for the Reimer–Tiemann reaction was used in each preparation.

For the 4,6-dihydroxy-2-thiopyrimidine-5-carboxaldehyde, the reaction mixture was cooled for a few hours in a refrigerator and filtered to give a mixture of potassium salts which included the salt of the aldehyde. This buff colored salt mixture was suspended in water to form a thick slurry and acidified with 6 *N* sulfuric acid until the color change to orange-red was complete. This suspension was heated to 60°, cooled, and filtered. The product was washed with approximately 1 *N* sulfuric acid and finally cold water until free of potassium. A final washing involving ether was used to facilitate drying the product. This unrecrystallized product was used in preparing derivatives.

TABLE II

HYDRAZONE DERIVATIVES OF 2-THIO-SUBSTITUTED PYRIMIDINE-5-CARBOXALDEHYDES

Aldehyde of[a]	Reagent used[b]	Procedure[c]	M.p., °C.	% N Calcd.	% N Found
A	2-Benzothiazolyl(H)	I	237 dec.	23.09	23.32
A	*p*-Bromo(PH)	II	261 dec.	17.29	17.43
A	*o*-Carboxy(PH)	II	242 dec.	19.30	19.28
A	*p*-Chloro(PH)	II	270 dec.	19.96	19.96
A	2,4-Dinitro(PH)	I	305 dec.	24.98	25.02
A	1,1-Diphenyl(H)	II	258 dec.	17.38	17.45
A	*p*-Fluoro(PH)	II	250 dec.	21.20	21.19
A	Nitroaminoguanidine[d]	I	>360	38.12	37.90
A	*p*-Nitro(PH)[e]	I	370 dec.	24.04	23.80
A	4-Phenylsemicarbazide	II	221 dec.	24.20	24.38
A	1-Naphthyl(H)	II	235 dec.	18.89	18.72
A	Benzoyl(H)	I	285 dec.	20.42	20.33
A	2,4-Dinitrophenyl-semicarbazide	I	260 dec.	25.91	25.75
A	*m*-Nitrobenzhydrazide	I	260 dec.	21.92	22.39
A	*p*-Nitro(PH)	II	297 dec.	24.04	24.15
A	*p*-Carboxy(PH)	I	297 dec.	19.30	19.70
B	*o*-Carboxy(PH)	II	322 dec.	18.41	18.61
A	2,4-Dinitro(PH)[f]	I	305 dec.	23.98	23.73
B	*p*-Nitro(PH)	I	322 dec.	22.94	23.13
C	2,4-Dinitro(PH)	I	319 dec.	22.21	21.90
C	*p*-Nitro(PH)	I	313 dec.	21.01	21.14

[a] A, 4-Hydroxy-2-thiopyrimidine; B, 4-hydroxy-6-methyl-2-thiopyrimidine; C, 4-hydroxy-6-propyl-2-thiopyrimidine. [b] H, Hydrazine; P, phenyl; D, hydrazide. [c] The following procedures were used in preparing derivatives: (1) all of the derivatives were prepared from solutions of the unisolated aldehyde. The Reimer–Tiemann reaction mixture was cooled and filtered to remove any precipitated salts. The filtrate was acidified with acetic acid and refiltered if necessary. To the hot, acidified filtrate was added an excess of the hydrazine in dilute acetic acid. The reaction mixture was boiled for 5–10 min. and then cooled. The product was collected on a filter, dried, and recrystallized. All derivatives were recrystallized from dimethylformamide and water unless otherwise specified. (2) The same procedure (1) except the hydrazine was first liberated from the hydrazine hydrochloride by treatment with dilute sodium hydroxide. [d] Not recrystallized. The sample was prepared from filtered solutions and washed with hot water. [e] *Anal.* Calcd. for $C_{11}H_9N_5O_3S$: C, 45.35; H, 3.11. Found: C, 45.46; H, 3.42. [f] *Anal.* Calcd. for $C_{12}H_{14}N_6O_5S$: C, 41.15; H, 2.85. Found: C, 41.26; H, 2.97.

