β-(1-Azirdiinyl)ethyl Propionate (XX).—When methyl propionate was used in place of ethyl acetate in the procedure as described under XIX, XX was obtained.

β-(1-Aziridinyl)ethyl *n*-Butyrate (XXI).—Methyl *n*-butyrate was used instead of ethyl acetate in the synthesis of XIX; the butyrate was obtained as a colorless oil.

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Synthesis of N,N-Bis(2-chloroethyl)-DL-phenylalanine Hydrochloride[1]

William W. Lee, George L. Tong, Abelardo P. Martinez, Boris Weinstein, Marc G. M. Schelstraete, B. R. Baker, and Leon Goodman

Life Sciences Research, Stanford Research Institute, Menlo Park, California

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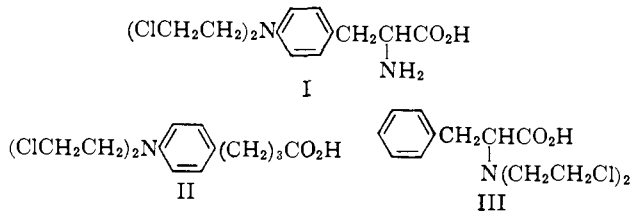
The conversion of methyl DL-phenylalanate (IV) to the α-mustard (III) of DL-phenylalanine is described. Reaction of methyl DL-phenylalanate with ethylene oxide gave 3-benzyl-4-(2-hydroxyethyl)morpholin-2-one. This reacted with ammonia to give 2-[bis(2-hydroxyethyl)amino]-3-phenylpropionamide. Chlorination followed by acid hydrolysis gave the α-mustard (III). Neither the α-mustard nor the morpholine mustard (VII) exhibited significant antitumor activity against Walker 256 Sarcoma, Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210. 2-[Bis(2-chloroethyl)]amino-3-phenylpropionamide was inactive against Walker 256 Sarcoma.

A biological rationale has been proposed[2] to account for the differences in antitumor activity of the clinically interesting nitrogen mustards, *p*-phenylalanine mustard (I)[3] and chlorambucil (II),[4] against a variety of transplanted tumors in mice. Briefly, it was suggested[2] that, in those systems where I was active and II was inactive, the mustard (I) fit an enzyme site normally



occupied by L-phenylalanine primarily by attachment through its amino and its carboxyl groups; on the other hand, in the Walker 256 sarcoma, which is affected by both I and II, only attachment of the alkylating agent through its carboxyl group was required. It was of interest to prepare the related compound III and to compare its antitumor activity with that of I and II as a test of the above hypothesis. However, it is possible that the difference in bulk and basicity of the bis (2-chloroethyl)amino group may obscure these comparisons. The α-mustard III is also of interest for comparison with the α-mustards of glycine and DL-alanine which Izumi[5] prepared and found to be more hydrophilic and less toxic than the simple nitrogen mustards like HN2. The synthesis of III, the first reported α-bis(2-chloroethyl)amino type of mustard of an aromatic amino acid, is the subject of this manuscript.

Reaction of ethylene oxide with methyl DL-phenylalanate (IV) in methanol gave the morpholone (VI) as a liquid that was characterized as the crystalline *p*-nitrobenzoate. Treatment of VI with alcoholic potassium hydroxide afforded the crystalline potassium salt (VIII) that recyclized to the morpholone (VI) on acidification. Ammonolysis of VI in liquid ammonia gave an excellent yield of the crystalline amide IX, which could also be obtained by the reaction of the amide V with ethylene oxide. The action of thionyl chloride on IX afforded the crystalline mustard amide (X) as the hydrochloride. Hydrolysis of X with 6 *N* hydrochloric acid at 85° for 4 hr. gave the hydrochloride of the α-aminomustard (III), which melts over a broad range, in a state of analytical purity. All attempts to recrystallize III failed, perhaps because of its tendency to lose hydrogen chloride. Thus, attempts to dry the hydrochloride of III *in vacuo* at 56° for 45 hours yielded essentially the morpholone (VII) hydrochloride. The free base III could apparently be formed by washing a chloroform solution of the hydrochloride of III with water but it slowly cyclized to the hydrochloride of VII in the chloroform solution. For comparison, the N-hydroxyethyl morpholone (VI) was converted to the morpholone (VII) hydrochloride with thionyl chloride.

