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STRUCTURE OF THE PRODUCT OF DEHYDRATION OF GLUTAMIC ACID

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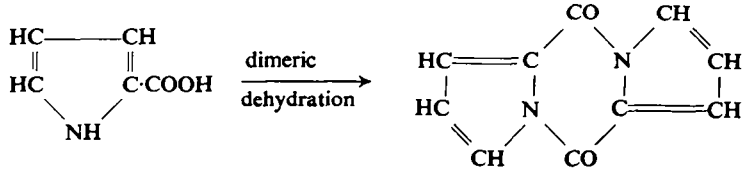
Abstract—Acylation of glutamic acid with glutaric anhydride instead of affording the expected N-glutaryl derivative yielded a high melting[1] substance (340° with dec) which was shown by X-ray diffraction, IR and UV to be identical with that obtained by King *et al.*, and Tetsuo *et al.*, from glutamic acid by dehydration. The tetraketo structure (I), suggested by King was shown by IR analysis to be untenable, while the lactone tricyclic structure (II) assigned by us is in keeping with the data obtained from the IR.

DURING work on the preparation of analogs of N-phthalylglutamimide "Thalidomide", the condensation of certain inner anhydrides with glutamic acid was achieved by heating an equimolecular mixture of the inner anhydride with glutamic acid in dry pyridine. Succinic anhydride and the α-substituted derivatives afforded the expected compounds[1] whereas with glutaric anhydride the expected N-glutaryl derivative was not produced and instead glutamic acid yielded a high melting crystalline substance of the empirical formula, $C_5H_5NO_2$ resulting from the abstraction of two molecules of water from one molecule of glutamic acid, or a high multiple of it. The high melting substance was also obtained by heating glutamic acid alone at 115° for 3½ hr. On the other hand when the amino acid was heated under reflux with pyridine—but in the absence of glutaric anhydride—it was recovered unchanged. These experiments show clearly that glutaric anhydride instead of acylating the amino acid brought about dehydration to the high melting substance.



Pyrrole-α-carboxylic acid — Pyrocoll

It has long been known that internal dehydration of glutamic acid affords 5-pyrrolidone-2-carboxylic acid[2–4] which is structurally analogous to pyrrole-α-carboxylic acid. This, coupled with the fact that pyrrole α-carboxylic acid on dimeric dehydration yields di-pyrrolo (a,d) pyrazine-5, 10-dione (pyrocoll) led King and McMillan in 1952[5] to assign to the high melting substance obtained by dehydration of glutamic acid, a structure analogous to that of pyrocoll. Furthermore, Beecham

[1] S. El-Zanfally, M. Khalifa and Y. M. Abou-Zeid, *J. Pharm. Sci.* **54**, 467 (1965).
[2] L. Wolff, *Liebigs Ann.* **260**, 164 (1890).
[3] Menozzi and Appiani, *Gazz. Chim. Ital.* **24**, 373 (1894).
[4] F. W. Foreman, *Biochem. J.* **8**, 481–93 (1914).
[5] J. A. King and F. H. McMillan, *J. Amer. Chem. Soc.* **74**, 2859 (1952).

in 1954[6] and Tetsuo in 1959[7] obtained the high melting substance by thermal dehydration of glutamic acid and they accepted King's tetraketo structure.

From the m.p. and method of preparation it was assumed that our high melting compound was identical with that of King's. Its preparation according to King's procedure gave a poor yield but it was obtained in a better yield adopting Tetsuo's method. After several recrystallizations from aqueous ethanol, King's and Tetsuo's compounds melted at 340° with dec as reported. To find out whether all high melting compounds were identical, spectroscopic methods were used. The IR spectra of the three compounds did not justify King's tetraketo structure.

