Preparation and Screening of Aminoacridines for Induction of Lung Tumor Fluorescence in Rats

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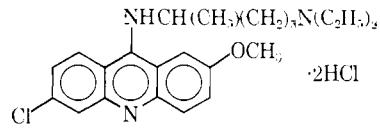
Certain aminoacridines induce fluorescence and are selectively concentrated in lung tumors in rats. To enable a more precise determination of the chemical configuration associated with these properties, a group of 86 acridine compounds was evaluated. Twenty-five aminoacridines produced intense fluorescence in lung tumors in rats following a single 1.5–20-mg subcutaneous dose. All active compounds contained a $NH–Y–NR_1R_2$ group attached to a 9-acridinyl, 9-acridinyl 10-oxide, benz[*b*]acridin-12-yl, benz[*c*]acridin-7-yl, or benzo[*b*][1,8]phenanthrolin-7-yl nucleus. The application of such compounds in fluorescent bronchoscopy or fluorescent exfoliative cytology are possibilities in lung cancer study. The potential use of radioisotope-tagged derivatives in scintillation scanning of organs such as the lung and liver also holds promise.

There has been a continuing search by many investigators for compounds that would localize to a greater extent in tumors than in surrounding normal tissues. If such a compound were made radioactive, it might be useful in the diagnosis and/or therapy of internal cancer.

Several years ago Ackerman and Shemesh[2] observed that certain aminoacridine compounds such as quinacrine (I) induce fluorescence in implanted lung tumors in rats and are concentrated selectively in tumor tissue. Thus, localization of quinacrine in Walker carcinosarcoma 256 and Novikoff hepatoma tumors implanted into rat lungs was noted by ultraviolet light visualization following the administration of a single 5-mg subcutaneous dose. Additional studies were performed with samples of radioactive "iodoquinacrine" of unknown structure which were prepared by the iodination of quinacrine with $^{125}I_2$ and $^{131}I_2$, respectively.[2] Once again the lung tumors fluoresced brightly and were clearly identifiable on radioautographs. Concentration of radioactivity in the lung tumor averaged five times higher than the concentration in the surrounding normal lung tissue, thus confirming earlier estimates of selective uptake based on fluorescence measurements.[2]



I

In order to define more precisely the chemical configuration that is associated with the induction of lung tumor fluorescence, a group of 86 acridine compounds was screened for this property.

Chemistry.—A majority of the aminoacridine compounds included in the present study (Tables I–IX) were described previously in connection with the synthesis of potential antimalarial,[3–14] antiamebic,[10,11,13–19] anthelmintic,[10,14,20] antibacterial,[14,21] and antifungal[10,14,22] agents. The other 9-(mono- and -dialkylaminoalkylamino)acridines (V) listed in Table X were prepared by the condensation of a substituted 9-chloroacridine (IV)[4,10] with the appropriate diamine, or by ring-closure of an N-(mono- or -dialkylaminoalkyl)-2-anilinobenzamide (III). The latter route was especially useful for the preparation of the 3,6-disubstituted 9-aminoacridines, since the N-(*m*-substituted phenyl)anthranilamides with bulky side chains ring-closed predominantly in the *para* position, whereas the

(1) (a) This investigation was supported in part by the Jane Coffin Childs Memorial Fund for Medical Research. (b) Faculty Research Associate of the American Cancer Society.

(2) N. B. Ackerman and A. Shemesh, *J. Am. Med. Assoc.*, **187**, 832 (1964).

(3) F. Y. Wiselogle, "A Survey of Antimalarial Drugs, 1941-1945," Vol. II, J. T. Edwards, Ann Arbor, Mich., 1946.

(4) A. Albert, "The Acridines," 2nd ed, Edward Arnold Ltd., London, 1966.

(5) E. R. Shepard and H. A. Shonle, *J. Am. Chem. Soc.*, **70**, 1979 (1948).

(6) G. B. Bachman and G. M. Picha, *ibid.*, **68**, 1599 (1946).

(7) J. H. Burckhalter, E. M. Jones, W. F. Holcomb, and L. A. Sweet, *ibid.*, **65**, 2012 (1943).

(8) J. H. Burckhalter, F. H. Tendick, E. M. Jones, P. A. Jones, W. F. Holcomb, and A. L. Rawlins, *ibid.*, **70**, 1363 (1948).

(9) W. Huber, R. K. Bair, and S. C. Laskowski, *ibid.*, **67**, 1619 (1945).

(10) E. F. Elslager, R. E. Bowman, F. H. Tendick, D. J. Tivey, and D. F. Worth, *J. Med. Pharm. Chem.*, **5**, 1159 (1962).

(11) E. F. Elslager and F. H. Tendick, *ibid.*, **5**, 1153 (1962).

(12) H. Medenwald, *Med. Chem. Abhandl. Med-Chem. Forschungsstaetten Farbenfabriken Bayer*, **5**, 206 (1956).

(13) E. F. Elslager, E. A. Weinstein, and D. F. Worth, presented in part at the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964.

(14) E. F. Elslager, F. W. Short, F. H. Tendick, and D. F. Worth, unpublished data.

(15) E. F. Elslager, E. L. Benton, F. W. Short, and F. H. Tendick, *J. Am. Chem. Soc.*, **78**, 3453 (1956).

(16) N. Fisher, C. S. Franklin, E. N. Morgan, and D. J. Tivey, *J. Chem. Soc.*, 1411 (1958).

(17) E. F. Elslager and F. H. Tendick, *J. Med. Pharm. Chem.*, **5**, 546 (1962).

(18) E. F. Elslager, A. M. Moore, F. W. Short, M. J. Sullivan, and F. H. Tendick, *J. Am. Chem. Soc.*, **79**, 4699 (1957).

(19) F. W. Short, E. F. Elslager, A. M. Moore, M. J. Sullivan, and F. H. Tendick, *ibid.*, **80**, 223 (1958).

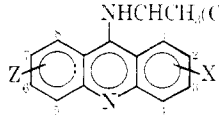
(20) F. H. Tendick, P. E. Thompson, and E. F. Elslager, U. S. Patent 3,012,036 (1961).

(21) E. F. Elslager and F. H. Tendick, *J. Med. Pharm. Chem.*, **5**, 1149 (1962).

(22) F. H. Tendick, P. E. Thompson, and E. F. Elslager, U. S. Patent 3,017,413 (1962).

