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The absolute configurations of the alkaloids of *Physostigma venenosum* seeds*

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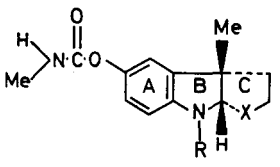
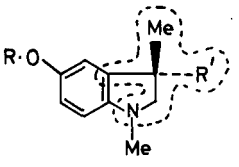
The absolute configuration of physostigmine has been established by correlating the configuration of its C-3a atom with that of the asymmetric carbon atom in (+)-3-ethyl-3-methoxycarbonyl-3-methylpropionic acid. Comparison of the optical rotatory dispersion spectra of physostigmine, N_a-norphysostigmine, geneserine, physovenine and eseramine have shown that all five alkaloids have the same absolute configurations.

In physostigmine, the major alkaloid of *Physostigma venenosum* seeds, and in the four minor alkaloids physovenine, N_a-norphysostigmine, geneserine and eseramine (Robinson, 1968), the aromatic ring A and the ring B form a near-planar system. It may therefore be deduced that the B/C ring junction is represented by a *cis* fusion (Jackson, 1954, see also Witkop & Hill, 1955; McFarland, Inoue & Nakanishi, 1969; Spande, Wilchek & Witkop, 1968). This deduction is supported by the X-ray crystallographic determination of the structures and absolute configurations of the alkaloids echitamine (Hamilton, Hamor, & others, 1962; Manohar & Ramaseshan, 1961), chimonanthine (Grant, Hamor & others, 1965) and hodgkinsine (Fridrichsons, Mackay & Mathieson, 1967), all of which contain the physostigmine ring system with the B/C rings *cis*-fused, and it has recently been verified by the detection of an internal nuclear Overhauser effect between the protons of the 3a-methyl group and the 8a-proton (Newkome & Bhacca, 1969). Thus physostigmine and its above-mentioned related minor alkaloids are represented either by I [R = Me, X = N—Me; R = Me, X = O; R = H, X = N—Me; R = Me, X = (N→O) Me and R = Me, X = N—CONHMe, respectively] or the mirror images.



I



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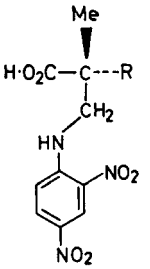
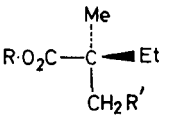


III



IV

With a view to obtaining C-3a of physostigmine in a form (β-amino-acid) suitable for comparison with a compound of known absolute configuration, the following preliminary oxidative experiments were made on model compounds. 5-Methoxy-1,3,3-trimethylindoline (II, R = R′ = Me) was prepared by sodium borohydride

* Alkaloids of *Physostigma venenosum*, Part VIII; for Part VII see Robinson & Robinson (1968): for a preliminary communication of this work see Longmore & Robinson (1969).

reduction of 5-methoxy-1,3,3-trimethyl-3*H*-indolium iodide (Ahmed & Robinson, 1967). Ozonolysis of this indoline followed by treatment with hydrogen peroxide afforded a low yield of the desired β-amino-acid, containing the moiety outlined in (II), which was isolated by the method of Rao & Sober (1954) as its 2,4-dinitrophenyl derivative (III, R = Me). (±)-5-Ethoxy-3-ethyl-1,3-dimethylindoline (±)-(II, R = R′= Et) was prepared from (±)-5-hydroxy-1,3-dimethyloxindole (Robinson, 1965; Longmore & Robinson, 1967) by *O*-ethylation, 3-ethylation and lithium aluminium hydride reduction respectively. Ozonolysis of the indoline as before gave (±)-III (R = Et), the structure of which was verified by the following synthesis. (±)-3-Ethyl-3-methoxycarbonyl-3-methylpropionic acid, (±)-IV (R = Me, R′ = COOH) (Ställberg-Stenhagen, 1951) was converted into its amide (±)-IV (R = Me, R′ = $CONH_2$) via the anhydride. Treatment of the amide with sodium hypobromite, conditions which also effected hydrolysis of the ester group, yielded (±)-IV (R = H, R′ = NH_2) which was isolated by the method of Rao & Sober (1954) as its 2,4-dinitrophenyl derivative (±)-III (R = Et). This oxidative route was then applied to the problem of the establishment of the absolute configuration of physostigmine as follows.

