465. *A Novel Synthesis of Histidine.*

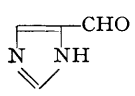
By A. C. Davis and A. L. Levy.

Dithiohydantoin and 2-phenyl-4-ethoxymethyleneoxazolone condense at room temperature in presence of triethylamine and sodium methoxide to give *methyl α-benzamido-β-(2 : 4-dimercapto-5-glyoxalinyl)acrylate* (VII) (93%), the intensely coloured *oxazolone* (VI) being an intermediate. (VII) is simultaneously reduced and desulphurised by Raney nickel at room temperature to give DL-N^{α}-*benzoylhistidine methyl ester* (42%), hydrolysed by acid to DL-histidine. The amino-acid is also made in 53% overall yield by reducing, with red phosphorus and hydriodic acid, the condensation product of 5-formylglyoxaline with 2-mercaptothiazol-5-one (X).

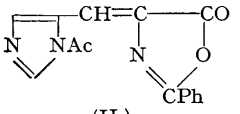
A NEW synthetic approach to the glyoxaline nucleus was demonstrated (Cook, Heilbron, and Levy, " Studies in the Azole Series," Part II, *J.*, 1947, 1598) by the desulphurisation of 2 : 4-dithio-5-phenylhydantoin with Raney nickel to give 5(4)-phenylglyoxaline. With the exception of the purines, which have already been considered in the papers of the Azole Series, the most important natural compounds containing the glyoxaline nucleus are those related to histidine (see Fox, *Chem. Reviews*, 1943, **32**, 47), and the present communication concerns a synthesis of this amino-acid from dithiohydantoin.

Three previous synthetic routes to histidine are recorded in the literature. Pyman's classical synthesis (*J.*, 1911, **99**, 1386) employed 5(4)-hydroxymethylglyoxaline, built up in five stages from citric acid, the derived chloromethyl compound being condensed with sodiochloromalonic ester; hydrolysis and decarboxylation, followed by amination, produced histidine in 21% yield from hydroxymethylglyoxaline.

Pyman subsequently (*J.*, 1916, **109**, 186) modified the later stages by oxidation of the 5(4)-hydroxymethylglyoxaline to the corresponding aldehyde (I) (50% yield) and condensed this compound with hippuric acid by an Erlenmeyer synthesis to give the corresponding oxazolone (II) (68—76% yield). Treatment with sodium carbonate caused deacetylation and ring opening of the oxazolone to give the acid (III) (88% yield). (III) was reduced by sodium amalgam to DL-N^{α}-benzoylhistidine (42% yield) which was then hydrolysed to histidine with concentrated hydrochloric acid (overall yield 20% from formylglyoxaline).



(I.)



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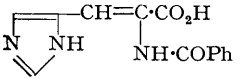
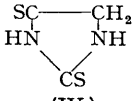


(III.)

The third and most recent synthesis, due to Albertson (*J. Amer. Chem. Soc.*, 1945, **67**, 308 and 502), was similar in principle to Pyman's earlier method, except that the amino-group was introduced at an early stage as acetamido-malonic or -cyanoacetic ester. The former was condensed with 4(5)-chloromethylglyoxaline (now more readily available in two stages from fructose), and the product simultaneously hydrolysed and decarboxylated to histidine, in 18% yield from cane sugar.

In " Studies in the Azole Series," Part III, Cook, Heilbron, and Levy (*J.*, 1948, 207) recorded the preparation of 2 : 4-dithiohydantoin (IV) in 67% yield from aminoacetonitrile, and demonstrated the presence of an active methylene group in this compound by its ready condensation with acetone to yield 2 : 4-dithio-5-*iso*propylidenehydantoin. For the analogous



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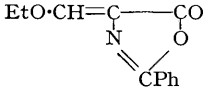

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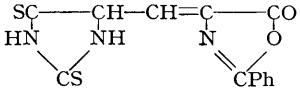

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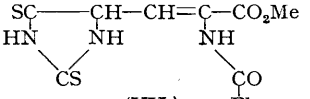
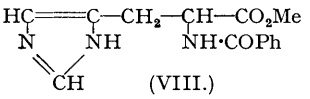


(VII.)

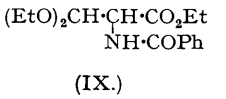
introduction of an amino-acid residue, the aldehyde OHC·CH(NH_2)·CO_2H would be required, and, as a result of chemical studies on penicillin, this was readily available in the form of its derivative, 2-phenyl-4-ethoxymethyleneoxazolone (V).

