taneous) tumor at 50.0 mg/kg/day. However, introduction of substituents in the phenyl ring of this compound either retains or increases its activity against Dunning leukemia but lowers the activity against Walker 256 tumor.

Experimental Section

Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were taken in an open capillary tube in sulfuric acid bath and are uncorrected.

Ethyl N-(4-*p*-Tolyl-2-thiazolyl)malonamate.—A mixture of 1.9 g (0.01 mole) of 2-amino-4-*p*-tolylthiazole[13] and 9.6 g (0.06 mole) of diethyl malonate was refluxed for 2.5 hr in an oil bath at 160°. The reaction mixture was cooled, diluted with hexane, and kept in an ice box for 1 hr. The compound which separated on cooling was collected and recrystallized from EtOH to give 1.9 g (63%) of the ester, mp 147–148°. *Anal.* ($C_{15}H_{18}N_2O_3S$) C, H, N.

N-(4-*p*-Tolyl-2-thiazolyl)malonamic Acid Hydrazide.—A solution of 1.5 g (0.005 mole) of ethyl N-(4-*p*-tolyl-2-thiazolyl)-malonamate in a small quantity of EtOH was treated with 0.6 ml of 58% hydrazine hydrate and the solution was heated under reflux for 10 min. The reaction mixture gradually deposited a crystalline solid which was filtered off and washed with a little EtOH to give 1.1 g (76%) of the crude hydrazide. It was crystallized from EtOH–H_2O (1:1), mp 270° dec. *Anal.* ($C_{13}H_{14}N_4O_2S$) C, H, N.

Other malonamic acid esters and hydrazides were similarly prepared and are listed in Table I.

The following hydrazides were prepared as described in the literature: 4-nitrobenzoic acid hydrazide,[14] 3-chlorobenzoic acid hydrazide,[15] 3,4,5-trimethoxybenzoic acid hydrazide,[16] nicotinic acid hydrazide,[17] isonicotinic acid hydrazide,[18] picolinic acid hydrazide,[19] and 2-hydroxy-4,6-dimethylnicotinic acid hydrazide.[20,21]

4-[N,N-Bis(2-chloroethyl)amino]-*o*-anisaldehyde.—To 22 ml of DMF cooled in an ice bath was added 14 ml of $POCl_3$ with stirring at 7–10°. Then a mixture of 10.5 g (0.05 mole) of N,N-bis(2-hydroxyethyl)-*m*-anisidine[22] dissolved in 30 ml of DMF was added slowly at 5–10°. The mixture was then heated for 1 hr on a water bath and poured onto ice and kept overnight at 4°. The solid was filtered off, washed thoroughly with ice water, and dried. Crystallization from hexane yielded 11.0 g (80%) of the aldehyde mustard, mp 96–97°. *Anal.* ($C_{12}H_{15}Cl_2NO_2$) C, H, N.

The 2,4-dinitrophenylhydrazone, prepared in EtOH, was recrystallized from Me_2CO, mp 215–216°. *Anal.* ($C_{18}H_{19}Cl_2N_5O_5$) C, H, N.

Other aldehyde mustards employed in the present work are reported in the literature and were prepared according to the known methods.[5,12]

N-(4-Phenyl-2-thiazolyl)malonamic Acid {*p*-[N,N-Bis(2-chloroethyl)amino]benzylidene}hydrazide.—To a solution of 0.20 g (0.001 mole) of N-(4-phenyl-2-thiazolyl)malonamic acid hydrazide in a minimum of EtOH at 70° was added a solution of 0.25 g (0.001 mole) of the 4-[N,N-bis(2-chloroethyl)amino]-benzaldehyde[12] in EtOH. Two drops of concentrated HCl were then added to this solution and the mixture was allowed to stand. Within a short time, a crystalline solid separated out. This was filtered off and washed with a little EtOH to give 0.30 g (60% yield) of product, mp 223–224°. *Anal.* ($C_{23}H_{23}Cl_2N_5O_2S$) C, H, N.

All other benzylidenehydrazides were similarly prepared and are recorded in Tables II and III.

Acknowledgments.—The authors wish to thank Drs. H. B. Wood and H. W. Bond of the Cancer Chemotherapy National Service Center for their cooperation and for making available the screening data and Mr. M. T. Jaokar for the microanalysis. We are also grateful to Dr. H. I. Jhala, Director, Haffkine Institute, Bombay, for providing facilities to carry out the present work. One of the authors (M. G. D.) is greatly indebted to the Ministry of Education, Government of India, for the award of a scholarship.

