CXIV. SYNTHESIS OF THE *d*- AND *l*-3:4-DIHYDROXYPHENYLALANINES.

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ALTHOUGH *dl*-3:4-dihydroxyphenylalanine has been synthesised on several occasions [Funk, 1911; Stephen and Weizmann, 1914; Harington and McCartney, 1927], the optically active forms of this amino-acid have not so far been artificially obtained. *l*-3:4-Dihydroxyphenylalanine was first isolated by Guggenheim [1913] from *Vicia faba*; renewed biological interest attaches to this amino-acid as the result of the recent work of Raper [1926], who has shown that it represents the first stage in the formation of melanin from tyrosine by the enzyme tyrosinase. It therefore appeared to us desirable to complete the synthesis of *l*-3:4-dihydroxyphenylalanine by resolution of the *dl*-amino-acid into its optically active components.

We attempted first to formylate the *dl*-amino-acid with the intention of subsequently resolving the formyl derivative by means of suitable alkaloidal salts. The formyl derivative turned out, however, to be an excessively soluble substance which was also difficult to handle owing to the extreme ease with which it was oxidised.

We then turned our attention to the preparation of a triacetyl derivative of dihydroxyphenylalanine which would be free from the objection of ready oxidisability. Having failed, in this case, to obtain any good result by the elegant method of acetylation with ketene, recently described by Bergmann [1930], we proceeded as follows. Protocatechuic aldehyde was condensed with acetyl glycine in presence of acetic anhydride and sodium acetate. The azlactone formed in this reaction is much less stable than is generally the case, and on standing in contact with water is converted into β -3:4-diacetoxyphenyl- α -acetaminoacrylic acid. Reduction of this compound to the corresponding propionic acid derivative by means of sodium amalgam in the ordinary way was excluded by the ease with which the acetoxy-groups were hydrolysed in alkaline solution. It could, however, be effected readily and in good yield by a catalytic method. The resulting β -3:4-diacetoxyphenyl- α -acetaminopropionic acid was treated with one equivalent of hydrated brucine in concentrated alcoholic solution. Crystallisation set in very slowly and was not complete until a fortnight had elapsed. During the crystallisation a smell of ethyl acetate

developed and it was found in fact that the salt which had separated was the brucine salt of *l*-β : 3 : 4-dihydroxyphenyl-α-acetaminopropionic acid, elimination of the O-acetyl groups having occurred during the protracted crystallisation. The salt of the corresponding *d*-acid was obtained from the mother-liquor.

The free acids, as was to be expected, were excessively soluble compounds and very readily oxidisable. They could not be isolated satisfactorily and the solutions recovered from the salts after removal of the alkaloid in the usual way were, therefore, submitted directly to hydrolysis with hydrochloric acid. In this way there were obtained preparations of the *d*- and *l*-amino-acids having respectively $[\alpha]_D + 11.9^\circ$ and $- 12.0^\circ$. The *l*-amino-acid corresponded in all its properties with a sample of the natural acid isolated from *Vicia faba*.

EXPERIMENTAL.

β-3 : 4-Diacetoxyphenyl-α-acetaminoacrylic acid. Protocatechuic aldehyde (21.6 g.), acetylglycine (18.4 g.) and freshly fused sodium acetate (12.8 g.) were ground together to form an intimate mixture. This was treated with freshly distilled acetic anhydride (60 cc.) and the whole was heated on the steam-bath for 2 hours. After cooling, the dark-coloured viscous solution was poured into cold water (750 cc.). The sticky yellow gum which separated hardened on keeping overnight. It was collected, washed with water and crystallised from ethyl acetate. A further crop was obtained by concentrating the ethyl acetate mother-liquor, stirring up the residue with water, keeping overnight and then proceeding as before. The total yield was 18 g. For analysis the acid was recrystallised from water, decoloration being effected with charcoal. It separated in glistening white plates. The air-dried material sintered at 115° – 120° and melted at 183.4° . On heating at 100° it lost $1\text{H}_2\text{O}$ and the anhydrous product melted at 187 – 8° .

(Found for the anhydrous product: C, 56.1; H, 4.8; N, 4.4 %. $\text{C}_{15}\text{H}_{15}\text{O}_7\text{N}$ requires: C, 56.1; H, 4.7; N, 4.4 %. 300 mg. of the air-dried material lost 17.3 mg. in 3 hours at 100° ; whence loss of weight = 5.8 %; calculated for $1\text{H}_2\text{O}$ 5.3 %.)

β-3 : 4-Diacetoxyphenyl-α-acetaminopropionic acid. The above-described unsaturated acid (28 g.) was dissolved in warm water (4000 cc.) The solution was cooled to the ordinary temperature, and, after addition of 1 g. of palladium chloride (dissolved in water with the aid of the minimum amount of hydrochloric acid), was shaken in an atmosphere of hydrogen. The uptake of hydrogen ceased after 2 hours, by which time 2600 cc. had been absorbed. On keeping the solution overnight the catalyst flocculated and could easily be removed by filtration. The filtrate was digested with a suspension of well-washed silver carbonate; after removal of the precipitate the solution was freed from excess of silver with hydrogen sulphide and concentrated under diminished pressure to about 800 cc. when separation of crystalline material commenced. The crystals were collected after the solution had stood overnight and amounted to 17.4 g.; a further crop of 3 g. was obtained by concentrating the mother-liquor to about 75 cc.

The acid was recrystallised from water from which it separated in small colourless blunt prisms, m.p. 171–172°. It is readily soluble in hot water, sparingly so in cold. It does not absorb bromine nor does it decolorise alkaline permanganate immediately.

