2-*Mercaptoglyoxalines. Part VIII.* The Preparation of 2-Mercaptoglyoxalines from Glutamic Acid.*

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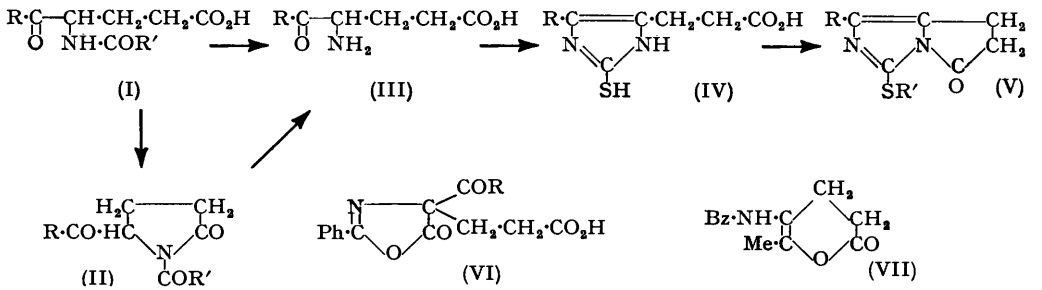
Benzoylglutamic acid has been acylated by several acid anhydrides in the presence of a basic catalyst (Dakin and West, *J. Biol. Chem.*, 1928, **78**, 745). Acylation precedes the formation of the pyrrolidone (II) which on hydrolysis gives the amino-ketone from which the corresponding 2-mercaptoglyoxaline (IV) is obtained by means of thiocyanate. Under certain conditions the reduction of ethyl glutamate according to the Akabori procedure leads to a product which on treatment with thiocyanate gives a 2-mercaptoglyoxaline which is either 1 : 4- or 1 : 5-disubstituted.

The reaction of glutamic acid with acetic anhydride in the presence of pyridine was shown by Dakin and West (*J. Biol. Chem.*, 1928, **78**, 745) to yield only 15—20% of one molecular equivalent of carbon dioxide. King and McMillan (*J. Amer. Chem. Soc.*, 1952, **74**, 2859) showed that the ω-carboxyl group of the amino-acid caused pyrrolidone ring-closure, 1 : 5-diacetyl-2-pyrrolidone, 1-acetyl-5-oxopyrrolidine-2-carboxylic acid and 3 : 5 : 8 : 10-tetraketoperhydrodipyrrolo[*a*,*d*]pyrazine being isolated. The formation of the last two compounds accounts for the low yield of carbon dioxide and consequently of ketone.

* Part VII, *J.*, 1953, 1046.

As in the case of aspartic acid (Lawson, *J.*, 1953, 1046), by the use of the benzoyl derivative instead of the free amino-acid, one molecular equivalent of carbon dioxide and simultaneous high yields of ketone have been obtained. It was found that decarboxylative acetylation in the presence of pyridine, 2-picoline, or sodium acetate occurred even at 0°, when the crystalline product isolated was 4-benzamido-5-oxohexanoic acid (I; R = Me, R′ = Ph). At 110°, the product was shown to be 5-acetyl-1-benzoyl-2-pyrrolidone (II; R = Me, R′ = Ph), which was also obtained from I; R = Me, R′ = Ph by hot acetic anhydride and pyridine, both the dehydrating and basic reagents apparently being necessary.



The pyrrolidone structure (II) was substantiated as King and MacMillan (*loc. cit.*) did that of 1 : 5-diacetyl-2-pyrrolidone (II; R = R′ = Me). The substance was neutral but slowly absorbed one equivalent of alkali when titrated to phenolphthalein. An acetyl group was indicated by a positive iodoform test, and a monosemicarbazone was prepared. These observations exclude the oxazolone (VI) and the lactone structure (VII).

