and then cooled in ice. The product that separated on crystallization from nitrobenzene afforded 1.313 g. (84%) of orange-red needles, m.p. 385°, $\lambda^{benzene}$ 277 (15,600), 289 (23,500) 300 mμ (32,500).

Anal. Calcd. for $C_{24}H_{12}O_4N_2$: C, 73.47; H, 3.08; N, 7.14. Found: C, 73.63; H, 2.97; N, 7.22.

1,2-Phthaloylanthraquinone (IV). A suspension of 420 mg. of 5,8-dinitrodibenzpyrene in 40 ml. of acetic acid was treated gradually with stirring with a solution of 600 mg. of chromic anhydride in 1.2 ml. of water and the mixture was heated for 1 hr. in an oil bath at 115–120° under reflux. The solution was cooled to about 50°, diluted with 80 ml. of hot water, and let cool. The product, crystallized from xylene, afforded 220 mg. (61%) of yellow needles, m.p. 330° (lit.[12] m.p. 325), $\lambda^{benzene}$ 250 (23,100), 276 mμ (46,200).

Anal. Calcd. for $C_{22}H_{10}O_4$: C, 78.10; H, 2.98. Found: C, 78.31; H, 2.89.

1,2,6,7-Tetrahydro-3,4,9,10-dibenzpyrene (VII). A solution of 650 mg. of dibenzpyrene in 180 ml. of isoamyl alcohol was refluxed and treated with a total of 8 g. of sodium, added in the course of 4 hr. The solution was cooled, water was added, and the organic layer was diluted with ether and washed repeatedly with water and dilute acid. After removal of solvent in vacuum, the residue was dissolved in benzene and chromatographed on alumina. Some unchanged dibenzpyrene was recovered. The product eluted by benzene on crystallization from cyclohexane gave 276 mg. (40%) of yellow leaflets, m.p. 248°, $\lambda^{ethanol}$ 229 (58,500), 250 (70,300), 261 (100,000), 309 (41,800), 322 (62,900), 328 (50,500) 336 (39,200), 352 mμ (36,100).

Anal. Calcd. for $C_{24}H_{18}$: C, 94.08; H, 5.92. Found: C, 93.74; H, 5.95.

1,2-Dihydro-3,4,9,10-dibenzpyrene (X). A solution of 200 mg. of dibenzpyrene in 200 ml. of ethyl acetate and 50 ml. of acetic acid when treated with 70 mg. of platinum oxide and shaken with hydrogen absorbed 1 mole of gas in 2 hr. Chromatography of the product gave, in the benzene eluates, a substance which on crystallization from cyclohexane afforded 130 mg. (65%) of light greenish yellow leaflets, m.p. 224–225°, $\lambda^{ethanol}$ 233 (40,400), 256 (63,000), 274 (86,300), 286 (114,100), 302 (40,200), 317 (29,200), 331 mμ (27,800).

Anal. Calcd. for $C_{24}H_{16}$: C, 94.70; H, 5.30. Found: C, 94.61; H, 5.48.

3,4,9,10-Di-(tetrahydrobenz)-pyrene (VII). A solution of 341 mg. of dibenzpyrene in 300 ml. of ethyl acetate and 50 ml. of acetic acid in the presence of 80 mg. of platinum oxide absorbed 4 moles of hydrogen in 10 hr. Crystallization of the product from cyclohexane gave pinkish white needles, m.p. 190–193°. Chromatography and recrystallization gave 212 mg. (62%) of bluish white needles, m.p. 193–194°, $\lambda^{ethanol}$ 242 (47,700), 250 (98,900), 262 (21,300), 272 (29,500), 284 (40,900), 310 (6,700), 323 (11,000), 338 (24,900), 354 mμ (34,100).

Anal. Calcd. for $C_{24}H_{22}$: C, 92.86; H, 7.14. Found: C, 92.89; H, 7.10.

Acknowledgment. This work was supported by grants from the National Cancer Institute of the National Institutes of Health (CY 1696 Endo), the National Science Foundation, Research Corporation, and the Higgins Fund of Harvard University.

CAMBRIDGE 38, MASS.

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

Potential Anticancer Agents.[1] LXX. Some Simple Derivatives of the Actinomycins

BORIS WEINSTEIN,[2a] OSBORNE P. CREWS, MORRIS A. LEAFFER, B. R. BAKER,[2b] AND LEON GOODMAN[3]

Received October 5, 1961

A new and simpler preparative route is described for 3-(benzyloxy)-2-nitro-*p*-toluic acid (III), a key intermediate in the synthesis of the actinocinyl-L-threonine peptides (II, XXIII, and XXIV). In contrast to previously reported results, the peptide ester (II), obtained by synthesis, is microbiologically inactive against *Staph. aureus*.

The actinomycins are a group of closely related compounds produced by certain species of *Streptomyces* and possessing antibiotic and cytostatic activity.[4] The involved chemistry of these materials has been elucidated chiefly by Brockmann and Johnson during the past decade.[5] The work in this area was crowned recently by the total synthesis of Actinomycin C_3 (I).[6]

The statement that the dimethyl ester of actinocinyl-bis-L-threonine (II) was active against *Staph. aureus* to a dilution of 1:700,000[7] suggested that the

---

(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 the preceding paper in this series, see J. DeGraw, L. Goodman, B. Weinstein, and B. R. Baker, *J. Org. Chem.*, **27**, 576 (1962).

(2) (a) Department of Chemistry, Stanford University. (b) School of Pharmacy, University of Buffalo.