(Found: C, 55.6; H, 5.3; N, 4.4 %. $C_{15}H_{17}O_7N$ requires: C, 55.7; H, 5.3; N, 4.3 %.)

Brucine l-β-3 : 4-dihydroxyphenyl-α-acetaminopropionate. dl-β-3 : 4-Diacetoxyphenyl-α-acetaminopropionic acid (27 g.) and brucine (38.5 g. of the hydrated alkaloid) were dissolved together in absolute alcohol (270 cc.) and the solution was set aside. Crystallisation commenced in about 24 hours. After 14 days the solid, which appeared not to be increasing in amount any further, was filtered off, washed with a little alcohol and dried. The yield was 19 g. The salt was recrystallised from 95 % alcohol from which it separated in colourless needles containing $2H_2O$; attempts to remove the water of crystallisation failed owing to decomposition of the salt at the high temperature required; on heating in a capillary tube it sintered at 157°, and melted with effervescence at 176°.

It was readily soluble in water, sparingly so in absolute alcohol, and insoluble in other organic solvents.

After two recrystallisations the rotation became constant

$$\alpha_{5461}^{20} = -1.89^\circ (c = 2.04, l = 2);$$

whence

$$[\alpha]_{5461}^{20} = -46.3^\circ.$$

The strong smell of ethyl acetate which had developed during the slow separation of this salt had suggested the possibility of de-acetylation. That such de-acetylation had indeed occurred was shown by the analysis of the salt, the figures agreeing well with those required by the brucine salt of *N*-acetyl-3 : 4-dihydroxyphenylalanine with $2H_2O$. (Found: C, 61.48; H, 6.47; N, 6.64 %. $C_{34}H_{38}O_9N_3, 2H_2O$ requires: C, 61.1; H, 6.43; N, 6.28 %.) Further, the salt gave with ferric chloride in neutral aqueous solution an intense green coloration which passed into reddish-purple on addition of sodium bicarbonate, indicating that the adjacent hydroxyl groups attached to the benzene ring were free.

Brucine d-β-3 : 4-dihydroxyphenyl-α-acetaminopropionate. The alcoholic mother-liquor after removal of the crude insoluble brucine salt was concentrated under diminished pressure to a syrup. This was kept for a week, during which time it partly solidified. The pasty mass was boiled out three times in succession with ethyl acetate and then triturated with ether until it became wholly granular. The crude dried material (24 g.) was crystallised from water, in which it was very soluble at the boiling point but sparingly so in the cold. After two recrystallisations the optical rotation became constant

$$\alpha_{5461}^{20} = +0.31^\circ (c = 2.04, l = 2);$$

whence

$$[\alpha]_{5461}^{20} = +7.6^\circ.$$

The salt formed long narrow prisms containing $2\text{H}_2\text{O}$, which sintered at 150° and melted with effervescence at 154° .

(Found: C, 61.46; H, 6.51; N, 6.35 %. $\text{C}_{34}\text{H}_{39}\text{O}_9\text{N}_3, 2\text{H}_2\text{O}$ requires: C, 61.1; H, 6.43; N, 6.28 %.)

d-3 : 4-Dihydroxyphenylalanine. The laevorotatory brucine salt described above was dissolved in water and the solution, cooled in ice, was treated with excess of saturated aqueous barium hydroxide. The mixture was stirred vigorously with a glass rod for about 5 minutes whilst still in the ice in order to accelerate the separation of the alkaloid. This was filtered off rapidly and the last traces were removed by extraction with chloroform, the air in the separating-funnel being displaced with hydrogen. With as little delay as possible the aqueous solution was treated with just sufficient dilute sulphuric acid to precipitate all the barium, brought to the boil and filtered. The filtrate was concentrated under diminished pressure to about 15 cc. for every 5 g. of salt taken, treated with strong hydrochloric acid to make a final concentration of 10 % HCl and boiled under reflux for 1 hour. The solution was then evaporated to dryness under diminished pressure; the residue was dissolved in a little water, and ammonia was cautiously added, under a layer of light petroleum, until present in slight excess. The whole was then rapidly evaporated to dryness under diminished pressure in a stream of hydrogen. The crystalline residue was purified by dissolving in boiling water containing a trace of sulphur dioxide and treatment with charcoal; the filtered solution deposited stout colourless prisms of the amino-acid on cooling. The amino-acid melted at 282° with decomposition. In 5.01 % solution in *N* hydrochloric acid the observed rotations were $\alpha_{5461} + 1.33^\circ$ and $\alpha_D + 1.20^\circ$ respectively in a 2 dm. tube; whence $[\alpha]_{5461} = + 13.2^\circ$ and $[\alpha]_D = + 11.9^\circ$.

l-3 : 4-Dihydroxyphenylalanine. This was obtained in a precisely similar manner from the alcohol-soluble dextrorotatory brucine salt and formed colourless prisms, m.p. 282° (decomp.). In 5.04 % solution in *N* hydrochloric acid the observed rotations in a 2 dm. tube were $\alpha_{5461} - 1.30^\circ$ and $\alpha_D - 1.21^\circ$ respectively, whence $[\alpha]_{5461} = - 12.9^\circ$ and $[\alpha]_D = - 12.0^\circ$. A sample of natural *l-3 : 4-dihydroxyphenylalanine*, kindly supplied by Dr M. Guggenheim, had m.p. 281° (decomp.); this m.p. was unaltered by admixture with the synthetic material. The natural acid had $[\alpha]_{5461} - 13.6^\circ$ and $[\alpha]_D - 12.0^\circ$ ($c = 5.3$, and $l = 2$). The identity of the two acids is, therefore, established.

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