125. *The Synthesis of* β-(3 : 4-*Dihydroxyphenyl*)-N-*methylserine* (*Adrenalinecarboxylic Acid*).

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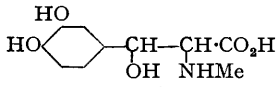
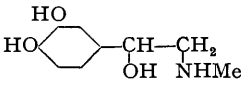
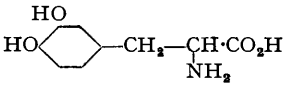
The above amino-acid has been synthesised by the interaction of 3 : 4-diethylcarbonatobenzaldehyde and sarcosine ethyl ester in the presence of sodium. A possible mechanism of the reaction is suggested, and a number of intermediate derivatives are described.

β-(3 : 4-*Dihydroxyphenyl*)-N-*methylserine* (I) is a compound of considerable pharmacological interest in view of its intermediate relationship to adrenaline (II) and " dopa " (III), and we have consequently investigated its synthesis.

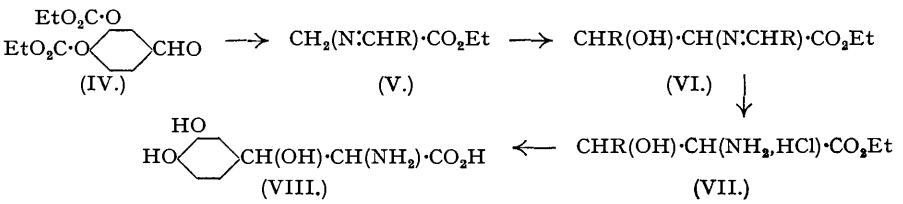
Erlenmeyer (*Annalen*, 1895, **284**, 36) has shown that when certain aromatic aldehydes, for example benzaldehyde, react with glycine in aqueous sodium hydroxide solution, a Schiff's base of type $CH_2(N{:}CHPh)\cdot CO_2H$ is first formed and then undergoes an aldol condensation with a second molecule of aldehyde to give compounds of type $CHPh(OH)\cdot CH(N{:}CHPh)\cdot CO_2H$; hydrolysis then furnishes the α-amino-β-hydroxy-acid $CHPh(OH)\cdot CH(NH_2)\cdot CO_2H$. This condensation is markedly affected by substituents in the phenyl group; Erlenmeyer and Bade


(I.)


(II,)


(III.)

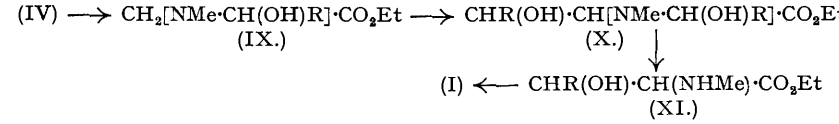
(*ibid.*, 1904, **337**, 235) showed that, although the methyl ether of salicylaldehyde readily underwent the above series of reactions, *p*-anisaldehyde failed to do so. Even when methoxyphenyl derivatives can be obtained by this method, demethylation without dehydration or deamination would be difficult (cf. Barger and Jowett, *J.*, 1905, **87**, 967). To prepare hydroxyphenyl analogues, Rosenmund and Dornsaft (*Ber.*, 1919, **52**, 1734) therefore converted protocatechualdehyde into the dicarbethoxy-derivative (IV), which was treated in ethereal solution with glycine ethyl ester and sodium. They adduced evidence that in this reaction also the initial reaction was the formation of the Schiff's base (V, R = 3 : 4-diethylcarbonatophenyl), which


(IV.) (V.) (VI.) (VII.) (VIII.)

then underwent a similar aldol condensation to (VI). By hydrolysis with alcoholic hydrogen chloride they isolated the crystalline β-(3 : 4-diethylcarbonatophenyl)serine ethyl ester hydrochloride (VII), which after alkaline hydrolysis in a hydrogen atmosphere yielded β-(3 : 4-dihydroxyphenyl)serine (VIII).

