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Diiodo-*m*-tyrosine, Three Isomeric Dinitro-*m*-tyrosines and Some of their Derivatives

BY ERNEST L. JACKSON

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Iodination of *m*-tyrosine yields β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine, the structure of which was proved as follows: acetylation to N-acetyl- β -(3-acetoxy-4,6-diiodophenyl)-D,L-alanine; removal of the O-acetyl group to form N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine; methylation of the latter compound to N-acetyl- β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine which was deacetylated to β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine, permanganate oxidation of which afforded 3-methoxy-4,6-diiodobenzoic acid identical with the authentic compound prepared from known 3-methoxy-4,6-dinitrobenzoic acid. N-Acetyl- β -[3-(*p*-nitrobenzyloxy)-4,6-diiodophenyl]-D,L-alanine was prepared. Nitration of *m*-tyrosine produced three isomers of β -(3-hydroxydinitrophenyl)-D,L-alanine. Acetylation of these isomers yielded the N-acetyl derivatives, methylation of two of which with diazomethane gave two isomeric N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl esters. Hydrolysis and deacetylation of these esters afforded two isomers of β -(3-methoxydinitrophenyl)-D,L-alanine. The hydrocarbostyryl derivative (I or II) resulted from the catalytic reduction of the nitro groups of one N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester. One isomer of N-acetyl- β -[3-(*p*-methoxyphenoxy)-dinitrophenyl]-D,L-alanine ethyl ester was prepared.

The nitration of L-tyrosine yields 3,5-dinitro-L-tyrosine from which Chalmers, Dickson, Elks and Hems¹ have synthesized L-thyroxine. Similarly, the iodination of D,L-tyrosine affords 3,5-diiodo-D,L-tyrosine.² The iodination of *m*-tyrosine has been shown by Dickinson and Marshall³ to yield a diiodo derivative of unknown structure and there appears to be no record of the nitration of *m*-tyrosine. Diiodo and dinitro derivatives of *m*-tyrosine provide starting material for the synthesis of a new type of thyroxine analog.

Diiodo-*m*-tyrosine³ now is shown to be β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine. This compound upon acetylation either with acetic anhydride-sodium hydroxide or with acetic anhydride-sulfuric acid mixture yielded N-acetyl- β -(3-acetoxy-4,6-diiodophenyl)-D,L-alanine (m.p. 218°), the O-acetyl group of which was removed readily by 2 *N* sodium hydroxide solution at 25° to form N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine (m.p. 200°). The latter compound was methylated by methyl sulfate to yield N-acetyl- β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine (m.p. 217–218°) which was deacetylated by a hot mixture of sulfuric and acetic acids to produce β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine (m.p. 245–246°). Degradative oxidation of the side chain of this amino acid by alkaline potassium permanganate yielded a crystalline 3-methoxydiiodobenzoic acid melting at 235–236°. This compound obviously differed from 3-methoxy-2,6-diiodobenzoic acid (m.p. 137–138°) which was prepared by permanganate oxidation of Hodgson and Smith's⁴ 3-methoxy-2,6-diiodobenzaldehyde. The 3-methoxydiiodobenzoic acid melting at 235–236° was proved to be 3-methoxy-4,6-diiodobenzoic acid by synthesis from 3-methoxy-4,6-dinitrobenzoic acid, the structure of which has been established by Hodgson and Beard⁵ and by

Tröger and Eicker.⁶ Reduction of the nitro groups of 3-methoxy-4,6-dinitrobenzoic acid in ethyl acetate solution with hydrogen and a Raney nickel catalyst, followed by tetrazotization of the product in a mixture of concentrated sulfuric acid and acetic acid and subsequent reaction of the tetrazo derivative with iodine and sodium iodide yielded crystalline 3-methoxy-4,6-diiodobenzoic acid melting at 235–236° alone or mixed with the compound from β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine. Although the diamine resulting from the reduction of 3-methoxy-4,6-dinitrobenzoic acid was not isolated in pure condition, heating the crude reduction product with acetic anhydride afforded a crystalline compound (m.p. 215–216°) which, from analytical data, appeared to be slightly impure 3-methoxy-4,6-di-acetamidobenzoic anhydride.

Woolley⁷ has reported that the *p*-nitrobenzyl and certain other ethers of N-acetyl-3,5-diiodo-L-tyrosine antagonize the action of thyroxine on tadpoles and also show a weak thyroxine-like activity both in tadpoles and in mice. N-Acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine through reaction with *p*-nitrobenzyl bromide and sodium hydroxide yielded the crystalline sodium salt of N-acetyl- β -[3-(*p*-nitrobenzyloxy)-4,6-diiodophenyl]-D,L-alanine. Both the sodium salt and its parent acid, which does not crystallize readily from solvents, gave low iodine analyses. The crystalline ethyl ester (m.p. 215°), prepared by refluxing the chloroform-ethanol solution of the acid in the presence of *p*-toluenesulfonic acid and azeotropic removal of water, proved to be suitable for characterization of the compound. An attempt to prepare N-acetyl- β -[3-(*p*-methoxyphenoxy)-4,6-diiodophenyl]-D,L-alanine ethyl ester by reaction of *p*-methoxyphenol in pyridine solution with N-acetyl- β -(3-*p*-toluenesulfonyloxy-4,6-diiodophenyl)-D,L-alanine ethyl ester was unsuccessful.⁸ Crystalline N-acetyl- β -(3-*p*-

(1) J. R. Chalmers, G. T. Dickson, J. Elks and B. A. Hems, *J. Chem. Soc.*, 3424 (1949); E. T. Borrows, J. C. Clayton and B. A. Hems, *ibid.*, S199 (1949).

