[CONTRIBUTION FROM THE WARNER INSTITUTE FOR THERAPEUTIC RESEARCH]

The Reaction of Glutamic Acid with Acetic Anhydride and Pyridine[1]

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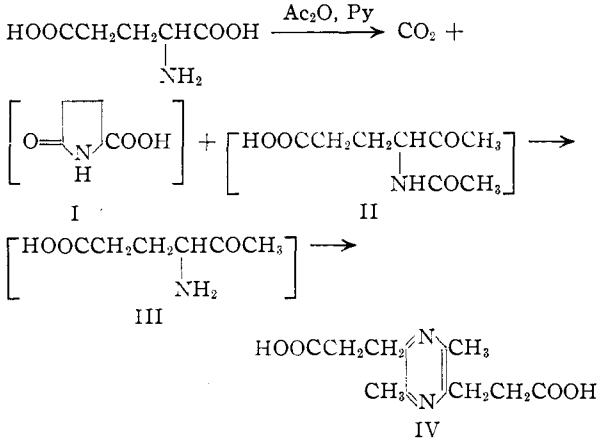
The decarboxylative acylation of glutamic acid by acetic anhydride in the presence of pyridine has been shown to be complicated by the ω-carboxyl group of the α-amino acid. The previously unreported products isolated from the reaction mixture have been identified as 3,5,8,10-tetraketoperhydrodipyrrolo[a,d]pyrazine, 1,5-diacetyl-2-pyrrolidone and 1-acetyl-5-pyrrolidone-2-carboxylic acid probably formed by rearrangement during distillation of α-acetamidoglutaric anhydride.

It was reported by Dakin and West[2] that when glutamic acid was heated with acetic anhydride and pyridine there was evolved 15 to 20% of one molecular equivalent of carbon dioxide, the remainder of the amino acid having been assumed to have been converted by the dehydrating action of the acetic anhydride to 5-pyrrolidone-2-carboxylic acid (I). The authors assumed the reaction product derived from that portion of the glutamic acid which evolved carbon dioxide to be α-acetamido-γ-carboxypropyl methyl ketone (II) (although it could not be isolated as such) because the residual solution from steam distillation of the reaction mixture gave reactions characteristic of such a substance and repeated butanol extraction of this solution yielded



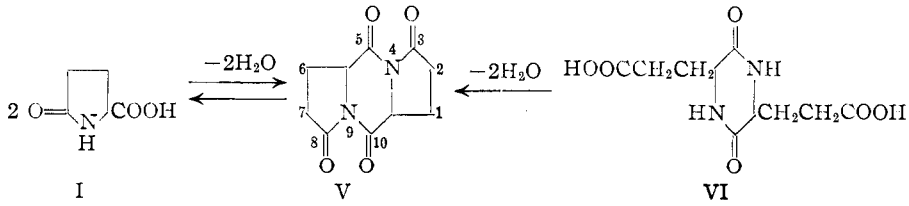
a sirup which after acid hydrolysis (to the salt of III) and basification (with accompanying air oxidation) furnished a small amount of 3,6-dimethylpyrazine-2,5-dipropionic acid (IV).

We wished to learn if the conversion of glutamic acid to 5-pyrrolidone-2-carboxylic acid was the only reason for the cessation of the decarboxylative acylation reaction after it had proceeded to the extent of 15 to 20% and we therefore carried out the reaction under forcing conditions and examined the products from it in somewhat greater detail than did Dakin and West. Our examination of these products forms the subject of the present paper.

It was easily verified that when glutamic acid was refluxed with excess acetic anhydride and pyridine for periods of time ranging from 30 to 60 minutes only 15 to 20% of a molecular equivalent of carbon dioxide was evolved. In separate experiments we verified that 5-pyrrolidone-2-carboxylic acid gave no carbon dioxide when it was refluxed with acetic anhydride and pyridine and we similarly verified that the analogous desoxy cyclic amino acid *l*-proline yielded no carbon dioxide when it was refluxed with acetic anhydride and pyridine; from the latter acid there was isolated, in the form of a high-boiling liquid, a practically quantitative yield of racemic acetylproline.

From the glutamic acid reaction mixture the first solid product isolated was a high-melting (m.p. *ca.* 340°, dec.) neutral crystalline substance of the empirical formula $C_5H_5NO_2$. Although the substance was non-acidic it behaved in a manner reminiscent of a lactone on titration with 0.1 *N* alkali, in that a short time interval was required for neutralization of each added increment of base; at the completion of the titration the neutralization equivalent of the material was found to be the same as the empirical formula weight. From a solution which had been neutralized there was isolated, after acidification, 5-pyrrolidone-2-carboxylic acid. It was possible to reform the high-melting substance from its hydrolysis product: when crystalline 5-pyrrolidone-2-carboxylic acid was heated with acetic anhydride in either the presence or absence of pyridine the high-melting material was formed in good yield and no carbon dioxide was evolved.

Because intramolecular dehydration of 5-pyrolidone-2-carboxylic acid seemed highly unlikely attention was turned to the possibilities for intermolecular loss of two molecules of water from two molecules of 5-pyrrolidone-2-carboxylic acid. Among the more obvious possibilities is symmetrical dehydration to yield the tricyclic compound 3,5,-



8,10-tetraketoperhydrodipyrrolo[a,d]pyrazine[3] (V), containing as its central portion a diketopiperazine nucleus. A brief review of the literature revealed that formation of such a ring system by dehydration with acetic anhydride is not without prece-

(1) Presented at the 4th Annual Meeting-in-Miniature of the North Jersey Section of the A. C. S., January 28, 1952.