(13) L. C. King and R. J. Hlavacek, *J. Am. Chem. Soc.*, **72**, 3722 (1950).
(14) M. Claesen, P. van Dijck, and H. Vanderhaeghe, *J. Pharm. Pharmacol.*, **6**, 127 (1954).
(15) T. Curtius and F. Foerster, *J. Prakt. Chem.*, **64**, 326 (1901).
(16) I. A. Pearl and D. L. Beyer, *J. Am. Chem. Soc.*, **77**, 3660 (1955).
(17) T. Curtius and E. Mohr, *Ber.*, **31**, 2493 (1898).
(18) H. Meyer and J. Mally, *Monatsh.*, **33**, 400 (1912).
(19) D. Libermann, N. Rist, F. Grumbach, M. Moyeux, B. Gauthier, A. Ronaix, J. Maillard, J. G. Himbert, and S. Cals, *Bull. Soc. Chim. France*, 1430 (1954).
(20) J. L. Greene, Jr., and J. A. Montgomery, *J. Med. Chem.*, **7**, 17 (1964).
(21) T. A. Geissman, M. J. Schlatter, I. D. Webb, and J. D. Roberts, *J. Org. Chem.*, **11**, 741 (1946).
(22) M. Freifelder and G. R. Stone, *ibid.*, **26**, 1477 (1961).

Some New Salts of Lucanthone as Potential Anticancer Agents

BENJAMIN PRESCOTT

U. S. Department of Health, Education and Welfare, Public Health Service, Laboratory of Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Received June 29, 1967

Since the report by Mauss, *et al.*,[1a] that lucanthone, 1-(2-diethylaminoethylamino)-4-methylthioxanthen-9-one,[1b] possessed schistosomicidal activity, numerous analogs have been synthesized. Many of the derivatives have also been tested against a variety of experimental tumors *in vitro* and *in vivo*. Hirschberg and coworkers[2] have reported that lucanthone exhibits antitumor activity against a variety of transplantable mouse tumors such as Sarcoma 180, lymphoid leukemia L1210 ascites, and Adenocarcinomas 755 and E0771. More recently, Blanz and French[3] also showed that lucanthone hydrochloride possessed antitumor activity when tested with a number of structural analogs in three tumor (Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210) mouse screening experiments. However, the hydrochloride of the chemotherapeutic agent is somewhat limited in usefulness by its high toxicity. For a number of years, we have studied the effects of numerous chemicals as potential detoxifying adjuvants for toxic chemotherapeutic agents. The results have indicated that certain sulfonic acids[4] possessed significant detoxifying action when administered concomitantly with the toxic chemotherapeutic agent (streptomycin), so that mice tolerated twice the lethal dose. This study stimulated our interest in the possibility of sulfonic acid salts of lucanthone as potential anticancer agents with maximum therapeutic effectiveness and with little or no toxicity. This report includes the preparation of five sulfonic acid salts of lucanthone with analyses and tests for acute toxicity in mice and *in vivo* antitumor activity of certain derivatives against Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210.

(1) (a) H. Mauss, H. Kolling, and R. Gönnert, *Med. Chem. Abhandl. Med.-Chem. Forschungsstaetten Farbenfabriken Bayer*, **5**, 185 (1956); (b) Miracil D®.
(2) E. Hirschberg, A. Gellhorn, M. R. Murray, and E. F. Elslager, *J. Natl. Cancer Inst.*, **22**, 567 (1959).
(3) E. Blanz and F. French, *J. Med. Chem.*, **6**, 185 (1963).
(4) B. Prescott and H. J. Stone, *Farmaco* (Pavia), *Ed. Sci.*, **21**, 471 (1966).