(2) E. T. Borrows, J. C. Clayton and B. A. Hems, *ibid.*, S185 (1949); P. Block and G. Powell, *THIS JOURNAL*, **65**, 1430 (1943); A. Y. Savitskii, *J. Gen. Chem., U. S. S. R.*, **9**, 1342 (1939); *C. A.*, **34**, 741 (1940); H. Bauer and E. Strauss, *Ber.*, **68**, 1108 (1935); C. R. Harington, *Biochem. J.*, **22**, 1434 (1928); H. L. Wheeler and G. S. Jamieson, *Am. Chem. J.*, **33**, 365 (1905).

(3) W. P. Dickinson and P. G. Marshall, *J. Chem. Soc.*, 2289 (1930).

(4) H. H. Hodgson and E. W. Smith, *ibid.*, 76 (1937).

(5) H. H. Hodgson and H. G. Beard, *ibid.*, 2381 (1937).

(6) J. Tröger and C. Eicker, *J. prakt. Chem.*, [2] **116**, 17 (1927).

(7) D. W. Woolley, *J. Biol. Chem.*, **164**, 11 (1946); D. W. Woolley, "A Study of Antimetabolites," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 178; see also E. Frieden and R. J. Winzler, *J. Biol. Chem.*, **179**, 423 (1949); T. C. Bruce, R. J. Winzler and N. Kharasch, *ibid.*, **210**, 1 (1954); J. H. Barnes, J. Elks, F. F. Stephens and G. J. Waller, *J. Chem. Soc.*, 764 (1953).

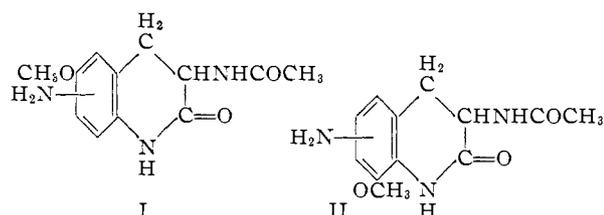
(8) Cf. C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927); A. Canzanelli, C. R. Harington and S. S. Randall, *ibid.*, **28**, 68 (1934); E. T. Borrows, J. C. Clayton, B. A. Hems and A. G. Long, *J. Chem. Soc.*, S190 (1949); E. T. Borrows, J. C. Clayton and B. A. Hems, ref. 2.

toluenesulfonyloxy-4,6-diiodophenyl)-D,L-alanine ethyl ester (m.p. 133–134°) resulted from the reaction of *p*-toluenesulfonyl chloride with sirupy N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine ethyl ester in alkaline acetone solution.

The nitration of *m*-tyrosine with a mixture of nitric and sulfuric acids under selected conditions produced, in 50% yield, three crystalline isomers of β -(3-hydroxydinitrophenyl)-D,L-alanine which presumably are β -(3-hydroxy-2,6-dinitrophenyl)-D,L-alanine, β -(3-hydroxy-4,6-dinitrophenyl)-D,L-alanine and β -(3-hydroxy-2,4-dinitrophenyl)-D,L-alanine. The isomers were separated partially by fractional recrystallization of their barium salts and purification was completed by recrystallization of the acids from water. Isomer A and isomer B are not distinguished reliably by their melting points but isomer C, which was isolated in about 5% yield, is characterized by an explosive decomposition point of 197°. Acetylation of each of the isomers in aqueous medium by a mixture of acetic anhydride and sodium hydroxide afforded the crystalline N-acetyl derivatives which could be distinguished readily by their melting points (N-acetyl-A, 200–201°; N-acetyl-B, 210–211°; N-acetyl-C, 194–195°). Methylation of N-acetyl-A with diazomethane yielded N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester melting at 128–129° and similar methylation of N-acetyl-B gave N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester melting at 172–173°. Methyl sulfate failed to methylate these compounds satisfactorily. Hydrolysis and deacetylation of the two methyl esters by a hot mixture of sulfuric and acetic acids produced from the former ester β -(3-methoxydinitrophenyl)-D,L-alanine melting at 234–235° and from the latter ester β -(3-methoxydinitrophenyl)-D,L-alanine melting at 231–232°. Oxidation of the first of these amino acids by alkaline potassium permanganate afforded a crystalline product melting at 202–203° and similar oxidation of the second amino acid gave a crystalline product melting at 188–189°. Although the structures of these oxidation products have not yet been established, the possibility of their identity with 3-methoxy-2,6-dinitrobenzoic acid^{5,6} (m.p. 199°) and 3-methoxy-4,6-dinitrobenzoic acid^{5,6,9} (m.p. 188°) is eliminated, since mixtures with these reference compounds showed depressions in melting points. Reduction of the nitro groups of N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester (m.p. 172–173°) in either ethyl acetate or methanol solution at 25° and atmospheric pressure in the presence of a Raney nickel catalyst produced, through reaction of an *ortho*-amino group with the carbomethoxy group in the side chain, a hydrocarbostyryl derivative (m.p. 245–246°): 3-acetamido-6-methoxy-(amino)-hydrocarbostyryl (I) or 3-acetamido-8-methoxy-(amino)-hydrocarbostyryl (II). This result is consistent with the instability of β -(2-aminophenyl)-propionic acid and its methyl ester which form hydrocarbostyryl quite readily.¹⁰ The formation of the hydrocarbostyryl derivative precluded the substitution of

iodine atoms for the two nitro groups and possible correlation in this way of the structure of the dinitro compound with β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine or one of its derivatives.