A number of unsuccessful routes to III were explored. Thus, the Strecker synthesis utilizing bis(2-chloroethyl)amine, cyanide ion, and the bisulfite addition product of phenylacetaldehyde failed to give the nitrile precursor of III. This method, successful in the glycine and alanine mustard synthesis of Izumi,[5] was unsuccessful when applied to the synthesis of higher homologs here and elsewhere.[6]

Izumi[5] also successfully condensed chloroacetic acid with diethanolamine to yield N,N-bis(2-hydroxyethyl)-glycine, the precursor of glycine mustard. In our hands, the condensation of diethanolamine with 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 are not necessarily those of the Cancer Chemotherapy National Service Center.

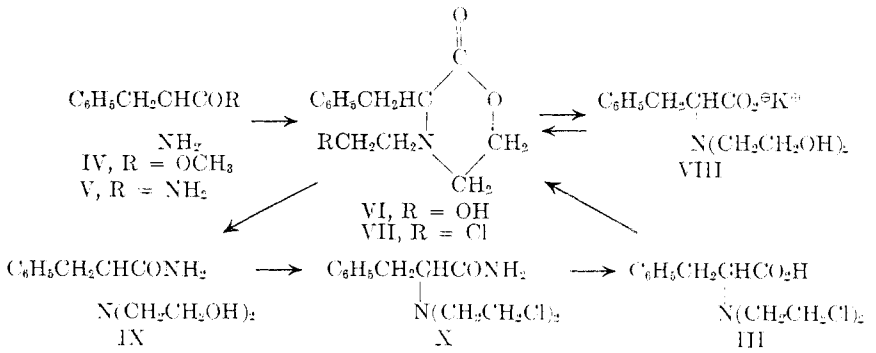
(2) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(3) F. Bergel, V. C. E. Burnop, and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); F. Bergel and J. A. Stock, *ibid.*, 2409 (1954); L. F. Larinov, *Lancet*, **269**, 169 (1955).

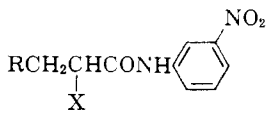
(4) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(5) M. Izumi, *Chem. Pharm. Bull.* (Tokyo), **2**, 275 (1954).

(6) The work of M. Ishidate, Y. Sakurai, and I. Aiko, *ibid.*, **8**, 732 (1960), became available to us after we had tried the same reaction.



α-haloanilides (XI and XII) was unsuccessful. However, the α-bromopropionanilide (XVII), under the



XI, X = Cl, R = C_6H_5
XII, X = Br, R = C_6H_5
XIII, X = $NHCO_2CH_2C_6H_5$, R = C_6H_5
XIV, X = NH_2, R = C_6H_5
XV, X = $N(CH_2CH_2OH)_2$, R = C_6H_5
XVII, X = Br, R = H
XVIII, X = $N(CH_2CH_2OH)_2$, R = H
XIX, X = $N(CH_2CH_2Cl)_2 \cdot HCl$, R = H

conditions that led to the dehydrohalogenation of XII to N-(*m*-nitrophenyl)cinnamide (XVI), afforded the crystalline bis(2-hydroxyethyl)amine (XVIII). This gave the crystalline mustard (XIX), but hydrolysis of XIX did not yield the crystalline α-alanine mustard.[5]

The bis(hydroxyethyl)amine (XV) was not obtained by the reaction of ethylene oxide with the amine (XIV) under a variety of conditions. The amine (XIV) was prepared by hydrogen bromide cleavage of the carbobenzoxy derivative (XIII). The *m*-nitroanilide group was employed in the above synthetic efforts as a potentially hydrolyzable group that gave distinctive infrared absorption bands and permitted paper chromatographic identification of derivatives by examination with ultraviolet light.