We have repeated this synthesis in the hope that *N*-methylation of (VII) would be practicable. (VII) was treated in absolute alcoholic solution first with one equivalent of sodium ethoxide to liberate the free amine and then with diazomethane: no methylation occurred, however, and the amine was ultimately recovered unchanged. (VII) was also treated with benzenesulphonyl chloride in various solvents in the hope that the sulphonamido-compound could subsequently be methylated and the benzenesulphonyl groups then removed catalytically: the benzenesulphonyl derivative formed a glass, however, and could not be purified. It is noteworthy that Rosenmund and Dornsaft's reaction is also critically affected by the nature of the substituents in the benzene ring; we have been unable to condense glycine ester with either veratraldehyde or piperonal under the above conditions.

We have consequently investigated the condensation of various aldehydes in ethereal solution with sarcosine ethyl ester in the presence of sodium, although the absence of a primary amino-group in sarcosine would prevent a series of reactions strictly analogous to those adduced by Rosenmund and Dornsaft. In earlier experiments we could not detect any reaction between veratraldehyde and sarcosine ester or nitrile under these conditions. 3 : 4-Diethylcarbonatobenzaldehyde did, however, react with sarcosine ester in the presence of sodium, the reaction at room temperature requiring about 36 hours. When the product was hydrolysed with alcoholic hydrogen chloride, the hydrochloride of β-(3 : 4-diethylcarbonatophenyl)-N-methylserine ethyl


(IV) (IX.) (X.) (I) (XI.)

ester (XI, R as before) was isolated as a gum which could not be crystallised. It gave a crystalline *picrate*, and when treated in aqueous solution with potassium oxalate gave a crystalline

monohydrated *oxalate*. Recrystallisation of the crude oxalate gave the highly crystalline monohydrated *hydrogen oxalate*, the yield of which, based on the esterified aldehyde and allowing for recovered aldehyde, was 6%.

Alkaline hydrolysis of the hydrogen oxalate in an atmosphere of hydrogen (cf. Rosenmund and Dornsaft), using either sodium or barium hydroxide, failed to give the pure acid. Hydrolysis with dilute acetic acid in an inert atmosphere, however, funished the required β-(3 : 4-dihydroxyphenyl)-*N*-methylserine (I), as cream-coloured crystals, m. p. 233° (decomp.), the yield of the recrystallised acid being 65% based on the hydrogen oxalate used, and 3·9% based on (IV) and allowing for recovered aldehyde.

Further work is required before the mechanism of the above condensation is elucidated. It is significant, however, that the synthesis appears to be successful only if at least two molecules of the esterified aldehyde (IV) are used for each molecule of the sarcosine ester employed. This suggests that the initial stage in the reaction may be the combination of sarcosine ester with one molecule of the aldehyde to give a compound of type (IX), which then unites with a second molecule to give the compound (X); finally the hydrolysis with alcoholic hydrogen chloride removes the first molecule of the aldehyde, giving the esterified amino-acid (XI).

It is noteworthy that throughout our synthesis no indication was obtained of the existence of more than one racemate, and it is at present impossible to say whether our amino-acid has the same configuration as that of naturally occurring adrenaline. In the synthesis of compounds such as $CHPh(OH)\cdot CHPh\cdot NH_2$, different racemates are obtained according to the method employed (cf. Dodds, Lawson and Williams, *Nature*, **1944**, **154**, 514; *Proc. Roy. Soc.*, 1944, *B*, **132**, 119; McPhee and Erickson, *J. Amer. Chem. Soc.*, 1946, **68**, 621).

Experimental.

When duplicate analyses are given they are analyses of different preparations of the compound, and not merely repeated analyses on the same sample.

