

the elimination of MeOH and the formation of ( $\pm$ )-12-HCl in the NMR spectrum. Anal. ( $C_{21}H_{24}ClN$ ) C, H, N.

A sample of this salt was treated with aqueous  $NaHCO_3$  and extracted with  $Et_2O$ . The  $Et_2O$  solution was dried over anhydrous  $MgSO_4$  and 1.0 mL of  $CH_3I$  was added. The reaction mixture was allowed to stand for 1 h at 21 °C and evaporated to dryness on a rotary evaporator (the NMR spectrum was consistent with the methyl iodide salt derived from structure 12). This salt was refluxed in ethanolic KOH for 30 min. The reaction mixture was then added to  $H_2O$  and extracted with pentane. The pentane layer was dried and evaporated to an oily residue. The NMR and IR spectra of this residue are identical with that of an authentic sample of 1-phenyl-naphthalene.<sup>28</sup>

(28) C. J. Pouchert and J. R. Cambell, "Aldrich Library of NMR Spectra", Vol 4, Aldrich Chemical Co., Milwaukee, WI, 1974, p 28C.

*cis*-3-[*N*-(Cyclopropylmethyl)-*N*-methylamino]-1-phenyltetralin Hydrochloride (13b-HCl). Hydrogenation of a sample of 12-HCl (0.1 g) with 5% Pd/C in EtOH and workup by evaporation, conversion to the free amine with 5%  $NaHCO_3$ , and  $Et_2O$  extraction gave an oil which would not crystallize. The NMR spectrum indicated an approximate 9:1 mixture of 13b/13a. This oil was dissolved in  $Et_2O$ , and HCl gas was passed into the flask. The resulting precipitate was recrystallized from ether-ethanol to give 0.06 g (60%) of 13b-HCl: mp 152-154 °C; IR and NMR spectra differed from those of ( $\pm$ )-13a (see chemistry section for details); EIMS,  $m/e$  291 ( $M^+$ ).

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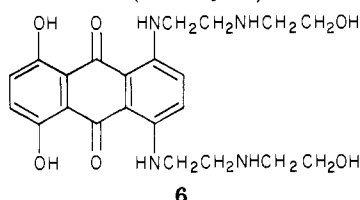
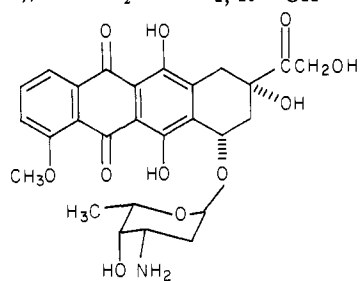
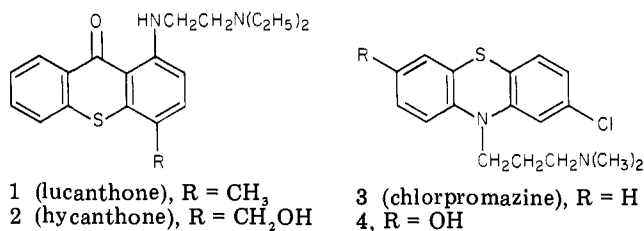
## Ring-Hydroxylated Analogues of Lucanthone as Antitumor Agents

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A series of ring-alkoxylated and ring-hydroxylated analogues of lucanthone was prepared and tested for antitumor activity. The most biologically interesting members of this group were the 7-hydroxylucanthone derivatives, 50 and 51, which gave T/C values in the NCI P-388 antitumor screen of 188 and 265, respectively. The apparent association constants and  $\Delta T_m$  values for a number of analogue-DNA complexes were determined to ascertain whether there was any quantitative correlation with biological activity. The most that can be said is that intercalation may be a necessary but far from sufficient condition for antitumor activity.

Several years ago Hirschberg reported that the antitumor activity of lucanthone (1) in L 1210 mice was abolished



by pretreatment of the animals with the mixed-function oxidase inhibitor SKF-525A.<sup>1</sup> This result indicated that

biotransformation of 1 was required for in vivo antitumor activity. Several analogues of lucanthone were tested as antitumor agents, but none showed interesting activity. Some years ago it was reported that the active metabolite of lucanthone in schistosomiasis was the hydroxylated derivative, hycanthone (2).<sup>2</sup> This compound is an antitumor agent also, but, like lucanthone, the antitumor activity of 2 in L1210 mice was also abolished by pretreatment of the animals with SKF-525A.<sup>1</sup> The identity of the mysterious antitumor biotransformation product of lucanthone remains unknown to this day.

Chlorpromazine (3) bears a structural resemblance to lucanthone in the sense that both drugs have tricyclic aromatic systems, the middle ring of which contains divalent sulfur and a dialkylaminoalkyl group attached directly to one of the ring atoms. The metabolism of 3 has been well studied. Just as in the case of 1, the corresponding sulfoxide is a metabolite and another major metabolite is the 7-hydroxy derivative (4).<sup>3</sup> It has been shown that lucanthone (1) and hycanthone (2) intercalate into DNA.<sup>4</sup> Many naturally occurring intercalating antitumor agents, such as dactinomycin and adriamycin (5),<sup>5</sup> as well as synthetic anthraquinones, such as 6,<sup>6</sup> have oxygen substituents on their planar (or nearly planar) polycyclic ring systems. Ring hydroxylation increased the

(1) E. Hirschberg, "Antibiotics", Vol. III, J. W. Corcoran and F. E. Hahn, Eds., Springer-Verlag, Berlin and Heidelberg, 1974, p 274.

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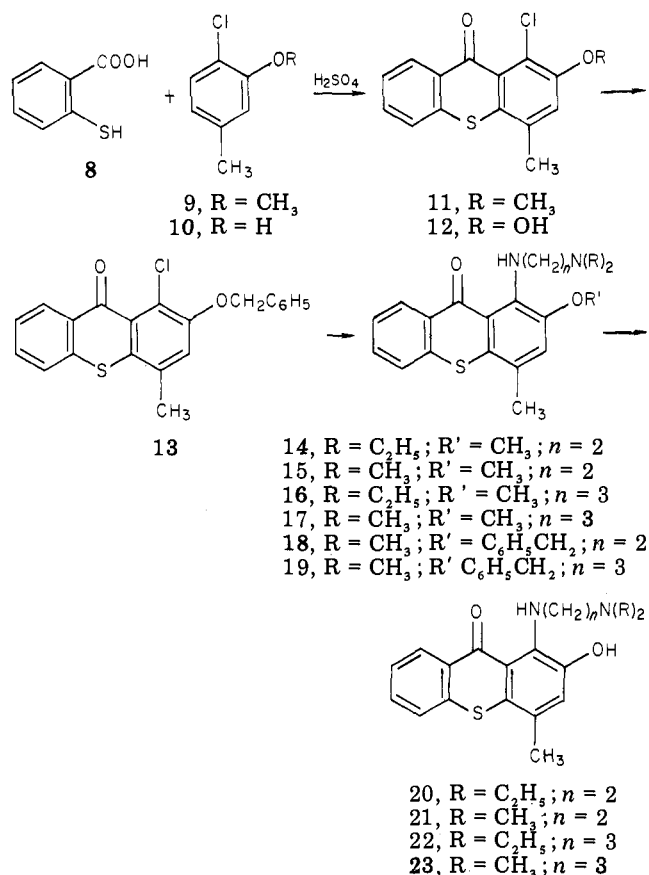
(3) D. A. Buyske and D. Dvornik, *Annu. Rep. Med. Chem.*, 1, 247 (1965).

