
Ring CH_3	2.29(3H,s)	2.29(3H,s)	2.40(3H,s)	2.38(3H,s)	2.42(3H,s)	2.23(3H,s)
H_3CN				2.90(3H,s)		
Ethyl: CH_3	1.08(6H,t)	1.11(6H,t)	1.10(6H,t)	1.00(6H,t)	1.10(6H,t)	1.08(12H,t)
CH_2	2.64(q) (6H)	3.06-3.55 (asym. q,	2.40-3.00 (asym. quint,	2.32-2.82 (asym. q,	2.36-2.86 (asym. quint,	2.40-2.95 (asym. quint,
13-CH_2	2.82(t)	6H overlap)	6H overlap)	6H overlap)	6H, overlap)	12H overlap)
12-CH_2	3.24(2H,t)	3.90(2H,t)	3.30(2H,t)	3.28(2H,t)	3.21(2H,t)	3.24(4H,t)
H-2	6.50(1H,d, J=8Hz)	6.44(1H,d, J=8Hz)		6.94(1H,d, J=8Hz)		
H-2,H-5,H-7					6.36(2H, asym. q.)	6.60(3H, asym. t)
H-2,H-4			6.60(3H, asym. t)			
H-8	8.3-8.55 (1H,m)	8.3-8.55 (1H,m)	8.25-8.50 (1H,dd, J=8Hz,2Hz)	8.2-8.5 (1H,m)		
other aromatic protons	7.08-7.55 (4H,m)	7.11-7.66 (4H,m)	7.10-7.50 (3H,m)	7.16-7.64 (4H,m)	6.90-7.10 (3H,m)	6.96-7.36 (2H,m)
NH bonded (exchangeable with D_2O	10.15 (1H,t)	10.15 (1H,t)	10.15 (1H,m)	-	8.88 (1H,m)	9.80 (2H,m)

the splitting patterns arising from the five aromatic protons giving rise to a multiplet. However, in the mono-piperazinyl compounds (184) and (185) it was possible to identify a split triplet centred at δ 7.0 occurring as part of the multiplet at δ 6.90-7.53, as shown in Fig. 26.

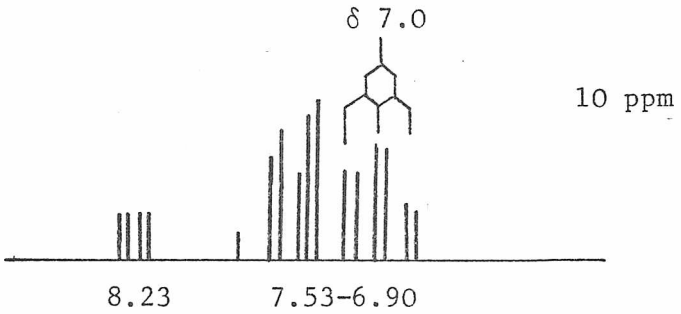
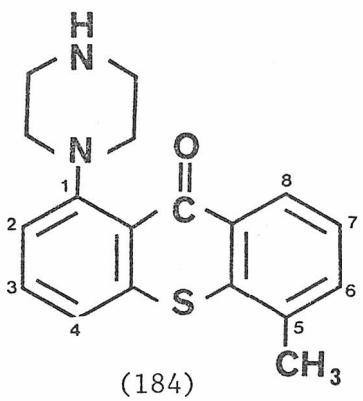


Fig. 26. Diagram of the aromatic splitting pattern of compound (184)

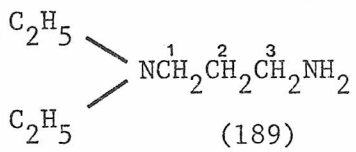


(184)

The splitting of the triplet arises from an overlap of the 2-H and 4-H ortho-meta triplets; the remaining multiplet contains the 3-H and 7-H diortho triplets and the 6-H ortho-meta doublet of doublets.

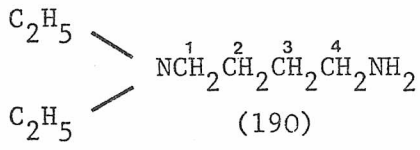
2. Side chain resonances

In the compounds containing dimethylaminopropylamino and diethylaminobutylamino side chains considerable overlapping of the methylene splitting patterns were observed and occasionally overlap of the ring methyl group singlet. This is in agreement with the spectra of the pure diamines (189) and (190) given below and the position of the C-4 methyl group in compounds (165) and (167).



(189)

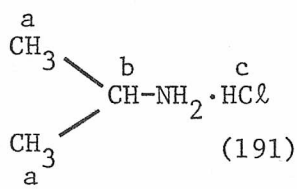
δ (CDCℓ$_3$)[93] 1.01 (6H, t), 1.53 (4H, 2-CH_2, t superimposed on NH_2, 2H, s), 2.3-2.9 (8H, 8-line pattern, 2CH_2 ethyl and 1-CH_2, 3-CH_2).



(190)

δ (CDCℓ$_3$)[93] 0.97 (6H, t), 1.50 (6H, m, 2-CH_2, 3-CH_2, NH_2) 2.50-2.98 (8H, m, 1-CH_2, 4-CH_2, 2CH_2 ethyl)

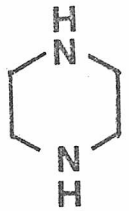
Similarly the isopropylamino compounds show the characteristic methyl doublet and methine multiplet similar to isopropylamine (191).



(191)

δ (DMSO-d_6)[93] a = 1.29 (6H, d); b = 3.33 (1H, sextet) c = 8.35 (2H, s).

The eight ring hydrogens of piperazine base (192) absorb as a



(192)

singlet at δ 2.83 [93] however substitution results in the protons becoming dissimilar and in the case of the N-methylpiperazinyl compounds (178) and (185) the protons adjacent to the N-methyl group are slightly shielded giving a four proton triplet at δ 2.69 in compound (185) and an eight proton triplet at δ 2.65 in compound (178). In the piperazinyl-thioxanthenones (177) and (184) a singlet is seen at δ 3.10 (16H and 8H, respectively).

The piperazine amino protons of compounds (177) and (184) resonate at δ 1.81 (1H, s) and 1.76 (2H, s) respectively compared with the hydrogen-bonded amino protons of the open chain compounds which resonated between 9.70-10.20. Coupling of the N-H proton with the adjacent methylene groups would be expected to give rise to a triplet, however due to the small intensity of this resonance only a multiplet was observed.

In compounds (172) and (179) containing an aminoethylamino group, the NH_2 protons resonated at δ 1.23 (4H, s) and 1.20 (2H, s) respectively, in keeping with the spectrum of 1,2-diaminoethane (193).

$$NH_2CH_2CH_2NH_2$$

(193)

δ ($CDC\ell_3$)[93] 1.19 (4H, s), 2.60 (4H, s).

Mass Spectrometry of 1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and Related Compounds

Four diethylaminoethylaminomethylthioxanthenones have been studied and their fragmentation patterns used to interpret the spectra of other substituted thioxanthenones synthesized (chemical structures, p. 159).

The mass spectrum obtained for lucanthone (1) is shown in Fig. 27.

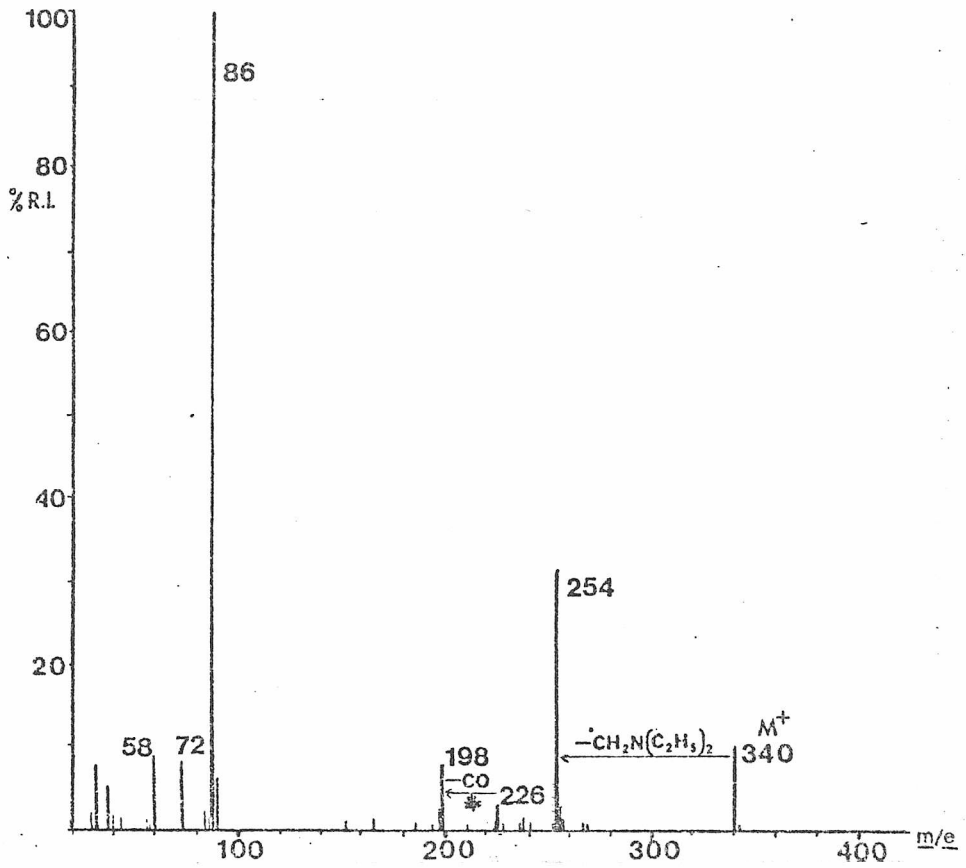
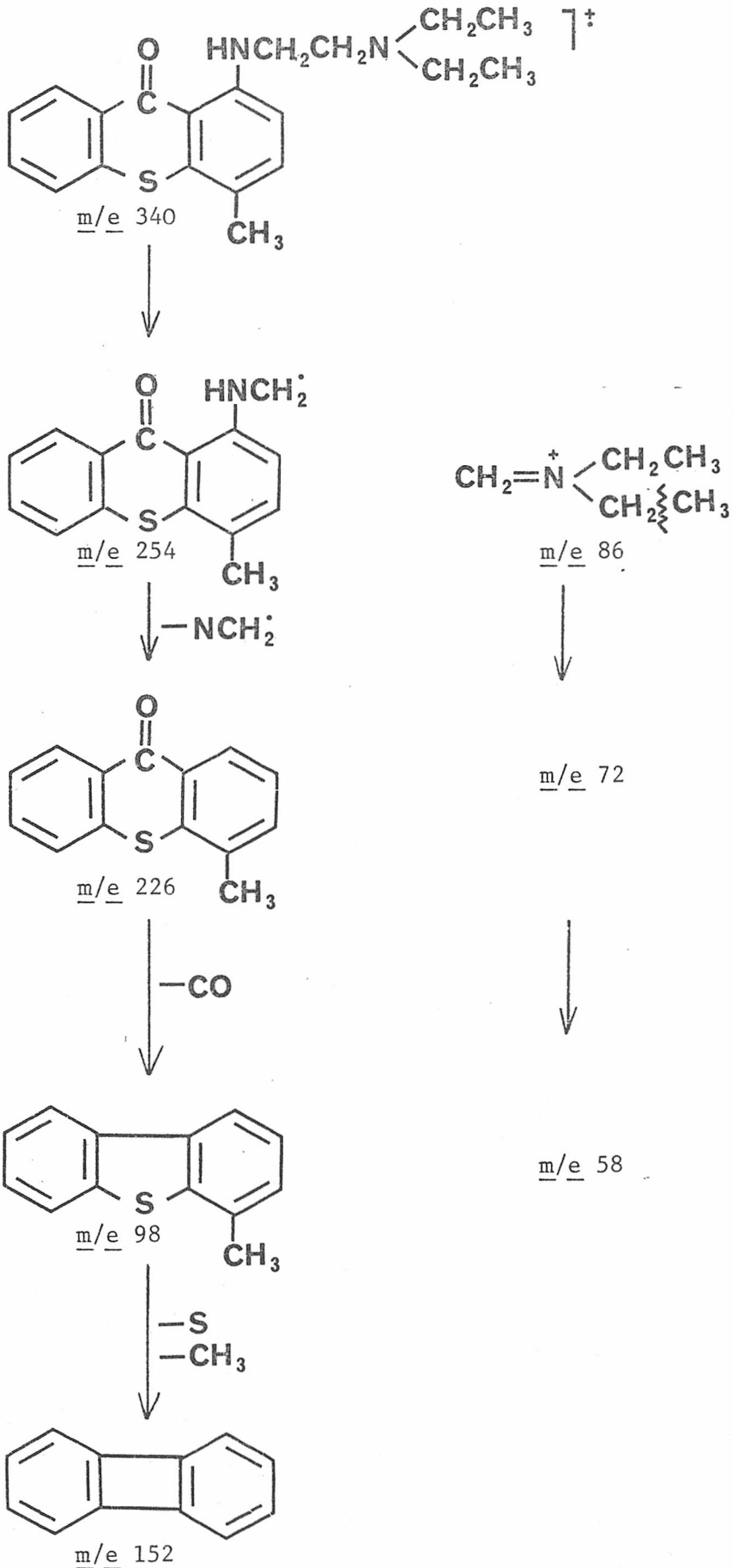


Fig. 27. Diagram of the mass spectrum of lucanthone (1)

The loss of part of the side chain corresponding to 86 mass units is the major fragmentation in this compound, consistent with β-cleavage in aliphatic amines.[105] An ion at m/e 72 and 58 suggest further degradation of this fragment (Scheme 4). Ion m/e 58 is particularly common in aliphatic amine degradations and the structure (194) has been assigned to it.[106]



Scheme 4

CH_2-NH-CH_2-CH_3

m/e 58

(194)

1-Chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (166) shows the primary loss of the m/e 86 ion followed by the loss of the halogen as indicated by the isotope abundance ratio. This provided evidence that the material of R_f 0.55 from the Ullmann condensation (p.102) was chloro-substituted.

In contrast the spectrum of 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one (167), R_f 0.52, shows a similar fragmentation pattern to lucanthone (1). The spectrum showed no evidence of a chlorine atom and this suggested a dehalogenation had occurred in the modified Ullmann reaction, (p.103).

1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) shows a $(M-86)^+$ fragmentation predominating; however, m/e 86 and m/e 100 ions occur as joint base peaks. The $(M-100)^+$ ion was not seen but the fragmentation was indicated by the presence of a metastable peak at m/e 276.0 (m/e 454 → 354). Fragmentation $(M-(CH_2N(C_2H_5)_2)_2)^+$ gives m/e 282 (Fig. 28).

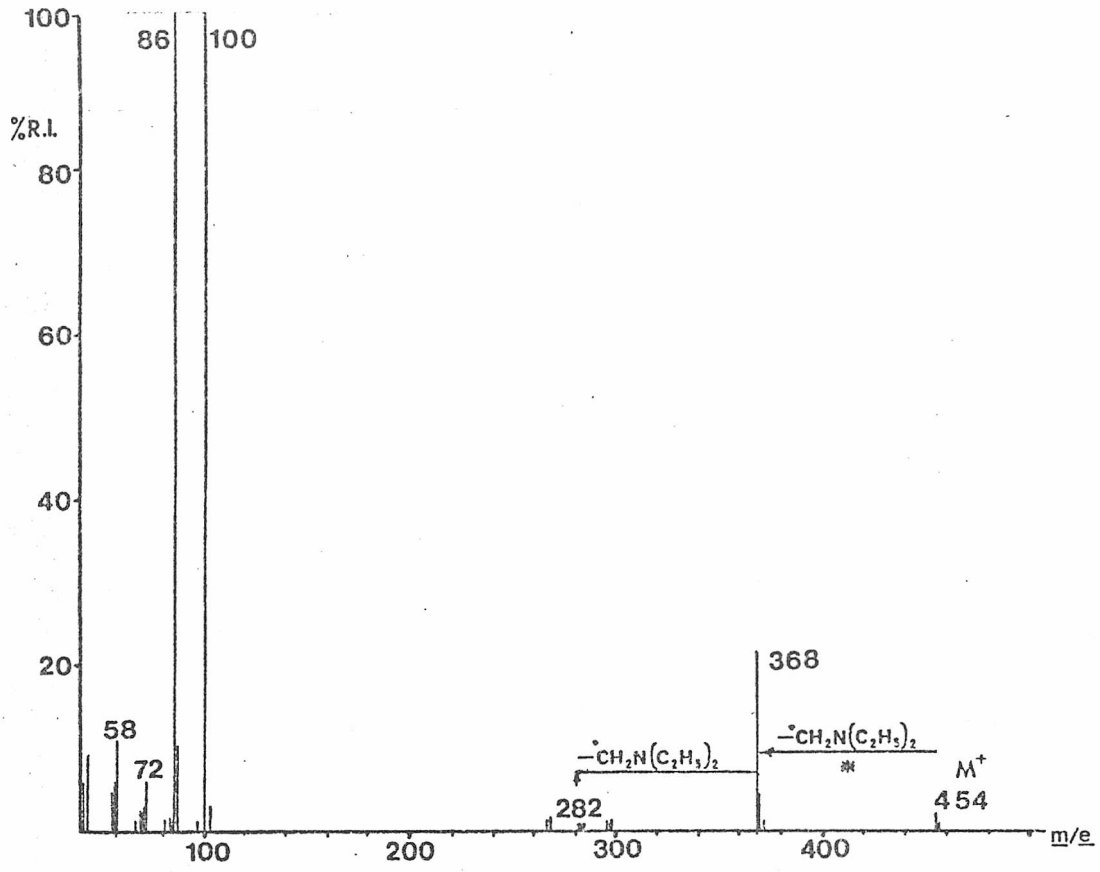


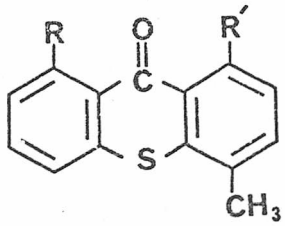
Fig. 28. Diagram of the mass spectrum of 1,8-di[[2-(diethylamino)ethyl]-amino]-4-methylthioxanthen-9-one (165)

The main mass spectral fragmentation pathways of the other diethylaminoalkylamino compounds studied are recorded in Table 28.

