scribed. When a cyclohexyl group was substituted for one of the phenyl groups on C-1, and the phenyl group on C-2 had a fluorine atom in the *ortho* position, activity was retained. Shifting the fluorine atom to the *para* position caused a loss of activity.

In the light of these findings it seemed likely that in the compounds of this series, antigonadotropin activity was associated with the triphenylethylene structure. To maximize this effect and minimize the estrogenic activity which was also a frequent accompaniment, the study was extended in the direction which retained the basic triphenylethylene structure and changed only the substituent groupings on the benzene rings.

The compounds reported in this study fall into two general categories; namely, the triphenylethanol and the triphenylethylene derivatives. They are all new compounds and were all prepared by the general methods previously described.[1,2] The triphenylethanol derivatives are listed in Table I and the triphenylethylene derivatives in Table II.

Pharmacology.—The antigonadotropin and estrogenic activity of the compounds was determined by the methods previously described.[1,2] The triphenylethanol derivatives were all inactive as antigonadotropins though some of them had weak estrogenic activity. Five of the triphenylethylene derivatives were active as antigonadotropins and as estrogens. The latter compounds are listed in Table III.

Synthesis of 6- and 7-Bis(2-chloroethyl)amino-DL-tryptophan[1a]

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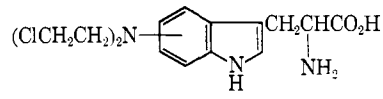
The interesting antitumor activity[2] of 5-bis(2-chloroethyl)amino-DL-tryptophan[1c,3] (IIIa) stimulated us to prepare two other substitution isomers (IIIb and IIIc) of IIIa with the hope of synthesizing a better antitumor drug. In the case of another aromatic nitrogen mustard system, that derived from phenylalanine, the *meta* and *para* isomers have significantly different antitumor activity.[4] The route used to prepare IIIb and IIIc was the same as that used in preparing IIIa. Thus the appropriate substituted gramine I was converted to the acetamidomalonate II that was reduced catalytically to the amine IV. Reaction of IV with ethylene oxide yielded the bis(2-hydroxyethyl)amine V which with methanesulfonyl chloride in pyridine afforded the bis(2-chloroethyl)amine VI. The hydrolysis of both VIb and VIc to IIIb and IIIc, respectively, required a careful control of conditions to obtain the pure nitrogen mustards.

In the course of the work 6-nitro-L-tryptophan[5] was reduced catalytically to the hitherto unreported 6-amino-L-tryptophan (VII).

The antitumor activity[6] of the three tryptophan mustards against the Walker 256 (subcutaneous) tumor in rats is presented in Table I. The increase in therapeutic index of IIIc as compared to IIIa is evident. The 7-mustard IIIc also shows activity against the Sarcoma 180 tumor in contrast with IIIa which does not respond to that tumor. All three tryptophan mustards givea good response in the leukemia L1210 system.

TABLE I

ANTITUMOR ACTIVITY OF DL-TRYPTOPHAN NITROGEN MUSTARDS



Isomer	LD_{10}, mg./kg./day[a]	MED, mg./kg./day[b]	T.I.[c]
5-Mustard	1.2	0.31	4
6-Mustard	1.3	0.15	9
7-Mustard	4.8	0.35	14

[a] The LD_{10} is the dose that kills more than 10% of the animals and is the maximum tolerated dose. [b] The minimum effective dose (MED) is that dose which gives a ratio of 0.10 of tumor weight in treated animals to tumor weight in control animals. [c] The therapeutic index (T.I.) is defined as LD_{10}/MED.