The mustards III, VII, and X,[7] all as the hydrochlorides, were found to have insignificant activity against Walker Sarcoma 256 in the rat. The first two, III and VII, also showed insignificant activity against the three mouse tumors, Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210. Compounds III and VII were quite toxic; for example in the S-180 testing, the non-toxic but inactive doses were of the order of 5–10 mg./kg./day.

The inactivity of III as compared with I and II does not permit a satisfactory test of the biological rationale[2] proposed for the preparation of III. Possibly the bulky α-mustard group of III interferes with enzyme binding. In comparison with glycine and alanine mustards which are active against S-180 and L-1210,[8] the α-phenylalanine mustard (III) is inactive.

(7) Compounds III, VII, and X were screened under the auspices of the Cancer Chemotherapy National Service Center according to its protocols.

(8) *Cancer Chemotherapy Rept.*, **7**, 99 (1960).

Experimental[9]

3-Benzyl-4-(2-hydroxyethyl)morpholin-2-one (VI).—A solution of 15.0 g. (0.083 mole) of methyl DL-phenylalanate (IV), 100 ml. of methanol, and 15 ml. of ethylene oxide (0.30 mole) in a stoppered flask was stirred at 25° for 64 hr. Evaporation of the solvent *in vacuo* left 21.1 g. (108%) of product as a light oil sufficiently pure for preparation of the amide (IX) and the morpholone mustard (VII): $\lambda_{max(\mu)}^{film}$ 2.95, 9.4–9.7 (OH), 5.75 (carbonyl). The morpholone (VI) appeared to contain essentially no monohydroxylation product since the acetylation product showed no amide carbonyl absorption in the infrared.

Reaction of 1.50 g. (5.60 mmoles) of the morpholone (VI) with 2.15 g. (11.6 mmoles) of *p*-nitrobenzoyl chloride in pyridine gave 1.33 g. (62%) of the mono-*p*-nitrobenzoate, m.p. 125–128°. Trituration with boiling ether gave an analytical sample, m.p. 133–134°; $\lambda_{max(\mu)}^{Nujol}$ 5.74, 5.82 (C=O of benzoate and morpholone), 6.51, 7.39, (NO_2); R_f 0.88 in A, 0.06 in B.[9]

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: C, 62.5; H, 5.24; N, 7.29. Found: C, 62.5; H, 5.58; N, 7.20.

Potassium 2-[Bis(2-hydroxyethyl)amino]-3-phenylpropionate (VIII).—A solution of 0.62 g. (2.30 mmoles) of the morpholone (VI) and 4.7 ml. of 0.5 *N* ethanolic potassium hydroxide was heated at reflux for 15 min., the solution was evaporated to dryness, and the residue was triturated with acetone to leave 0.70 g. (103%) of white crystals, m.p. 183–188°. Recrystallization from absolute ethanol gave 0.61 g. (89%) of the potassium salt (VIII) as white crystals, m.p. 192–193°; $\lambda_{max(\mu)}^{Nujol}$ 3.15, 9.30, 9.70 (OH), 6.24, 7.12 (COO^-); R_f 0.24 in A, 0.75 in C,[9] using mercuric chloride–potassium iodide spray for detection.

Anal. Calcd. for $C_{13}H_{18}KNO_4$: C, 53.6; H, 6.23; K, 13.4; N, 4.82. Found: C, 52.8; H, 6.10; K, 13.5; N, 5.06.

2-[Bis(2-Hydroxyethyl)amino]-3-phenylpropionamide (IX). A. From the Morpholone VI.—A precooled stainless steel bomb was charged with 0.72 g. (3.1 mmoles) of the morpholone (VI) and 5 ml. of liquid ammonia, sealed, and allowed to stand at room temperature for 66 hr. The bomb was cooled and the contents were dissolved in 200 ml. of absolute ethanol. Evaporation *in vacuo* of this solution left 0.75 g. (97%) of an oil that crystallized. Two recrystallizations from methylene chloride gave 0.60 g. (78%) of the product, m.p. 100–101.5°. $\lambda_{max(\mu)}^{Nujol}$ 3.01, 3.09, 3.19 (OH, NH), 5.95 (amide C=O), 9.39, 9.50 (C–OH); R_f 0.67 in A, and 0.66 in D.[9]

Anal. Calcd. for $C_{13}H_{20}N_2O_3$: C, 61.9; H, 7.98; N, 11.1. Found: C, 61.8; H, 8.24; N, 11.2.