N-Acetyl- β -(3-hydroxydinitrophenyl)-D,L-alanine (N-acetyl-A) yielded N-acetyl- β -(3-hydroxydinitrophenyl)-D,L-alanine ethyl ester (m.p. 145–146°). The reaction of this ethyl ester with *p*-toluenesulfonyl chloride in pyridine solution at 100° and subsequent heating of the resulting solution of the 3-*p*-toluenesulfonyloxy derivative with *p*-methoxyphenol produced, in 23% yield, crystalline N-acetyl- β -[3-(*p*-methoxyphenoxy)-dinitrophenyl]-D,L-alanine ethyl ester (m.p. 135°).



Experimental

Except where otherwise stated, melting points are corrected and were determined with the bath of the Hershberg apparatus preheated to 20–30° below the melting point. *m*-Tyrosine was prepared according to Sealock, Speeter and Schweet.¹¹

N-Acetyl- β -(3-acetoxy-4,6-diiodophenyl)-D,L-alanine.—A mixture of 1 g. of Dickinson and Marshall's³ diiodo-*m*-tyrosine with 2.5 ml. of water and 1.5 ml. of 2 *N* sodium hydroxide solution was kept at 0–5° during the alternate addition of six 1.0-ml. portions of 2 *N* sodium hydroxide and six 0.17-ml. portions of acetic anhydride.¹² After one hour at 25°, the addition of 2.8 ml. of 6 *N* sulfuric acid precipitated a gum that soon crystallized; yield 1.2 g. melting at 208–210°. Recrystallized from acetone-benzene, the pure air-dried, colorless crystals melted at 218°.

A mixture of 0.4 g. of powdered diiodo-*m*-tyrosine, 4 ml. of acetic anhydride and 0.1 ml. of concentrated sulfuric acid was kept at 25° for 24 hours, then mixed with ice-water and neutralized partially by sodium bicarbonate. The diacetyl derivative (0.2 g.), after purification, melted at 218°.

Anal. Calcd. for C₁₃H₁₃I₂NO₃: C, 30.19; H, 2.53; I, 49.09. Found (dried at 100° *in vacuo*): C, 30.32; H, 2.36; I, 48.85.

N-Acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine.—A solution of 0.75 g. of N-acetyl- β -(3-acetoxy-4,6-diiodophenyl)-D,L-alanine in 2.2 ml. of 2 *N* sodium hydroxide was kept at 25° for 5 hours and then treated with 4.4 ml. of *N* hydrochloric acid. The precipitated sirup crystallized readily; yield 0.65 g. Purified from ethanol-petroleum ether (b.p. 30–75°) it melted at 200°.

Anal. Calcd. for C₁₁H₁₁I₂NO₃: C, 27.81; H, 2.33; I, 53.43. Found (dried at 100° *in vacuo*): C, 28.14; H, 2.71; I, 53.45.

N-Acetyl- β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine.—To a solution of 0.5 g. of N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine in 3 ml. of ethanol at 25–30° were added alternately with shaking, during 20 minutes, three 0.17-ml. portions of 5 *N* sodium hydroxide and three 0.07-ml. portions of redistilled methyl sulfate. The solution was kept at 40–42° for 15 minutes and then for 20 minutes longer while three 0.17-ml. portions of 5 *N* sodium hydroxide and three 0.07-ml. portions of methyl sulfate were added. After an additional 30 minutes at 40–42°, the solution was cooled and treated with 1.02 ml. of 5 *N* hydrochloric acid. The product was precipitated by mixing the solution with 15 ml. of water containing 0.3 ml. of 5 *N* hydrochloric acid. The solid was dissolved in 5 ml. of water by rendering the

(9) M. Giua, *Gazz. chim. ital.*, **45**, 352 (1915).

(10) F. Mayer, H. Philipps, F. W. Ruppert and A. T. Schmitt, *Ber.*, **61**, 1966 (1928); E. R. Blout and D. C. Silverman, *THIS JOURNAL*, **66**, 1442 (1944).

(11) R. R. Sealock, M. E. Speeter and R. S. Schweet, *THIS JOURNAL*, **73**, 5386 (1951).

(12) Cf. V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 305 (1932).

mixture alkaline to litmus with sodium hydroxide. Precipitation from the filtered solution by addition of hydrochloric acid yielded 0.48 g. or 94% of the methoxy derivative melting at 211–213°. Recrystallized as well-formed, colorless prisms from ethanol or from ethanol–petroleum ether, it melted at 217–218°.

Anal. Calcd. for $C_{12}H_{13}I_2NO_4$: C, 29.47; H, 2.68; I, 51.90. Found (dried at 57° *in vacuo*): C, 29.72; H, 2.70; I, 52.13.