TABLE I

EFFECTS OF 9-(4-DIETHYLAMINO-1-METHYLBUTYLAMINO)ACRIDINES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS

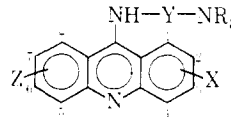


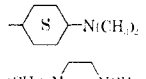
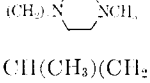
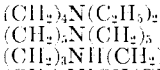
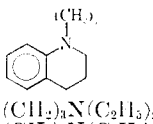
No.	X, Z	Formula	Ref	Activity[c]
1	2-Br, 4-CH_3	$C_{23}H_{30}BrN_3 \cdot 2HCl$[a]	5	++
2	3-Cl, 6-CH_3	$C_{23}H_{30}ClN_3 \cdot 2HCl \cdot 0.5H_2O$	Table X	+++
3	2-OCH_3, 6-Cl (quinacrine)	$C_{23}H_{30}ClN_3O \cdot 2HCl$[b]	4, 5	+++
4	3-Cl, 6-OCH_3	$C_{23}H_{30}ClN_3O \cdot 2HCl \cdot 0.5H_2O$	Table X	+++
5	2-OCH_3, 6-I ("iodoquinacrine")	$C_{23}H_{30}IN_3O \cdot 2HCl \cdot 0.5H_2O$	12	+++
6	2,3-$(CH_3)_2$, 7-OCH_3	$C_{25}H_{35}N_3O$	3	+++
7	3-Cl, 6-OC_6H_4-*p*-Cl	$C_{28}H_{31}Cl_2N_3O \cdot 2HCl$	Table X	±
8	3-Cl, 6-OC_6H_5	$C_{28}H_{32}ClN_3O \cdot 2HCl \cdot 0.5H_2O$	Table X	–

[a] Obtained through the courtesy of Dr. John A. Leighty, The Lilly Research Laboratories, Indianapolis, Ind. [b] Sample provided through the courtesy of Dr. R. O. Clinton, Sterling-Winthrop Research Institute, Rensselaer, N. Y. [c] Activity rating is assigned as follows: –, no fluorescence at 5 mg; ±, questionable fluorescence at 5 mg; +, no fluorescence at 5 mg, intense fluorescence at 20 mg; ++, intense fluorescence at 5 mg; +++, intense fluorescence at 1.5 mg.

TABLE II

EFFECTS OF SUBSTITUTED 9-(MONO- AND -DIALKYLAMINOALKYLAMINO)ACRIDINES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS



No.	YNR$_1$R$_2$	X, Z	Formula	Ref	Activity[h]
9	$(CH_2)_2NH(CH_2)_2OH$	2-OCH_3	$C_{18}H_{21}N_3O_2$	Table X	–
10	$(CH_2)_3NHCH(CH_3)_2$	3,6-Cl_2	$C_{19}H_{21}Cl_2N_3 \cdot 2HCl$	Table X	–
11	$(CH_2)_3NH(CH_2)_2OH$	2-OCH_3	$C_{19}H_{23}N_3O_2 \cdot 2HCl \cdot 0.25H_2O$	Table X	–
12	$(CH_2)_3N(C_2H_5)_2$	3,6-Cl_2	$C_{20}H_{23}Cl_2N_3 \cdot 2HCl$	Table X	++
13	$CH_2CHOHCH_2N(C_2H_5)_2$	3,6-Cl_2	$C_{20}H_{23}Cl_2N_3O \cdot 2HCl \cdot H_2O$	Table X	+++
14	$(CH_2)_3N(C_2H_5)CH_2CH_2OH$	3,6-Cl_2	$C_{20}H_{23}Cl_2N_3O \cdot 2HCl \cdot 0.5H_2O$	Table X	–
15	$(CH_2)_3NH(CH_2)_3NH_2$	2-OCH_3, 6-Cl	$C_{20}H_{25}ClN_4O \cdot C_6H_8O_7$[a]	3	–
16	$(CH_2)_3N(CH_2CH_2OH)_2$	3-Cl, 6-CF_3	$C_{21}H_{23}ClF_3N_3O_2 \cdot 2HCl \cdot 1.5H_2O$	Table X	±
17	$CH_2CHOHCH_2N(C_2H_5)_2$	2-OCH_3, 6-NO_2	$C_{21}H_{26}N_4O_4 \cdot 2HCl \cdot 2H_2O$	Table X	+++
18		2-OCH_3, 6-Cl	$C_{22}H_{26}ClN_3O \cdot 2HCl$[b]	3	++
19		2-OCH_3, 6-Cl	$C_{22}H_{27}ClN_4O \cdot 3HCl \cdot 2H_2O$	Table X	–
20		3,6-Cl_2	$C_{22}H_{27}Cl_2N_3O \cdot 2HCl \cdot H_2O$	10	+++
21	$(CH_2)_4N(C_2H_5)_2$	2-OCH_3, 6-Cl	$C_{22}H_{28}ClN_3O \cdot 2HCl \cdot 2H_2O$[c,d]	9	++
22	$(CH_2)_5N(CH_3)_5$	3,6-Cl_2	$C_{23}H_{27}Cl_2N_3 \cdot 2HCl$	Table X	+++
23	$(CH_2)_3NH(CH_2)_7CH_3$	3,6-Cl_2	$C_{24}H_{31}Cl_2N_3 \cdot 2HCl \cdot H_2O$	Table X	–
24	$(CH_2)_4N[CH(CH_3)_2]_2$	2-OCH_3, 6-Cl	$C_{24}H_{32}ClN_3O \cdot 2HCl \cdot 2H_2O$[e]	3	++
25	$(CH_2)_3N(C_2H_5)_2$	2-$(CH_2)_3CH_3$	$C_{24}H_{33}N_3 \cdot 2HCl \cdot 0.5H_2O$	*f*	+++
26	$(CH_2)_3NH(CH_2)_7CH_3$	3-Cl, 6-CF_3	$C_{25}H_{31}ClF_3N_3 \cdot 2HCl \cdot 0.5H_2O$	Table X	–
27		2-OCH_3, 6-Cl	$C_{26}H_{26}ClN_3O$	3	–
28	$(CH_2)_4N(C_2H_5)_2$	3-Cl, 6-OC_6H_5	$C_{26}H_{28}ClN_3O \cdot 2HCl \cdot 0.5H_2O$	Table X	–
29	$(CH_2)_3N(C_2H_5)(CH_2)_3NHC_{13}H_6Cl_2N$[g]	3,6-Cl_2	$C_{34}H_{31}Cl_4N_5 \cdot 3HCl$	Table X	–

[a] Monocitrate. [b] Obtained through the courtesy of Mr. F. J. Murray, The Wm. S. Merrill Co., Cincinnati, Ohio. [c] Supplied through the courtesy of Dr. R. O. Clinton, Sterling-Winthrop Research Institute, Rensselaer, N. Y. [d] W. Huber, R. K. Bair, and S. C. Laskowski, *J. Am. Chem. Soc.*, **67**, 1619 (1945). [e] Supplied through the courtesy of Dr. John A. Leighty, The Lilly Research Laboratories, Indianapolis, Ind. [f] Personal communication, Dr. Alfred Campbell, Parke, Davis and Co., Ann Arbor, Mich. [g] $C_{13}H_6Cl_2N$ represents the 3,6-dichloroacridin-9-yl radical. [h] See footnote *c*, Table I.

