large mass of polymeric pot residue. *Anal.* ($C_{16}H_{20}ClN_3O$) C, H, N.

N-(6-Chloro-2-methoxy-9-acridinyl)-N-[3-(diethylamino)propyl]acetamide (XX).—A solution of 46.3 g (0.1 mole) of 6-chloro-9-{[3-(diethylamino)propyl]amino}-2-methoxyacridine dihydrochloride hydrate[16] in warm H_2O was made basic with NH_4OH and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried ($MgSO_4$) and treated with 25 ml of AcCl. The mixture was boiled under reflux for 48 hr, filtered hot, and cooled to give 11.9 g of yellow solid, mp 266–274°. This material was presumed to be starting material from its lack of C=O absorption in the ir. The filtrate was concentrated to dryness to give a yellow semisolid. Crystallization from 95% EtOH gave 6.0 g of a solid of indeterminate melting point which was discarded. The EtOH filtrate was concentrated to dryness, and the residue was dissolved in H_2O, filtered, and made basic with NaOH. The gummy material which formed solidified on standing and was crystallized twice from heptane. The bright yellow crystals thus obtained, mp 113–114.5°, weighed 11.2 g (27%) and showed strong C=O absorption in the ir at 1665 cm^{-1}. *Anal.* ($C_{23}H_{28}ClN_3O_2$) C, H, N.

1-{5-[(7-Chloro-4-quinolyl)amino]salicyl}-4-piperidinol Dihydrochloride.—To a mixture of 4′-hydroxyacetanilide (45.3 g, 0.3 mole) and 30.4 g (0.3 mole) of 4-piperidinol in 200 ml of *i*-PrOH was added dropwise during 1 hr 22.5 ml of 40% CH_2O. The mixture was heated under reflux for 5 hr, the solvent was removed *in vacuo*, and 100 ml of H_2O was added. The mixture was heated on a steam bath for 3 hr, cooled, and neutralized until just acid to congo red. 4,7-Dichloroquinoline (59.4 g, 0.3 mole) and 50 ml of EtOH were added and the resulting mixture was heated on a steam bath for 3 hr. The pasty mass was stirred with 2 l. of H_2O and filtered. The filtrate was diluted to 4 l. and made alkaline with NH_4OH. The solid was collected by filtration, digested with a mixture of boiling MeOH–EtOH, and filtered. The residue was suspended in hot EtOH and treated with concentrated HCl. The suspension of the hydrochloric acid salt was diluted with Me_2CO and filtered to yield 97.0 g (70%) of product, mp 298–300° dec. *Anal.* ($C_{21}H_{22}ClN_3O_2 \cdot 2HCl \cdot 0.25H_2O$) C, H, N, H_2O.

N-(2-Cyanoethyl)-N-ethylacetamide.—To a mixture of 147 g of HOAc and 225 g of Ac_2O was added dropwise 236 g (2.4 moles) of 3-(ethylamino)propionitrile.[19] Heat was evolved and the temperature was maintained at 50–60° by controlling the rate of addition. The solvent was removed *in vacuo* and the residue was distilled to give 278 g (83%) of product, bp 95° (0.3 mm), $n^{25}D$ 1.4640. *Anal.* ($C_7H_{12}N_2O$) C, H, N. The ir spectrum contained a carbonyl band at 1647 cm^{-1}.

N-(3-Aminopropyl)-N-ethylacetamide.—N-(2-Cyanoethyl)-N-ethylacetamide (272 g, 1.94 moles) was hydrogenated in 500 ml of toluene over 60 g of Raney cobalt in the presence of 60 ml of Et_3N at 100° and an initial hydrogen pressure of 140.6 kg/cm^2. The solvent was removed *in vacuo* and the residue was distilled to give 170 g of product, bp 90–99° (0.2 mm). The ir spectrum showed a strong carbonyl peak at 1635 and a shoulder at 1670 cm^{-1}; in CCl_4 a split peak at 1678, 1652 cm^{-1} was present. The material was redistilled through a 30-cm Vigreux column and yielded 42 g (15%) of fraction A, bp 73° (0.2 mm), $n^{25}D$ 1.4725, and 77 g (27%) of fraction B, bp 83° (0.2 mm), $n^{25}D$ 1.4668. The ir spectrum of fraction A showed C=O at 1640 and a split primary amine band at 3300, 3370 cm^{-1} and was presumed to be the desired N-(3-aminopropyl)-N-ethylacetamide. *Anal.* ($C_7H_{16}N_2O$) C, H, N.

Fraction B exhibited a C=O peak at 1650, a strong NH absorption at 3300, and an amide II band at 1560 cm^{-1} and was presumed to be the rearranged terminal amide N-[3-(ethylamino)-propyl]acetamide. *Anal.* ($C_7H_{16}N_2O$) C, H, N.