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(5) F. Arcamone, "Doxorubicin", Academic Press, New York, London, Toronto, Sydney, and San Francisco, 1981, pp 103-112.

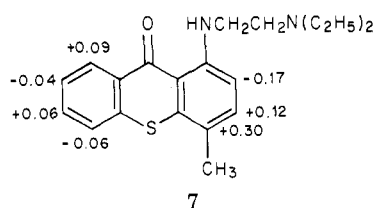
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Scheme I



apparent association constants ( $K_{\text{app}}$ ) of some anthraquinone-DNA complexes.<sup>7</sup>

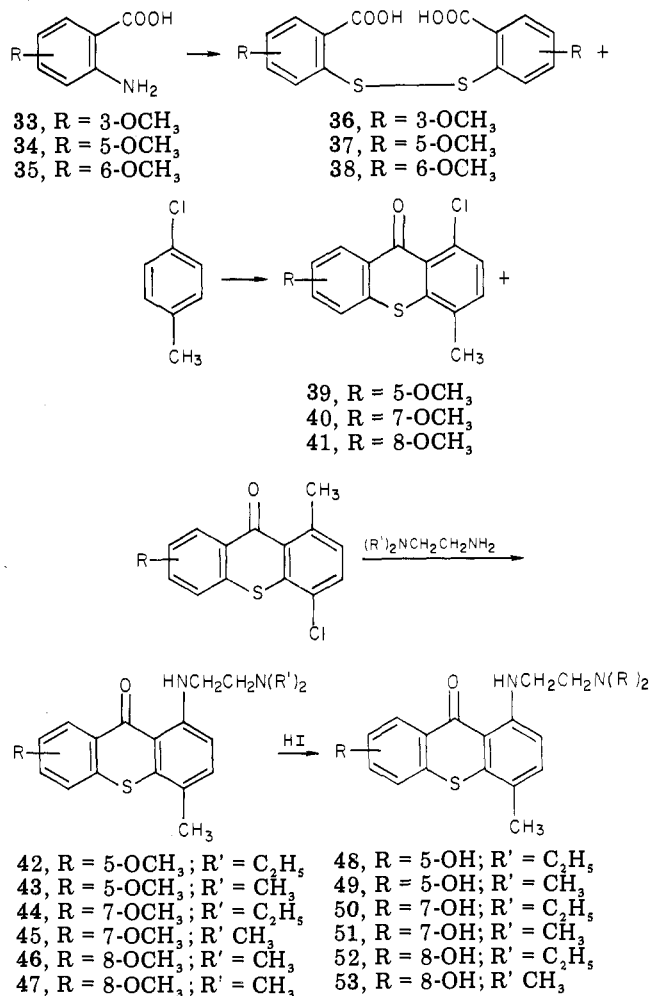
On the basis of these and other considerations, we postulate that the unknown active metabolite of lucanthone is a ring-hydroxylated analogue. It is known that mixed-function oxidase systems attack sites of high electron density on aromatic rings.<sup>8</sup> With the aid of a MINDO/3 program, an electron density map of lucanthone was constructed as shown in 7. The calculation confirmed our



belief that C-2 was the site of highest electron density and also indicated that C-5 and C-7 are plausible sites for electrophilic attack. For this reason it was deemed prudent to synthesize and test for antitumor activity a group of 2-, 5-, and 7-monohydroxylated lucanthone derivatives and, if any were of biological interest, to expand the program to include other ring-hydroxylated analogues as well. In addition, the apparent association constants ( $K_{\text{app}}$ ) and  $\Delta T_m$  values of a few lucanthone derivatives complexed with purified calf thymus DNA were to be determined in order to see whether any correlation existed between these biophysical and antitumor properties.

**Chemistry.** The preparation of the 2-hydroxylucanthone analogues is shown in Scheme I.

Scheme II



Thiosalicylic acid and 4-chloro-3-methoxytoluene reacted in sulfuric acid to give the thioxanthene (11), which condensed with *N,N*-diethylethylenediamine to give 14.<sup>9</sup> Demethylation of the 2-methoxythioxanthene (14) with 48% HI gave 20. The demethylation route to (20-23) proceeded smoothly in some cases but was unreliable in others, particularly where R = CH<sub>3</sub>. The 2-(benzyloxy)thioxanthene (13) was prepared from 12, which in turn was obtained by condensing 8 with the phenol 10. Demethylation proved to be more reliable than demethylation in the conversion of 18 and 19 to 21 and 23, respectively. The antitumor activities of these compounds are recorded in Table II.

Similar difficulties were encountered in the 4-methoxy series. For example, 1-[[2-(diethylamino)ethyl]amino]-4-methoxythioxanthene (24) was obtained as described by Blanz and French,<sup>10</sup> but could not be demethylated smoothly. Accordingly, 1-chloro-4-hydroxythioxanthene (25) was prepared from 8 and *p*-chlorophenol. It was benzylated with benzyl chloride to afford 1-chloro-4-(benzyloxy)thioxanthene (26), which upon treatment with the requisite diamines gave the 4-(benzyloxy)thioxanthenes 27-29 (Table I). Demethylation with HI gave the corresponding 4-hydroxythioxanthenes 30-32 (Table II).

The preparation of the 5-, 7-, and 8-hydroxylucanthone analogues was carried out as shown in Scheme II.<sup>9</sup>

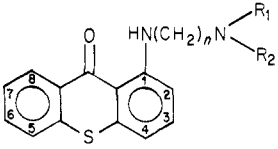
(7) J. C. Double and J. R. Brown, *J. Pharm. Pharmacol.*, **27**, 502 (1975).

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(9) S. Archer and C. M. Suter, *J. Am. Chem. Soc.*, **74**, 4296 (1952).