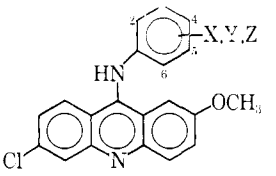
corresponding acid chlorides gave a mixture of the 1,6- and 3,6-disubstituted 9-chloroacridines which was difficult to separate (Scheme I).

Condensation of the potassium salt of the appropriate *o*-chlorobenzoic acid with the requisite aniline derivative gave the corresponding N-phenylanthranilic acids (II).[4,10] Although earlier attempts[23] to prepare 3,6-dichloro-9-aminoacridines *via* 4-chloro-N-(*m*-chlorophenyl)anthranilic acid were abandoned because of poor yields (5.8%) encountered in the Ullmann procedure,[23] this route was used extensively in the current work following the discovery that 4-chloro-N-(*m*-chlorophenyl)anthranilic acid could be readily prepared in good yield (42–53%) utilizing a modification of the

(23) D. P. Spalding, G. W. Moersch, H. S. Mosher, and F. C. Whitmore, *J. Am. Chem. Soc.*, **68**, 1596 (1946).

TABLE III

EFFECTS OF 9-ANILINO-6-CHLORO-2-METHOXYACRIDINE DERIVATIVES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS

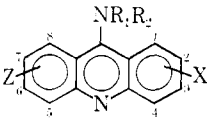


No.	X, Y, Z	Formula	Ref	Activity[a]
30	4-OH	$C_{20}H_{15}ClN_2O_2$	3, 8	–
31	4-$N(C_2H_5)_2$	$C_{24}H_{24}ClN_3O$	3, 7	–
32	3-$CH_2N(CH_2CH_2Cl)_2$, 4-OH	$C_{25}H_{24}Cl_3N_3O_2 \cdot 2HCl$	14	–
33	3-$CH_2N(C_2H_5)_2$, 4-OH	$C_{25}H_{26}ClN_3O_2 \cdot 2HCl$	3, 8	–
34	3-$CH_2N(C_2H_5)_2$, 4-OCH_3	$C_{26}H_{28}ClN_3O_2 \cdot 2HCl \cdot 0.5H_2O$	3, 8	–
35	3-$CH_2N(C_2H_5)_2$, 4-OH, 5-$CH_2CH{=}CH_2$	$C_{28}H_{30}ClN_3O_2 \cdot 2HCl$	3, 8	–
36	3-$CH_2N(CH_2)_5$, 4-OH, 5-$CH_2CH{=}CH_2$	$C_{29}H_{30}ClN_3O_2$	3, 8	–
37	3-$CH_2N[(CH_2)_3CH_3]_2$, 4-OH	$C_{29}H_{34}ClN_3O_2 \cdot 2HCl$	3, 8	–
38	2-OH, 3,5-$[CH_2N(C_2H_5)_2]_2$	$C_{30}H_{37}ClN_4O_2 \cdot 3HCl$	3, 8	–
39	3-$CH_2N[(CH_2)_5CH_3]_2$, 4-OH	$C_{33}H_{42}ClN_3O_2 \cdot 2HCl$	3, 8	–

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

TABLE IV

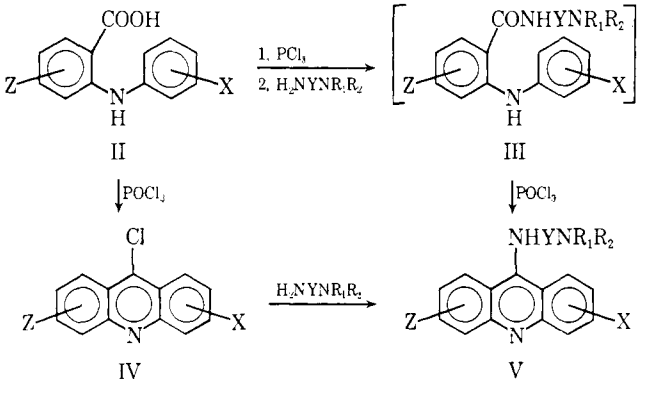
EFFECTS OF OTHER 9-AMINOACRIDINES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS



No.	NR_1R_2	X, Z	Formula	Ref	Activity[b]
40	NH_2	H	$C_{13}H_{10}N_2 \cdot HCl$[a]	4	–
41	$NH(CH_2)_3NHN(CH_3)_2$	2-OCH_3, 6-Cl	$C_{15}H_{23}ClN_4O \cdot 2HCl \cdot H_2O$	13	–
42	$NH(CH_2)_2N(CH_2CH_2OH)COCHCl_2$	2-OCH_3, 6-Cl	$C_{20}H_{26}Cl_3N_3O_2 \cdot 1.5H_2O$	15	–
43	$NHCH_2C_6H_4$-*o*-Cl	2-OCH_3, 6-Cl	$C_{21}H_{18}Cl_2N_2O \cdot HCl$	*c*	–
44	$NHNHSO_2C_6H_4$-*p*-CH_3	2-OCH_3, 6-Cl	$C_{21}H_{18}ClN_3O_3S \cdot HCl$	14	–
45	$NCOCH_3(CH_2)_3N(C_2H_5)_2$	2-OCH_3, 6-Cl	$C_{23}H_{28}ClN_3O_2$	14	–

[a] Obtained through the courtesy of Dr. R. O. Clinton, Sterling-Winthrop Research Institute, Rensselaer, N. Y. [b] See footnote *c*, Table I. [c] See Experimental Section.

