426. *The Configuration of Adrenaline and of its* p-*Hydroxyphenyl Analogue.*

By P. Pratesi, A. La Manna, A. Campiglio, and V. Ghislandi.

Reduction of optically active mandelamides by lithium aluminium hydride to the corresponding amines without appreciable racemisation permits deduction of the configuration of sympathomimetic amines from that of suitably substituted mandelic acids. In this way the configuration of adrenaline and of its *p*-hydroxyphenyl analogue has been correlated with that of the mandelic acid, through 3-amino-4-methoxy- and 4-hydroxymandelic acid. The configuration of D(−)-mandelic acid is found for natural lævorotatory adrenaline and its lævorotatory *p*-hydroxyphenyl analogue.

Knowledge of configurational relationships in the field of optically active sympathomimetic amines is an important element in interpreting and forecasting the specific biological properties of these bases. We here report a purely chemical determination of the configuration of adrenaline and its *p*-hydroxyphenyl analogue.

Freudenberg [1] suggested, but without experimental evidence, that (—)-adrenaline had configuration (I) by analogy with (—)-ephedrine and (—)-ψ-ephedrine whose steric configuration had been unambiguously established (all formulæ in this paper are Fischer projections). Blaschko, Holton, and Stanley [2] later found that Dalgliesh and Mann's racemic 3 : 4-dihydroxyphenylserine [3] was decarboxylated by an L-amino-acid decarboxylase from *Streptococcus faecalis* to (—)-noradrenaline in 50% yield; Dalgliesh [4] suggested that the serine used had the *erythro*-configuration and consequently that the configuration of adrenaline was the opposite of (I), basing this on the well-known biogenetic relation of noradrenaline to adrenaline. However, from infrared spectrophotometric studies Bolhofer [5] ascribed to the same substrate the *threo*-configuration, and Hartman, Pogrund, Drell, and Clark [6] showed that the other racemic form of 3 : 4-dihydroxyphenylserine, to which they ascribe the *erythro*-configuration, is enzymically decarboxylated to (+)-noradrenaline. Thus, Drell [7] reached the same conclusion as Freudenberg as to the configuration of natural adrenaline, but pointed out that the configuration of the *threo*-serine derivative is not unequivocally established, so that the configurational assignment for adrenaline is not definitive.

Reduction of optically active mandelamides by lithium aluminium hydride to the corresponding amines without appreciable racemisation,[8] makes it possible to deduce the configuration of sympathomimetic amines from that of suitably substituted mandelic acids. This method has now been applied to adrenaline. The chemical transformations were first carried out on the racemic compounds [9] and they have now been applied to the optically active substances.

DL-4-Methoxy-3-nitromandelic acid (V) was synthesised from 4-methoxy-3-nitrobenzaldehyde [9] and resolved by means of cinchonine and brucine into the optical antipodes. The dextrorotatory acid gave the methyl ester (VI) which was catalytically reduced to methyl (+)-3-amino-4-methoxymandelate (VII); this was transformed by nitrous acid and copper hydride into methyl (+)-4-methoxymandelate (IX), identical with that

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[1] Freudenberg, " Stereochemie," Deuticke, Leipzig, 1932, pp. 697–698.

[2] Blaschko, Holton, and Stanley, *Brit. J. Pharmacol.*, 1948, **3**, 315.

[3] Dalgliesh and Mann, *J.*, 1947, 658.

[4] Dalgliesh, *J.*, 1953, 3323.

[5] Bolhofer, *J. Amer. Chem. Soc.*, 1954, **76**, 1322.

[6] Hartman, Pogrund, Drell, and Clark, *ibid.*, 1955, **77**, 816.