Other pertinent experimental details for the aldehyde and derivative preparations are given as footnotes to Table I. The compounds were dried at 150° (1 mm.) for 8 hr. prior to analysis. In addition to the derivatives listed in the Tables a few other were prepared for which nonconfirmatory nitrogen analyses were obtained.

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Some Dichloroacetyl Derivatives and Their Antitumor Activity[1,2]

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Feitelson[3] and co-workers have shown that replacing the dichloroacetyl group with an acetyl group in chloramphenicol produces a sevenfold decrease in the potency of this antibiotic. It has also been shown that the compounds synthesized by Surrey,[4] with a dichloroacetyl group present, were strong amebicides. This evidence indicates the specificity of the dichloroacetyl group at specific receptor sites in the biological system.

Taking advantage of the increased potency potential of the dichloroacetyl group, Levi, *et al.*,[5] prepared N-dichloroacetyl-DL-serine and showed that it depressed the growth of Sarcoma 37 in mice, and in some cases the tumors sloughed off. Recent studies[6] report that this compound was effective in treating human tumors in combination with irradiation. Therefore, it was decided to prepare compounds which are related to physiologically active substances but which contain the dichloroacetyl group, and test them for carcinostatic activity on Sarcoma 180.

Inositol, a naturally occurring sugar in both plant and animal organisms, was tested by Laszlo and Leuchtenberger[7] and found to be effective in inhibiting the growth of Sarcoma 180 in mice. The hexadichloroacetate of inositol was prepared most effectively by the use of dichloroacetic anhydride.

Another compound which has shown anticancer possibilities is 9,10-phenanthraquinone. According to Powell,[8] when incorporated in the diet at the level of 1–2%, 9,10-phenanthraquinone inhibits the growth of several types of transplanted mouse tumors. In order to attach the dichloroacetyl group to the molecule, 2-amino-9,10-phenanthraquinone was first synthesized according to the procedure of Schmidt and Spoun[9] and the amine was then allowed to react with dichloroacetyl chloride.

Anthranilic acid has been demonstrated to be a precursor in the metabolic formation of tryptophan.[10] Therefore, the N-dichloroacetyl derivative of methyl anthranilate was prepared using dichloroacetyl chloride and methyl anthranilate.

(1) Parts of this work were first presented at the 10th and 11th Meetings in Miniature of the New York Association of the American Chemical Society Student Affiliates at St. Johns University, April, 1962, and Hofstra University, April, 1963, respectively.

(2) This work was supported by grants from the New York City Cancer Committee of the American Cancer Society, Inc., the Carl and Lily Pforzheimer Foundation, Inc., and the George N. Shuster Faculty Fellowship Fund.

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TABLE I

WEIGHT CHANGE AND TUMOR DIAMETER CHANGE IN S-180 AT DIFFERENT DOSAGES OF CHEMICALS TESTED

	Compd.	Dose, mg./kg./day	Av. tumor diam., cm. (T/C × 100)[a]	Wt. change, g. (T/C)[a]	Survivors
1	II	60	84	+1.9/+3.1	5/5
2	II	250	56	+1.1/+2.8	5/5
3	X[b]	500	81	+2.3/+1.9	5/5
4	XII	60	100	−0.7/+3.1	5/5
5	XII	125	86	+2.0/+2.8	5/5
6	XII	250	90	+0.2/+2.2	5/5
7	XII	500	63	+2.9/+1.9	5/5
8	VIII	60	72	−3.0/+3.1	4/4
9	VIII	125	60	+1.9/+1.2	5/5
10	VIII	125	60	+1.9/+1.2	5/5
11	II	30	138	+2.2/+3.1	5/5
	X	30			
12	II	63	55	+1.8/+1.9	5/5
	XII	63			
13	II	84	86	+0.6/+1.9	5/5
	XII	42			
14	II	42	80	+0.1/+1.8	5/5
	XII	84			
15	XI	125	84	+2.6/+1.9	5/5
16	II	63	91	+2.5/+2.8	5/5
	VIII	63			
17	XIV	125	102	+3.6/+1.2	4/4
18	XIV	250	73	0.0/+1.2	5/5
19	II	125	125	+1.3/+1.2	5/5
	XII	125			
20	II	42			
	XII	42	73	+2.6/+1.2	5/5
	VII	42			
21	II	42			
	X	42	74	+1.4/+1.2	4/4
	VII	42			
22	I	63	102	+3.7/+1.2	5/5
	XI	63			
23	II				
	XII	42	71	+3.0/+1.2	5/5
	XV	42			
24	XV	60	51	+1.8/+3.1	4/4
25	XV	60	30	−1.0/+2.8	4/4