(2) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 745 (1928).

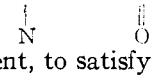
(3) Ring Index No. 1394. A. M. Patterson and L. T. Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940, p. 196.

dent.[4] Lack of a strongly basic nitrogen atom in 5-pyrrolidone-2-carboxylic acid cannot be considered an argument against its dimeric dehydration to a diketopiperazine derivative inasmuch as pyrrole-α-carboxylic acid forms pyrocoll and the nitrogen atom in pyrrole itself is well known to be so feebly basic that it forms an alkali metal salt under conditions no more forcing than those required for the similar metalation of an amidic nitrogen. As final evidence of structure V for the high-melting material, which should and did show on alkali titration a behavior typical of a diacylated nitrogen compound,[5] as mentioned above, it was found that the material could also be prepared by hot acetic anhydride dehydration of 3,6-diketopiperazine-2,5-dipropionic acid (VI), which itself was prepared by two independent methods.

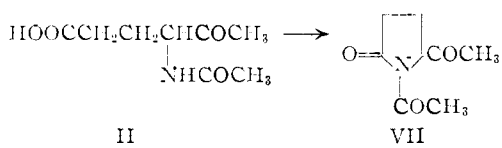
When glutamic acid was thermally dehydrated[6] to prepare a sample of 5-pyrrolidone-2-carboxylic acid (I) for comparison with the hydrolysis product of V there was obtained, in addition to the expected product, an appreciable yield of VI, which was considerably more soluble than I in water and from which solvent it crystallized in massive prisms. Further, the α-monoethyl ester of glutamic acid was prepared by the procedure of Le Quesne and Young[7] and when this material was heated in an oven at 100° for 24 hours there was obtained about a 10% yield of the diketopiperazine VI, identical with the product obtained by thermal dehydration of glutamic acid.

The next material isolated from the glutamic acid–acetic anhydride–pyridine reaction, by fractional distillation of the filtrate from which V had been removed, was a liquid which later crystallized to a neutral solid, m.p. 68–69°, that did not decolorize acid permanganate. This material, which could be obtained in as high as 29% yield, had the empirical formula $C_8H_{11}NO_3$. On titration with standard alkali the substance behaved in a lactone- or imide-like manner, just as did V; at the completion of the titration the neutral equivalent of the material was found to be in agreement with the molecular weight calculated from the empirical formula. In contrast, the saponification equivalent obtained on refluxing the material with base gave a value only half as great, indicating that a second acidic group was being liberated by this treatment. The ketonic nature of the substance was demonstrated by its formation of a neutral monosemicarbazone which still contained a latent acidic group as evidenced by its behavior on titration with standard alkali; the neutral equivalent of the semicarbazone was in agreement with the molecular weight calculated from the empirical formula $C_9H_{14}N_4O_3$. A methyl ketone was denoted by the occurrence of a positive iodoform test. The partial carbon skeleton of the material was indicated by strong acid hydrolysis of the substance, followed by basification, which produced 2,5-dimethylpyrazine-3,6-dipropionic acid (IV); this can only mean that the ultimate hydrolysis product was the amino keto acid III or its isomer in which the positions of the amino and keto groups in the six-carbon chain were reversed and the positive iodoform test on the original material excludes this latter possibility. The cumulative effect of all of these facts has been to provide a proof of structure of the original substance.

Vigorous hydrolysis of $C_8H_{11}NO_3$ to γ-amino-δ-ketocaproic acid shows that six of the eight carbon atoms are contiguous and the formation of a semicarbazone with the loss of no atoms other than those in the one molecule of water essential for its formation shows that the carbonyl group, shown by the iodoform test to be either CH_3CHOH- or CH_3CO-, actually is the latter (not an enolic ester or other derivative) and that there is no carbonyl group in the β- or γ-position to this ketonic function (lack of formation of a disemicarbazone, a pyrazole or a dihydropyridazine derivative). Thus, $C_8H_{11}NO_3$ can now be represented as



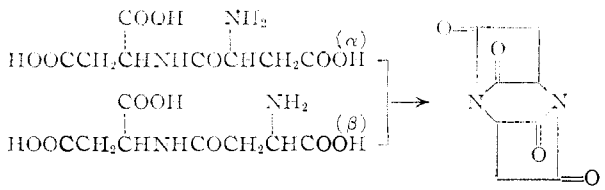
(C_2H_3O); there must be one ring present, to satisfy valency requirements (no carbon–carbon unsaturation), and the hydrolytic formation of γ-amino-δ-ketocaproic acid shows the ring to be a γ-lactam. The structure has thereby been reduced to 5-acetyl-2-pyrrolidone carrying a C_2H_3O group as a nuclear substituent; the C_2H_3O group can show no ketonic properties, but must be responsible for the lactone- or imide-like behavior of the material on titration (2-pyrrolidones are stable to cold alkali); only one group in one location satisfies these requirements; namely, the $-COCH_3$ group in the 1-position, structure VII, where it would be imidic, devoid of ketonic character, and would be expected



