Synthesis of a Metabolic Product of Histidine

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RECEIVED JUNE 28, 1954

A crystalline barium salt containing equimolar quantities of alkali-labile ammonia, formic acid and L-glutamic acid has been isolated from the urine of folic acid deficient rats and shown to be derived from histidine.[1-3]

Products of similar composition have been reported to accumulate in the enzymatic degradation of histidine by liver preparations[4-8] and by partially purified extracts of histidine-adapted *Pseudomonas fluorescens*.[3] The enzymatic products isolated in this Laboratory have been shown to be identical with the urinary compound, and appear to have the same properties as the compound recently reported by Borek and Waelsch.[7] We are now reporting a synthesis of this material.

Experimental

Twenty grams of L-glutamic acid and 9.7 g. of ethyl formimino ether (free base, freshly prepared[9,10] from the hydrochloride[11]) were shaken in the cold in 450 ml. of cold absolute methanol. After 16 hours the solution was filtered, and the precipitate discarded. The methanol solution was evaporated to dryness *in vacuo;* the residue was extracted repeatedly with 50-ml. portions of absolute ethanol and 1 to 2 g. of a micro-crystalline product was obtained by the addition of absolute ether. This free acid was compared with the natural compound liberated from the barium salt. Both materials exhibited the same infrared spectra and the same lability toward heat and alkali as previously described for the natural material.[2,7,8] Both compounds were hygroscopic and the melting point was ill-defined; both materials (in sealed evacuated tubes) melted at 70–100° with decomposition and a pink color developed at 120° (Borek and Waelsch[7] 78–85°). Chromatography on Dowex-50 and on paper[2,8] showed no differences between the synthetic and natural (liver) compounds. Both compounds were degraded at the same rate by *Pseudomonas* extracts, which specifically hydrolyze histidine and its metabolic products to L-glutamic acid, ammonia and formic acid.

The crystalline barium salt of the synthetic compound was prepared from the free compound as previously described.[2,8] The X-ray diffraction diagram was identical with that of the liver and urinary derivatives. *Anal.* (After drying at 55° *in vacuo* for 37 hours.) Calcd. for $(C_6H_9N_2O_4)_2Ba$: C, 29.80; H, 3.75; N, 11.58; Ba, 28.41. Found: C, 29.59; H, 3.95; N, 11.32; Ba, 28.32.

Since it seems unlikely that cyclization would occur under the conditions of the synthesis, it is probable that the compound is α-formamidinoglutaric acid (N-formiminoglutamic acid), as proposed by Borek and Waelsch[7] on the basis of elementary analysis and titration data showing two acidic and one alkaline dissociation groups. Synthesis of analogous N-formimino derivatives of glycine, alanine, and leucine has been reported by Micheel and Flitsch.[12] However, unequivocal proof of the structure of the glutamic acid derivative must still await the synthesis of the corresponding cyclic compounds.

The authors acknowledge their indebtedness to Mr. W. White for X-ray diffraction studies, Mrs. I. Siewers for infrared absorption spectra and Dr. W. C. Alford for microanalysis. The interest and many helpful suggestions given by Dr. DeWitt Stetten, Jr., and Dr. Floyd S. Daft are also gratefully acknowledged.

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Stobbe Condensation of 3,4,5-Trimethoxybenzaldehyde and Ethyl 3,4-Methylenedioxybenzylsuccinate

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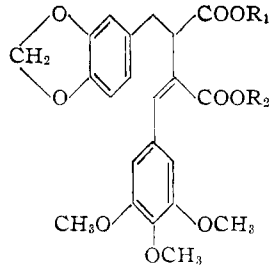
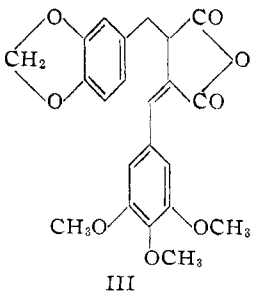
RECEIVED AUGUST 2, 1954

The reaction indicated in the title was carried out in the presence of sodium ethoxide according to a method exploited previously.[1] After hydrolysis of the ester-acid, compound I was obtained in 17% yield. The corresponding anhydride, III, was obtained by action of acetic anhydride on I.

