Synthesis of Potential Anticancer Agents. XV. Nitrogen Mustards from Indole Derivatives[1,2]

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Application of the Mannich reaction with bis-2-chloroethylamine and formaldehyde to isatin, carbazole, phthalimide, and succinimide gave the corresponding nitrogen mustard derivatives. Condensation of *p*-[*N*,*N*-bis(2-chloroethyl)amino]-benzaldehyde with 3-indolepropiohydrazide, 3-indoleglyoxylhydrazide, and 3-indoleacetohydrazide gave the corresponding benzylidene derivatives. Reduction of the benzylidene derivative of 3-indoleacetohydrazide gave the corresponding hydrazine. The Schiff base of tryptamine and *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde is reported.

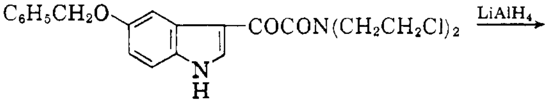
In the search for chemotherapeutic agents capable of selectively interfering with the growth of neoplastic cells as compared with normal cells, the idea of incorporating an alkylating function into a derivative of indole has attractive implications. If one accepts the so-called "carrier" hypothesis as it relates to mechanism of drug action, the observation of Cole and Matthews[4] that labelled tryptophan is localized in a metastatic human tumor to a considerably greater extent than in certain normal tissues becomes of interest and suggests that this scheme may be used to direct the pharmacodynamic mustard function selectively to malignant cells.

Also of interest is a theory recently put forward by Baker and co-workers[5] which suggests that the selective action of some agents against neoplastic disease may be due to the capacity of normal cells to metabolize a drug to a less toxic substance whereas certain neoplastic cells may be deficient in enzymes necessary to carry out such detoxification. The enzyme, tryptophan pyrrolase, catalyzes the conversion of tryptophan to *N*-formylkynurenine[6] and it has been noted that tryptophan pyrrolase activity is absent or quite low in certain rat and mouse hepatomas.[7] The further observation has been made that tryptophan pyrrolase activity was depressed during the period of most rapid tumor growth but that activity of the enzyme increased in the terminal stages of the disease.[8] Udenfriend and co-workers conclude that the carcinoid tumor is parasitic on the tryptophan stores of the patient and that, as a result of this, less of the amino acid may be available for formation of other metabolites.[9]

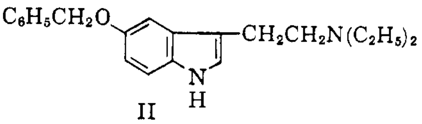
It is therefore strongly suggested that tryptophan pyrrolase or one or more of the other enzymes associated with tryptophan (or indole) metabolism may be able to detoxify an indole mustard in normal cells while the toxic properties of the agent remain substantially undiminished in the tumor system.

Apart from biochemical considerations, an investigation of mustards prepared from indole derivatives presents some interest from a purely organic chemical point of view. At the time this work was begun, only one mustard derived from a pyrrole derivative, 1-(2-chloroethyl)-2-chloromethylpyrrolidine,[10] has been reported and none derived from indoles. In the meantime, synthesis of I, which on reduction with lithium aluminum hydride gave II,[11] of III[12] and of IV[13] has been described. In this communication we wish to report the synthesis of a number of nitrogen mustard derivatives of indole, isoindole, and pyrrole.

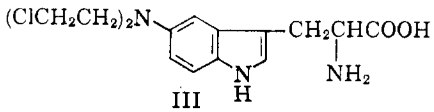
At the outset, application of the Mannich re-



I



II



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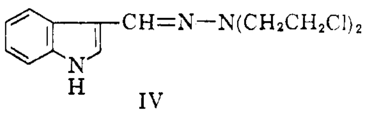

IV

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[2] For the preceding paper in this series see R. C. Elderfield, R. N. Prasad, and T. K. Liao, *J. Org. Chem.*, **27**, 573 (1962).

[3] USPHS Predoctoral Fellow.

[4] J. W. Cole and L. M. Matthews, *A.M.A. Arch. Surg.*, **76**, 912 (1958); *Chem. Abstr.*, **52**, 13961 (1958).

[5] A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 6050 (1960).

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[7] J. Claudatus and S. Ginori, *Science*, **125**, 394 (1957).

[8] S. Wood, Jr., R. S. Rivlin, and W. E. Knox, *Cancer Research*, **16**, 1053 (1956).