α-Cleavage does not occur to any significant degree in aliphatic amines but ions corresponding to γ- and δ-cleavages become more abundant as the size of the carbon chain increases;[107] thus ions corresponding to $(M-100)^+$ cleavage are seen in the diethylamino-propylamino (175), (182) and diethylaminobutylamino compounds (176), (183) and $(M-114)^+$ in compounds (182) and (183). Because of the abundance of m/e 86 and 58 ions is the 1,8-disubstituted compounds the relative intensities of other ions above m/e 114 are small and consequently further analysis beyond the initial β-cleavage is difficult.

β-Cleavage was found to be the predominant mechanism of fragmentation in other substituted thioxanthenones synthesised, (Tables 41 and 42), but

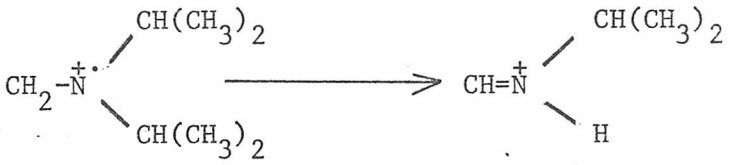
Table 28. Main mass spectral fragmentation pathways of the diethylaminoethylamino-4-methylthioxanthen-9-ones



No.	R	R'	Fragmentation M^+	$(M-72)^+$	$(M-86)^+$	$(M-100)^+$	$(M-114)^+$	m/e (%) 58	72	86	100	114
(1)	H-	$-NHCH_2CH_2N(C_2H_5)_2$	340(100)	268(0.6)	254(32)		226(3)	(9)	(8)	(100)		
(167)	$(C_2H_5)_2NCH_2CH_2NH-$	-H	340(10)	268(0.8)	254(40)		226(0.8)	(22)		(100)		
(166)	$(C_2H_5)_2NCH_2CH_2NH-$	-Cℓ	374(0.3)		288(0.3)					(100)		
(165)	$(C_2H_5)_2NCH_2CH_2NH-$	$-HNCH_2CH_2N(C_2H_5)_2$	454(2.5)		368(22) (m-86-86) 282(0.6)			(11)	(6)	(100)	(100)	
(182)	$(C_2H_5)_2N(CH_2)_3NH-$	-H	354(18)		268(18)	254(18)	240(3) 238(25)	(32)	(40)	(100)	(79)	(56)
(175)	$(C_2H_5)_2N(CH_2)_3NH-$	$-HN(CH_2)_3N(C_2H_5)_2$	482(3)		396(50)	382(6)		(10)	(11)	(100)	(11)	(28)
(183)	$(C_2H_5)_2N(CH_2)_4NH-$	-H	368(0.2)		282(1.5)	268(5) 267(7)	254(63)	(100)	(10)	(35)	(32)	(1.5)
(176)	$(C_2H_5)_2N(CH_2)_4NH-$	$-HN(CH_2)_4N(C_2H_5)_2$	510(0.3)		424(1)	410(8) 409(25)		(100)	(8)	(10)	(79)	

where deviations from this pattern occurred the fragmentations are discussed.

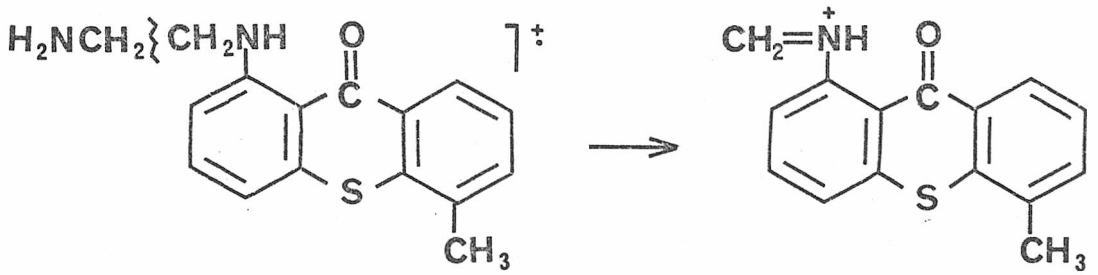
In the diisopropylaminoethylamino compounds (174) and (181) the base peak is m/e 114 derived from a β-cleavage of the side chain; this ion further fragments to give m/e 72.



m/e 114 m/e 72

Similarly in the dimethylaminoethylamino compounds (173) and (180) the main fragmentation is $(M-58)^+$. Cleavage giving $(M-72)^+$ was not apparent, however the m/e 72 ion occurred as the joint base peak in (173); there was however no metastable transition evidence for this fragmentation.

The aminoethylamino compounds (172) and (179) show a fragmentation $(M-30)^+$ which is consistent with β-cleavage of an aliphatic amine.



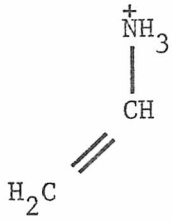
m/e 284 m/e 254

(179)

+ $\dot{C}H_2NH_2$

m/e 30

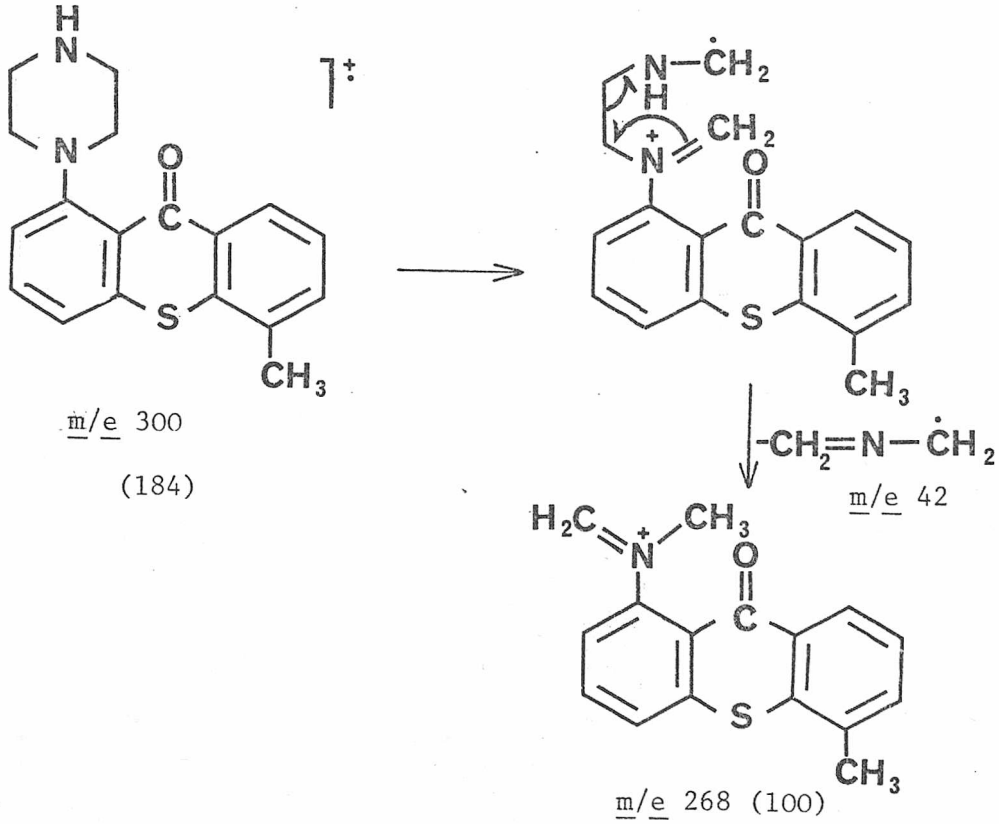
The ion m/e 44 is also seen and is common to this type of amine; the structure (195) has been suggested.[107]



m/e 44

(195)

The main fragment ion in the piperazinylthioxanthenone (184) is formed by the loss of 42 mass units. This could occur by a McLafferty rearrangement with transfer of an N-bonded hydrogen atom in a similar way to the fragmentation of piperazine (192), (Scheme 5).[108]



m/e 300

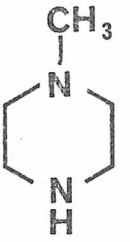
(184)

m/e 42

m/e 268 (100)

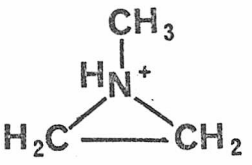
Scheme 5.

The fragmentation pathway is more complex in the methylpiperazinyl compounds (178) and (185) and may arise by three routes, (Scheme 6). In the spectrum of N-methylpiperazine (196) the ion $C_3H_8N^+$ (m/e 58) is

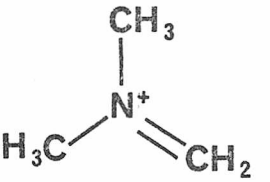


(196)

reported as producing the base peak[108] and the structure (197) and (198) have been suggested. A metastable transition is observed

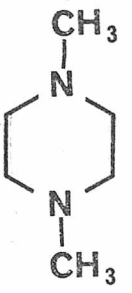


(197)



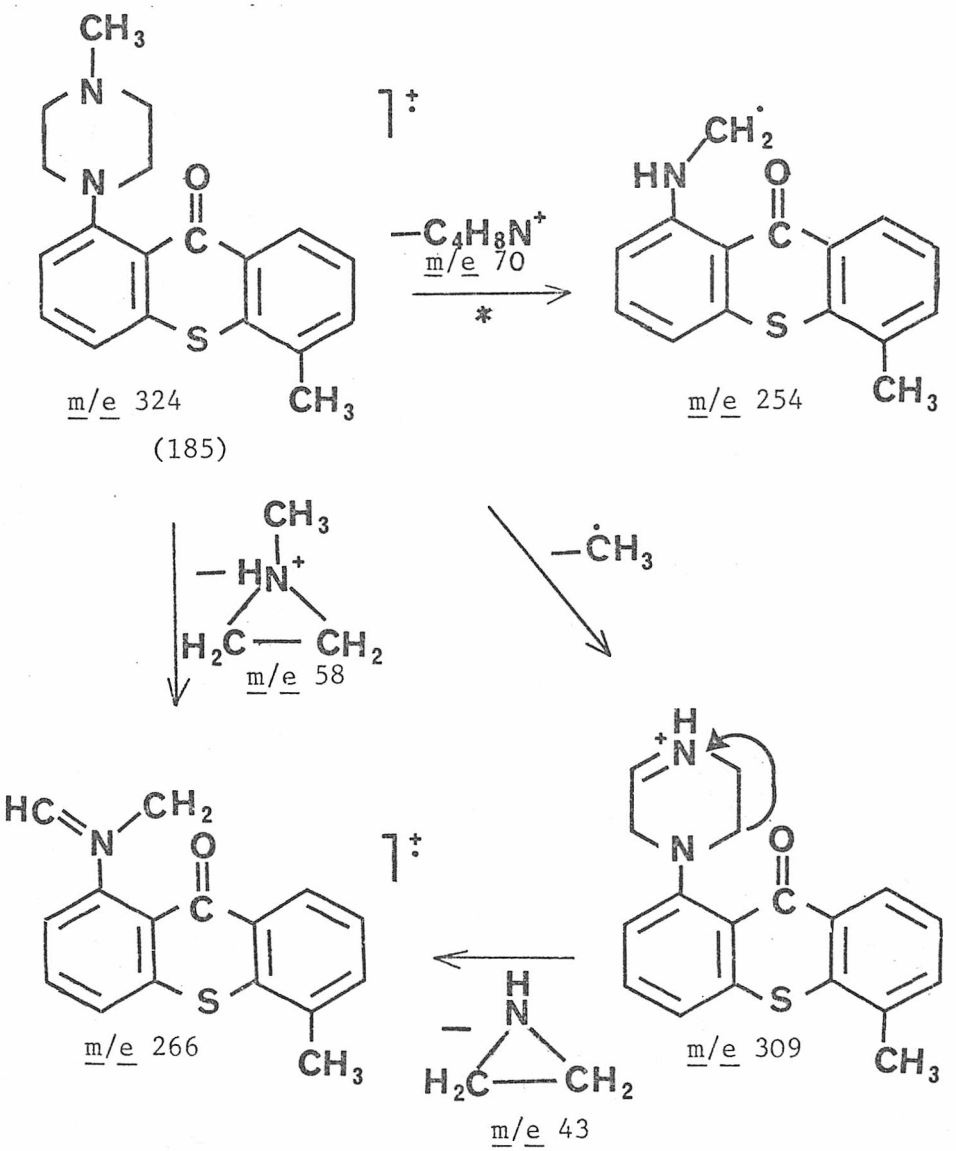
(198)

showing the ion to be produced directly from the parent ion, however in N,N'-dimethylpiperazine (199) the fragmentation occurs via the intermediate ion $(p\text{-}CH_3)^+$.[108]



(199)

A small intensity ion at m/e 407 $(M-15)^+$ was observed in the dipiperazinyl compound (178) but no such fragmentation was detected in compound (185). This type of fragmentation could account for the ions m/e 366 (185) and 364 (178), (Scheme 6), however these fragments could arise through either route and no metastable transitions were observed to confirm either pathway. The more important route in compound (185) arises from the fragmentation $(M-70)^+$ to give the ion m/e 254.



Scheme 6

EXPERIMENTAL

General Experimental Details

I.r., u.v., n.m.r. and mass spectra were recorded on the same instruments as Chapter 1. Similarly melting point determinations and t.l.c. were the same as on pages 86-87.

High Pressure Liquid Chromatography (h.p.l.c.)

An A.R.L. Model 750 liquid chromatograph with constant pressure pump and fixed wavelength (254 nm) detector having an 8 μℓ flow cell of path length 10 mm was used. The column was a 300 x 43 mm Partisil ODS, a chemically bonded reverse phase C-18 packing 10 μm in diameter (Reeve Angel).

Experimental

Lucanthone (1)

A sample of 1-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one hydrochloride (lucanthone) was supplied by Burroughs Wellcome and Co., m.p. 195-198° (Lit.,[15] 195-196°); $\nu_{max.}^{KBr}$ 3260 (N-H stretching, Lit.,[64] 3250), 3020-2850 (C-H stretching, Lit.,[64] 3060-2870), 2580 (ammonium band of t-amine, Lit.,[64] 2650-2490), 1611 (C=O) cm^{-1}; $\lambda_{max.}^{EtOH}$ 206 (log ε 4.40), 256 (4.56), 335 (3.88) and 440 nm (3.81); R_f 0.54 (ammonia-methanol 1.5:100; Lit.,[109] 0.53); δ 1.11 (6H, t, CH_3(2)), 2.29 (3H, s, CH_3), 3.06-3.55 (6H, asym. q., 13-CH_2, CH_2(2)), 3.90 (2H, t, 12-CH_2), 6.44 (1H, d, J=8Hz, H-2), 7.11-7.66 (4H, m, H-3,5, 6 and 7), 8.3-8.55 (1H, m, H-8) and 10.15 (1H, t, N-H bonded, exchangeable

with D_2O. The free base was obtained from a sample of the hydrochloride by dissolving the salt in warm water, making the solution alkaline with sodium carbonate solution and then extracting with ether. The ethereal solution was dried, concentrated under vacuum and the resulting oily base recrystallised from ethanol, m.p. 62.5-64° (Lit.,[109] 66°). $\nu_{max.}^{KBr}$ 3460 (N-H st.), 3020-2850 (C-H st.), 1616 (C=O), 1591, 1512, 1385, 1225, 795 and 763 cm^{-1}; $\lambda_{max.}^{EtOH}$ 206 (log ε 4.30), 256 (4.60), 330 (3.90), and 440 nm (3.80); (Lit.,[41] 255, 330 and 444 nm); R_f 0.50 (chloroform-ethanol, 4:1), 0.66 (dioxane-acetone-water, 7:7:1), 0.42 (ethanol-n-hexane, 1:1), 0.74 (ethanol-ammonia, 77:23); δ 1.08 (6H, t, CH_3(2)), 2.29 (3H, s, CH_3), 2.64 (6H, q, CH_2(2), 2.82 (2H, t, 13-CH_2), 3.24 (2H, t, 12-CH_2), 6.50 (1H, d, J=8Hz, H-2), 7.08-7.55 (4H, m, H-3, 5 and 7), 8.3-8.55 (1H, m, H-8), 10.15 (1H, t, N-H bonded, exchangeable with D_2O). m/e (%) 340(10), 254(32), 237(1.5), 226(3), 198(8), 197(2.5), 165(1.5), 152(1), 87(6), 86(100), 85(1), 83(2), 72(8), 58(9), 56(1.5), 42(1.5). m^* 173.5 (226 → 198).

