Alkylating Agents Derived from Indole. IV. Synthesis of the 5-Nitrogen Mustard of Indole-3-carboxylic Acid[1a]

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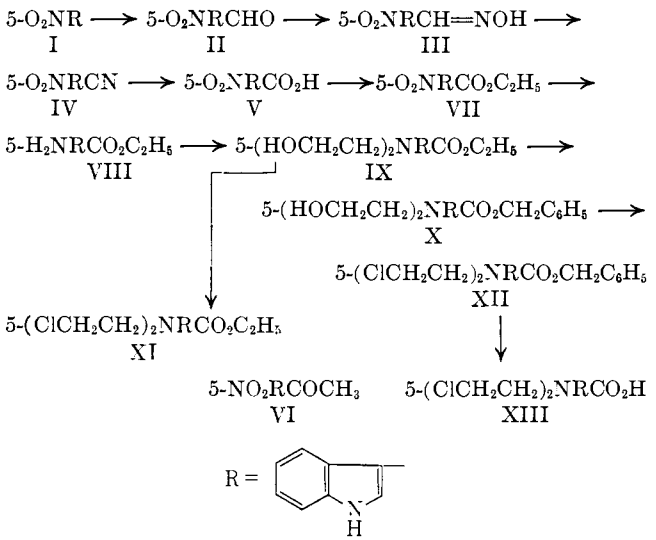
The preparation of 5-[bis(2-chloroethyl)amino]indole-3-carboxylic acid (XIII) is described. A comparison of the antitumor activity of XIII with some related 5-bis(2-chloroethyl)amino derivatives of indole is also presented.

In a previous communication[2] the preparation of the 5-nitrogen mustard of DL-tryptophan was described. The interesting antitumor activity of this compound stimulated the synthesis of the 5-nitrogen mustards of indole-3-acetic and propionic acids.[3] As a continuation of this series, the preparation of the 5-nitrogen mustard of indole-3-carboxylic acid (XIII) is described in this manuscript.

For the synthesis of XIII, utilization of the general method for preparing the indolealkanoic acid mustards[3] required a practical preparation of 5-nitroindole-carboxylic acid (V). Treatment of 5-nitroindole (I) with phosphoryl chloride in N,N-dimethylformamide afforded the 3-aldehyde (II) in 97% yield.[4] Efforts to oxidize II directly to the acid (V) were unsuccessful and a three-step process was employed for the conversion of II to V. The aldehyde (II) was converted to the oxime (III) which was dehydrated with thionyl chloride to afford the nitrile (IV). Hydrolysis of IV with strong alkali gave the acid (V).[5] The ketone (VI), prepared by stannic chloride mediated acetylation of I,



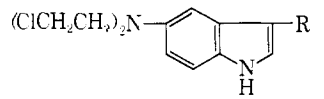
was considered as an alternative source of V, but hypochlorite oxidation of VI was unsuccessful. The acid (V) was readily decarboxylated by boiling hydrochloric acid yielding, apparently, a polymeric form of 5-nitroindole.

The ester (VII) was prepared from V with ethanol under acid catalysis and was converted to the amino ester (VIII) by catalytic hydrogenation. The reaction of VIII with ethylene oxide in ethanol gave the bis(2-hydroxyethyl)amine (IX) as a crystalline solid that, with methanesulfonyl chloride in pyridine,[2,3] afforded the mustard ester (XI). All attempts to convert XI to XIII with hot, concentrated hydrochloric acid led to decarboxylation. An alternative, and successful, route to XIII was available by first transesterifying IX with sodium benzyloxide in benzyl alcohol to give a rather low yield of the ester (X). Chlorination using methanesulfonyl chloride converted X to XII, and hydrogenolysis of the benzyl ester over platinum oxide in acidic tetrahydrofuran afforded the desired mustard acid (XIII).

A comparison of the antitumor activity of XIII with a number of related indole mustards is presented in Table I. These data indicate the superiority of the propionic acid mustard in this series. It would be of interest to prepare and test 5-[bis(2-chloroethyl)amino]indole-3-butyric acid, since in an analogous series of *p*-[bis(2-chloroethyl)amino]phenylalkanoic acids tested against the Walker tumor, the

TABLE I

ANTITUMOR ACTIVITY OF INDOLE 5-NITROGEN MUSTARDS[a,b]



