815. *The Configuration of Noradrenaline.*

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The stereochemical configuration of noradrenaline has been correlated by chemical means with that of the mandelic acid, through 3-hydroxy-4-methoxymandelic acid. The configuration of D(−)-mandelic acid is found for natural lævorotatory noradrenaline. Thereby the steric correspondence between natural noradrenaline and adrenaline has been definitively established. Deductions are possible also regarding the configuration of the β-carbon atom in the 3,4-dihydroxyphenylserines.

Knowledge of the optical configuration of noradrenaline is an important element in interpreting the biochemical behaviour and the biological properties of this catecholamine. Moreover it makes it possible to establish the configurational relations between this base and other substances which are in biogenetic relationship with noradrenaline, such as adrenaline and 3,4-dihydroxyphenylserine.

Attempts to solve the problem of the configuration of noradrenaline have been made on the basis of biochemical data, namely, the decarboxylation of 3,4-dihydroxyphenylserine by means of L-amino-acid decarboxylase.[1,2] These attempts are based on the assumption that the decarboxylase will only attack the isomer with L-configuration at the α-carbon atom and on knowledge of the configuration of the substrate. But since at present the latter cannot be considered to be unequivocally established,[2] it follows that the configurational assignment for noradrenaline is not definitive.

We here report a purely chemical determination of the configuration of noradrenaline.[3] The method followed is similar to that used by us for determining the configuration of adrenaline.[4] It consists of correlating the configuration of the catecholamine with that of a suitably substituted mandelic acid of known configuration, through transformations which exclude racemization or inversion of configuration at the centre of asymmetry.

3-Hydroxy-4-methoxymandelic acid was taken as the reference substance, its configuration having already been determined.[4] Methyl L(+)-3-hydroxy-4-methoxymandelate (XI) was transformed by means of diazomethane into the oily methyl L(+)-3,4-dimethoxymandelate (XII), which with gaseous ammonia gave the L(+)-amide (XIII). By reduction with lithium aluminium hydride in tetrahydrofuran, the latter gave L(+)-O^3O^4-dimethylnoradrenaline (XIV) whose hydrochloride had m. p. 168—169° and $[\alpha]_D^{18}$ +33·6° (1·1% w/v in 50% EtOH).

The optical antipode (VII) of the amine (XIV) was obtained from D(−)-4-hydroxy-3-methoxymandelic acid (VIII), prepared by the method of Armstrong, McMillan, and

[1] Blaschko, Holton, and Stanley, *Brit. J. Pharmacol.*, 1948, **3**, 315; Dalgliesh, *J.*, 1953, 3323; Hartman, Pogrund, Drell, and Clark, *J. Amer. Chem. Soc.*, 1955, **77**, 816.

[2] Drell, *ibid.*, p. 5429.

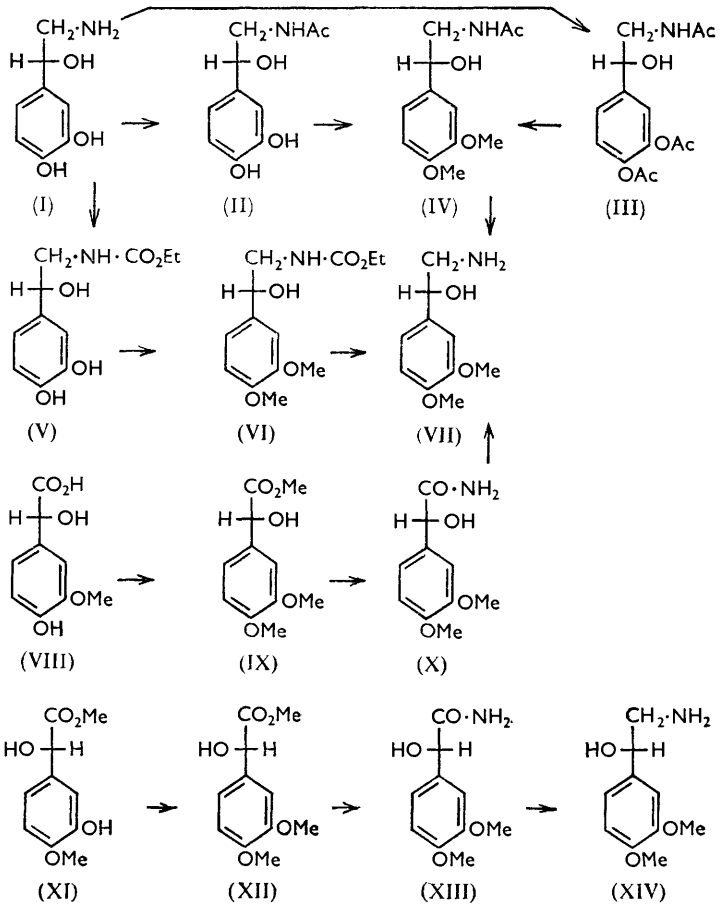
[3] Cf. Pratesi, La Manna, Campiglio, and Ghislandi, Preliminary Communication to the Istituto Lombardo, Accademia di Scienze e Lettere, Milano, Meeting of June 18th, 1959.