II VII

to react with alkali in the lactone-like manner characteristic of diacylamides with rupture of one of the acyl-nitrogen bonds and generation of a carboxyl group.[5] Vigorous hydrolysis would liberate both carboxyl groups, as shown by the saponification equivalent. It may therefore be considered that the structure of the substance has been established as VII. The formation of VII from

(4) Pyrocoll formation from pyrrole-α-carboxylic acid and its homologs: G. L. Ciamician and P. Silber, *Ber.*, **17**, 103 (1884); G. L. Ciamician and C. Zatti, *ibid.*, **21**, 1929 (1888); O. Piloty and K. Wilke, *ibid.*, **45**, 2586 (1912). Another closely related substance is the dilactam of 3,6-diketopiperazine-2,5-diacetic acid, prepared from both α- and β-aspartylaspartic acid by C. Ravenna and G. Bosinelli, *Gazz. chim. ital.*, **49**, II, 303 (1919); **50**, I, 281 (1920); C. Ravenna, *ibid.*, **51**, II, 28 (1921), by merely heating these isomeric peptides.



The parent ring system, perhydrodipyrrolo[a,d]pyrazine, has recently been obtained by E. Segel, THIS JOURNAL, **74**, 851 (1952), by the intermolecular cross-alkylation of 5-hydroxymethyl-2-pyrrolidone on high-pressure hydrogenation in the presence of copper–chromium oxide catalyst.

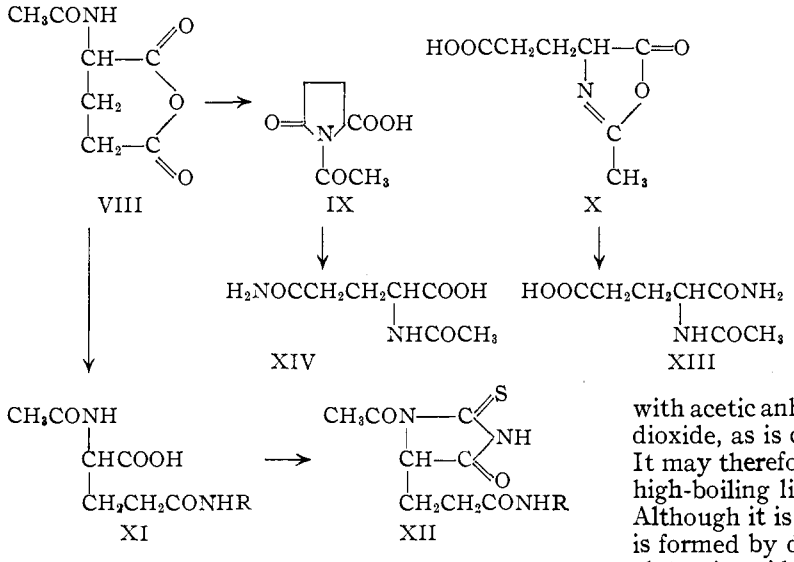
(5) A. W. Titherley and L. Stubbs, *J. Chem. Soc.*, **105**, 299 (1914).

(6) A. Menozzi and G. Appiani, *Gazz. chim. ital.*, **22**, II, 105 (1892); **24**, I, 370 (1894).

(7) W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950.)

II, the normally expected reaction product from glutamic acid, acetic anhydride and pyridine, probably is not due to thermal dehydration during distillation but occurs in the reaction mixture under the influence of the acetic anhydride, inasmuch as acidic dehydrating agents are known[8,9] to be capable of effecting imidic ring-closure between intramolecularly located carboxyl and amide groups. No attempt was made to isolate II directly from the reaction mixture.

The last product isolated from the reaction of glutamic acid, acetic anhydride and pyridine was a still higher-boiling (b.p. 160° at 0.25 mm.) liquid which could not be induced to crystallize and which had the empirical formula $C_7H_9NO_4$. From the carbon–oxygen ratio it was apparent that in the formation of this product glutamic acid had not undergone any decarboxylation but had suffered monoacetylation and loss of an additional molecule of water. This conclusion was verified by mild alkaline hydrolysis of the material to N-acetylglutamic acid. Three structures appear to fulfill the above requirements: α-acetamidoglutaric anhydride[10] (VIII), 1-acetyl-5-pyrrolidone-2-carboxylic acid (IX) and 2-methyl-5-oxazolone-4-propionic acid (X). Nicolet reported glutamic acid to be so insoluble in boiling acetic anhydride



that it was not practicable to acetylate it directly but that if the N-acetyl derivative were made by the Schotten–Baumann method this derivative could be readily converted to the acid anhydride VIII by warming it with acetic anhydride. He did not analyze his material ("a viscous oil") but proved its structure by treatment of it with amines to give amides (XI) on the carboxyl farthest removed from the acetamido group as proved by conversion of these amides by ammonium thiocyanate and acetic anhydride to crystalline thiohydantoins (XII), one of which (R = H) had previously been prepared[11] from glutamine.