Experimental[7]

Ethyl α-acetamido-α-carbethoxy-β-(6-nitro-3-indolyl)propionate (IIb) was prepared in 70% yield from 6-nitrogramine[8] using the procedure of Cavallini and Ravenna[9] described for preparation of IIa. The analytical sample, recrystallized from chloroform or acetonitrile, had m.p. 225–226°; λ_{max}^{Nujol} 3.00 and 6.61 (NH), 5.71 and 5.82 (ester C=O), 6.12 (amide C=O), 7.46 μ (NO_2). A lower melting polymorph (with a correct analysis), m.p. 196–197°, was also noted in some runs and could be converted to the higher melting form by recrystallization and seeding.

Anal. Calcd. for $C_{18}H_{21}N_3O_7$: C, 55.2; H, 5.37; N, 10.8. Found: C, 55.0; H, 5.46; N, 10.6.

Ethyl α-acetamido-α-carbethoxy-β-(7-nitro-3-indolyl)propionate (IIc) was prepared by the procedure described by DaSettimo.[10]

Ethyl α-acetamido-α-carbethoxy-β-(6-amino-3-indolyl)propionate (IVb) was prepared by the procedure described for IVa,[3] to give a 97% yield of product. The analytical sample, recrystallized from acetonitrile, had m.p. 176–177°; λ_{max}^{Nujol} 2.98, 3.02, and 6.55 (NH,NH_2), 5.73 (ester C=O), 6.00 μ (amide C=O).

Anal. Calcd. for $C_{18}H_{23}N_3O_5$: C, 59.8; H, 6.38; N, 11.6. Found: C, 59.9; H, 6.53; N, 11.5.

Ethyl α-acetamido-α-carbethoxy-β-(7-amino-3-indolyl)propionate (IVc) is described by Casini and Goodman.[11]

Ethyl α-Acetamido-α-carbethoxy-β-[6-bis(2-hydroxyethyl)-amino-3-indolyl]propionate (Vb) Hydrochloride.—Ethylene ox-

(1) (a) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center. (b) Holder of a NATO fellowship during 1962. (c) In the numbering in the text, the a series represent the 5-substituted compounds, the b series the 6-substituted, and the c series the 7-substituted.

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(4) (a) M. O. Greene, B. R. Baker, and J. Greenberg, *Cancer Res.*, **20**, 1160 (1960); (b) V. I. Trusheikina, *Vopr. Onkol.*, **6**, 63 (1960).

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(6) Antitumor testing was carried out under the auspices of the Cancer Chemotherapy National Service Center according to the protocols described in *Cancer Chemotherapy Rept.*, **25**, 11 (1962).

(7) Melting points, uncorrected, were obtained with the Fisher-Johns apparatus. Magnesium sulfate was used to dry organic extracts.

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(9) G. Cavallini and F. Ravenna, *Farmaco* (Pavia), *Ed. Sci.*, **13**, 105 (1958).

(10) A. DaSettimo, *Ann. Chim.* (Rome), **52**, 17 (1962).

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ide, 20 ml., was added to a cooled (0°), stirred solution of 6.50 g. (17.9 mmoles) of the amine IVb in 25 ml. of glacial acetic acid. The mixture was allowed to warm to room temperature and was stirred for 18 hr., then was concentrated *in vacuo* to half its volume. The solution was diluted with 20 ml. of dichloromethane and extracted with 20 ml. of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with two 50-ml. portions of dichloromethane, and the extracts were combined with the original dichloromethane solution. The dried organic solution was diluted with 12 ml. of absolute ethanol, cooled to 10°, and saturated with HCl. Dry ether was added to the cloud point and the solution was chilled, affording 6.68 g. (76%) of a white solid, m.p. 172–175°. The analytical sample, recrystallized from absolute ethanol, had m.p. 177–178°; λ_{max}^{Nujol} 2.90 and 6.60 (OH, NH_3^+), 5.72 (ester C=O), 6.00 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{31}N_3O_7 \cdot HCl$: C, 54.4; H, 6.60; Cl, 7.22; N, 8.64. Found: C, 54.2; H, 6.77; Cl, 7.50; N, 8.48.