[4] Pratesi, La Manna, Campiglio, and Ghislandi, *J.*, 1958, 2069.

Shaw.[5] This acid, treated with diazomethane, gave the oily methyl D(—)-3,4-dimethoxymandelate (IX), from the amide (X) of which D(—)-O^3O^4-dimethylnoradrenaline (VII) was obtained Its hydrochloride had m. p. 168—169° and $[\alpha]_D^{18}$ —35·7° (1·08% w/v in 50% EtOH).

Thus the configuration of (—)-4-hydroxy-3-methoxymandelic acid, a urinary metabolite of noradrenaline, which had been deduced by Armstrong *et al.*[5] by enzymic resolution of the DL-amide with leucine aminopeptidase, is chemically confirmed.

It still remained to prepare the O^3O^4-dimethyl ether of noradrenaline from the lævorotatory natural base. The latter was acetylated, as described by Bretschneider [6] for



racemic adrenaline, and gave the oily (—)-*N*-acetylnoradrenaline (II), which did not crystallize. By acetylation as described by Welsh [7] for (—)-adrenaline, (—)-noradrenaline gave an oily lævorotatory triacetate (III), which, unlike the racemic compound,[8] could not be obtained crystalline.

Both the crude compounds (II) and (III), treated with dry and wet diazomethane respectively, gave (—)-*N*-acetyl-O^3O^4-dimethylnoradrenaline (IV), which by alkaline hydrolysis, gave (—)-O^3O^4-dimethylnoradrenaline (VII), m. p. 96—97°, $[\alpha]_D^{20}$ —29·7°

[5] Armstrong, McMillan, and Shaw, *Biochem. Biophys. Acta*, 1957, **25**, 422.
[6] Bretschneider, *Monatsh.*, 1948, **78**, 77.
[7] Welsh, *J. Amer. Chem. Soc.*, 1952, **74**, 4967.
[8] Pratesi, La Manna, and Campiglio, *Il Farmaco, Ed. Sci.*, 1959, **14**, 645.

(0·72% w/v in absolute EtOH). Its hydrochloride had m. p. 168—169° and $[\alpha]_D^{18}$ −33·2° (1% w/v in 50% EtOH).

(−)-*N*-Ethoxycarbonylnoradrenaline (V) was also prepared from (−)-noradrenaline. With diazomethane it gave the oily (−)-*N*-ethoxycarbonyl-*O*^{3}*O*^{4}-dimethylnoradrenaline (VI) which, hydrolysed with alkali, gave the amine (VII) again with m. p. 96—97°, $[\alpha]_D^{21}$ −30·3° (0·75% w/v in absolute EtOH); the hydrochloride had m. p. 168—169° and $[\alpha]_D^{20}$ −34·8° (1·08% w/v in 50% EtOH).

As a result of these transformations lævorotatory natural noradrenaline possesses the configuration (I), and thus on the basis of the " sequence rule " [9] it is an (*R*)-form.

The steric correspondence between natural adrenaline [4] and noradrenaline is definitively established by the determination of the configuration of the two catecholamines.

Deductions are possible also regarding the configuration of the 3,4-dihydroxyphenylserines, because the configuration of the β-carbon atom is established. The *threo*-configuration for Dalgliesh and Mann's racemic 3,4-dihydroxyphenylserine, from which Blaschko *et al.*[1] obtained (−)-noradrenaline enzymically, is confirmed. For the same reason the racemate used by Hartman *et al.*,[1] which enzymically gave (+)-noradrenaline, has the *erythro*-configuration. These facts are of interest because of the possibility that 3,4-dihydroxyphenylserine may be a natural precursor of noradrenaline.

Experimental

M. p.s were determined on a Kofler block.

(+)-3,4-*Dimethoxymandelamide* (XIII).—Methyl (+)-3-hydroxy-4-methoxymandelate [4] (XI) (3·5 g.), m. p. 118—119°, $[\alpha]_D^{18}$ +129° (0·55% w/v in EtOH), in anhydrous methanol (50 ml.) was mixed with an ethereal solution of diazomethane (6 g.) and kept for 24 hr. The solution was evaporated *in vacuo*, and the crude methyl (+)-3,4-dimethoxymandelate (XII) taken up in a little dry methanol and saturated at 0° with anhydrous ammonia. After 24 hr. at 0° the mixture was concentrated *in vacuo* and the residue stirred with ethyl acetate. (+)-3,4-*Dimethoxymandelamide* (XIII) so obtained (2·9 g.) crystallized from ethyl acetate; it had m. p. 135—136°, $[\alpha]_D^{18}$ +112·1° (0·45% w/v in $CHCl_3$) (Found: C, 56·8; H, 6·4; N, 6·7. $C_{10}H_{13}O_4N$ requires C, 56·9; H, 6·2; N, 6·6%).