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Table I. Chemical and Biological Data on Alkoxythioxanthone Analogues



no.	ring substituents	n	R	R <sup>1</sup>	emp formula	mp, °C	in vivo antitumor act. vs. P-388 lymphocytic leukemia <sup>b</sup>	
							dose, mg/kg	T/C
14	2-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S·C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	142-143	200	toxic <sup>d</sup>
							100	117
							50	115
15	2-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S	90-91	200	129
							100	127
							50	96
16	2-OCH <sub>3</sub> , 4-CH <sub>3</sub>	3	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub> S·C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> <sup>c</sup>	148-149	200	toxic <sup>d</sup>
							100	toxic <sup>d</sup>
							50	102
17	2-OCH <sub>3</sub> , 4-CH <sub>3</sub>	3	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S·2HI·H <sub>2</sub> O	210-211	200	toxic <sup>d</sup>
							100	103
							50	100
18	2-OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>25</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S	127-128	NT <sup>e</sup>	NT <sup>e</sup>
24	4-OCH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S	87-88 <sup>f</sup>	200	100
							100	92
							50	92
27	4-C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> O	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>26</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub> S	103-104	NT	NT
28	4-C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> O	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>24</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S	174-175	NT	NT
29	4-C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> O	3	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>25</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S	130-132	NT	NT
42	5-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S	147-149	200	116
							100	100
							50	113
43	5-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S	176-177	NT	NT
44	7-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S	81-82	100	147
							50	115
							25	115
45	7-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S	139-141	200	toxic <sup>d</sup>
							100	135
							50	115
60	7-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	H	H	C <sub>17</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S	120-124	NT	NT
61	7-OCH <sub>3</sub> , 4-CH <sub>3</sub>	2	CH <sub>3</sub>	H	C <sub>18</sub> H <sub>20</sub> ON <sub>2</sub> O <sub>2</sub> S·C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	217-219	NT	NT

<sup>a</sup> Analyses for C, H, and N for all compounds listed are within  $\pm 0.4\%$  of the calculated values unless noted otherwise. All compounds were prepared by method A (Experimental Section). Compounds 17 and 61 were purified by crystallization from MeOH. All others were crystallized from EtOH. <sup>b</sup> The standard NCI protocols described in ref 17 were used. The dose listed was given once a day for 9 days. T/C = treated animal survival time/control animal survival time  $\times 100$ . I = inactive, i.e., T/C  $\leq 125$ . <sup>c</sup> Fumarate salt. <sup>d</sup> Toxic means deaths occurred at this dose. <sup>e</sup> NT = not tested. <sup>f</sup> Blanz and French<sup>10</sup> reported mp 89-90 °C.

3-Methoxy- (33),<sup>11</sup> 5-methoxy- (34),<sup>12</sup> and 6-methoxyanthranilic acid (35)<sup>13</sup> were diazotized and converted to the corresponding dithiosalicylic acids (36-38), and the unpurified acids were condensed with *p*-chlorotoluene to give the mixed thioxanthones. The isomers 39-41, which contained the more reactive 1-chloro substituent, were allowed to react with *N,N*-diethylethylenediamine and *N,N*-dimethylethylenediamine as described by Archer and Suter<sup>9</sup> to give the corresponding 1-[[dialkylamino]alkyl]amino]thioxanthones 42-47.

In the case of the preparation of 46, chromatography of the crude acid-soluble fraction gave a mixture of the desired methoxythioxanthone (46) and the corresponding 8-hydroxy compound (52). This result can be attributed to the presence of considerable amounts of 1-chloro-8-hydroxy-4-methylthioxanthone in the mixture of me-

thoxythioxanthones. Demethylation during the H<sub>2</sub>SO<sub>4</sub> cyclization step probably occurred during the preparation of 52 (see below). Demethylation of the methoxythioxanthones 42-47 with 48% HI proceeded smoothly in each case to give the target compounds 48-53, which were purified as the hydriodide salts.

Since the hydroxyethylaminoethylamino derivative 59 could not be prepared by HI demethylation of the corresponding methoxythioxanthone, the sequence shown in Scheme III was used to prepare 59 and a few other 7-hydroxy analogues.

5-Methoxydithiosalicylic acid (37) was reduced with zinc to give 5-methoxythiosalicylic acid<sup>14</sup> (54), which was condensed with 2-bromo-4-chlorotoluene (55) to give the phenylthio acid 56 using the procedure of Laidlaw et al.<sup>15</sup> Ring closure with H<sub>2</sub>SO<sub>4</sub> gave a mixture of the thioxanthones 57 and 58. NMR spectroscopy indicated that the demethylated compound 58 was present in substantial

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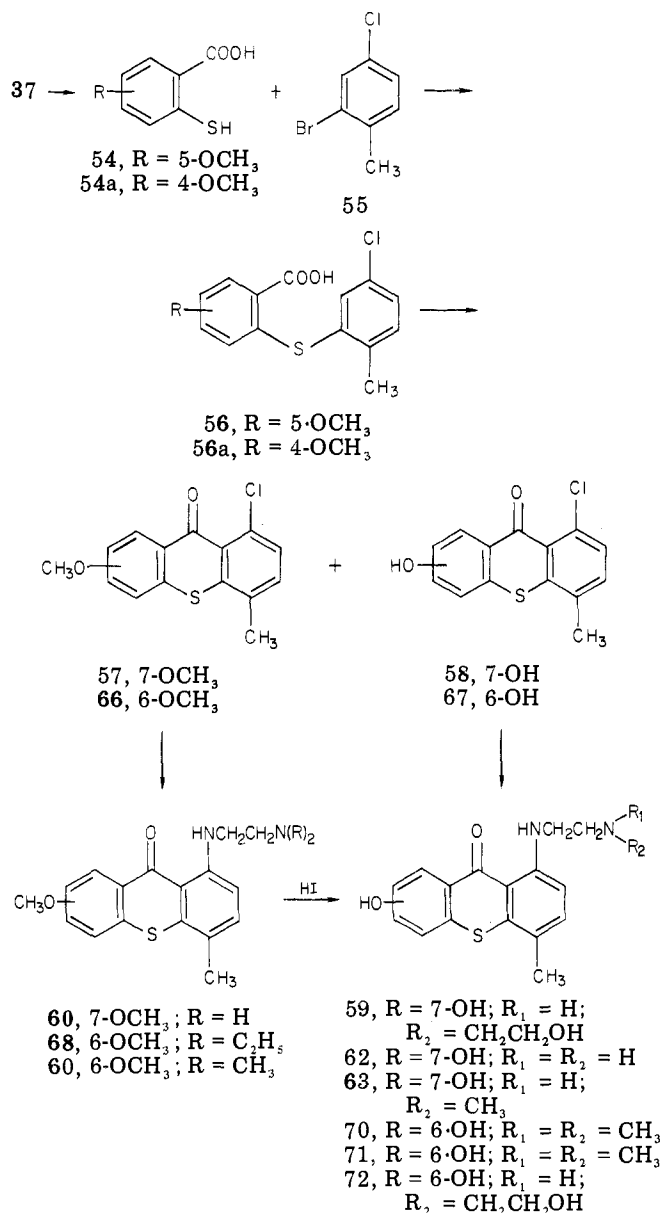
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Table II. Chemical and Biological Data on Hydroxylucanthone Analogues

