I and 0.012 g. of recovered XII. An additional recrystallization from water afforded an analytical sample of I as off-white needles of m.p. 138–139°; λ_{max} 239 mμ (log ε 4.60), λ_{max} of acridine[12] 250 mμ (log ε 5.02).

Anal. Calcd. for $C_{12}H_8N_2$: C, 80.0; H, 4.4; N, 15.5. Found: C, 80.1; H, 4.6; N, 15.5.

Pyrido[4,3-b]quinolin-10(5H)thione (XIII).—A solution of 0.250 g. (1.25 millimoles) of XI and 0.32 g. (1.44 millimoles) of phosphorus pentasulfide in 6 ml. of anhydrous pyridine was refluxed for 5 hr. The pyridine was removed under reduced pressure and the residue was extracted with hot dilute sodium hydroxide solution. The addition of acid to this extract precipitated crude XIII as an amorphous solid which contained phosphorus and which resisted purification. After extraction with carbon disulfide, followed by dilute sodium bicarbonate solution, the residue was partially soluble in ethanol. Evaporation of this ethanol extract gave a solid which was recrystallized from methanol; XIII was isolated in the form of red needles, 0.010 g. (4%), m.p. 298–301°.

Anal. Calcd. for $C_{12}H_8N_2S$: N, 13.2; S, 15.1. Found: N, 13.0; S, 14.9.

Attempted Synthesis of I from Pyrido[4,3-b]quinolin-10(5 H)-thione (XIII).—An ethanol solution of crude thione XIII and Raney nickel[13] was heated to reflux. The reaction was followed by withdrawing aliquots and measuring the optical density at 239 mμ. The spectral changes indicated rapid and extensive reduction of the ring system. Less active Raney nickel failed to remove the sulfur.

Model experiments with thioacridone indicated that a maximum of only 25% of acridine was formed after 5 min. and that after 1 hr., when desulfurization was complete, only 2–5% of acridine was present in the mixture.

Attempted Synthesis of 10-Chloro[4,3-b]quinoline.—A mixture of 0.050 g. of XI and 5 ml. of phosphorus oxychloride was refluxed for 3 hr. and then poured on crushed ice. Only unreacted XI (0.040 g.) could be isolated. A mixture of phosphorus pentachloride and oxychloride was also used but without success.

(13) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE]

The Synthesis of *m*-[Di-(2-chloroethyl)-amino]-DL-phenylalanine

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The synthesis of *m*-[di-(2-chloroethyl)-amino]-DL-phenylalanine in five steps from *m*-nitrobenzyl bromide and acylamino malonic esters is reported. The compound was prepared as a potential tumor inhibitor.

The interest in aromatic nitrogen mustard derivatives as tumor inhibiting substances has led to the development of various new types. One of the most interesting is *p*-[di-(2-chloroethyl)-amino]-phenylalanine (I), which was synthesized independently by Bergel and his collaborators[1] who made the DL-, D- and L-forms, and also by Larionov, *et al.*,[2] who prepared the DL-form only. This compound has been reported to have pronounced carcinostatic activity against certain tumors.[3] Luck and his co-workers[4] have studied I and a number of related homologs. Most interesting is their finding that at least a temporary regression of the Cloudman S-91 melanoma in mice is obtained on treatment with I, since the melanomas are generally resistant to nitrogen mustards. The foregoing findings have led to the use of this drug in clinical trials at various centers as an anti-cancer drug.

Phenylalanine can be hydroxylated *in vivo* in the *para* position to give the amino acid tyrosine,[5] a possibility which has been eliminated in I by the presence of the nitrogen mustard group in that position. Since this hydroxylation is of importance in a number of metabolic functions of phenylalanine, it was reasoned that a derivative of I should be made in which hydroxylation in the para position could occur. Therefore, the synthesis of *m*-[di-(2-chloroethyl)-amino]-DL-phenylalanine (II) was undertaken.