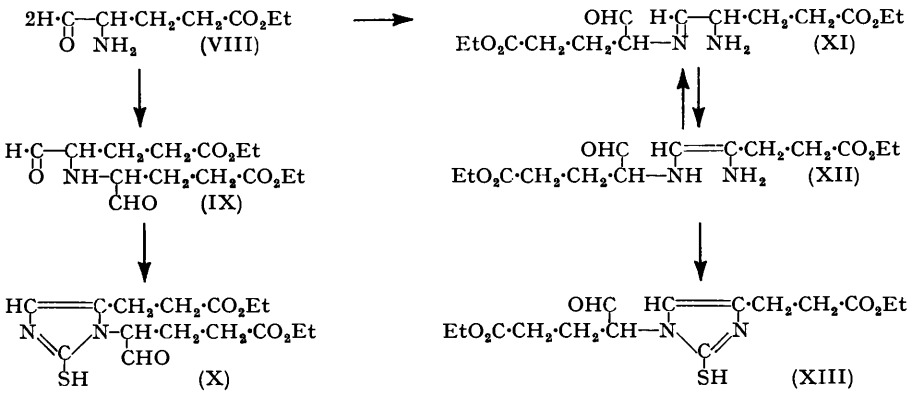
Unsuccessful attempts were made to prepare 1-benzoyl-2-oxopyrrolidine-5-carboxylic acid in order to ascertain whether it also underwent decarboxylative acetylation. Even without such evidence, however, isolation of the benzamido-ketone (I; R = Me, R′ = Ph) at 0° eliminates the possibility of intermediate pyrrolidone formation before *C*-acetylation of benzoylglutamic acid. Further work is being carried out to discover how far our observations are consistent with Cornforth and Elliot's view (*Science*, 1950, **112**, 534) that ketone synthesis occurs by acetylation of an oxazolone.

By employing propionic, butyric, hexanoic, and benzoic anhydrides in place of acetic, analogous pyrrolidones (II) were obtained, but in lower yield. The amino-ketones (III) obtained by hydrolysis of some of the corresponding pyrrolidones were condensed with thiocyanate, to give the 2-mercaptoglyoxalines (IV).

Reducing diethyl glutamate with sodium amalgam at pH 2—2·5 and without delay boiling the resulting solution of the amino-aldehyde (III; R = H) with potassium thiocyanate (cf. Bullerwell and Lawson, *J.*, 1951, 3030) gave β-(2-mercapto-5-glyoxalinyl)-propionic acid (IV; R = H). The action of hot acetic anhydride then produced an acetyl-anhydro-derivative (V; R = H, R′ = Ac) which was rapidly hydrolysed by hot water with loss of the acetyl group. Relatively prolonged boiling with water was required to reconvert the anhydro-compound (V; R = R′ = H) into the 2-mercaptoglyoxalinylpropionic acid. The formation of an *S*-acetyl derivative of a 2-mercaptoglyoxaline having a labile acetyl group has been reported by Heath, Lawson, and Rimington (*J.*, 1951, 2219). Cook, Downer, and Heilbron (*J.*, 1948, 1262) prepared a diacetyl derivative of 5-benzamido-2-mercaptoglyoxaline, thus demonstrating that the ring is capable of accommodating *S*- and *N*-acyl groups simultaneously. This evidence is the basis for the glyoxalino-pyrrolidone structure (V).

When, however, diethyl glutamate was reduced with sodium amalgam at pH 3—5 and the resulting solution kept for some time before the addition of thiocyanate, two neutral substances (separated by benzene) were obtained, both being 2-mercaptoglyoxalines as shown by their reactions with sulphur dioxide and gold chloride and by their high-intensity absorption at 258 mμ. The less-soluble substance, $C_8H_{12}O_2N_2S$, gave on hydrolysis β-(2-mercapto-5-glyoxalinyl)propionic acid (IV; R = H) and was, therefore, the ethyl ester

of (IV; R = H). The benzene-soluble product, $C_{15}H_{22}O_5N_2S$, produced in variable yield, gave aldehydic reactions and lost on hydrolysis two ethyl groups, to give the corresponding aldehydo-dicarboxylic acid. This substance was therefore ethyl β-[1-(3-ethoxycarbonyl-1-formylpropyl)-2-mercapto-5-glyoxalinyl]propionate (X) or the isomer (XIII) depending on whether the original aldehyde (VIII) lost ammonia to give the secondary amino-aldehyde (IX) or lost water to give the Schiff's base (XII). The former seems probable since Siegfried