The same reaction run in methanol at room temperature for for 72 hr. gave a 35% yield of IX.

B. From Phenylalanamide (V).—The reaction of 50 ml. of ethylene oxide and 0.65 g. (3.96 mmoles) of phenylalanamide (V) for 72 hr. by the procedure used for preparing VI gave 0.35 g. (35%) of IX, m.p. 97.5–98.5°, identical in all respects with IX prepared by procedure A. Optimum reaction conditions were not established.

3-Benzyl-4-(2-chloroethyl)morpholin-2-one (VII) Hydrochloride.—To a stirred suspension of 28.7 g. (0.113 mole) of the

(9) Melting points were obtained with a Fisher-Johns apparatus. Paper chromatograms were run by the descending technique on Whatman No. 1 paper and the spots were detected visually under ultraviolet light, unless otherwise noted. When an R_f value is given, the material is homogeneous unless otherwise indicated. The solvent systems used were: A, water-saturated 1-butanol; B, benzene–water–methanol (2/1/6) Schleicher and Schuell No. 2496 paper; C, benzene–water–methanol (2/1/6) Whatman No. 1 paper; D, 1-butanol–acetic acid–water (5/3/2); E, 5% disodium hydrogen phosphate.

hydroxymorpholone (VI), in 120 ml. of methylene chloride cooled in an ice bath was added dropwise 72.6 g. (0.610 mole) of thionyl chloride. The clear amber solution was stirred at room temperature for 60 min., then at reflux temperature for 30 min., and was finally evaporated *in vacuo* to a dark oil. The evaporation was repeated after each of three additions of 200-ml. portions of methylene chloride. The residue was refluxed in ether, decanted, and the gummy residue taken up in 150 ml. of methylene chloride. After 1 day at 5°, the white crystals were collected, washed with cold solvent, and dried to afford 14.8 g. (45.2%) of VII·HCl, m.p. 114–119° (softening at 100°); $\lambda_{max(\mu)}^{Nujol}$ 4.4 (NH^+), 5.72 (C=O).

Anal. Calcd. for 95% $C_{13}H_{16}ClNO_2$·HCl (the salt) and 5% $C_{13}H_{16}ClNO_2$ (the free base): C, 54.2; H, 5.93; Cl, 23.9; Cl^-, 11.6; N, 4.86. Found: C, 54.5; H, 5.83; Cl, 23.8; Cl^-, 12.2; N, 4.69.

2-[Bis(2-Chloroethyl)amino]-3-phenylpropionamide (X) Hydrochloride.—To 10 ml. of thionyl chloride, stirred and cooled in an acetone–Dry Ice bath, was added 1.0 g. (3.96 mmoles) of the bis(2-hydroxyethyl)amide IX. The mixture, protected from moisture, was removed from the cooling bath and allowed to stir for 0.5 hr. at room temperature. During this time some precipitate appeared and redissolved. The solution was diluted with methylene chloride, stirred for 1 hr. more, and evaporated to dryness *in vacuo* (25°, 15 mm.), the evaporation being repeated after the addition of 25 ml. of 1,2-dichloroethane. The dark residue was triturated with 25 ml. of 1,2-dichloroethane to afford 0.87 g. (68%) of a white, crystalline material, m.p. 123–128°. Trituration with ethyl acetate left 0.70 g. (55%) of solid, m.p. 136–139°; $\lambda_{max(\mu)}^{Nujol}$ 3.00, 3.16 (N—H), 4.15 (NH^+), 5.90 (amide C=O); R_f 0.85 in A, 0.93 in D, detected with the mercuric chloride–potassium iodide spray.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O$·HCl: C, 47.9; H, 5.88; Cl, 32.6; Cl^-, 10.9; N, 8.64. Found: C, 47.4; H, 5.87; Cl, 32.0, Cl^-, 10.9; N, 8.64.

This mustard amide appears to be a potent skin irritant and should be handled with proper precautions.