(+)-O^{3}O^4-*Dimethylnoradrenaline* (XIV).—(+)-3,4-Dimethoxymandelamide (1 g.) in hot anhydrous tetrahydrofuran (75 ml.) was added dropwise during 30 min. to a stirred suspension of lithium aluminium hydride (0·8 g.) in tetrahydrofuran (25 ml.) at 70—75°. Stirring was continued for 8 hr. at 70—75°. The next day the mixture was treated with a saturated solution of Rochelle salt, then filtered, and the tetrahydrofuran distilled off *in vacuo*. The residue was dried (P_2O_5) and taken up in a little ethanolic hydrochloric acid. By cooling and rubbing, (+)-O^{3}O^4-*dimethylnoradrenaline hydrochloride* (0·35 g.) was obtained; crystallized from anhydrous 1 : 1 ethanol–ether it had m. p. 168—169°, $[\alpha]_D^{18}$ +33·6° (1·1% w/v in 50% EtOH) (Found: C, 51·6; H, 7·2; N, 6·2. $C_{10}H_{16}O_3NCl$ requires C, 51·4; H, 6·9; N, 6·0%).

(−)-3,4-*Dimethoxymandelamide* (X).—(−)-4-Hydroxy-3-methoxymandelic acid (VIII) (3·2 g.) (obtained as described by Armstrong *et al.*[5]), m. p. 156—157°, $[\alpha]_D^{18}$ −128·9° (0·86% w/v in H_2O), was dissolved in anhydrous methanol (15 ml.) and treated with ethereal diazomethane as described above. By treatment with anhydrous ammonia (−)-3,4-*dimethoxymandelamide* (2·3 g.) was obtained which, crystallized from ethyl acetate, had m. p. 135—136°, $[\alpha]_D^{20}$ −115·4° (0·45 w/v in $CHCl_3$) (Found: C, 56·7; H, 6·2; N, 6·7%).

(−)-N-*Acetyl*-O^{3}O^4-*dimethylnoradrenaline* (IV).—(*a*) (−)-Noradrenaline [10·1 g.; m. p. 215—217°, $[\alpha]_D^{18}$ −36·8° (5% w/v in 0·1N-HCl)] was acetylated as described by Bretschneider [6] for (±)-adrenaline. By concentration of ethyl acetate extract, oily (−)-*N*-acetylnoradrenaline (II) (9·3 g.) was obtained, which did not crystallize even after chromatography in ethanol on alumina and elution with the same solvent. This oily crude (−)-*N*-acetylnoradrenaline (6·0 g.) in anhydrous methanol (100 ml.) was treated in several portions with a large excess of ethereal diazomethane, with cooling and shaking. After 24 hr. the mixture was evaporated *in vacuo*. The oily residue was chromatographed in methanol–ether (1 : 10) on alumina. Elution with the same solvent mixture gave the oily product, which, cooled and stirred with a little ethyl

[9] Cahn, Ingold, and Prelog, *Experientia*, 1956, **12**, 81.

acetate, crystallized. (−)-N-*Acetyl*-O^{3}O^4-*dimethylnoradrenaline* (IV) so obtained (1·4 g.) recrystallized from ethyl acetate: it had m. p. 77—78°, $[\alpha]_D^{19}$ −52·5° (0·95% w/v in $CHCl_3$) (Found: C, 60·1; H, 7·4; N, 5·9. $C_{12}H_{17}O_4N$ requires C, 60·2; H, 7·2; N, 5·8%).

(*b*) The oily crude (−)-*O*^{3}*O*^{4}*N*-triacetylnoradrenaline (III) (3·0 g.) [obtained by direct acetylation of (−)-noradrenaline as described by Welsh [7] for (−)-adrenaline], which did not crystallize even after chromatography, was treated in methanol (35 ml.) with wet ethereal diazomethane, as described above. After 48 hr. the mixture was evaporated *in vacuo* and the oily residue dissolved in methanol–ether (1 : 10) and chromatographed on alumina. By concentration of the eluates, (−)-*N*-acetyl-*O*^{3}*O*^{4}-dimethylnoradrenaline (IV) (1·1 g.) was obtained; it had m. p. and mixed m. p. 77—78°, $[\alpha]_D^{19}$ −51·8° (0·94% w/v in $CHCl_3$) (Found: C, 60·3; H, 7·3; N, 5·9%).