N-Methyllucanthone (82)

1-[Methyl[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one dihydrochloride was supplied by Dr. D.F. Worth, Warner-Lambert/Parke-Davis Ann Arbor, Michigan; m.p. 155-157° (Lit.,[103] 155-156°); $\nu_{max.}^{KBr}$ 3060-2870 (C-H st.), 2650-2490 (quart. ammonium), 1615 (C=O), 1360, 790 and 760 cm^{-1}; $\lambda_{max.}^{EtOH}$ 253, 335, 374, 430 nm; R_f 0.52 (ammonium hydroxide-ethanol 1.5:100). The free base was obtained from a solution of the dihydrochloride by the method described for lucanthone, and recrystallised from ethanol, m.p. 70-72°; $\nu_{max.}^{KBr}$ 1612 (C=O), 1320, 795 and 750 cm^{-1}; $\lambda_{max.}^{EtOH}$ 193 (log ε 4.45) and 262 nm (3.60); R_f 0.49 (chloroform-ethanol, 4:1); δ 1.00 (6H, t, CH_3(2)), 2.38 (3H, s, ring CH_3), 2.90 (3H, s, N-CH_3), 2.32-2.82

(6H, asym. q, CH_2(2), 13-CH_2), 3.28 (2H, t, 12-CH_2), 6.94 (1H, d, J=8Hz, H-2), 7.16-7.64 (4H, m, H-3, 5, 6 and 7) and 8.2-8.5 (1H, m, H-8).

1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) (Method A)

1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one was prepared by a method analagous to that of Archer and Suter.[15] A mixture of 1,8-dichloro-4-methylthioxanthen-9-one (2 g), diethylaminoethylamine (5 g) and pyridine (10 g) was refluxed at 121-123° for 20 hours. The mixture was allowed to cool, treated with 50% aqueous potassium hydroxide (1 mℓ) and steam distilled to remove the volatile base; the residue was cooled and the supernatant liquid carefully decanted. The solid remaining was boiled successively with two 5 mℓ portions of 10% acetic acid; the solid being filtered off after each extraction. The acid filtrates were combined, made alkaline with sodium carbonate solution (20%) and the thioxanthenones were extracted using chloroform. After drying, the extract was concentrated in vacuo and the residue redissolved in chloroform (2 mℓ).

The solution was separated by preparative t.l.c. on silica gel plates run in chloroform-ethanol (4:1). The separated thioxanthenone bands (R_f 0.55 and 0.13) were removed and extracted with acetone. The acetone was removed by distillation and the residue dissolved in dry ether, filtered and the filtrate treated with a slight excess of alcoholic hydrogen chloride. The solids were filtered and dried. Material from the band of R_f 0.55 of the preparative t.l.c. plates yielded 1-chloro-8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one hydrochloride (46%), a yellow crystalline solid, m.p. 168-170°; $\nu_{max.}^{KBr}$ 3290 (N-H st.), 2980 (C-H st.), 2805 ($N-H^+$) and 1632 (C=O) cm^{-1}; $\lambda_{max.}^{EtOH}$ 206, 228, 256, 338 and

431 nm. A sample of the base was obtained from the hydrochloride by the method described for lucanthone and recrystallised from ethanol, m.p. 96-98°; $\nu_{max.}^{KBr}$ 3290 (N-Hst.), 3060-2860 (C-Hst.), 1630 (C=O), 1563, 1504, 1430, 1385, 1245, 1194, 855, 815, 805, 770 cm^{-1}; $\lambda_{max.}^{EtOH}$ 206 (log ε 4.37), 230sh (4.20), 257 (4.41), 337 (3.69), and 435 nm (3.65); δ 1.10 (6H, t, CH_3(2)), 2.42 (3H, s, CH_3), 2.36-2.86 (6H, asym. q, CH_2(2) and 13-CH_2), 3.21 (2H, t, 12-CH_2), 6.36 (2H, asym. q, H-2 and H-4), 6.90-7.10 (3H, m, H-3, 6 and 7), and 8.88 (1H, m, N-H bonded, exchangeable with D_2O); R_f 0.55 (chloroform-ethanol, 4:1), 0.74 (ethanol-ammonia, 77:23); m/e (%) 376(0.1), 374(0.3, M^+), 290(0.1), 288(0.3), 253(0.1), 197(0.1), 99(0.1), 97(0.1), 86(100), 85(1), and 83(2). Found: M (mass spectrometry) 374.1219. $C_{20}H_{23}ClON_2S$ requires 374.1219. Found: C, 62.16; H, 5.52; N, 8.21; S, 9.38 and Cl, 10.56; $C_{20}H_{23}ClON_2S$ requires C, 62.34; H, 5.48; N, 8.08; S, 9.24 and Cl, 10.25%.

Material from the band of R_f 0.13 of the preparative t.l.c. plates gave a yellow solid, 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one hydrochloride hydrate (29%), m.p. 174-177;[(d)] $\nu_{max.}^{KBr}$ 3280 (N-H st.), 2930, 2805, 2650-2490 (N^+-H), 1612 (C=O), 1567, 1516, 1390, 1227, 1085, 850, 815 and 769 cm^{-1}; $\lambda_{max.}^{EtOH}$ 204, 255, 333 and 435 nm; Found: C, 55.28; H, 7.66; N, 9.61; S, 5.57; Cl, 15.74; $C_{26}H_{38}N_4OS.2HCl.2H_2O$ requires C, 55.42; H, 7.82; N, 9.95; S, 5.68; Cl, 12.61%.

A sample of the base was prepared by the method described for lucanthone and recrystallised from ethanol, m.p. 40-41.5°; $\nu_{max.}^{KBr}$ 3280 (N-Hst.), 2930-2800, 1612 (C=O), 1567, 1516, 1395, 1227, 1087, 850, 815 and 769 cm^{-1}; $\lambda_{max.}^{EtOH}$ 205.5 (log ε 4.48), 231sh (4.26), 258 (4.45), 338 (3.76), and 422 nm (4.04); δ 1.08 (12H, t, CH_3(4)), 2.23 (3H, s, CH_3), 2.40-2.95 (12H, asym. quint. CH_2(4), 13-CH_2 and 13'-CH_2), 3.24 (4H, t, 12-CH_2,

12'-CH_2), 6.60 (3H, asym. t, H-2, H-5 and H-7), 6.96-7.36 (2H, m, H-3 and H-6), and 9.80 (2H, m, N-H bonded (2), exchangeable with D_2O); R_f 0.13 (chloroform-ethanol, 1:4); m/e (%) 454(2.5), 369(4), 368(22), 297(1.5), 295(1.5), 282(0.6), 101(3), 100(100), 97(15), 87(10), 86(100), 85(1.5), 84(1), 83(2), 72(6), 71(2.5), 70(1.5), 69(1.5), 58(11), 57(6), 56(6), 55(4), 44(9), 43(5), 42(6); m^* 298.3 (454 → 368), 276.0 (454 → 354) Found: M (mass spectrometry) 452.2610. $C_{26}H_{38}N_4OS$ requires 452.2608. Found: C, 68.64; H, 8.44; N, 12.28; S, 7.12; $C_{26}H_{38}N_4OS$ requires C, 68.69; H, 8.43; N, 12.33; S. 7.04%.

1,8-Di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) (Method B)

1,8-Dichloro-4-methylthioxanthen-9-one (5 g) was dissolved in diethylaminoethylamine (12 g) and anhydrous potassium carbonate (5 g) and copper bronze powder (0.5 g) were added. The mixture was refluxed under nitrogen for 10 hours, cooled and poured into dilute hydrochloric acid solution (5%). The solution was filtered and made alkaline with sodium carbonate and extracted with chloroform. The chloroform layer was dried over anhydrous magnesium sulphate and the chloroform removed by distillation under vacuum, yielding a brown oil. The oil was dissolved in chloroform and separated by preparative t.l.c. using silica gel plates run in chloroform-ethanol (4:1). Two bands were removed at R_f 0.13 and 0.52 and each extracted with dilute hydrochloric acid solution (5%), the solutions filtered, made alkaline with sodium carbonate and extracted with chloroform. The chloroform was dried over anhydrous magnesium sulphate and the solvent removed on a rotary evaporator. The residues were taken up in dry chloroform (5 mℓ) and dry hydrogen chloride gas bubbled into the solution until a red precipitate formed. The precipitates were

collected and dried under vacuum. The solid hydrochlorides were washed with acetone (x2) and dried under vacuum to give crystalline products.

The thioxanthenone (R_f 0.52) was recrystallised from acetone giving a yellow crystalline salt, 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one hydrochloride, (23%), m.p. 195-197°; $\nu_{max.}^{KBr}$ 3600-3350 (N-H st.), 3060-2850 (C-H st.), 2470 (quaternary ammonium), 2125, 1610 (C=O), 1520, 1341, 1232, 795 and 763 cm^{-1}; $\lambda_{max.}^{EtOH}$ 204, 223, 255, 333, 435 nm; Found: C, 60.65; H, 6.68; N, 6.93; S, 8.53; Cℓ, 10.17; $C_{20}H_{24}N_2OS.HC\ell.H_2O$ requires C, 60.84; H, 6.84; N, 7.10; S, 8.11; Cℓ, 9.00%.

A sample of the base was obtained from the hydrochloride by the method described for lucanthone and was recrystallised from ethanol, giving a yellow crystalline product, m.p. 116-118°; $\nu_{max.}^{KBr}$ 3600-3150 (N-H st.), 2925 (C-H st.), 1610 (C=O), 1563, 1505, 1381, 1260, 1242, 1192, 812, and 803 cm^{-1}; $\lambda_{max.}^{EtOH}$ 205 (log 4.29), 228 (4.33), 256.5 (4.61), 332 (3.90), and 436 nm (3.92); δ 1.10 (6H, t, CH_3(2)), 2.40 (3H, s, CH_3), 2.40-3.00 (6H, asym. quint, CH_2(2) and 13-CH_2), 3.30 (2H, t, 12-CH_2), 6.60 (2H, asym. t, H-2 and H-4), 7.10-7.50 (3H, m, H-3, 6 and 7), 8.25-8.50 (1H, dd, J=8Hz and 2Hz, H-8), and 10.15 (1H, m, N-H bonded, exchangeable with D_2O); R_f 0.52 (chloroform-ethanol, 4:1), 0.74 (ethanol-ammonia, 77:23), 0.65 (dioxane-acetone-water, 7:7:1), 0.43 (ethanol-n-hexane, 1:1); m/e (%) 340(5), 268(1), 255(4), 254(40), 226(1), 198(1), 165(5), 87(40), 86(100), 85(1), 72(6), 58(22); m^* 189.8 (340 → 254); Found: M (mass spectrometry) 340.1607. $C_{20}H_{24}N_2OS$ requires 340.1609; Found: C, 70.38; H, 7.08; N, 8.10; S, 9.57. $C_{20}H_{24}N_2OS$ requires C, 70.57; H, 7.06; N, 8.23; S, 9.41%.

Recrystallisation from ethanol-acetone (1:1) of the material from

the band of R_f 0.13 gave 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one hydrochloride hydrate (55%), m.p. 174-177°; the base was prepared from the hydrochloride by the method described for lucanthone and showed the same spectral properties and t.l.c. as described in Method A (p. 139).

1-[[2-(Diethylamino)ethyl]amino-5-methylthioxanthen-9-one (167)

2-Toluidine (50 g, Aldrich Chemical Co. Ltd) was diazotised and reacted with potassium xanthate (65.5 g) as described on p. 95. Hydrolysis with potassium hydroxide (80.3 g) gave 2-methylthiophenol (33.1 g, 57%). The 2-methylthiophenol (12 g) was reacted with the potassium salt of 2,6-dichlorobenzoic acid (15 g), sodium methoxide (3.6 g) and copper bronze powder (0.1 g) under the melt conditions described on p. 96, giving 6-chloro-2-[(2-methylphenyl)thio]benzoic acid (26.1 g). This was cyclised with concentrated sulphuric acid (260 g, S.G. 1.84), for 2 hours and the mixture then poured into water containing ice. The yellow precipitate was filtered off and boiled with ammonia solution (10%, 100 mℓ) for 1 hour. The solid was filtered off, washed with water and dilute ethanol and dried at 70° (8.6 g, 51%).

The crude 1-chloro-5-methylthioxanthen-9-one was recrystallised from acetic acid (10%), m.p. 181.5-183°; $\nu_{max.}^{KBr}$ 1640 (C=O), 1600, 1470-1430, 1300, 860-800 and 800-600; $\lambda_{max.}^{EtOH}$ 205 (log ε 4.12) 222 (4.10), 258 (4.50), 304 (3.73) and 381 nm (3.66); δ 2.42 (3H, s, CH_3), 7.14-7.66 (5H, m, aromatic H), 8.08-8.43 (1H, dd, J=8Hz and 2Hz, H-8); R_f 0.40 (chloroform); m/e (%) 262(31), 261(10), 260(100,M^+), 234(2), 232(6), 231(4), 197(27), 152(12), 98(3); m^* 207.0 (260 → 232), 167.3 (232 → 197); Found: C, 64.54; H, 3.62; Cℓ, 13.48; S, 12.40. $C_{14}H_9C\ell SO$ requires C, 64.47; H, 3.45; Cℓ, 13.62; S, 12.32%.

1-Chloro-5-methylthioxanthen-9-one (5 g) was refluxed with diethylaminoethylamine (10 g) in pyridine (20 g) for 20 hours. The mixture was cooled, treated with potassium hydroxide solution (2 mℓ, 50%) and then steam distilled to remove volatile material. The residue was cooled, the supernatant decanted and the solid boiled with acetic acid (10%, 200 mℓ). The insoluble residue was filtered off and boiled successively with acetic acid (50 mℓ, 10%, x 2). The acid filtrates were combined, made alkaline with sodium carbonate and the thioxanthenone extracted with ether. The hydrochloride was formed by the method described on page 141 and recrystallised from acetone (5 g, 66%), m.p. 195-197.5°.

The base, prepared by the method described for lucanthone, showed the same spectral properties and t.l.c. as described for 1-[[2-(diethylamino)ethyl]amino]-5-methylthioxanthen-9-one in Method B (p. 142).

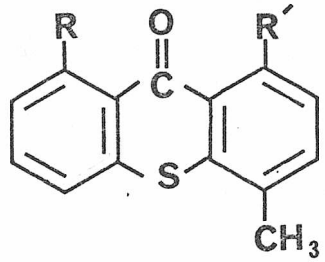
Synthesis of 1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds

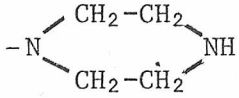
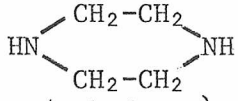
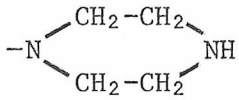
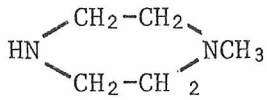
(Method C)

1,8-Dichloro-4-methylthioxanthen-9-one (0.02 mol) was dissolved in the diamine (0.1 mol) and treated with anhydrous potassium carbonate (0.04 mol) and copper bronze powder (0.01 mol). The mixture was refluxed under nitrogen, monitored at 30 minute intervals by t.l.c., cooled, and poured into 5% hydrochloric acid solution. The reaction products were then worked-up by the technique described on p. 141 (Method B). The reaction conditions are recorded in Table 29.