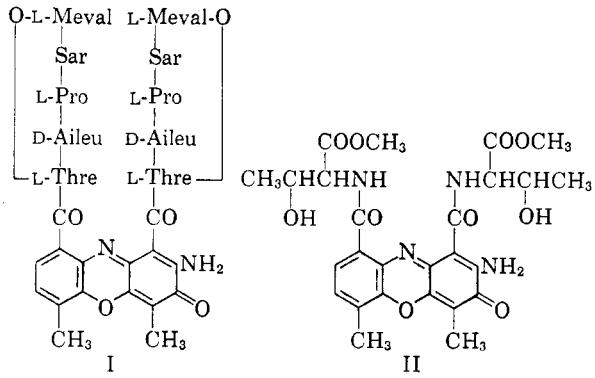
(3) To whom request for reprints should be addressed.

(4) For leading references, see "The Actinomycins and Their Importance in the Treatment of Tumors in Animals and Man," S. A. Waksman, ed., The New York Academy of Sciences, New York, 1960; and S. Farber in "Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity," Little, Brown and Company, Boston, 1958, p. 138.

(5) For pertinent reviews see H. Brockmann, *Angew. Chem.*, **72**, 939 (1960), and H. Brockmann, *Fortschr. Chem. Org. Naturstoffe*, **18**, 1 (1960).

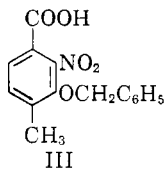
(6) H. Brockmann and H. Lackner, *Naturwissenschaften*, **47**, 230 (1960).

(7) H. Brockmann and H. Grove, Ger. Patent **1,001,685** (July 11, 1957); *Chem. Abstr.*, **54**, 1345 (1960).



synthesis of simple actinomycin analogs might yield effective antibacterial compounds or effective antitumor agents, possibly less toxic than the natural actinomycins. In particular it seemed important to prepare compound II synthetically to verify its activity, as the sample reported to be active was obtained by chemical degradation of the natural antibiotic.

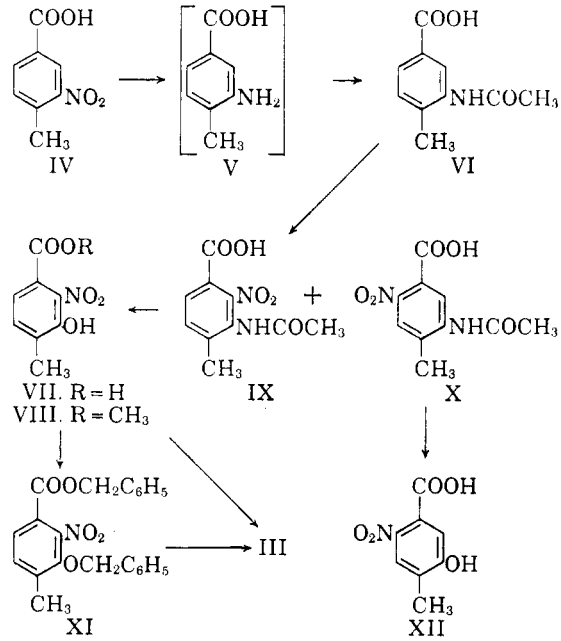
The two existing literature routes for the preparation of the *p*-toluic acid (III), a key precursor to the desired actinomycin analogs, are practically identical and utilize the sulfonation of *p*-toluic acid as the first step of the seven-step synthesis.[8] Several steps of this sequence were inconvenient,



in particular the fusion of the first-formed sulfonic acid with potassium hydroxide as a means of introducing a phenolic group. An alternative route to III was devised, also starting from *p*-toluic acid, which gave a good over-all yield of the substituted *p*-toluic acid (III) in a six-step sequence.

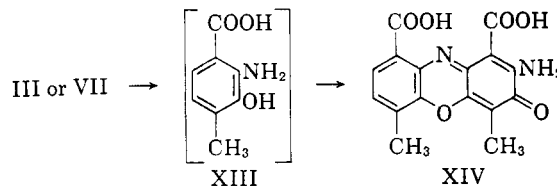
Treatment of *p*-toluic acid with hot concentrated nitric acid gave a good yield of 3-nitro-*p*-toluic acid (IV). Either catalytic hydrogenation over platinum oxide or, more conveniently, reduction with hydrazine mediated by palladium-on-carbon served to convert IV to the amine (V), which, without isolation, was converted to 3-acetamido-*p*-toluic acid (VI).

Nitration of VI with fuming nitric acid afforded two isomeric acids, easily separated by crystallization from glacial acetic acid. The less soluble, desired isomer (IX) was the predominant material being favored over X in a ratio of 4 to 1. Both IX and X were hydrolyzed with aqueous alkali to afford good yields of the phenols VII and XII,[9] respectively. Mixed melting point determinations and infrared spectral comparison with the known compounds VII[10] and XII thus served to establish the structures of the isomers from the nitration of VI.



Treatment of the disodium salt of VII with excess benzyl chloride gave the crystalline benzyl ester, benzyl ether (XI) which could be saponified to yield III. The acid (III) was also obtained directly from VII by eliminating the isolation of XI and saponifying the crude benzylation product. Compound VII was esterified with methanol, giving VIII, whose melting point was in agreement with the literature value.[8]

Catalytic hydrogenation of either III or VII yielded the *o*-aminophenol (XIII), which was not isolated but was converted to actinocinylbiscarboxylic acid (XIV) either by simple exposure to



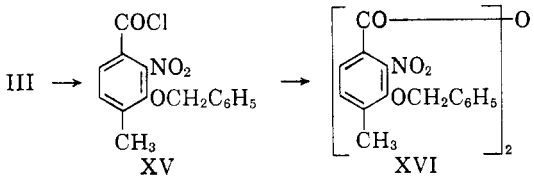
atmospheric oxygen or by oxidation with *p*-benzoquinone.