3 : 4-*Diethylcarbonatobenzaldehyde* (IV).—This was prepared by the action of ethyl chloroformate on a solution of protocatechualdehyde in cold aqueous sodium hydroxide, as Rosenmund and Dornsaft (*loc. cit.*) direct. When the product, isolated by ether extraction and dried, was distilled even at low pressure (<0·1 mm.), 3 : 4-carbonyldioxybenzaldehyde distilled first and crystallised in the receiver, the liquid aldehyde (IV) following; meanwhile the liquid-air trap often became blocked with solid ethyl carbonate. To reduce this decomposition of the aldehyde (IV) to a minimum it was distilled rapidly in moderate quantities, using a high-capacity pump with large traps to avoid choking. The distillation could then be continued without interruption, and the required product, b. p. 186°/0·03 mm., 193°/0·1 mm., (65% yield), could ultimately be either obtained as a separate fraction or, if decomposition was unusually extensive, decanted from the carbonyl-aldehyde which had crystallised in the receiver. It was characterised as the 2 : 4-*dinitrophenylhydrazone*, orange crystals from ethyl acetate, m. p. 165° (Found: N, 12·6. $C_{19}H_{18}O_{10}N_4$ requires N, 12·2%). The aldehyde (IV), after recrystallisation from benzene, had m. p. 122°.

Derivatives of β-3 : 4-*Dihydroxyphenylserine* (VIII).—The aldehyde (IV) (67·5 g.), freshly prepared glycine ethyl ester (12·5 g.), and sodium wire (5·5 g.) in ether (200 c.c.) were shaken vigorously together at intervals over a period of 36 hours (cf. Rosenmund and Dornsaft, *loc. cit.*). Alcoholic hydrogen chloride was added to the filtered solution whereupon the hydrochloride (VII) was deposited as an oil. This was twice extracted with water, and the aqueous extract again extracted with ether. A portion of the aqueous extract was treated with sodium picrate, giving *β-(3 : 4-diethylcarbonatophenyl)serine ethyl ester picrate*, yellow needles from alcohol, m. p. 152° (Found: N, 9·3. $C_{15}H_{23}O_9N,C_6H_3O_7N_3$ requires N, 9·5%). The remainder of the extract was evaporated in a vacuum, and the residue, crystallised from alcohol, gave colourless needles of the corresponding hydrochloride (VII), m. p. 152—153°; 14·2 g. [14% based on the aldehyde (IV)].

Alkaline hydrolysis of (VII) gave *β*-(3 : 4-dihydroxyphenyl)serine (VIII), colourless crystals from water, m. p. 219—221° (Found: N, 6·4. Calc. for $C_9H_{11}O_5N$: N, 6·5%); yield, 28% based on the hydrochloride (VII). Rosenmund and Dornsaft (*loc. cit.*) give m. p. 208—210°. The acid gives a *picrate*, orange crystals, m. p. 90—92° (Found: N, 12·5. $C_9H_{11}O_5N,C_6H_3O_7N_3$ requires N, 12·6%); it also gives a marked and ready ninhydrin reaction on warming.

It is probable that the acid hydrolysis subsequently devised for the *N*-methyl compound (XI), if applied to (VII), would give considerably greater yields of the amino-acid (VIII).

The hydrochloride (VII) when treated in aqueous solution with potassium oxalate gave a sticky precipitate which on crystallisation from alcohol containing a small proportion of water afforded the *ester oxalate monohydrate*, colourless crystals, m. p. 140—141° (decomp.) (Found: C, 49·4; H, 6·0; N, 3·6. $2C_{17}H_{23}O_9N,C_2H_2O_4,H_2O$ requires C, 49·2; H, 5·7; N, 3·2%). This oxalate was treated in aqueous solution with silver nitrate, and the solution, filtered to remove silver oxalate, deposited white leaflets: these, when recrystallised first from alcohol and then water, gave the *ester monohydrate*, m. p. 138—139° (Found: C, 50·6; H, 5·7; N, 3·5. $C_{17}H_{23}O_9N,H_2O$ requires C, 50·6; H, 6·2; N, 3·5%). It is clear from this experiment that the intermediate ester nitrate must have undergone dissociation to liberate the free ester.