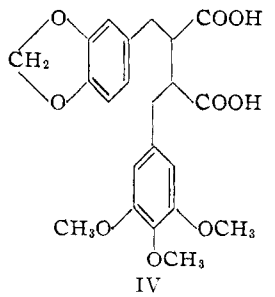
When I was treated with warm absolute ethanol containing 5% of sulfuric acid, the main product was an acid ester. This compound was assigned structure II, since the infrared spectrum indicated the presence of a conjugated acid group and an unconjugated ester group. It is apparent that treatment of diacids of this type with alcohol and mineral acid under mild conditions[1] leads to preferential esterification of the carboxyl group attached at



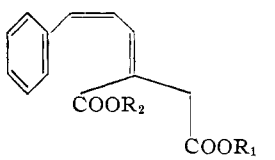
I, $R_1 = R_2 = H$
II, $R_1 = Et$; $R_2 = H$



III



IV



V, $R_1 = R_2 = H$
VI, $R_1 = Et$; $R_2 = H$

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the methylene position, while the cinnamic acid group is not affected appreciably. Further evidence for the course of this reaction was obtained by semi-esterification of V, the product of Stobbe condensation of cinnamaldehyde with ethyl succinate and subsequent hydrolysis.[2] The product, VI, is more highly conjugated than II, and the infrared spectrum showed more clearly in this case than in the case of II that a conjugated acid group and an unconjugated ester group were present. Relatively high reactivity of the carboxyl group indicated by R_1 can be anticipated since that group is similar electronically to the carboxylic acid group of phenylacetic acid.

Hydrogenation of I afforded a mixture of two forms of IV, possibly the *dl*-forms, only one of which was crystalline. Attempts to cyclize I and IV by various methods were not promising.

Experimental[3-5]

α-(3,4-Methylenedioxybenzyl)-α'-(3,4,5-trimethoxybenzylidene)-succinic Acid (I).—Trimethylgallaldehyde (32 g., 0.163 mole) and ethyl 3,4-methylenedioxybenzylsuccinate (58 g., 0.188 mole; prepared by Stobbe condensation of piperonal with ethyl succinate, hydrogenation and esterification with ethanol) were added to a solution of 10.1 g. (0.44 g.-atom) of sodium in 200 ml. of absolute ethanol. The mixture was stirred and refluxed for 3 hours, and 145 ml. of ethanol was distilled. Water (100 ml.) was added to the cooled suspension. The solution was distilled until 145 ml. of solvent had been collected, and was refluxed for four hours. The cooled solution was diluted with 200 ml. of water and acidified with a solution of 22 ml. of concd. hydrochloric acid in 30 ml. of water. The gummy material was removed by washing with ethyl acetate, and the solution was acidified strongly at ice temperature. The crude product was extracted with ethyl acetate–ether, and was isolated by evaporation of the solvents. The material (60 g.) crystallized partly after 2 weeks. Trituration with ether gave 12.0 g. (17%) of colorless crystals, m.p. 147–152° dec. Recrystallization from ethyl acetate raised the m.p. to 162–164° dec. The infrared spectrum (chloroform) had a broad band at 3.2–3.5 μ, and peaks at 5.80–5.86 and 6.10 μ (carboxyl groups and double bond, respectively).

Anal. Calcd. for $C_{22}H_{22}O_9$: C, 61.39; H, 5.15. Found: C, 61.28; H, 5.26.

The **anhydride**, III, was prepared by refluxing I with acetic anhydride for an hour; yellow crystals from ethanol, m.p. 117–119°. The infrared spectrum (chloroform) had peaks at 5.43 and 5.60 μ (anhydride) and a weak band at 5.98 μ (double bond).

Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 63.71; H, 4.91.

The acid was destroyed by warm polyphosphoric acid, and recovered unchanged from attempts to cyclize in the presence of boron trifluoride etherate.

