Potential Anticancer Agents.[1] LXXIV. Alkylating Agents Derived from Indole. III. Synthesis of Some Indolealkanoic Acid Nitrogen Mustards

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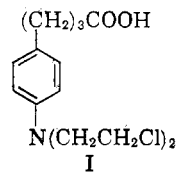
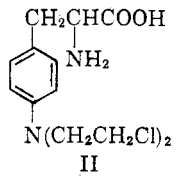
The synthesis of two indole alkylating agents, 5-bis(2-chloroethyl)aminoindole-3-acetic acid (VII) and 5-bis(2-chloroethyl)-aminoindole-3-propionic acid (XV), is described.

One of the most useful alkylating agents in the clinic[2] is chlorambucil, 4-*p*-[bis(2-chloroethyl)-amino]phenylbutyric acid (I).[3] Although chlorambucil (I) is highly effective against the Walker rat Sarcoma 256, it shows little activity against the standard three-tumor screen of Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210 in the mouse. On the other hand, sarcolysin (II), also of considerable clinical interest, is very active against the standard three-tumor system as well as against Walker Sarcoma 256.[4] Recently the synthesis of the 5-substituted mustard (III) of tryptophan was



I



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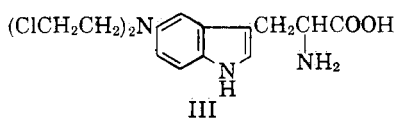
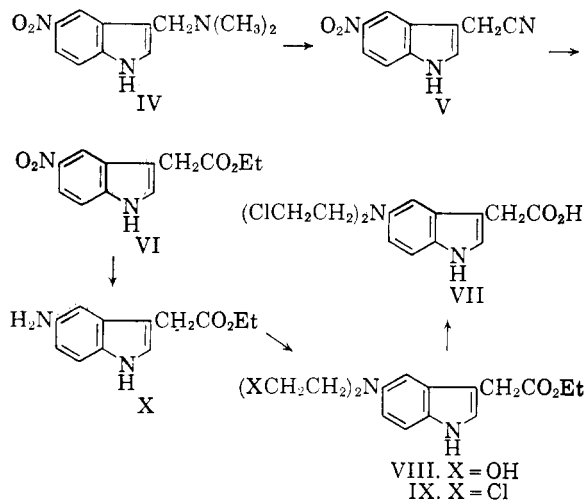


III

announced[5]; it was of interest to prepare the analogous indolealkanoic acid mustards for comparison of their biological activity with that of III in the same manner that phenylalkanoic acid nitrogen mustards and II have been compared. This manuscript reports the synthesis of two such indolealkanoic acid nitrogen mustards.

The conversion of 5-nitrogramine (IV)[6] to 5-nitro-3-indoleacetonitrile (V) was best effected with sodium cyanide and methyl iodide in aqueous methanol at room temperature.[7] The previously described[6] preparation of V from the methiodide of IV was less convenient and, in the present work, gave a mixture of IV and V. Refluxing ethanolic hydrogen chloride served to convert V to the ester (VI) and hydrogenation of VI over platinum oxide



gave an 87% yield of the amino ester (X). The bis(2-hydroxyethyl)amine (VIII) was obtained as a crystalline solid by the reaction of X with excess ethylene oxide in ethanol containing a catalytic quantity of *p*-toluenesulfonic acid. The blocked mustard (IX) was obtained as a crystalline solid in low yield by the reaction of VIII with methanesulfonyl chloride in pyridine[5] and was hydrolyzed to the acetic acid (VII) with refluxing concentrated hydrochloric acid.

The preparation of the propionic acid mustard (XV) also started from 5-nitrogramine (IV). Reaction of IV with a large excess of diethyl malonate in toluene containing powdered sodium hydroxide afforded the substituted malonic ester (XI) as a sirup. The use of smaller amounts of diethyl malonate gave diethyl bis(5-nitroindol-3-ylmethyl)-malonate as a major product. Saponification of XI, followed by acidification, afforded a solid assumed to be the diacid XII, which was decarboxylated at

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(2) R. W. Rundles, J. Grizzle, W. N. Bell, C. C. Corley, W. B. Frommeyer, B. C. Greenberg, C. M. Huguley, G. W. James, III, R. Jones, Jr., W. E. Larsen, V. Loeb, L. A. Leone, J. G. Palmer, W. H. Riser, Jr., and S. J. Wilson, *Am. J. Med.*, **27**, 424 (1959).