Attempted Methylation.—Sodium ethoxide solution (sodium, 0·23 g.: alcohol, 5 c.c.) was added to a solution of the hydrochloride (VII) (4·2 g.) in alcohol (15 c.c.). The mixture was rapidly filtered and treated with an ethereal solution of diazomethane [30 c.c., 0·5 g. CH_2N_2 (1·2 mols.)]. The solution was

subsequently treated with alcoholic hydrogen chloride, but ultimately only the unchanged ester (as VII), isolated as its picrate, could be identified.

When ethereal solutions of 3 : 4-carbonyldioxybenzaldehyde and glycine ester were mixed in the absence of sodium, a turbidity rapidly developed and a yellow oil was deposited. In the absence of ether, the two compounds reacted vigorously with much heat evolution, and the orange-coloured resin formed a glass which could not be purified.

Sarcosine Nitrile and Ethyl Ester.—Staudt's method (*Z. physiol. Chem.*, 1925, **146**, 286) for the preparation of sarcosine nitrile was found to be most effective, carbon dioxide being passed through a stirred, cooled aqueous solution of methylamine sulphate, formaldehyde, and potassium cyanide. Contrary to published statements, the nitrile could be distilled with very little decomposition as a colourless viscous liquid, b. p. 95—105°/0·2 mm., which remained unchanged after several months. Yield, 68% based on the methylamine. It gave a *picrate*, yellow needles from alcohol, m. p. 142—143° (Found : N, 23·4. $C_3H_6N_2,C_6H_3O_7N_3$ requires N, 23·4%). The nitrile was converted into the ester hydrochloride by Staudt's method (*loc. cit.*), and the free ester isolated by treating the hydrochloride in ice-cold aqueous solution with 30% sodium hydroxide solution, followed by rapid ether extraction and distillation; colourless liquid, b. p. 52°/15 mm.; yield, 37% based on the nitrile, 25% on the methylamine employed.

Derivatives of β-(3 : 4-Dihydroxyphenyl)-N-methylserine (I).—Fine sodium wire (7 g., 7 atoms) was added to a mixture of the aldehyde (IV) (90 g., 2 mols.) and freshly distilled sarcosine ethyl ester (17·3 g., 1 mol.) in dry ether (450 c.c.); glass beads or fragments of porcelain were also added. Effervescence occurred, and the sodium became covered with a yellow film. The mixture was vigorously shaken at intervals, the beads assisting the removal of this film from the sodium. No detectable reaction had occurred after 12 hours, but the occasional shaking was continued for 36 hours; a shorter period caused a decreased yield. Ether was then added until deposition of a yellow material (in small amount) ceased, and alcoholic hydrogen chloride was then added to the filtered solution until precipitation of the crude hydrochloride of the ester (XI) was complete. This yellow viscous hydrochloride was twice extracted with water, and the extract, after being shaken with ether, was evaporated under reduced pressure, depositing the hydrochloride as an uncrystallisable gum. A portion of this gum, dissolved in water and treated with sodium picrate, gave *β-(3 : 4-diethylcarbonatophenyl)-N-methylserine ethyl ester picrate*, yellow plates from alcohol, m. p. 144° (Found : C, 46·3; H, 4·8; N, 9·1. $C_{18}H_{25}O_9N,C_6H_3O_7N_3$ requires C, 45·9; H, 4·5; N, 8·9%).