In order to prepare the L-threonine peptides from III, the acid chloride (XV) was first investigated. It had been reported,[6] without experimental details, that the Schotten-Baumann reaction between XV and L-threonine afforded the desired peptide. When this reaction was attempted in a

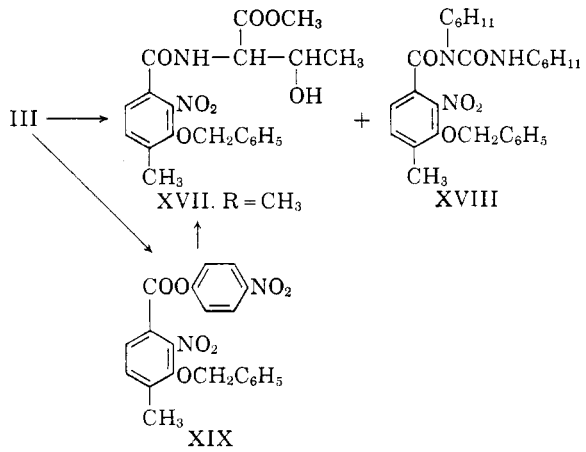
(8) (a) S. J. Angyal, E. Bullock, W. E. Hanger, W. C. Howell, and A. W. Johnson, *J. Chem. Soc.*, 1592 (1957). (b) H. Brockmann and H. Muxfeldt, *Chem. Ber.*, 91, 1242 (1958).

(9) D. Gardner and J. F. Grove, *J. Chem. Soc.*, 3649 (1953). We wish to thank Mr. J. F. Grove, Imperial Chemical Industries, The Frythe, Welwyn, Herts, England, for the sample.

(10) We wish to thank Dr. H. Brockmann, University of Göttingen, for this and other reference samples.



single experiment in the present work, the major product was the acid (III) accompanied by a small amount of the anhydride (XVI). Two alternative methods, however, afforded the methyl L-threonate peptide (XVII). The direct reaction of III with methyl L-threonate,[11] using *N,N'*-dicyclohexylcarbodiimide (DCC) in dichloromethane, gave an excellent yield of the ester peptide (XVII). When the reaction of III and methyl L-



threonate was carried out in methanol-tetrahydrofuran, the urea (XVIII) was a major by-product. Although a formal structure proof for XVIII was not carried out, its analysis and spectral properties were completely consistent with structure XVIII and the presence of such ureas has been previously reported in DCC coupling carried out in tetrahydrofuran.[12]

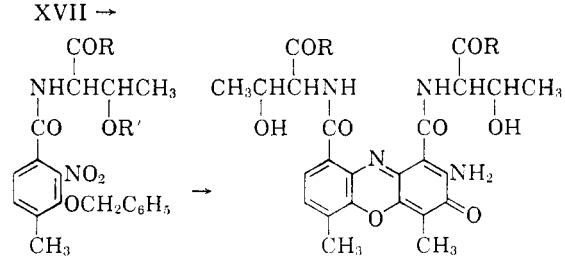
The use of the "activated ester" (XIX)[13] provided the second route to XVII. Compound XIX was prepared by coupling III and *p*-nitrophenol in ethyl acetate with DCC. The reaction between XIX and methyl L-threonate in warm chloroform gave a fair yield of XVII with optical rotation slightly lower than that of XVII prepared by the direct coupling (see above).

Mild saponification of XVII afforded the free acid XX and ammonolysis of XVII yielded the amide XXI. The reaction of XXI with propionyl chloride in refluxing benzene gave a good yield of the ester, amide (XXII); the use of propionyl chloride in pyridine gave a much lower yield of XXII, accompanied by a large amount of a nitrile-containing material.

(11) K. Vogler and P. Lanz, *Helv. Chim. Acta*, **43**, 270 (1960).

(12) J. C. Sheehan, M. Goodman, and G. P. Hess, *J. Am. Chem. Soc.*, **78**, 1367 (1956).

(13) M. Bodansky and V. du Vigneaud, *Nature*, **183**, 1324 (1959).



XX. R = OH; R' = H
XXI. R = NH_2; R' = H
XXII. R = NH_2; R' = C_2H_5CO

XXIII. R = OH
XXIV. R = NH_2

Catalytic reduction of XVII, XX, and XXI over platinum oxide followed by oxidation of the product *o*-aminophenols, either with *p*-benzoquinone or potassium ferricyanide, afforded the desired simple actinomycin analogs II, XXIII, and XXIV, respectively, in fair yields. The similar reduction-oxidation procedure with XXII gave only tars, probably because of interaction between the amine group formed by reduction and the ester group, which prevented a smooth oxidation of an *o*-aminophenol. The phenoxazone product from XXII would have been a closer analog of the actinomycin (I), as both the hydroxyl and carboxyl groups of the L-threonine moieties would have been derivatized as in I.

Compounds II, XXIII, and XXIV were completely inactive against *Staph. aureus* (Strain AD 175N209) and *E. coli* (Strain S) when tested by the standard dilution technique. In contrast, Actinomycin D gave a graded series of inhibition zones in an identical dilution sequence.[14] These results differ from those previously reported for II,[7] obtained by degradation. A direct microbiological comparison of the samples of II obtained by synthesis and by degradation was not available so that differences in the assaying methods can not be ruled out as an explanation for the different results reported. However, the possibility exists that the sample of II obtained by degradation may have contained a microbiologically active impurity that gave rise to the activity against *Staph. aureus*.

EXPERIMENTAL[15]

3-Nitro-p-toluic acid (IV). Solid *p*-toluic acid (500.0 g., 3.680 moles) was added with stirring over a 20-min. period

(14) We wish to thank Dr. J. Mandell and Dr. D. M. Powelson for the microbiological results.