TABLE I

SULFONIC ACID SALTS OF LUCANTHONE. CHEMICAL AND PHYSICAL PROPERTIES

–SO$_3$H	Mp, °C[a]	Formula	Analyses
p-$NH_2C_6H_4$	183	$C_{26}H_{31}N_3O_4S_2$	C, H, N
m-$NH_2C_6H_4$	96–98	$C_{26}H_{31}N_3O_4S_2$	C, N; H[b]
o-$NH_2C_6H_4$	106	$C_{26}H_{31}N_3O_4S_2$	C, H, N
p-HOC_6H_4	141–143	$C_{26}H_{30}N_2O_5S_2$	C, H, N
1-$CH_3C_6H_2$-3-OH-4-$(CHCH_3)_2$	258	$C_{30}H_{38}N_2O_5S_2$	C, H, N

[a] All melting points are uncorrected and were determined on a Fischer-Johns melting point apparatus. [b] *Anal.* H: calcd, 6.08; found, 6.53.

Experimental Section

General Procedure.—A solution of the sodium salt of the sulfonic acid (0.1 mole) in distilled H_2O (200 ml) was added with stirring to an equimolecular proportion of lucanthone hydrochloride dissolved in distilled H_2O (2000 ml) cooled to 10–15°. The resulting mixture was stirred for 1 hr. In all instances crystals of the sulfonic acid salt of lucanthone began to separate immediately. The mixture was kept overnight in the cold room (4°). The crystalline precipitate was collected, washed with cold, distilled H_2O, and dried in air. One crystallization from 70% EtOH sufficed to give a pure product. The salts appeared as yellow-orange, fine, crystalline powders with consistently good yields from 90 to 95%. Table I gives a summary of the sulfonic acids used and the analytical results.

Acute Toxicity.—Intraperitoneal toxicity studies for the compounds were performed in the DBA strain of mice, as maintained at the National Institutes of Health, Bethesda, Md., according to a procedure described previously.[5] The results were judged by 72-hr survival. The mice treated with the *m*-aminobenzenesulfonic acid salt of lucanthone tolerated 1.5 g/kg of body weight. All of the mice tested with the other four preparations tolerated a dose of 2 g/kg. Thus, the compounds were of low toxicity compared with the parent compound, lucanthone hydrochloride. The highest dose of lucanthone hydrochloride tolerated by DBA mice was 250 mg/kg. It may be that the decrease in toxicity is due to the insolubility of the sulfonates. However, significant amounts of these compounds were detected in the blood plasma of the mice 24 hr after administration.

Antitumor Studies.—The compounds were tested for antitumor activity in the mouse screening program of the Cancer Chemotherapy National Service Center. The testing procedures employed have been described previously.[6] Among the five compounds, three were found to exhibit significant antitumor activity. The *p*-aminobenzenesulfonic acid, *m*-aminobenzenesulfonic acid, and the 5-hydroxy-*p*-cymene-2-sulfonic acid salts of lucanthone showed good inhibition against the L1210 mouse tumor systems. One preparation, the *p*-aminobenzenesulfonic

TABLE II

ANTITUMOR ACTIVITY OF CERTAIN SULFONIC ACID SALTS OF LUCANTHONE AGAINST SARCOMA 180, ADENOCARCINOMA 755, AND LEUKEMIA L1210

Salt of acid	Dose, mg/kg	Survivors	Animal wt dif, g test/control	Tumor wt, mg, or (survival, days) test/control	T/C, %
		Sarcoma 180			
HCl	65	4/6	−1.0	483/1209	39
	32.5	5/6	−0.8	1006/1209	83
	16.2	5/6	1.5	1148/1209	94
m-$NH_2C_6H_4SO_3H$	125	6/6	−1.4	593/1996	29
	125	5/6	−1.7	639/1890	33
		Adenocarcinoma 755			
HCl	90	7/10	−9.4	101/2092	4
	60	10/10	−7.0	200/2092	9
	40	10/10	−4.9	422/2092	20
	27	10/10	−2.9	1217/2092	58
	18	10/10	−3.2	1332/2092	63
p-$NH_2C_6H_4SO_3H$	110	10/10	−2.7	240/881	27
	82	10/10	−5.3	220/1321	16
		Leukemia L1210			
HCl	90	6/6	−4.2	(12.5/8.5)	147
	65	6/6	−2.7	(11.5/6.5)	176
	32.5	6/6	−0.8	(7.7/6.5)	118
	16.2	6/6	−0.8	(7.5/6.5)	115
p-$NH_2C_6H_4SO_3H$	165	6/6	−3.0	(17.5/9.1)	192
	110	6/6	−2.3	(14.3/9.1)	157
	73	6/6	−3.2	(8.8/9.1)	96
	51	6/6	−0.6	(11.0/9.1)	120
m-$NH_2C_6H_4SO_3H$	125	6/6	−2.9	(20.3/9.7)	209
	82.5	6/6	−2.9	(14.7/8.7)	168
	55	6/6	−1.7	(15.0/9.4)	159
	36.6	6/6	0.0	(9.7/8.7)	111
	24	6/6	0.3	(9.8/8.7)	112
1-$CH_3C_6H_2$-3-OH-4-$(CHCH_3)_2$-6-SO_3H	150	6/6	−0.5	(16.5/11.3)	146
	75	6/6	−0.3	(15.3/11.3)	135
	37.5	6/6	−0.5	(12.5/11.3)	110
	18.7	6/6	−0.9	(11.8/11.3)	104