After treatment of the high-boiling material with aniline under Nicolet's conditions no anilide could be isolated nor could a thiohydantoin be isolated after treatment of this mixture with ammonium thiocyanate. On repetition of the procedure of Nicolet it was found that the thiohydantoin was indeed easily prepared and isolated after reaction of his "viscous oil" with aniline and also that vacuum distillation of his oil gave a product, b.p. 160° at 0.25 mm., which appeared to be identical with the high-boiling material isolated from the glutamic acid–acetic anhydride–pyridine reaction. Similarly, it was not possible to isolate either an anilide after treatment of this distillate with aniline or a thiohydantoin after further treatment with ammonium thiocyanate. Since there is no reason to doubt the structure of Nicolet's anhydride (VIII) (treatment of acylated glutamic acids with acetic anhydride is now well known to give the corresponding acylamidoglutaric anhydrides[7,12]) it seems certain that some change must have occurred during the distillation. Titration behavior of the high-boiling material indicated that it was an acid but it was not possible to obtain a good end-point because of the presence of a (second) latent acidic grouping, which behavior would be expected of either structure IX or X. It seemed certain[13,14] that treatment of X with aqueous ammonia would give acetylisoglutamine[15] (XIII) whereas it could not be predicted with any certainty if IX would even react with aqueous ammonia. It was found that treatment of the high-boiling material with aqueous ammonia gave, after acidification, a compound $C_7H_{12}N_2O_4$; this proved to be acetylglutamine (XIV) and *not* acetylisoglutamine (XIII); it showed no depression in melting point when mixed with an authentic sample of acetylglutamine[16] and when it was heated with acetic anhydride and pyridine it evolved carbon dioxide, as is characteristic of α-acetamidoacids.[2,17] It may therefore be considered established that the high-boiling liquid is represented by structure IX. Although it is possible that a certain amount of IX is formed by direct imidic ring closure of N-acetylglutamic acid (XV), analogously to the previously discussed conversion II to VII, it is highly probable that the bulk of IX is produced by thermal rearrangement of VIII and as proof that this was possible the product obtained after distillation of Nicolet's oily anhydride (VIII) was treated with aqueous ammonia and found to yield acetylglutamine. The mechanism of the formation of acetylglutamine from 1-acetyl-5-pyrrolidone-2-carboxylic

(8) (a) N. E. Searle (to E. I. du Pont de Nemours and Company), U. S. Patent 2,444,536, July 6, 1948; (b) S. Wilkinson, *J. Chem. Soc.*, 104 (1951).

(9) M. Berenbom and J. White, THIS JOURNAL, **71**, 2246 (1949).

(10) B. H. Nicolet, *ibid.*, **52**, 1192 (1930).

(11) H. Thierfelder, *Z. physiol. Chem.*, **114**, 192 (1921).

(12) C. R. Harrington and T. H. Mead, *Biochem. J.*, **29**, 1602 (1935).

(13) H. E. Carter, "Azlactones" in R. Adams, "Organic Reactions," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1946.

(14) J. W. Cornforth, "Oxazolones," in H. T. Clarke, J. R. Johnson and R. Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1948.

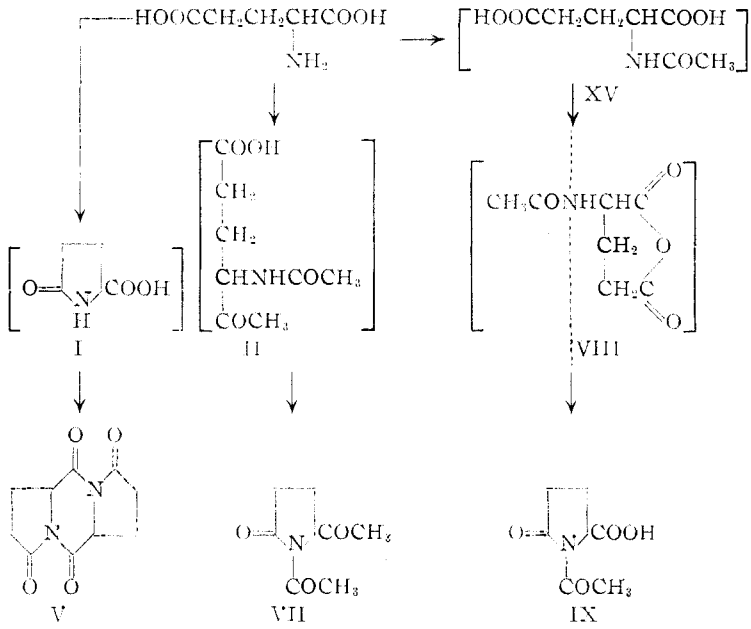
(15) E. Mohr and T. Geis, *Ber.*, **41**, 798 (1908).

(16) P. Karrer, K. Escher and R. Widmer, *Helv. Chim. Acta*, **9**, 301 (1926).

(17) P. A. Levene and R. E. Steiger, *J. Biol. Chem.*, **79**, 95 (1928).

acid on treatment with aqueous ammonia can be considered to be a reversal of the mechanism suggested[18] for the formation of 3-ketoöctahydropyrrocoline from β-(2-piperidyl)-propionamides.