(15) Melting points are uncorrected and were obtained with the Fisher-Johns apparatus. Optical rotations were measured on 1% solutions with a standard polarimeter Model D attachment to the Beckman DU spectrophotometer, calibrated with standard sucrose solutions. Paper chromatography was run by the descending technique on Whatman No. 1 paper in *n*-butyl alcohol–pyridine–water (35:35:30) (Solvent A) or on Schleicher and Schuell No. 2496 acetylated paper in benzene–*n*-butyl alcohol (2:1) saturated with water (Solvent B), and the spots were detected by visual examination under ultraviolet light. *p*-Toluic acid was used as the standard and the spots were located relative to R_{TA} 1.00.

to 3500 ml. of concd. nitric acid at room temperature. The suspension was warmed to 80° to dissolve the toluic acid and the resulting solution was heated at 90° for 2 hr., then was allowed to stand at room temperature for 14 hr. The nitric acid was decanted from the crystalline cake, which was collected and washed with three 1000-ml. portions of water to afford 500.5 g. of product, m.p. 187–188°. Dilution of the mother liquors with the combined aqueous washes yielded a further 32.0 g. of product, m.p. 184–185°, for a total yield of 80.1%. A sample recrystallized from aqueous ethanol had m.p. 187–188° (lit. m.p. 189–190° [16a] and 186–187° [16b]); $\lambda_{max(\mu)}^{Nujol}$ 5.85 (C=O), 6.50 and 7.38 (NO_2), 13.56 (substituted phenyl). On paper chromatography in solvent A, the compound moved as a single spot, with R_{TA} 1.05.

3-Acetamido-p-toluic acid (VI). A. *By hydrazine reduction.* A mixture of 135.0 g. (0.746 mole) of 3-nitro-*p*-toluic acid (IV), 1.50 g. of 5% palladium-on-charcoal, and 900 ml. of 95% ethanol was heated to reflux and 40 ml. of 95% hydrazine was added dropwise over a period of 15 min., producing a heavy precipitate which largely dissolved after the mixture was heated about 30 min. more. A second portion of hydrazine (30 ml.) was added to the refluxing solution, resulting in complete solution of the solid, and finally, after 1.25 hr. of heating, 10 ml. more of hydrazine was added. The solution was heated 1 hr. more at reflux, then was cooled and filtered through Celite. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in 750 ml. of warm, glacial acetic acid. Acetic anhydride (475 ml.) was added in a steady stream to the stirred acetic acid solution and, on standing overnight, the solution solidified to a crystalline mass. The product was collected and washed with three 200-ml. portions of acetic acid, then with four 200-ml. portions of ether, yielding 127.0 g. (88.2%) of product, m.p. 275–276° (lit. m.p. 277° [17] and 279–281° [18]); $\lambda_{max(\mu)}^{Nujol}$ 3.13 (NH), 5.90 (carboxyl C=O), 6.05 (amide C=O), 13.08 (substituted phenyl). The compound moved as a single spot on paper chromatography, using solvent A, with R_{TA} 0.72.

B. *By catalytic reduction.* A suspension of 10.7 g. (59.2 mmoles) of IV, 0.100 g. of platinum oxide, and 100 ml. of glacial acetic acid was shaken with hydrogen for 14 hr. at 40 p.s.i. The reaction mixture was filtered through Celite and 50 ml. of acetic anhydride was added to the filtrate. Within a few minutes the solution solidified to a cake, which was collected and recrystallized from glacial acetic acid, yielding 6.55 g. (57.4%) of product, m.p. 275–276°; this product was spectrally and chromatographically identical with that prepared by Method A (see above).

3-Acetamido- (IX) *and 5-acetamido-* (X) *2-nitro-p-toluic acid.* Solid 3-acetamido-*p*-toluic acid (VI) (125.0 g., 0.647 mole) was added portionwise over a period of 1 hr. to 500 ml. of stirred and chilled (*ca.* 5°) 90% fuming nitric acid. The solution gradually changed to a heavy slurry during the addition; at the end of the addition, 170 ml. more of the nitric acid was added. Stirring was continued for an additional hour, then the reaction solution was allowed to warm to room temperature and was quenched by being poured into 2 l. of an ice-water mixture. The mixture was filtered and the light yellow precipitate was washed with five 1000-ml. portions of water, then was stirred overnight with 2000 ml. of water. After it had been collected by filtration, the solid weighed 118.0 g. and had m.p. 225–244°. Recrystallization of the solid from 1200 ml. of boiling glacial acetic acid gave 80.2 g. (52.1%) of IX, m.p. 255–256°; $\lambda_{max(\mu)}^{Nujol}$ 3.02 (NH), 5.80 (carboxyl C=O), 6.07 (amide C=O), 6.43 and 7.20 (NO_2), 12.92 (substituted phenyl). The compound moved as a single spot on chromatography in solvent A, with R_{TA} 0.72

Anal. Calcd. for $C_{10}H_{10}N_2O_5$: C, 50.4; H, 4.23; N, 11.8. Found: C, 50.8; H, 4.78; N, 11.4.

The acetic acid mother liquors remaining after the crystallization of IX were concentrated, to afford 20.0 g. of solid, m.p. 220–235°. This was recrystallized three times from boiling glacial acetic acid, to afford 12.3 g. (8.0%) of compound (X), m.p. 247–248°, mixed melting point with IX, 217–222°; $\lambda_{max(\mu)}^{Nujol}$ 3.04 (NH), 5.83 (carboxyl C=O), 5.99 (amide C=O), 6.49 and 7.27 (NO_2), 12.72 (substituted phenyl). On paper chromatography in solvent A, compound X moved as a single spot, with R_{TA} 0.78.

Anal. Found: C, 50.3; H, 4.46; N, 11.3.