(5) B. Prescott and C. P. Li, *J. Med. Chem.*, **7**, 383 (1964).

(6) J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *Cancer Res.*, **20**, 734 (1960).

acid salt, also showed inhibition against Ca755, and another, the *m*-aminobenzenesulfonic acid salt, possessed antitumor activity against the S180 mouse tumor system with the latter compound showing confirmed activity. Table II lists the antitumor testing data for the three active compounds, supplied by the Cancer Chemotherapy National Service Center.

Steroids. XXX.[1a] Some Indolocholestanes and Indoloandrostanes[1b,c]

NORMAN J. DOORENBOS[1d] AND MU TSU WU

School of Pharmacy, University of Maryland, Baltimore, Maryland 21201

Received September 13, 1967

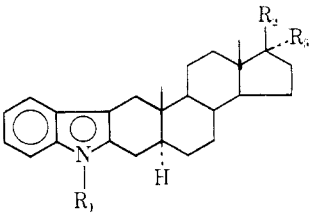
Interest in steroids to which heterocyclic rings are fused or attached developed in our laboratory[2] during a study of heterocyclic steroids of potential therapeutic value. One derivative of this type, 1′,4′,5′,6′-tetrahydropyrimidino[4,3-*a*]-5-cholestene,[2d] possesses significant antimicrobial,[3] antiinflammatory, hypocholesterolemic, hypotensive, and diuretic activities.[2d] Another derivative of this type, pyrazolo[3,2-*c*]-17α-methyl-5α-androstan-17β-ol,[4] possesses a high degree of separation of anabolic from androgenic activity and has been in clinical use for several years. Its discovery stimulated the synthesis of analogous heterocyclic steroids in several laboratories.[5] We wish to report the synthesis and preliminary biologic study of some indolocholestanes and indoloandrostanes.

Doree[6a] obtained indolo[3,4-*b*]-5β-cholest-3-ene in 1909 when he attempted to prepare the phenylhydrazone of 5β-cholestan-3-one (I) by reaction in hot glacial acetic acid. In 1935, indolo[3,2-*b*]-5α-cholest-2-ene (II) was synthesized in a similar manner.[6b] Fusion of the indole ring at positions 2,3 in the 5α series and 3,4 in the 5β series was confirmed recently by identifying the ozonolysis products of these compounds.[6c]

Indolo[3,2-*b*]-17α-methyl-5α-androst-2-en-17β-ol (VI) was prepared by a procedure similar to that used by Doree in the synthesis of indolocholestanes. The N-methyl derivatives (III, VII, and VIII) of II, VI, and I were prepared similarly by the reaction of N-methyl-N-phenylhydrazine with the appropriate steroid ketone (Tables I and II). Identical products were obtained in somewhat lower yields by adding methyl iodide to solutions of II, VI, or I and sodamide in dry dioxane–liquid ammonia. N-Aminoindolo[3,2-*b*]-5α-cholest-2-ene (V) and N-aminoindolo[3,4-*b*]-5β-cholestene (X) were prepared from II and I by nitrosation and $LiAlH_4$ reduction. X was characterized further by reaction with acetic anhydride, N-acetamidoindolo-[3,4-*b*]-5β-cholest-3-ene (XI) being obtained.