Aqueous potassium oxalate solution was added to a concentrated solution of the gummy hydrochloride, precipitating an oil which crystallised after the mixture had been set aside overnight. This crude oxalate was collected, and the filtrate then slowly deposited white crystals of the pure *oxalate monohydrate* of the above ester (XI), m. p. 147° (decomp.) (Found : C, 50·5; H, 5·9; N, 3·2. $2C_{18}H_{25}O_9N,C_2H_2O_4,H_2O$ requires C, 50·3; H, 5·9; N, 3·1%). The crude oxalate, when recrystallised from alcohol containing a small proportion of water, afforded the *hydrogen oxalate monohydrate*, glistening white leaflets, m. p. 157° (decomp.) (Found : C, 47·5, 47·5; H, 5·8, 5·7; N, 3·0, 2·9. $C_{18}H_{25}O_9N,C_2H_2O_4,H_2O$ requires C, 47·3; H, 5·7; N, 2·8%); yield, 3·2 g. (2% based on initial esterified aldehyde, 6% allowing for recovered aldehyde). This oxalate was unchanged when heated at 80°/0·1 mm. (Found : C, 47·5; H, 5·6%); m. p. 157° (decomp.). It is probable that the crude oxalate initially precipitated consisted mainly of the hydrogen oxalate. Had it consisted of the normal oxalate, recrystallisation must have involved dissociation to the hydrogen oxalate and the free base; yet the mother-liquor from the recrystallisation, when treated with oxalic acid, gave no further precipitation of the hydrogen oxalate, although with picric acid it furnished the above picrate.

A portion of the hydrogen oxalate in aqueous solution was treated with silver nitrate (2 mols.), as in the experiment with the unmethylated product, but neither the ester nitrate nor the free ester could be isolated.

The ethereal residues from the preparation of the crude hydrochloride were united, dried, and distilled. After removal of the ether, an unidentified fraction, b. p. 110—130°/0·2 mm., was obtained, but the residual esterified aldehyde (60 g.) could not be distilled without decomposition. It was therefore hydrolysed by refluxing with 5% hydrochloric acid (charcoal) for 3 hours, and the filtered solution was then evaporated under reduced pressure until crystallisation of protocatechualdehyde began; the mixture was cooled and the aldehyde collected, dried, and re-esterified for subsequent preparations.

Hydrolysis. A solution of the pure hydrogen oxalate monohydrate (2·3 g.) in acetic acid (30 c.c.) and water (70 c.c.) was refluxed in nitrogen atmosphere for 6 hours. The solution was concentrated under reduced pressure, and then taken to dryness in a vacuum desiccator. The residue, crystallised from water containing a small proportion of alcohol, gave cream-coloured crystals of *β-(3 : 4-dihydroxypheny)-N-methylserine* (I), m. p. 233° (decomp., with darkening above 220°) (Found : C, 53·1; 53·1; H, 5·4, 5·5; N, 6·1. $C_{10}H_{13}O_5N$ requires C, 52·9; H, 5·7; N, 6·2%). Yield of recrystallised acid, 0·65 g. [65% based on oxalate, 3·5% based on (IV) allowing for recovered aldehyde].

Alternatively, the crude unrecrystallised oxalate was hydrolysed as above, and the solution then concentrated under reduced pressure, cooled, and diluted with acetone. The acid (I) was deposited as colourless needles, m. p. 233° (decomp.), which, however, darkened slightly on recrystallisation. This method is quicker and the yield rather higher (1·3% based on initial esterified aldehyde, 3·9% allowing for recovered aldehyde).

Attempted alkaline hydrolysis of the oxalate with sodium hydroxide caused much oxidation, and with barium hydroxide gave a brown product from which total removal of the barium was difficult. Attempts to hydrolyse the crude hydrochloride directly, by boiling it, either alone in aqueous solution or with dilute acetic acid containing sodium acetate, did not prove satisfactory.

The amino-acid (I) is moderately soluble in cold water, and oxidises readily in aqueous solution, especially in the presence of alkalis. It is almost insoluble in cold, but slightly soluble in hot, alcohol. Its aqueous solution does not precipitate a picrate with picric acid solution. It gives a ninhydrin reaction, the aqueous solution on boiling becoming first red and then blue; this reaction occurs more slowly than with the unmethylated acid (VIII).

Attempts to condense veratraldehyde with sarcosine ester (*a*) in ether in the presence of sodium, (*b*) in a mixture of pyridine and acetic anhydride, (*c*) in a mixture of pyridine and piperidine (cf. Boyd and Robson, *Biochem. J.*, 1935, **29**, 542) all failed. Similarly, veratraldehyde could not be condensed with sarcosine nitrile in the presence of sodium.

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