β -(3-Methoxy-4,6-diiodophenyl)-D,L-alanine.—A solution of 0.34 g. of N-acetyl- β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine in 7.3 ml. of warm acetic acid was mixed with 9.2 ml. of 5.76 *N* aqueous sulfuric acid solution. After the solution had been refluxed for 3 hours, the solvent was evaporated *in vacuo* (bath 65°). Most of the product soon crystallized from a solution of the residue in 15 ml. of water. Neutralization of the filtrate to litmus with ammonium hydroxide precipitated additional product. The compound was recrystallized by dissolving it in a hot mixture of 30 ml. of acetic acid and 10 ml. of water, and concentrating the solution *in vacuo*; yield 0.28 g. or 90%, m.p. 245–246° dec. It is only slightly soluble in water and the usual organic solvents.

Anal. Calcd. for $C_{10}H_{11}I_2NO_3$: C, 26.87; H, 2.48. Found (dried at 57° *in vacuo*): C, 26.98; H, 2.69.

3-Methoxy-4,6-diiodobenzoic Acid from Permanganate Oxidation of β -(3-Methoxy-4,6-diiodophenyl)-D,L-alanine.—To a solution of 62 mg. of β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine in 10 ml. of 5.05% aqueous potassium hydroxide solution at 30° was added, during one hour, 4 ml. of 0.7% aqueous potassium permanganate solution in several portions. After one hour at 50–54°, 9 ml. of 0.7% permanganate solution was added and the mixture was held at 50–54° for 8 hours. Saturation of the mixture with sulfur dioxide dissolved the hydrated manganese dioxide and precipitated 32 mg. of 3-methoxy-4,6-diiodobenzoic acid melting at 232–233°. Recrystallized from benzene as colorless prisms, the compound melted at 235–236° alone or mixed with authentic crystals of 3-methoxy-4,6-diiodobenzoic acid which was prepared from 3-methoxy-4,6-dinitrobenzoic acid.

Anal. Calcd. for $C_8H_8I_2O_3$: C, 23.79; H, 1.50; I, 62.8. Found: C, 23.85; H, 1.60; I, 62.4.

3-Methoxy-4,6-diiodobenzoic Acid from 3-Methoxy-4,6-dinitrobenzoic Acid.—A solution of 0.28 g. of 3-methoxy-4,6-dinitrobenzoic acid (m.p. 188–189°) in 36 ml. of ethyl acetate was shaken for 19 hours with hydrogen and a Raney nickel catalyst at 25° and atmospheric pressure, 6 moles of hydrogen being absorbed. The catalyst was filtered off in an atmosphere of carbon dioxide and the solvent was evaporated at 40° *in vacuo*, nitrogen being introduced to minimize oxidation. A solution of the residual solid in 12 ml. of glacial acetic acid was added, with shaking and cooling at ca. 10°, to 6 ml. of concentrated sulfuric acid. A solution of 0.2 g. of sodium nitrite in 6 ml. of concentrated sulfuric acid was diluted at 0° with 12 ml. of glacial acetic acid and this solution was stirred at –2 to –4° while the solution of the reduction product was added dropwise during 2 hours. The stirring was continued at 0° for 1.5 hours and the ice-cold solution then was added during 10 minutes to an efficiently stirred solution of 0.9 g. of sodium iodide, 0.8 g. of iodine and 0.12 g. of urea in 16 ml. of water covering 10 ml. of chloroform, the mixture becoming warm from the heat of the reaction. After being stirred for one hour, the chloroform layer was separated and the aqueous layer was extracted thrice with chloroform. The chloroform solution was washed with water, after which free iodine was removed by adding an aqueous solution of 1.2 g. of sodium sulfite and treating the mixture with sulfur dioxide. The chloroform solution was washed again with water and the solvent was removed at 35–40° to deposit a brown solid, which was extracted repeatedly with boiling benzene. The hot benzene solution was treated with Norit, filtered and concentrated to give 24 mg. of slightly impure 3-methoxy-4,6-diiodobenzoic acid. A solution of these crystals in chloroform was extracted thrice with 10% aqueous sodium carbonate solution. Acidification of the aqueous solution with hydrochloric acid followed by thorough extraction with chloroform, drying the chloroform solution over anhydrous sodium sulfate, treating it with Norit and removal of the solvent gave crystals, which were recrystallized from benzene as colorless prisms, m.p. 235–236°, not depressed when mixed with crystals of 3-methoxy-4,6-diiodobenzoic acid from per-

manganate oxidation of β -(3-methoxy-4,6-diiodophenyl)-D,L-alanine.

3-Methoxy-4,6-diacetamidobenzoic Anhydride.—The solid product resulting from the catalytic reduction of 0.15 g. of 3-methoxy-4,6-dinitrobenzoic acid was dissolved in 20 ml. of hot acetic anhydride, the mixture being heated on the steam-bath for a short time. The solution was left at room temperature for 16 hours; the acetic anhydride was evaporated *in vacuo* with the bath at 65°; some benzene was added and evaporated *in vacuo*. A solution of the residual crystals in benzene was diluted with *n*-hexane to turbidity and some amorphous solid was removed. Concentration of the solution and dilution with *n*-hexane then yielded crystals, which were recrystallized from ethyl acetate as light-yellow prismatic needles, m.p. 215–216° (uncor.). The analytical data, obtained on a sample dried in an evacuated desiccator over calcium chloride, indicated the compound to be somewhat impure 3-methoxy-4,6-diacetamidobenzoic anhydride.

Anal. Calcd. for $C_{24}H_{26}N_2O_9$: C, 56.02; H, 5.09; N, 10.89. Found: C, 56.91; H, 4.92; N, 10.81.