(3) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

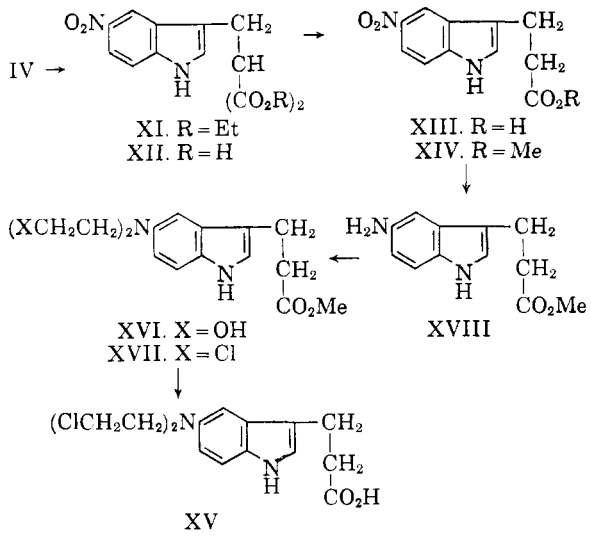
(4) For a summary of data concerning sarcolysin and related compounds, see Cancer Chemotherapy Reports, No. **6**, p. 61 (1960), published by the Cancer Chemotherapy National Service Center, National Cancer Institute.

(5) J. DeGraw and L. Goodman, *Chem. & Ind.* (London), 1448 (1961).

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

(7) Adapted from a procedure used to prepare indole-3-acetonitrile; *cf.* H. Henbest, E. Jones, and G. Smith, *J. Chem. Soc.*, 3796 (1953).

180–190° to the propionic acid (XIII). The overall yield of XIII from IV was 47%.



Conventional esterification, then catalytic reduction, yielded the amino ester (XVIII) and the remaining steps to XV followed the procedures that were used to prepare the acetic acid mustard (VII). The stability of these indole derivatives (VII and XV) to strong acid was previously noted in the preparation of III.

Experimental[8]

5-Nitroindole-3-acetonitrile (V).—To a solution of 200 g. (0.91 mole) of 5-nitrogramine (IV)[6] in 4 l. of methanol was added 92 g. (1.88 moles) of sodium cyanide in 200 ml. of water. Then 146 ml. of methyl iodide was added slowly with stirring, using a cold water bath to maintain the temperature below 40°. The mixture was stirred overnight at room temperature and was evaporated to dryness *in vacuo*. After the residue was stirred with 2 l. of water, the gummy crystals were collected by filtration and washed with water. The damp material was recrystallized twice from 95% ethanol to afford 108 g. (59%) of yellow crystals, m.p. 174–176° (lit.,[6] m.p. 180–182°); $\lambda_{max(\mu)}^{Nujol}$ 2.95 (NH), 4.45 (C≡N), 7.51 (NO_2). The compound moved as a single spot on paper chromatography[9] in *n*-butyl alcohol–acetic acid–water (5:2:3), with R_{Ad} 1.64.

Ethyl 5-Nitroindole-3-acetate (VI).—To 5 ml. of 95% ethanol, previously saturated with hydrogen chloride, was added 0.50 g. of the nitrile (V) and the mixture was refluxed with stirring for 6.5 hr., then was cooled and poured into 10 ml. of ice water. The mixture was extracted with two 10-ml. portions of chloroform and the combined chloroform extracts were washed with 10 ml. of saturated aqueous sodium bicarbonate and then dried over magnesium sulfate. The chloroform was evaporated *in vacuo* to give 0.48 g. of a yellow solid, which was recrystallized from 2 ml. of benzene to yield 0.27 g. (44%) of bright yellow crystals, m.p. 109–110°; $\lambda_{max(\mu)}^{Nujol}$ 3.03 (NH), 5.82 (ester C=O), 7.52 (NO_2).

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.1; H, 4.87; N, 11.3. Found: C, 58.3; H, 4.70; N, 11.2.