Acknowledgments.—The authors express their appreciation to Dr. Paul E. Thompson and coworkers for the antimalarial studies, Mrs. Eva Gold and Miss Maria Limson for assistance in the chemical work, Mr. William Pearlman for carrying out the hydrogenations, Mr. C. E. Childs and associates for the microanalyses, and Dr. J. M. Vandenbelt and coworkers for determination of the ir and uv absorption spectra.

Comparison of Schistosomicidal Activity of Xanthenones and 4-Methyl-3-chloroanilines and Their Hydroxymethyl Analogs in Swiss Mice and Syrian Hamsters Infected with *Schistosoma mansoni*

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The schistosomicidal activity of a number of xanthenones, 4-methyl-3-chloroanilines, and their hydroxylated derivatives were tested against *Schistosoma mansoni*. It was demonstrated that hydroxylation enhanced schistosomicidal activity one- to sixfold in the mouse and two- to 33-fold in the hamster.

A series of xanthenones initially synthesized in the thirties by Mauss[1] and designated as "miracils" were demonstrated by Kikuth and Gönnert[2] to be orally effective against *Schistosoma mansoni* infection in white mice and monkeys. Of the several xanthenones, lucanthone (Miracil D) was found to be effective not only in experimental animal infections but also in natural infections of humans. Although highly efficacious in the monkey, lucanthone was less effective in humans and mice. Of the hundreds of xanthenones synthesized during the past quarter of a century a few were more active than lucanthone in experimental infections in animals but less effective when field tested against schistosome infections of man. The erratic and unpredictable activity in different hosts was found to be related to the ability of the host to hydroxylate the 4-methyl group *para* to the alkylamino side chain. Hycanthone, the hydroxymethyl analog of lucanthone, was the most active of the several metabolites produced by each host species. It was also demonstrated that the variety and the proportion of lucanthone metabolites whether urinary excretion products or products obtained after incubation with liver microsomes were different for each host species.[3–6]

Swiss mice and Syrian hamsters infected experimentally with *S. mansoni* were treated with xanthenones, 4-

(1) H. Mauss, *Chem. Ber.*, **81**, 19 (1948).

(2) W. Kikuth and R. Gönnert, *Z. Tropenmed. Parasitol.*, **1**, 234 (1949).

(3) D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, and S. Archer, *Nature*, **208**, 1005 (1965).

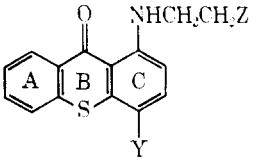
(4) D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, and S. Archer, *J. Med. Chem.*, **10**, 867 (1967).

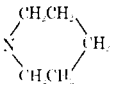
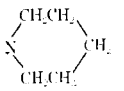
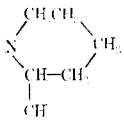
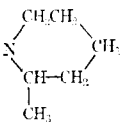
(5) D. Rosi, T. R. Lewis, R. Lorenz, H. Freele, D. A. Berberian, and S. Archer, *ibid.*, **10**, 877 (1967).

(6) D. Rosi, A. J. Merola, and S. Archer, *Life Sci.*, **6**, 1433 (1967).

TABLE I

COMPARATIVE SCHISTOSOMICIDAL ACTIVITY OF XANTHENONES AND THEIR HYDROXYMETHYL ANALOGS

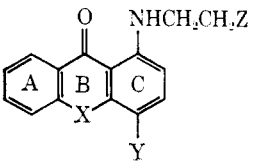


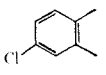
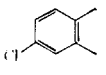
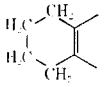
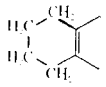
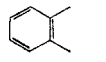
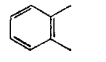
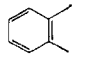
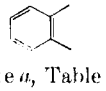
Compd[a]	Y	Z	Mice: $ED_{50} \pm SE$, mg/kg/day × 5	Mice: Act. index	Hamsters: $ED_{50} \pm SE$, mg/kg/day × 5	Hamsters: Act. index
1* (lucanthone)	CH_3	$N(C_2H_5)_2$	44.5 ± 4.5	1	9.5 ± 1.1	1
2* (hycanthone)	CH_2OH	$N(C_2H_5)_2$	15.9 ± 0.9	3	1.1 ± 0.1	9
3*	CH_3		86.0 ± 15.8	1	25.0 ± 5.5	1
4*	CH_2OH		26.7 ± 5.1	3	5.0 ± 1.6	5
5*	CH_3		43.0 ± 7.4	1	7.5 ± 1.1	1
6*	CH_2OH		22.2 ± 3.3	2	1.8 ± 0.5	4

[a] Compounds marked with an asterisk were reported previously; see Results section.