Glutamic acid being both an α-amino, and γ-amino acid, dehydration can follow either of two possible routes: (a) the intermolecular dehydration—characteristic of α-amino acids—with the formation of 3,6-diketopiperazine-2,5-dipropionic acid (IVa) the enol form of which being a δ-hydroxy acid (IVb) readily loses water to form the δ-lactone tricyclic compound 2,7-dioxo-3,4,8,9-tetrahydro-2H, 7H-dipyrano-(3,2-b:2′,3′-e)-dihydropyrazine (II); and (b) the intramolecular dehydration—characteristic of γ-amino acids—with the formation of 5-pyrrolidone-2-carboxylic acid (III). Further dehydration of III and whether it takes place internally or by intermolecular loss of two molecules of water from two molecules of the acid seems highly unlikely because of the lack of a strongly basic nitrogen in pyrrolidone carboxylic acid. In our opinion, glutamic acid on dehydration can afford either the pyrrolidone carboxylic acid (III) or the lactone tricyclic compound (II) or a mixture of the two according to experimental conditions. The literature reports[2–4] on the formation of III as well as on the formation of a high melting substance either alone or together with III[5–7].

In the present investigation, the high melting substance was prepared according to three different procedures. IR analysis and X-ray diffraction revealed that King's and Tetsuo's compounds alone were identical, but the difference with our compound, possibly due to impurities,[8] was removed by heating our compound under reflux with a mixture of pyridine and acetic anhydride. As a result the IR spectra of all three compounds were identical and strongly favour the lactone structure (II) and not King's structure (I).

King's structure is built up of two saturated γ-lactam rings fused to a central diketopiperazine ring. The carbonyl stretching frequency of diketopiperazine was found to be at 1670–1700 cm^{-1} (broad) whereas the carbonyl frequency of the γ-lactam in the methyl ester of the lactam of benzoyl-homopenicilloic acid (V) is reported to be at 1709 cm^{-1}.[9] This latter compound contains a fused γ-lactam ring similar to that in King's structure.

On the other hand the observed frequency of about 1760 cm^{-1} agrees well with our structure (approximately 1740 cm^{-1} for δ-lactones.[10] The shift from 1740 to 1760 cm^{-1} may be attributed to the structure CO—O—C=N in II (approximately 1770 cm^{-1} for vinyl acetates[11]. Furthermore, the strong band at 1220 cm^{-1} may be assigned to the

[6] A. F. Beecham. *J. Amer. Chem. Soc.* **76,** 4612 (1954).

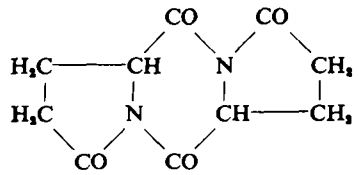
[7] O. Tetsuo and A. Takekazu, *Nippon Nege Kagakukaishi* **59,** 194–6 (1955); *Chem. Abstr.* 53, 1323 (1959).

[8] H. Gilman, *Organic Chemistry* Vol. III, p. 130. Wiley, New York, N.Y. (1953).

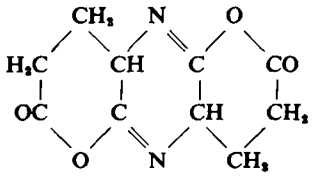
[9] H. H. Wasserman, F. M. Precopioa and L. Tien-Chuan, *J. Amer. Chem. Soc.* **74,** 4093 (1952).

[10] R. S. Rasmussen and R. R. Brattain, *J. Amer. Chem. Soc.* **71,** 1073 (1949).

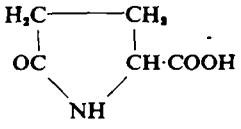
[11] E. J. Hartwell, R. E. Richards and H. W. Thompson, *J. Chem. Soc.* 1436 (1948).



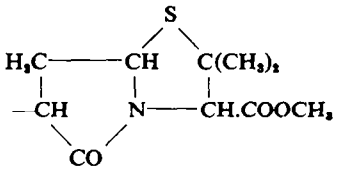
I



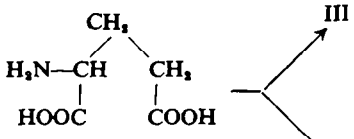
II



III

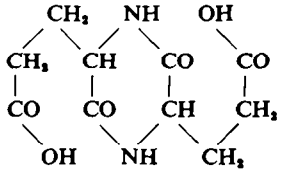


V

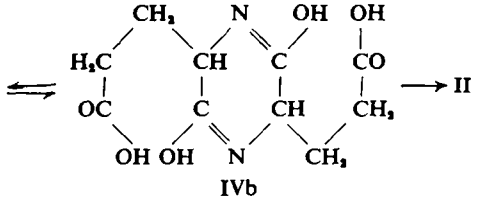


Glutamic acid

III



IVa



IVb

C—O—C ester band, fulfilling the linear relationship[12] established for carbonyl stretch and C—O—C ester bands. Finally, the stretching frequency of the C=N in non-conjugated ring systems is reported to occur within the range 1690–1640 cm^{-1}.[13] Thus the observed bands at 1675, 1685 and 1690 cm^{-1} in the spectra of the investigated compounds may be assigned to the C=N stretching vibration.