R	LD_{10} (mg./kg./day)[c]	MED (mg./kg./day)[d]	T. I.[e]
CO_2H	13.0	4.7	3
$CO_2C_2H_5$	25.0	4.3	6
CH_2CO_2H[f]	7.1	1.5	5
$CH_2CH_2CO_2H$[f]	3.8	0.15	25
$CH_2CH(NH_2)CO_2H$[g]	1.2	0.31	4

[a] The observations are for the Walker 256 (subcutaneous) tumor in rats. The drugs were administered intraperitoneally following the standard Cancer Chemotherapy National Service Center protocol, *Cancer Chemotherapy Rept.*, **25**, 11 (1962). [b] We are indebted to Miss Ann Morris Smith, Southern Research Institute, Birmingham, Alabama for many of these testing results. [c] The LD_{10} is that dose that kills more than 10% of the animals and is the maximum tolerated dose. [d] The minimum effective dose (MED) is that dose which gives a ratio of tumor weight in treated animals to tumor weight in control animals of 0.10. [e] The therapeutic index (T. I.) is defined as LD_{10}/MED. [f] See ref. 3. [g] See ref. 2.

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(2) J. DeGraw and L. Goodman, *J. Org. Chem.*, **27**, 1395 (1962).

(3) J. DeGraw and L. Goodman, *ibid.*, **27**, 1728 (1962).

(4) J. DeGraw and L. Goodman, *J. Med. Chem.*, in press.

(5) Subsequent to this work a much more efficient preparation of V from I was described: M. Melzer, *J. Org. Chem.*, **27**, 496 (1962).

carboxylic acid was essentially inactive, the acetic acid had a higher activity than the propionic acid, and the butyric acid was more active than the acetic acid.[6]

Experimental[7]

Oxime of 5-Nitroindole-3-carboxaldehyde (III).—To 20 ml. of pyridine was added 2.00 g. (10.5 mmoles) of 5-nitroindole-3-carboxaldehyde (II), 1.80 g. (26 mmoles) of hydroxylamine hydrochloride, and 10 ml. of methanol. The solution was refluxed for 4.5 hr. and evaporated to dryness *in vacuo*. The residue was stirred with 20 ml. of water until it solidified. The solid was collected, washed with water, and sucked as dry as possible. The damp material was recrystallized from 27 ml. of 67% ethanol to give 1.64 g. (76%) of yellow crystals, m.p. 217–223°. An analytical sample, m.p. 214–218°, was obtained by recrystallization from ethanol.

Anal. Calcd. for $C_9H_6N_2O_4$: C, 52.4; H, 2.93; N, 13.6. Found: C, 52.7; H, 3.16; N, 13.7.

Methyl 5-Nitroindol-3-yl Ketone (VI).—To an ice-cold suspension of 0.81 g. (5 mmoles) of 5-nitroindole in 10 ml. of benzene containing 0.80 ml. (8 mmoles) of acetic anhydride, was added, during 15 min. with stirring, a solution of 1.8 ml. (15 mmoles) of anhydrous stannic chloride in 3 ml. of benzene. Stirring was continued for another hr. at 0–5°, and then the mixture was poured onto 50 g. of ice with stirring. The mixture was stirred for 30 min. and filtered. The cake was washed with water and air-dried to leave 0.77 g. of solid. The crude material was recrystallized from dimethylformamide–acetonitrile to give 0.40 g. of pale yellow crystals, m.p. >305°. A second crop of 0.24 g. was obtained by dilution of the mother liquors with water; total yield 0.64 g. (63%); λ_{max}^{Nujol} 3.1–3.2 (NH), 6.15 (C=O), 7.48 (NO_2) μ.

Anal. Calcd. for $C_{10}H_8N_2O_3$: C, 58.8; H, 3.95; N, 13.7. Found: C, 58.6; H, 4.08; N, 13.5.

Ethyl 5-Nitroindole-3-carboxylate (VII).—To 600 ml. of absolute ethanol, containing 12 ml. of ethanesulfonic acid was added 37.0 g. of the carboxylic acid (V), and the mixture was refluxed with stirring for 24 hr. The resulting solution was evaporated to dryness *in vacuo*, and the residue was stirred with 300 ml. of saturated sodium bicarbonate for 1 hr. The solid was collected by filtration, washed with water, and dried. The material was recrystallized from absolute ethanol to yield 25.2 g. (59%) of yellow crystals, m.p. 229–230°. An analytical sample, m.p. 230–232°, was obtained similarly; λ_{max}^{Nujol} 3.00 (NH), 5.99 (ester C=O), 7.45 (NO_2) μ.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 52.7; H, 3.44; N, 20.5. Found: C, 52.4; H, 3.35; N, 20.3.