no.	ring substituents	n	R	R <sub>1</sub>	emp formula <sup>a</sup>	mp, °C	method of preparation	solvent of recrystn	in vivo antitumor act. vs. P-388 lymphocytic leukemia <sup>b</sup>	
									dose, mg/kg	T/C
1	(lucanthone)								100	158 <sup>b</sup>
20	2-OH, 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S	79-80	B	EtOH	200 100 100 50	100 104 99
21	2-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S <sup>e</sup>	119-120	B	MeOH	200 100 50	100 101 100
22	2-OH, 4-CH <sub>3</sub>	3	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub> S	84-85	B	EtOH	200 100 50	100 94 94
23	2-OH, 4-CH <sub>3</sub>	3	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S·H <sub>2</sub> O <sup>c</sup>	138-140	B	EtOH	200 100 50	115 126 108
30	4-OH	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S·2HI <sup>e</sup>	223-224	B	EtOH	50 25 12.5	103 99 89
31	4-OH	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>17</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S <sup>e</sup>	179-180	B	MeOH	200 100 50	toxic <sup>d</sup> 90 94
32	4-OH	3	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S·2HI <sup>e</sup>	243-245	B	MeOH	200 100 3.13-50	toxic <sup>d</sup> toxic <sup>d</sup> <125
48	5-OH, 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S·HI	251-253	B	MeOH	200 100 50	104 100 104
49	5-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S·HI	272-275	B	MeOH	200 100 50	97 95 100
50	7-OH, 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S·HI	254-255	B	MeOH	100 50 25 12.5	123 188 159 142
51	7-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S·HI	273-275	B	MeOH	128 64 32 16 8 4 2 1	toxic <sup>d</sup> 265 191 170 180 141 139 130
52	8-OH, 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S·HI	242-244	B	MeOH	200 100 50	<125 181 143
53	8-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S·HI	278-280	B	MeOH	200 100 50	<125 <125 166
59	7-OH, 4-CH <sub>3</sub>	2	CH <sub>2</sub> CH <sub>2</sub> OH	H	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S	193-195	C	EtOH	25 12.5 6.25	103 169 150
62	7-OH, 4-CH <sub>3</sub>	2	H	H	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> S·HI	277-278	B	MeOH	100 50 25	148 135 117
63	7-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	H	C <sub>17</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S·HI	278-280	B	MeOH	100 50 25	toxic <sup>d</sup> toxic <sup>d</sup> 93
70	6-OH, 4-CH <sub>3</sub>	2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S·HI	231-234	B	EtOH	200 100 50	106 108 107
71	6-OH, 4-CH <sub>3</sub>	2	CH <sub>3</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S·HI	265-266	B	MeOH	200 100 50	126 110 110
72	6-OH, 4-CH <sub>3</sub>	2	CH <sub>2</sub> CH <sub>2</sub> OH	H	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S	193-195	C	EtOH	100 50 25 12.5	154 146 133 130

<sup>a</sup> Analyses for C, H, and N for all compounds listed are within ±0.4% of the calculated unless noted otherwise. <sup>b</sup> The standard NCI protocols were used. See Table I. See ref 18. <sup>c</sup> Anal. Calcd: C, 63.30; H, 6.70; N, 7.77. Found: C, 63.49; H, 6.12; N, 7.68. Prepared by 30-min reflux of 19 without purification of 19. <sup>d</sup> Deaths occurred at this dose. <sup>e</sup> Prepared by refluxing the corresponding benzyl ether with 48% HI for 30 min.

## Scheme III



amounts. Methylation of the crude mixture, followed by crystallization, proved to be the most convenient way to secure pure 57, which in turn was demethylated smoothly with HI in acetic acid to give pure 58. The latter was allowed to react with hydroxyethylaminoethylamine to give 59 accompanied by a high-melting, acetic acid-insoluble, unidentified byproduct.

Condensation of 57 with ethylenediamine gave 60, which on demethylation gave 62. When the same reaction was carried out with *N*-methylthylenediamine, a mixture of bases was obtained; 1-[[methylamino]ethyl]amino]-7-methoxy-4-methylthioxanthone (61) was the minor component. Demethylation of 60 afforded 63. The major product was tentatively assigned structure 65 on the basis of the spectroscopic and analytical data, which indicated that a mole of H<sub>2</sub>O had been lost.

The formation of 65 can be rationalized by postulating that 57 reacted with *N*-methylthylenediamine at the secondary amine site to give 64, which at the elevated temperature of the reaction cyclized to give the diazepine 65 (Scheme IV).

The 6-hydroxy analogues 70 and 71 were prepared by the sequence shown in Scheme III. 4-Methoxyanthranilic

## Scheme IV

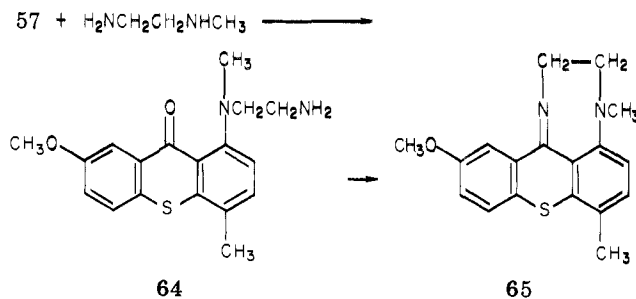


Table III. Association Constants and  $\Delta T_m$  of Some Drug-DNA Complexes

compd	$\Delta T_m$ , °C	$K_{app}$
lucanthone	20	$2.2 \times 10^6$
20	6	$2.3 \times 10^6$
48	20	$2.2 \times 10^6$
42		$2.7 \times 10^6$
50	20	$4.3 \times 10^6$
44		$1.8 \times 10^7$

acid<sup>16</sup> was used as the starting material to prepare 4-methoxythiosalicylic acid (54a), which was converted to 2-[(5-chloro-2-methylphenyl)thio]-4-methoxybenzoic acid (56a). Cyclization in H<sub>2</sub>SO<sub>4</sub> gave 66, which, with the requisite *N,N*-dialkylethylenediamines, gave 68 and 69, which were smoothly demethylated to give 70 and 71.

Demethylation of 66 with HI in acetic acid furnished 67, which was converted to 72 with the aid of *N*-(2-hydroxyethyl)ethylenediamine.

## Biological Results

The target thioxanthones related to lucanthone were submitted to the National Cancer Institute for evaluation in the P-388 screen.<sup>17</sup> The results are recorded in Tables I and II. The historical data on lucanthone are included in Table II for comparative purposes.<sup>18</sup> None of the methoxy analogues in Table I was of sufficient biological interest to warrant further consideration. The 7-methoxy analogue (44) showed a T/C = 147 at 100 mg/kg and 45 was weakly active (T/C = 135 at 100 mg/kg). The others were either ineffective, toxic, or both.

The hydroxy analogues reported in Table II are of greater interest. The results in the 2-hydroxy and 5-hydroxy series were uniformly disappointing. However, in the 7-hydroxy series at least two compounds, 50 and 51, are of considerable biological interest; the former showed T/C = 188 at 50 mg/kg and the latter showed T/C = 265 at 64 mg/kg. Activities for both compounds were confirmed in repeated tests. It should be noted that 59, which has the same side chain as 6, was less active (possibly more toxic) than 50 and 51, whereas in the dihydroxyanthracenedione series, Cheng found the reverse to be true.<sup>6</sup>

Compounds in the 6-hydroxy (e.g., 19, T/C = 154 at 100 mg/kg) and 8-hydroxy series (e.g., 52, T/C = 181 at 100 mg/kg) were active in the P-388 screen, but no other compounds in this series were as active as 51.