Table 29

Reaction conditions



No.	Thioxanthenone (RR')	Diamine	Reflux time	Reaction temperature	% Yield* Mono-	% Yield* Di-substitution
(172)	$-NH(CH_2)_2NH_2$	$NH_2(CH_2)_2NH_2$	½ hr.	117-119°	5	10
(173)	$-NH(CH_2)_2N(CH_3)_2$	$NH_2(CH_2)_2N(CH_3)_2$	10 hrs.	131-135°	18	22
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	$NH_2(CH_2)_2N(C_2H_5)_2$	10 hrs.	121-123°	23†	55†
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	$NH_2(CH_2)_2N(CH(CH_3)_2)_2$	20 hrs.	166-168°	5	9
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	$NH_2(CH_2)_3N(C_2H_5)_2$	10 hrs.	159-160°	8	12
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	$NH_2(CH_2)_4N(C_2H_5)_2$	6 hrs.	165-167°	5	8
(177)		 (anhydrous)	10 hrs.	145-146°	15	5
(178)			16 hrs.	138-139°	22	30

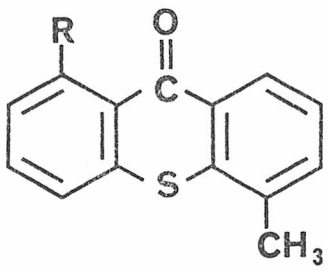
* 5 g scale (5 experiments) † 5 g scale (20 experiments)

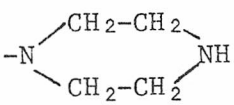
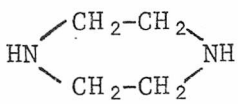
Synthesis of 1-Dialkylaminoalkylamino-5-methylthioxanthen-9-one and related compounds

(Method D)

1-Chloro-5-methylthioxanthen-9-one (0.02 mol) and the diamine (0.1 mol) were dissolved in pyridine (15 mℓ) and the mixture refluxed under nitrogen (Table 30). The reaction was monitored by t.l.c. and worked up by the technique described for 1-[[2-(diethylamino)ethyl]-amino]-5-methylthioxanthen-9-one (p. 144).

Table 30 Reaction conditions



No.	Thioxanthenone R	Diamine	Reflux time (hrs.)	% Yield
(179)	$-NH(CH_2)_2NH_2$	$NH_2(CH_2)_2NH_2$	6	44
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	$NH_2(CH_2)_2N(C_2H_5)_2$	20	66
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	$NH_2(CH_2)_2N(CH(CH_3)_2)_2$	6	22
(184)		 (anhydrous)	6	20

H.p.l.c. study of the modified Ullmann reaction involving 1,8-dichloro-4-methylthioxanthen-9-one (136)

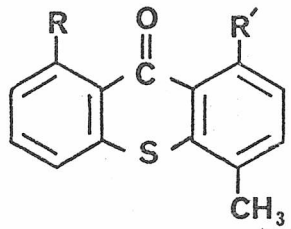
1,8-Dichloro-4-methylthioxanthen-9-one was reacted with diethylaminoethylamine in the presence of copper catalyst and anhydrous potassium carbonate using the conditions described in Method B (p. 141). A sample was removed from the reaction after 4 hours and extracted with chloroform, evaporated to dryness and redissolved in acetonitrile.

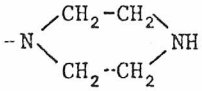
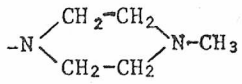
The solvent used to elute the column was acetonitrite-water 45:55 at a flow rate of 1 $cm^3 min^{-1}$ giving retention times (t_r) for the sample and reference chloromethylthioxanthenones shown in Table 31, with resolution in all cases to baseline. A sample of 4,8-dichloro-1-methylthioxanthen-9-one was used as an internal standard.

Table 31. Retention times

No.	Compound	t_r relative to internal standard
(138)	1-chloro-4-methylthioxanthen-9-one	1.94
(159)	1-chloro-5-methylthioxanthen-9-one	1.76
(136)	1,8-dichloro-4-methylthioxanthen-9-one	1.99
	sample from Ullmann reaction (4 hrs)	1.78

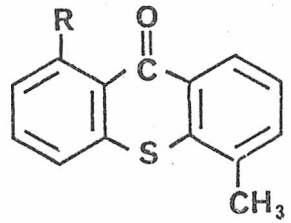
Table 32. Properties of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds



No.	Thioxanthenone RR'	Method	Recrystallisation solvent	m.p.°	Formula	Analysis: Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found	Sulphur % Calcd.	Sulphur % Found
(172)	$-NH(CH_2)_2NH_2$	C	Ethanol	140-143(d)	$C_{18}H_{22}N_4OS$	63.14	63.19	6.48	6.29	16.37	16.30	9.35	9.51
(173)	$-NH(CH_2)_2N(CH_3)_2$	C	Ethanol	81.5-83	$C_{22}H_{30}N_4OS$	66.33	66.12	7.59	7.85	14.06	13.36	8.03	8.22
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	A,B	Ethanol	40-41.5(d)	$C_{26}H_{38}N_4OS$	68.69	68.64	8.43	8.44	12.33	12.28	7.04	7.12
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	C	Ethanol	103-106(d)	$C_{30}H_{46}N_4OS$	70.57	70.41	9.02	8.93	10.98	10.79	6.27	6.13
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	C	Ethanol	46.2-48.2	$C_{28}H_{42}N_4OS$	69.67	69.45	8.77	8.72	11.61	10.37	6.63	6.76
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	C	Ethanol	168-170(d)	$C_{30}H_{46}N_4OS$	70.55	70.23	9.08	8.81	10.97	10.80	6.27	6.04
(177)		C	Ethanol	150-154	$C_{22}H_{26}N_4OS$	66.98	66.85	6.64	6.46	14.21	14.42	8.11	8.30
(178)		C	Ethanol	150-151	$C_{24}H_{30}N_4OS$	68.22	68.01	7.11	6.93	13.26	12.46	7.57	7.31

(d) = melted with decomposition

Table 33. Properties of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds



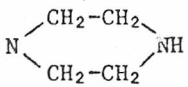
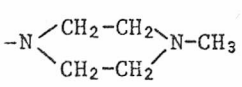
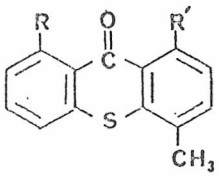
No.	Thioxanthenone R	Method	Recrystallisation solvent	m.p.°	Formula	Analysis: Carbon% Calcd.	Carbon% Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found	Sulphur % Calcd.	Sulphur % Found
(179)	$-NH(CH_2)_2NH_2$	D	Ethanol	143-146(d)	$C_{16}H_{16}N_2OS$	67.59	67.97	5.67	5.79	9.86	9.88	11.26	10.55
(180)	$-NH(CH_2)_2N(CH_3)_2$	C	Ethanol	81-82.5	$C_{18}H_{20}N_2OS$	69.23	68.95	6.45	6.59	8.97	8.93	10.24	10.12
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	B	Ethanol	116-118	$C_{20}H_{24}N_2OS$	70.57	70.38	7.06	7.08	8.23	8.10	9.41	9.57
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	D	Ethanol	84.5-86	$C_{22}H_{28}N_2OS$	71.71	71.54	7.66	7.36	7.60	7.51	8.70	8.56
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	C	Ethanol	84.5-86.0	$C_{21}H_{26}N_2OS$	71.16	71.20	7.39	7.51	7.91	7.32	9.03	8.88
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	C	Ethanol	97.0-99.0	$C_{22}H_{28}N_2OS$	71.71	71.57	7.66	7.47	7.60	7.57	8.70	8.54
(184)		D	Ethanol	152-153	$C_{18}H_{18}N_2OS$	69.66	69.58	5.85	5.95	9.03	8.84	10.32	9.97
(185)		C	Ethanol	129-130	$C_{19}H_{20}N_2OS$	70.35	70.37	6.22	6.25	8.64	8.67	9.87	10.02

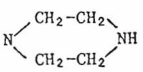
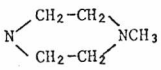
Table 34 Thin-layer chromatography of thioxanthen-9-ones

1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds

Thioxanthenone

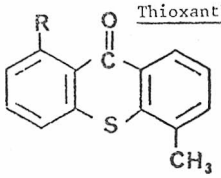


Solvent system [64]

No.	RR'	Chloroform-Ethanol (4:1) R_f	Ethanol-n-Hexane (1:1) R_f	Dioxane-Acetone-Water (7:7:1) R_f	Ethanol-Ammonia (77:23) R_f
(172)	$NH(CH_2)_2NH_2$	0.01	0.01	0.07	0.60
(173)	$NH(CH_2)_2N(CH_3)_2$	0.06	0.02	0.07	0.76
(165)	$NH(CH_2)_2N(C_2H_5)_2$	0.13	0.01	0.03	0.76
(174)	$NH(CH_2)_2N(CH(CH_3)_2)_2$	0.04	0.27	0.03	0.62
(175)	$NH(CH_2)_3N(C_2H_5)_2$	0.02	0.03	0.04	0.75
(176)	$NH(CH_2)_4N(C_2H_5)_2$	0.01	0.01	0.01	0.69
(177)		0.01	0.01	0.01	0.72
(178)		0.06	0.03	0.04	0.74

1-Dialkylaminoalkylamino-5-methylthioxanthenones and related compounds

Thioxanthenone



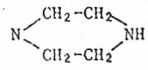
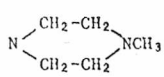
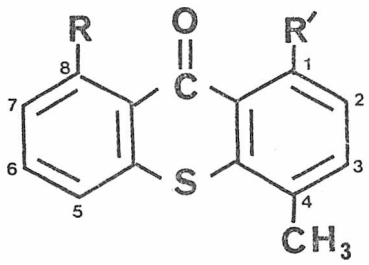
No.	R	96% 34%
(179)	$NH(CH_2)_2NH_2$	0.19 0.01 0.55 0.61
(180)	$NH(CH_2)_2N(CH_3)_2$	0.49 0.25 0.48 0.74
(167)	$NH(CH_2)_2N(C_2H_5)_2$	0.52 0.43 0.65 0.74
(181)	$NH(CH_2)_2N(CH(CH_3)_2)_2$	0.62 0.62 0.81 0.66
(182)	$NH(CH_2)_3N(C_2H_5)_2$	0.22 0.16 0.21 0.73
(183)	$NH(CH_2)_4N(C_2H_5)_2$	0.15 0.08 0.11 0.70
(184)		0.11 0.03 0.10 0.77
(185)		0.34 0.19 0.27 0.74
(1)	Lucanthone	0.50 0.42 0.66 0.74

Table 35. Infrared spectra of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds



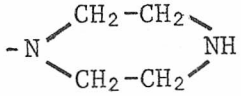
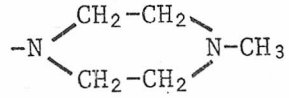
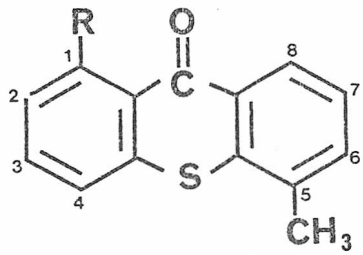
No.	Thioxanthenone RR'	N-Hst.	C-Hst.	C=Ost.	Other absorptions
(172)	$-NH(CH_2)_2NH_2$	3600-3400 3380,3240	2960-2810	1610	1570,1515, 1395,1250, 812,768
(173)	$-NH(CH_2)_2N(CH_3)_2$	3600-3300	2980-2790	1610	1567,1510, 1390,1250-1210,805, 758
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	3280	2930-2800	1612	1567,1516, 1395,1227, 1087,850, 815,769
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	3600-3120	2980-2940	1605	1565,1507, 1395,1225, 1118,1087, 809,767
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	3640-3120	2920	1603	1585,1506, 1445,1405, 1280,1195, 1080,765
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	3620-3310	2930	1619	1565,1517, 1393,1260, 1230,1085, 1020,957, 810
(177)		3520-3130	3040-2800	1607	1580,1508, 1460,1370, 1285,1180, 1130,995, 970,920, 850,800
(178)			2940-2800	1605	1450,1375, 1285,1185, 1135,996, 975,920, 891,800

Table 36. Infrared spectra of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds



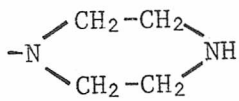
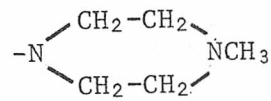
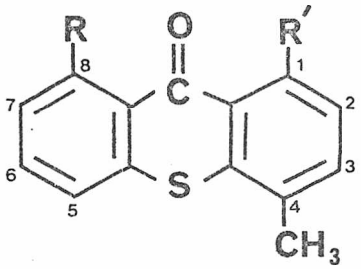
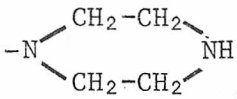
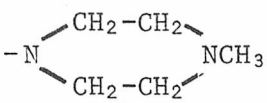
No.	Thioxanthenone R	N-Hst.	C-Hst.	C=Ost.	Other absorptions
(179)	$-NH(CH_2)_2NH_2$	3600-3300	3100-2940	1604	1570,1500, 1450,1312, 1067,775,714
(180)	$-NH(CH_2)_2N(CH_3)_2$	3600-3260	2910	1615	1568,1505, 1384,1255, 1110,798,775
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	3600-3150	2925	1610	1563,1505, 1381,1260, 1242,1192, 812,803
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	3620-3320	2620	1605	1583,1570, 1498,1430, 1390,1320, 1255,1170, 772,730
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	3600-3200	2920	1600	1565,1498, 1435,1255, 1170,1095, 780,712
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	3615-3100	2980-2790	1609	1585,1560, 1505,1465, 1383,1240, 1190,1080, 955,815, 770,763
(184)		3550-3040	3020-2520	1608	1583,1508, 1443,1325, 1275,1173, 1060,997, 950,910,845, 819,792
(185)		3630-3120	3060-2800	1620	1585,1445, 1325,1250, 1187,1140, 1062,970, 940,795
(1)	Lucanthone	3460	3020-2850	1616	1591,1512, 1385,1225, 795,763

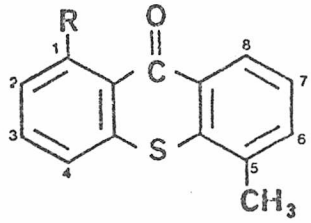
Table 37. Ultraviolet spectra of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds



No.	Thioxanthenone RR'	$\lambda_{max.}^{EtOH}$	Log ε
(172)	$-NH(CH_2)_2NH_2$	206	4.42
		231(sh)	4.30
		258.5	4.47
		337	3.78
		420	4.05
(173)	$-NH(CH_2)_2N(CH_3)_2$	206.5	4.50
		231(sh)	4.38
		258	4.55
		338	3.92
		420	4.18
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	205.5	4.48
		231(sh)	4.26
		258	4.45
		338	3.76
		422	4.04
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	206	4.46
		231(sh)	4.30
		258	4.44
		337	3.77
		425	3.91
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	206	4.53
		232(sh)	4.35
		258	4.53
		338	3.84
		424	4.13
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	205	4.49
		232(sh)	4.30
		258	4.43
		338	3.81
		424	4.01
(177)		206	4.38
		257	4.52
		309	3.64
		366	3.75
(178)		206	4.34
		256.5	4.49
		308	3.59
		364	3.72

(sh = shoulder)

Table 38. Ultraviolet spectra of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds



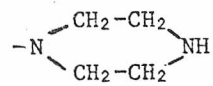
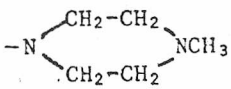
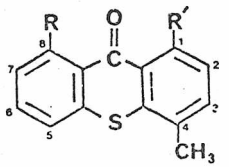
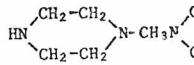
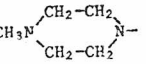
No.	Thioxanthenone R	$\lambda_{max.}^{EtOH}$	Log ε
(179)	$-NH(CH_2)_2NH_2$	205	4.12
		228	4.23
		255.5	4.47
		330	3.81
		435	3.77
(180)	$-NH(CH_2)_2N(CH_3)_2$	206	4.31
		229	4.34
		256	4.61
		333	3.93
		438	3.89
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	205	4.29
		228	4.33
		256.5	4.61
		332	3.90
		436	3.92
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	205.5	4.18
		222	4.18
		256	4.47
		333	3.72
		438	3.66
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	205.5	4.22
		229	4.24
		256	4.50
		333	3.80
		442	3.79
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	206	4.28
		230	4.26
		256	4.53
		330	3.87
		446	3.81
(184)		205	4.09
		226(sh)	4.06
		259	4.43
		320	3.60
		375	3.44
		423	3.52
(185)		205	4.05
		226(sh)	4.02
		260	4.40
		320	3.56
		363	3.40
		420	3.49
(1)	Lucanthone	206	4.30
		256	4.60
		330	3.90
		440	3.88

Table 39. N.m.r. spectroscopy of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds



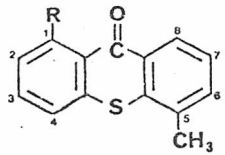
$R = HN\overset{12}{C}H_2\overset{13}{C}H_2\overset{14}{C}H_2\overset{15}{C}H_2N$

(values in δ).