2-[Bis(2-Chloroethyl)amino]-3-phenylpropionic Acid (III) Hydrochloride.—A mixture of 15.0 g. (0.046 mole) of the mustard amide (X) hydrochloride and 75 ml. of 6 *N* hydrochloric acid was heated at 85° for 4 hr. The resultant solution was evaporated *in vacuo* to give a tan gum which was extracted with ten 150-ml. portions of methylene chloride. The combined extracts were dried, evaporated to 75 ml. *in vacuo* at 30–40°, and cooled to 5°. The precipitate was collected, washed with cold methylene chloride, and dried at room temperature *in vacuo* to afford 9.22 g. (61%) of crystalline product, m.p. 98–110°; $\lambda_{max(\mu)}^{Nujol}$ 3.4–4 (NH^+ and COOH), 5.75 (acid C=O); R_f 0.77 in E,[9] detected by mercuric chloride–potassium iodide spray.

Anal. Calcd. for $C_{13}H_{17}Cl_2NO_2$·HCl: C, 47.9; H, 5.55; Cl, 32.6; Cl^-, 10.9; N, 4.29. Found: C, 47.7; H, 5.32; Cl, 32.7; Cl^-, 11.0; N, 4.50.

No suitable solvent for recrystallization was found, and no crystalline derivative was obtained, using picric, picrolonic, and styphnic acids. Optimum hydrolysis conditions were determined by following the disappearance of the amide band in the infrared spectrum. A sample of III heated at 56° (0.1 mm.) for 45 hr. afforded a gum whose infrared spectrum was similar to that of the morpholone hydrochloride (VII·HCl).

Anal. Calcd. for VII·HCl: Cl, 24.4; Cl^-, 12.2. Found: Cl, 23.0; Cl^-, 12.4.

2-Chloro-*m*′-nitro-3-phenylpropionanilide (XI).—A suspension of 5.36 g. (0.039 mole) of *m*-nitroaniline in 50 ml. of methylene chloride was slowly added to a stirred solution of 3.94 g. (0.019 mole) of 2-chloro-3-phenylpropionyl chloride[10] in 25 ml. of methylene chloride. The mixture solidified within a few minutes and the cake was collected and washed with three 50-ml. portions of methylene chloride. Hydrogen chloride was bubbled through the combined filtrates for a few min., the mixture was filtered, and the filtrate evaporated to dryness *in vacuo*. The residue was recrystallized from benzene to afford 3.87 g. (65%) of product, m.p. 98–99°; $\lambda_{max(\mu)}^{Nujol}$ 3.07 (NH), 5.93 (amide C=O), 6.44, 6.50 (amide II, NO_2), 740 (NO_2); R_f 0.19 in B. This failed to react with diethanolamine under a variety of conditions.

Anal. Calcd. for $C_{15}H_{13}ClN_2O_3$: C, 59.1; H, 4.30; Cl, 11.6; N, 9.20. Found: C, 59.0; H, 4.12; Cl, 11.5; N, 9.47.

2-Bromo-*m*′-nitro-3-phenylpropionanilide (XII).—From 21.2 g. (0.086 mole) of 2-bromo-3-phenylpropionyl chloride[11] and 27.6 g. (0.20 mole) of *m*-nitroaniline was obtained 23.9 g. (80%) of crude product by the procedure used to prepare XI. This was suitable for use in the next experiment.

Three crystallizations from benzene gave an analytical sample, m.p. 137–139°; $\lambda_{max(\mu)}^{Nujol}$ 3.09 (N—H), 5.94 (amide C=O), 7.35 (NO_2); R_f 0.17 in B.

Anal. Calcd. for $C_{15}H_{12}BrN_2O_3$: C, 51.6; H, 3.75; Br, 22.9; N, 8.02. Found: C, 51.4; H, 3.39; Br, 22.8; N, 8.03.