The  $\Delta T_m$  and  $K_{app}$  values for some of the lucanthone analogues are recorded in Table III.

(16) J. M. L. Stephen, I. M. Tonkin, and J. Walker, *J. Chem. Soc.*, 1034 (1947).

(17) R. L. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3(2), 1 (1972).

(18) We thank Drs. Harry Wood and B. J. Abbott of the NCI for furnishing these data.

It should be noted that all of the compounds listed in Table III have  $K_{app}$  values equal to or greater than lucanthone. The 7-methoxy analogue (44) appears to bind better to DNA than lucanthone yet is no more active as an antitumor agent and far less active than 50. Compound 48 has a  $\Delta T_m$  value and a  $K_{app}$  approximately the same as lucanthone yet is completely devoid of antitumor activity. It is apparent from these few examples that there is no quantitative correlation between the ability of these thioxanthenones to bind to DNA and their antitumor activity. On the other hand, compound 50 is more active than lucanthone as an antitumor agent and has a somewhat better  $K_{app}$  also. If the data in Table III may be interpreted to indicate that, like lucanthone, these compounds are intercalating agents, then it may be concluded that in this series, intercalation is a necessary but not sufficient condition for antitumor activity.

It is important to note that the antitumor activity of lucanthone was increased significantly by hydroxylation at C-7, a possible site of metabolism by a mixed-function oxidase which is susceptible to inhibition by SKF-525-A. Work in progress indicates that 50 may not be the ultimate biotransformation product which is responsible for the antitumor activity of lucanthone but that 7-hydroxylation is an essential step in the metabolic pathway.

### Experimental Section

Melting points were taken on a laboratory device Mel-Temp apparatus and are corrected. Infrared spectra were obtained on a Perkin-Elmer Model 137 spectrometer, and NMR spectra were run on a Varian T-60A spectrometer in either  $CDCl_3$  or  $(CD_3)_2SO$  solution with  $Me_4Si$  as an internal standard. Elementary analyses were determined by Instranal Laboratories, Rensselaer, NY, and Spang Microanalytical Laboratory, Eagle Harbor, MI.

**1-[[2-(Diethylamino)ethyl]amino]-2-methoxy-4-methylthioxanthone (14).** Method A. A mixture of 31.3 g (0.2 mol) of 4-chloro-3-methoxytoluene, 15.4 g (0.1 mol) of thiosalicylic acid, and 250 mL of concentrated  $H_2SO_4$  was stirred at room temperature for 16 h and then at 60 °C for 2 h before being poured onto ice. During the early stages of the reaction the solution turned dark red and  $SO_2$  was evolved. The yellow solid that separated was filtered and suspended in 7%  $NH_4OH$ . The suspension was warmed on the steam bath for about 30 min and filtered. The solid was washed with  $H_2O$ , EtOH, and acetone and dried. After crystallization from pyridine, the 1-chloro-2-methoxy-4-methylthioxanthone weighed 11.0 g (38%), mp 177–178 °C.

A mixture of 21.75 g (0.075 mol) of the thioxanthone, 10 mL of  $N,N$ -diethylethylenediamine, and 3.0 mL of dry pyridine was heated under reflux for 20 h. The dark mixture was cooled, treated with 5.0 mL of 50% KOH solution, and steam distilled. The mixture was allowed to cool and the aqueous supernatant was carefully decanted. The residue was heated to boiling with 10% acetic acid solution and filtered. The dark filtrate was made alkaline and the base was dissolved in  $CHCl_3$ . The  $CHCl_3$  solution was washed with  $H_2O$ , dried, and concentrated to leave an oil, which did not crystallize. In most of the cases listed in Table I, crystallization occurred at this point, and the bases were purified by recrystallization from the solvents indicated in Table I. In other instances when the base did not crystallize readily, it was converted to a crystalline salt.

In this case, the base (14) readily formed a fumarate salt in absolute EtOH. After two recrystallizations from the same solvent, there was obtained 15.0 g (41%) of analytically pure material.

**1-[[2-(Diethylamino)ethyl]amino]-2-hydroxy-4-methylthioxanthone (20).** Method B. A suspension of 18.5 g of the 2-methoxythioxanthone (14) and 50 mL of 48% HI was refluxed under  $N_2$  for 20 h and cooled. The HI salt which separated was collected. In most cases the salt was recrystallized. The properties of these compounds are recorded in Table II. In some instances the hydroxythioxanthenones were obtained as free bases as described in the present instance. The crude HI salt was suspended in  $H_2O$ , and the suspension was made alkaline with  $K_2CO_3$  and

shaken thoroughly with  $CHCl_3$ . The organic layer was separated, washed with  $H_2O$ , dried, and concentrated to furnish an oil, which crystallized. Recrystallization from EtOH gave the pure base: yield 11.6 g (65%).

**1-Chloro-2-hydroxy-4-methylthioxanthone (12).** A solution of 20 g (0.13 mol) of thiosalicylic acid and 35.7 g (0.25 mol) of 2-chloro-5-methylphenol in 1500 mL of concentrated  $H_2SO_4$  was stirred overnight and then poured into a large volume of ice-water. The yellow solid was collected and then suspended in 7%  $NH_4OH$ . The suspension was heated with stirring to 90 °C and filtered hot. The thioxanthone was crystallized from MeOH: mp 188–189 °C; yield 9.0 g (25%). Anal. ( $C_{14}H_9ClO_2S$ ) C, H, N.

**1-Chloro-2-(benzyloxy)-4-methylthioxanthone (13).** A suspension of 5.5 g (0.02 mol) of the 2-hydroxythioxanthone (12), 2.5 mL (0.02 mol) of benzyl chloride, and 1.52 g (0.011 mol) of  $K_2CO_3$  in 300 mL of MeOH was refluxed for 16 h. The cooled mixture was filtered. The solid that was collected was washed with  $H_2O$ , dried, and recrystallized from MeOH to give 4.0 g (55%) of the benzyl ether, mp 141–143 °C. Anal. ( $C_{21}H_{15}ClO_2S$ ) C, H, N.

**1-Chloro-4-hydroxythioxanthone (25).** A solution of 52.0 g (0.30 mol) of thiosalicylic acid, 65.0 g (0.50 mol) of *p*-chlorophenol, and 750 mL of concentrated  $H_2SO_4$  was stirred overnight and poured into a large volume of ice-water. The solid that separated was washed with  $H_2O$  and then suspended in 7%  $NH_4OH$ . The suspension was heated with stirring on the steam bath and filtered hot. The solid was washed with  $H_2O$  and recrystallized from pyridine to give 30.4 g (39%) of the thioxanthone, mp, 260–262 °C. Anal. ( $C_{13}H_7ClO_2S$ ) C, H, Cl.