Ethyl 5-Aminoindole-3-acetate (X).—To 290 ml. of absolute ethanol was added 29.3 g. of the nitro ester (VI) and 2.9 g. of platinum oxide. The mixture was treated with hydrogen at 3 atmospheres; the theoretical amount of hydrogen was absorbed after 3 hr. Two other identical runs were made and the combined reaction mixtures were filtered through Celite. The filtrate was evaporated to dryness *in vacuo* and the brown, sirupy residue was recrystallized from benzene, using decolorizing carbon, to yield 63.2 g. (87%) of white crystals, m.p. 98.5–99.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.96, 3.02, 3.17 (NH_2, NH), 5.79 (ester C=O).

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.47; N, 12.8. Found: C, 65.5; H, 6.39; N, 12.8.

Ethyl 5 - Bis(2 - hydroxyethyl)aminoindole - 3 - acetate (VIII).—To an ice-cold solution of 59 g. of the amino ester (X) in 590 ml. of absolute ethanol, containing 0.3 g. of *p*-toluenesulfonic acid, was added 88 ml. of ethylene oxide. The solution was allowed to stand overnight at room temperature, then was neutralized with saturated sodium bicarbonate and evaporated to dryness *in vacuo*. The residue was diluted with 600 ml. of water and the mixture was extracted with three 400-ml. portions of dichloromethane. The combined dichloromethane extracts were washed twice with 300 ml. of water, dried over magnesium sulfate, and evaporated to dryness *in vacuo*, leaving 73.6 g. (89%) of tan crystals, m.p. 89–90°. An analytical sample, m.p. 87.0–87.5°, was obtained by recrystallization of the crude material from benzene–ethyl acetate; $\lambda_{max(\mu)}^{Nujol}$ 3.0, 3.15 (NH, OH), 5.83 (ester C=O), 9.7 (C—OH).

Anal. Calcd. for $C_{16}H_{22}N_2O_4$: C, 62.7; H, 7.24; N, 9.14. Found: C, 63.0; H, 7.12; N, 9.33.

Ethyl 5 - Bis(2 - chloroethyl)aminoindole - 3 - acetate (IX).—To a solution of 5.0 g. (16 mmoles) of the bishydroxyethyl compound (VIII) in 20 ml. of pyridine was added 4.1 g. (36 mmoles) of methanesulfonyl chloride over a period of 4 min., using a cold water bath to prevent the temperature from rising above 90°. The resulting solution was heated at 90–95° for 7 min., cooled and, with stirring, diluted with 200 ml. of water. The aqueous supernatant was decanted from the precipitated gum. The gum was stirred for 1 hr. with another 50 ml. of water to cause crystallization. The crystals were collected, washed with water, and dried to give 1.74 g. (31%) of product, m.p. 64–66°. An analytical sample, m.p. 67.0–67.5°, was obtained by recrystallization from Skellysolve B of material from another run; $\lambda_{max(\mu)}^{Nujol}$ 3.00 (NH), 5.78 (ester C=O), 8.38 (C—O—C); the strong hydroxyl absorptions at 2.9–3.0 and 9.7 μ in the starting material were absent.

Anal. Calcd. for $C_{16}H_{20}Cl_2N_2O_2$: C, 56.0; H, 5.87; Cl, 20.7; N, 8.16. Found: C, 56.1; H, 6.00; Cl, 20.5; N, 8.27.

5 - Bis(2 - chloroethyl)aminoindole - 3 - acetic Acid (VII).—A solution of 2.5 g. of the ester (IX) in 15 ml. of concd. hydrochloric acid was heated at reflux for 4 hr. The solution was cooled and adjusted to *p*H 5–6 with saturated aqueous sodium bicarbonate. The supernatant was decanted from the gummy precipitate that formed and the residue was stirred with another 50 ml. of water to cause solidification of the gum. The material was collected, washed with water, and dried to afford 1.8 g. (79%) of product, m.p. 117–121°. The material was recrystallized from benzene, using decolorizing carbon, to yield 0.99 g. (43%) of white crystals, m.p. 122–123°; $\lambda_{max(\mu)}^{Nujol}$ 2.93 (NH), 5.83 (COOH).

