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.

(8) L. F. Cavalieri, J. F. Tinker and A. Bendich, THIS JOURNAL, **71**, 533 (1949).

(9) A. Pinner, *Ber.*, **16**, 357 (1883).

(10) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(11) F. Micheel and W. Flitsch, *Ann.*, **577**, 234 (1952).

(12) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publ. Corp., New York, N. Y., 1943, p. 454.

(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

RECEIVED AUGUST 17, 1954

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.

(1) M. S. Newman and H. S. Lowrie, THIS JOURNAL, **76**, 4598 (1954).

(2) M. Carmack and M. A. Spielman in R. Adams, "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1946, pp. 93, 97–98.

(3) D. T. Mowry and E. L. Ringwald, THIS JOURNAL, **72**, 2037 (1950). We thank Dr. Mowry of the Monsanto Chemical Co. for a generous sample of triacetylbenzene, m.p. 157°.

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

equiv., 84.1. Found: C, 57.45; H, 4.80; neut. equiv., 83.5, 83.9.

Triethyl 1,3,5-Benzenetriacetate (II).—In the best of several runs, 57.1 g. (0.227 mole) of I, m.p. 197–204°, was refluxed with 500 ml. of ethanol, 250 ml. of benzene and 5 ml. of sulfuric acid. Working up in the usual way yielded 68.7 g. (90%) of II as a light yellow oil, b.p. 165–193° at 0.05 mm. Redistillation of a portion for analysis gave II as a water-white oil, b.p. 166–167° at 0.1 mm.

Anal.[4] Calcd. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.22; H, 7.20.

A solution of 2.0 g. of II in 10 ml. of acetic acid and 20 ml. of concd. hydrochloric acid was slowly distilled to a volume of 5 ml. After addition of 10 ml. of acetic acid and cooling an almost quantitative yield of I was obtained. The melting point and neutral equivalent were identical to that above mentioned.

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Phosphorylation of Adenylic Acid by the Phosphate Anhydride of Leucine and Chromatographic Analysis of the Resulting Products

By M. Paecht and A. Katchalsky

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The phosphate anhydrides of amino acids prepared recently in this Laboratory[1] are labile, reactive compounds. It was of interest to determine whether they would phosphorylate lower-energy phosphates, *e.g.*, adenylic acid, to adenosine diphosphate or triphosphate.

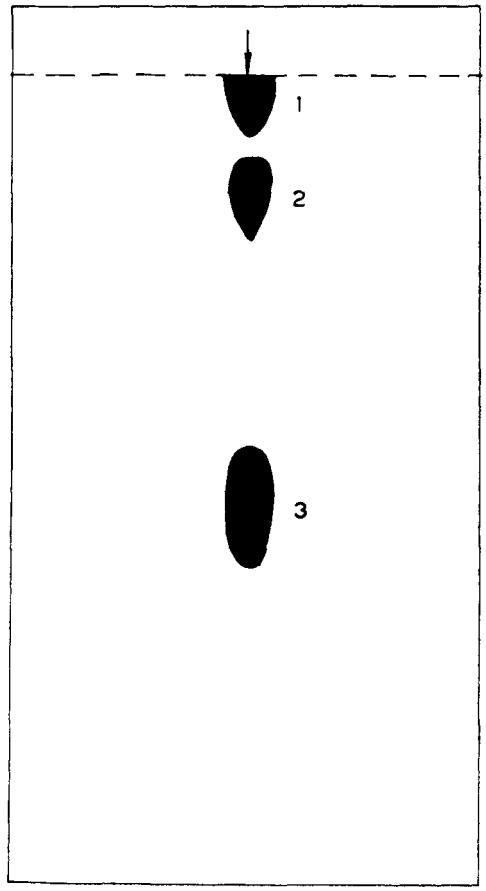

Fig. 1.—Ascending chromatogram in acid solvent, developed with phosphorus reagents; reaction mixture, leucine phosphoanhydride + adenylic acid: Spot 1, ATP + ADP + peptides; spot 2, AMP + inorganic pyrophosphate: spot 3, inorganic orthophosphate.

(1) A. Katchalsky and M. Paecht, This Journal, **76**, 6042 (1954).

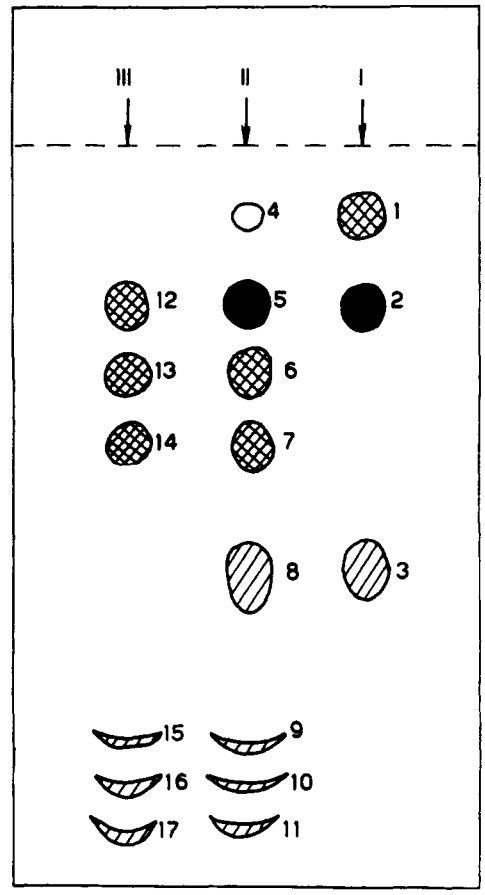

Fig. 2.—Descending chromatogram in alkaline solvent, after the orthophosphate has been cut off, developed with phosphorus reagents. Column I: control mixture, ATP + ADP + AMP + inorg. pyrophosphate + inorg. orthophosphate. Spot 1, ATP; spot 2, ADP + inorg. pyrophosphate; spot 3, AMP. Column II: reaction mixture, phosphate anhydride of leucine + adenylic acid in water solution. Spot 4, ATP; spot 5, ADP + inorg. pyrophosphate; spots 6, 7, 9, 10, 11, peptides; spot 8, AMP. Column III: control solution, phosphate anhydride of leucine in water solution. Spot 12, inorg. pyrophosphate; spots 13, 14, 15, 16, 17, peptides.

The phosphorylation reaction in aqueous solutions was analyzed chromatographically. Since, during this reaction, numerous other reactions take place which lead to the formation of polypeptides and inorganic phosphates, the reaction mixture is a rather complicated system. It can however be shown that adenosine diphosphate is obtained, and that even traces of adenosine triphosphate are detectable. This indicates that the phosphoanhydrides of amino acids should be classified as reactive phosphates.

Experimental

Phosphorylation.—In 1 ml. of an aqueous solution of $m/50$ adenylic acid, 1 equivalent of leucine phosphate anhydride was dissolved and allowed to stand at room temperature for about an hour. Then the mixture was analyzed chro-