3-Methoxy-2,6-diiodobenzoic Acid.—To a thoroughly stirred suspension of 0.1 g. of 3-methoxy-2,6-diiodobenzaldehyde⁴ in 6 ml. of water at 25° were added 4 ml. of 5.05% potassium hydroxide solution and 10 ml. of 0.37% potassium permanganate solution in several portions. After 3 hours at 50° the mixture was saturated with sulfur dioxide which precipitated 70 mg. of almost pure 3-methoxy-2,6-diiodobenzoic acid. Recrystallized from benzene as colorless plates and then from benzene–petroleum ether, it melted at 137–138°.

Anal. Calcd. for $C_8H_8I_2O_3$: C, 23.79; H, 1.50; I, 62.84. Found: C, 24.00; H, 1.55; I, 62.90.

N-Acetyl- β -[3-(*p*-nitrobenzyloxy)-4,6-diiodophenyl]-D,L-alanine Ethyl Ester.—To a solution of 0.5 g. of N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine in 2.3 ml. of *N* sodium hydroxide at steam-bath temperature was added portionwise during 5 minutes, with stirring, 0.23 g. of *p*-nitrobenzyl bromide. After 15 minutes 5 ml. of absolute ethanol was added and the solution was refluxed for 15 minutes. At room temperature the sodium salt of N-acetyl- β -[3-(*p*-nitrobenzyloxy)-4,6-diiodophenyl]-D,L-alanine crystallized as needles and concentration of the solution increased the yield to 0.5 g. After recrystallization from water the air-dried sodium salt melted at 254–255°.

Anal. Calcd. for $C_{18}H_{15}I_2N_2O_6Na + 3.5 H_2O$: H_2O , 9.07; C, 31.10; H, 3.19; I, 36.51. Found: H_2O (dried at 100° *in vacuo*), 9.23; C, 31.51; H, 3.05; I, 35.13.

The acid was precipitated by addition of 0.5 ml. of *N* hydrochloric acid to an aqueous solution (27 ml.) of 0.3 g. of the sodium salt. The air-dried acid melted first at 124–126°, then solidified and melted again at 229–230°. It loses weight at 100° *in vacuo* and becomes quite hygroscopic.

Anal. Calcd. for $C_{18}H_{15}I_2N_2O_6$: C, 35.43; H, 2.64; I, 41.6; N, 4.59. Found (dried at 100° *in vacuo*): C, 35.37; H, 3.06; I, 39.9; N, 4.59.

The crystalline ethyl ester proved to be more suitable for characterization of the compound than the sodium salt or the acid. A mixture of 185 mg. of the acid, 20 mg. of *p*-toluenesulfonic acid monohydrate, 75 ml. of chloroform and 25 ml. of absolute ethanol was refluxed for 9 hours in a flask fitted with a water-separator, 60 ml. of condensate being collected. After filtration the solvent was removed and the ester was purified by recrystallization from ethanol as almost colorless prismatic needles, yield 140 mg., m.p. 215°. The analytical sample was dried in an evacuated desiccator over calcium chloride.

Anal. Calcd. for $C_{20}H_{20}I_2N_2O_6$: C, 37.64; H, 3.16; I, 39.77. Found: C, 37.75; H, 3.25; I, 39.79.

N-Acetyl- β -(3-*p*-toluenesulfonyloxy)-4,6-diiodophenyl)-D,L-alanine Ethyl Ester.—A mixture of 0.8 g. of N-acetyl- β -(3-hydroxy-4,6-diiodophenyl)-D,L-alanine, 0.06 g. of *p*-toluenesulfonic acid monohydrate, 30 ml. of absolute ethanol and 200 ml. of chloroform was refluxed for 12 hours, 160 ml. of condensate being collected. The solvent was evaporated *in vacuo* and the solution of the sirupy ester in 5 ml. of acetone was mixed with a solution of 0.34 g. of *p*-toluenesulfonyl chloride in 5 ml. of acetone. After the addition of 1.68 ml. of *N* sodium hydroxide, the solution was refluxed for one hour and then concentrated to ca. 3 ml. The sirup which separated was taken up in ethyl acetate and combined with the ethyl acetate extract of the aqueous layer.

The solution was extracted with 10% sodium carbonate solution, washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent left a sirup which proved to be difficult to crystallize, although some fine colorless needles separated very slowly from a solution of the sirup in benzene-petroleum ether. After a second recrystallization from the same solvents and drying in an evacuated desiccator over calcium chloride, the crystals melted at 91–92°. Found: C, 37.35; H, 3.20. The low melting point of these crystals may be due to a small amount of combined benzene or other impurity, but possibly the crystals are an unstable second form of the compound. After the crystals had been kept for 14 months in a stoppered vial at room temperature, the melting point had increased to 123–125°. The principal part of the sirupy product was purified further by extracting its chloroform solution with 2 *N* sodium hydroxide solution, then washing it with water and drying it over anhydrous sodium sulfate. The sirup left by evaporation of the chloroform crystallized readily from benzene-petroleum ether; yield 0.35 g., m.p. 133–134°. The melting point was unchanged after the crystals had been kept for 11 months at room temperature.

Anal. Calcd. for $C_{20}H_{21}N_2NO_6S$: C, 36.55; H, 3.22; I, 38.62. Found (dried at 79° *in vacuo*): C, 36.84; H, 3.14; I, 38.43.