In summary, one can now say that on treatment with acetic anhydride and pyridine glutamic acid undergoes the acylative decarboxylation characteristic of α-amino acids to the extent of 15 to 20%, leading to the acetamidoketo acid II, which is dehydrated further in the reaction mixture to the diacetylpyrrolidone, VII. The cessation of the reaction is partially due, as thought by Dakin and West, to conversion of the starting material to pyrrolidone carboxylic acid I, but in the reaction mixture this substance is likewise dehydrated, in this case bimolecularly, to the tricyclic piperazine derivative, V. The bulk of the starting material,



however, is N-acetylated to XV which is internally dehydrated to acetamidoglutaric anhydride, VIII, and this under the conditions of isolation used by us, was rearranged to the acetylpyrrolidone carboxylic acid, IX, a portion of which may also have been formed in the reaction mixture by direct imidic ring-closure of XV.

Experimental[19,20]

Reaction of Glutamic Acid with Acetic Anhydride and Pyridine.—A mixture of glutamic acid (7.35 g. 0.05 mole), acetic anhydride (50 cc.) and pyridine (50 cc.) was refluxed for one-half hour, by which time carbon dioxide evolution (395 cc., not N.T.P.) had ceased. The reaction mixture was evaporated under vacuum to a viscous residue (8.8 g.) which on crystallization from 50 cc. of absolute alcohol gave 1.0 g. of crystalline solid (A). After recrystallization from aqueous alcohol this material melted at 340° (dec.).

Anal. Calcd. for $C_5H_5NO_2$: C, 54.10; H, 4.54; N, 12.62. Found: C, 54.35; H, 4.22; N, 12.77.

The filtrate from which the crystalline solid (A) had been removed was evaporated under vacuum to a viscous residue (7.8 g.) which on fractional distillation yielded 1.1 g. of liquid (B), b.p. 100° (0.25 mm.), and 1.0 g. of liquid (C), b.p. 160° (0.25 mm.).

Liquid (B) subsequently crystallized and, after recrystallization from Skellysolve C, melted at 68–69°. This same material could be obtained in 29.6% yield (15.2 g. from 44.1 g. glutamic acid) in a later and larger run.

Anal. Calcd. for $C_8H_{11}NO_3$: C, 56.79; H, 6.55; N, 8.28. Found: C, 57.27; H, 7.05; N, 8.29.

Liquid (C) was never obtained in the solid state.

Anal. Calcd. for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.19. Found: C, 48.98; H, 5.44; N, 8.01.

Identification of Fraction (A). 3,5,8,10-Tetraketoperhydrodipyrrolo[a,d]pyrazine (V). A. Neutralization Equivalent.—Titration of an aqueous suspension of the material with standard alkali proceeded with slow consumption of base; the time interval required for neutralization of added increments of alkali became increasingly greater as the titration progressed, rendering it difficult to determine the exact end-point. 93.2 mg. of sample required 9.85 ml. of 0.0948 *N* sodium hydroxide to give a permanent end-point (phenolphthalein), corresponding to a neutral equivalent of 100; $C_5H_5NO_2$ requires 111; the deviation from theoretical is probably due to the fact that sodium 5-pyrrolidone-2-carboxylate was starting to consume alkali.

B. Hydrolysis to 5-Pyrrolidone-2-carboxylic Acid.—Addition of normal sodium hydroxide solution (11 ml., 0.011 mole) to a flask containing 1.11 g. (0.010 equivalent) of the material resulted in an exothermic reaction. After the reaction mixture had stood for one hour it was acidified with concentrated hydrochloric acid and then refrigerated overnight. The resultant crystalline precipitate melted, after recrystallization from water, at 180–181° with no depression on admixture with an authentic sample of 5-pyrrolidone-2-carboxylic acid.

C. Preparation from 5-Pyrrolidone-2-carboxylic Acid.—A mixture of 5-pyrrolidone-2-carboxylic acid (5.8 g., 0.045 mole), acetic anhydride (50 cc.) and pyridine (50 cc.) was refluxed for one hour (no carbon dioxide evolution) and then chilled. The resultant precipitate weighed 3.0 g. (60% yield) and melted, after recrystallization from water, at 337–341° (dec.), undepressed on admixture with fraction (A) obtained from glutamic acid. Results were the same when the pyridine was omitted from the reaction mixture.

D. Preparation from 3,6-Diketopiperazine-2,5-dipropionic Acid.—One gram of 3,6-diketopiperazine-2,5-dipropionic acid was refluxed for one hour with 5 cc. of acetic anhydride. The cooled reaction mixture deposited a good yield of crystalline product, m.p. 336–337° (dec.), undepressed when mixed with fraction (A) obtained from glutamic acid.

Thermal Dehydration of Glutamic Acid. Preparation of 5-Pyrrolidone-2-carboxylic Acid (I) and 3,6-Diketopiperazine-2,5-dipropionic Acid (VI).—Glutamic acid (59.8 g., 0.4 mole) was placed in a 500-cc. round-bottomed flask and heated in a bath at 175–180° until the flask contents were liquid, at which point the pressure in the flask was lowered to about 100 mm. and the bath temperature was lowered to and held at 150° until there was no more bubbling in the melt. The melt was then cooled and crystallized from water (40 cc.) to give a crystalline material which weighed 22.3 g. and melted at 145–155° (fraction 1). The filtrate from fraction 1 was evaporated to dryness to leave a residue that was boiled with 500 cc. of dry acetone and filtered. The residue after evaporation of the acetone filtrate weighed 19.6 g. and melted at 125–180° (fraction 2); the acetone-insoluble material was discarded. Fraction 1 was fractionally recrystallized from water (20 cc.) to give three subfractions: 1A, 9.0 g. (18% yield), m.p. 180–183° (5-pyrrolidone-2-carboxylic acid); 1B, 2.0 g., m.p. 155–159° (massive prisms); 1C, 7.0 g., m.p. 150–153° (massive prisms).