**4-(Benzyloxy)-1-chlorothioxanthone (26).** A suspension of 9.48 g (0.04 mol) of (25), 5.0 mL (0.04 mol) of benzyl chloride, and 0.45 g (0.025 mol) of  $K_2CO_3$  in 50 mL of MeOH was refluxed with stirring for 20 h. The cooled mixture was filtered, and the solid that was collected was washed with  $H_2O$ , dried, and crystallized from pyridine to afford 8.3 g (59%) of the benzyl ether, mp 140–141 °C. Anal. ( $C_{20}H_{13}ClO_2S$ ) C, H, Cl.

**1-[[2-(Dimethylamino)ethyl]amino]-4-hydroxythioxanthone (31).** Method B. A suspension of 5.0 g (0.014 mol) of 4-(benzyloxy)-1-chlorothioxanthone, 15 mL of  $N,N$ -dimethylethylenediamine, and 5 mL of pyridine was refluxed overnight and worked up as described above. The crude base (28) melted at 174–175 °C after recrystallization from EtOH: yield 1.71 g (30%). A solution of 1.616 g (0.004 mol) of 28 and 10 mL of 48% HI was refluxed for 30 min, cooled, and filtered. The crude HI salt was suspended in  $H_2O$  and treated with  $K_2CO_3$  and  $CHCl_3$ . The organic phase was separated, dried, and concentrated to give the free base, which melted at 177–178 °C after crystallization from MeOH: yield 430 mg (34%).

**3-Methoxydithiosalicylic Acid (36).** A solution of 37.2 g (0.26 mol) of 2-amino-3-methoxybenzoic acid<sup>11</sup> (33) in 45 mL of concentrated HCl and 112 mL of  $H_2O$  was cooled to 5 °C and diazotized with a solution of 18.0 g of  $NaNO_2$  in 63 mL of  $H_2O$ . The diazonium solution was filtered and added slowly to a cold solution of  $Na_2S_2$  prepared from 58 g of  $Na_2S \cdot 9H_2O$  dissolved in 65 mL of  $H_2O$  to which was added 7.6 g of S and 9.0 g of NaOH in 23 mL of  $H_2O$ . The mixture was left overnight, filtered, and cautiously acidified with 35 mL of concentrated HCl, whereupon 33.5 g (80%) of the dithio acid, suitable for the next step, was obtained. A small sample was recrystallized twice from EtOH, mp 200–205 °C. Anal. Calcd for  $C_{16}H_{14}O_6S_2$ : C, 52.46; H, 3.83. Found: C, 52.34; H, 4.32.

**1-[[2-(Dimethylamino)ethyl]amino]-5-methoxy-4-methylthioxanthone (43).** A mixture of 32.2 g of 3-methoxydithiosalicylic acid (36), 89 mL of *p*-chlorotoluene, and 340 mL of concentrated  $H_2SO_4$  was stirred overnight at room temperature and then at 60 °C for 2 h before being poured into ice-water. The neutral fraction which was a mixture of isomeric 5-methoxythioxanthenones was used directly in the next step.

A suspension of 1.5 g of the above thioxanthenones and 5 mL of  $N,N$ -dimethylethylenediamine was refluxed overnight and then worked up as usual to give the crystalline base, mp 176–177 °C after crystallization from EtOH: yield 300 mg.

**5-Methoxydithiosalicylic Acid (37).** Ten grams of 5-methoxyanthranilic acid<sup>12</sup> (34) was converted to the corresponding dithio acid as described above. There was obtained 8.5 g (78%)

of the desired dithio acid, mp 302–305 °C after two crystallizations from 2-methoxyethanol. Anal. (C<sub>16</sub>H<sub>14</sub>O<sub>6</sub>S<sub>2</sub>·0.5H<sub>2</sub>O) C, H.

1-[[2-(Dimethylamino)ethyl]amino]-7-methoxy-4-methylthioxanthene (45) and 1-[[2-(Dimethylamino)ethyl]amino]-7-hydroxy-4-methylthioxanthene (51). A mixture of 12.3 g of 5-methoxydithiosalicylic acid (37), 34 mL of *p*-chlorotoluene, and 130 mL of concentrated H<sub>2</sub>SO<sub>4</sub> furnished 9.0 g of a mixture of isomeric 7-methoxythioxanthenes. A suspension of 3.2 g of this mixture in 8 mL of *N,N*-dimethylethylenediamine was refluxed overnight. The excess diamine was removed by distillation with steam. The residue was dissolved in glacial acetic acid, diluted with H<sub>2</sub>O, and filtered to remove the unreacted 4-chloro-7-methoxy-1-methylthioxanthene. Basification of the filtrate furnished the desired product (45): mp 141–143 °C after crystallization from EtOH; yield 1.55 g.

A suspension of 1.55 g of 45 in 12 mL of 48% HI was refluxed for 3 h, cooled, and filtered. Recrystallization from MeOH gave 1.7 g of the HI salt of 51, mp 273–275 °C.

1-Chloro-7-methoxy-4-methylthioxanthene (57). A solution of 28.3 g of 5-methoxyanthranilic acid<sup>12</sup> (34) in 34.0 mL of concentrated HCl and 85 mL of H<sub>2</sub>O was diazotized with a solution of 11.8 g of NaNO<sub>2</sub> in 48 mL of H<sub>2</sub>O. The filtered diazonium solution was added to a solution of Na<sub>2</sub>S<sub>2</sub> prepared from 45 g of Na<sub>2</sub>S·9H<sub>2</sub>O, 5.8 g of S, 6.9 g of NaOH, and 70 mL of H<sub>2</sub>O. The crude dithio acid (26.7 g) was suspended in 140 mL of glacial acetic acid and stirred under reflux with 6.5 g of Zn dust. During the course of the reduction, which took 5 h, two additional portions of 6.0 g of Zn dust in 20 mL of acetic acid were added. The suspension was cooled and filtered. The filter cake was washed with H<sub>2</sub>O and then suspended in hot H<sub>2</sub>O and treated with a solution of 10.0 g of NaOH in H<sub>2</sub>O. The suspension was filtered through a bed of Celite, and the filtrate on acidification with HCl gave the crude thiosalicylic acid:<sup>19</sup> mp 172–175 °C after recrystallization from aqueous EtOH; yield 17.3 g.

A suspension of 14.0 g of the above acid, 17.3 g of 2-bromo-4-chlorotoluene, 16.2 g of K<sub>2</sub>CO<sub>3</sub>, 700 mg of KI, and 750 mg of Cu bronze in 190 mL of DMF was stirred under reflux for 18 h, cooled, and poured onto ice. The cold suspension was filtered (Celite) and extracted with ether. The aqueous phase was acidified to give 18.5 g of 2-[(5-chloro-2-methylphenyl)thio]-5-methoxybenzoic acid (56), mp 143–145 °C after two recrystallizations from aqueous EtOH. Anal. Calcd for C<sub>15</sub>H<sub>13</sub>ClO<sub>3</sub>S: C, 58.34; H, 4.24. Found: C, 58.77; H, 4.13.