When (IV) and (V) were brought together in cold methanol containing one equivalent of triethylamine, an intense colour at once developed, due to the cyanine-like condensation product, *2-phenyl-4-(2′ : 4′-dimercapto-5′-glyoxalinylmethylene)oxazolone* (VI) which separated, when the mixture was kept, as a *monotriethylamine* salt in fine purple-red needles. The parent thiol was readily obtained as a purple-black microcrystalline powder on acidification of an

aqueous solution. When either of these compounds was treated with Raney nickel at room temperature, desulphurisation occurred, as shown by rapid loss of colour, but the product was firmly bound to the nickel and could not be removed by extraction with solvents, including pyridine or aqueous sodium hydroxide. This difficulty was overcome when it was observed that another condensation of (IV) and (V) under more vigorous conditions yielded, instead of (VI), a yellow compound isolated as its *triethylamine* salt. This analysed for (VI) with the addition of the elements of methanol, and was evidently the methyl ester (VII). (VII) also was rapidly desulphurised by Raney nickel in ethanol, but in this case a colourless crystalline product was recovered, in 42% yield, of m. p. 151°. This was saturated, free from sulphur, gave a strong Pauly reaction for the glyoxaline nucleus, and was identified as DL-N$^{\alpha}$-*benzoylhistidine methyl ester* (VIII), and it was therefore apparent that reduction of the double bond in (VII) had accompanied desulphurisation.



(VIII.)



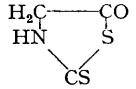
(IX.)

(VII) could also be obtained from (VI) by warming with methanol containing triethylamine, but it was most conveniently prepared in 93% yield directly from (IV) and (V) by treatment with triethylamine followed by sodium methoxide. Further evidence that condensation with dithiohydantoin had preceded opening of the oxazolone ring in such circumstances was given by the failure of ethyl norpenaldate diethyl acetal (IX) to condense under comparable conditions. Treatment of (VI) with methyl sulphate and alkali gave a *tetramethyl* derivative; under the same conditions (VII) also afforded this compound, whilst dithiohydantoin itself gave a dimethyl derivative, formulated as 2 : 4-*dithio*-1 : 3-*dimethylhydantoin* since alkaline hydrolysis did not yield methanethiol.

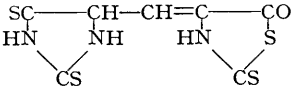
While this material was available, it was thought of interest to treat DL-N$^{\alpha}$-benzoylhistidine, obtained readily by hydrolysis of the ester with alkali, with aniline in the presence of cysteine-activated papain (Bergmann and Fraenkel-Conrat, *J. Biol. Chem.*, 1937, **119**, 707), but no asymmetric synthesis of the anilide took place, possibly owing to the basic nature of the glyoxaline ring hindering its separation from a solution of low pH. Further hydrolysis with hot 20% hydrochloric acid yielded DL-histidine dihydrochloride (70%; Pyman, *loc. cit.*), prepared in this case directly (83%) by hydrolysis of the ester (VIII). Thus, the overall yield of histidine in this three-stage synthesis from dithiohydantoin is 32%.

It is known that serine, in certain peptide-like combinations, is readily transformed into an ethoxymethyleneoxazolone (Copp, Duffin, Smith, and Wilkinson, "The Chemistry of Penicillin," Princeton Univ. Press, p. 747), so that in the above synthesis histidine is in effect constructed from glycine, ammonia, carbon disulphide, and serine. This result suggests that the amino-acid may arise *in vivo* by the elaboration of glycine, carbon dioxide, and ammonia into hydantoin, followed by condensation with serine and reduction to a glyoxaline. 1-Methylhistidine would be derived in this way from sarcosine.

It was shown ("Studies in the Azole Series," Part III, *loc. cit.*) that 5-amino-2-mercaptothiazole yielded dithiohydantoin with bases and 2-mercaptothiazol-5-one (X) with acids, and it seemed therefore an attractive possibility to condense these two substances through ethyl orthoformate, and, proceeding as above, to effect a synthesis of histidine almost entirely from aminoacetonitrile and carbon disulphide. Indeed, 2-mercapto-4-ethoxymethylenethiazol-5-one



(X.)