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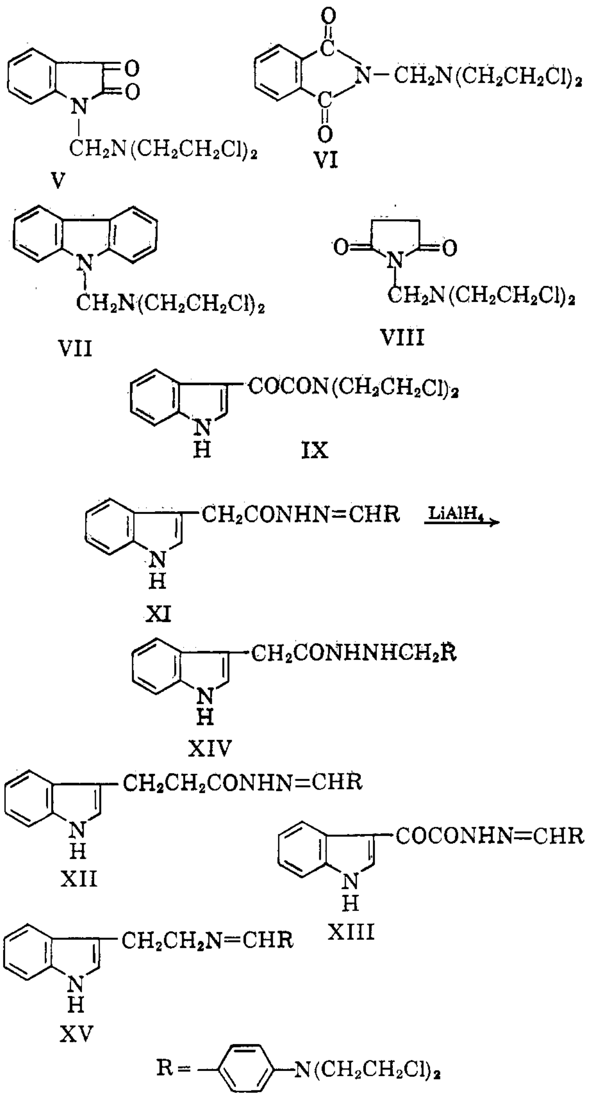
[10] A. M. Rutenberg, L. Persky, O. M. Friedman, and A. M. Seligman, *J. Pharmacol. and Exptl. Therap.*, **111**, 483 (1954).

action with formaldehyde and bis-β-chloroethylamine was investigated.[14] Well defined crystalline products (V–VIII) were obtained from isatin, phthalimide, succinimide, and carbazole. No workable products have been obtained thus far with indole derivatives themselves.

Analytical data for I as reported[11] leave something to be desired. We have therefore prepared IX by reaction of 3-indoleglyoxyl chloride with bis-β-chloroethylamine. Attempts at reduction of IX to the tryptamine mustard did not lead to a tangible product and the reaction was not investigated further in view of the dechlorination of I under similar conditions reported by Kondo.[11] This dechlorination is discussed further below.



Finally, incorporation of the mustard function into selected indole derivatives was accomplished by use of the readily available *p*-(bischloroethyl)-aminobenzaldehyde (X).[15] Condensation of X with 6-ethoxythioindoxyl[16] and with oxindole[17] has been described. Condensation of the appropriate hydrazide with X gave the benzylidene hydrazides XI–XIII. Reduction of XI with lithium aluminum hydride gave XIV. It is interesting that in this instance the chloroethyl groups did not undergo dechlorination as has been noted with I. It has been our experience as well as that of others[18] that so-called "aromatic" nitrogen mustards are considerably less reactive than "aliphatic" or amide mustards. The inertness of hydrazide carbonyl groups to reduction with lithium aluminum hydride has been discussed by Hinman.[19]

Finally, tryptamine on condensation with X readily gave the Schiff base (XV).

Results of tests of these compounds against experimental tumors will be reported elsewhere.

Experimental[20,21]

1-Bis(2-chloroethyl)aminomethylisatin (V).—The Mannich condensations were done by the following typical procedure. A mixture of 14.7 g. (0.1 mole) of isatin, 9.0 ml. of 37% formalin (0.11 mole), bis-2-chloroethylamine (prepared from 17.9 g. (0.1 mole) of the hydrochloride,[22] and 1250 ml. of benzene was refluxed for 25 min. After filtering while still hot, the filtrate was concentrated at the aspirator to one third its volume, dried over magnesium sulfate, treated with decolorizing carbon, and taken to dryness. The residue was recrystallized from chloroform–petroleum ether (b.p. 40–60°) and, after removal of two high-melting crops, gave 15.4 g. (51%) of crystalline material, m.p. 78–80°. Further recrystallization from ethyl acetate–petroleum ether (60–75°) gave analytically pure material, m.p. 82.5–83°.

