Synthesis of a Metabolic Product of Histidine

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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*.[8] 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.

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

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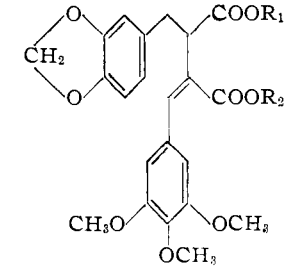
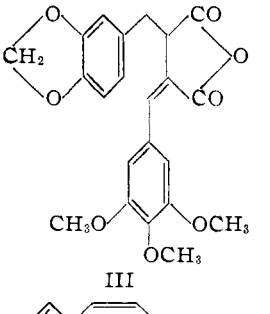
The reaction indicated in the title was carried out in the presence of sodium ethoxide according to a method exploited previously.[1] After hydrolysis of the ester-acid, compound I was obtained in 17% yield. The corresponding anhydride, III, was obtained by action of acetic anhydride on I.

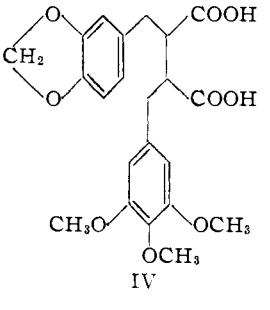
When I was treated with warm absolute ethanol containing 5% of sulfuric acid, the main product was an acid ester. This compound was assigned structure II, since the infrared spectrum indicated the presence of a conjugated acid group and an unconjugated ester group. It is apparent that treatment of diacids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



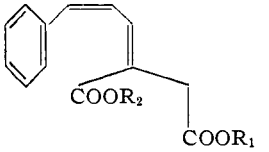
I, $R_1 = R_2 = H$
II, $R_1 = Et$; $R_2 = H$



III



IV



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

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