Anal. Calcd. for $C_{14}H_{16}Cl_2N_2O_2$: C, 53.4; H, 5.12; Cl, 22.5; N, 8.88. Found: C, 53.5; H, 5.16; Cl, 22.5; N, 9.15.

Diethyl (5-Nitroindol-3-ylmethyl)malonate (XI).—To 30 ml. of toluene was added 3.0 g. (13.7 mmoles) of 5-nitrogramine (IV), 10.4 g. (65 mmoles) of diethyl malonate, and 0.56 g. (14 mmoles) of powdered sodium hydroxide. The mixture was refluxed for 24 hr. with vigorous stirring and then cooled and filtered. The filtrate was extracted with 15 ml. of 1 *N* hydrochloric acid and washed with 10 ml. of water. The toluene extract was dried over magnesium sulfate and evaporated *in vacuo*, finally at 70° and 1 mm. for 4 hr., leaving 4.8 g. (104%) of a yellow sirup; $\lambda_{max(\mu)}^{film}$ 2.98 (NH), 5.75 (ester C=O), 7.48 (NO_2). The material could

(8) Melting points are uncorrected and were obtained with the Fisher-Johns apparatus.

(9) Paper chromatography was done by the descending technique on Whatman No. 1 paper, using ultraviolet examination to locate the spots. The spots were located relative to adenine, *R* of adenine = 1.0.

not be further purified and was used directly in the next step. A negligible amount of unchanged 5-nitrogramine was precipitated when the above acid wash was made basic.

When this reaction was conducted with equimolar amounts of nitrogramine and diethyl malonate, the major product isolated was a compound, m.p. 239–241°, postulated to be diethyl bis(5-nitroindol-3-ylmethyl)malonate.

Anal. Calcd. for $C_{25}H_{24}N_4O_8$: C, 59.1; H, 4.76; N, 11.0. Found: C, 59.6; H, 5.05; N, 11.4.

5-Nitroindole-3-β-propionic Acid (XIII).—A solution of 3.7 g. of the malonate ester (XI) in 20 ml. of ethanol and 30 ml. of 10% sodium hydroxide was refluxed for 3 hr. and the ethanol evaporated *in vacuo*. The aqueous residue was diluted with 75 ml. of water to dissolve the crystallized sodium salt. The resulting solution was made strongly acidic with conc. hydrochloric acid and extracted with three 30-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over magnesium sulfate and evaporated to dryness *in vacuo* to give 2.5 g. of yellow crystals presumed to be the diacid (XII). The material was heated at 180–190° for 30 min., after which time no more carbon dioxide was evolved. The melt was treated with 40 ml. of boiling ethylene dichloride and rapidly centrifuged. The residue was extracted with another 10 ml. of ethylene dichloride in the same manner. The combined extracts were allowed to stand overnight and the yellow crystals were collected, to afford 1.07 g., m.p. 172–174°. A second crop of 0.10 g. was obtained by concentrating the filtrate; total, 1.17 g. (45%). Another recrystallization from ethylene dichloride gave an analytical sample, m.p. 176.0–176.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.92 (NH), 5.86 (carboxyl C=O), 7.52 (NO_2).

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.4; H, 4.30; N, 12.0. Found: C, 56.7; H, 4.23; N, 12.0.

Methyl 5-Nitroindole-3-β-propionate (XIV).—A solution of 1.00 g. of the acid (XIII) in 10 ml. of methanol containing 170 mg. of *p*-toluenesulfonic acid was refluxed for 4.5 hr. and evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml. of dichloromethane and the solution washed with 5 ml. of saturated sodium bicarbonate. The dichloromethane extract was dried over magnesium sulfate and evaporated *in vacuo* to leave 0.86 g. of yellow crystals, which were recrystallized from 6 ml. of benzene to yield 0.72 g. of product, m.p. 118.0–119.5°. A second crop of 0.04 g. was obtained; total, 0.76 g. (72%). An analytical sample, m.p. 119–120°, was obtained similarly from another run; $\lambda_{max(\mu)}^{Nujol}$ 3.00 (NH), 5.82 (ester C=O), 7.55 (NO_2), 8.36 (C—O—C).

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.1; H, 4.87; N, 11.3. Found: C, 58.1; H, 4.89; N, 11.3.