[a] T/C = Test/Control. [b] These data are supported by tests at lower dosages.

Furst and Freedlander[11] tested 2-aminobiphenyl for possible carcinostatic activity on Sarcoma 37 but obtained negative results. The N-dichloroacetyl derivative of 2-aminobiphenyl was prepared to see if the presence of a dichloroacetyl group would encourage carcinostatic capabilities. This derivative was prepared by adding dichloroacetyl chloride to 2-aminobiphenyl.

Some authors[12,13] have suggested that cancer may be caused by metabolic aberration of specific hormones. Among these is Crile's "signal substance" theory.[14] Conversion of estriol to 16-ketoestradiol *in vivo* has been demonstrated.[15] Work on chick embryo tissue has shown that 16-ketoestradiol inhibits cell division.[16] The effectiveness of diethylstilbestrol and hexestrol in cancer chemotherapy has been demonstrated.[17–20] We have now prepared the dichloroacetyl derivatives of these synthetic hormones.

Experimental[21,22]

Inositol Hexadichloroacetate (II).—Inositol (I) (2 g.) was resublimed under 2 mm. pressure. This material was mixed with 25 g. of dichloroacetic anhydride (III).[23] The mixture was refluxed for 40 hr. and then poured into 100 ml. of water and 100 g. of ice. II settled out of this aqueous solution in 90% yield. Recrystallization from various solvents was not satisfactory but sublimation according to the method of Sweeny, *et al.*,[24] was found to be effective. After two successive sublimations a pure II was obtained in the form of colorless needles melting at 195.5–198°. The infrared spectrum[25] exhibited the expected strong halogen ester band at 1765 cm.$^{-1}$, the C–Cl stretch at 715–785 cm.$^{-1}$, and the acetate band from 1225–1250 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{12}Cl_{12}O_{12}$: C, 25.56; H, 1.44; Cl, 50.30. Found: C, 26.04; H, 1.45; Cl, 49.92.

N-2-Dichloroacetamido-9,10-phenanthraquinone (V).—2-Amino-9,10-phenanthraquinone (IV)[8] (2 g.) and 8 g. of dichloroacetyl chloride (VI) were added to 100 ml. of dioxane and the mixture was refluxed for 20 hr. It was then poured over 300 ml. of ice. The red precipitate of V which separated was recrystallized from hot dioxane–methanol solution with the addition of Norite A (yield 85%). The crystals so obtained melted at 269–276°. Further recrystallization from ethanol yielded pink needle-like crystals melting at 272.5–275.0° dec. The infrared spectrum exhibited the strong α-halogen ester band at 1745 cm.$^{-1}$, the strong C–Cl stretch from 700–800 cm.$^{-1}$, the acetate absorption from 1220–1250 cm.$^{-1}$, and strong secondary amide absorption of N–H linkage at 1665 cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_8Cl_2NO_5$: Cl, 21.22; Found: Cl, 21.21.

N-Dichloroacetamido Methyl Anthranilate (VIII).—Methyl anthranilate (VII) was purified by vacuum sublimation as mentioned above. A mixture of 5 g. of VII and 14.8 g. of VI was refluxed for 5 hr., and then poured over 300 ml. of ice and water. A dark brown oil separated, which upon stirring gave a white precipitate (VIII) (yield 91%), m.p. 54–55°. VIII was recrystallized from ethanol yielding white crystals which melted at 55–56°. The infrared spectrum exhibited the expected strong secondary amide absorption at 1665 cm.$^{-1}$, strong absorption at 700–800 cm.$^{-1}$ for C–Cl, and moderate to strong absorption for the acetate linkage at 1200–1240 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_9Cl_2NO_3$: Cl, 27.06; N, 5.35. Found: Cl, 27.01; N, 5.58.