Results and Discussion

The synthetic approach to II closely paralleled that employed by Bergel, *et al.*,[1] and the Russian workers[2] for the synthesis of I. Two syntheses were achieved, the only difference between them being in the utilization of different protecting groups on the α-amino nitrogen introduced in the first stage of the synthesis. In series "a" the acetyl group was used while in series "b" the benzoyl group was used. Ethyl acetamidomalonate or ethyl benzamidomalonate was condensed with *m*-nitrobenzyl bromide to give ethyl acetamido-(*m*-nitrobenzyl)-malonate (IIIa) or ethyl benzamido-(*m*-nitrobenzyl)-malonate (IIIb) in 75% yields. Compounds IIIa and IIIb were then reduced by catalytic hydrogenation to the corresponding amino derivatives, ethyl acetamido-(*m*-aminobenzyl)-malonate (IVa) or ethyl benzamido-(*m*-aminobenzyl)-malonate (IVb) in almost quantitative yields. The aromatic amino group was then hydroxyethylated with ethylene oxide in 50% acetic acid solution. The ethyl acetamido-[*m*-di-(2-hydroxyethyl)-aminobenzyl]-malonate (Va) and the ethyl benzamido-[*m*-di-(2-hydroxyethyl)-aminobenzyl]-malonate (Vb) were obtained initially as gums. A seed crystal of Va was obtained from Dr. B. R. Baker[6] which made it possible to isolate the crystal-

(1) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); F. Bergel, V. C. Burnop and J. A. Stock, *ibid.*, 1223 (1955).

(2) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaja, O. S. Vasina, V. I. Trooshetkina and M. A. Novikova, *Lancet, Lond.*, **2**, 169 (1955).

(3) See, for example: P. C. Koller and U. Veronesi, *Brit. J. Cancer*, **10**, 703 (1956); J. F. Holland and W. Regelson, *Ann. N. Y. Acad. Sci.*, **68**, 1122 (1958); R. Papac, D. A. G. Galton, M. Till and E. Wiltshaw, *ibid.*, **68**, 1126 (1958); N. Blokhin, L. Larionov, N. Perevodchikova, L. Chebotareva and N. Merkulova, *ibid.*, **68**, 1128 (1958).

(4) J. M. Luck, *Science*, **123**, 984 (1956); J. M. Luck, *Cancer Research*, **17**, 1071 (1957); H. E. Smith and J. M. Luck, **23**, 837 (1958).

(5) A. B. Lerner, *Adv. Enzymology*, **14**, 73 (1953).

(6) During the course of this work we learned from Dr. Howard W. Bond of the Cancer Chemotherapy National Service Center that Dr.

line compound, although some early experiments were carried out with this gum. The compound Vb was finally obtained as a solid by treatment with ether. The yield of purified materials varied from 50–70%.

Conversion of the hydroxy groups to chloro groups was attempted using phosphorus oxychloride in benzene. The desired intermediates, ethyl acetamido-[*m*-di-(2-chloroethyl)-aminobenzyl]-malonate (VIa) or ethyl benzamido-[*m*-di-(2-chloroethyl)-aminobenzyl]-malonate (VIb) should undergo hydrolysis and decarboxylation in boiling hydrochloric acid[2] to yield the alpha-amino acid. However, the materials obtained after treatment with hydrochloric acid in this instance did not give a positive ninhydrin test indicating absence of an α-amino acid and showing that the reaction had taken a different course. Since the materials had an intense green fluorescence under ultraviolet light, it is suggested that a Bischler–Napieralski type ring closure had taken place during treatment with phosphorus oxychloride.

The conversion was next attempted using thionyl chloride in boiling chloroform for 18 minutes. The products after treatment with hydrochloric acid contained material which gave a positive ninhydrin reaction, although extensive decomposition was indicated by the presence of at least seven other components detected by paper chromatography. Most important, however, was the absence of any green fluorescent materials indicating that ring closure had been avoided. By using methylene chloride as a solvent, the decomposition was largely eliminated and the desired intermediates VIa and VIb were obtained in moderate yields. The excess thionyl chloride was decomposed by pouring the reaction mixture onto ice. Both the use of methylene chloride as solvent for the reaction and the hydrolysis of excess thionyl chloride with ice (rather than removal of thionyl chloride and methylene chloride under reduced pressure) were critical points in the synthesis. The products VIa and VIb were obtained at this stage as gums which could be directly hydrolyzed and decarboxylated to II, or alternatively, isolated and purified.

For the final step involving hydrolysis and decarboxylation 6 *N* hydrochloric acid was used. In early experiments the acid solution was then neutralized with a saturated sodium acetate solution followed by extraction of the resulting gum with chloroform. As purer samples of VI were processed in this manner, it became clear that the presence of unidentified impurities in the earlier experiments were enhancing the chloroform solubility of the desired product (II). Thus, it became necessary to modify the extraction procedure by using a mixture of chloroform and ethanol (2:1). In our most recent preparations, which are reported in the Experimental section, the most satisfactory procedure was found to be neutralization with concentrated ammonium hydroxide to *p*H 6.1–6.5 when the final product precipitated as a solid of high purity in about 30–60% yield. Further purification was carried out by dissolving the product in acetone and precipitating with petroleum ether.

