[CONTRIBUTION FROM THE WARNER INSTITUTE FOR THERAPEUTIC RESEARCH]

Anhydrophenacetylglutamic Acid

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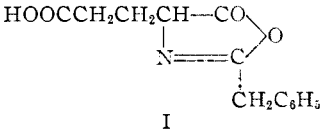
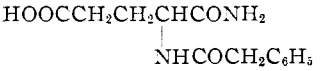
The acetic anhydride dehydration product of N-phenylacetylglutamic acid has been shown to be an acid anhydride and not an oxazolone. Its ammonolysis product has been shown to be inhomogeneous and the question of homogeneity is raised in connection with several other of its transformation products.

Baker and Jones recently reported[1] that N-phenylacetyl-L-glutamic acid, prepared by the action of phenylacetyl chloride on L-glutamic acid in alkaline solution, was converted by the action of acetic anhydride into a crystalline optically inactive anhydro derivative which was readily hydrolyzed by cold aqueous sodium bicarbonate to N-phenylacetyl-DL-glutamic acid. Since racemization occurred they believed the anhydro derivative to be 2-benzyl-DL-4,2′-carboxyethyloxazol-5-one (I) and the amide obtained from it by treatment with ammonia in chloroform to be the α-amide of N-phenylacetyl-DL-glutamic acid (II).

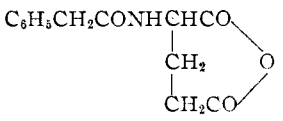
In view of the work of Le Quesne and Young[2] and ourselves[3] on anhydroderivatives of N-acylglutamic acids it seemed to us that the anhydro derivative of Baker and Jones probably was α-phenylacetylaminoglutaric anhydride (III), and that its ammonolysis product was a mixture of the α-amide (II) and the γ-amide (IV). The latter conclusion was indicated by the chemical and physical properties of the ammonolysis product.



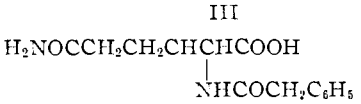
I



II



III



IV

We repeated the preparation of anhydrophenacetylglutamic acid and ammonolyzed it as described[1]; the crude reaction product (A), before any recrystallization, melted at 131–136° and on treatment with acetic anhydride and pyridine, which is diagnostic for an α-amino or α-acylamido acid with a free carboxyl group,[3] it evolved about a fifth of a molar equivalent of carbon dioxide. After several recrystallizations the product had the m.p. 144–146° reported by Baker and Jones and this material (B) on treatment with acetic anhydride and pyridine evolved about a tenth of a molar equivalent of carbon dioxide. Completely pure material (C), melting at 148–149°, evolved no carbon dioxide on such treatment.

Confirmation of these approximate purities of the three samples was obtained from their solubility analysis,[4-6] the results of which are portrayed as a composite in Fig. 1.

Since there is no evidence to indicate that an oxazolone undergoes ammonolysis other than by acyl–oxygen fission[7a] the above physical and chemical behavior of the ammonolysis product of anhydrophenylacetylglutamic acid constitute presumptive evidence for III as the structure of the anhydro derivative. This conclusion was confirmed by the infrared spectrum[8] of the anhydro derivative, presented in Fig. 2.

The 6.06 μ (1649 cm.$^{-1}$) band is considered to be the amide I band, the 3.03 μ (3297 cm.$^{-1}$) band to be due to N–H stretching vibration, and the 6.57 μ (1522 cm.$^{-1}$) band to be the amide II band.[9] The two acid anhydride bands are those at 5.50 μ (1817 cm.$^{-1}$) and 5.64 μ (1774 cm.$^{-1}$),[9] while a strong carboxyl carbonyl vibration band at 5.77–5.93 μ[9-11] is missing, showing that our compound cannot contain a carboxyl group, except perhaps as a trace impurity causing the very weak band at 5.88 μ (1700 cm.$^{-1}$). The bands which could possibly be assigned to the C—O bond and C—N bond of a saturated oxazolone[7b,9,12] are excluded from such assignment by other above considerations.

We have shown that the amide of Baker and Jones is inhomogeneous and that anhydrophenacetylglutamic acid is the anhydride III instead of the oxazolone I. The implication of our results is that their hydrazide, prepared both by hydrazinolysis of III and by methanolysis followed by treatment of the methyl ester with hydrazine, as well as the derived azide may be mixtures of the α-

(1) W. Baker and P. G. Jones, *J. Chem. Soc.*, 1143 (1951).

(2) W. J. Le Quesne and G. T. Young, *ibid.*, 1954, 1959 (1950).

(3) J. A. King and F. H. McMillan, THIS JOURNAL, **74**, 2859 (1952).

(4) J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, **13**, 781 (1930).

(5) T. J. Webb, *Anal. Chem.*, **20**, 100 (1948).

(6) R. M. Herriott, *Federation Proc.*, **7**, 479 (1948).

(7) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, New Jersey, 1949: (a) J. W. Cornforth, chapter on "Oxazoles and Oxazolones"; (b) H. W. Thompson, section of chapter on "Infrared Spectroscopic Studies of Penicillin."

(8) This determination was carried out on a Nujol mull of the material, under the direction of Dr. Robert L. Bohon, by the Anderson Physical Laboratory, Champaign, Illinois, using a Perkin-Elmer Model 12-B Infrared Spectrometer.

(9) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

(10) M. St. C. Flett, *J. Chem. Soc.*, 962 (1951).

(11) J. F. Grove and H. A. Willis, *ibid.*, 877 (1951).

(12) E. D. Bergmann, E. Zimkin and S. Pinchas, *Rec. trav. chim.*, **71**, 168 (1952); see also H. E. Carter and J. W. Hinman, *J. Biol. Chem.*, **178**, 403 (1949).