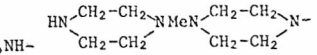
Side chain RR'	$NH_2(CH_2)_2NH-$	$(CH_3)_2N(CH_2)_2NH-$	$(C_2H_5)_2N(CH_2)_2NH-$	$((CH_3)_2)_2N(CH_2)_2NH-$	$(C_2H_5)_2N(CH_2)_3NH-$	$(C_2H_5)_2N(CH_2)_4NH-$		
solvent	DMSO	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
Group No.	(172)	(173)	(165)	(174)	(175)	(176)	(177)	(178)
Methyl:								
piperazine 4-CH_3	2.18(3H,s)	2.24(3H,s)	2.23(3H,s)	2.28(3H,s)	2.28(3H,s)	2.25(s) overlapping	2.16(3H,s)	2.37(9H,s) overlapping
$N(CH_3)_2$		2.32(12H,s)				15-CH_2 and CH_2 ethyl [19H]		
ethyl			1.08(12H,t)		1.11(12H,t)	1.08(12H,t)		
isopropyl				1.08(24H,d) J=6Hz				
Methine:								
isopropyl				3.12(8H,m) overlapping 12-CH_2				
Methylene:								
ethyl			2.40-2.95 (12H,quint) overlapping 13-CH_2		2.54(12H,q)	2.25 overlapping CH_3		
12-CH_2	3.20(4H,t)	3.24(4H,t)	3.24(4H,t)	overlap with 4CH isopropyl	3.25(4H,q)	3.22(4H,q)	3.10(16H,s)	3.19(8H,t) 2.65(8H,t)
piperazine 13-CH_2	2.58-3.05 (4H,m)	2.64(4H,t)	qverlap with CH_2 ethyl	2.90(4H,t)	1.92(4H,q) overlapping CH_2 ethyl	1.77(8H,m)		
14-CH_2								
15-CH_2						2.55 overlap with methyl		
Amino:								
NH bonded*	9.80(2H,m)	9.95(2H,m)	9.80(1H,m)	9.92(1H,m)	9.80(2H,m)	9.85(2H,m)		
NH_2*	1.23(4H,s)							
NH unbonded*							1.76(2H,s)	
Aromatics:								
H-2,H-5,H-7	6.62(3H,m)	6.50(3H,t)	6.60(3H,t)	6.56(3H,t)	6.50(3H,t)	6.55(3H,t)		
H-3	7.22(1H,d) J=8Hz	7.09(1H,d) J=8Hz	7.10(1H,d) J=8Hz	7.13(1H,d) J=8Hz	7.05(1H,d) J=8Hz	7.02(1H,d) J=8Hz	6.8-7.4 (5H,m)	6.8-7.45 (5H,m)
H-6	7.31(1H,t)	7.22(1H,t)	7.20(1H,t)	7.26(1H,t)	7.20(1H,t)	&.23(1H,t)		

* exchangeable with D_2O

Table 40. N.m.r. spectroscopy of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds



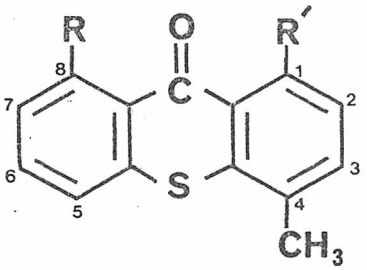
R= $HN^{12}CH_2{}^{13}CH_2{}^{14}CH_2{}^{15}CH_2N$

Side chain R	$NH_2(CH_2)_2NH-$	$(CH_3)_2N(CH_2)_2NH-$	$(C_2H_5)_2N(CH_2)_2NH-$	$((CH_3)_2)_2N(CH_2)_2NH-$	$(C_2H_5)_2N(CH_2)_3NH-$	$(C_2H_5)_2N(CH_2)_4NH-$		
solvent	DMSO	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
Group No.	(179)	(180)	(167)	(181)	(182)	(183)	(184)	(185)
Methyl:								
piperazine								2.36(3H,s)
4-CH_3	2.40(3H,s)	2.40(3H,s)	2.40(3H,s)	2.36(3H,s)	2.41(3H,s)	2.40(3H,s)	2.42(3H,s)	2.42(3H,s)
$N(CH_3)_2$		2.31(6H,s)						
ethyl			1.10(6H,t)		1.34(6H,t)	1.25(6H,t)		
isopropyl				1.07(6H,d) J=11Hz				
Methine:								
isopropyl				4.10(1H,m)				
Methylene:								
ethyl			2.40-3.00 (6H,quint) overlapping 13-CH_2		3.10(q)‡	3.20(q)†		
12-CH_2	3.19(2H,t)		3.30(2H,t)		3.34(t)‡	3.19(t)† †overlap (6H)	3.10(8H,s)	3.19(4H,t) 2.69(4H,t)
piperazine								
13-CH_2	2.83(2H,t)				2.27(2H,t)	1.77(4H, broad s)		
14-CH_2					3.20(t)‡ ‡overlap integral=8H			
15-CH_2						2.28(2H,m)		
Amino:								
NH-bonded*	9.95(1H,m)	10.17(1H,m)	10.15(1H,m)	10.18(1H,m)	10.20(1H,m)	9.70(1H,m)		
NH_2*	1.20(2H,s)							
NH-unbonded*							1.81(1H, broad s)	
Aromatics:								
H-2,H-4	6.70(2H,t)	6.55(2H,t)	6.60(2H,t)	6.55(2H,t)	6.61(2H,t)	6.57(2H,t)	7.00(2H,t)	6.98(2H,t)
H-3,H-6,H-7	7.16-7.60 (3H,m)	7.05-7.55 (3H,m)	7.10-7.50 (3H,m)	7.02-7.55 (3H,m)	7.07-7.59 (3H,m)	7.05-7.55 (3H,m)	7.18-7.53 (3H,m)	7.15-7.53 (3H,m)
H-8	8.21(1H,dd)	8.46(1H,m)	8.38(1H,dd J=8hz,2Hz)	8.41(1H,m)	8.32(1H,dd J=8Hz,2Hz)	8.45(1H,m)	8.23(1H,m)	8.23(1H,dd J=8Hz,2Hz)

Key: dd = doublet of doublets; q = quartet; t = triplet (in the case of the aromatics, not a true triplet, but a composite splitting pattern giving an apparent triplet)

* exchangeable with D_2O.

Table 41. Mass spectrometry of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds



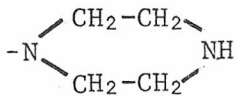
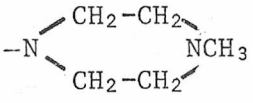
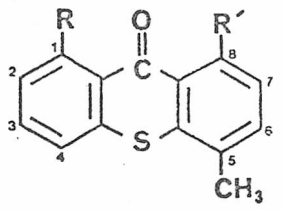
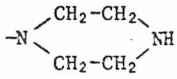
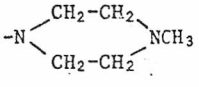
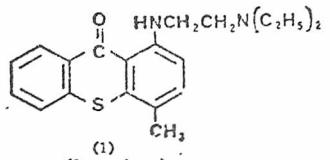
No.	RR'	m/e (%)
(172)	$-NH(CH_2)_2NH_2$	342(8), 313(2.5), 312(56), 299(5), 295(7), 294(25), 283(9), 282(3), 281(3), 280(3), 265(3.5), 255(6), 77(2), 64(5), 58(5), 48(8), 44(100), 41(7)
(173)	$-NH(CH_2)_2N(CH_3)_2$	398(9), 341(8), 340(56), 297(1.5), 295(3), 283(2), 267(1.5), 252(1), 242(1), 197(0.6), 78(28), 72(100), 58(100) m^* 290.5 (398 → 340)
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	454(2.5), 369(4), 368(22), 297(1.5), 295(1.5), 282(0.6), 101(3), 100(100), 97(15), 87(10), 86(100), 85(1.5), 84(1), 83(2), 72(6), 71(2.5), 70(1.5), 69(1.5), 58(11), 57(6), 56(6), 55(4), 44(9), 43(5), 42(6) m^* 298.3 (454 → 368), 276.0 (454 → 354)
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	510(1), 396(1.5), 128(3.5), 115(3), 114(100), 86(2), 83(2.5), 72(7)
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	482(3), 397(9), 396(50), 382(6), 269(5), 267(2), 252(1), 114(28), 100(11), 98(7), 87(5), 86(100), 85(2.5), 84(4.5), 83(5), 72(11), 71(2), 70(2), 58(10), 56(4)
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	510(0.3), 424(1), 410(8), 409(25), 392(1.5), 391(8), 365(3), 364(18), 363(2), 347(2.5), 346(10), 323(4), 270(3), 100(79), 86(10), 85(2.5), 84(10), 72(8), 58(100)
(177)		394(0.8), 378(1.5), 352(5.5), 350(3), 334(2.5), 295(3), 282(8), 276(3), 266(3), 265(3), 263(3), 254(4), 252(2), 85(3.5), 83(7), 72(9), 71(3), 70(10), 58(4.5), 56(2), 42(13), 38(20), 36(100), 35(5)
(178)		423(13), 422(79), 407(0.4), 394(10), 378(1.5), 364(1.5), 352(10), 350(3), 334(3), 332(2), 291(2), 281(8), 280(2), 277(3), 268(2), 267(3), 265(1.5), 264(2.5), 254(3.5), 252(2), 238(1), 226(1), 197(1.5), 152(0.5), 97(1), 86(6), 83(3.5), 72(11), 71(3), 70(8), 58(16), 56(3), 43(25), 42(10), 38(18), 36(100)

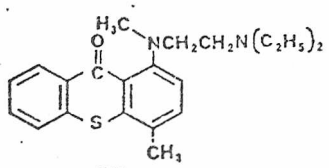
Table 42. Mass spectrometry of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds



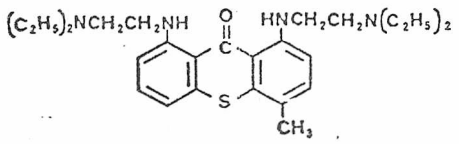
No.	R	R'	m/e (%)
(179)	$-NH(CH_2)_2NH_2$	H	284(4), 254(100), 238(1), 226(3), 210(0.6), 197(1.5), 184(1), 165(1), 152(0.8), 44(20)
(180)	$-NH(CH_2)_2N(CH_3)_2$	H	312(3), 254(56), 226(1), 197(1), 165(0.5), 72(0.4), 58(100), 42(1.5)
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	H	340(5), 268(1), 255(4), 254(40), 226(1), 198(1), 165(5), 87(40), 86(100), 85(1), 72(6), 58(22) m* 189.8 (340 → 254)
(166)	$-NH(CH_2)_2N(C_2H_5)_2$	Cl	376(0.1), 374(0.3,M^+), 290(0.1), 288(0.3), 253(0.1), 197(0.1), 99(0.1), 97(0.1), 86(100), 85(1), 83(2)
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	H	368(0.2), 254(2), 184(0.3,M^{++}), 115(2.5), 114(100), 83(0.7), 72(8)
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	H	354(18), 280(2), 268(18), 255(4.5), 254(18), 240(3), 238(25), 227(3), 210(2.5), 197(3), 176(1), 165(1.5), 152(1.5), 114(56), 100(79), 98(22), 86(100), 85(13), 84(10)
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	H	368(0.2), 340(13), 310(1.5), 282(1.5), 268(5), 267(7), 254(63), 241(50), 240(5), 238(2), 227(3), 226(13), 212(13), 198(2.5), 197(8), 195(2), 184(4), 165(4), 153(2), 152(4.5), 149(2.5), 127(1), 114(1.5), 100(32), 98(32), 97(3), 86(35), 83(4), 72(10), 58(100), 57(20), 56(8), 55(10)
(184)		H	310(8), 294(32), 280(0.4), 281(14), 269(9), 268(100), 254(84), 252(16), 251(4), 239(4.5), 238(14), 237(4), 227(3), 226(18), 225(2), 210(3), 198(3), 197(10), 184(1), 165(3.5), 152(2.5), 141(2), 131(2), 69(2), 56(7), 46(1.5), 42(2) m* 278.8 (310 → 294), 251.1 (310 → 279), 231.7 (310 → 268)
(185)		H	324(2.5), 293(1), 281(13), 280(40), 267(11), 266(13), 254(100), 238(5), 226(7), 209(1), 197(4), 165(2), 152(1), 131(1), 86(1.5), 70(9), 56(2.5), 43(22). m* 199.1 (324 → 254)
(1)	Lucanthone		340(10), 254(32), 237(1.5), 226(3), 198(8), 197(2.5), 165(1.5), 152(1), 87(6), 86(100), 85(1), 83(2), 72(8), 58(9), 56(1.5), 42(1.5) m* 173.5 (226 → 198)



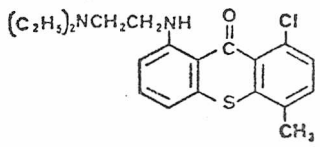
(1)
(lucanthone)



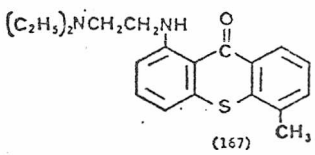
(82)
(N-methyllucanthone)



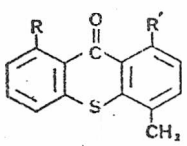
(165)

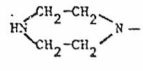
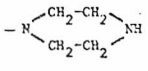
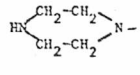
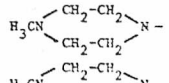
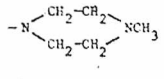
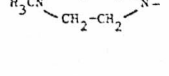


(166)



(167)



No.	R	R′
(172)	$NH_2(CH_2)_2NH-$	$-HN(CH_2)_2NH_2$
(179)	$NH_2(CH_2)_2NH-$	$-H$
(173)	$(CH_3)_2N(CH_2)_2NH-$	$-HN(CH_2)_2N(CH_3)_2$
(180)	$(CH_3)_2N(CH_2)_2NH-$	$-H$
(165)	$(C_2H_5)_2N(CH_2)_2NH-$	$-HN(CH_2)_2N(C_2H_5)_2$
(167)	$(C_2H_5)_2N(CH_2)_2NH-$	$-H$
(174)	$((CH_3)_2CH)_2N(CH_2)_2NH-$	$-HN(CH_2)_2N(CH(CH_3)_2)_2$
(181)	$((CH_3)_2CH)_2N(CH_2)_2NH-$	$-H$
(175)	$(C_2H_5)_2N(CH_2)_3NH-$	$-HN(CH_2)_3N(C_2H_5)_2$
(182)	$(C_2H_5)_2N(CH_2)_3NH-$	$-H$
(176)	$(C_2H_5)_2N(CH_2)_4NH-$	$-HN(CH_2)_4N(C_2H_5)_2$
(183)	$(C_2H_5)_2N(CH_2)_4NH-$	$-H$
(177)		
(154)		$-H$
(178)		
(155)		$-H$

CHAPTER 3

Biological Activity of Thioxanthenones

Chapter 3

Index

Part 1. Interaction of 1,8-Di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and Related Compounds with DNA

Introduction

To investigate one aspect of the *in vitro* activity of the prepared 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds the thioxanthenones have been evaluated in a test system involving the effect on the denaturation of DNA.

The primary structure of DNA consists of a polynucleotide chain with phosphodiester linkages.[110] The proposal by Watson and Crick[111] of a two stranded α-helical secondary structure for DNA provided a structural model that explained many of its observed biochemical and physical properties. Complementary base sequences in the two strands are held together by hydrogen bonds between cytosine-guanine and adenine-thymine base pairs and these bonds confer specificity on the base-pairing mechanism.[110] In addition to the hydrogen bonds the stability of the helix is maintained by base stacking forces.[112]

Denaturation of the DNA molecule results in the irreversible rupture of a substantial fraction of the hydrogen bonds between complementary base pairs, which leads to a collapse of the rigid secondary structure of the native DNA so that the DNA strands can unwind forming two flexible, loosely coiled polyelectrolyte chains.[113] This process of denaturation is referred to as the helix → coil transition and may be brought about by heat or alkaline conditions. When heat is used to bring about the transition the temperature at which the helix is ruptured is referred to as the transition or melting temperature (Tm).

Biological, physical and chemical methods have been employed to study the denaturation of DNA, the principle techniques being enzymatic ,[114]

spectrophotometry,[54] viscosity,[115] optical rotation[116] and light scattering.[117]

The spectrophotometric method of studying the denaturation of DNA has been the most widely employed. A hyperchromic change in the u.v. absorbance associated with the helix → coil transition arises from the removal of the suppression of absorbance due to the stacking of the base pairs. This hyperchromic effect is accompanied by only minor changes in the absorption maxima and shape of the absorption curve.[118]

The value of the melting temperature (Tm) obtained depends somewhat on the wavelength employed, since the more thermally stable guanine-cytosine base pairs, which raise the melting point, have a different absorption spectrum from the adenine-thymine pairs.[113] The ratio of guanine-cytosine to adenine-thymine varies with the biological source of the DNA.[54]

Extensive studies have been carried out on the binding of acridines with DNA *in vitro*[119,120] and these have led to the intercalation concept of Lerman,[48] previously outlined. The combination of both acridines and phenanthridines with double stranded DNA results in a stabilisation of the helix as measured by the increase in the melting temperature (ΔTm) required to denature the molecule.