(*Z. physiol. Chem.*, 1906, **73**, 196, 201) showed that iminodiacetic acid is formed from glycine by mercuric chloride solution at 40° and Fischer (*Ber.*, 1893, **26**, 194) observed the production of ammonia from aminoacetaldehyde in slightly acid solution. On the other hand, the conditions do not exclude the formation of a Schiff's base, which might condense with thiocyanate as does *o*-phenylenediamine (Frerichs and Hupka, *Arch. Pharm.*, 1903, **241**, 165).

A number of other amino-acid esters, in particular alanine ethyl ester, when reduced under less acid conditions than usual, give analogous glyoxalines with an aldehyde-containing substituent and further work is in progress.

Experimental

Benzoyl-DL-glutamic Acid.—The following modification of Fischer's method (*Ber.*, 1899, **32**, 2464) was advantageous. DL-Glutamic acid (10 g.) was dissolved in water (150 ml.) containing sodium hydrogen carbonate (50 g.). Benzoyl chloride (22 ml.) was added dropwise with vigorous stirring during 45 min. Stirring was continued for a further 30 min., and the filtered solution was acidified with concentrated hydrochloric acid (58 ml.) without cooling, and again filtered quickly to remove benzoic acid, then kept at 0°. The crude product which separated was dried at 80° for 3 hr. and extracted with light petroleum (b. p. 100—120°) to remove final traces of benzoic acid. Recrystallisation from water gave benzoyl-DL-glutamic acid monohydrate, m. p. 98°, which was converted into the anhydrous acid (13·0 g.; m. p. 152—154°) by heating it at 80°.

Benzoyl-DL-glutamic Anhydride.—Benzoyl-DL-glutamic acid (5 g.) was warmed with acetic anhydride (20 ml.) on the steam-bath for 15 min. The solution so obtained was cooled and large prisms of the *anhydride* separated. This product was recrystallised from ethyl acetate (Found: C, 61·8; H, 4·6. $C_{12}H_{11}O_4N$ requires C, 61·9; H, 4·7%).

5-Oxopyrrolidine-2-carboxylic Acid.—DL-Glutamic acid (10 g.) heated at 150—160° in a stream of nitrogen for 3 hr. gave 5-oxopyrrolidine-2-carboxylic acid, m. p. 180—183° (from water). DL-Benzoylglutamic acid was heated in the same way for 6 hr. The residue was dissolved in water and the solution extracted with ether to remove benzoic acid. After concentration, 5-oxopyrrolidine-2-carboxylic acid (identified by mixed m. p.) separated. This acid, benzoylated as described above, gave DL-benzoylglutamic acid.

5-Acetyl-1-benzoyl-2-pyrrolidone.—Benzoyl-DL-glutamic acid (3 g.), anhydrous pyridine (10 ml.), and freshly distilled acetic anhydride (10 ml.) were heated at 110° for 45 min. Evolution of carbon dioxide (determined as barium carbonate) was quantitative. The solution was then concentrated under reduced pressure to a semi-solid mass which crystallised upon trituration with ethanol to give *5-acetyl-1-benzoyl-2-pyrrolidone* (2·5 g., 90%), m. p. 148° (from water or

ethyl acetate) (Found: C, 67·3; H, 5·5; N, 5·0. $C_{13}H_{13}O_3N$ requires C, 67·5; H, 5·6; N, 6·1%). The same product was obtained by using benzoylglutamic anhydride in place of the acid, but there was no advantage in this modification.

Refluxing benzoylglutamic acid (2·5 g.), anhydrous sodium acetate (1·6 g.), and acetic anhydride (20 ml.) for 30 min., gave 5-acetyl-1-benzoyl-2-pyrrolidone in 65% yield. The mother-liquor was then poured into water; benzoic acid (0·4 g.) slowly separated.