5-Nitroindole-3-carbonitrile (IV).—To a stirred solution of 1.20 g. of the aldoxime (III) in 15 ml. of tetrahydrofuran was added 3.0 ml. of thionyl chloride during 7 min. while maintaining the temperature below 30° with a cold-water bath. A precipitate, which initially formed, redissolved after about half of the reagent was added. The solution was stirred for another 15 min. at room temperature and was evaporated to dryness *in vacuo*. The residue was recrystallized from 20 ml. of 75% methanol to afford 0.71 g. (65%) of pale yellow crystals, m.p. 220–227°. An analytical sample, m.p. 223–225°, was obtained by recrystallization from ethyl acetate; λ_{max}^{Nujol} 2.97 (NH), 4.50 (C≡N), 7.46 (NO_2) μ.

Anal. Calcd. for $C_9H_5N_3O_2$: C, 57.8; H, 2.69; N, 22.5. Found: C, 57.5; H, 2.91; N, 22.4.

5-Nitroindole-3-carboxylic Acid (V).—A solution of 50 g. of the nitrile (IV) in 500 ml. of 25% sodium hydroxide was refluxed for 20 hr. The red solution was cooled and some crystalline material was filtered. The filtrate was acidified with concentrated hydrochloric acid, and the yellow precipitate was collected and dried to yield 26.1 g. of solid. The crystalline material, obtained by cooling the hydrolysate, was extracted with boiling water and filtered, and the filtrate acidified to give another 20.0 g. of product; total 46.1 g. (84%). An analytical sample, m.p. >300°, was prepared by recrystallization from acetone–water; λ_{max}^{Nujol} 2.96 (NH), 5.92 (carboxyl C=O), 7.40 (NO_2) μ.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.4; H, 4.30; N, 12.0. Found: C, 56.6; H, 4.25; N, 12.4.

Ethyl 5-Aminoindole-3-carboxylate (VIII).—A suspension of 25.0 g. of the nitro ester (VII) and 3.5 g. of platinum oxide in 300 ml. of absolute ethanol was shaken with hydrogen at 3 atmospheres, absorption of the theoretical amount of hydrogen requiring about 15 hr. The catalyst was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. Only the residue was crystallized from benzene to afford 19.0 g. (88%) of yellowish needles, m.p. 103–104°; λ_{max}^{Nujol} 2.95, 3.01, 3.20 (NH), 6.00 (ester C=O) μ.

Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.7; H, 5.92; N, 13.7. Found: C, 65.2; H, 6.06; N, 13.9.

Ethyl 5-[Bis(2-hydroxyethyl)amino]indole-3-carboxylate (IX).—To an ice-cold solution of 220 mg. of the amino ester (VIII) in 3 ml. of absolute ethanol containing 5 mg. of *p*-toluenesulfonic acid was added 0.5 ml. of ethylene oxide. The solution was allowed to stand at room temperature for 15 hr. and was evaporated to dryness *in vacuo*. The crystalline residue was stirred for 5 min. with 5 ml. of water containing 1 ml. of saturated sodium bicarbonate, and the solid was collected, washed with water, and dried to leave 220 mg. (70%) of tan crystals, m.p. 115–117°. One recrystallization from 50% ethanol afforded an analytical sample, m.p. 124–125°: λ_{max}^{Nujol} 3.07 (OH, NH), 5.99 (ester C=O), 9.55 (C–OH) μ.

Anal. Calcd. for $C_{15}H_{20}N_2O_4$: C, 61.6; H, 6.90; N, 9.58. Found: C, 61.5; H, 6.97; N, 9.78.

Ethyl 5-[Bis(2-chloroethyl)amino]indole-3-carboxylate (XI).—To a solution of 2.00 g. (6.9 mmoles) of the bis(hydroxyethyl) compound (IX) in 10 ml. of pyridine (dried over potassium hydroxide) was added 1.81 g. (15.8 mmoles) of methanesulfonyl chloride. When the vigorous exothermic reaction began to subside, the solution was heated for 10 min. on the steam bath, then was cooled and diluted with 100 ml. of water to produce a gummy precipitate. The mixture was stirred for 1 hr. to cause crystallization and then filtered. The cake was washed with water and dried to give 1.10 g. (49%) of white crystals, m.p. 115–117°. An analytical sample, m.p. 115.0–116.5°, was obtained by recrystallization from benzene; λ_{max}^{Nujol} 3.05 (NH), 5.95 (ester C=O) μ.