Lucanthone (1) has also been shown to bind strongly to DNA and stabilise it against heat denaturation.[45] In buffer solutions of low ionic strength the Tm of calf thymus DNA (56°) increased by 15° and 23° on addition of 6×10^{-6} mol ℓ^{-1} and 12×10^{-6} mol ℓ^{-1} lucanthone (1), respectively.[45]

Weinstein *et al.*[46] have found that in the presence of lucanthone (1), (6×10^{-6} mol ℓ^{-1}), the total hyperchromicity for the denaturation profile of calf thymus DNA was increased. This increase cannot be totally accounted for by subtracting the absorption at 260 nm of an equivalent

amount of lucanthone (1), measured at 90°. It is possible therefore that lucanthone (1) produces a small increase in hyperchromicity by binding to the denatured nucleic acid.

In the present study the interaction of native DNA with 1,8-di [[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) and its analogues has been studied in comparison with lucanthone (1). The methods reported in the literature for the measurement of Tm of native DNA have largely been based on the work of Marmur and Doty[54] and for this reason the type of DNA and buffer system employed has been based on their work.

Results

The results of the studies on the heat denaturation profile of native DNA are recorded in Table 43. All the compounds tested, with the exception of N-methyllucanthone (82) produced an appreciable increase in the temperature required to produce denaturation of the DNA. A similar result to that of Hirschberg et al.[44] was obtained for lucanthone (1), (ΔTm = 13.0°, Lit.[44] 11-14°) and N-methyllucanthone (82), (ΔTm = 3.5°, Lit.,[44] 3°).

Modification of the lucanthone (1) molecule by substituting a second diethylaminoethylamino group at the 8 position to form 1,8-di[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) resulted in a highly significant increase in ΔTm (ΔTm = 13.0° and 18.2°, respectively; P = 0.01, t = 26.99, d.f. = 76).

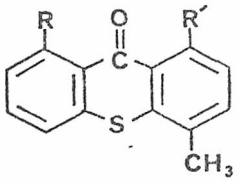
In the 1,8-disubstituted methylthioxanthenone series tested the results in Table 43 show a highly significant progressive decrease in ΔTm with compounds of increasing side chain length but with the same terminal ethyl groups [(165), ΔTm = 18.2°; (175), ΔTm = 10.4°; P = 0.01, t = 40.43, d.f. = 76; and (175), ΔTm = 10.4°; (176), ΔTm = 6.7°; P = 0.01, t = 18.92, d.f. = 76].

If the terminal alkyl groups are changed but the length of the side chain kept at two methylene groups between the two nitrogen atoms, then an increase in the alkyl group size from ethyl (165) to isopropyl (174) caused a highly significant decrease in ΔTm (ΔTm = 18.2° and 6.7°, respectively; P = 0.01, t = 56.77, d.f. = 76).

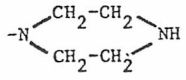
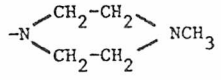
A decrease in the alkyl group size from ethyl (165) to methyl (173) gave an apparent increase in activity but this was not statistically significant (ΔTm = 18.2° and 18.9°, respectively; P = 0.01, t = 3.52, d.f. = 76). The primary amine (172) shows a decreased ΔTm compared with

Table 43. Effect of thioxanthenones on the heat denaturation profile of calf thymus DNA.

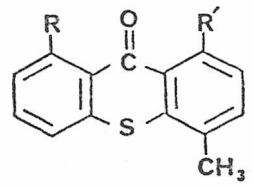
1,8-Disubstituted-4-methylthioxanthen-9-ones

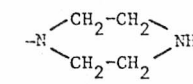
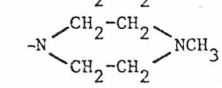


Mean Tm (calf thymus DNA) = 60.9° (std. dev. = 0.06)

No.	RR'	mean ΔTm°	std. dev.	no significant difference with compound No. :-
(172)	$-NH(CH_2)_2NH_2$	14.4	0.23	(179),(180)
(173)	$-NH(CH_2)_2N(CH_3)_2$	18.9	0.36	(165)
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	18.2	0.36	(173)
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	7.2	0.36	(176),(181)
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	10.4	0.23	(182)
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	6.7	0.16	(174),(181),(183)
(177)		15.7	0.30	
(178)		13.7	0.23	(166),(167),(180)

1-Substituted-5-methylthioxanthenon-9-ones



No.	R	R'	mean ΔTm°	std. dev.	no significant difference with compound No. :-
(179)	$-NH(CH_2)_2NH_2$	H	14.7	0.31	(172)
(180)	$-NH(CH_2)_2N(CH_3)_2$	H	14.0	0.24	(167),(172),(178)
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	H	13.6	0.29	(1),(166),(178),(180)
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	H	6.8	0.32	(174),(176),(183)
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	H	9.8	0.24	(175)
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	H	6.4	0.36	(176),(181)
(184)		H	8.9	0.57	(185)
(185)		H	8.2	0.29	(184)
(166)	$-NH(CH_2)_2N(C_2H_5)_2$	Cℓ	13.5	0.28	(1),(167),(178),(180)
(1)	lucanthone		13.0	0.16	(166),(167)
(82)	N-methyllucanthone		3.5	0.37	

* Multiple Student 't' test, 76 degrees of freedom, 1%, t = 3.65

the diethylamino analogue (165), (ΔTm = 14.4° and 18.2°, respectively; P = 0.01, t = 19.34, d.f. = 76).

Replacement of the entire side chain by piperazinyl groups (177) led to a significant decrease in ΔTm compared with the diethylamino-ethylamino compound (165), (ΔTm = 15.7° and 18.2°, respectively; P = 0.01, t = 13.03, d.f. 0 76) and introduction of N-methyl groups onto the piperazinyl rings (178) also caused a further decrease in ΔTm, (ΔTm = 13.7°).

In the monosubstituted methylthioxanthenone series the diethylamino-ethylamino compound (167) showed no significant difference in ΔTm from lucanthone (1), (ΔTm = 13.6° and 13.0°, respectively; P = 0.01, t = 3.31, d.f. = 76). Variation of the diethylaminoethylamino side chain resulted in a similar pattern of decreased activity to the disubstituted series; the diethylamino compounds with increased chain length had significantly decreased Tm's from the diethylaminoethylamino compound (167), [(167), ΔTm = 13.6°; (182), ΔTm = 9.8°; (183), ΔTm = 6.4°; P = 0.01, t = 17.48, d.f. = 76]. Increase in the size of the N-alkyl terminal groups from ethyl (167) to isopropyl (181) while keeping the side chain length at two methylene groups, also led to a significant decrease in ΔTm (ΔTm = 13.6° and 6.8°, respectively; P = 0.01, t = 35.16, d.f. = 76). There was no significant difference, however, between the diethyl compound (167) and the dimethyl compound (180), (ΔTm = 13.6° and 14.0°, respectively; P = 0.01, t = 1.86, d.f. = 76).

Unlike the disubstituted series the monosubstituted aminoethylamino compound (179) was slightly more active than the dimethylaminoethylamino compound (180), (ΔTm = 14.7° and 14.0°, respectively; P = 0.01, t = 3.72, d.f. = 76, a significant difference).

The piperazine compound (184) shows a highly significant decrease in

ΔTm compared with the diethylaminoethylamino compound (167), (ΔTm = 8.9° and 13.6°, respectively; P = 0.01, t = 24.30, d.f. = 76); however, there was no significant difference between compound (184) and its N-methyl analogue (185), (ΔTm = 8.9° and 8.2°, respectively; P = 0.01, t = 3.52, d.f. = 76).

The 8-chloro compound (166) showed no significant difference in ΔTm from lucanthone (1), (ΔTm = 13.5° and 13.0°, respectively; P = 0.01, t = 2.79, d.f. = 76).

EXPERIMENTAL

The buffer solution used contained disodium hydrogen phosphate (3.3×10^{-4} mol ℓ^{-1}), ethylenediamine tetra-acetic acid (EDTA, 1×10^{-4} mol ℓ^{-1}) and sodium chloride (3×10^{-3} mol ℓ^{-1}). The pH was adjusted to 7.0 by the addition of dilute hydrochloric acid.

The DNA was calf thymus Type 1, sodium salt, highly polymerised, No. D-1501, supplied by Sigma Ltd. A stock solution of the DNA containing 50 μg $m\ell^{-1}$ was prepared by gentle stirring for a period of 12 hours. The prepared solution was stored in the dark at 4°.

Stock solutions of the thioxanthenones (1 mg $m\ell^{-1}$) were prepared by dissolving the thioxanthenone in dilute hydrochloric acid and then neutralising the excess acid with sodium hydroxide solution (5%). All solutions were prepared fresh on the day of the experiment. The absorbances of the solutions were measured on a Unicam S.P. 1800 spectrophotometer and the temperature of the cell varied using a Unicam S.P. 870 cell holder assembly. The temperature drop between the thermostat and the cuvette was 0-6° over the range 25-100°. The temperature in the reference cell was monitored using a R.S. Th-B11 bead thermister mounted in the cuvette cap and immersed in the buffer solution. The thermister was calibrated over the range 20-100° using 0-50° and 50-100° mercury thermometers fitted in the sample cell and immersed in buffer solution. The cuvettes were quartz 3 cm^3 cells with a path length of 10 mm. Loss of water by evaporation was minimised by the use of lightly greased cell caps. Measurements of the absorbance of a solution of lucanthone in contact with the grease over a period of one hour revealed no change in absorbance.

The resistance of the thermister was measured on a Schlumberger

Solation digital ohmmeter 4440 (20Ω - 2MΩ) accurate to 1 in 1000Ω. Changes in absorbance were recorded on a potentiometric chart recorder.

The Tm of calf thymus DNA (25 μg $m\ell^{-1}$) was determined at 260 nm fixed wavelength, by initially measuring the absorbance of the solution at 25°, and then recording the heat denaturation profile on the chart recorder between 25° and 95°, during a one hour period. The thermister resistance was read at one minute intervals initially up to 50° and then at 25 second intervals until the absorbance became constant. An increase in absorbance for calf thymus DNA of 0.28 to 0.41 was observed (46% shift) giving an average value for Tm of 60.5° (s.d. 0.06, 5 determinations), (Lit., 56°,[41] 65°[54]).

The Tm of DNA in the presence of the thioxanthenones was determined by preparing a solution (100 mℓ) containing DNA (25 μg $m\ell^{-1}$) and thioxanthenone ($6x10^{-6}$ mol ℓ^{-1}) from the appropriate stock solutions. A sample was placed in the cuvette and the cell contents allowed to equilibrate for 10 minutes to allow binding to occur before measuring the absorbance of the solution at 25°. The heat denaturation profile was then recorded as before.

Changes in temperature over the range 25-95° were not found to affect the absorbance of the thioxanthenones.

A graph was plotted for each thioxanthenone of the ratio of absorbance at temperature T° to absorbance at 25° ($A_{T°}/A_{25°}$) against the thermister resistance representing temperature. The Tm values was calculated from this graph as the temperature at which 50% of the total hyperchromicity was observed. The degree of interaction of the compounds with DNA was expressed as ΔTm, i.e. the Tm observed with DNA (25 μg $m\ell^{-}$) in the presence of thioxanthenone ($6x10^{-6}$ mol ℓ^{-1}) minus the Tm obtained with DNA in the absence of thioxanthenone.

Five measurements were made for each thioxanthenone and the mean and standard error calculated and analysed using a multiple Student 't' test on a Dec System 22 computer. This programme (Edpack), based on the Duncan multiple F test[121] and Bartlett's Test of Homogenicity,[122] gives an analysis of variance and contrasts each of the respective treatment means giving the 5% and 1% values of significance for the 't' test. The analysis of variance confirmed that the 'within treatments' variances were due to chance and the 't' test provides information as to whether the mean ΔTm for each drug was significantly different from any other.

Part 2. Schistosomicidal Activity of 1,8-Dialkylaminoalkylamino-4-methylthioxanthen-9-ones and Related Compounds

Introduction

Schistosomiasis testing of new compounds in laboratory animals is usually performed in two stages. In the first one, the compounds are screened in a model usually involving S. mansoni infection in mice. Once a compound is found to display some schistosomicidal activity additional information is obtained from further preclinical tests in mice, hamsters, and monkeys.[123]

The selection of mammalian host, method of infection and the preferred route and regimen of medication used in schistosomicidal screening varies with the laboratory. A number of techniques for the in vivo evaluation of schistosomicidal compounds in rodents have been reviewed by Schubert,[124] Standen,[24] Pellegrino and Katz.[123,125] Yarinsky[126] suggests that a test system should be employed which reasonably mimics the situation in humans so that a compound can be evaluated for curative purposes.

In designing a screening programme attention must be given to the life cycle of the parasite. The disease is transmitted to man by contact with cercariae, one of the free-living water-borne stages of the life cycle, which may be male or female and have the rudiments of adult organs. The cercariae enter the primary host, man, through the skin and are transported to the liver by the circulatory system. After maturing in the liver the adult schistosomes migrate via the portal system to the mesenteric or vesical veins. During this period the schistosomes pair and the female worms produce large quantities of eggs which pass through the wall of the bladder or intestine into the urine

or faeces, depending on the species. Further development takes place, usually in fresh water, to produce ciliated larvae or miracidia which swim about until they make contact with their secondary host; the aquatic snails of the genera Biomphalaria (S. mansoni), Bulinus (S. haematobium) or Oncomelania (S. japonicum). The miracidia attach themselves to the surface of the snail and enter through the skin, whereupon they develop into mother sporocysts from which balls of cells are budded off. From these develop daughter sporocysts which migrate to the snails digestive gland where they grow and develop into the final larval stage or cercariae,[58,127] (Fig. 29).

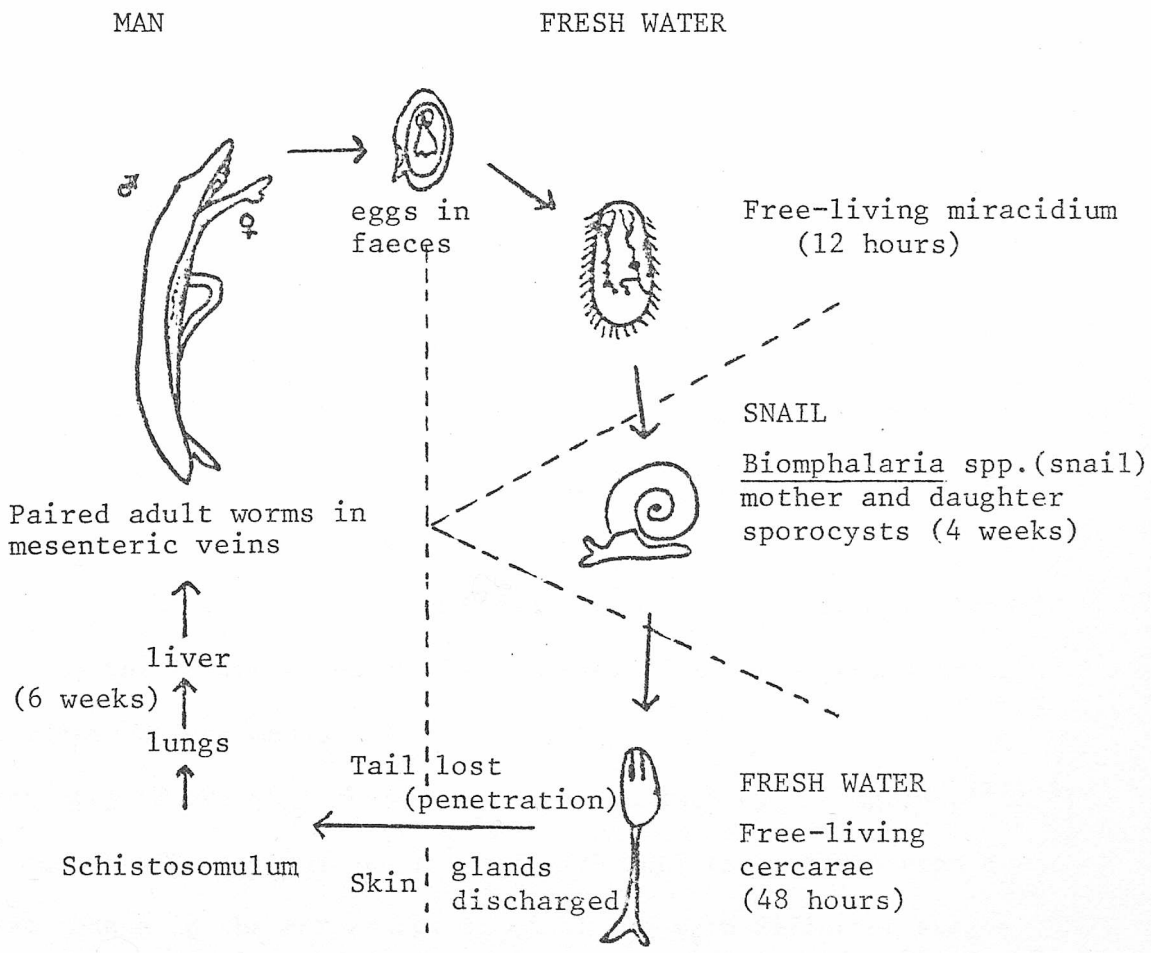


Fig. 29. Life cycle of Schistosoma mansoni

The stages of development which are particularly susceptible to chemotherapeutic attack are the young schistosomulum, the immature schistosomes, and the female worms during the deposition of eggs.[128]

The three schistosomes that are infective in man can be successfully maintained in the laboratory, however initial screening of chemical compounds in the Western World is usually directed against S. mansoni. If significant activity is obtained and confirmed, supplementary tests may be conducted with S. haematobium and S. japonicum.