Preliminary experiments on the Cloudman S-91 melanoma in mice indicate *m*-[di-(2-chloroethyl)-amino]-DL-phenylalanine is an inhibitor of that tumor.

B. R. Baker and his group at Stanford Research Institute were engaged in the synthesis of the same compound II. We are indebted to Dr. Baker for the exchange of some of the final products from the two laboratories (at this point our product was still impure). We would further like to thank him for his kindness in delaying presentation of his manuscript while we completed a reinvestigation of certain stages in our synthesis and purification. Certain other relevant information also was exchanged.

Experimental[7]

Ethyl Acetamido-(*m*-nitrobenzyl)-malonate (IIIa).—Clean sodium metal, 2.5 g. (0.109 mole), was dissolved in 200 ml. of absolute ethanol, and 21.8 g. (0.101 mole) of ethyl acetamidomalonate was added to the solution, which then was boiled under reflux for 15 minutes, after which 22.3 g. (0.105 mole) of *m*-nitrobenzyl bromide was added in small portions. After the vigorous reaction abated, the mixture was boiled under reflux for 4 hr., and the solvent was removed using a rotary evaporator. The residual mixture was added to an excess of crushed ice and after scratching the crystalline product was obtained, m.p. 154–155°. Recrystallization from absolute ethanol gave colorless crystals, m.p. 159–161°; yield 37.3 g. (75%). The ultraviolet spectrum (ethanol) had λ_{max} 268 mμ (log ϵ 3.88).

Anal. Calcd. for $C_{16}H_{20}N_2O_7$: C, 54.54; H, 5.72; N, 7.95. Found: C, 54.49; H, 5.80; N, 8.11.

Ethyl Benzamido-(*m*-nitrobenzyl)-malonate (IIIb).—This compound was made identically to that described in the preceding preparation (IIIa) using 4 g. (0.174 mole) of sodium in 500 ml. of absolute ethanol, 35.65 g. (0.165 mole) of *m*-nitrobenzyl bromide and 46.2 g. (0.164 mole) of ethyl benzamidomalonate. The product, which was recrystallized from absolute ethanol as large colorless prisms, had a m.p. 100–100.5°; yield 50 g. (75%). The ultraviolet spectrum (ethanol) had λ_{max} 268 mμ (log ϵ 3.89).

Anal. Calcd. for $C_{21}H_{22}N_2O_7$: C, 60.86; H, 5.35; N, 6.76. Found: C, 60.92; H, 5.43; N, 6.89.

Ethyl Acetamido-(*m*-aminobenzyl)-malonate (IVa).—Fifty grams (0.142 mole) of IIIa was suspended in a mixture of 300 ml. of ethyl acetate and 10 ml. of methanol, and 350 mg. of 10% palladium on charcoal was added. The suspension was shaken with hydrogen in a Parr hydrogenator for 2 hr. at which time no more hydrogen was absorbed. The colorless solution was filtered free from catalyst and the solvent was removed using a rotary evaporator. The product was recrystallized from absolute ethanol and had a m.p. 164–165°; yield 44.8 g. (98%). The ultraviolet spectrum (ethanol) had λ_{max} 290 and 238 mμ (log ϵ 3.24 and 3.93, respectively).

Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.53; H, 6.84; N, 8.57.

Ethyl Benzamido-(*m*-aminobenzyl)-malonate (IVb).—This compound was prepared as described for IVa; 50 g. (0.121 mole) of IIIb was dissolved in 250 ml. of ethyl acetate and 10 ml. of methanol to which 300 mg. of 10% palladium on charcoal was added. The product obtained after concentration was a gum which solidified on scratching. Recrystallization from aqueous ethanol gave the product, m.p. 118–118.5°; yield 45 g. (97%). The ultraviolet spectrum (ethanol) had λ_{max} 290 and 231 mμ (log ϵ 3.26 and 4.27, respectively).

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.48; H, 6.19; N, 7.54.