N-(*m*-Nitrophenyl)cinnamamide (XVI). A. From XII.—A solution of 1.75 g. (5 mmoles) of the bromoanilide XII and 1.16 g. (11 mmoles) of diethanolamine in 50 ml. of ethanol was heated at reflux for 3.5 hr., the solvent was removed *in vacuo*, and the gummy residue was digested with a small volume of boiling benzene to give 0.73 g. (54%) of XVI, m.p. 160–163°. Two recrystallizations from benzene gave the analytical sample of XVI, m.p. 170–171°; $\lambda_{max(m\mu)}^{95\%\ EtOH}$ 222 (ϵ 22,400), 227 (ϵ 20,800) shoulder, and 296 (ϵ 39,500); $\lambda_{max(\mu)}^{nujol}$ 3.01 (N—H) 5.94 (amide), 7.38 (NO_2); R_f 0.21 in B.

Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.2; H, 4.51; N, 10.4. Found: C, 67.3; H, 4.24; N, 10.4.

B. From Cinnamoyl Chloride.—From 1.67 g. (10 mmoles) of cinnamoyl chloride and 3.04 g. (22 mmoles) of *m*-nitroaniline there was obtained, by the procedure used for preparing XI, 0.22 g. (8.2%) of XII, identical in all respects with that prepared above.

2-Bromo-*m*-nitropropionanilide (XVII).—From 14.5 g. (0.105 mole) of *m*-nitroaniline and 9.80 g. (0.052 mole) of 2-bromopropionyl chloride,[12] using the procedure for preparing XI, there was obtained 11.5 g. (81%) of product, m.p. 99–100°, and a second crop of 1.15 g. (8%), m.p. 92–95°. The analytical sample, m.p. 104–105°, was obtained by sublimation *in vacuo*; $\lambda_{max(\mu)}^{Nujol}$ 3.16 (NH), 6.01 (amide), 7.41 (NO_2) and 13.5 (phenyl); R_f 0.30 in B.

Anal. Calcd. for $C_9H_9BrN_2O_3$: C, 39.6; H, 3.32; N, 10.3. Found: C, 39.5; H, 3.38; N, 10.6.

2-[Bis(2-Hydroxyethyl)amino]-*m*-nitropropionanilide (XVIII).—A solution of 7.79 g. (0.029 mole) of the bromoanilide (XVII) and 5.68 g. (0.054 mole) of diethanolamine in 50 ml. of ethanol was heated at reflux for 4 hr., evaporated to dryness, and the residue partitioned between 50 ml. each of benzene and water. The water layer was repeatedly extracted with benzene and ether. From the total organic extracts was obtained 4.14 g. (49%) of product, m.p. 90–93°. Recrystallization from ethyl acetate gave an analytical sample, m.p. 95–98°; R_f 0.55 in B.

Anal. Calcd. for $C_{13}H_{19}N_3O_5$: C, 52.5; H, 6.44; N, 14.2. Found: C, 52.8; H, 6.70; N, 13.9.

2-[Bis(2-Chloroethyl)amino]-*m*-nitropropionanilide (XIX) Hydrochloride.—To an ice-cooled, stirred portion of 1.45 g. (4.90 mmoles) of the bis(2-hydroxyethyl)anilide XVIII was added dropwise 5.0 ml. (69 mmoles) of thionyl chloride. The mixture was allowed to warm to room temperature (during which time XVIII dissolved), refluxed for 30 min., and evaporated *in vacuo* to leave an amber gum. The evaporation was repeated each time after the addition of two 15-ml. portions of chloroform and three 15-ml. portions of methylene chloride. The residue was triturated with 5 ml. of methylene chloride, collected, and washed with methylene chloride to afford after drying, 1.19 g. (66%) of XIX as a tan powder, m.p. 144.5–150.5° (softens at 140°). Recrystallization from acetone gave white crystals, m.p. 140.5–153.5°; $\lambda_{max(\mu)}^{Nujol}$ 4.1–4.2 (NH^+), 5.88 (amide), 6.4, 6.5 (amide, NO_2) 7.36 (NO_2); no absorption at 9–10 (OH); R_f 0.89 in A, 0.14 in B.

Anal. Calcd. for 95% $C_{13}H_{17}Cl_2N_3O_2$·HCl (the salt) and 5% $C_{13}H_{17}Cl_2N_3O_3$ (the free base): C, 42.3; H, 4.91; Cl, 28.3; Cl^-, 9.07. Found: C, 42.1; H, 4.61; Cl, 27.9; Cl^-, 9.32.