The most commonly used mammalian host for initial screening has been the mouse, Mastomys natalensis[125] although the hamster, Cricetus auratus is also a suitable host for S. mansoni.[2] The mice are infected by the subcutaneous, percutaneous or intraperitoneal administration of cercariae. Yarinsky[126] has reported that the route of infection does not appear to influence the assessment of chemotherapeutic agents against adult worms residing in the mesenteric veins and hepatic portal system.

Test compounds may be administered orally by intubation or inclusion in the diet or parenterally by infection. The period of medication usually varies from five to ten days depending on the choice of the route of administration. The criteria for drug efficacy include the reduction in the number of eggs deposited in the tissues, increases in survival time of medicated animals, the hepatic shift of the schistosomes; reduction in live worm burden, and oögram changes.

The hepatic shift is a migration of the schistosomes from the hepatic portal vein to the liver caused by the presence of the test compound in the blood stream of the mouse.[129]

In the case of the oögram assessment, as devised by Pellegrino,[129,130] mice infected with S. mansoni and treated with schistosomicides show a progressive change in the percentage of viable eggs in different stages of

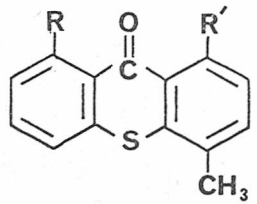
maturation in their intestinal walls. Normally, within a few weeks after the commencement of egg laying, a dynamic equilibrium occurs between eggs which are being laid and those which are continuously being eliminated in the faeces. The relative proportion of eggs at different stages of development found in intestine or liver fragments is defined as the oögram. Slight variations in the progressive development of the eggs is readily observed by this measurement. The first sign of activity of a schistosomicide generally consists of a simple increase in the percentage of mature eggs. When the activity of compounds is not pronounced or when active compounds are administered in very low dosage schedules, the oögram can return to normal within a few days.[126]

Results

The results of the *in vivo* evaluation of the 1,8-disubstituted and 1-substituted methylthioxanthenones against experimentally induced *S. mansoni* infection in mice are recorded in Tables 44 and 45.

The results show that the established optimum activity of a diethylaminoethylamino side chain on the thioxanthenone ring system is upheld when the compound contains two side chains at positions 1 and 8. 1,8-Di-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-ones (165) the most active member of the series, gave a 100% kill of schistosomes at 50 $mgkg^{-1}$ dose for 5 days and 86% kill at 25 $mgkg^{-1}$ for 5 days. All the mice survived the test, but yellow pigmentation of organs and skin was observed. This pigmentation was similar to that caused by anti-

Table 44. Activity of 1,8-di(dialkylaminoalkylamino)-4-methylthioxanthen-9-ones and related compounds against S. mansoni in the mouse



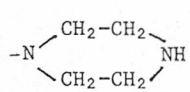
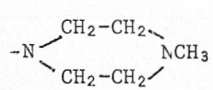
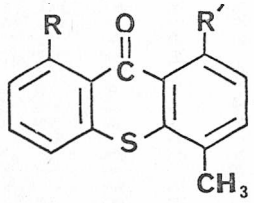
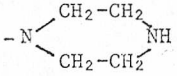
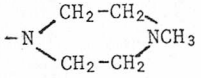
No.	RR'	Dose mg kg^{-1} b.i.d. x days	Total dose mg kg^{-1}	Post treatment to autopsy (days)	Survivors	% kill flukes	Oögram	Comments
(172)	$-NH(CH_2)_2NH_2$	50 x 5	500	14	4/5	0	++ 2 mice	No staining or pigmentation of organs
(173)	$-NH(CH_2)_2N(CH_3)_2$	50 x 5	500	14	1/5	38	N	Yellow pigmentation of gut, liver, kidneys, ears and feet. Necrosis of liver
		25 x 5	250	14	4/5	0	++ 2 mice N 2 mice	Yellow pigmentation of gut, liver, lymphatic glands, ears, and feet. No liver necrosis
(165)	$-NH(CH_2)_2N(C_2H_5)_2$	50 x 5	500	14	5/5	100	++++	Yellow pigmentation of gut, liver, kidneys, ears, and feet, lymphatic glands.
		25 x 5	250	14	5/5	86	++++ 4 mice ++ 1 mouse	As above but kidney not affected
(174)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	50 x 5	500	14	5/5	4	++ 3 mice N 2 mice	Yellow pigmentation of liver, lymphatic glands and ears
(175)	$-NH(CH_2)_3N(C_2H_5)_2$	50 x 5	500	14	4/5	8	++	Yellow pigmentation of gut only. V. faint pigmentation of ears and feet
(176)	$-NH(CH_2)_4N(C_2H_5)_2$	50 x 5	500	14	4/5	0	++ 2 mice N 2 mice	Yellow pigmentation of liver, lymphatic glands and ears
(177)		50 x 5	500	14	3/5	0	N	No colouration
(178)		50 x 5	500	14	1/5	0	N	No colouration

Table 45. Activity of 1-dialkylaminoalkylamino-5-methylthioxanthen-9-ones and related compounds against S. mansoni in the mouse.



No.	R	R'	Dose mg kg^{-1} b.i.d. x days	Total dose mg kg^{-1}	Post treatment to autopsy days	Survivors	% Kill flukes	Ögram	Comment
(179)	$-NH(CH_2)_2NH_2$	-H	50 x 5	500	14	4/5	0	N	No colouration
(180)	$-NH(CH_2)_2N(CH_3)_2$	-H	50 x 5	500	14	3/5	0	N	No colouration
(167)	$-NH(CH_2)_2N(C_2H_5)_2$	-H	50 x 5	500	14	4/5	9	N	No colouration
(181)	$-NH(CH_2)_2N(CH(CH_3)_2)_2$	-H	50 x 5	500	14	2/5	0	N	No colouration
(182)	$-NH(CH_2)_3N(C_2H_5)_2$	-H	50 x 5	500	14	5/5	0	N	No colouration
(183)	$-NH(CH_2)_4N(C_2H_5)_2$	-H	50 x 5	500	14	5/5	0	N	No colouration
(184)		-H	50 x 5	500	14	4/5	0	N	No colouration
(185)		-H	50 x 5	500	14	5/5	0	N	No colouration
(166)	$-NH(CH_2)_2N(C_2H_5)_2$	-Cℓ	50 x 5	500	14	5/5	0	N	No colouration
(1) (lucanthone)	-H	$-NH(CH_2)_2N(C_2H_5)_2$	50 x 5	500	14	5/5	41	++++	No colouration
			25 x 5	250	14	5/5	27	++	No colouration

malarial compounds such as mepacrine (3).

Schistosomicidal activity falls off when the character of the diethylaminoethylamino side chain is varied; thus an increase in the number of methylene groups between the two nitrogens, (175) and (176), reduced activity. The same effect was observed by Archer[15] in the lucanthone series. Similarly variation of the substituents on the terminal nitrogen reduced activity; the primary amino compound (172) was virtually inactive, non-toxic and caused no staining or pigmentation of the organs at the dose employed in the test. The dimethyl compound (173), at a dose of 50 $mgkg^{-1}$ b.i.d. x 5 days orally, killed 38% of the schistosomes and was extremely toxic killing four out of the five mice used in the test; the value of 38% is therefore statistically suspect under these circumstances. However, at a dose of 25 $mgkg^{-1}$ b.i.d. x 5 days, no schistosomes were killed and 80% of the mice survived, compared with the diethyl compound (165) at the same dosage which gave an 86% kill of schistosomes and was not lethal to the mice employed in the test. At the higher dose the dimethyl compound (173) caused intense yellow staining and pigmentation of the gut, liver, kidneys, lymphatic glands, ears and feet and severe necrosis of the liver. At the reduced dosage no liver necrosis was observed, however the yellow pigmentation of the organs was still present.

The introduction of diisopropyl terminal groups (174) produced a further reduction in schistosomicidal activity. In this case all of the animals survived the test but pigmentation of the liver, lymphatic glands and ears was observed.

Replacement of the dialkylaminoalkylamino side chains by piperazinyl groups, (177) and (178), abolished schistosomicidal activity completely. Both compounds (177) and (178) showed toxicity causing the death of 40%

and 80% of the mice respectively, but no pigmentation of the organs was observed. In the monosubstituted series only the diethyl compound (167) showed schistosomicidal activity but one of the five mice did not survive the test. The dimethylaminoethylamino and diisopropylaminoethylamino compounds (180) and (181) were more toxic killing 40% and 60% of the mice respectively. The piperazinyl compounds (184) and (185) showed no schistosomicidal activity and were not as toxic to the mice as their 1,8-disubstituted analogues, (177) and (178).

The 8-chloro compound (166) showed no activity against schistosomes at the 50 $mgkg^{-1}$ b.i.d. dosage level, nor did it show lethal toxicity to the mice, unlike its deschloro analogue (167).

EXPERIMENTAL

The *in vivo* screening programme of the synthesised thioxanthenones against experimental *S. mansoni* infection in mice was undertaken by Mr. G. Dickerson of the Parasitology Unit of the Wellcome Research Laboratories, under the direction of Mr. J.E.D. Keeling.

S. mansoni (Egyptian strain) maintained in the laboratory by the technique of Standen[24] was used for the screening of the compounds.

The snails, *Biomphalaria glabrata*, were reared in aquaria maintained at 23-28° containing purified water and including the aquatic weed *Valisneria spiralis* which provided aeration and suitable egg laying sites for the snails.

Lettuce was supplied as food and *Daphnia sp.* added to control excessive harmful microflora and fauna. Snail faeces and decaying vegetable materials were removed by oligochaete worms such as *Tubifex sp.* present in the sand at the bottom of the aquaria. Artificial incandescent light was provided for about 12 hours per day.

Such aquaria supported about fifty adult snails or several hundred juveniles. Miracidia were obtained from infected mice faeces which contained schistosome eggs. Mass infection was carried out on 50-100 snails with 15 miracidia per snail, showing 100% infection after 6 weeks. Cercariae for infection of animals were obtained by transference of infected snails to shallow containers and stimulation with intense light and temporarily raising the temperature to 28-30°. Exposure for 1.5 to 2 hours was usually sufficient to obtain an adequate suspension of cercariae after which the snails were returned to their aquaria.

For the infection of the mice the cercariae were administered by percutaneous exposure. Mice, 3-4 weeks old, were allowed to run in 1.25 cm

of water to stimulate voidance of faeces and urine and were then placed in wide mouth glass jars and the cercarial suspension introduced by a spring-loaded syringe, allowing 130-150 cercariae per mouse. After exposure to the cercariae for 20 minutes the mice were removed and dried in warm wood-wool to prevent death through exposure to water.

An average of 10-20 worms per mouse developed from this infection and provided a convenient number for chemotherpaeutic work. The animals were not used for nine weeks after exposure to the cercariae to allow the infection to develop.

Compounds were given by stomach tube, 50 $mgkg^{-1}$, twice daily for 5 days and five mice per test were used in the primary screen. No preliminary toxicity test was carried out.

The mice were killed fourteen days after the last dose by cervical dislocation. The autopsy involved counts of live and dead worms in mesenteric, portal and intrahepatic veins. The worms were counted as they were removed from the former two sites and the entire liver was then removed and crushed between two rectangular glass plates and examined under a dissecting microscope for both living and dead schistosomes. The male and female worms were identified and counted and note taken of the proportion of worms paired and the proportion of worms ensheathed in inflammatory tissue. Immobilisation of worms by inflammatory tissue may occur as early as 3-4 days after hepatic shift. Too long a period before autopsy leads to phagocytosis occurring so that dead worms may be unrecognisable.

The oögram was also determined for each test using the following scoring assignment:

N = normal egg laying and development pattern.

++ = reduction in egg laying; very few 1st stage eggs, mainly later immature stages of mature eggs.

+++ = no new eggs, older and mature eggs only.

++++ = no egg laying, mature and dead eggs only.

Infected, non-medicated animals were included in the screening programme to assure that deaths of schistosomes in the treated animals was due to the medications and that deaths of animals was not due to the infection.

DISCUSSION

Discussion

The synthesis of 1,8-dichloro-4-methylthioxanthen-9-one (136) has been undertaken by two distinct routes and the resulting compound used to prepare a series of 1,8-di(dialkylaminoalkylamino)- and 1,8-dipiperazinyl-4-methylthioxanthen-9-ones. The biological activity, *in vitro* and *in vivo*, of these compounds and a series of related monosubstituted dialkylaminoalkylamino and piperazinyl methylthioxanthenones have been examined.

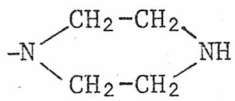
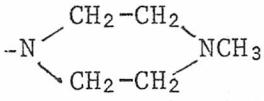
Results in the *in vitro* studies on the heat denaturation profile of native DNA (p. 165) have confirmed the reported activity of lucanthone (1) in stabilising the DNA helix, (ΔTm = 13.0°; Lit.[44] 11-14°); however the results also demonstrated that activity was retained when the diethylaminoethylamino side chain was not in the *para* position to the methyl group [(167), ΔTm = 13.6°]. Hirschberg *et al.*[44] have shown that desmethyllucanthone (63) had a lower ΔTm value than lucanthone (1), (ΔTm = 9° and 13°, respectively). Therefore these results would suggest that a ring methyl group is required for increased heat stabilisation of the DNA, but not necessarily in the position *para* to the basic side chain.

When the dimensions of the diethylaminoethylamino side chain are changed by lengthening, as in compounds (182) and (183), or the size of the substituents on the terminal nitrogen are increased (181) then the ΔTm value is decreased.

Similarly replacement of the entire side chain by piperazinyl groups as in compounds (184) and (185) leads to a decreased ΔTm compared with its diethylaminoethylamino analogue (167).

Table 46 shows a comparison of the ΔTm results of the 1,8-series of compounds with their monosubstituted analogues.

Table 46. Comparison of the ΔTm results for the 1,8-disubstituted methylthioxanthenones with their monosubstituted analogues.

Side chain	disubstituted No.	disubstituted mean ΔTm°	monosubstituted No.	monosubstituted mean ΔTm°	't'	significant difference
$-HN(CH_2)_2NH_2$	(172)	14.4	(179)	14.7	1.24	n.s.
$-HN(CH_2)_2N(CH_3)_2$	(173)	18.9	(180)	14.0	25.34	s.
$-HN(CH_2)_2N(C_2H_5)_2$	(165)	18.2	(167)	13.6	23.68	s.
$-HN(CH_2)_2N(CH(CH_3)_2$	(174)	7.2	(181)	6.8	2.07	n.s.
$-HN(CH_2)_3N(C_2H_5)_2$	(175)	10.4	(182)	9.8	2.90	n.s.
$-HN(CH_2)_4N(C_2H_5)_2$	(176)	6.7	(183)	6.4	1.45	n.s.
	(177)	15.7	(184)	8.9	34.95	s.
	(178)	13.7	(185)	8.2	28.23	s.

n.s. = not significant; s = significant difference

Significance for Students 't' (0.01, 76 d.f.) = 3.65

(two-tailed test)

The results in Table 46 show no significant difference between mono and disubstitution when the side chains are aminoethylamino (172) and (179), diisopropylaminoethylamino (174) and (181), and diethylaminobutylamino, (176) and (183); however, disubstitution does enhance the stabilisation of the DNA to heat denaturation when the side chain is dimethylaminoethylamino (173), diethylaminoethylamino (165), dipiperazinyl (177) or di(methyl-piperazinyl) (178). Thus the proposed hypothesis (p. 60) that 1,8-disubstitution in the thioxanthenones could lead to enhanced activity over

the 1-substituted methylthioxanthenones was found to be true in the heat denaturation system for the latter four types of compound.

These results suggest that an optimum length and size of side chain exists for DNA interaction and the introduction of a second identical side chain into the thioxanthenone ring at the 8 position leads to an increased ΔTm; however, if the nature of the side chain is not close to the optimal requirements then the introduction of a second side chain will not increase the ΔTm, as shown in the results for compounds (172), (174), (175) and (176).

The models proposed by Hirschberg *et al.*[44] and Carchman *et al.*[56] for the interaction of lucanthone (1) with DNA have already been discussed (p. 46). Heller, Tu and Maciel[131] have recently reported n.m.r. spectroscopy studies on the interaction of lucanthone (1) with double stranded poly(adenylic acid)-poly(uridylic acid). Their results suggest that the best fit for lucanthone (1) into the double-helical structure of the nucleic acid is when the thioxanthenone ring is inserted between the base pairs in such a way that the diethylaminoethylamino side chain extends from the major groove side of the double helix, with the 4 methyl group pointing out of the minor groove side. This orientation allows the intercalation of the thioxanthenone ring system with the stacked base pairs and also brings the terminal nitrogen on the lucanthone (1) side chain into proximity with the ionised oxygen atoms of the phosphate group.