Physostigmine (I, R = Me, X = N — Me) was converted, via eserethole and eserethole methiodide, into eserethole methine (Hoshino & Kobayashi, 1934) which upon catalytic hydrogenation in acid solution followed by quaternization with methyl iodide afforded dihydroeserethole methine methiodide [II, R = Et, R′ = $(CH_2)_2N^+Me_3I^-$] (Polonovski, 1918). This was subjected to Hofmann degradation to give II (R = Et, R′ = CH = CH_2) which on hydrogenation yielded

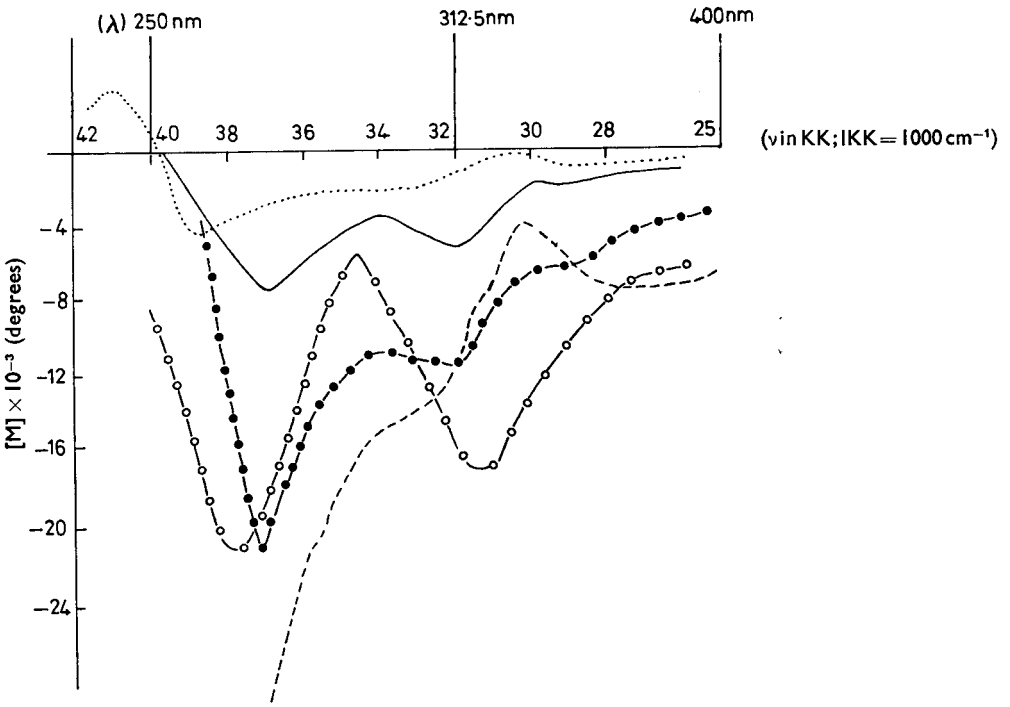


FIG. 1. Optical rotatory dispersion spectra of the alkaloids of *Physostigma venenosum* seeds. ———, Physostigmine (rotations ×4); ·····, Na-norphysostigmine; ●—●—●—●, eseramine; ○—○—○—○, geneserine and — — — — —, physovenine.

II (R = R′ = Et). When the oxidative procedure described above was applied to this indoline the 2,4-dinitrophenyl derivative III (R = Et) was obtained.

The absolute configuration of this compound was established by the synthesis of its enantiomer from (−)-3-ethyl-3-methoxycarbonyl-3-methylpropionic acid (IV, R = Me, R′ = COOH) (Ställberg-Stenhagen, 1951), by the route described above for the racemic compound. In turn the absolute configuration of IV (R = Me, R′ = COOH) follows from its hydrolysis to (−)-2-ethyl-2-methylsuccinic acid (IV, R = H, R′ = COOH), the absolute configuration of which has been established using the quasi-racemate technique (Porath, 1951; Fredga, 1960; see also Cox, Koch & others, 1967). These results have been consolidated by relating (Harris, Robertson & Whalley, 1958; Cox, Ellestad & others, 1965) the configuration of (+)-2-ethyl-2-methylsuccinic acid to the asymmetric C-13 atom of rosenonolactone, the absolute configuration of which has been determined by X-ray crystallography (Scott, Sutherland & others, 1964). The absolute configuration of physostigmine is therefore established as being as shown in I, (R = Me, X = N—Me).