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

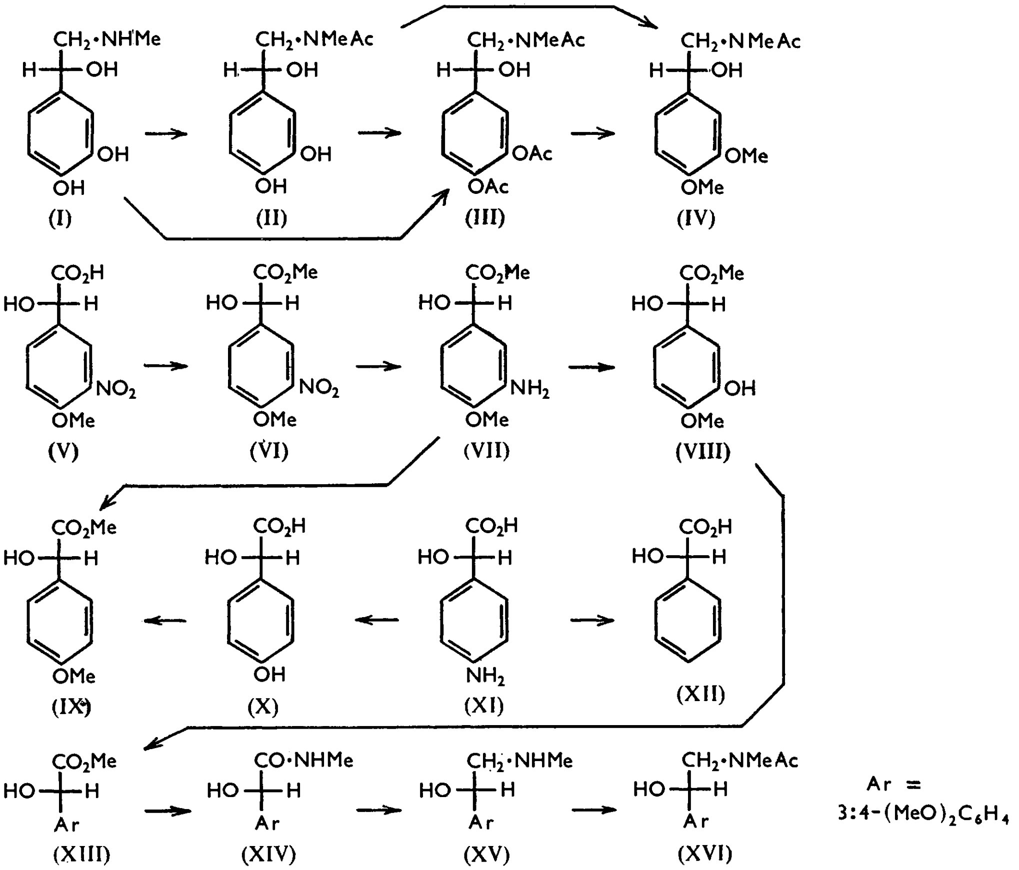
[8] Pratesi and Grassi, *Il Farmaco, Ed. Sci.*, 1953, **8**, 86.

[9] Pratesi, La Manna, Campiglio, and Ghislandi, 16th Internat. Congr. Pure Appl. Chem., Paris, 1957, Abs. Papers, Vol. II, p. 143; *Il Farmaco, Ed. Sci.*, 1957, **12**, 993.

obtained from L(+)-4-hydroxymandelic acid (X) by means of diazomethane. We know that (+)-4-hydroxymandelic acid (X) has the L-configuration because it was obtained[10] from L(+)-4-aminomandelic acid (XI) which Fredga and Andersson[11] converted into L(+)-mandelic acid (XII).

Treating the ester (VII) with nitrous acid and copper sulphate gave methyl L(+)-3-hydroxy-4-methoxymandelate (VIII), which was methylated by diazomethane to oily methyl L(+)-3 : 4-dimethoxymandelate (XIII); when treated with methylamine, this furnished the L(+)-*N*-methylamide (XIV), reduced by lithium aluminium hydride in tetrahydrofuran to oily L(+)-O^3O^4-dimethyladrenaline (XV) (characterised as chloroplatinate).