A solution of 1.0 g of the acid 56 in 5.0 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was heated with stirring on the steam bath for 1.5 h. It was poured into ice-water to give a solid, which melted at 230–265 °C after treatment with warm 7% NH<sub>4</sub>OH. After recrystallization from acetic acid it melted at 280–287 °C: yield 320 mg. The NMR and IR spectra indicated that it was 1-chloro-4-methyl-7-hydroxythioxanthene (58) prepared as described below. Concentration of the filtrate furnished 200 mg of 1-chloro-7-methoxy-4-methylthioxanthene (57), mp 155–157 °C after recrystallization from EtOH. The NMR spectrum showed a signal for CH<sub>3</sub>O. Anal. Calcd for C<sub>15</sub>H<sub>11</sub>ClO<sub>2</sub>S: C, 61.96; H, 3.81. Found: C, 62.70; H, 3.80.

In another experiment 12.4 g of the crude thioxanthene mixture was added to a suspension of 12.0 g of K<sub>2</sub>CO<sub>3</sub> in 300 mL of MeOH. The suspension was stirred under reflux while a total of 25 mL of CH<sub>3</sub>I was added over a period of 6 h. At the end of this time, the solvent was removed and the residue was washed with H<sub>2</sub>O and crystallized from ethanol: mp 155–157 °C: yield 11.1 g. This material was used in the preparation of 60 and 61 (Table I).

1-[[2-(Methylamino)ethyl]amino]-7-methoxy-4-methylthioxanthene (61). A suspension of 3.0 g of 1-chloro-7-methoxy-4-methylthioxanthene (57) in 10 mL of *N*-methylethylenediamine was refluxed for 14 h. The mixture was cooled, diluted with 100 mL of H<sub>2</sub>O, and distilled until about 50 mL of H<sub>2</sub>O was collected. The residue was treated with a few milliliters of 50g KOH, and the gummy solid that separated was taken up in CHCl<sub>3</sub>. The extract was dried and then chromatographed on silica gel. The CHCl<sub>3</sub> eluates were combined and evaporated to

leave a crystalline substance, mp 152–155 °C after recrystallization from EtOH; yield 1.6 g. The diazepine structure (65) was tentatively assigned to this compound. Anal. (C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>OS) C, H, N.

On further elution using CHCl<sub>3</sub>–3% MeOH, another crystalline substance was obtained: yield 518 mg; mp 94–98 °C. This is the desired product (61). It formed a fumarate, mp 217–219 °C after crystallization from MeOH. Refluxing the 7-methoxythioxanthene (518 mg) with 3 mL of 48% HI for 3 h gave the corresponding 7-hydroxy derivative (63) as the HI salt, mp 278–280 °C after crystallization from MeOH: yield 550 mg.

1-[[2-(2-Hydroxyethyl)amino]ethyl]amino]-7-hydroxy-4-methylthioxanthene (59). Method C. A suspension of 1.0 g of 1-chloro-7-methoxy-4-methylthioxanthene (57) in 10 mL of acetic acid and 10 mL of 48% HI was refluxed for 2 h, cooled, and filtered. The solid was crystallized from 2-methoxyethanol to give 650 mg of 1-chloro-7-hydroxy-4-methylthioxanthene (58), mp 297–299 °C dec. Anal. (C<sub>14</sub>H<sub>9</sub>ClO<sub>2</sub>S) C, H.

A solution of 300 mg of 58, 3.0 mL of *N*-(hydroxyethyl)ethylenediamine, and 3.0 mL of dry pyridine was refluxed for 16 h. It was diluted with H<sub>2</sub>O and distilled until about 20 mL of distillate was collected. The cooled suspension was filtered, and the collected crystals were washed with H<sub>2</sub>O, dried, and crystallized from EtOH: mp 189–191 °C; yield 200 mg.

1-[[2-(Diethylamino)ethyl]amino]-8-methoxy-4-methylthioxanthene (46). A solution of 8.7 g of 6-methoxyanthranilic acid<sup>13</sup> (35) in 10.5 mL of concentrated HCl and 25.0 mL of H<sub>2</sub>O was converted to the corresponding diazonium salt with a solution of 3.6 g of NaNO<sub>2</sub> in 15.0 mL of H<sub>2</sub>O. The filtered solution was converted to the dithiosalicylic acid (38) by addition to a solution of Na<sub>2</sub>S<sub>2</sub> prepared from 13.8 g of Na<sub>2</sub>S·9H<sub>2</sub>O, 2.1 g of NaOH, and 1.5 g of S in 20 mL of H<sub>2</sub>O, followed by cautious acidification. The crude acid, 38, so obtained<sup>14</sup> weighed 7.0 g, of which 5.4 g was converted to a mixture of 8-methoxythioxanthenes by stirring overnight with 19 mL of *p*-chlorotoluene and 64 mL of H<sub>2</sub>SO<sub>4</sub>. After the customary workup, there was obtained 3.5 g of a mixture of thioxanthenes.

A suspension of 5.7 g of the above mixture of thioxanthenes and 15 mL of *N,N*-diethylethylenediamine was refluxed for 17 h, cooled, diluted with H<sub>2</sub>O, and subjected to distillation. After about 25 mL was collected, the cooled suspension was filtered. The gummy solid was dissolved in glacial acetic with warming, diluted with H<sub>2</sub>O, and filtered. The basified filtrate yielded an oil, which was taken up in CHCl<sub>3</sub>, dried, and chromatographed on silica gel using CHCl<sub>3</sub> and CHCl<sub>3</sub>–MeOH as the eluates. The first crystalline fraction weighed 300 mg after crystallization from EtOH, mp 73–75 °C. The NMR spectrum showed all the expected signals except for a CH<sub>3</sub>O group. The elementary analyses were those expected for the corresponding 8-hydroxy analogue 52. Anal. (C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>S·0.25H<sub>2</sub>O) C, H, N.

The major fraction was eluted with CHCl<sub>3</sub> and CHCl<sub>3</sub>–2% MeOH: yield 1.45 g; mp 93–96 °C after crystallization from EtOH. The NMR spectrum and elementary analyses were in agreement with the assigned structure (46).

1-[[2-(Dimethylamino)ethyl]amino]-6-methoxy-4-methylthioxanthene (69). A suspension of 20.2 g of 4-methoxythiosalicylic acid<sup>20</sup> (54a), 25.0 g of 2-bromo-4-chlorotoluene, 23.4 g of K<sub>2</sub>CO<sub>3</sub>, 1.0 g of KI, and 1.1 g of Cu bronze in 275 mL of DMF was refluxed for 18 h. The mixture was cooled, diluted with H<sub>2</sub>O, and extracted with ether. The aqueous phase was acidified to give the desired acid (56a), which, after crystallization from acetic acid, melted at 192–194 °C: yield 25.3 g.