The absolute configurations of the other four alkaloids follows from comparison of their optical rotatory dispersion spectra with that of physostigmine. Fig. 1 shows that all five alkaloids have closely similar spectra, comprising a negative Cotton effect for the absorption band at *ca* 300 nm, followed by a second, stronger negative Cotton effect, centred at *ca* 250 nm. Further details of the spectra are given in Table 1.

Table 1. *Salient features of the optical rotatory dispersion spectra of the alkaloids of* Physostigma venenosum *seeds*

	1st Trough		1st Peak		2nd Trough		2nd Peak		3rd Trough		Crossover	
Alkaloid	λ nm	[M]°	λ nm	[M]°	λ nm	[M]°	λ nm	[M]°	λ nm	[M]°	λ nm	[M]°
Physostigmine[a] ..	344	−520	336	−480	313	−1560	294	−940	272	−2180	250	0
N_a-Norphysostigmine	350	−400	328	−100	303	−1100[b]	280	−1900[b]	258	−3700	252	0[c]
Physovenine	355	−7600	332	−4100	313	−14000[b]	294	−15800[b]	270	−28000[d]	—	—
Geneserine	[e]	[e]	[e]	[e]	318	−17200	288	−5700	263	−21000	—	—
Eseramine[f]	333	−6400[b]	333	−6400[b]	313	−11300	297	−10700	270	−21300	255	0

[a] Rotations for physostigmine have been multiplied by 4. [b] Inflection. [c] Further data; 242 nm, [M] = +3650° (peak); 230 nm, [M] = 0°; 222 nm, [M] = −1500° (lowest point observed). [d] Lowest wavelength of observation. [e] The spectrum for geneserine does not show a peak, trough or inflection in this region, though a marked change of slope occurs—typical values are 357 nm, [M] = −7600°; 335 nm, [M] = −13000°. [f] An inflection occurs in this spectrum at 279 nm, [M] = −15000° (see Fig. 1).

For eseramine and physovenine, the Cotton effect at longer wavelength is observed only as inflections on the steeply falling background dispersion curve, but the close similarity of all five spectra is clearly established. Only for N_a-norphysostigmine have both extrema of the second Cotton effect been observed (these arise from the absorption band at *ca* 250 nm), but the negative extrema at *ca* 265 nm is clear in the other cases. The observed Cotton effects, which show a correspondence of sign for corresponding transitions throughout the series, show that all five alkaloids have the same absolute configurations. This conclusion can also be independently reached for physostigmine, geneserine and physovenine since the former alkaloid has been chemically converted into the latter two by reactions which cannot cause optical inversion at the asymmetric centres (Robinson, 1963; Longmore & Robinson, 1966, respectively). The absolute configurations of the four minor alkaloids are therefore identical with that of physostigmine about their B/C ring junctions.

EXPERIMENTAL

Melting-points were recorded on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were measured in ethanolic solution on a Perkin-Elmer model 137

spectrophotometer, infrared spectra were recorded as Nujol mulls or liquid films on a Perkin-Elmer model 237 spectrophotometer and the mass spectrum was recorded on an A.E.I. MS.9 spectrometer. Optical rotatory dispersion spectra were obtained in 95% ethanol using a Bendix-N.P.L. "Polarmatic" spectropolarimeter; concentrations were varied from 4×10^{-4} to $0{\cdot}8 \times 10^{-4}$M, all spectra were checked at several different concentrations and results are reproducible within 3%. Solutions were dried with anhydrous magnesium sulphate and solvents were removed on a steam-bath (unless otherwise stated) under reduced pressure (water pump). Solid analytical samples were dried (6 h) at room temperature/0·1 mm over phosphorus pentoxide.