2-Nitro-3,4-cresotic acid (VII). A solution of 75.4 g. (0.316 mole) of IX, 142.0 g. (2.54 moles) of potassium hydroxide, and 1000 ml. of water was heated at reflux for 48 hr., after which time ammonia could not longer be identified in the exit gases. The dark red solution was cooled and acidified to *p*H 1 with conc. hydrochloric acid, giving a yellow solution which was continuously extracted with ethyl acetate for 18 hr. The ethyl acetate extract was washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, and, after filtration, evaporated to dryness *in vacuo*. The residue was recrystallized from water, affording 57.3 g. (92.0%) of product, m.p. 185–186° (lit. m.p. 185.5–186.5° [8a] and 182–183° [8b]); $\lambda_{max(\mu)}^{Nujol}$ 3.27 (OH), 5.87 (C=O), 6.46 and 7.38 (NO_2), 13.62 (substituted phenyl). On paper chromatography in solvent A, the material moved as a single spot, with R_{TA} 0.87. The product was identical, on the basis of spectral and chromatographic properties and mixed melting point comparison, with an authentic sample of VII.[10]

6-Nitro-3,4-cresctic acid (XII). A solution of 23.8 g. (0.100 mole) of X, 40.0 g. (1.00 mole) of sodium hydroxide, and 200 ml. of water was heated at reflux for 3.5 days. The hot solution was acidified to *p*H 1 with concd. hydrochloric acid, causing the deposition on standing of 11.6 g. of long needles. The solid was sublimed *in vacuo*, furnishing 11.6 g. (48.9%) of product, m.p. 179.5–180.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.01 (OH), 5.83 (C=O), 6.52 and 7.48 (NO_2), 13.18 (substituted phenyl). The compound moved as a single spot on paper chromatography in solvents A and B, with R_{TA} 0.78 and 0.90, respectively, and was identical, on spectral, chromatographic, crystallographic, and mixed melting point comparison, with an authentic sample of XII.[9]

Methyl 2-nitro-3,4-cresotate (VIII). A solution of 1.30 g. (0.066 mole) of the cresotic acid (VII) in 35 ml. of 5% methanolic hydrogen chloride was heated at reflux for 24 hr., then was poured into 200 ml. of water. The resulting suspension was extracted with four 100-ml. portions of ether and the combined ether extracts were washed with 50 ml. of 2 *M* aqueous sodium bicarbonate solution and with 50 ml. of water. After being dried over sodium sulfate and filtered, the ether solution was evaporated *in vacuo* and the residue was recrystallized from methanol-water, giving 0.404 g. (29.2%) of needles, m.p. 116–117°. A sample obtained by sublimation had m.p. 116.0–116.5° (lit. m.p. 116.0–116.5° [8a] and 115–116° [8b]); $\lambda_{max(\mu)}^{Nujol}$ 2.96 (OH), 5.80 (C=O), 6.43 and 7.58 (NO_2), 12.88 (substituted phenyl). The compound moved as a single spot in solvents A and B, with R_{TA} 0.75 and 1.12, respectively.

Acidification of the bicarbonate washes and extraction of the resulting solution with ethyl acetate gave, after drying and evaporation, a residue which was recrystallized from water to yield 0.437 g. (31.5%) of a solid, m.p. 101–102°. This appears to be a crystallographic modification of VIII, as its infrared spectrum in solution was identical with that of the 116° material, as was its paper chromatographic behavior.

Benzyl 3-(benzyloxy)-2-nitro-p-toluate (XI). The disodium salt of 2-nitro-3,4-cresotic acid (VII) was prepared by mixing 106.4 g. (0.541 mole) of VII with a solution of 63.5 g. (1.17 moles) of sodium methoxide in 500 ml. of methanol. After all the solid had dissolved, 500 ml. of benzene was

(16) (a) H. King and W. O. Murch, *J. Chem. Soc.*, **127**, 2632 (1925); (b) W. O. Kermack, *J. Chem. Soc.*, **125**, 2285 (1923).

(17) M. Yasue, Y. Takai, and S. Yoshizawa, *Yakugaku Zasshi*, **77**, 1048 (1957).

(18) W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **21**, 455 (1915).

added and the mixture was evaporated *in vacuo*. Benzene (300 ml.) was added to the residue and evaporated *in vacuo;* after repeating this treatment with benzene and re-evaporating, a red powder remained. The powder was suspended in 1100 ml. of an acetone–methyl ethyl ketone mixture (8:3); 163 g. (1.29 moles) of benzyl chloride and 8.0 g. of sodium iodide was added, and the resulting suspension was heated at reflux for 2 weeks. At this time all traces of the red sodio salt were gone. The mixture was filtered and the salts washed with three 100-ml. portions of acetone. The combined filtrate and washings were evaporated *in vacuo* and the residue was dissolved in 400 ml. of boiling methanol. The solution, on standing, deposited 187.6 g. (89.2%) of long needles, m.p. 95–96°; $\lambda_{max(\mu)}^{Nujol}$ 5.80 (C=O), 6.44 (NO_2), 13.32 and 14.39 (substituted phenyl). The compound traveled as a single spot on paper chromatography in solvents A and B, with R_{TA} 0.71 and 1.20, respectively.

Anal. Calcd. for $C_{22}H_{19}NO_5$: C, 70.0; H, 5.07; N, 3.71. Found: C, 70.0; H, 5.18; N, 4.20.

3-(Benzyloxy)-2-nitro-p-toluic acid (III). A. *From* XI. A mixture of 188 g. (0.497 mole) of XI in 1500 ml. of 10% methanolic potassium hydroxide solution and 1000 ml. of water was heated at reflux 1 hr., then allowed to digest overnight on the steam bath. Water (600 ml.) was added, the solution was brought to boiling, and concd. hydrochloric acid was slowly added to the stirred solution until no more precipitate was produced. The cooled mixture was filtered and the residue washed with three 100-ml. portions of water. The solid was crystallized from benzene to afford 127 g. (89.1%) of product which was identical in all properties with that prepared by Method B (see below).