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_2$: C, 51.84; H, 4.69; N, 9.30. Found: C, 51.97; H, 4.72; N, 9.32.

***N*-Bis(2-chloroethyl)aminomethylphthalimide (VI).**—The residue after removal of the benzene was extracted with several portions of boiling ether leaving unchanged phthalimide as an insoluble portion. The ether extracts were treated with decolorizing carbon and diluted with petroleum ether (b.p. 40–60°). On refrigeration 10.1 g. (67%) of material, m.p. 76–79°, separated. Further recrystallization from the same solvents raised the m.p. to 82–83°.

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_2$: C, 51.84; H, 4.69; N, 9.30. Found: C, 51.95; H, 4.75; N, 9.40.

9-Bis(2-chloroethyl)aminomethylcarbazole (VII). The crude product was crystallized from ether–petroleum ether (b.p. 40–60°) to give 13.4 g. (42%) of colorless crystals and 5.2 g. of tan material which was easily separated by hand.

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(11) H. Kondo, H. Kataoka, and T. Dodo, *Itsuu Kenkyusho Nempo*, **11**, 53 (1960); *Chem. Abstr.*, **55**, 17619 (1961). However, Y. Kuwada [*Chem. Pharm. Bull.* (Tokyo), **8**, 77 (1960)], reports the reduction of a number of amides of bis-2-chloroethylamine with lithium aluminum hydride with retention of the chlorines.

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

(13) Von W. Schulze and G. Letsch, *J. prakt. Chem.*, **14**, 11 (1961).

(14) For citations of earlier applications of the Mannich reaction with bis-β-chloroethylamine, see R. C. Elderfield and J. R. Wood, *J. Org. Chem.*, **26**, 3042 (1961).

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(16) R. M. Anker and J. H. Cook, *J. Chem. Soc.*, 489 (1944).

(17) Brit. Patent 595,571, December 9, 1947.

(18) H. H. Liu and C. C. Price, *J. Org. Chem.*, **26**, 264 (1961).

(19) R. L. Hinman, *J. Am. Chem. Soc.*, **78**, 2463 (1956).

(20) All melting points are corrected for stem exposure.

(21) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(22) F. G. Mann, *J. Chem. Soc.*, 461 (1934).

Further recrystallizations of the crystalline material raised the m.p. to 56–58°.

Anal. Calcd. for $C_{17}H_{18}Cl_2N_2$: C, 63.56; H, 5.65; N, 8.72. Found: C, 63.55; H, 5.58; N, 8.84.

***N*-Bis(2-chloroethyl)aminomethylsuccinimide (VIII).**—The procedure was standard except that the reactants were stirred in chloroform (110 ml.) at room temperature for 45 min. After drying and treatment with decolorizing carbon, the solution was concentrated to 60 ml. under reduced pressure. Addition of petroleum ether (b.p. 40–60°) to turbidity and refrigeration with further addition of petroleum ether gave 20.4 g. (73%) of material, m.p. 65–68°. After three recrystallizations from chloroform–petroleum ether (b.p. 40–60°), the m.p. was 69–71°.

Anal. Calcd. for $C_9H_{14}Cl_2N_2O_2$: C, 42.70; H, 5.58; N, 11.07. Found: C, 42.68; H, 5.65; N, 11.12.

***N*,*N*-Bis-2′-chloroethyl-3-indoleglyoxylamide (IX).**—A solution of bis-2-chloroethylamine (from 40.6 g.) (0.23 mole) of the hydrochloride and 31.5 g. (0.227 mole) of triethylamine in 200 ml. of chloroform was stirred and cooled in an ice bath. 3-Indoleglyoxyl chloride[23] (47.1 g., 0.23 mole) was added gradually at such a rate that the temperature did not exceed 10°. After the addition was complete, stirring with cooling was continued for 15 min. After washing three times with water, the chloroform solution was dried over anhydrous magnesium sulfate, treated with decolorizing carbon, and diluted with petroleum ether (60–75°) at the b.p. until turbid. On cooling 37.02 g. (52%) of material, m.p. 120–122°, separated. After recrystallization from ethyl acetate–petroleum ether (b.p. 60–75°), the m.p. was 121–122°.