Fractional recrystallization of fraction 2 from water (15 cc.) similarly gave three subfractions: 2A, 2.2 g., m.p. 158–162°; 2B, 3.0 g., m.p. 151–155° (massive prisms);

(18) F. H. McMillan and J. A. King, THIS JOURNAL, **73**, 3165 (1951).

(19) Melting points and boiling points are uncorrected.

(20) Microanalyses were carried out in the Microanalytical Laboratory of this Institute under the direction of Dr. F. A. Buehler.

2C, 1.5 g. (3% yield, m.p. 160–161°[21] (massive prisms, 3,6-diketopiperazine-2,5-dipropionic acid). 109 mg. of sample required 8.85 ml. of 0.0948 *N* sodium hydroxide to give a permanent end-point (phenolphthalein), corresponding to a neutral equivalent of 130; $C_6H_7NO_3$ requires 129. Cryoscopic molecular weight determinations were carried out using purified glacial acetic acid (depression constant 3.9) as the solvent.

Anal. Calcd. for $C_6H_7NO_3$: 129. Found, fraction 1A: 128. Calcd. for $C_{10}H_{14}N_2O_6$: 258. Found, fraction 2C: 250.

Preparation of 3,6-Diketopiperazine-2,5-dipropionic Acid (VI) from α-Ethyl Glutamate.—α-Ethyl glutamate[7] (0.3 g.) was heated in an oven at 100° for 24 hours and the resultant dark brown material was leached with 10 cc. of water (there was considerable water-insoluble material). The aqueous extract was evaporated nearly to dryness and on standing deposited the characteristic large prisms obtained when this compound was prepared by thermal dehydration of glutamic acid. The prisms weighed 30 mg. (about 10% yield) and melted at 156–158°, undepressed in melting point when mixed with fraction 2C described above.

Identification of Fraction (B). 1,5-Diacetyl-2-pyrrolidone (VII). A. Neutralization Equivalent.—The crystalline material, m.p. 68–69°, dissolved in water to form a neutral solution. On titration with alkali, however, it slowly consumed one equivalent in much the same manner as a lactone, with a few seconds being required for the consumption of each few drops of alkali. Eighty-three mg. of sample required 5.00 cc. of 0.0996 *N* sodium hydroxide to give a permanent end-point (phenolphthalein), corresponding to a neutral equivalent of 172. $C_8H_{11}NO_3$ requires 169.

B. **Saponification Equivalent.**—One hundred and thirty-five mg. of the substance was refluxed one hour with 2.00 cc. of 5% sodium hydroxide and then back-titrated with 0.1149 *N* hydrochloric acid, the difference consumed between the sample and a blank being 13.10 cc., corresponding to a saponification equivalent of 89.5. One-half of $C_8H_{11}NO_3$ requires 84.5.

C. **Semicarbazone.**—A sample of the material was treated with semicarbazide hydrochloride and sodium acetate in aqueous solution in the usual manner and yielded the monosemicarbazone which melted at 220–222° (dec.) after recrystallization from water.

Anal. Calcd. for $C_9H_{14}N_4O_3$: N, 24.78. Found: N, 25.02, 25.06.

This semicarbazone had its neutral equivalent determined and behaved entirely analogously to the original material, in that considerable time was required for the consumption of each added increment of alkali. One hundred and thirteen mg. of substance required 5.00 cc. of 0.0966 *N* sodium hydroxide to give a permanent end-point (phenolphthalein), corresponding to a neutral equivalent of 234. $C_9H_{14}N_4O_3$ requires 226.

D. **Conversion to 2,5-Dimethylpyrazine-3,6-dipropionic Acid (IV).**—A few hundred mg. of the substance was refluxed one hour with 10 cc. of 1:1 hydrochloric acid and the solution was then evaporated to dryness under vacuum. Ten cc. of concentrated ammonium hydroxide was added to the residue, the solution was let stand for one hour and then evaporated to dryness under vacuum. The residual sirup crystallized on trituration with 5 cc. of water containing a few drops of acetic acid; the washed crystals melted at 209–211°; reported,[1,2] 211–213°.

This pyrazine derivative had its neutral equivalent determined in the usual manner. Thirty-eight and one-half mg. of substance was dissolved in 13.80 cc. of 0.0966 *N* sodium hydroxide and back-titrated to a phenolphthalein end-point with 8.80 cc. of 0.1148 *N* hydrochloric acid, corresponding to a neutral equivalent of 120. One-half of $C_{12}H_{16}N_2O_4$ requires 126.

Identification of Fraction (C). 1-Acetyl-5-pyrrolidone-2-carboxylic Acid (IX). A. Neutralization Equivalent.—One hundred and forty-eight mg. of the high-boiling liquid consumed 19.5 cc. of 0.0948 *N* sodium hydroxide to a permanent end-point (phenolphthalein), corresponding to a neutral equivalent of 80. One-half of $C_7H_9NO_4$ requires 85.5.

