recrystallized first from ethyl acetate, then from benzene, affording 0.645 g. (46.2%) of orange needles, m.p. 245.5–247.5° (lit. m.p. 238–240°[21] and 251–253°[22]); $\lambda_{max(\mu)}^{Nujol}$ 2.96 and 3.11 (NH, OH, NH_2), 5.67, 5.78, and 5.83 (ester and quinone C=O), 6.09 (amide C=O); $\lambda_{max(m\mu)}^{EtOH}$ 239 (ε 30,600), 426 (ε 19,400), and 445 (ε 20,300); $[\alpha]^{22}D$ +90.0° (chloroform) [lit.[22] $[\alpha]D$ +130° (chloroform)] and −22.4° (*N,N*-dimethylformamide).

Anal. Calcd. for $C_{26}H_{30}N_4O_{10}$: C, 55.9; H 5.41; N, 10.0. Found: C, 55.6; H, 5.59; N, 9.79.

N,N'-(2-Amino-4,6-dimethyl-3-oxo-3H-phenoxazine-1,9-diyldicarbonyl)di-L-threonine [actinocinyl-bis(L-threonine)] (XXIII). A mixture of 0.854 g. (2.20 moles) of XX, 0.100 g. of platinum oxide, and 15 ml. of absolute ethanol was treated with hydrogen for 3 hr. as in the preparation of II (see above), then was filtered, and to the filtrate was added 0.389 g. (3.60 mmoles) of *p*-benzoquinone. The oxidation proceeded as in the preparation of II and the crude product, 0.187 g. (32.1%), was recrystallized from *N,N*-dimethylformamide–water to give a quantitative recovery of solid, m.p. 233–235°; $\lambda_{max(\mu)}^{Nujol}$ 2.88, 2.98, and 3.11 (NH, OH), 5.79 (carboxyl C=O), 6.02 (amide C=O); $\lambda_{max(m\mu)}^{EtOH}$ 238 (ε 40,000), 426 (ε 24,400), and 446 (ε 25,700).

Anal. Calcd. for $C_{24}H_{26}N_4O_{10}.1/2H_2O$: C, 53.2; H, 5.40; N, 10.4. Found: C, 53.1; H, 5.48; N, 10.2.

2-Amino-N,N'-bis(L-1-carbamoyl-2-hydroxypropyl)-4,6-dimethyl-3-oxo-3H-phenoxazine-1,9-dicarboxamide [actinocinyl-bis(L-threonamide)] (XXIV). A mixture of 3.76 g. (9.69 mmoles) of the amide (XXI), 0.200 g. of platinum oxide, and 35 ml. of absolute ethanol, treated as in the preparation of II, absorbed the theoretical amount of hydrogen in 8 hr. The intermediate *o*-aminophenol was oxidized with 1.62 g. (15.0 mmoles) of *p*-benzoquinone as in the preparation of II, to afford 1.340 g. (52.3%) of the crude phenoxazine (XXIV). The analytical sample, obtained by recrystallization from *N,N*-dimethylformamide–water, had m.p. 256.5–258.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.97, 3.12 (NH, OH, NH_2), 5.91 and 6.02 (amide C=O); $\lambda_{max(m\mu)}^{EtOH}$ 239 (ε 34,700), 427 (ε 24,000), and 447 (ε 25,000); $[\alpha]^{22}D$ + 25.6° (*N,N*-dimethylformamide

Anal. Calcd. for $C_{24}H_{26}N_6O_8.H_2O$: C, 52.7; H, 5.53; N, 15.4. Found: C, 53.2; H, 5.70; N, 15.2.

Acknowledgment. The authors are indebted to Dr. Peter Lim for infrared interpretations and to his staff for the paper chromatography and optical rotations.

MENLO PARK, CALIF.

(21) H. Brockmann and P. Boldt, *Naturwissenschaften*, **46**, 262 (1960).

(22) H. Brockmann, P. Boldt, and H. S. Petras, *Naturwissenschaften*, **47**, 62 (1960).