TABLE II

COMPARATIVE SCHISTOSOMICIDAL ACTIVITY OF XANTHENONES AND THEIR HYDROXYMETHYL ANALOGS

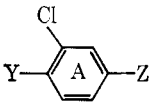


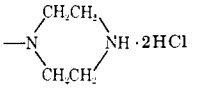
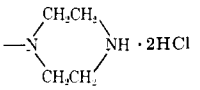
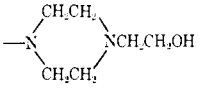
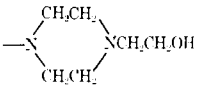
Compd[a]	A	X	Y	Z	Mice: $ED_{50} \pm SE$, mg/kg/day × 5	Mice: Act. index	Hamsters: $ED_{50} \pm SE$, mg/kg/day × 5	Hamsters: Act. index
7		S	CH_3		21.5 ± 3.6	1	51.0 ± 13.9	1
8		S	CH_2OH		18.9 ± 2.3	1	13.4 ± 2.3	4
9*		S	CH_3	$N(C_2H_5)_2$	50.0 ± 11.4	1	76.5 ± 11.8	1
10*		S	CH_2OH	$N(C_2H_5)_2$	>25		4.8 ± 1.9	16
11*		O	CH_3	$N(C_2H_5)_2$	88.0 ± 17.6	1	24.5 ± 4.4	1
12*		O	CH_2OH	$N(C_2H_5)_2$	39.6 ± 7.8	2	10.6 ± 5.3	2
13*		SO	CH_3	$N(C_2H_5)_2$	39.0 ± 6.4	1	42.0 ± 7.9	1
14*		SO	CH_2OH	$N(C_2H_5)_2$	>12.5		3.1 ± 1.1	14

[a] See footnote *a*, Table I.

TABLE III

COMPARATIVE SCHISTOSOMICIDAL ACTIVITY OF 4-METHYL-3-CHLOROANILINES AND THEIR HYDROXYMETHYL ANALOGS

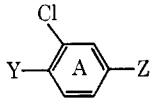


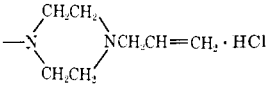
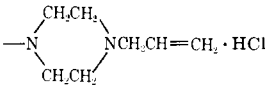
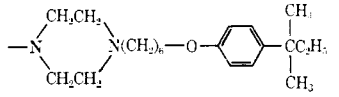
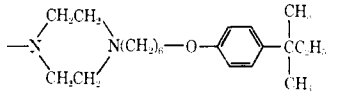
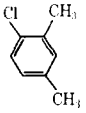
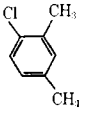
Compd[a]	Y	Z	Mice: ED_{50} ± SE, mg/kg/day × 5	Mice: Act. index	Hamsters: ED_{50} ± SE, mg/kg/day × 5	Hamsters: Act. index
15* (Mirasan)	CH_3	$NHCH_2CH_2N(C_2H_5)_2 \cdot HCl$	12.1 ± 2.1	1	53.0 ± 13.4	1
16*	CH_2OH	$NHCH_2CH_2N(C_2H_5)_2 \cdot HCl$	15.0 ± 2.8	1	7.0 ± 0.9	7
17*	CH_3		5.2 ± 0.7	1	>100.0	1
18*	CH_2OH		2.1 ± 0.3	2	3.0 ± 0.4	>33
19	CH_3		14.6 ± 2.1	1	>400.0	1
20	CH_2OH		40.0 ± 9.3	2	73.0 ± 15.5	>5

[a] See footnote *a*, Table I.

TABLE IV

COMPARATIVE SCHISTOSOMICIDAL ACTIVITY OF 4-METHYL-3-CHLOROANILINES AND THEIR HYDROXYMETHYL ANALOGS



Compd[a]	A	Y	Z	Mice: ED_{50} ± SE, mg/kg/day × 5	Mice: Act. index	Hamsters: ED_{50} ± SE, mg/kg/day × 5	Hamsters: Act. index
21		CH_3		7.5 ± 1.7	1	>400.0	1
22		CH_2OH		9.0 ± 1.9	1	94.0 ± 38.8	>4
23*[b]		CH_3		>200.0	1	>400.0	1
24*		CH_2OH		36.2 ± 7.2	6	273.0 ± 63.7	2
25[c]		CH_3	$-NHCH_2CH_2N(C_2H_5)_2 \cdot HCl$	4.0 ± 0.9	1	42.5 ± 9.5	1
26		CH_2OH	$-NHCH_2CH_2N(C_2H_5)_2 \cdot (CH_3C_6H_4SO_3H)_2$	5.7 ± 1.0	2	5.1 ± 1.3	10

[a] See footnote *a*, Table I. [b] Also reported as Abbott A-16612. [c] Also reported as Hoechst S-616.

methyl-3-chloroanilines, and their hydroxylated derivatives. It was found that hydroxylation caused a one- to six fold enhancement of schistosomicidal activity in the mouse and a two- to 33-fold enhancement in the hamster. The purpose of this publication is to present the findings relating to the schistosomicidal activity in mice and hamsters of a number of xanthenones, 4-methyl-3-chloroanilines, and their hydroxylated derivatives.