5-*Methoxy*-1,3,3-*trimethylindoline* (II, R = R′ = Me). To an ice-cold solution of 5-methoxy-3,3-dimethyl-3-*H*-indole methiodide (Ahmed & Robinson, 1967) (16·0 g) in methanol (300 ml) sodium borohydride (10·0 g) was added in portions with occasional swirling. The solution was then kept at room temperature overnight, water (150 ml) added, the methanol evaporated and the liberated oil extracted into ether (3 × 150 ml). Evaporation of the combined dried ethereal extracts afforded an oil (8·8 g) which upon distillation gave a colourless oil (6·44 g; 67%), b.p. 80°/0·4 mm (Millson & Robinson, 1955, b.p. 118°/0·5 mm). The picrate crystallized from ethanol in yellow leaflets, m.p. 147–149° (with sweating at 135°). Found: C, 51·4; H, 4·95. $C_{18}H_{20}N_4O_8$ requires C, 51·4; H, 4·8%.

Ozonolysis of 5-*Methoxy*-1,3,3-*trimethylindoline* (II, R = R′ = Me). Ozone-enriched oxygen was bubbled (24 h) through a solution of 5-methoxy-1,3,3-trimethylindoline (502 mg) in glacial acetic acid (50 ml). Hydrogen peroxide solution (6% v/v, 50 ml) was then added and the mixture stood at room temperature for 4 h. The excess hydrogen peroxide was decomposed by the addition of finely-divided platinum (50 mg) and after filtration the solution was concentrated on a rotary evaporator. Ethanol (25 ml) was added and evaporated again to remove all traces of acetic acid. The residue, in 0·88 ammonia (40 ml), was treated with hydrogen peroxide (6% v/v, 40 ml) and the effervescing solution was kept at room temperature overnight. Finely-divided platinum (50 mg) was added to the solution which was heated (5 min) on a steam-bath, filtered and evaporated on a rotary evaporator. The residue was dissolved in ethanol and the solution evaporated to remove the last traces of water. The resulting semicrystalline residue was triturated with 95% ethanol (10 ml) and the colourless crystalline ammonium oxalate (159 mg) filtered off. Evaporation of the filtrate afforded a gum (324 mg) which was dissolved in 50% aqueous ethanol (40 ml), sodium bicarbonate (1·0 g) and 2,4-dinitrofluorobenzene (1·0 g) were added and the mixture was shaken (3 h) at room temperature. The ethanol was removed at room temperature on a rotary evaporator and the excess 2,4-dinitrofluorobenzene was removed by extraction into ether (3 × 25 ml). The aqueous solution was then acidified to approximately pH 1·3 with 6N hydrochloric acid, and the insoluble material was extracted into chloroform (3 × 15 ml). Evaporation of the dried chloroform extracts gave III (R = Me) (26 mg; 3:5%) which was recrystallized three times from ether-light petroleum (b.p. < 40°) to give yellow needles, m.p. 189–191° (with sweating from 160°). The high resolution mass spectrum had a molecular ion at m/e = 283·0807 ($C_{11}H_{13}N_3O_6$ requires 283·0804) and a base peak at m/e = 196 [2,4-(diNO_2) — C_6H_3 — N^+H = CH_2 produced by cleavage of the methylene group-quaternary carbon C–C bond in III (R = Me)].

(±)-5-*Ethoxy*-3-*ethyl*-1,3-*dimethyloxindole.* (±)-5-Ethoxy-1,3-dimethyloxindole (Julian & Pikl, 1935) (42·9 g) was dissolved in a solution of sodium (7·22 g) in dry ethanol (500 ml). Ethyl iodide (83·2 g) was then added dropwise over 30 min, with stirring, at room temperature. After a further 1 h the solution was boiled under reflux (2 h). The ethanol was removed, water (100 ml) was added to the residue, and the resulting oil was extracted into chloroform (3 × 100 ml). The dried combined chloroform extracts were evaporated to leave a light-brown oil which upon distillation (b.p. 130–135°/0·5 mm) gave a pale yellow oil which soon crystallized. Recrystallization from light petroleum (b.p. < 40°) afforded pale yellow plates (38·7 g; 79%), m.p. 35·5–37°. Found: C, 71·55; H, 8·1. $C_{14}H_{19}NO_2$ requires C, 72·05; H, 8·2%.