[CONTRIBUTION FROM THE LIFE SCIENCES DIVISION, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LXXII. Alkylating Agents Derived from Indole. II.[2] Synthesis of a Nitrogen Mustard Derived from DL-Tryptophan

JOSEPH DEGRAW AND LEON GOODMAN

Received October 16, 1961

A synthesis of the 5-nitrogen mustard of DL-tryptophan (VIII) from the previously reported[3] diethyl acetamido(5-nitro-3-indolylmethyl)malonate (III) is described. The preparation of 5-amino-DL-tryptophan (X) is also reported.

Since the demonstration of the antitumor activity of the *meta*-[4,5] and *para*-[6,7] nitrogen mustards of phenylalanine, there has existed an interest in mustards derived from other naturally occurring aromatic amino acids.[8] This manuscript reports the preparation of one such mustard derived from DL-tryptophan, namely, 5-bis(2-chloroethyl)amino-DL-tryptophan (VIII).

At the start of this research, it was felt that the notorious acid sensitivity of the indole nucleus might present many difficulties, or even prevent the preparation of compounds such as VIII by the conventional routes.[4–7] These suspicions were later realized in part, so that certain modifications of the usual routes to such mustards were found to be necessary. The key intermediate, diethyl acetamido(5-nitro-3-indolylmethyl)malonate (III) was prepared according to the procedure of Cavallini and Ravenna,[3] starting from 5-nitroindole (I) and proceeding through 5-nitrogramine (II).

The nitro diester (III) was hydrogenated over platinum oxide as a suspension in ethanol, to give the amino diester (IV) as a crystalline solid

(1) 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. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center. For Part LXXI in this series, see K. A. Hyde, E. M. Acton, B. R. Baker, and L. Goodman, *J. Org. Chem., in press.*

(2) A prior communication on this work has appeared in *Chem. and Ind.*, 1448 (1961).

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

(4) T. Osdene, D. Ward, W. Chapman, and J. Rakoff, *J. Am. Chem. Soc.*, **81**, 3100 (1959).

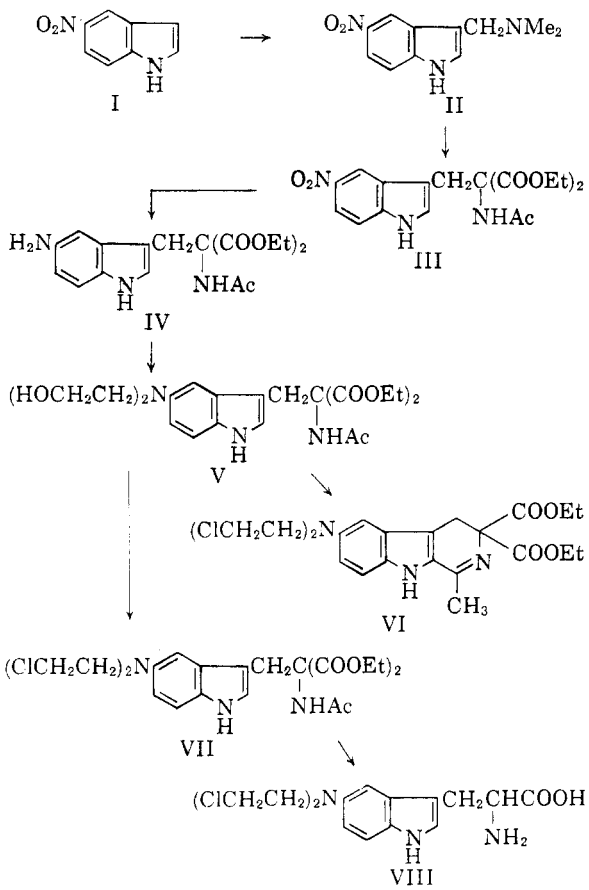
(5) H. Gram, C. Mosher, and B. R. Baker, *J. Am. Chem.* **81**, 3103 (1959).