The UV absorption spectra of the three compounds (in 95% EtOH) showed maximum absorption at the wavelength 220 mμ with ε_{max} 10,000.

EXPERIMENTAL

M.p.'s were determined by a Koflar hot plate microscope.

X-ray diffraction analysis was carried out using an X-ray diffractometer with CuKα radiation. The records for the diffraction patterns were obtained after calibrating the diffractometer using LiF monochromator. Several runs were made at various tube voltages and currents.

[12] R. N. Jones and Sandorfy, *Chemical applications of Spectroscopy* p. 482 in *Technique of Organic Chemistry* IX (Edited by A. Weissberger).

[13] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules* p. 226. Wiley, New York, N.Y. (1956).

The IR spectra were determined on a 300 mg KBr disk containing 1 mg of sample. The spectra were run on a Perkin–Elmer model 21.

2,7-*Dioxo*-3,4,8,9-*tetrahydro*-2H,7H-*dipyrano*-(3,2-b : 2′,3′-e)-*dihydropyrazine*. This compound was prepared from glutamic acid by two procedures:

(a) A mixture of glutamic acid (7g) and glutaric anhydride (6·5 g) was suspended in dry pyridine (30 ml) and heated under reflux to a clear solution (2 hr). The pyridine was distilled off *in vacuo* and the residue boiled with Ac_2O (15 ml) for 5 min and the solution concentrated under red. press. until crystals appeared. After one recrystallization from aqueous EtOH, it melted at 340° (dec) with a yield of 20%.

Repeating the above experiment to the point of pyridine distillation, and then extracting the residue with dry benzene, the extract afforded an acid m.p. 96–97°, undepressed when admixed with an authentic sample of glutaric acid. The viscous mass left after the benzene extraction yielded the high melting compound after being boiled with Ac_2O for about 5 min.

(b) Glutamic acid (3 g) was gently heated to its m.p. and the heating continued with occasional shaking for 3½ hr in an oil bath at 110–120°. The clear homogeneous melt thus obtained was then boiled with Ac_2O for about 3 min. On cooling the product separated out as colourless platelets. A single recrystallization from aqueous EtOH yielded 0·8 g (40%) of the high melting substance.

The high melting substance was also prepared according to two reported methods:

(a) *King's method* consists of dehydrating glutamic acid with Ac_2O in presence of pyridine. The reported yield is 15% while in our hands the method gave only 5%.

(b) *Tetsuo's method* consists of the thermal dehydration under red. press. of the amino acid. The method afforded a 40% yield (lit., no reported yield).

The three samples m.p. 340° (dec) gave the following microanalytical data:

	C% Found	H% Found	N% Found[a]
Our product	53·96	4·71	12·59
King's product	54·20	4·64	12·47
Tetsuo's product	54·03	4·60	12·47

[a] Calc. for $C_{10}H_{10}N_2O_4$: C, 54·06; H, 4·50; N, 12·61%. Analyses performed by Alfred Bernhardt, Germany.

2,5-*Diketopiperazine*. This compound was prepared by condensing the ethylglycine hydrochloride in alkaline medium.[14] The glycine ester hydrochloride was obtained from methyleneaminoacetonitrile by hydrolysis with ethanolic HCl adopting Curtius procedure.[15] Using formalin, sodium cyanide and ammonium chloride as starting materials, the synthesis of the nitrile was carried out as described by Amundsen *et al.*[16] the yields of intermediates and of the final product closely approximated those found by the above authors.

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[14] E. Fischer, *Ber. Dtsch. Chem. Ges.* **39**, 2930 (1906).
[15] T. Curtius, *Ber. Dtsch. Chem. Ges.* **27**, 60 (1894).
[16] L. H. Amundsen and R. Velitzkin, *J. Amer. Chem. Soc.* **61**, 212 (1939).