Longer chlorination times gave a darker product whose infrared spectrum indicated a decrease in amide absorption; shorter times gave incomplete reaction. Chlorination with thionyl chloride in refluxing methylene chloride was incomplete after 2.5 hr.

The free base from XIX had m.p. 93–95°, but was unstable and could not be recrystallized to analytical purity.

The hydrolysis of XIX could be followed by paper chromatography. Because of the insolubility of XIX, a mixture of hydrochloric acid–acetic acid (4:1) was required and hydrolysis

(10) L. Knunyants and N. P. Gambaryan, *Izv. Akad. Nauk. SSSR, Otd. Khim. Nauk*, 1037 (1955); *Chem. Abstr.*, **50**, 11277 (1956).

(11) E. Fischer, *Ber.*, **37**, 3062 (1904).

(12) K. Freudenberg and L. Markert, *ibid.*, **60**, 2447 (1927).

appeared to be more than 95% complete after 16 hr. at reflux temperature. Without recourse to alkaline conditions, most of the *m*-nitroaniline hydrochloride could be extracted from most of the mustard acid hydrochloride with acetonitrile followed by extraction with ether. Although paper chromatography indicated the presence of the DL-alanine *α*-mustard in the residue, it was not possible to obtain the crystalline mustard or the crystalline picrylsulfonate reported by Izumi.[3]

2-Benzyloxycarbonylamino-*m*'-nitro-3-phenylpropionanilide (XIII).—To a stirred and cooled (0°) suspension of 2.99 g. (10 mmoles) of N-benzyloxycarbonyl-DL-phenylalanine in 20 ml. of freshly distilled tetrahydrofuran was added 0.95 ml. (10 mmoles) of ethyl chloroformate followed by 1.39 ml. (10 mmoles) of triethylamine and finally after 15 min. by 1.38 g. (10 mmoles) of *m*-nitroaniline. After being stirred for 15 min., the cooling bath was removed and the reaction mixture stirred for 2.5 hr. at room temperature. The precipitate of 1.25 g. (91%) of triethylamine hydrochloride was collected and the filtrate was evaporated *in vacuo* to leave 4.31 g. (103%) of crude product. Extraction with two 50-ml. portions of hot ethyl acetate gave, on chilling, 2.28 g. (55%) of product, m.p. 179–182°. Recrystallization from benzene–cyclohexane gave an analytical sample, m.p. 183–183.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.05 (N—H), 5.85, 5.95 (C=O), 6.44, 6.50 (amide II, NO_2), 7.37 (NO_2).

Anal. Calcd. for $C_{23}H_{21}N_3O_5$: C, 65.9; H, 5.05; N, 10.0. Found: C, 66.2, 66.3, H, 5.33, 5.35; N, 9.47, 9.54.

2-Amino-*m*'-nitro-3-phenylpropionanilide (XIV) Hydrobromide.—A solution of 0.50 g. (1.20 mmole) of the carbobenzyloxy anilide XIII in 25 ml. of saturated hydrogen bromide in glacial acetic acid was allowed to stand at room temperature for 2 hr., then was poured into ether, and the precipitate collected to give 0.41 g. (93%) of the product as the hydrobromide, m.p. 270–273° dec., $\lambda_{max(\mu)}^{Nujol}$ 3.11 (N—H), 3.3–4.0 ($-NH_3^+$), 5.88 (C=O), 6.52 ($-NH_3^+$, NO_2), 7.40 (NO_2).

Anal. Calcd. for $C_{15}H_{15}N_3O_3 \cdot HBr$: C, 49.2; H, 4.40; Br, 21.8; N, 11.5. Found: C, 49.0; H, 4.63; Br, 22.0; N, 10.9.

To a solution of 0.80 g. (20 mmoles) of sodium hydroxide in 50 ml. of methanol was added 7.33 g. (20 mmoles) of the above hydrobromide. The mixture was stirred at room temperature for 15 min., filtered, and the filtrate evaporated *in vacuo* to give 5.08 g. (89%) of product as an oil. Digestion of this oil with ether and evaporation *in vacuo* of the ether solution left 3.34 g. of product as an oil; $\lambda_{max(\mu)}^{Nujol}$ 2.94, 3.03 (NH_2), 5.90 (amide C=O), 6.52 (amide II, NO_2), 7.40 (NO_2); R_f 0.34 in solvent B.

Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.2; H, 5.30; N, 14.7. Found: C, 63.3; H, 5.49; N, 13.6.

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Synthesis of β-Phenylserines

S. WAWZONEK AND W. G. GAFFIELD

Department of Chemistry, State University of Iowa, Iowa City, Iowa

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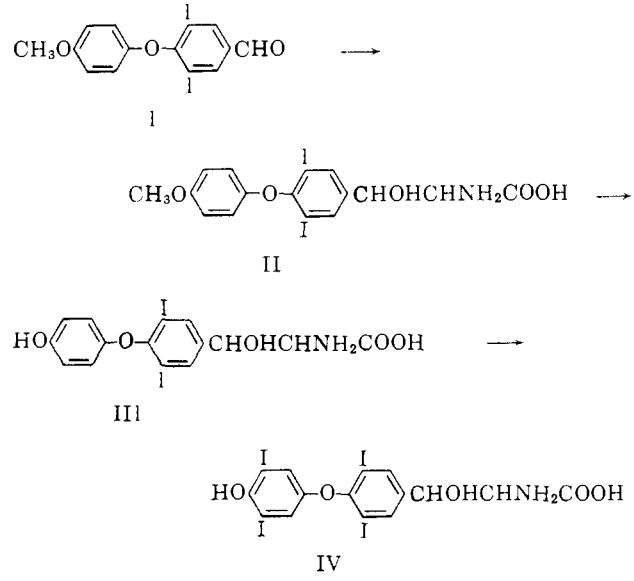
β-[3,5-Diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)phenyl]serine, β-[3,5-diiodo-4-(4-hydroxyphenoxy)phenyl]-serine, β-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]serine, the isomeric β-(4-hydroxy-3,5-diiodophenyl)serines, and the hydrochloride of β-(4-benzoyloxyphenyl)serine ethyl ester have been prepared for testing as possible antithyroids. None of the compounds was able to inhibit the activity of L-triiodothyronine on a thiouracil-induced goiter. The first compound, however, did show some thyronimetic activity.

The report that β-phenylserine is an antagonist for the utilization of phenylalanine in microorganisms[2] suggested the synthesis of the serine analog (IV) of thyroxine and simpler β-(4-hydroxyphenyl)serine derivatives for testing as possible antithyroids.

The analog of thyroxine was prepared from 3,5-diiodo-4-(4-methoxyphenoxy)benzaldehyde (I) by the following sequence of reactions.

The condensation of 3,5-diiodo-4-(4-methoxyphenoxy)benzaldehyde (I) with glycine was carried out by an adaptation of the method of Erhart and Ott[3] for various β-(hydroxyphenyl)serines and involved the reaction of two moles of the aldehyde (I) with one mole of glycine in the presence of sodium hydroxide. The precipitate which appeared upon standing was presumably the Schiff's base and was not characterized further but was hydrolyzed to the serine (II) with dilute hydrochloric acid. The product differed in melting point from the serine (II) obtained by Friedenberg and Nobles[4] by the condensation of equimolar amounts of the organic reactants with dilute alkali. The latter reaction, which may produce a different diastereoisomer, could not be repeated in this Laboratory; only starting materials were obtained.



I II III IV

The structure of the serine (II) obtained in this study was in agreement with its infrared spectrum.

(1) Abstracted in part from the Ph.D. thesis, February, 1963, of W. G. Gaffield.

(2) E. Beerstecher, Jr., and W. Shive, *J. Biol. Chem.*, **164**, 53 (1946).

(3) G. Ehrhart and H. Ott, U. S. Patent 2,737,526 (1956); *Chem. Abstr.*, **50**, 15587*c* (1956).

(4) R. Friedenberg and W. L. Nobles, *J. Am. Pharm. Assoc. (Sci. Ed.)*, **46**, 387 (1957).