SCHEME I



procedure employed by Hurd and Fancher[24] for related compounds.

The N-phenylanthranilic acids (II) were converted to the N-phenylanthraniloyl chlorides by the action of PCl_5, or ring-closed with $POCl_3$ to the 9-chloroacridines (IV). The 9-aminoacridines (V) were prepared by heating the appropriate 9-chloroacridine[4,10] and diamine in phenol (procedure I), or by allowing the acid chloride to react with the appropriate diamine followed by closure of the resulting amide III with $POCl_3$ (procedure II). The intermediate diamines are either commercially available or were described previously.[10,18,19]

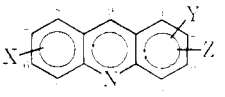
Pharmacological Method.—Studies were performed on female Sprague–Dawley albino rats using Novikoff hepatoma and Walker carcinosarcoma 256 tumors. Lung tumors were produced by intravenous injection of saline suspensions of homogenated tumors.[2]

The acridine compounds (Tables I–IX) were screened using two to four animals per drug. In most instances the drugs were evaluated against both tumors. Two per cent aqueous or propylene glycol solutions were prepared. In routine tests, the experimental animals were given a single 5-mg dose of drug subcutaneously and were sacrificed 24–48 hr later. In some instances, aminoacridine compounds which proved to be inactive at the 5-mg dose were tested at 20 mg. Compounds active at 5 mg were subsequently evaluated at a dose of 1.5 mg. Lungs containing tumor implants were removed and examined visually under ultraviolet light stimulation, using a Burton Model 1910 ultraviolet lamp which has a maximum emission at the long-wave band of 3660 Å. Control animals with lung tumors were not given aminoacridines but were examined in a similar manner. The color and intensity of any fluorescence present in the tumors were noted. Prior to *in vivo* studies, it was established that solutions of each of the aminoacridines emitted a bright yellow-green fluorescence under ultraviolet light stimulation.

(24) C. D. Hurd and O. E. Fancher, *J. Am. Chem. Soc.*, **69**, 716 (1947).

TABLE V

EFFECTS OF MISCELLANEOUS ACRIDINE DERIVATIVES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS

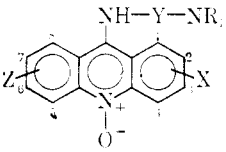


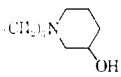
No.	X, Y, Z	Formula	Ref	Activity[b]
46	3,6-$(NH_2)_2$ (proflavine)	$C_{13}H_{11}N_3 \cdot 2HCl \cdot 2H_2O$	4	--
47	2-OCH_3, 6-Cl, 9-SH	$C_{14}H_{10}ClNOS$[a]	4	--
48	3,6-$[N(CH_3)_2]_2$ (acridine orange)	$C_{17}H_{19}N_3 \cdot 2HCl$	4	--
49	9-$(CH_2)_3N(CH_3)_2$	$C_{18}H_{29}N_2 \cdot 2HCl \cdot 2H_2O$	16	--
50	9-$(CH_2)_3N^+(CH_3)_3$	$C_{19}H_{33}N_2^+ \cdot OSO_2OCH_3^- \cdot H_2O$	16	--
51	9-$(CH_2)_4N^+(CH_3)_3$, 10-CH_3^-	$C_{21}H_{35}N_2^{2+} \cdot 2OSO_2OCH_3^- \cdot 0.5H_2O$	16	--

[a] Obtained through the courtesy of Mr. Julian E. Philip, Abbott Laboratories, North Chicago, Ill. [b] See footnote *c*, Table I.

TABLE VI

EFFECTS OF SUBSTITUTED 9-(MONO- AND -DIALKYLAMINOALKYLAMINO)ACRIDINE 10-OXIDES[19] ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS



No.	YNR_1R_2	X, Z	Formula	Activity[a]
52	$(CH_2)_2N(CH_3)_2$	2-OCH_3, 6-Cl	$C_{18}H_{29}ClN_4O_2 \cdot 2HCl \cdot 1.33H_2O$	+++
53	$(CH_2)_3N(C_2H_5)_2$	3-Cl	$C_{20}H_{24}ClN_3O \cdot 2HCl \cdot 0.75H_2O$	++
54		3-Cl	$C_{21}H_{24}ClN_3O_2$	--
55	$(CH_2)_2N(CH_2CH{=}CH_2)_2$	2-OCH_3, 6-Cl	$C_{22}H_{24}ClN_3O_2 \cdot 2HCl \cdot H_2O$	++
56	$(CH_2)_2N(CH_2)_4$	3-Cl	$C_{23}H_{26}ClN_3O \cdot 2HCl \cdot 0.75H_2O$	--
57	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$ (quinacrine 10-oxide)	2-OCH_3, 6-Cl	$C_{24}H_{30}ClN_3O_2 \cdot 2HCl$	+++
58	$CH(CH_3)(CH_2)_3N(CH_2CH_2OH)_2$	2-OCH_3, 6-Cl	$C_{24}H_{30}ClN_3O_4 \cdot 2HCl \cdot 0.5H_2O$	+
59	$(CH_2)_3NHCH[CH_2N(CH_3)_2]_2$	2-OCH_3, 6-Cl	$C_{24}H_{34}ClN_5O_2 \cdot 4HCl \cdot 3.25H_2O$	--
60	$(CH_2)_3NH(CH_2)_7CH_3$	2-OCH_3, 6-Cl	$C_{25}H_{44}ClN_3O_2 \cdot 2HCl$	--
61	$(CH_2)_3N(CH_3)(CH_2)_9CH_3$	3-Cl	$C_{27}H_{38}ClN_3O \cdot 2HCl \cdot H_2O$	--
62	$(CH_2)_3NH(CH_2)_3N(CH_2CH_2OH)_2$	3-Cl	$C_{23}H_{31}ClN_4O_3 \cdot 3HCl \cdot 0.5H_2O$	--

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

Results.—Twenty-five aminoacridines among the 86 compounds tested produced intense yellow-green fluorescence in lung tumors following a single 1.5–20-mg subcutaneous dose (Tables I–IX). No fluorescence was induced in lung tumors by the other 61 acridine compounds. The bright yellow-green fluorescence was not present in any of the control animals that did not receive a drug. Similar results were obtained in all studies with Walker and Novikoff tumor systems. An analysis of structure–activity relationships has enabled a preliminary determination of the chemical configuration associated with lung tumor fluorescence in rats.