5-Acetyl-1-benzoyl-2-pyrrolidone dissolved in hot water to form a neutral solution. On titration with alkali (phenolphthalein) it slowly reacted with one equivalent in a similar manner to a lactone (Found: equiv., 227. $C_{13}H_{13}O_3N$ requires equiv., 231 as monobasic acid). When refluxed for 1 hr. with excess of 0·1N-sodium hydroxide and then back-titrated with 0·1N-hydrochloric acid, it gave a saponification equivalent of 114.

5-Acetyl-1-benzoyl-2-pyrrolidone semicarbazone, m. p. 229° (from water), was readily formed (Found: C, 58·5; H, 5·4; N, 19·3. $C_{14}H_{16}O_3N_4$ requires C, 58·3; H, 5·6; N, 19·4%).

4-Amino-5-oxohexanoic Acid.—5-Acetyl-1-benzoyl-2-pyrrolidone (1 g.) was refluxed with 20% hydrochloric acid (25 ml.) for 1 hr. After cooling in ice, the solution was filtered from benzoic acid and concentrated to dryness. The residue was recrystallised from ethanol–ether to give a product m. p. 60—65°, which after drying at 60° *in vacuo* over phosphoric oxide was *4-amino-5-oxohexanoic acid hydrochloride*, m. p. 162° (Found: C, 39·5; H, 7·0; N, 7·7. $C_6H_{11}O_3N$,HCl requires C, 39·7; H, 6·6; N, 7·7%).

3 : 6-Dimethylpyrazine-2 : 5-dipropionic Acid.—4-Amino-5-oxohexanoic acid hydrochloride (1 g.) was dissolved in concentrated aqueous ammonia (20 ml.). After 1 hr. the solution was concentrated to dryness under vacuum. The residue, crystallised from water containing a few drops of acetic acid, gave 3 : 6-dimethylpyrazine-2 : 5-dipropionic acid, m. p. 211—213° (Found: C, 57·4; H, 7·35. Calc. for $C_{12}H_{16}O_4N_2$: C, 57·1; H, 6·35%).

β-(2-Mercapto-4-methyl-5-glyoxalinyl)propionic Acid.—Crude 4-amino-5-oxohexanoic acid hydrochloride obtained from 5-acetyl-1-benzoyl-2-pyrrolidone (2 g.) was dissolved in water (10 ml.) together with potassium thiocyanate (1 g.) and was heated on the steam-bath until crystals separated. After recrystallisation from water the *propionic acid* (0·9 g.) had m. p. 268° (Found: C, 44·7; H, 5·4; N, 15·2. $C_7H_{10}O_2N_2S$ requires C, 45·1; H, 5·4; N, 15·1%).

4-Benzamido-5-oxohexanoic Acid.—Benzoyl-DL-glutamic acid (4·5 g.), acetic anhydride (10 ml.), and 2-picoline (5 ml.) were shaken at room temperature until all went into solution, which was then stored at 0° for 24 hr. Crushed ice was added, followed by 17·5% hydrochloric acid until acid to Congo-red (final vol. of solution, 60 ml.). Overnight *4-benzamido-5-oxohexanoic acid* (2·7 g.) separated. Recrystallised from ethyl acetate it had m. p. 152—153° (Found: C, 62·3; H, 5·8; N, 6·5. $C_{13}H_{15}O_4N$ requires C, 62·7; H, 6·0; N, 5·6%). The *semicarbazone* had m. p. 212° (from water) (Found: C, 54·1; H, 5·9; N, 17·4. $C_{14}H_{18}O_4N_4$ requires C, 54·9; H, 5·9; N, 18·3%).

1-Benzoyl-5-propionyl-2-pyrrolidone.—Benzoyl-DL-glutamic acid (3 g.), propionic anhydride (12 ml.), and pyridine (10 ml.) were refluxed together for 45 min. The solution was then concentrated to a syrup under vacuum. Ethanol (5 ml.) was added and, after concentration again, the residue was dissolved in hot ethyl acetate. During 1 week at 0°, *1-benzoyl-5-propionyl-2-pyrrolidone* (2·2 g.), m. p. 104° (from ethanol), separated (Found: C, 68·1; H, 6·3. $C_{14}H_{15}O_3N$ requires C, 68·6; H, 6·1%). The *semicarbazone*, recrystallised from water, had m. p. 201° (Found: C, 59·8; H, 6·1. $C_{15}H_{18}O_3N_4$ requires C, 59·6; H, 6·0%).