It was next necessary to prepare this dimethyl ether from an optically active adrenaline. But natural adrenaline was too insoluble for methylation by diazomethane: racemic adrenaline is somewhat more soluble and can be thus methylated, though in very poor yield.[9] L(+)-O^3O^4-Dimethyladrenaline (XV) was therefore acetylated under the conditions described by Welsh[12] for adrenaline to yield the amide (XVI), m. p. 123—124°, $[\alpha]_D^{18}$ +77·4°. Natural (—)-adrenaline was acetylated by the method used by Bretschneider[13] for the racemic compound, yielding (+)-*N*-acetyladrenaline (II), which with diazomethane gave the optical antipode (IV) of (XVI); this had m. p. 123—124°, $[\alpha]_D^{17}$ —77·4°.



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[10] Pratesi, La Manna, and Grassi, *Il Farmaco, Ed. Sci.*, 1955, **10**, 563.

[11] Fredga and Andersson, *Arkiv Kemi, Mineralog. Geol.*, 1941, **14**, *B*, 38.

[12] Welsh, *J. Amer. Chem. Soc.*, 1952, **74**, 4967.

[13] Bretschneider, *Monatsh.*, 1948, **78**, 77.

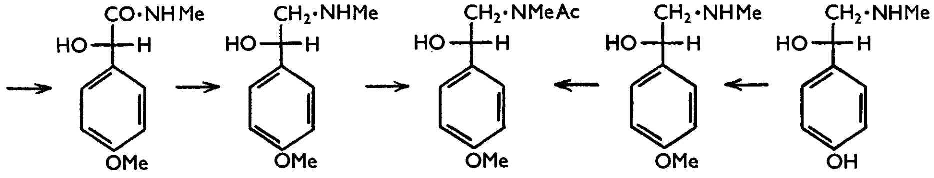
Under the acetylation conditions described by Welsh,[12] the amide (II) was transformed into (—)-O^3O^4N-triacetyladrenaline (III), identical with that which Welsh obtained by direct acetylation of (—)-adrenaline. With wet diazomethane compound (III) also gave the antipode (IV) of (XVI).

As a result of these transformations, natural lævorotatory adrenaline clearly possesses the configuration (I).

The configuration of the *p*-hydroxyphenyl analogue (" Sympatol," " Synephrine ") was determined similarly, methyl L(+)-4-methoxymandelate (IX) being transformed into L(+)-4-methoxy-*N*-methylmandelamide (XVII), which by reduction with lithium aluminium hydride gave the oily L(+)-O^4-methyl derivative (XVIII). On acetylation this yielded the L(+)-acetamide (XIX), m. p. 119—121°, $[\alpha]_D^{17}$ +93·9°. The same compound, m. p. 119—121°, $[\alpha]_D^{17}$ +91·3°, was obtained from the (+)-amine (XXI) by methylation (⟶ XX) and acetylation. Therefore, the configuration of the lævorotatory drug is the same as that of natural adrenaline (I) [the opposite of (XXI)].

On the basis of the sequence rule,[14] natural lævorotatory adrenaline and its lævorotatory *p*-hydroxyphenyl analogue are (*R*)-forms.

(IX)



(XVII) (XVIII) (XIX) (XX) (XXI)

Our work also provides a method of establishing the configuration of the β carbon atom in the 3 : 4-dihydroxyphenylserines, and of the sympathomimetic amines and related compounds in general, some of which have recently become of increased biological interest in connection with central action and the metabolism of adrenaline and noradrenaline.

EXPERIMENTAL

Resolution of DL-4-*Methoxy*-3-*nitromandelic Acid* (V).—Cinchonine (58·9 g., 0·2 mole) was added to a solution of 4-methoxy-3-nitromandelic acid (45·4 g., 0·2 mole) in boiling 1 : 4 (v/v) aqueous ethanol (560 ml.). The hot solution was treated with charcoal and filtered, and crystals (*A*) (55·3 g.) were collected 24 hr. later. Fraction A had m. p. 166—167°, $[\alpha]_D^{18}$ +156·0° (0·3—0·4% w/v in $CHCl_3$) and gave an acid with $[\alpha]_D^{18}$ +63·4° (0·2—0·3% w/v in EtOH). Four crystallisations from 80% ethanol gave *cinchonine* (+)-4-*methoxy*-3-*nitromandelate* (29·5 g.) of constant m. p. (190—191°) and rotation {$[\alpha]_D^{18}$ +175·2° (0·3—0·4% w/v in $CHCl_3$)} (Found: C, 62·0; H, 6·4; N, 7·8; H_2O, 3·4. $C_{19}H_{22}ON_2,C_9H_9O_6N,H_2O$ requires C, 62·3; H, 6·2; N, 7·8; H_2O, 3·3%).