B. *From* VII. The disodium salt of 35.7 g. (0.181 mole) of VII was prepared as in the preparation of XI. A mixture of the salt, 46.0 ml. (0.400 mole) of benzyl chloride, 7.5 g. of sodium iodide, and 500 ml. of methyl ethyl ketone was heated at reflux for 5 days, then filtered, and the salts washed with two 100-ml. portions of boiling solvent. The combined filtrate and washings were evaporated *in vacuo* and the oily residue was dissolved in 250 ml. of 10% methanolic potassium hydroxide and 25 ml. of water. The solution was stirred at room temperature for 3 hr., diluted with 300 ml. of water, and boiled with decolorizing carbon. After being filtered, most of the methanol was removed from the solution by evaporation *in vacuo* and the largely aqueous mixture was adjusted to *p*H 1 with conc. hydrochloric acid. The product (45.2 g.) was collected and washed with water, then crystallized twice from benzene, giving 34.1 g. (65.6%) of yellow plates, m.p. 174–175° (lit. m.p. 174–175° [8b]); $\lambda_{max(\mu)}^{Nujol}$ 5.88 (C=O), 6.42 and 7.53 (NO_2), 13.25 and 14.35 (substituted phenyl). There was no melting point depression with authentic III.[11]

2-Amino-4,6-dimethyl-3-oxo-3H-phenoxazine-1,9-dicarboxylic acid (Actinocinyl-bis-carboxylic acid) (XIV). A mixture of 2.873 g. (10.0 mmoles) of III, 0.200 g. of platinum oxide, and 50 ml. of absolute ethanol was treated with hydrogen at 1 atm., the theoretical quantity of hydrogen being absorbed in 8 hr. The solution was filtered and the filtrate was added to 1.62 g. (0.150 mole) of freshly crystallized *p*-benzoquinone. The mixture, exposed to the atmosphere, was stirred for 1 hr. and the solid was separated by filtration. The crude product (1.65 g.) was dissolved in 250 ml. of boiling *N,N*-dimethylformamide and the solution, on cooling, deposited 1.137 g. (61.1%) of red crystals whose infrared spectrum was identical with that of the analytical sample (see below).

From another preparation, an analytical sample was obtained which had m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 3.13 (NH_2), 5.83 and 5.97 (C=O); $\lambda_{max(m\mu)}^{MeOH}$ 236, 433, and 446 (the material was too insoluble to obtain accurate ϵ values).

Anal. Calcd. for $C_{15}H_{12}N_2O_6$: C, 58.5; H, 3.68; N, 8.53. Found: C, 58.33; H, 3.33; N, 8.26.

3-(Benzyloxy)-2-nitro-p-toluyl chloride (XV). A mixture of 0.150 g. (0.524 mmole) of the benzyloxy acid (III), 3 ml. of thionyl chloride, and 5 ml. of benzene was heated at reflux for 1 hr., then evaporated *in vacuo*. Benzene (5 ml.) was added to the oily residue and the solution was evaporated to dryness *in vacuo*, leaving a powder which was recrystallized from *n*-heptane to afford 0.085 g. (53.0%) of product, m.p. 101.5–102.0°; $\lambda_{max(\mu)}^{Nujol}$ 5.65 (C=O), 6.43 and 7.27 (NO_2) 13.30 and 14.21 (substituted phenyl).

Anal. Calcd. for $C_{15}H_{12}ClNO_4$: Cl, 12.0; N, 4.74. Found: Cl, 11.9; N, 4.83.

3-(Benzyloxy)-2-nitro-p-toluic anhydride (XVI). In an attempt to acylate 1.31 g. of L-threonine in 1 *M* aqueous sodium hydroxide with the acid chloride (XV) prepared from 2.87 g. (10.0 mmoles) of III, a solid was obtained by filtration of the reaction mixture after it had been stirred 2 hr. at room temperature. The solid was recrystallized from benzene to afford 0.067 g. of anhydride (XVI), m.p. 152–155°; $\lambda_{max(\mu)}^{Nujol}$ 5.58 and 5.76 (C=O), 6.43 and 7.29 (NO_2), 13.01 and 14.26 (substituted phenyl). The compound moved as a single spot on paper chromatography in solvent B, with R_{TA} 0.48.

Anal. Calcd. for $C_{30}H_{24}N_2O_7$: C, 64.7; H, 4.35; N, 5.03. Found: C, 64.8; H, 5.04; N, 5.54.

Methyl L-threonate hydrochloride and methyl L-threonate. Hydrogen chloride was bubbled through a chilled (0–5°) suspension of 11.91 g. (0.100 mole) of L-threonine in 50 ml. of methanol until all the solid dissolved. The solution was heated at reflux for 3 hr., then was cooled to 0° and saturated again with hydrogen chloride. After it had stood for 2 days, the solution was evaporated *in vacuo* and the resulting sirup was maintained in vacuum over phosphorus pentoxide to yield 16.2 g. (95%) of product; $\lambda_{max(\mu)}^{film}$ 3.01 (OH), 3.36 (NH_3^+), 5.72 (C=O); $[\alpha]^{26}D$ −7.8° (absolute ethanol) and −13° (water). This product was identical in infrared spectrum and optical properties with a commercial sample.[19]

A mixture of 11.85 g. (70.00 mmoles) of methyl L-threonate hydrochloride, 7.85 g. (70.0 mmoles) of triethylamine, and 25 ml. of tetrahydrofuran was stirred for 1 hr., then filtered. The triethylamine hydrochloride was washed with three 10-ml. portions of tetrahydrofuran and the combined filtrate and washings were evaporated *in vacuo*. The residual powder was recrystallized from ethyl acetate-pentane, yielding 10.76 g. of a solid, m.p. 40–42°. This crystalline solid was sublimed *in vacuo* twice, affording 8.975 g. (96.3%) of white plates, m.p. 63.5–64.5° (lit. m.p. 70–72° [11]); $[\alpha]^{22}D$ 0.0° (absolute ethanol) (lit.[11] $[\alpha]D$ +5.00° (*c* = 3, methanol)). The compound moved as a single spot in solvent B, with R_{TA} 1.08. A comparison sample[20] had m.p. 63–64° and mixed melting point of 63–64° with the preparation described above.

