for eight days. The bomb was cooled and the brown liquid was concentrated in an air jet. Trituration with ethanol gave 4.08 g. of crude solid which was recrystallized from ethanol to yield 3.15 g. (61%) of light tan prisms, m.p. 97–99°. A portion was sublimed for analysis, m.p. 97–99°.

Anal. Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.18; H, 5.23; N, 5.48.

The ultraviolet absorption spectrum (ethanol) showed maxima at 234 mμ (log ε 3.68) and 288 mμ (log ε 3.59).

3-Amino-4-(3,4-methylenedioxyphenyl)-cyclohexene Hydrochloride (II).—By the procedure of Wildman and Norton,[1] 3.0 g. of 4-(3,4-methylenedioxyphenyl)-4-nitrocyclohexene was reduced with excess lithium aluminum hydride to give 2.21 g. (72%) of amine hydrochloride, m.p. 230–238°. The analytical sample was recrystallized twice from ethanol, m.p. 240–242°.

Anal. Calcd. for $C_{13}H_{15}NO_2 \cdot HCl$: C, 61.54; H, 6.36; N, 5.52. Found: C, 61.37; H, 6.43; N, 5.72.

The ultraviolet absorption spectrum (ethanol) showed maxima at 238 mμ (log ε 3.60) and 288 mμ (log ε 3.60).

1,4,5,6,13,14-Hexahydro-8,9-methylenedioxyphenanthridine (III).—A solution of 0.80 g. of II in 27 ml. of water was made basic with 10% sodium hydroxide solution and the free base was extracted three times with ether. The ether was removed and the residual oil was treated with 1.1 ml. of formalin. The mixture was heated on a steam-bath for 20 minutes with occasional swirling. The mixture was extracted with benzene and the oily Schiff base was obtained upon concentration of the benzene solution. A solution of 2 ml. of 19% hydrochloric acid was added to the Schiff base and the solution was allowed to stand overnight. The product crystallized during this time and was removed by filtration, 0.71 g., m.p. 253–265° dec. Concentration of the filtrate gave an additional 0.03 g., m.p. 271–282° dec., after one recrystallization from methanol–ethanol. This was combined with the first crop and recrystallized from methanol–ethanol, 0.49 g. (59%), m.p. 279–282° dec. A small portion was recrystallized from the same solvent for analysis, m.p. 280–282° dec.

Anal. Calcd. for $C_{14}H_{15}NO_2 \cdot HCl$: C, 63.27; H, 6.07; N, 5.27. Found: C, 63.09; H, 6.01; N, 5.38.

The ultraviolet absorption spectrum (water) showed maxima at 234 mμ (log ε 3.55) and 292 mμ (log ε 3.65).

1,4,5,6,13,14-Hexahydro-5-methyl-8,9-methylenedioxyphenanthridine Hydrochloride (IV).—By the procedure of Wildman and Norton,[1] 0.489 g. of III gave 0.317 g. (62%) of IV, m.p. 239–243° dec. A portion was recrystallized from methanol–ether for analysis, m.p. 240–243° dec.

Anal. Calcd. for $C_{15}H_{17}NO_2 \cdot HCl$: C, 64.39; H, 6.48; N, 5.01. Found: C, 64.14; H, 6.49; N, 4.75.

The ultraviolet absorption spectrum (ethanol) showed maxima at 238 mμ (log ε 3.53) and 292 mμ (log ε 3.66).

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α-L-Formamidinoglutaric Acid, an Intermediate in Histidine Metabolism[1]

BY ALEXANDER MILLER AND HEINRICH WAELSCH

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Isolation of a crystalline compound obtained upon incubation of histidine or urocanic acid with a phosphate extract of cat liver has recently been reported from this Laboratory.[2,3] Although not attacked by mammalian tissue preparations, the compound was rapidly degraded by cell-free extracts of histidine-adapted *Pseudomonas fluorescens* prepared according to Tabor and Hayaishi.[4] On the basis of the analytical data and properties of the crystalline compound and its mercury derivative, it was suggested that the compound was α-L-formamidinoglutaric acid. A compound with similar properties has recently been isolated from incubation mixtures of histidine and extracts of *Pseudomonas fluorescens*.[5]

Further characterization of the compound isolated from enzymatic digests was dependent upon comparison with synthetic α-L-formamidinoglutaric acid. Because of the necessity of distinguishing the formamidino compound from its tentatively postulated metabolic precursor, 4(5)-imidazolone-5(4)-propionic acid,[3,6] the synthesis used had to be one where cyclization of the formamidino compound to the imidazolone was unlikely. Compounds of these types are little known.