B. **Hydrolysis to Acetylglutamic Acid.**—Two and fifty-eight hundredths grams (about 0.032 equivalent) of the material was refluxed for one hour with 32 cc. of *N* sodium hydroxide, after which the solution was cooled and neutralized with 32 cc. of *N* hydrochloric acid. The resultant neutral solution was evaporated to a volume of 8 cc. and then chilled. The crystals which formed melted at 162–168° and after three recrystallizations (from about 2 cc. of water each time) the melting point was 183–190°; further recrystallization did not improve the melting point. A mixture of this material and an authentic sample of N-acetylglutamic acid[10] (m.p. 191–192°) melted at 185–190°.

Seventy-four mg. of the hydrolysis product of m.p. 183–190° was titrated to a phenolphthalein end-point with 8.30 cc. of 0.0948 *N* sodium hydroxide, corresponding to a neutral equivalent of 94.1. One-half of $C_7H_{11}NO_5$ requires 94.5.

C. **Attempted Thiohydantoin Formation.**—A mixture of 1.7 g. of the material and 1.9 g. of aniline was warmed at 50° for a few minutes and then allowed to stand at room temperature for three days. Dry ammonium thiocyanate (1.0 g.) and acetic anhydride (8 cc.) were then added and the mixture was heated at 100° for 1.5 hours. The resulting solution was poured into 50 cc. of water and the aqueous mixture was stirred until all the acetic anhydride had been decomposed. Prolonged cooling of the mixture gave only a small amount of crystalline material, m.p. 103–110°, which, after two recrystallizations from water, melted at 116–118°, undepressed when mixed with an authentic sample of acetanilide.

Authentic α-acetamidoglutaric anhydride[10] readily furnished 1-acetyl-2-thiohydantoin-5-β-propionanilide (XII, R = C_6H_5), m.p. 197°, when treated as described in the literature (essentially as described in the preceding paragraph). When α-acetamidoglutaric anhydride was subjected to distillation there came over a viscous liquid at 160° (0.25 mm.) which had the appearance of fraction (C) from the glutamic acid–acetic anhydride–pyridine reaction and which on treatment with aniline followed by treatment with ammonium thiocyanate and acetic anhydride furnished as the only recognizable product a small amount of acetanilide, just as did fraction (C).

The redistillation of either fraction C or the rearrangement product of α-acetamidoglutaric anhydride was quite unsatisfactory. A constant boiling distilled sample would yield on redistillation only a small fraction of the original weight in the distillate, the remainder of the material undergoing some sort of degradation or polymerization. We believe that this is the reason for the rather unsatisfactory material balance reported in the first paragraph of the Experimental section; namely, that the bulk of the unaccounted for material was derived secondarily from liquid (C).

D. **Ammonolysis to Acetylglutamine (XIV).**—Upon mixing 1.0 g. of the material with 2.0 cc. of concentrated ammonium hydroxide an exothermic reaction occurred. After the reaction mixture had stood for one-half hour it was chilled and acidified with concentrated hydrochloric acid. The resultant crystalline precipitate melted at 189–193° after two recrystallizations from water followed by one recrystallization from alcohol.

Anal. Calcd. for $C_7H_{12}N_2O_4$: C, 44.67; H, 6.43; N, 14.87. Found: C, 45.00; H, 6.46; N, 14.97.

Sixty-four mg. of the product consumed 3.60 cc. of 0.0948 *N* sodium hydroxide on titration to a phenolphthalein end-point, corresponding to a neutral equivalent of 187.5. $C_7H_{12}N_2O_4$ requires 188.

When 1.0 g. of the product was refluxed with 5 cc. each of acetic anhydride and pyridine, there was evolved 48 cc. of carbon dioxide, corresponding to nearly one-half a molar equivalent. Fraction (C) itself evolved no carbon dioxide under the same conditions.

As final proof of its identity, a mixture of the product (m.p. 189–193°) with a sample of authentic acetylglutamine[16] (m.p. 190–192°) was found to melt at 190–192°.

Reaction of *l*-Proline with Acetic Anhydride and Pyridine.—A mixture of *l*-proline (2.3 g., 0.02 mole), acetic anhydride (20 cc.) and pyridine (20 cc.) was refluxed for one hour, during which time no carbon dioxide was evolved. After the readily volatile materials had been removed from the re-

(21) M. A. Blanchetiere, *Bull. soc. chim. France*, [4] **31**, 1045 (1922), reported a compound, m.p. 151°, obtained by heating glutamic acid in the presence of glycerol which from elemental analyses, neutralization equivalent and non-identity with 5-pyrrolidone-2-carboxylic acid he concluded to be 3,6-diketopiperazine-2,5-dipropionic acid. Because of the similarity of his description and our observation of solubility properties and crystallization characteristics and because of our molecular weight determination we consider his conclusion to be correct.

action mixture by distillation under vacuum on a steam-cone the residual material resisted all attempts at crystallization and its aqueous solution showed a specific rotation of less than one degree. Neuberger has reported[22] that acetyl-*l*-proline melts at 115° and that its aqueous solution shows a specific rotation of −104°. Fractional vacuum distillation of the aqueous solution gave 2.5 g. (80% yield) of acetyl-*dl*-proline, b.p. 146–153° (0.9 mm.).

Anal. Calcd. for $C_7H_{11}NO_3$: N, 8.92; neut. equiv., 157. Found: N, 8.71; neut. equiv., 165.