This cinchonine salt (12·5 g.), dissolved in 0·1N-sulphuric acid (18 ml.) and extracted with ether, gave the (+)-*acid* (V) (4·9 g.), m. p. 96—97°, $[\alpha]_D^{17}$ +119·0° (0·26% w/v in EtOH) (Found: C, 47·3; H, 4·0; N, 6·1. $C_9H_9O_6N$ requires C, 47·6; H, 4·0; N, 6·2%).

The mother-liquor from fraction *A* was concentrated *in vacuo* below 40° to a syrup (*B*) from which crude (—)-4-methoxy-3-nitromandelic acid (18 g.), $[\alpha]_D^{17}$ —79·5° (0·44% w/v in EtOH), was obtained. This acid (17·45 g.) in boiling 80% ethanol (100 ml.) was treated with brucine (30·2 g.), and the solution cooled, seeded, and left overnight. The *brucine salt* obtained (31·3 g.) had m. p. 95—100°, $[\alpha]_D^{17}$ —58·1° (0·7% w/v in $CHCl_3$); crystallised to constant m. p. and rotation from 80% ethanol, it had m. p. 106—107°, $[\alpha]_D^{17}$ —60·3° (0·7% w/v in $CHCl_3$) (Found: C, 60·0; H, 6·1; N, 6·5; H_2O, 2·7. $C_{23}H_{26}O_4N_2,C_9H_9O_6N,H_2O$ requires C, 60·1; H, 5·8; N, 6·6; H_2O, 2·8%). The (—)-*acid* (V) (2·05 g.), obtained from the brucine salt (6·2 g.), had m. p. 96—97°, $[\alpha]_D^{18}$ —118·5° (0·25% w/v in EtOH) (Found: C, 47·5; H, 4·1; N, 6·3%).

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

Methyl (+)-3-*Amino*-4-*methoxymandelate* (VII).—Pure (+)-4-methoxy-3-nitromandelic acid (50·0 g.) was boiled in methanol (100 ml.) with concentrated sulphuric acid (5 ml.) for 8 hr. The solution was then concentrated *in vacuo* and the crystals (45·5 g.) were collected and washed with saturated aqueous sodium carbonate. Two crystallisations from benzene gave *methyl* (+)-4-*methoxy*-3-*nitromandelate* (VI), m. p. 81—82°, $[\alpha]_D^{17}$ +104·5° (0·42% w/v in EtOH) (Found: C, 49·6; H, 4·7; N, 5·9. $C_{10}H_{11}O_6N$ requires C, 49·8; H, 4·6; N, 5·8%).

The ester (44 g.) in ethanol (230 ml.) was reduced by hydrogen over 5% palladium–alumina at room temperature and 4 kg./cm.^{2}. The catalyst was removed and the solution concentrated *in vacuo* under nitrogen. The *amino-ester* (VII), recrystallised twice from ethanol (yield 30·1 g.), had m. p. 112—113°, $[\alpha]_D^{17}$ +134·3° (0·42% w/v in EtOH) (Found: C, 57·1; H, 6·1; N, 6·6. $C_{10}H_{13}O_4N$ requires C, 56·9; H, 6·2; N, 6·6%).