Experimental Section

Procedures used for the infection of snails (*Australorbis gla-*

bratus Puerto Rican origin) and rodents (Swiss mice and Syrian hamsters) with *S. mansoni* (Puerto Rican strain) and for the testing and evaluating of schistosomicidal activity of compounds administered *via* the oral and/or parenteral routes have been previously described by Berberian and Freele.[7]

Results

Data obtained from 50 tests in Swiss mice and from 39 tests in hamsters are summarized in Tables I–IV. The tables present (a) structural formulas of parent compounds and their hydroxymethyl analogs, (b) calculated $ED_{50} \pm SE$ values derived from tests carried out in mice and hamsters, and (c) activity indices in mice and hamsters. Although results obtained with compounds marked with an asterisk were reported previously[4,5] they are included in the tables for purposes of comparison. Furthermore, after publication of the earlier results, many more animals were treated with these compounds and the revised $ED_{50} \pm SE$ values are more meaningful.

Infection Controls.—A total of 6868 live and seven dead schistosomes were recovered at autopsy from 371 infection control mice utilized in 50 tests; the average number of live schistosomes per mouse was 18.5. A total of 4564 live and no dead schistosomes were found at autopsy from 189 infection control hamsters utilized in 39 tests; the average number of schistosomes per hamster was 23.8.

Schistosomicidal Activity.—Data on the schistosomicidal activity for xanthenones and their hydroxymethyl analogs are presented in Tables I and II, and for 4-methyl-3-chloroanilines and their hydroxymethyl analogs in Tables III and IV.

Discussion

Since the discovery in 1918 by Christopherson[8] that tartar emetic was an effective schistosomicidal agent, the search for more active and less toxic compounds has been relentlessly pursued at an ever increasing tempo with the *S. mansoni* infection in Swiss mice being used as a working model for routine *in vivo* screening. Standen[9] estimated that probably as many as 250,000 chemicals have been tested to date in laboratory animals. Three nonmetallic schistosomicides of merit have emerged from the extensive researches of the past three decades: niridazole,[10] lucanthone hydrochloride, and hycanthone, the hydroxymethyl analog of lucanthone.

Data presented in this paper constituted an extension of our observations on the schistosomicidal activity of a number of hydroxylated derivatives of xanthenones and 4-methyl-3-chloroanilines. The findings have lent further support to the thesis that hydroxylation of an essential methyl group in the *para* position produces a significant degree of enhancement in schistosomicidal activity, *i.e.*, a one- to six fold enhancement of schistosomicidal effect in the mouse and a two- to 33-fold enhancement in the hamster.

Although active orally, lucanthone is totally ineffective parenterally, whereas hycanthone is effective parenterally as well as orally in mice, hamsters, and also in humans. Field studies in Brazil and South Africa have fully confirmed the results obtained in the laboratory. Katz, *et al.*,[11] treated 253 Brazilians infected with *S. mansoni*. They administered hycanthone to 52 patients as capsules, to 86 as enteric-coated tablets, to 55 intramuscularly in the form of its sulfamate salt, and to 59 patients intramuscularly in the form of its methanesulfonate salt. They reported hycanthone to be a "powerful and safe" antischistosomal agent regardless of the route of administration. Argento, *et al.*,[12] treated 211 patients with single or multiple intramuscular doses of hycanthone (2–3.5 mg/kg) and concluded that the intramuscular route was "the most promising because of the good tolerance and high rate of cure." More recently Clarke, *et al.*,[13] reported the results of their trials on a total of 97 South African Rhodesians who were found to be infected with either *Schistosoma hematobium* or *S. mansoni* or both. They obtained a highly satisfactory cure rate on both hematobium and mansoni infections. They considered the toxicity of hycanthone as "negligible" and concluded that a single intramuscular injection was the preferred mode of administration. They stated that hycanthone was one drug which could be widely used in mass chemotherapy and control of hematobium and mansoni infections. Both Brazilian and South African studies published hitherto and those underway have also confirmed our laboratory findings that a single intramuscular dose of hycanthone is as effective as a 3–5-day course of oral medication. Although ineffective against *Schistosoma japonicum* hycanthone comes close to fulfilling the speculative criteria of Fairley[14] and Newsome[15] for an ideal schistosomicide.

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