(1) A $NHYNR_1R_2$ function is essential for activity (Tables I, II, VI, VIII, IX) where Y represents CH_2-$CHOHCH_2$, $(CH_2)_{2-5}$, $CHCH_3(CH_2)_3$, or $CH[(CH_2)_2]_2$-CH and NR_1R_2 is a lower *tertiary* amine group including $N(CH_3)_2$, $N(C_2H_5)_2$, $N[CH(CH_3)_2]_2$, $^-ON^+(C_2H_5)_2$, $N(CH_2)_5$, $N(CH_2CH{=}CH_2)_2$, $N(CH_2CH_2OH)_2$, or N-$(CH_2CH_2Cl)_2$. The presence of a third nitrogen atom anywhere in the side chain abolishes activity.

(2) The $NHYNR_1R_2$ function can be attached to a 9-acridinyl, 9-acridinyl 10-oxide, benz[*b*]acridin-12-yl, benz[*c*]acridin-7-yl, or benzo[*b*][1,8]phenanthrolin-7-yl nucleus (Tables I, II, VI, VIII, IX).

(3) The acridine nucleus can be substituted at positions 2-7 with one or more groups including CH_3, $(CH_2)_3CH_3$, Cl, Br, I, OCH_3, and NO_2 (Tables I, II, VI, VIII, IX). Derivatives with bulky substituents such as OC_6H_5 or OC_6H_4-*p*-Cl were inactive.

(4) All other acridine compounds studied gave negative results, including 9-anilinoacridines (Tables III, VII, VIII, IX), other 9-aminoacridines of diverse structure (Tables IV, VII), and miscellaneous acridine derivatives such as proflavine and acridine orange (Table V).

Many of the 9-(dialkylaminoalkylamino)acridines, 4-(9-acridinylamino)-α-amino-*o*-cresols, and their N-oxides are potent antimalarials,[3,4,10,11,14] and both basic types stain and retard the growth of tumors in mice.[25] Therefore, it was surprising to find in the present study that only the 9-(dialkylaminoalkylamino)-acridines induced lung tumor fluorescence in rats, while the 4-(9-acridinylamino)-α-amino-*o*-cresols were inactive.

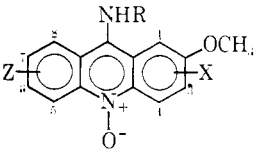
Discussion.—It has been conclusively demonstrated that various aminoacridines interact with the nucleic acids. Peacocke and Skerrett[26] proposed that profla-

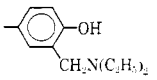
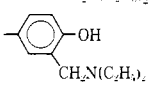
(25) M. R. Lewis and P. P. Gojanel, *Proc. Soc. Exptl. Biol. Med. Sci.*, **215**, 282 (1948).

(26) A. R. Peacocke and J. N. H. Skerrett, *Trans. Faraday Soc.*, **52**, 261 (1956).

TABLE VII

EFFECTS OF OTHER 2-METHOXY-9-AMINOACRIDINE 10-OXIDES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS

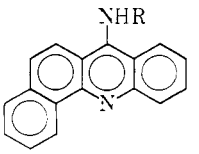


No.	R	X, Z	Formula	Ref	Activity[a]
63	$CH_2C_6H_4$-*o*-Cl	6-Cl	$C_{21}H_{16}Cl_2N_2O_2$	10	−
64	$(CH_2)_7CH_3$	6-Cl	$C_{22}H_{27}ClN_2O_2 \cdot 2HCl$	10	−
65		6-Cl	$C_{25}H_{26}ClN_3O_3 \cdot 2HCl \cdot 0.25H_2O$	11	−
66		3-OCH_3, 6-NO_2	$C_{26}H_{28}N_4O_6 \cdot 2HCl \cdot 0.75H_2O$	21	−

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

TABLE VIII

EFFECTS OF 7-AMINOBENZ[*c*]ACRIDINE DERIVATIVES ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS



No.	R	Formula	Ref	Activity[c]
67	H	$C_{17}H_{12}N_2$	4	−
68		$C_{23}H_{14}Cl_2N_2 \cdot HCl$	*d*	−
69	$(CH_2)_5CH_3$	$C_{23}H_{24}N_2$	18	−
70	$(CH_2)_3N(CH_2CH_2Cl)_2$	$C_{24}H_{25}Cl_2N_3 \cdot 2HCl$	14	+
71	$(CH_2)_3N(C_2H_5)_2$	$C_{24}H_{27}N_3 \cdot 2HCl \cdot 3H_2O$	6	++
72	$(CH_2)_3NHN(CH_2)_5$	$C_{25}H_{28}N_4 \cdot 2HCl \cdot H_2O$	13	−
73	$(CH_2)_3NH(CH_2)_3N(CH_3)_2$	$C_{25}H_{30}N_4 \cdot 3HCl \cdot 1.5H_2O$	14	−
74	$(CH_2)_5N(CH_2)_5$	$C_{27}H_{31}N_3$	18	++
75	$(CH_2)_3NH(CH_2)_7CH_3$	$C_{28}H_{35}N_3 \cdot 2C_7H_6O_3$[a]	19	−
76	$(CH_2)_3N[(CH_2)_2]_2NC_6H_5$	$C_{30}H_{30}N_4 \cdot 3HCl \cdot H_2O$	14	−
77	$(CH_2)_3NHC_{17}H_{10}N$[b]	$C_{39}H_{32}N_4 \cdot 2HCl \cdot 2.5H_2O$	14	−
78	$(CH_2)_3NH(CH_2)_4NHC_{17}H_{10}N$[b]	$C_{41}H_{37}N_5 \cdot 3HCl \cdot 4H_2O$	14	−
79	$(CH_2)_3O(CH_2)_2O(CH_2)_3NHC_{17}H_{10}N$[b]	$C_{42}H_{38}N_4O_2 \cdot 2HCl \cdot H_2O$	14	−
80	$(CH_2)_3N[CH_2CH_2N(C_2H_5)_2](CH_2)_3NHC_{17}H_{10}N$[b]	$C_{46}H_{48}N_6 \cdot 4HCl \cdot 4.5H_2O$	14	−

[a] Salicylate salt. [b] $C_{17}H_{10}N$ represents the benz[*c*]acridin-7-yl radical. [c] See footnote *c*, Table I. [d] See Experimental Section.