(6) F. Bergel, V. Burnop, and J. Stock, *J. Chem. Soc.*, 1233 (1955); F. Bergel and J. Stock, *J. Chem. Soc.*, 2409 (1954).

(7) L. Larionov, A. Khoklov, E. Shkodinskaia, O. Vasina, V. Trusheikina, and A. Novikova, *Lancet*, **269**, 169 (1955).

(8) For a complete summary of chemical, biological, and clinical data for *p*-phenylalanine mustard and related compounds, see *Cancer Chemotherapy Reports*, 1960, No. 6, p. 61 (a publication of the Cancer Chemotherapy National Service Center).

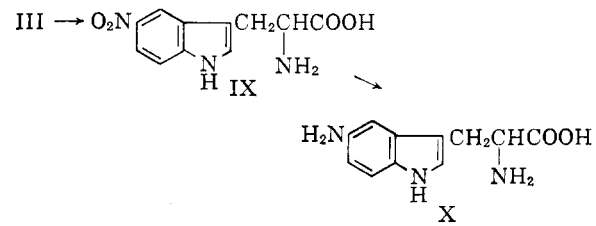
in an 80% yield. When IV was allowed to react overnight with ethylene oxide in 50% aqueous acetic acid, a considerable amount of tarry material was obtained. However, when this reaction was conducted in methanol with a catalytic amount of *p*-toluenesulfonic acid present, the bishydroxyethylated compound (V) was obtained as a sirup. The material could not be differentiated from the starting amine (IV) by various paper chromatographic systems, but its infrared spectrum possessed strong hyroxyl absorptions near 3.0 and 9.5 μ.



It was suspected that chlorination of the bishydroxy compound (V) with the usual reagents, *i.e.*, thionyl chloride or phosphoryl chloride, might lead to significant amounts of the harman-type compound (V) rather than the desired blocked mustard (VII). At least one apparent case of a thionyl chloride-promoted ring closure has been reported.[5] When V was treated with these reagents under a variety of conditions, no compounds related to VI were observed as shown by infrared spectra, but only dark, intractable products were obtained. However, if V was allowed to react with methanesulfonyl chloride in pyridine at room temperature, the desired blocked mustard (VII) was surprisingly obtained as the sole isolated product and as a crystalline solid. It was later found that higher yields, 40–50%, could be realized by carrying out the reaction at 80–90° for twenty minutes.

Hydrolysis of the blocking groups with concentrated hydrochloric acid at reflux proceeded very smoothly, to afford the tryptophan mustard (VIII) as a crystalline precipitate. The amino acid was best precipitated by adjustment of the hydrolysate to *p*H 5–6 with saturated sodium bicarbonate. When saturated sodium acetate was used for this purpose, the product was found to contain about one-fourth mole of acetic acid.

It was of interest also to prepare the previously unreported[9] 5-aminotryptophan. The nitroacetamido ester (III) was completely hydrolyzed with aqueous-alcoholic sodium hydroxide followed by acidification and decarboxylation to give 5-nitro-DL-tryptophan (IX). Cavallini and Ravenna[3] also prepared this compound by a different hydrolysis procedure. When IX was hydrogenated as an aqueous suspension over platinum oxide, the 5-aminotryptophan (X) was obtained in 73% yield as pink crystals, which could be recrystallized from



water. If the reduction was performed in acid solution followed by neutralization with sodium acetate, no product could be obtained; the solution acquired an intense purple coloration.

EXPERIMENTAL[10]

Diethyl acetamido(5-amino-3-indolylmethyl)malonate (IV). To 150 ml. of absolute ethanol was added 10.0 g. of the nitro diester (III) and 0.60 g. of platinum oxide. The mixture was treated with hydrogen at 3 atm. in a Parr shaker, consuming the theoretical amount of hydrogen after 15 hr. The product, which had crystallized from solution, was collected by filtration along with the catalyst and the mixture dried to give 9.6 g. of solid. This was treated with 90 ml. of hot acetonitrile, filtered to remove the catalyst, and the filtrate chilled, to give 6.75 g. of pale yellow crystals, m.p. 196–198°. A second crop of 0.67 g., m.p. 194–196°, was obtained by concentration of the mother liquors to a volume of 20 ml. The total yield was 7.42 g. (80%). An analytical sample, m.p. 198–199°, was obtained by ethanol recrystallization of material from another run; $\lambda_{max(\mu)}^{Nujol}$ 3.05, 3.10, 3.15

(9) This compound was used in a biological investigation, but no preparative details are given; see V. Erspamer, A. Glasser, C. Pasini, and G. Stoppani, *Nature*, **189**, 483 (1961).