Diethylstilbestrol Bisdichloroacetate (X).—Diethylstilbestrol (IX) (2.5 g.) and 12.5 g. of III were stirred, and pyridine was added slowly through a dropping funnel. At the end of the reaction, ethanol was added until a white precipitate (X) formed (yield 85%). The precipitate was recrystallized from ethanol to furnish a product which melted at 128.5–129°. The infrared spectrum exhibited strong absorption of α-halogen esters at 1754 cm.$^{-1}$, moderate acetate absorption at 1230–1250 cm.$^{-1}$, and a C–Cl stretch from 700–800 cm.$^{-1}$.

Anal. Calcd for $C_{22}H_{20}Cl_4O_4$: Cl, 28.93. Found: Cl, 28.84.

Hexestrol Bisdichloroacetate (XII).—This was prepared from hexestrol (XI) in the same manner as X (yield 85%), m.p. 149°. The infrared spectrum was the same as X.

Anal. Calcd. for $C_{22}H_{22}Cl_4O_4$: C, 53.68; Cl, 28.82. Found: C, 53.30; Cl, 28.36.

N-2-Dichloroacetamidobiphenyl (XIV).—2-Aminobiphenyl (XIII) was purified by sublimation at 2 mm. pressure. XIII (1.8 g.), 4.5 g. of VI, and 100 ml. of dioxane were refluxed for 5 hr.

(11) A. Furst and B. F. Freedlander, *Stanford Med. Bull.*, **10**, 308 (1952).
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(16) "Little and Ives Complete Book of Science" (adaptation of Van Nostrand's "Scientific Encyclopedia," 3rd Ed., 1958), Little and Ives, New York, N. Y., 1960, p. 1833.
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(21) Analyses by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.
(22) Melting points were taken on a Thomas-Hoover capillary melting point apparatus (Unimelt) and are corrected.
(23) Obtained from Distillation Products Industries, Rochester, N. Y.
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(25) Infrared work by Philip Pfeffer.

The resulting pale yellow solution was poured into 600 ml. of ice and water and XIV precipitated out (yield 95%). It was dried and sublimed at 3 mm. pressure. White crystals of XIV were obtained which melted at 104–105.5°. The infrared spectrum exhibited the expected strong secondary amide linkage at 1665 cm.$^{-1}$, strong acetate absorption at 1220 cm.$^{-1}$, and C–Cl absorption from 700–800 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{11}Cl_2NO$: Cl, 25.32. Found: Cl, 25.19.

Biological Results.—Screening of the compounds prepared was done with 2-month old Swiss Albino mice.[26] The compounds listed in Table I were screened in the Biology Department of Hunter College.[27] The procedure used was the established technique of the Sloan–Kettering Institute,[28] except that instead of injecting 24 hr. after implantation of tumors, injections began between 72 and 96 hr. after implantation. The vehicle used was Planters Peanut Oil. The mice were sacrificed the eighth day after implantation. Carcinostatic activity was obtained with II, VIII, XII, and a combination of II and XII at the specified dosages. Since we have had regression with 2-fluoro-9,10-phenanthraquinone (XV),[25] it was of interest to try a combination of XV, XII, and II. In a test using VIII at 125 mg./kg./day, the tumor on one of the test mice, which was definitely measurable, completely regressed by the eighth day. Dr. H. Christine Reilly of Sloan-Kettering Institute has obtained a tumor diameter measurement of T/C in cm. of 75% for a testing of XII at 250 mg./kg./day and 92% for V at 50 mg./kg./day.[29]

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(26) Mice, (18–20 g.) were obtained from Davies and Nagle Inc., New York, N. Y.