Acid-ester, II.—A solution of 0.6 g. of I and 40 ml. of absolute ethanol containing 3.5 ml. of concd. sulfuric acid was refluxed for two hours. After evaporation of most of the ethanol, the solution was diluted with cold water, and the product was extracted with ether. The ether solution was washed with 5% sodium hydroxide solution. Acidification of the alkaline solution gave 0.4 g. of crystals, m.p. 142–145°, after filtration and washing with water. Recrystallization from cyclohexane–ethyl acetate afforded gleaming, pale yellow crystals, m.p. 148–150°. The infrared spectrum (chloroform) had peaks at 5.73 (ester), 5.90 (conjugated acid), and 6.10 μ (double bond).

Anal. Calcd. for $C_{24}H_{26}O_9$: C, 63.01; H, 5.51. Found: C, 63.31; H, 5.84.

α-(3,4-Methylenedioxybenzyl)-α'-(3,4,5-trimethoxybenzyl)-succinic Acid (IV).—A solution of 2.0 g. of acid I in 100 ml. of glacial acetic acid containing 1.2 g. of 8% palladium–charcoal catalyst was shaken under hydrogen (40 lb.) at 75° for 1.5 hours. Filtration of the catalyst and evaporation of the solvent gave material which crystallized partly. Trituration with ether–ethyl acetate afforded 1.0 g. of crystals, m.p. 178–185°. Recrystallization from ethyl acetate gave colorless crystals, m.p. 195–196.5°. The infrared spectrum (Nujol) had an intense peak at 5.85 μ.

Anal. Calcd. for $C_{22}H_{24}O_9$: C, 61.10; H, 5.59. Found: C, 61.37; H, 5.73.

Evaporation of the filtrate from trituration gave 1.0 g. of very viscous, pale yellow glass, the infrared spectrum of which had an intense peak at 5.85 μ.

Anhydrides were prepared from these two materials by refluxing with acetic anhydride, but they were not crystalline. Polyphosphoric acid at 80° led to destruction of the compounds and formation of dark, alkali-soluble tars.

Cinnamenylitaconic Acid (V).—Stobbe condensation[1] of 65 g. (0.493 mole) of cinnamaldehyde with 88 g. (0.506 mole) of ethyl succinate in the presence of a solution of 23.5 g. (1.04 g.-atom) of sodium in absolute ethanol, followed by saponification with hot, 10% sodium hydroxide solution afforded, after trituration with ethyl acetate, 19.7 g. (17%) of pale yellow crystals, m.p. 214–219° dec. Recrystallization from ethyl acetate raised the m.p. to 218–220° dec. (reported[2] m.p. 215–218°). The infrared spectrum (Nujol) had peaks at 5.86 and 5.98 μ (unconjugated and conjugated carboxyl groups, respectively). The **anhydride** was prepared by action of polyphosphoric acid at 90° and was recrystallized from ethyl acetate; yellow crystals, m.p. 178–179.5°. The infrared spectrum (Nujol) had peaks at 5.47 and 5.65 μ.

Anal. Calcd. for $C_{13}H_{10}O_3$: C, 72.88; H, 4.71. Found: C, 72.84; H, 5.11.

Acid-ester, VI.—A solution of 18.5 g. of V in 400 ml. of absolute ethanol containing 3.5 ml. of concd. sulfuric acid was refluxed for 45 minutes. The cooled solution was poured into 500 ml. of cold water. The crystals were collected, washed with water, and air-dried. Trituration with methanol gave 16.0 g. (77%) of crystals, m.p. 188–190°, raised to 189–190.5° by recrystallization from methanol. The infrared spectrum (chloroform) had peaks at 5.79 and 5.99 μ (unconjugated ester and conjugated acid, respectively). The compound was soluble in sodium bicarbonate solution.

Anal. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.20. Found: C, 69.36; H, 6.36.

The acid ester was also obtained from the anhydride of V by the same procedure.

Hydrogenation of V in acetic acid at 70° in the presence of palladium afforded **β-carboxy-ε-phenylcaproic acid,** m.p. 108–110° from cyclohexane–ethyl acetate (reported[6] m.p. 112°). The infrared spectrum of this acid (chloroform) had an intense peak at 5.85 μ. The corresponding **anhydride** was prepared by treating the saturated acid with acetic anhydride; colorless crystals from ether, m.p. 66.5–69°, the infrared spectrum of which (chloroform) had peaks at 5.36 and 5.60 μ.

Anal. Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.60; H, 6.42.

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