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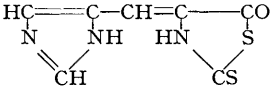

(XII.)

condensed readily with dithiohydantoin in methanol in presence of triethylamine as catalyst, to give the almost black compound (XI). With Raney nickel this was soon decolorised, but as in the case of (VI) the product could not be eluted from the nickel. It was noted that (XI) was remarkably stable, being recovered apparently unchanged after vigorous treatment with caustic alkali and with sodium amalgam.

It was possible, however, to effect a synthesis of histidine from 2-mercaptothiazol-5-one (X) by the method described in the Azole series, Parts VII and XIX (Chatterjee, Cook, Heilbron, and Levy, *J.*, 1948, 1337; Billimoria and Cook, in the press). 4(5)-Formylglyoxaline (I)

was condensed with 2-mercaptothiazol-5-one in glacial acetic acid to yield 80% of *2-mercapto-4-[4′(5′)-glyoxalinylmethylene]thiazol-5-one* (XII). When this compound was heated under reflux with red phosphorus and hydrogen iodide in acetic acid, DL-histidine was obtained in 64% yield (as the dipicrate). This represents double the yield and half the number of stages of the comparable Erlenmeyer synthesis (Pyman, *loc. cit.*).

Experimental.

Condensation of Dithiohydantoin and 2-Phenyl-4-ethoxymethyleneoxazolone.—Dithiohydantoin (4·3 g.) and 2-phenyl-4-ethoxymethyleneoxazolone (6·6 g.; Barber and Slack, *op. cit.*, p. 803) were shaken with water (25 c.c.) and triethylamine (7 g.) until dissolved (*ca.* 10 minutes) and the deep purple-red solution was set aside at room temperature for 1 hour. On acidification, *2-phenyl-4-(2′ : 4′-dimercapto-5′-glyoxalinylmethylene)oxazolone* (VI) (8·7 g.; 90%) was obtained as a reddish-purple, infusible powder (Found: N, 13·7. $C_{13}H_9O_2N_3S_2$ requires N, 13·9%).

Dithiohydantoin (5 g.) and 2-phenyl-4-ethoxymethyleneoxazolone (8·25 g.) were powdered finely and suspended in methanol (40 c.c.). On the addition of triethylamine (6 g.) all the solid dissolved and the temperature rose to 40°. After 36 hours the product (8·8 g.; 57%) was filtered off, washed with acetone and ether, and recrystallised from methanol to give the *mono-triethylamine* salt of (VI) as dark purple-red needles, m. p. 297—300° after darkening at 180° and sintering at 265° (Found: C, 56·3; H, 6·0; N, 14·2. $C_{19}H_{24}O_2N_4S_2$ requires C, 56·4; H, 6·0; N, 13·9%). Evaporation of the filtrate yielded an oily residue, which dissolved in water and was acidified, to yield 4·2 g. (36%) of the free thiol (VI).

Methyl α-Benzamido-β-(2 : 4-dimercapto-5-glyoxalinyl)acrylate (VII).—(*a*) Dithiohydantoin (2 g.) and 2-phenyl-4-ethoxymethyleneoxazolone (3·3 g.) were heated under reflux for 5 minutes in methanol (20 c.c.) containing water (1 c.c.) and triethylamine (3 c.c.). After the mixture had been cooled and kept overnight at room temperature, the crystalline mass was broken up, filtered off, and washed with methanol, leaving yellow needles (3·3 g.; 53%), m. p. 180° (decomp.). The *triethylamine* salt of methyl α-benzamido-β-(2 : 4-dimercapto-5-glyoxalinyl)acrylate was recrystallised from water (Found: C, 55·1; H, 6·45. $C_{20}H_{28}O_3N_4S_2$ requires C, 55·0; H, 6·5%).

(*b*) Dithiohydantoin (10·5 g.) and 2-phenyl-4-ethoxymethyleneoxazolone (17·3 g.) were treated with methanol (40 c.c.) and triethylamine (11 c.c.), whereupon the temperature rose almost to boiling point. After 0·5 hour at room temperature, 2N-methanolic sodium methoxide (80 c.c.) was added with ice-cooling, and the solution kept for 12 hours at 0°. Dilution with water (1 l.) and acidification with 2N-hydrochloric acid then gave the methyl ester (VII) (25 g.; 93·5%) as a fine yellow powder, m. p. 185—190°, undepressed by admixture with the compound obtained by acidification of an aqueous solution of the above triethylamine salt.