We wish to acknowledge the technical assistance of Messrs. Charles Anderson and Kenneth Hutton.

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(22) A. Neuberger, *Biochem. J.*, **32**, 1452 (1938).

[CONTRIBUTION FROM THE RESEARCH LABORATORY, DOMINION RUBBER CO., LTD.]

The Alkaloids of Fumariaceous Plants. XLVIII. The Structure of Corpaverine

BY RICHARD H. F. MANSKE

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An alkaloid (F24), now termed corpaverine, has been shown to be 2-methyl-6,7-dimethoxy-8-hydroxy-1-(4-methoxybenzyl)-tetrahydroisoquinoline. It is pointed out that it survives as a benzylisoquinoline in a plant which elaborates almost exclusively protoberberines because there is no activated position in the benzyl portion to permit further ring closure.

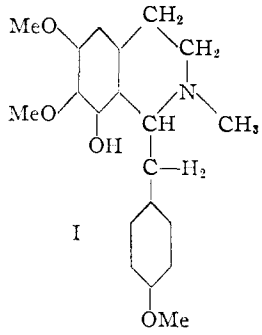
Alkaloid F24 has been isolated from *Corydalis aurea* Willd[1] in amounts less than one part per million. On mild oxidation it has yielded *p*-anisic acid and it is therefore obvious that this alkaloid, for which the name *corpaverine* is now proposed, is a benzylisoquinoline. Since it has four oxygen atoms—one phenolic hydroxyl and three methoxyls—its empirical formula must be $C_{20}H_{25}O_4N$ instead of $C_{19}H_{23}O_4N$ as previously given, the analytical figures being about equally good for both.

Evidently a number of *p*-methoxybenzylisoquinolines with two methoxyls and one hydroxyl in the isoquinoline nucleus are possible but bearing in mind the associated capaurine[2] it is quite evident that formula I is the most probable. When corpaverine was ethylated with diazoethane an O-ethyl ether was obtained which on treatment with methyl iodide yielded only a quaternary iodide. The nitrogen is therefore tertiary. This methiodide when degraded to the corresponding methine and the latter again methylated and heated with alkali yielded a neutral substance which on oxidation with permanganate yielded *p*-anisic acid and 3-ethoxy-4,5-dimethoxyphthalic acid. Corpaverine therefore has formula I if cognizance is taken of the fact that all natural trialkoxyisoquinolines have the substituents in the 6, 7 and 8-positions.

Corpaverine is of interest in connection with the biogenesis of the protoberberines. The plant in which it occurs is chiefly noted for the almost exclusive elaboration of protoberberines and the assumption that benzylisoquinolines are intermediates in their syntheses is virtually unavoidable. Such a last step however requires another ring closure and for this purpose a point of attack in a benzene ring must be activated by a hydroxyl or methoxyl in the ortho or para position. Such activation is lacking in corpaverine and in consequence this alkaloid is the end-product in the plant. It would be difficult to devise an experiment which would give clearer evidence of the nature of the ultimate intermediate in the biogenesis of the protoberberines.

(1) R. H. F. Manske, *Can. J. Research*, **B16**, 81 (1938).

(2) R. H. F. Manske and H. L. Holmes, THIS JOURNAL, **67**, 95 (1945).



Experimental

Owing to the extremely small amount of this alkaloid available, it was not possible to carry out the desirable isolation and characterization of the intermediates which were obtained in the various steps of its degradation.

Corpaverine is optically active; $[\alpha]^{20}D$ −154.2° (*c* 2.63 in chloroform).

Oxidation.—One hundred and fifty mg. of corpaverine was dissolved in 20 cc. of hot water to which had been added a small pellet of potassium hydroxide. The cooled solution was treated with small portions of potassium permanganate until the purple color remained permanent for 30 minutes. The decolorized solution was treated with calcium chloride, filtered, acidified, and extracted with ether. The residue from the ether extract was recrystallized twice from hot water and then consisted of colorless plates of *p*-anisic acid which melted sharply at 184°[3] either alone or in admixture with an authentic specimen.

Degradation.—A suspension of 0.4 g. of corpaverine in cold methanol was left for 24 hr. with an excess of an ethereal solution of diazoethane. The solvents were then removed from the clear solution and the residue dissolved in hot dilute hydrochloric acid. The filtered and cooled solution deposited colorless plates of a sparingly soluble hydrochloride which when recrystallized once from hot water melted at 247° when placed in the bath at 230°. The free *base* crystallized slowly from warm hexane in colorless plates and melted at 76°; calcd. for $C_{22}H_{29}O_4N$: C, 71.16; H, 7.81; N, 3.77. Found: C, 70.87; H, 7.70; N, 4.00. The methiodide separated as an oil when the base in ether was treated with excess methyl iodide. It dissolved only sparingly in hot water and separated as an oil on cooling the solution The addition of potassium hydroxide did not yield a turbidity in the cold. An excess of potassium hydroxide was added to the hot aqueous solution of the methiodide and the mixture heated for 12 hr. At intervals the separated methine was removed by extracting the cooled mixture with ether. The resulting methine was again converted to methiodide and the latter treated with an excess of silver oxide in hot aqueous solution. The filtrate from this mix-

(3) All melting points are corrected.