(10) Melting points are uncorrected and were obtained with the Fisher-Johns apparatus. Paper chromatography was done by the descending technique on Whatman No. 1 paper and the spots were detected by visual examination under ultraviolet light. Adenine was used as a standard and the spots were located relative to R_{Ad} 1.00. The solvent systems used were: A, isopropyl alcohol–2*N* hydrochloric acid; and B, *n*-butyl alcohol–acetic acid–water (5:2:3).

(NH), 5.80 (ester C=O), 6.06 (amide C=O); there was no NO_2 band near 7.5 μ.

Anal. Calcd. for $C_{18}H_{23}N_3O_5$: C, 59.8; H, 6.4; N, 11.6. Found: C, 59.5; H, 6.6; N, 11.6.

Diethyl acetamido [5-bis(2-hydroxyethyl)amino-3-indolyl-] methyl]malonate (V). To an ice-cold suspension of 7.5 g. of the amino diester (IV) in 100 ml. of methanol containing 50 mg. of *p*-toluenesulfonic acid was added 20 ml. of ethylene oxide and the mixture was stirred at room temperature for 18 hr. To the resulting solution was added 5 ml. of saturated sodium bicarbonate, and the mixture was evaporated to dryness *in vacuo*. The residue was extracted with 100 ml. of dichloromethane and the extract was washed with 20 ml. of water. The organic layer was dried over magnesium sulfate and evaporated *in vacuo*, to leave 9.2 g. (99%) of a sirup identified as V by its infrared spectrum; $\lambda_{max(\mu)}^{film}$ 3.0 (NH, OH), 5.73 (ester C=O), 6.02 (amide C=O), 9.3–9.5 (C—OH). The material could not be further purified and was used directly in the next step.

Diethyl acetamido [5-bis(2-chloroethyl)amino-3-indolylmethyl] malonate (VII). To a solution of 5.52 g. (12.3 mmoles) of the bishydroxyethyl compound (V) in 28 ml. of pyridine was added 3.6 g. (31.5 mmoles) of methanesulfonyl chloride. When the resulting exothermic reaction began to subside, the solution was heated in an 80–90° oil bath for 20 min. The solution was cooled and diluted with 250 ml. of water. The aqueous supernatant was decanted from the gum that separated and another 50 ml. of water was added to the residue. The mixture was stirred and chilled to cause solidification. The solid was collected, washed with water, and dried to yield 3.34 g. (56%) of crude product. The crude material was recrystallized twice from 95% ethanol to give 2.19 g. (37%) of white crystals, m.p. 156–157°. An analytical sample, m.p. 154–155°, was obtained similarly from another run; $\lambda_{max(\mu)}^{Nujol}$ 2.95 (NH), 5.75 (ester C=O), 6.0 (amide C=O); the strong hydroxyl absorption of the precursor was removed.

Anal. Calcd. for $C_{22}H_{29}Cl_2N_3O_5$: C, 54.3; H, 6.0; Cl, 14.6. Found: C, 54.6; H, 6.1; Cl, 14.7.