Ethyl Acetamido-[*m*-d-(2-hydroxyethyl)-aminobenzyl]-malonate (Va).—Sixty one grams (0.189 mole) of the amine IVa was dissolved in 300 ml. of glacial acetic acid, and the solution was diluted with an equal volume of water. Ninety ml. (80 g., 1.82 moles) of ethylene oxide was added with cooling, and the solution was allowed to stand at room temperature for 22 hr. The yellow liquor was neutralized with sodium carbonate solution when a gum was deposited. The mixture was extracted with three portions of ethyl acetate and the organic layer was washed twice with water and dried over anhydrous sodium sulfate. The solvent was removed using a rotary evaporator and the residual

(7) All melting points are uncorrected. Analyses are by Dr. A. Elek, Los Angeles, California.

yellow gum was repeatedly triturated with ether. The product obtained was a near white solid and this was removed by filtration and washed well with ether, m.p. 103–105°; yield 55.2 g. (71%). A sample for analysis was crystallized several times from a very small amount of alcohol, m.p. 104–106°. The ultraviolet spectrum (ethanol) had λ_{max} 305 and 260 mμ (log ε 3.48 and 4.21, respectively).

Anal. Calcd. for $C_{20}H_{30}N_2O_7$: C, 58.52; H, 7.37; N, 6.83. Found: C, 58.31; H, 7.24; N, 7.01.

Ethyl Benzamido-[*m*-di(2-hydroxyethyl)-aminobenzyl]-malonate (Vb).—This compound was prepared as described previously for Va using 40.4 g. (0.105 mole) of the amino derivative IVb in 200 ml. of glacial acetic acid and 200 ml. of water, to which was added 50 ml. (44.5 g., 1.01 moles) of ethylene oxide. The residual gum, which finally solidified on repeated treatment with ether, had a m.p. 100–101°; crude yield 50 g. Two recrystallizations from small volumes of ethyl acetate gave the pure material, m.p. 103–105°; yield 24 g. (48%). The ultraviolet spectrum (ethanol) had λ_{max} 305 and 259 mμ (log ε 3.50 and 4.23, respectively); there was also an ill-defined shoulder in the region 229–232 mμ.

Anal. Calcd. for $C_{26}H_{32}N_2O_7$: C, 63.54; H, 6.83; N, 5.93. Found: C, 63.68; H, 6.99; N, 6.06.

Ethyl Acetamido-[*m*-di(2-chloroethyl)-aminobenzyl]-malonate (VIa).—Sixteen and four tenths g. (0.04 mole) of the dihydroxyethylamino derivative Va was dissolved in 200 ml. of methylene chloride (dried over sodium hydroxide) and to the clear solution was added 24 ml. (0.33 mole) of redistilled thionyl chloride. The bright yellow solution was boiled under reflux for 1 hr. at which time evolution of gases had largely ceased and the color of the solution was deep orange. This was poured onto 500 g. of crushed ice and brought to *p*H 6 by addition of sodium carbonate solution. The heavier methylene chloride layer was separated and dried over a mixture of anhydrous sodium sulfate and potassium carbonate. Care must be taken at this stage since bad emulsions are formed, especially if the methylene chloride layer is washed with water. Addition of anhydrous potassium carbonate to the emulsion will usually cause satisfactory separation. The methylene chloride was removed using a rotary evaporator at a temperature below 50°. The residual yellow gum, after drying in a vacuum desiccator over paraffin wax, gave a yield of 13.6 g. (76%). Purification of the yellow gum was achieved by dissolving the gum in benzene and passing it through a short alumina column, followed by continuous elution with benzene. From 4.15 g. of gum, 1.5 g. of the product was obtained as a white solid, m.p. 87–88°. A yellow band was retained on the column. An analytical sample was recrystallized twice from cyclohexane m.p. 88–89°. The ultraviolet spectrum (ethanol) had λ_{max} 299 and 256 mμ (log ε 3.41 and 4.22, respectively).

Anal. Calcd. for $C_{20}H_{28}Cl_2N_2O_5$: C, 53.67; H, 6.31; Cl, 15.85; N, 6.26. Found: C, 53.68; H, 6.40; Cl, 15.92; N, 6.24.