Each of these steroids has the characteristic absorption of the indole ring at approximately 13.50 μ.[7] Each nitroso derivative has a strong peak at 6.95 μ which is consistent with NN=O.[8]

TABLE I



Compd	R_1	R_2	R_3	Method[a]	Yield, %	Solvent of recrystn	Mp, °C[b]	$[\alpha]^{25}D$, deg[c]	Formula	Analyses[d]
III	CH_3	C_8H_{17}	H	A	99	C_6H_6–MeOH	203–205	+64.0	$C_{34}H_{51}N$	C, H, N
IV	NO	C_8H_{17}	H	B	88	C_6H_6–petr ether (bp 30–60°)	130–132	+103.8	$C_{33}H_{48}N_2O$	C, H, N
V	NH_2	C_8H_{17}	H	C	89	C_6H_6–MeOH	223–224	+86.8	$C_{33}H_{50}N_2 \cdot H_2O$	H, N; C[e]
VI	H	OH	CH_3	D	70	MeOH–H_2O	233–234	+39.4	$C_{26}H_{35}NO$	H, N; C[f]
VII	CH_3	OH	CH_3	E	80	MeCN–MeOH	171–173	+48.3	$C_{27}H_{37}NO$	C, H, N

[a] Prepared as follows: A, treatment of 5α-cholestan-3-one with a sixfold excess of N-methyl-N-phenylhydrazine in HOAc at 95°; B, treatment of indolo[3,2-*b*]-5α-cholest-2-ene[6b] in HOAc–dioxane at 10° with a concentrated H_2O solution of KNO_2 in 10% excess; C, treatment of IV with $LiAlH_4$; D, treatment of 17α-methyl-5α-androstan-17β-ol-3-one with phenylhydrazine in HOAc at 95°; E, treatment of 17α-methyl-5α-androstan-17β-ol-3-one with N-methyl-N-phenylhydrazine in HOAc at 95°. [b] Melting points were taken on a Fisher-Johns apparatus and are corrected. [c] *c* 0.5, $CHCl_3$. [d] Analytical results for elements indicated were within ±0.4% of the theoretical values unless indicated otherwise. [e] C: calcd, 80.43; found, 79.93. [f] C: calcd, 82.71; found, 83.22.

Biological Testing.[9]—Each indole steroid (I-XI) described in this paper was screened for antimicrobial activity against gram-negative bacteria (*Escherichia coli*, *Salmonella typhosa*, and *Brucella abortus*), gram-

(1) (a) For paper XXIX in this series, see N. J. Doorenbos and R. K. Sharma, *J. Chromatog.*, **29**, 393 (1967). (b) Presented in part before the Division of Medicinal Chemistry at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 29, 1962. (c) Supported in part by a grant from Smith Kline and French Laboratories. (d) Address inquiries to N. J. D. at the School of Pharmacy, University of Mississippi, University, Miss. 38677.

(2) (a) N. J. Doorenbos and C. P. Dorn, *J. Pharm. Sci.*, **50**, 271 (1961); (b) N. J. Doorenbos and C. P. Dorn, *ibid.*, **51**, 414 (1962); (c) N. J. Doorenbos and C. P. Dorn, *ibid.*, **54**, 1219 (1965); (d) N. J. Doorenbos and M. T. Wu, *ibid.*, **54**, 1290 (1965); (e) N. J. Doorenbos and A. P. Shroff, *Steroids*, **5**, 399 (1965); (f) N. J. Doorenbos and L. Milewich, *J. Org. Chem.*, **31**, 3193 (1966).

(3) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Pharm. Sci.*, **53**, 1214 (1964).

(4) R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Am. Chem. Soc.*, **81**, 1513 (1959).

(5) P. de Ruggieri, G. Gandolfi, U. Guzzi, D. Chiaramonti, and C. Ferrari, *Farmaco* (Pavia), *Ed. Sci.*, **20**, 280 (1965).

(6) (a) C. Doree, *J. Chem. Soc.*, 638 (1909); (b) C. Doree and V. A. Petrow, *ibid.*, 1391 (1935); (c) Y. Ban and Y. Sato, *Chem. Pharm. Bull.* (Tokyo), **13**, 1073 (1965).

(7) A. F. Chaplin, D. H. Hey, and J. Honeyman, *J. Chem. Soc.*, 3191 (1959).

(8) R. N. Jones, Infrared Spectra of Organic Compounds, NRC Bulletin No. 6, National Research Council, Ottawa, Canada, 1959.

(9) (a) Antimicrobial assays were conducted by Dr. Rodney F. Smith in our laboratory. (b) The results of the endocrine assays were provided by Dr. Kerwin and Dr. Saunders of Smith Kline and French Laboratories.