Ethyl α-Acetamido-α-carbethoxy-β-[7-bis(2-hydroxyethyl)-amino-3-indolyl]propionate (Vc).—Ethylene oxide, 3 ml., was added to a chilled (0°), stirred solution of 0.69 g. (1.90 mmoles) of the amine IVc in 9 ml. of 50% aqueous acetic acid. The solution was stirred at 0° for 1 hr., then allowed to reach room temperature, when stirring was continued for 20 hr. more. The solution was poured into 30 ml. of water, then adjusted to pH 7 with solid $NaHCO_3$ and extracted with three 20-ml. portions of ethyl acetate. The dried extract was treated with decolorizing carbon, then filtered and evaporated, yielding 0.87 g. of oily residue. Crystallization from 100 ml. of water afforded 0.60 g. (70%) of white crystals, m.p. 64–69°, collected in two crops. The analytical sample, recrystallized from water, had m.p. 67–69°; λ_{max}^{Nujol} 2.95, 3.08 and 6.55 (OH, NH), 5.69 and 5.75 (ester C=O), 6.00 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{31}N_3O_7$: C, 58.8; H, 6.95; N, 9.35. Found: C, 59.1; H, 7.01; N, 9.16.

Ethyl α-Acetamido-α-carbethoxy-β-[6-bis(2-chloroethyl)-amino-3-indolyl]propionate (VIb).—A solution of 3.0 g. (6.2 mmoles) of Vb hydrochloride in 15 ml. of ethyl acetate was cooled to 10°, then treated with 20 ml. of cold saturated aqueous $NaHCO_3$. The organic layer was washed with 5 ml. of cold water, dried, and evaporated *in vacuo* to give Vb as a colorless foam.

To a chilled (0°), stirred solution of the free base in 24 ml. of dry pyridine was added dropwise 1.40 ml. (18.1 mmoles) of methanesulfonyl chloride. The reaction was stirred at room temperature for 18 hr., then was heated at 55–60° for 1.5–2 hr. The mixture was cooled to room temperature, then poured into 100 ml. of ice water. The oil which separated slowly crystallized yielding 1.65 g. (55%) of product, m.p. 135–142°. One recrystallization from 95% ethanol gave 1.33 g. of solid, m.p. 153–155°. The analytical sample had m.p. 157–158°; λ_{max}^{Nujol} 2.98, 3.07, and 6.59 (NH and possibly alcohol OH), 5.73 (ester C=O), 6.02 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{29}Cl_2N_3O_5 \cdot 0.5C_2H_5OH$: C, 54.2; H, 6.28; Cl, 14.0; N, 8.25. Found: C, 54.3; H, 6.26; Cl, 13.8; N, 8.32.

This reaction could not be scaled up satisfactorily.

Ethyl α-acetamido-α-carbethoxy-β-[7-bis(2-chloroethyl)-amino-3-indolyl]propionate (VIc) was prepared from Vc essentially as described for the preparation of VIb except that the reaction mixture was not heated before it was poured into ice water. The crude yield of material with m.p. 135–140° was 69%. The analytical sample was obtained by two recrystallizations from benzene-petroleum ether (30–60°) and had m.p. 139–141°; λ_{max}^{Nujol} 2.96, 3.01, and 6.56 (NH), 5.70 and 5.77 (ester C=O), 6.03 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{29}Cl_2N_3O_5$: C, 54.2; H, 6.01; Cl, 14.6; N, 8.64. Found: C, 54.4; H, 5.88; Cl, 14.5; N, 8.30, 8.37.

6-Bis(2-chloroethyl)amino-DL-tryptophan (IIIb).—A mixture of 2.0 g. of the blocked mustard VIb in 20 ml. of concentrated HCl was heated in an oil bath maintained at 120–130° for 2 hr., then was cooled to 10° and treated carefully with saturated aqueous sodium acetate. A yellow solid separated at pH 4–5. The solid was filtered, washed with ice water, then stirred with ice water that contained 5% acetic acid. After drying at room temperature, the product weighed 1.11 g. (67% yield); $\lambda_{max}^{cast\ film}$ 2.95 (NH), 3.1–3.6, 6.11, 6.50 (NH_3^+), 5.91 (acetic acid C=O), 6.40 and 7.10 μ (CO_2^-).