A solution of 8.0 g of the above acid in 44 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was heated with stirring on the steam bath for 90 min. It was cooled and poured into ice-water, and the solid was collected. It was warmed with 7% NH<sub>4</sub>OH, filtered, and dried: yield 5.0 g; mp 186–190 °C. Material of this quality was used to prepare the thioxanthenes 71 and 68 and 1-chloro-6-hydroxy-4-methylthioxanthene (67).

A mixture of 400 mg of the above 6-methoxythioxanthene (66) and 2.5 mL of *N,N*-dimethylethylenediamine was refluxed for 18 h and worked up in the usual way to give 300 mg of the

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(20) L. Katz, U.S. Patent 2767173 (Oct 16, 1956); *Chem. Absr.*, **51**, P6704b (1957).

desired base, mp 130-133 °C. After recrystallization from EtOH, the base (69) melted at 130-132 °C.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-6-hydroxy-4-methylthioxanthone (72). A solution of 1.0 g of 1-chloro-6-methoxy-4-methylthioxanthone in 10 mL of acetic acid and 10 mL of 48% HI was refluxed for 2 h and then allowed to stand overnight. After two crystallizations from acetic acid, the 6-hydroxythioxanthone (67) melted at 229-231 °C. Anal. (C<sub>14</sub>H<sub>9</sub>ClO<sub>2</sub>S) C, H.

A suspension of 1.3 g of the above 6-hydroxythioxanthone in 13.0 mL of *N*-(2-hydroxyethyl)ethylenediamine and 13.0 mL of pyridine was refluxed for 16 h. H<sub>2</sub>O was added to the cooled mixture, which was then set up to distill, and about 35 mL of distillate was collected. After cooling, the solid was collected by filtration and crystallized three times from EtOH to give the desired base: yield 1.0 g; mp 193-195 °C.

**Determination of Melting Temperatures of Drug-DNA Complexes.** All solutions used were prepared in a low ionic strength buffer of 3.3 × 10<sup>-4</sup> M Na<sub>2</sub>HPO<sub>4</sub>, 10<sup>-4</sup> M sodium ethylenediaminetetraacetate, and 3 × 10<sup>-3</sup> M NaCl at pH 6.8. The DNA solution concentration was approximately 20 µg/mL of calf thymus DNA (Sigma type 1). The exact concentration was determined spectrophotometrically.<sup>21</sup> Several milligrams of the drugs to be tested were weighed accurately and dissolved in 10 mL of the buffer solution. Varying volumes of the drug solutions ranging from 20 to 200 µL were added to 2.0-mL aliquots of the DNA solution so that the final drug concentrations ranged from 2 to 20 µmol/L.

The drug-DNA solutions were degassed at 50 °C under vacuum (20 torr) and placed in a water-jacketed cuvette of a Beckmann DB-G grating spectrophotometer. The temperature of the solution was raised at a rate of 1 °C/min, while absorbance was being read at 2 °C intervals at 260 nm. The results were plotted (*A*<sub>260nm</sub> vs. temperature), and the *T*<sub>m</sub> was taken as the midpoints of the curve

between the high and low temperature constant-absorbance regions. Under these conditions the *T*<sub>m</sub> of the uncomplexed DNA was 57.2 °C. Assuming that maximum intercalation occurs at a ratio of one drug molecule for every two base pairs, then at DNA concentrations of 20 µg/mL the maximum drug concentration for intercalation is 14.4 µmol/L. Beyond this limiting value, the drug does not intercalate but may bind electrostatically to phosphate residues. The secondary binding may cause a slight increase in the *T*<sub>m</sub> of the drug-DNA complex. A series of *T*<sub>m</sub> determinations were made at different drug-DNA ratios, and the *T*<sub>m</sub> values were plotted vs. drug concentration (µmol/L). The *T*<sub>m</sub> values reported in Table III are those read off at drug concentrations of 14.4 µmol/L or the point where the slope of the curve changed abruptly below this concentration.

**Determination of Drug-DNA Association Constants.** All solutions were prepared in a 0.009 M Tris-0.01 M NaCl buffer at pH 7.0. A crude DNA solution was prepared by dissolving 100 mg of the calf thymus DNA in 50 mL of buffer and dialyzing against 200-mL quantities of 4, 3, 2, and 1 M NaCl buffer and 0.1 M NaEDTA, and finally four times against the Tris buffer. After dialysis, the DNA solution was diluted to 100 mL with Tris buffer and frozen. Each week a new DNA portion was thawed, and the concentration was determined spectrophotometrically.<sup>21</sup>

Aliquots, 10 to 200 µL, of the DNA solution were added sequentially to 5 mL of a 5 × 10<sup>-5</sup> M drug solution at room temperature. After equilibration, absorption at 448 nm was determined on a Gilford 240 single-beam spectrophotometer. Approximately 15 aliquots were added per run so that about 1 mL of DNA solution was added per 5 mL of drug solution. The results were plotted and *K*<sub>app</sub>'s calculated as described by Double and Brown<sup>7</sup> and are recorded in Table III.

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## Synthesis and Peripheral Cardiovascular Action of *cis*- and *trans*-2-(3,4-Dimethoxybenzyl)cyclopentylamine Hydrochlorides

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The hydrochlorides of *cis*- and *trans*-2-(3,4-dimethoxybenzyl)cyclopentylamine have been synthesized. They are transient hypotensive agents with early and delayed depressor effects and antagonists of dopamine-induced vasodepression. In the atropinized and phenoxybenzamine-treated dog, the threshold dose for hypotension was 3-5 µmol/kg. The early depressor phases were attenuated variably by different types of antagonists, suggesting a nonspecific interaction with blood pressure regulation mechanisms. Cimetidine blocked the delayed depressor phases, consistent with endogenous histamine release. The *cis* amine hydrochloride was three to four times more potent than its *trans* isomer as a peripheral dopamine blocking agent. Cimetidine but not diphenhydramine interfered with this effect.

Dopamine cardiovascular pharmacology is of particular interest. The compound causes vasodepression in response to action at specific vascular receptors.<sup>1,2</sup> Vasodilation from inhibition of autonomic ganglionic transmission is attributed to specific dopamine receptors in sympathetic ganglia and postganglionic nerves.<sup>3-5</sup> The interaction of dopamine with α- and β-adrenergic receptors is also well documented,<sup>1-3</sup> and it has been postulated that there is a

composite dopamine-serotonin receptor in the canine vasculature.<sup>6</sup>

In the central nervous system, dopamine is of significant importance in modulating motor, behavioral, and neuroendocrine functions. Dopamine agonists and antagonists have been used therapeutically in those diseases that involve either its apparent deficiency or superabundance.<sup>7</sup> Several types of dopamine-sensitive sites and receptors have been identified by specific radioligand binding assays and by correlation with biological response patterns to dopaminergic drugs.<sup>8</sup> Evidence favors the existence of

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