Methyl N-[3-(benzyloxy)-2-nitro-p-toluyl]-L-threonate (XVII). A. *By direct coupling.* To a chilled (0°), stirred solution of 11.65 g. (87.3 mmoles) of methyl L-threonate, 25.05 g. (87.3 mmoles) of the benzoxytoluic acid (III), and 150 ml. of dichloromethane was added 18.00 g. (87.5 mmoles) of *N,N'*-dicyclohexylcarbodiimide (DCC). The mixture, protected from moisture, was stirred 1 hr. at 0°, allowed to stand at room temperature for 18 hr., and then filtered to remove dicyclohexylurea. The filter cake was washed with three 25-ml. portions of cold dichloromethane and the combined filtrate and washings were evaporated *in vacuo*. The residual sirup was dissolved in boiling ethanol and the resulting solution allowed to cool, causing the precipitation of 24.90 g. of product, m.p. 112.0–113.5°. A second crop, 0.672 g., was obtained by concentration of the alcoholic mother liquors, giving a total yield of 72.7%. The first crop had $[\alpha]^{20}D$ −46.2 ± 2.8° (absolute ethanol) and was identical with the product from DCC coupling in tetrahydrofuran (see below) according to infrared spectral comparison.

Methyl L-threonate was prepared *in situ* by treating 1.76 g. (10.5 mmoles) of methyl L-threonate hydrochloride with

(19) Cyclo Chemical Corporation, Los Angeles 1, Calif.

(20) We wish to thank Dr. K. Vogler, Hoffman-LaRoche and Co., Ltd., Basel, Switzerland, for this material.

a solution of 0.567 g. (10.5 mmoles) of sodium methoxide in 50 ml. of methanol-tetrahydrofuran (1:1). The sodium chloride formed was removed by filtration, then washed with two 10-ml. portions of boiling tetrahydrofuran. The combined organic extracts and washings were treated with 2.87 g. (10.0 mmoles) of III and 2.27 g. (11.0 mmoles) of DCC and stirred 1 hr. at room temperature. Acetic acid (0.30 ml.) was added, the dicyclohexylurea was collected and washed with 20 ml. of tetrahydrofuran, and the filtrate and washings were evaporated *in vacuo*. The residual oil was dissolved in boiling absolute ethanol, the solution was treated with decolorizing carbon and filtered, and the filtrate chilled to give two crops of dicyclohexylurea. The mother liquors were diluted with water, affording 0.939 g. (19%) of the trisubstituted urea (XVIII), m.p. 136.5–137.0°. The analytical sample, recrystallized from ethanol-water, had m.p. 137.3–137.8°; $\lambda_{max(\mu)}^{Nujol}$ 2.98 (NH), 5.84 (urea C=O), 6.05 (amide C=O), 7.33 (NO_2), 13.48 and 14.30 (substituted phenyl). The compound moved as a single spot on paper chromatography in solvent B, with R_{TA} 1.15.

Anal. Calcd. for $C_{28}H_{35}N_3O_5$: C, 68.2; H, 7.15; N, 8.51. Found: C, 68.3; H, 6.76; N, 8.54.

The mother liquors from crystallization of XVIII were evaporated *in vacuo*, giving a gum which solidified, on trituration with ether, to a solid, 2.592 g., m.p. 113–115°. This solid was recrystallized twice from benzene, affording 0.820 g. (20.4%) of product, m.p. 114–115°; $\lambda_{max(\mu)}^{Nujol}$ 2.83, 2.95, 3.02, 3.05 (NH, OH), 5.71 (ester C=O), 6.13 (amide C=O), 13.40 and 14.35 (substituted phenyl); $[\alpha]^{26}D$ −43.6 ± 3.0° (absolute ethanol). The compound moved as a single spot in solvent B, with R_{TA} 0.74.

Anal. Calcd. for $C_{20}H_{22}N_2O_7$: C, 59.7; H, 5.51; N, 6.96. Found: C, 59.8; H, 5.45; N, 7.01.

B. *From the "activated ester"* (XIX). Methyl L-threonate was prepared from 0.365 g. (2.15 mmoles) of methyl L-threonate hydrochloride and 0.222 g. (2.20 mmoles) of triethylamine in 55 ml. of chloroform and, after removal of the triethylamine hydrochloride by filtration, 0.810 g. (1.98 mmoles) of the "activated ester" (XIX) was added. The resulting solution was heated at 40° for 24 hr., then was evaporated *in vacuo*, and the residue was stirred with 20 ml. of ethyl acetate–water (1:1). The organic phase was separated and washed in turn with five 10-ml. portions of water, 10 ml. of 1 *M* ammonium hydroxide, 10 ml. of 1 *M* hydrochloric acid, and three 10-ml. portions of water. The extract was dried over sodium sulfate, filtered, and evaporated *in vacuo*, leaving a sirup that solidified upon beng triturated with ether. The solid, 0.158 g., m.p. 100–103°, was crystallized twice from benzene to give 0.138 g. (34.4%) of product, m.p. 113.0–114.5°; $[\alpha]^{24}D$ −36.1 ± 4.6°. The product was identical with the products from method A (see above), according to infrared spectral comparison and mixed melting point determination.

p-Nitrophenyl 3-(benzoxy)-2-nitro-p-toluate (XIX). To a chilled (0°), stirred solution of 1.474 g. (5.14 mmoles) of III, 0.858 g. (6.17 mmoles) of *p*-nitrophenol, and 20 ml. of ethyl acetate was added 1.06 g. (5.14 mmoles) of DCC. The mixture was stirred for 30 min. at 0° and for 90 min. at room temperature, then was treated with 0.30 ml. of glacial acetic acid, and the dicyclohexylurea was removed by filtration. Ethyl acetate (three 10-ml. portions) was used to wash the filter cake and the combined filtrate and washings were evaporated *in vacuo*, leaving a residual oil that was recrystallized from boiling absolute ethanol to afford 1.095 g. (52.2%) of product, m.p. 117.5–118.5°. The analytical sample was obtained after two recrystallizations from absolute ethanol and had m.p. 120.5–121.0°; $\lambda_{max(\mu)}^{Nujol}$ 5.70 (ester C=O), 6.43, 6.53, and 7.40 (NO_2), 13.35 and 14.29 (substituted phenyl). The compound moved as a single spot on paper chromatography in solvent B, with R_{TA} 0.46.