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot HC_2H_3O_2$: C, 50.6; H, 5.70; Cl, 17.5; N, 10.4. Found: C, 50.5; H, 5.71; Cl, 17.5; N, 10.6.

In an earlier run in which aqueous $NaHCO_3$ was used to neutralize the hydrolytic solution, a 57% yield of hydrated free base was obtained; the infrared spectrum was very similar to that of the acetic acid containing product but lacked the band at 5.91 μ.

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot 1.25H_2O$: C, 49.2; H, 5.74; Cl, 19.4; N, 11.5. Found: C, 49.1; H, 5.87; Cl, 19.7; N, 11.1.

7-Bis(2-chloroethyl)amino-DL-tryptophan (IIIc).—A solution of 4.0 g. (8.2 mmoles) of the blocked mustard VIc in 40 ml. of concentrated HCl was heated at reflux, under nitrogen, for 1.75 hr., then evaporated *in vacuo*, leaving a glassy residue (3.8 g.). A portion of this residue (2.69 g.) was dissolved in 55 ml. of dry pyridine. The solution was filtered to remove a small amount of insoluble material and the filtrate was treated with 150 ml. of dry ether. The precipitate was isolated by centrifugation and was thoroughly washed with dry ether three times using centrifugation to isolate the solid. The product, 1.92 g. (96%), carefully protected from atmospheric moisture, was dried *in vacuo* at 80° over P_2O_5; λ_{max}^{Nujol} 2.93 (NH), 3.6–4.0 and 6.21 (NH_3^+), 6.35 and 6.92 μ (CO_2^-).

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot 0.25H_2O$: C, 51.7; H, 5.66; Cl, 20.0; N, 12.1. Found: C, 51.6; H, 5.63; Cl, 20.4; N, 11.4, 11.8.

6-Amino-L-tryptophan (VII) was prepared from 0.30 g. (1.2 mmoles) of 6-nitro-L-tryptophan[5] over 0.20 g. of platinum oxide in 30 ml. of water in the manner described for the preparation of 5-amino-DL-tryptophan.[3] The product, recrystallized from water, was a white crystalline solid, 0.25 g. (98%), m.p. 208–211°. The analytical sample had m.p. 210–212°; $[\alpha]^{24}D$ +10° (1% in 1 *N* HCl); λ_{max}^{Nujol} 2.80, 2.95, and 3.1 (NH, NH_2), 3.65, 3.81, 6.12, and 6.50 (NH_3^+), 6.27 and 7.12 μ (CO_2^-).

Anal. Calcd. for $C_{11}H_{13}N_3O_2 \cdot 0.125H_2O$: C, 59.5; H, 6.40; N, 18.9. Found: C, 59.8; H, 6.21; N, 18.8.

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Carcinogenic Activity of 2-N,N-Dimethylamino-5-phenylazopyridine[1]

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We have previously synthesized and tested *p*-dimethylaminophenylazopyridines for rat hepatocarcinogenic activity.[3] They can be considered as pyridine analogs of Butter yellow, a well known carcinogen. The 3-pyridine analog has a modest activity[4] while the 2-isomer has no activity and the 4-isomer has a high activity.[1] It seemed of interest to synthesize the 3-isomer in which the dimethylamino group is on the pyridine instead of the benzene ring in order to investigate its activity.

The first synthesis which we undertook involved the Mills' reaction[5] using 2-dimethylamino-5-aminopyridine and nitrosobenzene. This failed, possibly due to instability on the part of the diamino compound.[6] In the second and successful method, 2-

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