(+)-3 : 4-*Dimethoxy*-N-*methylmandelamide* (XIV).—Methyl (+)-3-amino-4-methoxymandelate (10·5 g.) was diazotised at −5° in sulphuric acid (27 ml.; 25% v/v) with sodium nitrite (15·4 ml. of 25% solution), and the solution added dropwise to copper sulphate solution (150 g. in 300 ml. of water) at 80—85°. The whole was extracted with ether (8 × 250 ml.), and the extract concentrated, washed with sodium hydrogen carbonate solution and water, dried (Na_2SO_4), and evaporated. *Methyl* (+)-3-*hydroxy*-4-*methoxymandelate* (VIII) (3·7 g.) crystallised from benzene and had m. p. 118—119°, $[\alpha]_D^{17}$ +129·5° (0·56% w/v in EtOH) (Found: C, 56·8; H, 5·9. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7%). This ester (1·5 g.) in methanol (45 ml.) was kept for 24 hr. in an ethereal solution of diazomethane (6 g.). The solution was evaporated *in vacuo*, the residue taken up in a little methanol, and excess of anhydrous methylamine added. After 24 hr. at 0° the mixture was concentrated *in vacuo* and the residue stirred with benzene. (+)-3 : 4-*Dimethoxy*-N-*methylmandelamide* (XIV) so obtained crystallised from ethyl acetate; it had m. p. 153—154°, $[\alpha]_D^{20}$ +81·7° (0·9% w/v in $CHCl_3$), $[\alpha]_D^{18}$ +31·5° (0·87% w/v in EtOH) (Found: C, 58·4; H, 6·9; N, 6·5. $C_{11}H_{15}O_4N$ requires C, 58·7; H, 6·7; N, 6·2%).

The carbonate washings from the preparation of methyl (+)-3-hydroxy-4-methoxymandelate gave, on acidification and ten extractions with ether, a dextrorotatory oily acid which on treatment with diazomethane and then methylamine afforded more of the (+)-amide (XIV).

In another run the (+)-amide {m. p. 153—154°, $[\alpha]_D^{20}$ +81·7° (0·93% w/v in $CHCl_3$)} was obtained in 47·4% yield directly from the ester (VII) without isolation of the intermediates (Found: C, 58·6; H, 6·8; N, 6·3%).

(+)-N-*Acetyl*-O^{3}O^4-*dimethyladrenaline* (XVI).—(+)-3 : 4-Dimethoxy-*N*-methylmandelamide (3 g.) in hot anhydrous tetrahydrofuran (120 ml.) was added dropwise during 15 min. to a stirred suspension of lithium aluminium hydride (1 g.) in tetrahydrofuran 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 of *in vacuo*. The residual aqueous solution was extracted with chloroform (8 × 100 ml.). The chloroform extracts were washed with a little water, dried (Na_2SO_4), and evaporated *in vacuo*. The residual oily (+)-*O*^{3}*O*^{4}-dimethyladrenaline (XV) (1·9 g.) did not crystallise, but was characterised as its *chloroplatinate*, m. p. 152—153° (from ethanol) [Found: C, 31·9; H, 4·6; Pt, 23·7. $(C_{11}H_{17}O_3N)_2,H_2PtCl_6$ requires C, 31·7; H, 4·4; Pt, 23·5%].

(+)-*O*^{3}*O*^{4}-Dimethyladrenaline (0·544 g., 19·3%), obtained as an oil from the chloroplatinate by basification and chloroform-extraction, was suspended in sodium hydrogen carbonate solution (5·4 g. in 20 ml. of water) and treated with acetic anhydride (3·3 ml.). N-*Acetyl*-O^{3}O^4-*dimethyladrenaline* (XVI) (0·24 g.) was isolated by chloroform-extraction and crystallised from benzene (6 ml.); it had m. p. 123—124°, $[\alpha]_D^{18}$ +77·4° (1·13% w/v in $CHCl_3$) (Found: C, 61·6; H, 7·6; N, 5·6. $C_{13}H_{19}O_4N$ requires C, 61·6; H, 7·6; N, 5·5%).

(+)-N-*Acetyladrenaline* (II).—Natural (−)-adrenaline {22 g.; m. p. 211—212°, $[\alpha]_D^{17}$ −50·6° (0·3 g. in 1·56 ml. of N-HCl + 2·44 ml. of H_2O)} was acetylated as described by Bretschneider [13] for (±)-adrenaline. (+)-N-*Acetyladrenaline* (8·5 g.), recrystallised from water, had m. p. 162—163°, $[\alpha]_D^{20}$ +8·5° (2% w/v in H_2O) (Found: C, 58·5; H, 6·9; N, 6·3. $C_{11}H_{15}O_4N$ requires C, 58·7; H, 6·7; N, 6·2%).