A solution of 0.435 g. of sirupy *N*-acetyl- β -(3-*p*-toluenesulfonyloxy-4,6-diiodophenyl)-*D,L*-alanine ethyl ester and 0.66 g. of *p*-methoxyphenol in 0.3 ml. of dry pyridine, after being kept for one hour at 145–150° under reflux and subsequent treatment as described for *N*-acetyl- β -[3-(*p*-methoxyphenoxy)-dinitrophenyl]-*D,L*-alanine ethyl ester, yielded a sirup from which 0.2 g. of crystalline *N*-acetyl- β -(3-*p*-toluenesulfonyloxy-4,6-diiodophenyl)-*D,L*-alanine ethyl ester was isolated with the use of benzene-petroleum ether; m.p. 91–92°, not depressed when mixed with the authentic *p*-toluenesulfonyloxy ester melting at 91–92°.

Three β -(3-Hydroxydinitrophenyl)-*D,L*-alanines.—To a suspension of 4 g. of pure, powdered *m*-tyrosine in 8 ml. of water at 0–5° was added gradually, with stirring, 40 ml. of concentrated sulfuric acid. The stirred solution was kept at –5° during the dropwise addition of 8 ml. of 40% nitric acid in the course of 5 minutes. After being stirred at –5° for 35 minutes, the solution was poured into 600 ml. of ice-water. To this solution was added a hot solution of barium hydroxide until the mixture was slightly basic to congo red, about 232 g. of barium hydroxide octahydrate being required. The mixture was heated awhile on the steam-bath, the barium sulfate was removed and washed thoroughly with hot water. The pH of the combined solution and washings was adjusted, if necessary, to 6.0–6.5 (glass electrode) by the addition of barium hydroxide or sulfuric acid. The yellow solution, after being concentrated *in vacuo* to 115 ml., deposited at room temperature 1.6 g. of crystalline barium salts and upon further concentration yielded five additional crops to make the yield of the barium salts of the mixed isomers of β -(3-hydroxydinitrophenyl)-*D,L*-alanine 5.6 g. The combined first two crops were fractionally recrystallized from water and the more soluble crude salts were recrystallized similarly. The isomers of β -(3-hydroxydinitrophenyl)-*D,L*-alanine were prepared in somewhat mixed condition from the several fractions of recrystallized barium salts, most of isomer B being obtained from the least-soluble fractions of barium salts, and isomer A from the more soluble fractions; isomer C usually occurred mixed with isomer B but was obtained also in smaller amounts from some of the more soluble fractions. The following example illustrates the procedure for the preparation of an isomer of β -(3-hydroxydinitrophenyl)-*D,L*-alanine from its barium salt. A solution of 0.924 g. of air-dried barium salt of isomer A in 185 ml. of hot water was treated with 0.43 ml. of 5.757 *N* sulfuric acid solution, the barium sulfate was removed, washed thoroughly with hot water and the solution was concentrated *in vacuo* to give 0.7 g. of almost pure crystals of isomer A in two crops. The yield of the three isomers of β -(3-hydroxydinitrophenyl)-*D,L*-alanine was 3 g. or 50%. Further recrystallization of this somewhat mixed material from water gave the virtually pure isomers: 1.6 g. of A, 0.8 g. of B and 0.3 g. of C. The analytical samples were obtained by additional recrystallizations from water. Isomer A crystallized from water as slightly tan-colored needles melting at 234° (uncor., dec.). Isomer B usually crystallized as anhydrous, difficultly

soluble, yellow, rod-shaped prisms melting at 235–236° (uncor., dec.) but was observed to crystallize sometimes as prismatic needles of the monohydrate melting at 226–227° (uncor., dec.). Air-dried crystals of the monohydrate lost 6.1% in weight at 100° *in vacuo* (calcd. for $C_9H_9N_3O_7 \cdot H_2O$: H_2O , 6.23). Air-dried crystals of A and air-dried anhydrous crystals of B lost no weight at 100° *in vacuo*. Isomer C crystallized from water as light-yellow, elongated prisms which showed the explosive decomposition point of 197°; the air-dried crystals lost 15.6% in weight at 100° *in vacuo*.

Anal. Calcd. for $C_9H_9N_3O_7$: C, 39.86; H, 3.34; N, 15.50. Found (isomer A): C, 40.09; H, 3.40; N, 15.33. (Isomer B): C, 39.57; H, 3.49; N, 15.01. (Isomer C dried at 100° *in vacuo*): C, 39.84; H, 3.55; N, 15.37.

Three *N*-Acetyl- β -(3-hydroxydinitrophenyl)-*D,L*-alanines.—A solution of 0.3 g. of β -(3-hydroxydinitrophenyl)-*D,L*-alanine (isomer A) in 0.8 ml. of water and 0.84 ml. of 2 *N* sodium hydroxide was kept at 0–5° during the alternate addition of six 0.69-ml. portions of 2 *N* sodium hydroxide and six 0.11-ml. portions of acetic anhydride. After one hour at 25° the solution was treated with 1.83 ml. of 6 *N* sulfuric acid and then extracted thoroughly with ethyl acetate. The ethyl acetate solution, dried over anhydrous sodium sulfate and concentrated, yielded 0.23 g. of almost pure *N*-acetyl- β -(3-hydroxydinitrophenyl)-*D,L*-alanine (*N*-acetyl-A). After recrystallization from water as pale yellow prisms, it melted at 200–201°.

Anal. Calcd. for $C_{11}H_{11}N_3O_8$: C, 42.17; H, 3.54; N, 13.42. Found: C, 42.23; H, 3.51; N, 13.48.