vine (**46**) is bound to DNA by two mechanisms, namely a strong first-order reaction that reaches equilibrium at one proflavine molecule per four or five nucleotides, and a weaker higher order process that results in the fixation of one proflavine molecule per nucleotide. Lerman[27] showed that the strong binding site involves the intercalation of one acridine molecule between two layers of base pairs, with the weaker binding site on the exterior of the DNA model. This picture of intercalation is based on measurements of viscosity and sedimentation of the DNA–acridine complex in dilute aqueous solution, X-ray diffraction patterns,[27,28] polarization of fluorescent light, flow dichroism,[29] small-angle X-ray scattering,[30] kinetic diazotization studies,[31] and free-energy calculations based on thermal denaturation.[32] Intercalation of proflavine into RNA has also been observed.[4]

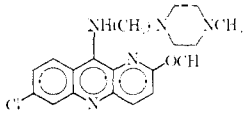
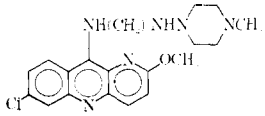
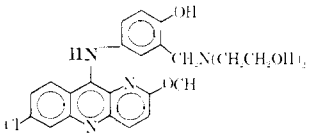
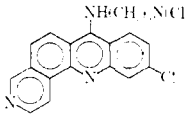
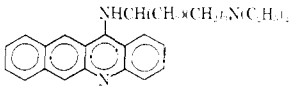
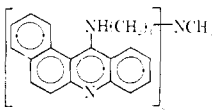
9-Aminoacridine (**40**) seems to intercalate as strongly as proflavine, whereas acridine orange (**48**) is much more weakly held than proflavine, presumably because of its lack of bondable hydrogen atoms.[4] Studies with a quinacrine–DNA complex are also compatible with the proflavine intercalation hypothesis.[29,33] However, the quinacrine–DNA complex is so tight as to prevent depolymerization of the DNA by deoxyribonuclease. Some of the aminoacridines have also been found to be mutagenic agents for bacteria, viruses, and yeast.[4,34]

Although simple aminoacridines (*i.e.*, proflavine, acridine orange, 9-aminoacridine) and quinacrine are all highly fluorescent and are known to interact with nucleic acids, only the basically substituted compounds induced lung tumor fluorescence in the present study. The reasons for the inability of simple acridines to induce lung tumor fluorescence are presently unknown, but presumably factors such as drug transport, binding strength, or quenching of fluorescence in tumor tissue are involved. Studies with labeled proflavine or acri-

(27) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961).
(28) D. M. Neville, Jr., and D. R. Davies, *ibid.*, **17**, 57 (1966).
(29) L. S. Lerman, *Proc. Natl. Acad. Sci. U. S.*, **49**, 94 (1963).
(30) V. Luzzati, F. Masson, and L. S. Lerman, *J. Mol. Biol.*, **3**, 634 (1961).
(31) L. S. Lerman, *ibid.*, **10**, 367 (1964).
(32) N. F. Gersch and D. O. Jordan, *ibid.*, **13**, 138 (1965).
(33) N. B. Kurnick and I. E. Radcliffe, *J. Lab. Clin. Med.*, **60**, 666 (1962).
(34) R. I. De Mars, *Nature*, **172**, 964 (1953).

TABLE IX

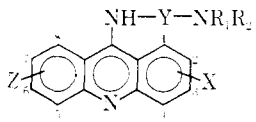
EFFECTS OF OTHER MONO- AND DIALKYLAMINOALKYLAMINO HETEROCYCLIC COMPOUNDS ON THE INDUCTION OF LUNG TUMOR FLUORESCENCE IN RATS

No.	Structure	Formula	Ref	Activity[a]
81		$C_{21}H_{26}ClN_5O \cdot 3HCl \cdot 2.5H_2O$	14	—
82		$C_{21}H_{27}ClN_6O \cdot 3HCl \cdot 3H_2O$	14	—
83		$C_{21}H_{25}ClN_4O_4 \cdot 2HCl \cdot H_2O$	14	—
84		$C_{26}H_{29}ClN_4 \cdot 3HCl \cdot 1.5H_2O$	17	++
85		$C_{26}H_{31}N_3 \cdot 2HBr \cdot H_2O$	14	++
86		$C_{41}H_{37}N_5 \cdot 3HCl \cdot 6.5H_2O$	14	—