(±)-5-*Ethoxy*-3-*ethyl*-1,3-*dimethylindoline* [(±)-II, R = R′ = Et]. Lithium aluminium hydride (650 mg) was added in small portions with stirring to a solution of (±)-5-ethoxy-3-ethyl-1,3-dimethyloxindole (2·0 g) in sodium-dried tetrahydrofuran (30 ml) at room temperature. The stirred mixture was boiled under reflux (3 h), water added to decompose excess lithium aluminium hydride and the resulting granular white precipitate removed by filtration and washed with ether. The combined filtrate and ether-washings were dried and evaporated to give a pale brown oil (1·9 g; 99%) which upon distillation afforded a pale yellow oil (1·3 g; 67%), b.p. 106°/0·2 mm. Found: C, 76·1; H, 9·4; N, 6·7. $C_{14}H_{21}NO$ requires C, 76·65; H,9·65; N, 6·4%. The picrate crystallized from 95% ethanol in yellow prisms, m.p. 148–150° (with sweating from 135°). Found: C, 53·6; H, 5·2. $C_{20}H_{24}N_4O_8$ requires C, 53·55; H, 5·4%.

Ozonolysis of (±)-5-*Ethoxy*-3-*ethyl*-1,3-*dimethylindoline* [(±)-II R = R′ = Et]. Ozone-enriched oxygen was bubbled through a solution of (±)-5-ethoxy-3-ethyl-1,3-dimethylindoline (2·0 g) in glacial acetic acid (150 ml) until the colour of the solution, which became very dark during the initial stages of the reaction, was bleached (about 4½ h). Hydrogen peroxide solution (30% v/v, 50 ml) was added and the solution kept at room temperature overnight, after which it was boiled under reflux for 30 min; platinum black (50 mg) was added and the boiling under reflux continued until oxygen-evolution ceased. After filtration and removal of the acetic acid on a rotary evaporator, the residue was dissolved in 4N hydrochloric acid (20 ml), the solution was again boiled under reflux (2 h) and again evaporated. The residue was dissolved in aqueous ethanol (50%, 40 ml), sodium bicarbonate (2·0 g) and 2,4-dinitrofluorobenzene (1·0 g) were added and the mixture was stirred (2 h) at room temperature. After pouring into water (50 ml), 2,4-dinitrophenol and excess 2,4-dinitrofluorobenzene were extracted into chloroform (6 × 20 ml) (Extract 1). The aqueous phase was acidified to approximately pH 1·3 with concentrated hydrochloric acid and the insoluble material extracted into chloroform (2 × 20 ml) (Extract 2). Evaporation of Extract 2 after drying gave an orange oil (150 mg) which was shown by thin-layer chromatography [on Eastman Chromagram silica gel sheets, type 6060 using methanol–chloroform (1 : 5 v/v) as developing solvent] to be a mixture of 2,4-*dinitrophenol* and (±)-2-*methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid*, (±)-III (R = Et). Authentic samples used as markers gave Rf = 0·37 and 0·70, respectively. The two components were separated by column chromatography on silica gel using ether–chloroform (1 : 10 v/v) as eluant and continuously monitoring (15 ml fractions) by thin-layer chromatography (as above).

A further yield of almost pure (±)-III (R = Et) was obtained by washing Extract 1 with saturated sodium bicarbonate solution (2 × 20 ml), washing the aqueous solution with chloroform (10 ml), acidifying with 4N hydrochloric acid and extracting the required product into chloroform (2 × 10 ml) (Extract 3).

The required eluates from the column chromatogram were combined with Extract 3 and evaporated to dryness to afford (±)-2-*methyl*-2-(2,4-*dinitrophenylaminomethyl*)-*butyric acid* as an oil (91 mg; 3·3%) which completely crystallized on trituration with ether. Recrystallization from ether containing a trace of chloroform afforded yellow needles (30 mg; 1%), m.p. 153–155° (with sweating from 141°), giving no depression on admixture with the authentic sample prepared below. Their behaviour on thin layers and their infrared spectra were likewise identical.