Anal. Calcd. for $C_{21}H_{16}N_2O_7$: C, 61.8; H, 3.95; N, 6.86. Found: C, 61.9; H, 3.66; N, 6.89.

N-[3-(Benzyloxy)-2-nitro-p-toluyl]-L-threonine (XX). A mixture of 2.01 g. (5.00 mmoles) of the methyl ester (XVII), 0.220 g. (5.50 mmoles) of sodium hydroxide, 10.5 ml. of tetrahydrofuran, and 0.5 ml. of water was stirred at room temperature for 5 hr. The solvent was evaporated *in vacuo*, the residue was dissolved in 10 ml. of water, and the solution extracted with two 10-ml. portions of ethyl acetate. The aqueous layer was adjusted to *p*H 1 by the dropwise addition of conc. hydrochloric acid, the oil which separated was extracted with two 15-ml. portions of ether, and the combined extracts were evaporated *in vacuo*. The residual oil was converted into a solid by digestion with 15 ml. of boiling benzene, giving 1.714 g. (88.3%) of product, m.p. 154.5–155.5°. The sample was recrystallized from acetonitrile, affording 1.364 g. of analytical product, m.p. 161.5–162.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.90, 2.95 (NH, OH), 5.80 (carboxyl C=O), 6.02 (amide C=O), 6.58, 7.25, and 7.31 (NO_2), 13.38 and 14.31 (substituted phenyl); $[\alpha]^{20}D$ −50.4° (absolute ethanol). The compound traveled as a single spot on paper chromatography in solvents A and B, with R_{TA} 1.01 and 1.17, respectively.

Anal. Calcd. for $C_{19}H_{20}N_2O_7$: C, 58.8; H, 5.19; N, 7.21. Found: C, 58.9; H, 5.42; N, 7.17.

N-(L-1-Carbamoyl-2-hydroxypropyl)-3-(benzyloxy)-2-nitro-p-toluamide (XXI). A suspension of 12.07 g. (30.00 mmoles) of the ester (XVII) and 150 ml. of conc. ammonium hydroxide was shaken in a closed bottle for 3 days, then filtered. The solid residue was washed with five 50-ml. portions of water, affording 8.32 g. (71.6%) of product, m.p. 170.5–171.5°. The analytical sample, recrystallized from water, had m.p. 173–174°; $\lambda_{max(\mu)}^{Nujol}$ 2.96, 3.12 (NH, OH, NH_2), 5.99 (amide C=O), 6.58, 7.25, and 7.31 (NO_2), 13.48 and 14.35 (substituted phenyl); $[\alpha]^{21}D$ +19.2° (absolute ethanol). The compound moved as a single spot in solvents A and B, with R_{TA} 0.83 and 1.18, respectively.

Anal. Calcd. for $C_{19}H_{21}N_3O_6$: C, 58.9; H, 5.46; N, 10.9. Found: C, 59.0; H, 5.10; N, 10.8.

N-[L-1-Carbamoyl-2-(propionoxy)propyl]-3-(benzyloxy)-2-nitro-p-toluamide (XXII). A mixture of 1.94 g. (5.00 mmoles) of the amide (XXI), 0.509 g. (5.50 mmoles) of propionyl chloride, and 50 ml. of benzene was heated at reflux for 24 hr., then cooled. The product that precipitated was recrystallized from ethanol, affording 1.475 g. (66.4%) of solid, m.p. 154–156°; $\lambda_{max(\mu)}^{Nujol}$ 2.98, 3.05, 3.12 (NH, NH_2), 5.75 and 5.81 (ester C=O), 5.94 and 6.03 (amide C=O), 6.49 and 7.26 (NO_2); $[\alpha]^{22}D$ −5.2° (absolute ethanol). The product moved as a single spot in solvents A and B, with R_{TA} 0.85 and 1.19, respectively.

Anal. Calcd. for $C_{22}H_{25}N_3O_7$: C, 59.6; H, 5.68; N, 9.48. Found: C, 59.3; H, 5.52; N, 9.02.

Dimethyl N,N'-(2-amino-4,6-dimethyl-3-oxo-3H-phenoxazine-1,9-diyldicarbonyl)di-L-threonate [dimethyl actinocinylbis(L-threonate)] (II). A mixture of 0.201 g. (0.500 mmole) of the ester (XVII), 0.050 g. of platinum oxide, and 20 ml. of absolute ethanol was treated with hydrogen at 1 atm. and room temperature for 15 hr. The solution was filtered through Celite and 0.054 g. (1.50 mmoles) of *p*-benzoquinone was added to the filtrate. The resulting solution, exposed to the atmosphere, was stirred for 2 hr., then was filtered, and the orange-red solid (0.052 g.) washed with three 5-ml. portions of cold ethanol. The solid was recrystallized from *N,N*-dimethylformamide-water, affording 0.037 g. (26.5%) of product, m.p. 249–251°, identical in spectral properties with the analytical sample (see below).

A mixture of 2.01 g. (5.00 mmoles) of XVII, 0.200 g. of platinum oxide, and 25 ml. of absolute ethanol was treated with hydrogen for 2 days as in the example above. The reaction mixture was filtered, the filtrate diluted with 200 ml. of ether, and saturated with hydrogen chloride at 0°. The solvents were removed *in vacuo* and the residual red oil was dissolved in 50 ml. of water and added to 500 ml. of an aqueous phosphate buffer (*p*H 7.8). A hot (50°) solution of 3.622 g. (11.00 mmoles) of potassium ferricyanide in 100 ml. of water was added in a steady stream to the stirred buffer solution. The resulting orange precipitate (1.130 g.) was

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).