Methylation Experiments.—The oxazolone (VI) (2 g.) was dissolved in 10% aqueous sodium hydroxide (50 c.c.); a colour change from purple to orange-yellow, occurring in 2—3 minutes, indicated opening of the oxazolone ring. Methyl sulphate (6 c.c.) was added gradually with shaking and ice-cooling. The crude *tetramethyl* derivative of (VI) which separated (2·3 g.; 96%) recrystallised from ethanol in thick colourless prisms, m. p. 176° (Found: C, 54·2; H, 5·2; N, 11·5. $C_{17}H_{19}O_3N_3S_2$ requires C, 54·1; H, 5·1; N, 11·1%). During the recrystallisation a *compound* was separated, which formed small felted yellow needles, m. p. 258—260°, crystallising from a large volume of ethanol (Found: C, 54·0; H, 4·15; N, 13·4. $C_{18}H_{16}O_3N_4S_2$ requires C, 54·0; H, 4·0; N, 14·0. $C_{28}H_{26}O_3N_6S_4$ requires C, 54·0; H, 4·2; N, 13·5%). The same tetramethyl compound was obtained from the methyl ester (VII) or the derived triethylamine salt, by methylation under the above conditions. The tetramethyl derivative added on bromine in chloroform, yielding colourless needles, m. p. 169—171° (decomp.), but was resistant to desulphurisation with Raney nickel.

Methylation of dithiohydantoin with an excess of methyl sulphate and alkali gave only a dimethyl derivative, m. p. 90°, which crystallised in fine colourless needles from light petroleum (b. p. 80—100°) (Found: C, 37·85; H, 5·2; N, 17·3. $C_5H_8N_2S_2$ requires C, 37·5; H, 5·0; N, 17·5%). In view of its failure to yield methanethiol when boiled with 2N-caustic alkali, the structure 1 : 3-*dimethyl*-2 : 4-*dithiohydantoin* was assigned to this substance.

DL-N^{α}-*Benzoylhistidine Methyl Ester.*—Methyl α-benzamido-β-(2 : 4-dimercapto-5-glyoxalinylmethylene)acrylate (VII) (4 g.) in boiling ethanol (10 c.c.) was treated with Raney nickel (prepared according to *Org. Synth.*, **21**, 15) (16·2 g.), the solution becoming colourless. After being heated under reflux for a further five minutes, the mixture was filtered through a Soxhlet thimble, and the nickel residue continously extracted with ethanol for 2 hours, in order to recover the maximum possible amount of product. The combined solutions were evaporated *in vacuo* to a syrup, dissolved in acetone (5 c.c.), filtered from insoluble material, and re-evaporated. After 1 day, crystallisation was complete, giving 1·4 g. (43%) of DL-N^{α}-*benzoylhistidine methyl ester*, which crystallised in sheaves of colourless needles, m. p. 150—151°, from acetone on the addition of light petroleum (Found: C, 61·4; H, 5·6; N, 15·7. $C_{14}H_{15}O_3N_3$ requires C, 61·5; H, 5·5; N, 15·4%).

The ester was hydrolysed by 10% aqueous sodium hydroxide at 100° to DL-N^{α}-benzoylhistidine, m. p. 248°, which was treated, under the conditions employed by Dekker and Fruton (*J. Biol. Chem.*, 1948, **173**, 471) for benzoylmethionine, with papain and aniline at pH 5. No separation of anilide occurred during 1 week at 40°, or when the experiment was repeated at pH 6 or 7. Seeding with DL-N^{α}-benzoylhistidine anilide, m. p. 250°, prepared by heating DL-N^{α}-benzoylhistidine at 100° with acetic anhydride and treating the resulting golden-yellow 2-phenyl-4-(1′-acetyl-5′-glyoxalinyl)oxazolone, m. p. 183—184°, with aniline in chloroform, was also without avail.

DL-*Histidine.*—DL-N^{α}-Benzoylhistidine methyl ester (0·515 g.) and concentrated hydrochloric acid (30 c.c.) were set aside overnight and then heated for 1·5 hours under reflux. Benzoic acid separated and was removed by ether-extraction. Evaporation *in vacuo* left a syrup, crystallising, on cooling, in sheaves of colourless needles, m. p. 200° (indefinite) (0·478 g., theoretical quantity). From the m. p.

it appears that these were substantially the dihydrochloride (m. p. 235° according to Pyman, *loc. cit.*). The hydrochloride was converted directly into the dipicrate dihydrate (1·03 g., 83%) which formed light orange-yellow needles, m. p. 90—100°; these were recrystallised from water (charcoal), yielding pale yellow needles, m. p. 103° after sintering at 99—100°, not depressed by admixture with an authentic specimen.