(±)-3-*Ethyl*-3-*methoxycarbonyl*-3-*methylpropionic anhydride*. A solution of (±)-3 ethyl-3-methoxycarbonyl-3-methylpropionic acid (Ställberg-Stenhagen, 1951) (1·0 g) in acetic anhydride (5 ml) was boiled under reflux (1½ h): acetic acid and excess acetic anhydride were then removed and the oily residue distilled to give the *anhydride* as a colourless viscous oil (720 mg; 76%), b.p. 204°/0·5 cm. Found: C, 58·15; H, 8·05. $C_{16}H_{26}O_7$ requires C, 58·15; H, 7·95%.

(−)-3-*Ethyl*-3-*methoxycarbonyl*-3-*methylpropionic anhydride* was likewise obtained from the (−) acid (Ställberg-Stenhagen, 1951) in an identical manner (86% yield), b.p. 190–195°/0·5 cm. The infrared spectrum was identical with that of the racemate prepared above.

(±) - 3 - *Ethyl* - 3 - *methoxycarbonyl* - 3 - *methylpropionamide* [(±) - IV, R = Me, R′ = $CONH_2$]. Ammonia, dried by passage over sodium hydroxide pellets, was bubbled through a solution of (±)-3-ethyl-3-methoxycarbonyl-3-methylpropionic anhydride (4·7 g) in dry ether (50 ml) (30 min) when a colourless crystalline deposit of ammonium (±)-3-ethyl-3-methoxycarbonyl-3-methylpropionate gradually formed. Water (25 ml) was then added, the aqueous layer was extracted with ether (20 ml) and the combined ether extracts were washed with water (2 × 25 ml). Evaporation of the dried ether extracts afforded the *amide* as a colourless oil (485 mg; 20%), b.p. 110° (bath temperature)/0·7 mm. Found: C, 55·45; H, 8·6. $C_8H_{15}NO_3$ requires C, 55·45; H, 8·75%.

(−)-3-*Ethyl*-3-*methoxycarbonyl*-3-*methylpropionamide* (IV, R = Me, R′ = $CONH_2$). The laevorotatory anhydride (1·6 g) was similarly converted into the (−)-*amide* (IV, R = Me, R′ = $CONH_2$) (807 mg; 97%), a colourless oil, b.p. 180°/0·5 cm, $[\alpha]_D^{23} = -7{\cdot}83°$, $[M]_D^{23} = -13{\cdot}57°$ (95% EtOH). The infrared spectrum was identical with that of the racemate prepared above.

(±)-2-*Methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid* [(±)-III, R = Et]. To a mixture of (±)-3-ethyl-3-methoxycarbonyl-3-methylpropionamide (200 mg) and bromine (185 mg) 10% aqueous sodium hydroxide was added until the colour of the mixture was pale yellow. Aqueous sodium hydroxide (5 ml, containing 2·25 g of sodium hydroxide) was then added and the mixture was warmed to 70° on a steam-bath for 30 min. After cooling, the solution was made weakly acidic by the careful addition of 4N-hydrochloric acid, excess sodium bicarbonate was added to neutralize the acid, the volume of the solution was doubled by the addition of absolute ethanol and 2,4-dinitrofluorobenzene (500 mg) was added. The mixture was then shaken vigorously (3 h); the ethanol was removed on a rotary evaporator at room temperature and unreacted excess 2,4-dinitrofluorobenzene was removed by extraction into ether (3 × 25 ml). The aqueous layer was acidified to approximately pH 1·3

with 6N hydrochloric acid and the solution extracted with chloroform (3 × 15 ml). Drying and evaporation of the combined chloroform extracts gave a yellow oil which crystallized and which was recrystallized from ether containing a trace of chloroform to give (±)-2-*methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid* in yellow needles (6·7 mg; 2%), m.p. 151–152° (with sweating from 142°). Found: C, 48·7; H, 5·1; N, 14·1. $C_{12}H_{15}N_3O_6$ requires C, 48·5; H, 5·1; N, 14·15%.