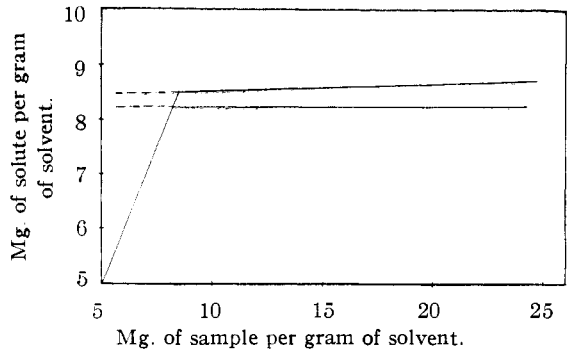


Fig. 1.—Solubility analysis of samples B (sloped line) and C (horizontal line).

and γ-isomers and that the reaction products of the azide with penicillamine and of III with penicillamine methyl ester may be mixtures of α- and γ-glutamyl derivatives instead of the substance constituting the title of their paper.

Experimental

N-Phenylacetyl-L-glutamic acid was prepared and dehydrated exactly as described.[1] The anhydro derivative was ammonolyzed exactly as described and the crude product melted at 131–136° (A). Several recrystallization of this material from acetone–petroleum ether (b.p. 60–80°) gave a product melting at 144–146° (B), as described. Pure material, m.p. 148–149° (C) was obtained as the insoluble residue in the equilibrated ampoules of (B) which were used in the solubility analysis.

Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C, 59.09; H, 6.06; N, 10.60. Found: Sample A: C, 60.33; H, 6.83; N, 10.12. Sample B: C, 58.75; H, 6.33; N, 10.88. Sample C: C, 59.09; H, 6.36; N, 10.54.

A mixture of 1.06 g. (4 millimoles) of sample, 4 cc. of pyridine and 4 cc. of acetic anhydride was refluxed until gas evolution ceased, the envolved gas being collected over water saturated with carbon dioxide. The volumes of carbon dioxide evolved, corrected for the blank on the apparatus, probably accurate within ±5 cc. were: sample A, 28 cc.; sample B, 9 cc.; sample C, none.

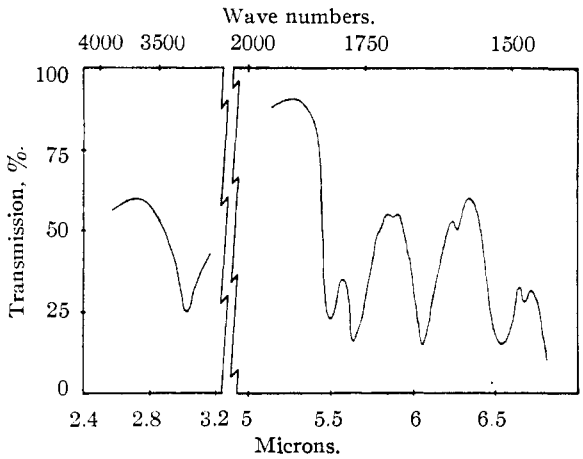


Fig. 2.—Infrared spectrum of anhydrophenacetylglutamic acid.

Solubility analyses were done in purified acetone, using about 8 g. of solvent in sealed glass ampoules which were constantly tumbled in a thermostatically controlled water-bath at 25.0 ± 0.1° for at least 44 hours. Approximately 2.5-g. aliquots of equilibrated solution were used for determination of the amount of dissolved sample. The purity of sample A was sufficiently low that the material gave erratic results of no analytical value in this determination. The values found and plotted in Fig. 1 are considered to be accurate within ±0.15 mg./g.

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NOTES

Synthesis of Dimethyl 6,7,8,9-Tetrahydro-5H-cycloheptabenzene-5-acetate-6-propionate[1,2]

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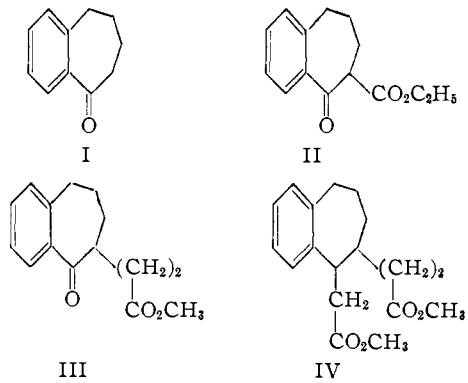
In the search for synthetic routes to compounds related to colchicine, we have carried out some model studies starting with 6,7,8,9-tetrahydro-5H-cycloheptabenzen-5-one (I). In the course of this work dimethyl 6,7,8,9-tetrahydro-5H-cycloheptabenzene-5-acetate-6-propionate (IV) has been synthesized. IV is of interest as a model compound in that an acyloin condensation of this diester followed by bromination and dehydrobromination according to known procedures[3] would afford a third ring having a tropolone structure and the resultant ring system would then be quite similar to that present in colchicine.



Carboethoxylation of I with diethyl carbonate in the presence of sodium hydride[4] gave ethyl 6,7,8,9-tetrahydro-5H-cycloheptabenzen-5-one-6-carboxylate (II) in 72% yield. The sodium salt of II was prepared by reaction with sodium hydride in anhydrous dioxane. Treatment of this salt with

(1) From the Ph.D. Thesis of Helen Frances Greef.

(2) Supported in part by State of Washington Initiative 171 funds for research in biology and medicine.

(3) D. J. Cram and J. D. Knight, THIS JOURNAL, **73**, 4136 (1951).

(4) F. S. Swamer and C. R. Hauser, *ibid.*, **72**, 1352 (1950).