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

TABLE X

PREPARATION OF 9-(MONO- AND -DIALKYLAMINOALKYLAMINO)ACRIDINES



No.	YNR$_1$R$_2$	X, Z	Mp, °C	Yield purified, %	Procedure	Purification solvent[a]	Formula[d]
9	$(CH_2)_2NH(CH_2)_2OH$	2-OCH_3	159–160	77	I	A	$C_{18}H_{21}N_3O_2$
10	$(CH_2)_3NHCH(CH_3)_2$	3,6-Cl_2	280 dec	44	II	B	$C_{19}H_{21}Cl_2N_3 \cdot 2HCl$
11	$(CH_2)_3NH(CH_2)_2OH$	2-OCH_3	215–217 dec	90	I	B	$C_{19}H_{23}N_3O_2 \cdot 2HCl \cdot 0.25H_2O$[b]
12	$(CH_2)_3N(C_2H_5)_2$	3,6-Cl_2	253 dec	72	II	B	$C_{20}H_{23}Cl_2N_3 \cdot 2HCl$[e]
14	$(CH_2)_3N(C_2H_5)CH_2CH_2OH$	3,6-Cl_2	239–240 dec	44	I	B	$C_{20}H_{23}Cl_2N_3O \cdot 2HCl \cdot 0.5H_2O$
87	$(CH_2)_3N(CH_2CH_2OH)_2$	3,6-Cl_2	227–228 dec	36	I	C	$C_{20}H_{23}Cl_2N_3O_2 \cdot 2HCl$
13	$CH_2CHOHCH_2N(C_2H_5)_2$	3,6-Cl_2	227–229 dec	62	II	C	$C_{20}H_{23}Cl_2N_3O \cdot 2HCl \cdot H_2O$
16	$(CH_2)_3N(CH_2CH_2OH)_2$	3-Cl, 6-CF_3	195 dec	36	I	D	$C_{21}H_{23}ClF_3N_3O_2 \cdot 2HCl \cdot 1.5H_2O$
88	$(CH_2)_3N(CH_2CH_2OH)_2$	3-Cl, 6-CH_3	237–239 dec	80	I	C	$C_{21}H_{26}ClN_3O_2 \cdot 2HCl$
22	$(CH_2)_5N(CH_2)_5$	3,6-Cl_2	278–279 dec	79	II	B	$C_{24}H_{27}Cl_2N_3 \cdot 2HCl$
2	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	3-Cl, 6-CH_3	240–241 dec	53	II	C	$C_{24}H_{30}ClN_3 \cdot 2HCl \cdot 0.5H_2O$
4	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	3-Cl, 6-OCH_3	215 dec	54	II	E	$C_{24}H_{30}ClN_3O \cdot 2HCl \cdot 0.5H_2O$
23	$(CH_2)_3NH(CH_2)_7CH_3$	3,6-Cl_2	283 dec	24	II	C	$C_{24}H_{31}Cl_2N_3 \cdot 2HCl \cdot H_2O$
26	$(CH_2)_3NH(CH_2)_7CH_3$	3-Cl, 6-CF_3	262–264	29	I	C	$C_{25}H_{31}ClF_3N_3 \cdot 2HCl \cdot 0.5H_2O$
28	$(CH_2)_3N(C_2H_5)_2$	3-Cl, 6-OC_6H_5	229–230 dec	53	II	F	$C_{28}H_{28}ClN_3O \cdot 2HCl \cdot 0.5H_2O$
7	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	3-Cl, 6-OC_6H_4-*p*-Cl	212–214 dec	50	II	B	$C_{28}H_{31}Cl_2N_3O \cdot 2HCl$[f]
89	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	3-Cl, 7-C_6H_5	75–80 dec	87	I	C	$C_{28}H_{32}ClN_3 \cdot 2HCl \cdot 1.5H_2O$
8	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	3-Cl, 6-OC_6H_5	246–247 dec	55	II	B	$C_{28}H_{32}ClN_3O \cdot 2HCl \cdot 0.5H_2O$
90	$(CH_2)_3NH(CH_2)_7CH_3$	3-Cl, 7-C_6H_5	253 dec	66	I	B	$C_{30}H_{36}ClN_3 \cdot 2HCl \cdot H_2O$[g]
29	$(CH_2)_3N(C_2H_5)((CH_2)_3NHC_{13}H_6Cl_2N^c$	3,6-Cl_2	294 dec	41	I	B	$C_{44}H_{51}Cl_4N_5 \cdot 3HCl$

[a] A, EtOH-*i*-PrOH; B, MeOH-Me_2CO; C, EtOH-Me_2CO; D, Me_2CO-Et_2O; E, *i*-PrOH-Et_2O; F, MeOH-C_6H_6. [b] *Anal.* H_2O: calcd, 1.12; found, 0.91. [c] $C_{13}H_6Cl_2N$ represents the 3,6-dichloroacridin-9-yl radical. [d] All compounds were analyzed for C, H, N. [e] *Anal.* C: calcd, 53.47; found, 53.93. [f] *Anal.* H: calcd, 5.84; found, 6.29. [g] *Anal.* N: calcd, 7.44; found, 7.86.

dine orange are planned to determine actual localization in the tumors.

There also appears to be a quenching of fluorescence of all aminoacridines by experimental tumors implanted in extrapulmonary sites. Recent studies[35] demonstrated an increased concentration of radioactivity in intrahepatic and in intragastric tumors following administration of radioiodinated quinacrine, in spite of the absence of observable fluorescent material in these tumors.

The aminoacridines, and particularly the basically substituted aminoacridines, may ultimately prove to be useful in the clinical diagnosis of cancer. Many of these compounds are relatively nontoxic[3,4,10,19] and several have been used in clinical medicine.[3,4] The application of fluorescent bronchoscopy or fluorescent exfoliative cytology are possibilities in lung cancer study. The use of radioisotope-tagged compounds in scintillation scanning of such organs as the lung and liver appears even more promising.

Experimental Section[36]

4-Chloro-N-(*m*-chlorophenyl)anthranilic Acid.—A mixture of 191 g (1 mole) of 2,4-dichlorobenzoic acid, 157 g (1.25 moles) of *m*-chloroaniline, 138 g (1 mole) of anhydrous K_2CO_3, 5 g of Cu powder, and 750 ml of dry 1-pentanol was heated at reflux with stirring for 5 hr. The mixture was cooled, 70 g of KOH and 500 ml of H_2O were added, and the mixture was steam distilled to remove volatile materials. The aqueous residue was filtered hot and the filtrate was made slightly acid with concentrated HCl. The crude acid was collected by filtration and was washed successively with warm water, hot 95% EtOH, and petroleum ether (bp 30–60°). The dried product was crystallized from chlorobenzene to give 144.5 g (51%) of nearly colorless crystals, mp 199–201° (lit.[23] mp 196–198°). In eight other similar 1-mole runs, the yields of purified acid ranged from 42 to 53%.

4-Chloro-N-(*m*-methoxyphenyl)anthranilic Acid.—Utilizing the general procedure described above for the preparation of 4-chloro-N-(*m*-chlorophenyl)anthranilic acid, 30.5 g (0.16 mole) of 2,4-dichlorobenzoic acid, 30.0 g (0.19 mole) of *m*-anisidine hydrochloride, and 55.0 g (0.44 mole) of anhydrous K_2CO_3 gave 7.5 g (14%) of product, pale yellow crystals from benzene, mp 163-165°. *Anal.* ($C_{14}H_{12}ClNO_3$) C, H.

4-Chloro-N-(α,α,α-trifluoro-*m*-tolyl)anthranilic Acid.—Utilizing the general procedure described above for the preparation of 4-chloro-N-(*m*-chlorophenyl)anthranilic acid, 573 g (3 moles) of 2,4-dichlorobenzoic acid, 483 g (3 moles) of *m*-aminobenzotrifluoride, and 207 g (1.5 moles) of anhydrous K_2CO_3 afforded 436 g (47%) of product, pale yellow crystals from $CHCl_3$, mp 208–210°. *Anal.* ($C_{14}H_9ClF_3NO_2$) C, H, N.

4-Chloro-N-(*m*-phenoxyphenyl)anthranilic Acid.—Utilizing the general procedure described above for the preparation of 4-chloro-N-(*m*-chlorophenyl)anthranilic acid, 80.0 g (0.42 mole) of 2,4-dichlorobenzoic acid, 77.3 g (0.42 mole) of 3-aminodiphenyl ether, and 58.0 g (0.42 mole) of anhydrous K_2CO_3 gave 49.4 g (35%) of product, pale green leaflets from chlorobenzene or aqueous ethanol (decolorizing charcoal), mp 168-169°. *Anal.* ($C_{19}H_{14}ClNO_3$) C, H, N.