(−)-*O*^{3}*O*^{4}*N*-Triacetyladrenaline (III), obtained by direct acetylation of (−)-adrenaline by Welsh's method,[12] had m. p. 95—96° (from benzene), $[\alpha]_D^{20}$ −87·4° (1% w/v in $CHCl_3$) (Found: C, 58·4; H, 6·4; N, 4·7. Calc. for $C_{15}H_{19}O_6N$: C, 58·2; H, 6·2; N, 4·5%). It was also obtained when (+)-*N*-acetyladrenaline (2 g.), suspended in aqueous sodium hydrogen carbonate (60 g. in 200 ml. of water), was treated with acetic anhydride (4 × 7·5 ml.): the triacetyladrenaline (1·6 g.), obtained by chloroform-extraction and recrystallisation from benzene, had m. p. 95—96°, $[\alpha]_D^{17}$ $-89·5°$ (0·96% w/v in $CHCl_3$).

(−)-N-*Acetyl*-O^3O^4-*dimethyladrenaline* (IV).—(+)-*N*-Acetyladrenaline (3 g.) in methanol (200 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 solid residue (2·7 g.) was treated with benzene and recrystallised from the same solvent. The product (2·1 g.) had m. p. 123—124°, $[\alpha]_D^{17}$ $-77·4°$ (1% w/v in $CHCl_3$) (Found: C, 61·4; H, 7·7; N, 5·7%).

The same product was obtained by treating (−)-triacetyladrenaline (10 g.) in methanol (70 ml.) with ethereal diazomethane provided a trace of water was present. Recrystallisation from benzene gave the pure product (6·7 g.).

Methyl (+)-4-*Methoxymandelate* (IX).—(*a*) Diazotised methyl (+)-3-amino-4-methoxymandelate (VII) (8·4 g. in 44 ml. of 25% v/v sulphuric acid) was added during 30 min. to a violently agitated suspension of copper hydride, which was prepared as follows: sodium hypophosphite (49·6 g.) in water (96 ml.) and sulphuric acid (132·8 ml.; 25% v/v) was heated to 50—60° and added during 30 min. to a stirred solution of copper sulphate (36·8 g.) in water (72 ml.) and the resulting suspension allowed to cool.

After being stirred for another hour the reaction mixture was extracted with ether (20 × 200 ml.). The combined extracts were concentrated *in vacuo*, then washed with saturated sodium hydrogen carbonate solution and a little water, dried (Na_2SO_4), and evaporated. The solid residue (1·65 g.) was recrystallised thrice from ligroin (b. p. 75—120°) and pure methyl (+)-4-methoxymandelate [15] (1·2 g.) obtained {m. p. 63—64°, $[\alpha]_D^{18}$ $+138·0°$ (3% w/v in EtOH)} (Found: C, 61·2; H, 6·4. Calc. for $C_{10}H_{12}O_4$: C, 61·2; H, 6·2%). The bicarbonate washings on acidification and ether-extraction gave (+)-4-methoxymandelic acid [16] (2·8 g.). Three crystallisations from benzene gave the pure acid, m. p. 105—106°, $[\alpha]_D^{18}$ $+141·5°$ (2·45% w/v in H_2O) (Found: C, 59·1; H, 5·8. Calc. for $C_9H_{10}O_4$: C, 59·3; H, 5·5%).

(*b*) (+)-4-Hydroxymandelic acid was obtained from (±)-4-hydroxymandelic acid [17] by precipitation of its brucine salt in 80% ethanol. After repeated crystallisation of the brucine salt from 80% ethanol, the acid was liberated by sulphuric acid, extracted with ether, and recrystallised from benzene.