Anal. Calcd. for $C_{15}H_{18}Cl_2N_2O_2$: C, 54.8; H, 5.52; N, 8.52; Cl, 21.5. Found: C, 54.9; H, 5.78; N, 8.30; Cl, 21.4.

Benzyl 5-[Bis(2-hydroxyethyl)amino]indole-3-carboxylate (X).—To 33 ml. of dry benzyl alcohol was added 1.20 g. (52.2 mg.-atoms of sodium which was warmed into solution at 95–100°. Then 12.4 g. (42.4 mmoles) of the ethyl ester (IX) was added, and the resulting solution was heated at 95–100° under nitrogen for 8 hr. The thick paste was neutralized with acetic acid, then partitioned between 60 ml. of water and 125 ml. of ethyl acetate. The aqueous portion was extracted with another 50 ml. of ethyl acetate. The combined ethyl acetate extracts were washed with 50 ml. of water, dried over magnesium sulfate, and evaporated *in vacuo*. The liquid residue was diluted with 600 ml. of Skellysolve B (b.p. 62–70°) and shaken vigorously. The supernatant was decanted from the oil which separated, and the oil was shaken with 170 ml. of ether, causing it to solidify. The ether supernatant was decanted into the previous Skellysolve B wash to give a white, crystalline precipitate. This material was collected, united with the above ether insoluble material, and dried *in vacuo* to leave 11.2 g. of solid. This residue was dissolved in 100 ml. of hot ethyl acetate and the solution cooled to cause a small amount of dark gum to deposit. The mixture was stirred with a little Norit and filtered, and the filtrate was allowed to stand for 2 days at room temperature. The white crystals were collected by filtration, washed with 10 ml. of ethyl acetate, and dried to yield 3.8 g. (25%), m.p. 130.5–134.5°. Concentration of the filtrate to 20 ml., followed by chilling for 20 hr., gave a second crop of 0.5 g., m.p. 127–133°. An analytical sample, m.p. 134.5–135.0°, was obtained from another run.

Anal. Calcd. for $C_{20}H_{22}N_2O_4$: C, 67.8; H, 6.26; N, 7.91. Found: C, 67.7; H, 6.47; N, 7.71.

Benzyl 5-[Bis(2-chloroethyl)amino]indole-3-carboxylate (XII).—To a solution of 3.75 g. (10.6 mmoles) of the benzyl ester (X) in 23 ml. of pyridine was added 2.72 g. (23.7 mmoles) of methanesulfonyl chloride during 1 min. with stirring. The temperature rose to a maximum of 60° and when it began to decline, the solution was heated for 15 min. on the steam bath. It was cooled and diluted with 200 ml. of water to give a gummy precipitate which solidified after the mixture was stirred for 2 hr. The crystals were collected, washed with two 10-ml. portions of water, and dried to afford 1.80 g. (43%) of product. The crude material

(6) W. C. J. Ross, "Biological Alkylating Agents," Butterworths, London, 1962, p. 123.

(7) Melting points, corrected, were obtained with a Fisher-Johns apparatus.

was recrystallized from 5 ml. of benzene to give 1.14 g. (27%) of product, m.p. 131–136°. An analytical sample, m.p. 137–138.5°, was obtained similarly.

Anal. Calcd. for $C_{20}H_{20}Cl_2N_2O_2$: C, 61.5; H, 5.17; N, 7.17; Cl, 18.2. Found: C, 61.8; H, 5.40; N, 6.95; Cl, 17.8.

5-[Bis(2-chloroethyl)amino]indole-3-carboxylic Acid (XIII).—To a solution of 300 mg. of the benzyl ester (XII) in 10 ml. of tetrahydrofuran was added 0.1 ml. of concentrated hydrochloric acid and 50 mg. of platinum oxide. The mixture was stirred under an atmosphere of hydrogen at room temperature, consuming the theoretical amount of gas during 35 min. The product, which had crystallized from solution as its hydrochloride, was collected along with the catalyst. The mixture was stirred in a solution of 5 ml. of water and 5 ml. of ethyl acetate, followed by removal of the catalyst by filtration. The ethyl acetate extract was evaporated to dryness *in vacuo* to leave 200 mg. (87%) of a sirup which crystallized when stirred with 2 ml. of water. The white crystals were collected, washed with water, and dried. Recrystallization of this material from 50% ethyl acetate–Skellysolve B afforded 93 mg. (40%), m.p. 150–152°. Another recrystallization gave an analytical sample, m.p. 151.5–152.5°.

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_2$: Cl, 51.8; H, 4.68; N, 9.30; Cl, 23.55. Found: C, 51.9; H, 5.21; N, 9.07; Cl, 23.16.