The synthesis of α-L-formamidinoglutaric acid with properties essentially identical with those of the isolated product has been accomplished. The γ-benzyl ester of L-glutamic acid suspended in formamide was treated with formamidine in the presence of silver carbonate. The resulting γ-benzyl ester of α-formamidinoglutaric acid was converted to free α-formamidinoglutaric acid by hydrogenolysis of the ester with palladium-black as a catalyst.

The analytical data and properties of synthetic α-L-formamidinoglutaric acid (A) and of the compound isolated from enzymatic digests (B) are given in Table I.

The synthetic compound, like that isolated from enzymatic digests,[2,3] was decomposed by extracts of *Pseudomonas fluorescens* more rapidly than urocanic acid.

TABLE I

PROPERTIES OF α-L-FORMAMIDINOGLUTARIC ACID

A synthetic; B from enzymatic digests[2,3]

Calcd. for	Found	
$C_6H_{10}N_2O_4 \cdot H_2O$	A	B
C 37.5	37.2[a]	37.5, 39.6
H 6.3	..	6.5, 6.7
N 14.6	14.6	14.0, 14.1
Alkali labile N, 7.3	7.3	7.0
pK_1'; pK_2'; pK_3'	2.7, 4.4, 11.3	2.4, 4.7, 11.1
$[\alpha]^{28}D$	−10.3[b]	−10.7
M.p., °C.	85–95	80–87
Infrared spectrum μ	3.1 (s),[c] 5.2 (w), 5.8(s), 6.2(s), 7.1 (m), 9.2 (w), 12.2 (w)	3.1 (s), 5.2 (w), 5.8 (s), 6.2 (s), 7.1 (m), 9.2(w), 12.2 (w)

[a] D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940). [b] 0.8% in 1 *N* HCl. [c] (s) strong, (w) weak, (m) medium.

The γ-benzyl ester of formamidinoglutaric acid

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was found to have $pK_1' = 2.5$.[7] Formamidinoacetic acid was found to have $pK_1' = 2.6$ and $pK_2' = 11.5$. It would appear that we are dealing with dipolar ions of the type $RCHNHCHNH_2^+$ with

$$\begin{array}{l} \\ | \\ COO^- \end{array}$$

a pK' value of about 2.5 for the carboxylate group and a pK' greater than 11 for the formamidinium group.

4(5)-Imidazolone-5(4)-propionic acid (or for that matter, any 2H,4-imidazolone derived from an α-amino acid) is as yet unknown, and the analytical data would correspond to a dihydrate of this compound. However, it would be difficult to assign the experimental pK' values to an imidazolone. Furthermore, the synthetic compound has an optical rotation equal to that of the natural product and the latter has been previously shown to be convertible to L-glutamic acid by acid hydrolysis.[2,3] One might expect, considering the behavior of azlactones and hydantoins, that an imidazolone possessing an α-hydrogen would racemize readily under the basic conditions used to make the synthetic compound.

On the basis of the data presented it may be concluded that the compound isolated from enzymatic digests of histidine or urocanic acid with cat liver extracts is α-L-formamidinoglutaric acid.

Experimental

Formiminoethyl ether hydrochloride was prepared by the procedure of Cavalieri, Tinker and Bendich[8] slightly modified in that liquid hydrogen cyanide (American Cyanamid), dried over calcium chloride was used.