4-Chloro-N-[*m*-(*p*-chlorophenoxy)phenyl]anthranilic Acid.—Utilizing the general procedure described above for the preparation of 4-chloro-N-(*m*-chlorophenyl)anthranilic acid, 84.0 g (0.44 mole) of 2,4-dichlorobenzoic acid, 96.3 g (0.44 mole) of 4′-chloro-3-aminodiphenyl ether, and 61.0 g (0.44 mole) of anhydrous K_2CO_3 afforded 40.7 g (25%) of product, pale yellow crystals from benzene, mp 162–163°. *Anal.* ($C_{19}H_{13}Cl_2NO_3$) C, H, N.

3,6,9-Trichloroacridine.—A mixture of 1 kg (3.55 moles) of 4-chloro-N-(*m*-chlorophenyl)anthranilic acid and 3.5 l. of $POCl_3$ in a 12-l. flask fitted with four large reflux condensers was *cautiously* warmed on a steam bath until the vigorous, exothermic reaction began. After the reaction had subsided, the mixture was stirred and heated on a steam bath for 3 hr and 3 l. of $POCl_3$ was removed *in vacuo*. The residue was poured slowly with vigorous stirring into a large excess of NH_4OH and ice. The crude trichloroacridine was collected by filtration, washed (H_2O), and dried *in vacuo* at 38°; weight 973 g. The product was extracted with several portions of boiling $CHCl_3$ and the combined extracts were concentrated, chilled, and filtered. The filter cake was washed thoroughly with petroleum ether and dried. Two crystallizations from chlorobenzene gave 256 g (26%) of pure product, mp 223–224° (lit.[23] mp 224-225°). In six smaller scale runs, the yields ranged from 22 to 31%.

3,9-Dichloro-6-(trifluoromethyl)acridine.—Utilizing the procedure described above for the preparation of 3,6,9-trichloroacridine, 50.0 g (0.16 mole) of 4-chloro-N-(α,α,α-trifluoro-*m*-tolyl)anthranilic acid, and 150 ml of $POCl_3$ gave 33.5 g of mixed chloroacridine isomers. Fractional crystallization of the mixture from benzene gave 20.1 g (40%) of pale yellow crystals, mp 159-160°. *Anal.* ($C_{14}H_6Cl_2F_3N$) C, H, N.

9-(Mono- and -Dialkylaminoalkylamino)acridines (Table X). Procedure I.—A mixture of 9.3 g (0.033 mole) of 3,6,9-trichloroacridine, 5.8 g (0.036 mole) of 2,2′-(3-aminopropylimino)diethanol, and 25 g of phenol was heated for 2 hr at 110° with stirring. The melt was allowed to cool to 75° and was diluted with a mixture of 20 ml of concentrated HCl and 160 ml of acetone. Several volumes of acetone were added, the mixture was chilled, and the supernatant was decanted. The residue was dissolved in H_2O, and the solution was treated with decolorizing charcoal and made alkaline with excess NH_4OH. After 1 hr, the waxy brown precipitate crystallized. Recrystallization from EtOH-Me_2CO-H_2O gave 5 g of yellow base, mp 158-160°. This was treated with excess ethanolic HCl to give 5.8 g (36%) of 2,2′-[3-(3,6-dichloroacridin-9-ylamino)propylimino]diethanol dihydrochloride (**87**), yellow crystals, mp 227-228° dec.

Procedure II.—4-Chloro-N-(*m*-chlorophenyl)anthranilic acid (16.9 g, 0.06 mole) was suspended in 140 ml of dry petroleum ether and treated portionwise with 13.8 g (0.066 mole) of PCl_5. The mixture was boiled under reflux for 30 min, decolorizing charcoal was added, and the mixture was filtered hot. Upon cooling, the crude 4-chloro-N-(*m*-chlorophenyl)anthraniloyl chloride crystallized and was collected by filtration and dried. Recrystallization from petroleum ether (decolorizing charcoal) gave 15.0 g (84%) of the purified material as canary yellow needles, mp 109-110°.

The acid chloride (15.0 g, 0.051 mole), N,N-diethyl-1,3-propanediamine (7.2 g, 0.056 mole), and 170 ml of dry C_6H_6 were heated under reflux for 40 min and cooled. $POCl_3$ (19 ml) was added dropwise with stirring and the mixture was boiled under reflux for 7 hr. A bright yellow solid began to separate in the first hr. The mixture was cooled, a few drops of water was added, and the benzene supernatant was decanted. The residue was taken up in 125 ml of boiling EtOH and diluted with 500 ml of ether. The mixture was chilled and the solid was collected by filtration and washed (Me_2CO). Crystallization from MeOH-Me_2CO gave 19.5 g (72%) of 3,6-dichloro-9-(3-diethylaminopropylamino)acridine dihydrochloride as fine yellow needles, mp 253° dec.

6-Chloro-9-(*o*-chlorobenzylamino)-2-methoxyacridine Monohydrochloride (43).—6,9-Dichloro-2-methoxyacridine (27.8 g, 0.1 mole) and *o*-chlorobenzylamine (14.0 g, 0.1 mole) were stirred and heated on a steam bath with 50 g of phenol for 3 hr, and the crude product was purified according to procedure I above. The hydrochloride salt was purified from $CHCl_3$–Me_2CO to give 16.5 g (39%) of yellow crystals, mp 300° dec. *Anal.* ($C_{21}H_{16}Cl_2N_2O\cdot HCl$) C, H, N.

7-(3,4-Dichloroanilino)benz[*c*]acridine Monohydrochloride (68).—7-Chlorobenz[*c*]acridine (15.8 g, 0.06 mole) and 3,4-dichloroaniline (9.7 g, 0.06 mole) were stirred and heated on a steam bath with 30 g of phenol for 3 hr, and the crude product was purified according to procedure I. The hydrochloride salt was purified from EtOH–Me_2CO to give 18.0 g (71%) of orange crystals, mp 300° dec. *Anal.* ($C_{23}H_{14}Cl_2N_2\cdot HCl$) H, N; C: calcd, 64.88; found, 64.43.

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(35) N. B. Ackerman, unpublished results.

(36) Melting points (corrected) were taken in open capillary tubes in a Thomas–Hoover capillary melting point apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.