The acetylation of 0.3 g. of isomer B was carried out as described for isomer A to yield 0.25 g. of slightly impure *N*-acetyl-B. Recrystallized from water as well-formed, yellow prisms, the pure compound melted at 210–211°. Found: C, 42.12; H, 3.56; N, 13.21.

Acetylation of 0.7 g. of a mixture of isomers C and B as described, extraction of the products into ethyl acetate, treatment of this solution with Norit and concentration to ca. 3 ml. afforded somewhat impure crystals of *N*-acetyl-B melting near 200°; dilution of the filtrate with petroleum ether gave a second crop of *N*-acetyl-B (yield 0.2 g.). The mother liquor yielded 0.22 g. of somewhat impure crystals of *N*-acetyl-C melting at 168–171° and a solid residue which, after being washed with petroleum ether, was recrystallized from water to yield an additional 0.12 g. of *N*-acetyl-C melting at 183–184°. The crystals of *N*-acetyl-B upon recrystallization from water gave prisms melting at 209° alone or mixed with crystals of the authentic compound. *N*-Acetyl-C was purified by recrystallization from water as small clusters of closely packed crystals; air-dried; m.p. 194–195° which varies with the rate of heating. Its melting point was depressed by mixture with crystals of either *N*-acetyl-A or *N*-acetyl-B. Found (dried at 100° *in vacuo*): C, 42.15; H, 3.71; N, 13.24.

Two *N*-Acetyl- β -(3-methoxydinitrophenyl)-*D,L*-alanine Methyl Esters.—To a dry ether solution of diazomethane, prepared¹³ from 1.7 g. of *N*-nitrosomethylurea and 17 ml. of ether, was added a cold solution of 150 mg. of *N*-acetyl- β -(3-hydroxydinitrophenyl)-*D,L*-alanine (*N*-acetyl-A) in 4 ml. of pure dioxane. The solution was kept overnight at 29–30° and the solvent was evaporated to give a sirup which was crystallized from benzene-petroleum ether; yield 134 mg. Purified from the same solvents, the pale yellow prismatic needles melted at 128–129°.

Anal. Calcd. for $C_{13}H_{15}N_3O_8$: C, 45.75; H, 4.43; N, 12.32. Found: C, 45.95; H, 4.49; N, 11.95.

A solution of 142 mg. of *N*-acetyl-B in 4.5 ml. of pure dioxane was mixed with a solution of diazomethane prepared from 1.5 g. of *N*-nitrosomethylurea and 15 ml. of ether. The solution was kept overnight at 28° and then decanted from 83 mg. of almost pure crystals of *N*-acetyl- β -(3-methoxydinitrophenyl)-*D,L*-alanine methyl ester. The filtered solution was evaporated to dryness at 25° and the residual sirup, upon evaporation of its dioxane solution and repeating the operation with ether, yielded 65 mg. of somewhat impure crystals. Recrystallized from methanol as nearly colorless prismatic needles and dried in an evacuated desiccator over calcium chloride, the pure compound melted at 172–173°. Found: C, 45.48; H, 4.55; N, 12.10.

(13) W. E. Bachmann and W. S. Struve in R. Adams, "Organic Reactions," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 50.

Two β -(3-Methoxydinitrophenyl)-D,L-alanines.—To a solution of 99 mg. of N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester (m.p. 128–129°) in 3 ml. of acetic acid was added 3.8 ml. of 5.76 N sulfuric acid. After being kept at 100° under reflux for 5 hours, the solution was diluted with 5 ml. of water and concentrated *in vacuo* to ca. 2 ml. After the addition of 20 ml. of water, the hot solution was treated with sufficient barium hydroxide solution to exactly remove sulfate ions as barium sulfate. The solution upon concentration *in vacuo* yielded crystals of β -(3-methoxydinitrophenyl)-D,L-alanine which, after being recrystallized from water as almost colorless needles and dried for 17 hours in the air at room temperature, melted at 234–235° (uncor., dec.). It lost 0.48% in weight at 100° *in vacuo*.

Anal. Calcd. for $C_{10}H_{11}N_3O_5$: C, 42.11; H, 3.89; N, 14.74; OCH_3 , 10.88. Found (dried at 100° *in vacuo*): C, 42.17; H, 3.88; N, 14.86; OCH_3 , 10.95.

A solution of 100 mg. of N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester (m.p. 172–173°) in 2.8 ml. of acetic acid and 3.2 ml. of 5.76 N sulfuric acid was treated as described above. The product (69 mg.) was recrystallized from water as slightly yellow prismatic needles and dried for 24 hours in the air at room temperature; m.p. 231–232° (uncor., dec.). These crystals lost 3.2% in weight at 100° *in vacuo*. Found (dried at 100° *in vacuo*): C, 42.10; H, 3.91; N, 14.54.

Permanganate Oxidation of the Two β -(3-Methoxydinitrophenyl)-D,L-alanines.—To a solution of 45 mg. of β -(3-methoxydinitrophenyl)-D,L-alanine (m.p. 234–235°) in 11.6 ml. of 5.05% aqueous potassium hydroxide solution at 25° was added, during 2 hours, 15 ml. of 0.7% potassium permanganate solution. After 2 hours at 50–55° and 16 hours at room temperature, the hydrated manganese dioxide was filtered off and the solution was treated with sufficient hydrochloric acid to make the pH 6.9. It was diluted with water to 100 ml., passed over Amberlite IR-120 to remove potassium ions and evaporated *in vacuo* to dryness. After addition of some benzene and evaporation *in vacuo*, the residue was extracted repeatedly with hot benzene. From this solution was isolated 12 mg. of product which was recrystallized from benzene or benzene-petroleum ether as fine slightly yellow needles, m.p. 202–203°, depressed when mixed with authentic crystals of 3-methoxy-2,6-dinitrobenzoic acid⁶ melting at 199°.