(27) The Sarcoma 180 was obtained from Dr. H. Christine Reilly. The screenings were assisted by Steven Rosenberg, Henry Clayman, and Virginia Wevurski.

(28) *Cancer Res.*, Suppl. No. 1, 91 (1953); Suppl. No. 2, 179 (1955); *Cancer Res.*, **18**, No. 8, 49 (1958).

(29) Private communication from Dr. H. Christine Reilly.

The Antileukemia Activity of Tylocrebrine

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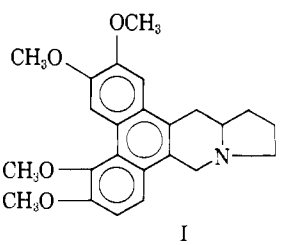
The isolation, structure determination, and synthesis of the alkaloid, tylocrebrine, from the North Queensland slender vine, *Tylophora crebriflora*, has been reported previously.[1] This levorotatory alkaloid was shown to have a phenanthroindolizidine structure (I).



I

Tylocrebrine has been tested against three tumor systems by the Cancer Chemotherapy National Service Center, U. S. Department of Health, Education, and Welfare, Bethesda, Md., in accordance with the protocols[2a] governing their screening program. The following is a report of the results obtained.

(1) E. Gellert, T. R. Govindachari, M. V. Lakshmikantham, I. S. Ragade, R. Rudzats, and N. Viswanathan, *J. Chem. Soc.*, 1008 (1962).

TABLE I

SARCOMA 180

Dose, mg./kg.	Survivors, X out of Y		Average tumor weight in mg. Test	Control	%, test/control
12.0	4	6	1185	1881	62

TABLE II

ADENOCARCINOMA 755

Dose, mg./kg.	Survivors, X out of Y		Average tumor weight in mg. Test	Control	%, test/control
10.0	9	10	735	1138	64

Methods

Test animals used as hosts were noninbred albino mice (Swiss) in the case of Sarcoma 180,[2b] and BDF_1 hybrid mice in the cases of Adenocarcinoma 755[2c] and Lymphoid Leukemia L1210.[2d]

All injections were administered intraperitoneally once a day except in the case of Sarcoma 180 when two injections per day were given. The solvent employed as vehicle of drug administration was carboxymethylcellulose with the exception of one series of tests involving Lymphoid Leukemia L1210, when another solvent (two drops of Tween 80 diluted to 20 ml. with saline) was used.

Mice were sacrificed after fourteen doses, *i.e.*, on the eighth day of administration in the case of Sarcoma 180; after eleven doses, *i.e.*, on the twelfth day in the case of Adenocarcinoma 755; while in the case of Lymphoid Leukemia L1210, administration of the drug was continued until death.

Results

Inactive Tests.—Against either Sarcoma 180 or Adenocarcinoma 755 administration of tylocrebrine at nontoxic dose levels showed insufficient decrease in the tumor weight of mice in comparison with the control animals.

Active Tests.—Administration of tylocrebrine to the Lymphoid Leukemia L1210 system considerably increased the survival time of the test animals. There are variations in the increases observed which appear to depend mainly upon the size of the administered dose, but occasional variations within the same dose range were also observed. The criterion of reproduc-

TABLE III

LYMPHOID LEUKEMIA L1210

Dose, mg./kg.	Survivors for at least 6 days, X out of Y		Average survival time of animals in days Test	Control	%, test/control
15.0	5	6	11.0	9.1	120
	6	6	15.0	9.9	151
10.0	6	6	14.0	9.9	141
	6	6	13.7	8.7	157
	6	6	14.0	8.3	168
	6	6	13.8	9.1	151
6.6	6	6	11.6	9.9	117
	6	6	11.7	9.1	128
4.4	6	6	12.5	9.9	126
	6	6	11.7	9.1	128
2.2	6	6	10.6	9.9	107

(2) (a) Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and other Biological Systems, in *Cancer Chemotherapy Rept.*, No. 25 (1962); (b) Protocol 1.301; (c) Protocol 1.302; (d) Protocol 1.303; (e) Protocol 12.303.