β-(4-Ethyl-2-mercapto-5-glyoxalinyl)propionic Acid.—1-Benzoyl-5-propionyl-2-pyrrolidone (2 g.) was refluxed with 20% hydrochloric acid (50 ml.) for 1 hr. The solution was cooled, filtered, and concentrated to dryness. The residue was washed with ether and dissolved in water (10 ml.). Potassium thiocyanate (2 g.) was added in three portions during 2 hours' heating on the steam-bath. The product, recrystallised from water, gave the *glyoxalinylpropionic acid* (0·6 g.), m. p. 229—230° (Found: C, 48·6; H, 6·0. $C_8H_{12}O_2N_2S$ requires C, 48·0; H, 6·0%).

1-Benzoyl-5-butyryl-2-pyrrolidone.—Benzoyl-DL-glutamic acid (5 g.), butyric anhydride (20 ml.) and 2-picoline (15 ml.) were heated for 1 hr. at 110°, then concentrated at 0·1 mm. The residue was dissolved in ether (50 ml.) and the solvent was allowed to evaporate at room temperature. *1-Benzoyl-5-butyryl-2-pyrrolidone* (1·6 g.) separated and after recrystallisation from water had m. p. 67° (Found: C, 69·8; H, 6·9. $C_{15}H_{17}O_3N$ requires C, 69·5; H, 6·6%).

β-(2-Mercapto-4-propyl-5-glyoxalinyl)propionic Acid.—1-Benzoyl-5-butyryl-2-pyrrolidone (0·5 g.) was refluxed with 20% hydrochloric acid (25 ml.) for 2 hr. The amino-ketone obtained was treated with potassium thiocyanate (1 g.) and *β-(2-mercapto-4-propyl-4-glyoxalinyl)propionic acid*, m. p. 217°, was obtained as above (Found: C, 51·0; H, 6·7. $C_9H_{14}O_2N_2S$ requires C, 50·5; H, 6·5%).

1-*Benzoyl*-5-*hexanoyl*-2-*pyrrolidone*.—Benzoyl-DL-glutamic acid (2 g.), hexanoic anhydride (8 ml.), and 2-picoline (8 ml.) were heated for 2 hr. at 120°. After cooling, ethanol (10 ml.) was added and the solution kept overnight. Solvents were removed at 0·1 mm Water (40 ml.) was added to the oily residue and the liquid was made just alkaline by addition of solid sodium hydrogen carbonate. The insoluble oil crystallised, to give 1-*benzoyl*-5-*hexanoyl*-2-*pyrrolidone* (0·7 g.), m. p. 83—84° (from water) (Found : C, 71·1; H, 7·4. $C_{17}H_{21}O_3N$ requires C, 71·1; H, 7·3%).

1 : 5-*Dibenzoyl*-2-*pyrrolidone*.—Benzoyl-DL-glutamic acid (2·5 g.), benzoic anhydride (5 g.), and 2-picoline (10 ml.) were heated at 90—100° for 1½ hr., then poured into water (100 ml.) which was then acidified to Congo-red with dilute hydrochloric acid. The precipitate was washed with sodium hydrogen carbonate solution to remove benzoic acid, and the residue, after being washed with water and then ethanol, consisted of 1 : 5-*dibenzoyl*-2-*pyrrolidone* (0·75 g.). Recrystallised from ethanol it had m. p. 167° (Found : C, 74·1; H, 5·4. $C_{18}H_{15}O_3N$ requires C, 73·7; H, 5·2%).

β-(2-*Mercapto*-4-*glyoxalinyl*)*propionic Acid*.—By Bullerwell and Lawson's method (*J.*, 1951, 3031), diethyl DL-benzoylglutamate (from DL-glutamic acid, 10 g.) was reduced at pH 2·3 and 0° by sodium amalgam (2·3%; 400 g.). Subsequent condensation with potassium thiocyanate (12 g.) gave β-(2-mercapto-4-glyoxalinyl)propionic acid (4·1 g., 35%), m. p. 203—205° (from water).