***m*-[Di-(2-chloroethyl)-amino]-DL-phenylalanine (II).**

a. By Hydrolysis of Purified Chloroester VIa.—To 3.5 g. (7.84 mmoles) of VIa was added 12 ml. of 6 *N* hydrochloric acid, and the mixture was boiled under reflux for 3 hr. The clear yellow solution was cooled in an ice-bath and was brought to *p*H 6.1 with concentrated ammonium hydroxide when a cream colored precipitate was obtained. This was removed by filtration, washed with ice-cold water and dried over phosphorus pentoxide in a vacuum desiccator. The product had a m.p. 157–158°; yield 1.3 g. (55%). The material was purified by dissolution in the minimum volume of warm acetone followed by filtration and addition of petroleum ether when the product was obtained as a white powder, m.p. 161–163°. Since the infrared data indicated some decomposition with drying at 100° (see below), the sample for analysis was dried at room temperature and retained some water.

The compound showed a single spot after paper chromatography in butanol saturated with water, R_f 0.56, examined either by ultraviolet absorption or ninhydrin reaction. The ultraviolet spectrum (*N* HCl) had λ_{max} 297 and 258 mμ (log ε 1.82 and 2.79, respectively). There was also a sharp shoulder at 267 mμ.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_2 \cdot 1/4H_2O$: C, 50.4; H, 6.01; N, 9.1; Cl, 22.9. Found: C, 50.2; H, 5.6; N, 9.2; Cl, 23.4.

b. From the Hydroxyethylated Benzamido Ester Vb with Intermediate Purification of the Chloroester.—The compound Vb 4.72 g. (0.01 mole) was dissolved in 50 ml. of dried methylene chloride and 6 ml. (0.0825 mole) of redistilled thionyl chloride was added. The yellow solution was boiled under reflux for 45 minutes and poured onto 125 g. of crushed ice and the mixture brought to *p*H 5 with sodium carbonate solution. The lower organic layer was separated, dried over a mixture of anhydrous sodium sulfate and potassium carbonate, and the solvent was removed using a rotary evaporator at a temperature below 50°. A yellow gum was obtained; yield of crude material 4.4 g. (93%). This gum was dissolved in 20 ml. of dry benzene, put on an alumina column (5 cm. × 3 cm.) and was eluted with a total of 800 ml. of benzene. (A band absorbing in ultraviolet light remained on the column and could be eluted by addition of one part of ethanol to 24 parts of benzene). The benzene eluate was evaporated to dryness yielding 2.65 g. (52%) of a colorless gum. On treatment with petroleum ether this material appeared to be slowly solidifying, but no attempt was made to isolate this product. The above gum was boiled with 8 ml. of 6 *N* hydrochloric acid under reflux for 4 hr. The solution was cooled, and the precipitated benzoic acid was removed by filtration. The solution was diluted with an equal volume of water, shaken with 1 g. of decolorizing charcoal and filtered, after which it was brought to *p*H 5 with concentrated ammonium hydroxide solution. A gum was deposited, the mother liquors were decanted and the gum was treated with 10 ml. of warm acetone. A solution was obtained which on cooling deposited the product, m.p. 160–162°, yield 0.15 g. On addition of petroleum ether to the solution and allowing it to stand a further 0.32 g. of material, m.p. 158–160° was obtained; combined yield was 33.8% (based on the chloroester).

Some additional material could be isolated from the aqueous mother liquors by extraction with a mixture of chloroform and ethanol (2:1), but this was impure as shown by paper chromatography.

Paper chromatography was carried out using the ascending method which gives R_f values slightly higher than the descending method. Ultraviolet absorption spectra were determined with a Beckman DK-2 Recording Spectrophotometer, using 10^{-4} to 10^{-5} *M* solutions in ethanol, with the exception of the final product which was analyzed using a 1.05×10^{-3} *M* solution in 1 *N* HCl. Molar extinction coefficients were estimated from the DK-2 graph values at the maxima.

The infrared spectrum of II was determined using a potassium bromide pellet.[8] The following assigned bands were present in the spectrum (numbers represent wave length in microns): 3.38, 3.90 (NH_3^+); 6.20 (zwitterion and aromatic ring); 6.67 (Ar, NH_3^+); 7.16 (CO_2^-); 12.87 (*m*-disubstituted phenyl); and 13.50 (C–Cl). All bands designated were moderate or strong bands. The band at 6.20 had a broad shoulder beginning at 6.08, indicating some hydration. After drying at 100° for 15 hr. this shoulder disappeared. However, a small band appeared at 5.71 (normal acid carbonyl of α-amino acid hydrochlorides)[9] indicating some decomposition occurred with the production of some of the hydrochloride form of the amino acid.

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(8) We wish to thank Dr. Tad Patton, of this Institution, for determination of the infrared spectra.

(9) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 202.