Formamidine hydrochloride was prepared by the method of Pinner.[9]

The γ-benzyl ester of L-glutamic acid was prepared by the method of Hanby, Waley and Watson.[10]

The γ-benzyl ester of α-L-formamidinoglutaric acid was prepared by a procedure similar to that of Micheel and Flitsch.[11] 1.99 g. (8 mmoles) of the γ-benzyl ester of glutamic acid suspended in 10 ml. of formamide, 0.64 g. (8 mmoles) of formamidine hydrochloride and 1.115 g. (4.05 mmoles) of silver carbonate were added to a 100-ml. three-necked pear-shaped flask equipped with a rubber-sealed stirrer and gas inlet and outlet tubes. The flask was placed in a bath kept at 50°, the suspension vigorously stirred and dry nitrogen passed through to sweep out ammonia formed during the reaction. The ammonia was trapped in borate buffer and titrated in order to follow the rate of reaction. After 26 hours, ammonia liberation had practically ceased and the reaction mixture was washed into a 50-ml. centrifuge tube with about 30 ml. of absolute methanol and centrifuged. The precipitate was washed with 10 ml. of methanol. The supernatant liquid and washing were combined and the methanol removed by distillation *in vacuo*. About 75 ml. of dry acetone was added and crystallization allowed to proceed in the cold overnight. The product was collected by centrifugation, washed twice with acetone, once with ether and dried *in vacuo* over potassium hydroxide; yield 1.0 g. (47%), m.p. 167–168° with decomposition.

Anal. Calcd. for $C_{13}H_{16}O_4N_2$: C, 59.1; H, 6.1; N, 10.6; alkali-labile, N, 5.3. Found: C, 58.8; H, 6.3; N, 10.8; alkali-labile, N, 5.4.

α-L-Formamidinoglutaric acid was prepared from the γ-benzyl ester by catalytic hydrogenation. One hundred mg. (0.38 mmole) of the ester was suspended in 4 ml. of methanol, 20 mg. of palladium oxide added, and hydrogen bubbled through. After one hour, the white solid appeared to dissolve. Hydrogenation was continued for an additional three hours, with periodic addition of methanol to maintain the volume at 4 ml. After removal of the catalyst by filtration, the product was precipitated by addition of ether. The solid was redissolved in 2 ml. of methanol and reprecipitated with ether; yield 62 mg. (94%). Formamidinoglutaric acid is extremely hygroscopic and has only been obtained as a monohydrate.

Formamidinoacetic acid was prepared according to Micheel and Flitsch.[11]

Titrations were carried out under nitrogen using an external glass electrode and Beckman model G *p*H meter. pK' values were calculated for each increment and averaged. For *p*H values greater than 10 and less than 4, blank titrations were run and hydrogen ion concentrations calculated as described by Edsall.[12]

Infrared spectra were obtained using potassium bromide discs with a potassium bromide disc as a blank with a Beckman infrared spectrophotometer, Model IR2T.[13]

The extract of *Pseudomonas fluorescens* used was made according to Tabor and Hayaishi.[4] Activity was determined by measuring ammonia liberated under conditions previously described.[3]

(7) The simultaneous saponification of the ester interfered with the titration of the formamidinium group.

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(13) We wish to thank Drs. S. Graff and L. May for performing these measurements.

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The Synthesis of 1,3,5-Benzenetriacetic Acid by a Triple-Willgerodt Reaction

BY MELVIN S. NEWMAN AND HARMAN S. LOWRIE

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As possible intermediates in an attempted synthesis of the adamantane skeleton,[1] 1,3,5-benzenetriacetic acid and its triethyl ester were prepared from 1,3,5-triacetylbenzene by means of the Kindler modification of the Willgerodt reaction.[2] To our knowledge, this is the first example of the preparation of a tricarboxylic acid from a triketone by this reaction.

Experimental

1,3,5-Benzenetriacetic Acid (I).—In the best of several runs, 26.4 g. (0.13 mole) of 1,3,5-triacetylbenzene,[3] 78.3 g. (0.9 mole) of morpholine and 28.8 g. (0.9 mole) of sulfur were refluxed together for 14 hours. The solution was then poured into water and the solid collected and hydrolyzed by refluxing for 7 hours with 100 ml. each of water, concentrated sulfuric acid, and glacial acetic acid. The resulting solution was made basic with sodium hydroxide then filtered, and extracted with ethyl ether. After acidification with sulfuric acid to congo red paper, the solution was extracted continuously for four days with ether. Removal of the ether left 24.6 g. (75%) of I as a yellow powder, m.p. 197–204°. Three recrystallizations from glacial acetic acid yielded I as fine, white needles, m.p. 215–216° with little loss.

Anal.[4] Calcd. for $C_{12}H_{12}O_6$: C, 57.14; H, 4.80; neut.

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(4) Analyses by Galbraith Microanalytical Laboratories, Knoxville, Tennessee.