Condensation of Dithiohydantoin and 2-Mercapto-4-ethoxymethylenethiazol-5-one.—2-Mercapto-4-ethoxymethylenethiazol-5-one (1·7 g.) (Cook, Heilbron, and Levy, *loc. cit.*) was dissolved in boiling methanol (10 c.c.) containing triethylamine (2 g.; 2 equivs.); dithiohydantoin (1·3 g.) (*idem, loc. cit.*) was added, and, after all the material had dissolved, the solution was cooled and kept for 2 hours at room temperature. By dilution with water (40 c.c.) and acidification with mineral acid, an oil was obtained which was solidified under methanol (20 c.c.). 2-Mercapto-4-(2′ : 4′-dimercapto-5-glyoxalinylmethylene)thiazol-5-one (1·5 g.; 79%) formed a purple-black infusible powder.

Similar intensely-coloured compounds were obtained by condensation of the following components, but were not analysed: 2-ethylthio-4-ethoxymethylenethiazolone and 2-thiohydantoin (red, m. p. above 290°), and 2 : 4-dithiohydantoin (purple-black, m. p. indefinite); 2-mercapto-4-ethoxymethylenethiazolone and 2-thio-3-methylthiazolid-2 : 4-dione [olive-green needles, m. p. 195° (decomp.)]. All these compounds had a green lustrous reflex in the solid state, showing their true colour when finely-ground or when viewed through the microscope. Similar compounds were obtained also from 2-phenyl-4-ethoxymethyleneoxazolone and 2-thiohydantoin [orange-yellow needles, m. p. 153° (decomp.)], and 2-thio-3-methylthiazolid-2 : 4-dione [yellow felted needles, m. p. 260° (decomp.)].

DL-*Histidine from* 4(5)-*Formylglyoxaline.*—(This synthesis was performed by Mr. G. F. Woods, to whom we express our thanks for permission to include it in the present paper.) 2-Mercaptothiazol-5-one (2·3 g.) and 4(5)-formylglyoxaline (2·6 g.) [prepared by the oxidation (Pyman, *loc. cit.*) of 4(5)-hydroxymethylglyoxaline (*Org. Synth.*, **24**, 64)] were together dissolved in glacial acetic acid (25 c.c.). The solution was heated to boiling, whereupon an exothermic reaction commenced and boiling maintained itself for several minutes. On cooling, 2-*mercapto*-4-(5′-*glyoxalinylmethylene*)*thiazol*-5-*one* (3·8 g.; 80%), m. p. 258—260°, separated and recrystallised from acetic acid as yellow-green plates, m. p. 260° (decomp.) (Found: C, 39·9; H, 2·2; N, 19·65. $C_7H_5ON_3S_2$ requires C, 39·8; H, 2·4; N, 19·85%).

The above thiazolone (1·5 g.) with red phosphorus (1·5 g.) and hydriodic acid (50% aq., 2·5 c.c.) in acetic acid (25 c.c.), and acetic anhydride (5 c.c.) was boiled under reflux for 1·5 hours. By this time all the soluble material had dissolved and the excess of phosphorus was removed by filtration through glass wool. The solution was then evaporated to leave a syrupy residue, extraction of which with ether left a semi-crystalline residue. This crude hydriodide was dissolved in water and converted into the dipicrate, m. p. 187—188° (after drying at 100°) (2·8 g., 64%), from which the free amino-acid was prepared by the method of Pyman (*loc. cit.*). Thus obtained, the amino-acid formed colourless quadrilateral plates, m. p. 281—283°, which rose to 283—284° after several recrystallisations from water and was not depressed by admixture with an authentic specimen (Found: N, 27·0. Calc. for $C_6H_9O_2N_3$: N, 27·1%).

We are deeply indebted to Sir Ian Heilbron, D.S.O., F.R.S., and Dr. A. H. Cook for their helpful interest in this work, to the Department of Scientific and Industrial Research for a Senior Research Award (A. L. L.), and to the Rockefeller Foundation for a grant (A. C. D.).

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[*Received, April 9th,* 1949.]