2′-*Mercaptoglyoxalino*(1′ : 5′-1 : 5)*pyrrolid*-2-*one*.—β-(2-Mercapto-4-glyoxalinyl)propionic acid (1 g.) was dissolved in boiling acetic anhydride (10 ml.). On cooling, 2′-*acetylthioglyoxalino*-(1′ : 5′-1 : 5)*pyrrolid*-2-*one* separated. Recrystallised from ethyl acetate it had m. p. 192° (Found : C, 49·4; H, 4·3. $C_8H_8O_2N_2S$ requires C, 49·0; H, 4·1%).

This was rapidly crystallised from boiling water, the clear solution being chilled in ice. 2′-*Mercaptoglyoxalino*(1′ : 5′-1 : 5)*pyrrolid*-2-*one*, m. p. 228°, separated (Found : C, 47·0; H, 4·4. $C_6H_6ON_2S$ requires C, 46·8; H, 3·9%).

β-(2-*Mercapto*-4-*glyoxalinyl*)*propionamide*.—2′-Acetylthioglyoxalino(1′ : 5′-1 : 5)pyrrolid-2-one (1 g.) was shaken with concentrated ammonia solution (10 ml.) until all went into solution. After 1 hr., the solution was evaporated to dryness *in vacuo*, and the residue was recrystallised from water containing a few drops of acetic acid. The *propionamide* (0·75 g.) so obtained had m. p. 210° (Found : C, 42·9; H, 5·4; N, 24·3. $C_6H_9ON_3S$ requires C, 42·1; H, 5·3; N, 24·6%).

Reduction of Diethyl Glutamate at pH 3—5.—Reduction of the ester (25 g.) with sodium amalgam (600 g.; 2·5%) was carried out as previously described, except that the pH was maintained between 3 and 5. When all the amalgam had been added, the solution was kept at pH 4·0 for 1 hr. at room temperature before being boiled with ammonium thiocyanate (33 g.) for 0·5 hr., then kept overnight at 0°; the crystals were filtered off, dried, and extracted with benzene. The residue, recrystallised from ethanol, gave colourless needles of *ethyl* β-(2-*mercapto*-5-*glyoxalinyl*)*propionate* (8·5 g.), m. p. 151° (Found : C, 48·3; H, 6·4. $C_8H_{12}O_2N_2S$ requires C, 48·1; H, 6·6%). Hydrolysis with concentrated hydrochloric acid gave 2-mercaptoglyoxalinylpropionic acid. On concentration of the benzene extract and addition of light petroleum (b. p. 60—80°), there were deposited, at 0°, colourless needles of *ethyl* β-1-(3-*ethoxycarbonyl*-1-*formylpropyl*)-2-*mercapto*- or -5-*glyoxalinylpropionate*, m. p. 99—100° (maximum yield, 3·3 g.) (Found : C, 52·5; H, 6·4; N, 8·2; S, 9·5. $C_{15}H_{22}O_5N_2S$ requires C, 52·6; H, 6·4; N, 8·2; S, 9·3%). The 2 : 4-*dinitrophenylhydrazone*, prisms from toluene, had m. p. 144° (Found : C, 48·9; H, 4·9. $C_{21}H_{26}O_8N_6S$ requires C, 48·2; H, 5·0%). This ester (0·3 g.) was hydrolysed by cold 3N-sodium hydroxide (10 ml.) for 24 hr. Addition of concentrated hydrochloric acid to pH 4, and recrystallisation of the precipitate from water gave light yellow needles of β-1-(3-*carboxy*-1-*formylpropyl*)-2-*mercapto*-4- or -5-*glyoxalinylpropionic acid*, m. p. 227—228° (0·12 g.) (Found : C, 45·4; H, 5·1; N, 9·8; S, 11·4. $C_{11}H_{14}O_5N_2S$ requires C, 46·2; H, 4·9; N, 9·8; S, 11·2%).

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