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Fluorine-Containing Potential Anticancer Agents. II.[1a] Syntheses of Some Trifluoromethylpurines and Trifluoromethylthiazolopyrimidines[1b]

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As part of a search for improved cancer chemotherapeutic agents, various trifluoromethylpyrimidines were prepared and cyclized by standard techniques to the corresponding trifluoromethyl analogs of purine and thiazolopyrimidine. The compounds were evaluated in the routine three-tumor mouse screen of the Cancer Chemotherapy National Service Center. All were ineffective as tumor inhibitors.

Paper I[1a] of this series described the synthesis of various trifluoromethylpyrimidines as the first stage in a program for the preparation of potential antimetabolites. This program has now been extended to include trifluoromethyl analogs of purines and thiazolopyrimidines.

The syntheses of 5-amino-4,6-dichloro-2-trifluoromethylpyrimidine (XXV) and 5-amino-2,4-dichloro-6-trifluoromethylpyrimidine (XXVI) were reported previously. These intermediates have been converted to various purines in the standard preparative sequence shown in Schemes A and B.

Refluxing XXV and XXVI in methanol with various alkylamines provided the corresponding 4-alkylaminopyrimidines (I–IV and XI–XIV). Ring closure of the 4-alkylaminopyrimidines to the 2- and 6-trifluoromethylpurines (V–VII and XV–XVII) was effected by heating either with a mixture of ethyl orthoformate and acetic anhydride (method A), with 97% formic acid (method B), or with pure ethyl orthoformate (method C).

Method A, applied to the 2-trifluoromethyl series gave the 5-acetamido-6-chloro-4-alkylamino-2-trifluoromethylpyrimidines as side products. With method B, the side products were the corresponding 5-formamidopyrimidines. No side product was isolated when using method C on the 2-trifluoromethyl compounds or when using any of the methods on the 6-trifluoromethyl series.

No attempt was made to cyclize the butylamino pyrimidines (III and XIII).

The 2- and 6-aminopurines (VIII–X and XVIII–XX) were obtained by heating the chloropurines in an autoclave with ethanolic ammonia. The 2-chloropurines (XV–XVII) were converted with potassium hydrogen sulfide in methanol to the 2-mercapto derivatives (XXI). These were difficult to purify. They were therefore treated with ethyl bromide and ethanolic potassium hydroxide, and isolated as the ethyl derivatives (XXII–XXIV).

The reaction of ethanolic ammonia with XXV and XXVI gave the corresponding 4,5-diaminopyrimidines (XXXI and XXVII). In Scheme B, cyclization of XXVII and XXXI, using method A, produced 2-chloro-6-trifluoromethylpurine (XXVIII) and 6-chloro-2-trifluoromethylpurine (XXXIV), respectively. When cyclization of XXVII was carried out with trifluoroacetic anhydride in trifluoroacetic acid the product was 2-chloro-6,8-bis(trifluoromethyl)purine (XXIX).

Thiourea and the chloropurine (XXXIV) provided 6-mercapto-2-trifluoromethylpurine (XXXV). Condensation of XXXIV with ethylamine, butylamine, aniline, and benzylamine gave the corresponding alkylamino derivatives (XXXVI–XXXIX).

Various trifluoromethyl[5,4-*d*]thiazolopyrimidines were prepared as outlined in Scheme C.

2,4-Dichloro-5-nitro-6-trifluoromethylpyrimidine (XL) was converted *via* XXVI to 5-amino-2-chloro-4-mercapto-6-trifluoromethylpyrimidine (XLI) as described previously.[1a] Ring closure of XLI to form 5-chloro-7-trifluoromethylthiazolo[5,4-*d*]pyrimidine (XLII) was brought about by heating with ethyl orthoformate. The conversion of 5-amino-6-chloro-4-mercapto-2-trifluoromethylpyrimidine (XLVI)[1a] to 7-chloro-5-trifluoromethylthiazolo[5,4-*d*]pyrimidine (XLVII) was carried out in the same fashion. Reaction of XL with 1 mole of potassium thiocyanate in acetic acid afforded the 4-thiocyano derivative (XLIII). Treatment of XLIII with aniline then gave the 2-

(1) (a) Paper I of this series: S. Inoue, A. J. Saggiomo, and E. A. Nodiff, *J. Org. Chem.*, **26**, 4504 (1961); (b) this investigation was supported by Research Grant CY-4270, from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service; (c) to whom inquiries should be addressed.