*5-Bis(2-chloroethyl)amino-*DL*-tryptophan* (VIII). A solution of 11.0 g. of the blocked mustard (VII) in 100 ml. of concd. hydrochloric acid was heated under reflux for 5 hr. The solution was cooled and the *p*H adjusted to 5–6 with 250 ml. of saturated sodium bicarbonate. Scratching caused the resulting gummy precipitate to solidify. The material was collected, washed with water, and dried to afford 4.1 g. (53%) of pale lavender crystals, which showed gradual decomposition above 200°. The material traveled as a single spot in solvent system A, R_{Ad} 1.58, identified by ultraviolet absorption and ninhydrin spray, and possessed a typical zwitterionic amino acid infrared spectrum; $\lambda_{max(\mu)}^{Nujol}$ 2.90, 3.08 (NH), 3.7–4.1 ($NH_3^{\oplus}$), 6.2 ($CO_2^{\ominus}$).

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2$: C, 52.3; H, 5.6; Cl, 20.6; N, 12.2. Found: C, 52.2; H, 5.7; Cl, 20.1; N, 12.4.

If saturated sodium acetate was used to precipitate the amino acid, the product so obtained appeared to contain one-fourth mole of acetic acid.

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot {}^1/_4HOAc$: C, 51.8; H, 5.6; Cl, 19.7; N, 11.7. Found: C, 51.8, 51.6; H, 5.6, 5.6; Cl, 19.1; N, 11.7.

*5-Nitro-*DL*-tryptophan* (IX). To 210 ml. of 95% ethanol was added 120 ml. of water and 22.0 g. of sodium hydroxide. To the resulting solution was added 26.0 g. of the acetamidomalonate (III), and the mixture was heated at reflux for 15 hr. The solution was evaporated nearly to dryness and 500 ml. of water was added with warming to dissolve the crystallized salts. The dark solution was filtered through Celite and the filtrate acidified to *p*H 1 with concd. hydrochloric acid. The solution was distilled until 200 ml. remained, then was heated at reflux to give a total heating time of 5 hr. The *p*H was adjusted to 5–6 with saturated sodium acetate, then the solution was chilled for 1 hr., filtered, and the cake was washed with cold water. The material was dried and recrystallized from 33% acetic acid to give the acetate salt hemihydrate.[3] The brown material was freed of acetic acid by refluxing it for 2 hr. in 700 ml. of water to give 12.8 g. (77%) of IX as yellow crystals, m.p. 270–272° dec. $\lambda_{max(\mu)}^{Nujol}$ 3.20 (NH), 3.7–4.1 ($NH_3^{\oplus}$), 6.37 ($CO_2^{\ominus}$), 7.50 (NO_2). The compound moved as a single spot in solvent system B, R_{Ad} 1.02, as shown by ultraviolet absorption and ninhydrin reagent.

Cavallini and Ravenna[3] observed a melting point of 284–285° dec.

*5-Amino-*DL*-tryptophan* (X). To a suspension of 500 mg. of 5-nitrotryptophan (IX) in 10 ml. of water was added 30 mg. of platinum oxide. The mixture was stirred under an atmosphere of hydrogen, taking up slightly more than the theoretical amount after 15 hr. The product crystallized during this time. The mixture was heated until the crystals dissolved and the catalyst was removed by filtration. The filtrate was chilled for an hour and the pink crystals were collected, washed with 5 ml. of water, and dried to give 320 mg. (73%) of product. The material showed gradual decomposition above 260°; $\lambda_{max(\mu)}^{Nujol}$ 2.88, 2.99, 3.05 (NH), 3.8 ($NH_3^{\oplus}$), 6.3 ($CO_2^{\ominus}$); there was no NO_2 absorption near 7.5 μ; $\lambda_{max(m\mu)}^{H_2O}$ 278 (ϵ 4675), 310 (ϵ 2920), which compares favorably with the ultraviolet spectrum reported for 5-aminoindole.[11]

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.3; H, 6.0; N, 19.2. Found: C, 60.0; H, 6.1; N, 18.9.

Acknowledgment. The authors are indebted to Dr. Peter Lim for infrared interpretations and to his staff for paper chromatography. They also wish to thank Mr. O. P. Crews and his staff for the large-scale preparation of certain intermediates.

MENLO PARK, CALIF.

(11) J. Harley-Mason and A. Jackson, *J. Chem. Soc.*, 1158 (1954).