Methyl 5-Aminoindole-3-β-propionate (XVIII).—To 10 ml. of absolute ethanol was added 0.70 g. of the nitro ester (XIV) and 70 mg. of platinum oxide. The mixture was treated with hydrogen at atmospheric pressure, absorbing the theoretical amount of gas in 2 hr. The catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The sirupy residue was crystallized from 7 ml. of toluene to give 0.43 g. of product, m.p. 111.5–113.5°, and a second crop of 0.02 g.; the total was 0.45 g. (74%) of white crystals. The first crop was recrystallized from toluene to afford an analytical sample, m.p. 113.5–114.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.92, 2.99, 3.17 (NH, NH_2), 5.75 (ester C=O).

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.47; N, 12.8. Found: C, 66.1; H, 6.53; N, 12.8.

Methyl 5-Bis(2-hydroxyethyl)aminoindole-3-β-propionate (XVI).—To an ice-cold solution of 0.64 g. of the amino ester (XVIII) in 6 ml. of methanol was added 1.0 ml. of ethylene oxide and 5 mg. of *p*-toluenesulfonic acid. The solution was allowed to stand overnight and was evaporated to dryness *in vacuo*. The sirupy residue was partitioned between 10 ml. of dichloromethane and 10 ml. of water containing 1 ml. of saturated sodium bicarbonate. The aqueous layer was extracted with another 5-ml. portion of dichloromethane. The combined dichloromethane extracts were washed with 5 ml. of water, dried over magnesium sulfate, and evaporated to dryness *in vacuo* to leave 0.79 g. (88%) of a sirup identified as XVI by its infrared spectrum; $\lambda_{max(\mu)}^{film}$ 2.9–3.0 (OH, NH), 5.75 (ester C=O), 9.4–9.6 (C—OH).

Methyl 5-Bis(2-chloroethyl)aminoindole-3-β-propionate (XVII).—To a solution of 10.0 g. (32.7 mmoles) of the bis-hydroxy ester (XVI) in 50 ml. of pyridine was added 7.5 g. (66 mmoles) of methanesulfonyl chloride. When the vigorous exothermic reaction subsided, the solution was heated for 5 min. on the steam bath, then was cooled, poured into 500 ml. of ice water, and the mixture stirred for 30 min. until the gummy precipitate solidified. The crystals were collected, washed with water, and dried to give 4.4 g. of solid product. The material was dissolved in 15 ml. of hot benzene and diluted with 20 ml. of Skellysolve B to precipitate some tars. The tars were separated by centrifugation and the supernatant was chilled overnight to give 3.76 g. (34%) of tan crystals, m.p. 103.5–108.5°. Recrystallization from benzene–Skellysolve B of material from another run gave an analytical sample, m.p. 108–109°; $\lambda_{max(\mu)}^{Nujol}$ 3.00 (NH), 5.80 (ester C=O); the strong C—OH bands at 9.4–9.6 μ in the starting material were absent.

Anal. Calcd. for $C_{16}H_{20}Cl_2N_2O_2$: C, 56.0; H, 5.87; Cl 20.7; N, 8.16. Found: C, 55.9; H, 5.84; Cl, 20.4; N, 8.13,

5-Bis(2-chloroethyl)aminoindole-3-β-propionic Acid (XV).—A solution of 1.00 g. of the ester (XVII) in 5 ml. of concd. hydrochloric acid was heated at reflux for 5 hr. The solution was cooled and diluted with 5 ml. of water, causing some dark gum to deposit. The supernatant was, decanted from the gum and adjusted to *p*H 5–6 with saturated aqueous sodium bicarbonate to give a gummy precipitate. After being chilled overnight, the material crystallized and was collected by filtration, washed with water, and dried to leave 0.46 g. of product. Recrystallization from benzene gave 0.31 g. (32%) of product, m.p. 118.5–119.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.96 (NH), 5.85 (carboxyl C=O).

Anal. Calcd. for $C_{15}H_{18}Cl_2N_2O_2$: C, 54.8; H, 5.51; Cl, 21.5; N, 8.51. Found: C, 55.2; H, 5.35; Cl, 20.8; N, 8.34.

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