Ellinger and Kotake [18] gave, for the anhydrous dextrorotatory acid, m. p. 103—104° and $[\alpha]_D$ $+144·4°$ (1·5% w/v in H_2O). According to Pratesi, La Manna, and Grassi [10] the acid has m. p. 114—115°, $[\alpha]_D^{21}$ $+154·7°$ (1·36% w/v in H_2O). The sample of (+)-acid used by us had m. p. 113—114°, $[\alpha]_D^{18}$ $+128·6°$ (1·2% w/v in H_2O) (Found: C, 57·0; H, 5·0. Calc. for $C_8H_8O_4$: C, 57·1; H, 4·8%).

(+)-4-Hydroxymandelic acid (0·94 g.) in methanol (20 ml.) was treated with an excess of ethereal diazomethane. After 70 hr. at room temperature, the solution was concentrated *in vacuo*. The solid residue was repeatedly crystallised from ligroin (b. p. 75—120°), giving *methyl* (+)-4-*methoxymandelate* (0·41 g., 37·3%), m. p. 63—64°, $[\alpha]_D^{18}$ $+136·1°$ (2·4% w/v in EtOH) (Found: C, 61·1; H, 6·3%).

(+)-4-*Methoxy*-N-*methylmandelamide* (XVII).—This *amide* was prepared from methyl (+)-4-methoxymandelate (5·5 g.) in methanol (50 ml.) by treatment with anhydrous methylamine as described for the 3 : 4-dimethoxymethylamide. The product (5·37 g.), recrystallised from benzene, had m. p. 107—108°, $[\alpha]_D^{18}$ $+94·6°$ (1% w/v in $CHCl_3$) (Found: C, 61·5; H, 6·9; N, 7·3. $C_{10}H_{13}O_3N$ requires C, 61·5; H, 6·7; N, 7·2%).

(+)-N-(2-*Hydroxy*-2-4′-*methoxyphenylethyl*)-N-*methylacetamide* (XIX).—(*a*) (+)-4-Methoxy-*N*-methylmandelamide (XVII) (2 g.) in anhydrous tetrahydrofuran (130 ml.) was reduced by lithium aluminium hydride (0·65 g.) as described above. The product (1·5 g.) was an oil. With acetic anhydride (7·5 ml.) and sodium hydrogen carbonate (12 g.) in water (40 ml.) it gave the (+)-N-*acetyl derivative* (50% crude; 16% pure, from EtOH), m. p. 119—121°, $[\alpha]_D^{17}$ $+93·9°$ (0·69% w/v in $CHCl_3$) (Found: C, 64·4; H, 7·8; N, 6·4. $C_{12}H_{17}O_3N$ requires C, 64·5; H, 7·7; N, 6·3%).

(*b*) (+)-2-Hydroxy-2-4′-hydroxyphenyl-*N*-methylethylamine was obtained by resolution of the racemic compound with ammonium bromocamphorsulphonate. In agreement with Legerlotz [19]

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[15] McKenzie and Pirie, *Ber.*, 1936, **69**, 869.

[16] Knorr, *Ber.*, 1904, **37**, 3172.

[17] Ladenburg, Folkers, and Major, *J. Amer. Chem. Soc.*, 1936, **58**, 1294.

[18] Ellinger and Kotake, *Z. physiol. Chem.*, 1910, **65**, 410.

[19] Legerlotz, D.R.-P. 543,529; *Chem. Zentr.*, 1932, I, 2867.

it had m. p. 179—180°, $[\alpha]_D^{17}$ +61·5° (0·12% w/v in H_2O). A suspension of it (1 g.) in methanol (50 ml.) was kept in ethereal diazomethane at room temperature. After 70 hr. the solution was concentrated *in vacuo*, and the residue taken up in chloroform, washed with aqueous sodium hydroxide, and dried (Na_2SO_4). The (+)-4-methoxy-derivative was obtained as an oil (0·71 g.). It was acetylated as described above, giving the (+)-*N*-acetyl derivative (18·7%), m. p. and mixed m. p. 119—121°, $[\alpha]_D^{17}$ +91·3° (0·73% w/v in $CHCl_3$) (Found: N, 6·4%).

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