(+)-2-*Methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid* [IV, R = H, R′ =2,4-(diNO_2)-C_6H_3-NH]. To a stirred solution of (−)-3-ethyl-3-methoxycarbonyl-3-methylpropionamide (410 mg) in methanol (10 ml) bromine (500 mg) was added followed by a solution of sodium (0·50 g) in methanol (10 ml). The mixture was allowed to stand at room temperature for 30 min, sodium hydroxide solution [5·4 g in water (12 ml)] was added and the mixture boiled under reflux (1 h). The solution was then acidified to pH 1·3 with 4N hydrochloric acid, excess sodium bicarbonate (1·5 g) and 2,4-dinitrofluorobenzene (1·0 g) added, the volume of the mixture was doubled by the addition of ethanol and the solution stirred (2 h) at room temperature. A further quantity of sodium bicarbonate (1·5 g) and 2,4-dinitrofluorobenzene (1·0 g) were added and the stirring continued (1 h). The product was isolated in an identical manner to the racemic compound above. (+)-2-*Methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid* (60 mg; 8·5%) was recrystallized from ether-light petroleum (b.p. < 40°) in yellow needles, m.p. 134–137° (with sweating from 128°). Found: C, 48·4; H, 5·2; N, 14·0. $C_{12}H_{15}N_3O_6$ requires C, 48·5; H, 5·1; N, 14·15%. Circular dichroism maxima at 199 nm ($\triangle\epsilon = +1{\cdot}0$).

(−)-5-*Ethoxy*-3-*ethyl*-1,3-*dimethylindoline* (II, R = R′ = Et). Dihydroeserethole methine methiodide (Polonovski, 1918; Hoshino & Kobayashi, 1934) (3·50 g) in a mixture of water (50 ml) and 95% ethanol (10 ml) was shaken (2 h) with freshly-prepared moist silver oxide (≡ 5 g silver nitrate). The mixture was filtered, the residue washed with 95% ethanol (20 ml) and the combined filtrate and washing evaporated to afford the *quaternary hydroxide* as a brown oil.

The oil was heated under reflux (3 h) at 120–130° (bath temperature)/10 mm and then partitioned between water (30 ml) and ether (3 × 50 ml). The combined ether extracts were dried and evaporated to give *dihydroeserethole methine methine* (II, R = Et, R′ = CH = CH_2) as a greenish-yellow oil (913 mg; 48%).

This was hydrogenated in ethanol (50 ml) at room temperature and atmospheric pressure over Adams' platinum oxide (50 mg). After the absorption of one mole equivalent of hydrogen, the platinum was removed by filtration and washed with ethanol (10 ml). The combined filtrate and washing were evaporated and the residue distilled to afford (−)-5-*ethoxy*-3-*ethyl*-1,3-*dimethylindoline* (II, R = R′ = Et) as a yellow oil (743 mg; 39%), b.p. 166° (bath temperature/2 mm. The picrate crystallized from ethanol in yellow needles, m.p. 147–150° (with sweating from 139°). Found: C, 52·9; H, 5·45. $C_{20}H_{24}N_4O_8$ requires C, 53·55; H, 5·4%. The free base recovered from the picrate had infrared spectrum and b.p. identical with those of the product obtained after distillation of the total reaction product and had $[\alpha]_D^{23} = -3{\cdot}46$, $[M]_D^{23} = -7{\cdot}58$ (95% EtOH). Found: C, 76·9; H, 9·6. $C_{14}H_{21}NO$ requires C, 76·65; H, 9·65%.

Ozonolysis of (−)-5-*ethoxy*-3-*ethyl*-1,3-*dimethylindoline* (II, R = R′ = Et) (2 g) was carried out by the method already described for the ozonolysis of the racemic indoline to give (−)-2-*methyl*-2-(2,4-*dinitrophenylaminomethyl*)*butyric acid* (III, R = Et) as yellow needles (107 mg; 4%) from ether-light petroleum (b.p. < 40°), m.p. 133–135°

(with sweating at 126°), m.p. (on admixture with an equal weight of the synthetic enantiomer prepared above), 152–154° (with sweating at 132°) [*cf.* the racemate, m.p. 151–152° (synthetic) and 153–155° (from degradation) (with sweating at 142° and 141°, respectively)]. Found: C, 48·5; H, 4·9; N, 13·8. $C_{12}H_{15}N_3O_6$ requires C, 48·5; H, 5·1; N, 14·15%. Circular dichroism maxima at 202·5 nm ($\Delta\epsilon = -1{\cdot}4$). The infrared spectra of the enantiomers and their behaviour on thin-layer chromatograms were identical.

Acknowledgements

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