Fundamental to this model is a knowledge of the ionisation state of the nitrogen atoms of the side chain of lucanthone (1). The tertiary amine group has a pKa of 8.25[104] and therefore at pH 7.0 some electrostatic interaction would be expected. Zilversmit[64] reports a pKa of -0.20±0.05 for the proximal nitrogen (Munro[104] pKa 3.40)[(a)] indicating that

(a) Determination carried out in ethanol-water 1:1

little electrostatic interaction with this nitrogen would be expected.

Hirschberg et al.[44] suggests that alkyl substitution of the proximal nitrogen in lucanthone (1) would be expected to alter the basicity of this nitrogen, which would account for the diminished activity in the DNA system of N-methyllucanthone (82). However, the pKa value of 3.41±0.04[64] for the proximal nitrogen of N-methyllucanthone (82) does not support this argument.

Elslager et al.[103] attributed the lower basicity of the secondary amino group in lucanthone (1) compared with the tertiary proximal nitrogen of N-methyllucanthone (82) to hydrogen bonding; however, Peters and Sumner[132] determined the influence of hydrogen bonding on the basic ionisation constants (Kb) of a series of amino, methylamino and dimethylamino anthraquinones and their results indicated that intramolecular hydrogen bonds affect Kb by a factor of ten or less. The basic ionisation constant of 1-dimethylaminoanthraquinone, in which the steric configuration is similar to that of N-methyllucanthone (82), shows a thousandfold increase over unhindered derivatives. Zilversmit[64] concludes that the enhanced basicity of N-methyllucanthone (82) compared to lucanthone (1) is caused by a steric effect preventing the lone pair of electrons of the proximal nitrogen from interacting with the π-electron cloud of the ring. He infers from this that coplanarity of the thioxanthenone ring and the amino side chain may be required for optimal biological activity.

N.m.r. studies show that the chemical shift of the aromatic proton ortho to the amino side chain in lucanthone (1) can give an indication of the degree of shielding of these protons by the lone pair of electrons of the proximal nitrogen of the side chain (p.114). In lucanthone (1) the ortho proton resonates at δ6.6 (H-2) but in N-methyllucanthone (82),

the H-2 resonance occurs at δ6.9 suggesting that the lone pair of electrons on the proximal nitrogen are not able to interact with the π-electron cloud of the aromatic ring to the same extent as in lucanthone (1). This data supports Zilversmit's conclusion[64] that the side chain and thioxanthenone ring of N-methyllucanthone (82) are non-planar.

Similarly in the piperazinyl compounds (184) and (185) the proton ortho to the piperazine ring resonates at δ7.00 and 6.98 respectively, indicating that the side chains in these molecules are non-planar with the thioxanthenone ring.

Since compounds (184) and (185) exhibit a reduced interaction with DNA (ΔTm = 8.9° and 8.2°, respectively), intermediate between lucanthone (1) (ΔTm = 13.0°) and N-methyllucanthone (82) (ΔTm = 3.5°), this indicates that lack of coplanarity is not the only factor in reducing the interaction of N-methyllucanthone (82) with DNA. Thus other steric factors associated with the N-methyl group of N-methyllucanthone (82) may play an important role in reducing the interaction.

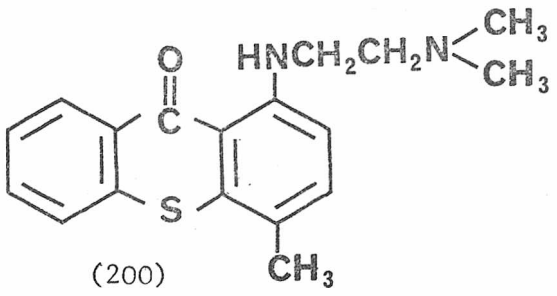
The diminished activity of the piperazinyl compounds (184) and (185) compared with lucanthone (1) may be partly accounted for by the shorter side chains of the piperazinylthioxanthenones. A determination of the distance between the nitrogen atoms in the chair conformation of piperazine (192), measured from an atomic model based on calculations by Schwarzenbach,[133] is 0.30 nm. In the more flexible diethylaminoethylamino side chain of lucanthone (1) and its analogues, the maximum distance between nitrogens in a planar molecule with orientation to provide a maximum separation between the two nitrogens, is 0.40 nm. In addition, the absence of a hydrogen atom on the proximal nitrogen would reduce the interaction by hydrogen bonding of these compounds with the base pairs of the DNA as suggested by the Hirschberg model (p. 46).

In the 1,8-series the diethylaminoethylamino compound (165) has the capability of intercalating with DNA in a similar manner to the model proposed by Heller, Tu and Maciel[131] for lucanthone (1). The thioxanthenone ring system and proximal amino groups can interact with the base pairs while the side chains extend from the major groove side of the DNA permitting electrostatic interaction of the two terminal nitrogen atoms with a phosphate grouping on each strand of the helix. In this way an interstrand bridge is created and this mechanism would account for the increased stabilisation of the DNA to heat denaturation produced by the 1,8-di(diethylaminoethylamino)- and 1,8-di(dimethylaminoethylamino)-4-methylthioxanthen-9-ones, (165) and (173), which have ΔTm values of the same order as mepacrine (3), (pKa 7.9 and 10.4[134]).

In the diethylaminopropylamino and diethylaminobutylamino compounds (175) and (176) where these longer side chains extend beyond the phosphate chains the degree of interaction is reduced. This major involvement of the terminal nitrogen would also explain the low activity of the diisopropyl compound (174) which would be more sterically hindered than the corresponding diethyl compound (165).

Hirschberg et al.[44] have found that features in the lucanthone series which favoured complexing with DNA, and inhibition of bacterial growth were also shown to be favourable in carcinostasis. Blanz and French[39] have studied the activity of lucanthone (1) against a variety of transplantable mouse tumours. They discovered that the structure-activity of the lucanthone series required a two carbon chain between the two nitrogens of the side chain with small terminal alkyl groups. The dimethylaminoethylamino side chain was found to be optimal and 1-[[2-(dimethylamino)ethyl]amino]-4-methylthioxanthen-9-one (200) was significantly

more active against tumours than lucanthone (1). An increase in life



(200)

span of leukaemic mice of up to 100%, and under optimal conditions, complete inhibition of growth of Adenocarcinoma 755, was observed during the test period. Other structural requirements were an intact thioxanthen-9-one ring, bearing a side chain containing a free hydrogen on the proximal nitrogen; and also a structurally small group, such as methyl, was required at position 4.

On these criteria our 1,8-disubstituted methylthioxanthenones should be of interest in cancer chemotherapy, and in particular 1,8-di[[2-(dimethylamino)ethyl]amino]-4-methylthioxanthen-9-one (173).

Hirschberg et al.[44] state that antitumour activity in mouse leukaemia L1210 requires the conversion of lucanthone (1) to an as yet unidentified carcinostatic metabolite. The mechanism of action for the selective toxicity of this metabolite may be completely different from that giving the in vitro results of the parent compound.

Both hycanthone (66) and lucanthone (1) have been found to be mutagenic in the E. coli T4 bacteriophage system; however, hycanthone (66) is 18-fold more mutagenic than lucanthone (1) in the Salmonella system.[135] The likely molecular features for mutagenicity in these compounds are the aromatic amino group and the substituent at position 4 of the ring.

Carcinogenicity of aromatic amines and amides is believed to depend on metabolic activation, N-hydroxylation being generally regarded as the

first step.[136] In some cases the inability of an animal species to N-hydroxylate a sufficient amount of the amine appears to be a crucial factor which prevents the amine from being a carcinogen in that species.[137]

Studies with 2-acetylaminofluorene and a hepatocarcinogenic amino-azo dye (MAB) suggest that the metabolic activation of the N-hydroxyl compound depends on esterification of the N-hydroxyl group.[138] The esters of N-hydroxy-2-acetylaminofluorene were shown to be very potent in initiating mutations and in inactivating transforming DNA from wild type Bacillus subtilis.[139]

In all the cases studied the electrophilic reactivities of the esters paralleled their inactivating and mutagenic effects on the transforming DNA; however this may not necessarily relate to their carcinogenic activities.[140] Miller suggests that most of the chemical carcinogens are either strong electrophilic reactants as administered or are converted in vivo into potent electrophilic reactants. It is presumed that these electrophilic reactants then initiate the carcinogenic process through certain of their reactions with nucleophiles in crucial tissue components, such as nucleic acids and proteins. This process would explain the low mutagenic activity of N-methyllucanthone (82) because of the inability to become N-hydroxylated and would also suggest that the piperazine substituted thioxanthenones would be likely to have reduced mutagenic activity; however, it would not explain the increased mutagenicity of hycanthone (66) over lucanthone (1) in the Salmonella system.

An alternative hypothesis for the mutagenic and carcinogenic activity of hycanthone (66) has been put forward by Hubert and Miller[141] who report that hycanthone (66) is an alkylating agent, since hycanthone (66) reacts

with 4-(4-nitrobenzyl)-pyridine (NBP), a reagent frequently used in the estimation of alkylating agents. The attack has been shown to occur at the hydroxymethyl group of hycanthone (66), thus implicating this chemical grouping in the biochemical mechanism of toxicity, and this may explain why hycanthone (66) is a far greater mutagen than lucanthone (1) in some systems.

Mildly acidic conditions are necessary for the hydroxymethyl group to become protonated and this renders it a better leaving group, Fig. 30.

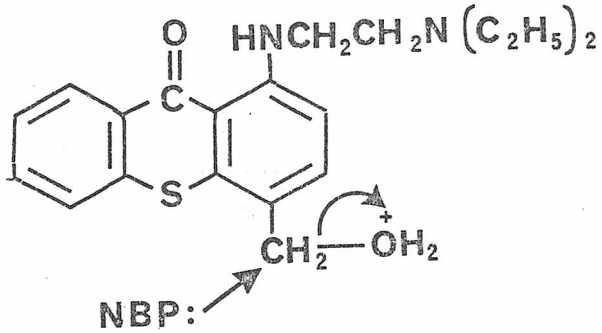


Fig. 30

Hycanthone acetate reacts with NBP at physiological pH and temperature with elimination of the acetate, and this ester is viewed as a model substance for the <u>in vivo</u> "reactive ester metabolite" of hycanthone (66). An hypothesis was made by Hulbert and Miller[141] that hycanthone (66) is converted <u>in vivo</u> into a potent alkylating agent such as a reactive ester. This is in accordance with current theories of chemical carcinogenesis in that chemical carcinogens act by being metabolised to electrophiles which then react to form covalent linkages with nucleophilic groups present in DNA.[47,140]

This latter hypothesis would better fit the findings of Strauss[41] (Table 17, p. 35) who showed that whilst 1-[1-methylpiperazinyl)]-4-

methylthioxanthen-9-one (54) had no detected mutagenicity its 4-hydroxymethyl analogue (90) was active. Similarly the 4-acetate derivative of lucanthone (64) was highly mutagenic. However, the fact that terminal nitrogen oxidation in lucanthone (1), hycanthone (66) and the benzothiopyranoindazoles significantly reduces mutagenicity (Table 20, p. 51) suggests that total mechanism of mutagenicity in these compounds may involve both the amino side chain and the substituent at the 4 position.

A recent World Health Organisation sponsored investigation[142] has suggested however, that the *in vitro* mutagenicity tests do not give a realistic indication of potential *in vivo* mutagenicity in the lucanthone series and thus the dangers associated with the use of these compounds may have been overemphasised. Extensive tests on transmitted genetic effects of hycanthone (66) in mammals have shown little mutagenic hazard even at dosages much higher than the clinical level. In a few cases where the results were definitely positive, there were none where the magnitude of the effect estimated at the clinical level reached as high as 10% of the spontaneous mutation rate or 10% of the frequency controls. This level has been suggested as an acceptable genetic risk for an efficaceaus schistosomicide.[142]

The results of the evaluation of the 1,8-disubstituted and 1-substituted alkylaminoalkylamino and piperazinyl methylthioxanthenones against experimentally induced *S. mansoni* infection in mice (p. 175) show that the optimal activity of the diethylaminoethylamino side chain is still apparent, even when disubstituted as shown in compound (165). This compound was found to be three to four times as active against schisosomes as lucanthone (1) at the oral dose employed in the tests. When the character of this side chain is altered, by increasing the chain length or by decreasing the size of the terminal groups, activity is

reduced or lost. Even the least active members of the 1,8-series had a slight effect on the egg laying capacity of the schistosomes, although only compound (165) completely prevented egg laying. Lucanthone (1) was also found to prevent egg laying in the test and did not cause the severe yellow pigmentation of the organs and skin of the treated mice. This feature of the 1,8-series indicates the tissue binding character of these compounds. The only exception being the primary amine (172) which produced no pigmentation of the organs or skin.

Only the diethylaminoethylamino compound (167) showed any schistosomicidal activity at the dose employed in the monosubstituted methylthioxanthenone series. Gönnert[43] reported that in the lucanthone series the 4-methyl group was essential for activity. This is consistant with the fact that lucanthone (1) is metabolically transformed to the active metabolite, hycanthone (66), thus establishing a biochemical basis for the necessity of the 4-methyl group. Archer and Yarinsky[2] report that desmethyllucanthone (63) showed no schistosomicidal activity in mice at a dosage of 400 mg kg^{-1} orally. The present results suggest that the methyl group need not be <u>para</u> to the amino side chain for schistosomicidal activity although improved activity is obtained when the two groups are situated in the 1 and 4 positions as in lucanthone (1).

The introduction of a chlorine atom <u>para</u> to the methyl group in compound (166) abolished schistosomicidal activity, suggesting that the nature of the substituents at the 1 and 8 positions is critical to activity.

In the other monosubstituted methylthioxanthenones variation of the length of the side chain and the nature of the terminal groups from diethylaminoethylamino led to loss of detectable schistosomicidal activity at the 50 mg kg^{-1} dose level. However, a similar gradation of activity

as seen in the 1,8-series may become apparent if the dose level of the order of that used in the testing of desmethyllucanthone (63)[2] is employed.

The results of the *in vivo* test also indicate that the structure-activity requirements for schistosomicidal activity and DNA heat denaturation stabilisation are different. Desmethyllucanthone (63) showed no activity against *S. mansoni* in mice yet like the monosubstituted methylthioxanthenone it does stabilise DNA to heat denaturation (p. 45). Similarly the piperazinyl compounds (184) and (185), which have been shown to interact with DNA, have no demonstrable activity against *S. mansoni* in mice. Further, 1-[1-(4-methylpiperazinyl)]-5-methylthioxanthen-9-one (185) shows similar activity in interacting with DNA to 1-[1-(4-methylpiperazinyl)]-4-methylthioxanthen-9-one (54) yet it was inactive against *S. mansoni* infection in mice at a dose level approximately equal to half that known to produce 100% kill by the latter compound (p. 20).[21]

The discovery of high schistosomicidal activity in 1,8-[[2-(diethylamino)ethyl]amino]-4-methylthioxanthen-9-one (165) has confirmed the basic hypothesis of this study that 1,8-disubstitution in the thioxanthenone molecule will lead to enhanced biological activity; this activity being demonstrated in two biological test systems.

The results from the DNA studies would indicate that the 1,8-di-(diethyl) compound (165) and the 1,8-di(dimethyl) compound (173) are strongly bound to DNA *in vitro* and the correlation between ΔTm and mutagenic activity put forward by Hirschberg *et al.*[44] would indicate that these compounds may also be mutagenic.

The work of Bueding[143] has shown that mutagenicity of lucanthone (1)

and hycanthone (66) can be dissociated from schistosomicidal activity by N-oxidation of the terminal amino group of the side chain. These compounds had negligible mutagenic activity *in vitro* while exhibiting the same or enhanced schistosomicidal activity. The possibility therefore exists of reducing any mutagenicity which may be demonstrated for compounds in the 1,8-series by the formation of di-N-oxides.

In addition, the formation of the 4-hydroxymethyl derivative of compound (165) poses an interesting future development for this work as enhanced schistosomicidal activities have been demonstrated for hydroxymethyl derivatives of thioxanthenones over their parent 4 methyl analogues.[2]

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Appendix 1

The following advanced studies in connection with the programme of work have been undertaken.

A course in organic spectroscopy in the Chemistry Department, University of Aberdeen has been completed together with attendance at post-graduate research seminars in that department.

I have participated in a series of research seminars held in the School of Pharmacy, Aberdeen and have given a lecture on recent advances in the chemotherapy of schistosomiasis in the Medical School, University of Aberdeen.

A continuous and comprehensive survey of the chemical and biological literature relative to the project has been made during the period and I have attended seminars and lectures relevant to the project in the Departments of Pharmacology, Zoology and Chemistry of the University of Aberdeen.

Finally, a visit has been made to the Wellcome Research Laboratories, Beckenham to attain first-hand knowledge of schistosome screening techniques and to gain information on the chemistry of the lucanthone-type compounds.