Oxidation of 69 mg. of β -(3-methoxydinitrophenyl)-D,L-alanine (m.p. 231–232°) with potassium permanganate as described gave 8 mg. of product which was recrystallized from benzene-petroleum ether as slightly yellow needles, m.p. 188–189°, depressed when mixed with authentic crystals of 3-methoxy-4,6-dinitrobenzoic acid^{6,9} melting at 188°.

3-Acetamidomethoxyaminohydrocarbostyryl (I or II).—A solution of 0.1 g. of N-acetyl- β -(3-methoxydinitrophenyl)-D,L-alanine methyl ester (m.p. 172–173°) in 6 ml. of ethyl acetate was shaken for 2.5 hours with hydrogen and a Raney nickel catalyst at 25° and atmospheric pressure. The filtered solution was concentrated *in vacuo* to dryness (bath 40°). A solution of the residual sirup in 2 ml. of methanol deposited 15 mg. of yellow needles. After recrystallization from methanol the crystals melted at 192–193°. These crystals probably are N-acetyl- β -(3-methoxy-4-aminonitrophenyl)-D,L-alanine methyl ester. Calcd. for $C_{13}H_{17}N_3O_6$: C, 50.15; H, 5.51. Found: C, 50.67; H, 5.67. The solvent was removed *in vacuo* from the mother liquor and the

residual sirup, upon being stirred with ether, yielded ca. 30 mg. of crystals melting near 225°. After recrystallization from ethyl acetate and drying in an evacuated desiccator over calcium chloride, the compound melted at 245–246° (uncor.) and showed analytical data in agreement with the hydrocarbostyryl derivative (I or II).

Anal. Calcd. for $C_{12}H_{15}N_3O_5$: C, 57.82; H, 6.07; N, 16.86. Found: C, 58.01; H, 6.04; N, 16.75.

Hydrogenation of 0.1 g. of the methyl ester (m.p. 172–173°) in 6 ml. of methanol, with the use of Raney nickel, at 25° and atmospheric pressure for 24 hours yielded ca. 40 mg. of the hydrocarbostyryl derivative which, after recrystallization from methanol, melted at 244–245°. Found: C, 57.51; H, 5.71.

N-Acetyl- β -(3-hydroxydinitrophenyl)-D,L-alanine Ethyl Ester.—A mixture of 0.76 g. of N-acetyl- β -(3-hydroxydinitrophenyl)-D,L-alanine (N-acetyl-A), 0.077 g. of *p*-toluenesulfonic acid monohydrate, 200 ml. of chloroform and 30 ml. of absolute ethanol was refluxed for 10 hours, 160 ml. of condensate being collected. The yellow solution, filtered and concentrated to ca. 5 ml., deposited pale yellow prismatic needles of the ethyl ester. The filtrate upon dilution with petroleum ether gave more crystals to make the yield 0.66 g. or 80%. After recrystallization from ethyl acetate and drying in an evacuated desiccator over calcium chloride, it melted at 145–146°.

Anal. Calcd. for $C_{13}H_{15}N_3O_8$: C, 45.75; H, 4.43; N, 12.32. Found (dried at 79° *in vacuo*): C, 45.85; H, 4.40; N, 12.51.

N-Acetyl- β -[3-(*p*-methoxyphenoxy)-dinitrophenyl]-D,L-alanine Ethyl Ester.—A solution of 0.258 g. (1.00 mol. equiv.) of N-acetyl- β -(3-hydroxydinitrophenyl)-D,L-alanine ethyl ester and 0.17 g. (1.18 mol. equiv.) of *p*-toluenesulfonyl chloride in 0.4 ml. of dry pyridine was kept at 100° under reflux for 30 minutes. After the addition of 0.4 g. (4.27 mol. equiv.) of *p*-methoxyphenol the solution was held at 145–155° for one hour. The red solution was diluted with chloroform and the solvents were evaporated *in vacuo* (bath 90°). A solution of the residue in 25 ml. of chloroform was extracted once with 2 N hydrochloric acid, twice with 2 N sodium hydroxide solution and once with water. After the solution had been dried over anhydrous sodium sulfate and the chloroform evaporated at 25°, the thick brown sirup was crystallized from a little ethyl acetate. The filtrate from the first crop of crystals upon dilution to 4 ml. with ethyl acetate and then to turbidity with petroleum ether deposited some amorphous solid, which was removed. The solvents were evaporated at 25° and the residual sirup was crystallized from a little absolute ethanol; yield 79 mg. or 23% melting at 127–129°. After recrystallization as yellow prismatic needles from ethanol-petroleum ether and drying in an evacuated desiccator over calcium chloride, the compounds melted at 135°.

Anal. Calcd. for $C_{20}H_{21}N_3O_9$: C, 53.69; H, 4.73; N, 9.39. Found: C, 53.63; H, 4.75; N, 9.00.

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BETHESDA 14, MARYLAND