(−)-N-*Ethoxycarbonylnoradrenaline* (V).—(−)-Noradrenaline (9·7 g.) was dissolved, under hydrogen, in 2N-sodium hydroxide (57·6 ml.); to this solution, at 5—10°, with stirring, was added every 10 min. 2N-sodium hydroxide (5 ml.) and ethyl chlorocarbonate in chloroform (5 ml.) (5·5 ml. of ethyl chlorocarbonate and 24·5 ml. chloroform). The mixture was stirred 30 min. and acidified with 4N-hydrochloric acid (57 ml.) and, after saturation with ammonium sulphate, extracted with ethyl acetate (4 × 150 ml.). The dried extract (Na_2SO_4), evaporated under hydrogen, gave an oil (12·0 g.). A sample of this was chromatographed in anhydrous ethanol on alumina. Elution with the same solvent gave (−)-N-*ethoxycarbonylnoradrenaline* (V), m. p. 43—44°, $[\alpha]_D^{19}$ −11·6° (0·7% w/v in anhydrous EtOH) (Found: C, 55·2; H, 6·4; N, 5·9. $C_{11}H_{15}O_5N$ requires C, 54·8; H, 6·3; N, 5·8%).

(−)-N-*Ethoxycarbonyl*-O^{3}O^4-*dimethylnoradrenaline* (VI).—(−)-*N*-Ethoxycarbonylnoradrenaline (3·0 g.) in methanol (25 ml.) was treated with ethereal diazomethane. After 24 hr. the mixture was evaporated *in vacuo*. The oily yellow residue (3·0 g.) was chromatographed in methanol on alumina. Elution with the same solvent and evaporation gave again oily (−)-*N*-ethoxycarbonyl-*O*^{3}*O*^{4}-dimethylnoradrenaline. This was dried and analysed. It had $[\alpha]_D^{18}$ −5·9° (1·51% w/v in anhydrous EtOH) (Found: C, 58·6; H, 7·3; N, 5·4. $C_{13}H_{19}O_5N$ requires C, 58·0; H, 7·1; N, 5·2%).

(−)-O^{3}O^4-*Dimethylnoradrenaline* (VII).—(*a*) (−)-3,4-Dimethoxymandelamide (X) (1·0 g.) in anhydrous tetrahydrofuran (75 ml.) was reduced by lithium aluminium hydride (0·8 g.) as described above for its optical antipode. (−)-O^{3}O^4-*Dimethylnoradrenaline hydrochloride* (0·48 g.) (from ethanol–ether) had m. p. 168—169°, $[\alpha]_D^{18}$ −35·7° (1·08% w/v in 50% EtOH) (Found: C, 51·3; H, 7·0; N, 6·1%).

(*b*) (−)-*N*-Acetyl-*O*^{3}*O*^{4}-dimethylnoradrenaline (IV) (2·1 g.) was dissolved in 2N-sodium hydroxide (15 ml.), and the solution was heated at 110° for 1 hr. After cooling, the alkaline mixture was extracted with ethyl acetate (4 × 150 ml.); the extract, when dried (Na_2SO_4) and evaporated, gave (−)-O^{3}O^4-*dimethylnoradrenaline* (VII) (0·5 g.) which, crystallized from ethyl acetate, had m. p. 96—97°, $[\alpha]_D^{20}$ −29·7° (0·72% w/v in absolute EtOH) (Found: C, 60·5; H, 7·6; N, 7·3. $C_{10}H_{15}O_3N$ requires C, 60·9; H, 7·7; N, 7·1%). From this base, by treatment with ethanolic hydrochloric acid, (−)-*O*^{3}*O*^{4}-dimethylnoradrenaline hydrochloride (from ethanol–ether) was obtained, having m. p. 168—169°, $[\alpha]_D^{18}$ −33·2° (1% w/v in 50% EtOH) (Found: C, 51·4; H, 7·1; N, 6·1%).

(*c*) (−)-*N*-Ethoxycarbonyl-*O*^{3}*O*^{4}-dimethylnoradrenaline (VI) (3·0 g.) was dissolved in 2N-sodium hydroxide (30 ml.) and heated as described above. (−)-*O*^{3}*O*^{4}-Dimethylnoradrenaline (0·61 g.) was obtained by concentration of ethyl acetate extracts; it had m. p. 96—97° (from ethyl acetate) and $[\alpha]_D^{21}$ −30·3° (0·75% w/v in absolute EtOH) (Found: N, 7·1%). Its hydrochloride (from ethanol–ether) had m. p. 168—169°, $[\alpha]_D^{20}$ −34·8° (1·08% w/v in 50% EtOH) (Found: C, 51·2; H, 6·9; N, 6·1%).

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