Anal. Calcd. for $C_{14}H_{14}Cl_2N_2O_2$: C, 53.69; H, 4.51; N, 8.95. Found: C, 53.84; H, 4.54; N, 9.01.

None of the hydrolyzed rearranged product of the type noted by Ross and Wilson[24] in similar reactions was observed, but in one run a small amount of bright yellow, water soluble, benzene insoluble material, m.p. 166–168°, was isolated. This was not investigated further.

3-Indoleaceto-[4′bis(2-chloroethyl)amino]benzylidene Hydrazide (XI).—A solution of 7.57 g. (0.04 mole) of the hydrazide of indole-3-acetic acid[25] and 9.85 g. (0.04 mole) of X in 740 ml. of absolute ethanol was refluxed for 3 hr. After cooling, the precipitate (15.2 g., 91%) was collected with absolute ethanol. The substance, m.p. 193–194°, was analytically pure. Recrystallization from nitromethane lowered the m.p. to 187–188°.

Anal. for the material, m.p. 193–194°. Calcd. for $C_{21}H_{22}Cl_2N_4O$: C, 60.43; H, 5.31; N, 13.43. Found: C, 60.42; H, 5.33; N, 13.50.

2-[3-Indolepropio-4′-bis(2′′-chloroethyl)amino]benzylidene Hydrazide (XII).—This was prepared by the above procedure from 2-(3-indole)propiohydrazide.[26] The substance, m.p. 167–168°, was obtained analytically pure in 79% yield directly on cooling the reaction mixture.

Anal. Calcd. for $C_{22}H_{24}Cl_2N_4O$: C, 61.25; H, 5.61; N, 12.99. Found: C, 61.11; H, 5.74; N, 12.95.

3-Indoleglyoxyl-[4′-bis(2-chloroethyl)amino]-benzylidene Hydrazide (XIII).—This was obtained in 92% yield from 3-indoleglyoxyl hydrazide[27] by the above procedure. The substance melted at 221–222°.

Anal. Calcd. for $C_{21}H_{20}Cl_2N_4O_2$: C, 58.47; H, 4.67; N, 12.99. Found: C, 58.52; H, 4.71; N, 12.95.

***N*-(3-Indoleacetyl)-*N*′-[4-bis(β-chloroethyl)aminobenzyl]-hydrazine (XIV).**—To a stirred suspension of 7.74 g. (0.19 mole) of lithium aluminum hydride in 300 ml. of tetrahydrofuran (distilled from lithium aluminum hydride) 27.1 g. (0.06 mole) of XI was added slowly in portions. Stirring was continued for 1 hr. after the addition was complete. After careful decomposition by dropwise addition of water, the solids were filtered off and washed thoroughly first with tetrahydrofuran and then with ethyl acetate. After drying the combined filtrates over anhydrous magnesium sulfate, treatment with decolorizing carbon, removal of most of the solvent under reduced pressure left a residue (11.4 g. or 42%) which was recrystallized from ethyl acetate–petroleum ether (b.p. 60–75°). The pure material melted at 139–140°.

Anal. Calcd. for $C_{21}H_{24}Cl_2N_4O$: C, 60.14; H, 5.77; N, 13.36. Found: C, 60.10; H, 5.71; N, 13.43.

4-Bis(2-chloroethyl)aminobenzylidenetryptamine. (XV).—To a solution of tryptamine prepared from 9.83 g. (0.05 mole) of the hydrochloride in 500 ml. of ether was added 12.31 g. (0.05 mole) of X. After stirring with slight warming until X was completely dissolved, the volume was reduced to 125 ml. and the mixture was refluxed for 60 min. Benzene (100 ml.) was added and the solution was taken to dryness under reduced pressure. Concentration was repeated twice with 250-ml. portions of benzene. The residual yellow oil was taken up in ethyl acetate and, after treatment with decolorizing carbon, petroleum ether (b.p. 60–75°) was added to the hot solution. On cooling, 12.77 g. (66%) of material separated. After further recrystallization from the same solvent, the m.p. was 113–114°.

Anal. Calcd. for $C_{21}H_{23}Cl_2N_3$: C, 64.95; H, 5.97; N, 10.82. Found: C, 64.98; H, 5.88; N, 10.88.

Acknowledgment.—We acknowledge the valuable assistance of James Hudson in the preparation of several intermediates.

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