Alkaloids of *Daphnandra* Species. Part VII.† Chemical Evidence for the Structure of Repanduline

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Chemical degradative evidence for the structure of repanduline is presented. It is concluded that the alkaloid is an unusually modified bisbenzylisoquinoline containing a cyclohexa-2,4-dienone system and a 1,4-benzodioxan system joined by a spiro-linkage. A close relationship to nortenuipine is suggested.

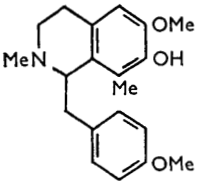
In an earlier Paper [1] a preliminary account was given of the chemistry of repanduline, but it was not then possible to advance a structure for the alkaloid. In this Paper further chemical transformations of repanduline are described, and these, together with the physical evidence and synthesis of the degradation product hemirepanduline described in the following two Papers,[2,3] now provide a solution to the structural problem.

It was earlier [1] concluded that repanduline, $C_{37}H_{36}N_2O_7$,‡ was a bisbenzylisoquinoline alkaloid containing two *N*-methyl groups, one methylenedioxy-group, and one methoxy-group, also that there was no hydroxy-group present. Repandulinic acid, 5,4′-dicarboxy-2,3-methylenedioxydiphenyl ether, was isolated after permanganate oxidation of repanduline, thus characterising the "lower half" of the molecule. The i.r. spectrum displays a sharp band at 1703 cm.$^{-1}$ which disappears on borohydride reduction being replaced by a band at 3560 cm.$^{-1}$ (OH) and is therefore assigned to a carbonyl group. The reduction product is colourless so that the carbonyl group is involved in the visible chromophore of the bright yellow alkaloid. No other functional groups could be detected by standard methods, and, in particular, Kuhn–Roth *C*-methyl determination was negative. Thus the three remaining oxygen atoms are presumably present as inert ether linkages.

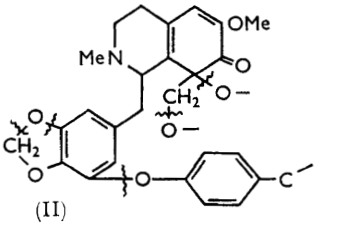
Only reduction with potassium in liquid ammonia proved of value in degrading the repanduline macrocycle. This led to the isolation in low and erratic yield of a colourless crystalline optically active degradation product, hemirepanduline, $C_{20}H_{25}NO_3$. Hemirepanduline exhibited a u.v. spectrum characteristic of a simple benzyltetrahydroisoquinoline and very similar to that of armepavine. The substance contained one *N*-methyl group, one *C*-methyl group, one hydroxy-group, and two methoxy-groups. The presence of two methoxy-groups is remarkable in that repanduline contains only one, and the second group can only arise by an unusual cleavage of the methylenedioxy-group. This is supported by the fact that potash fusion of hemirepanduline gave *p*-anisic acid, and this can only arise from the "lower half" of the molecule. Also the presence of a *C*-methyl group is noteworthy, since this is absent in repanduline; cleavage of a benzyl ether is a likely explanation, as in the case of insularine [4] and cissampareine.[5]

Despite the fact that hemirepanduline is insoluble in aqueous alkali, it was concluded that the hydroxy-group was phenolic since the material gave a positive reaction with the Soloway–Wilenol [6] ferric chloride–pyridine reagent and the *O*-acetyl derivative had a band at 1760 cm.$^{-1}$ in the i.r. spectrum. These facts, together with those derived from a study of the mass spectrum and n.m.r. spectrum,[2] as well as biogenetic considerations, suggested (I) as the most likely structure, and this was confirmed by the synthesis of the racemate.[3]

If it be assumed that the phenolic hydroxy-group in hemirepanduline was the carbonyl group in repanduline, then the partial structure (II) can be derived for repanduline, cleavages in the reduction occurring as indicated.



(I)



(II)

Various further transformations supporting this structure were carried out and are illustrated by partial formulae below. Borohydride reduction of repanduline gave repandulinol (III) $C_{37}H_{38}N_2O_7$, containing a hydroxy-group which could be acetylated, and which on Oppenauer oxidation regenerated repanduline.

Catalytic reduction of repandulinol gave dihydrorepandulinol (IV), $C_{37}H_{40}N_2O_7$, which was also obtained by catalytic reduction of repanduline to dihydrorepanduline (V) followed by borohydride reduction. Treatment of repandulinol with hot dilute acid gave a material, $C_{36}H_{36}N_2O_7$, in which the methoxy-group had been replaced by a carbonyl group appearing in the i.r. spectrum at 1724 cm.$^{-1}$. This reaction is clearly the

† Part VI, I. R. C. Bick, P. S. Clezy, and M. J. Vernengo, *J. Chem. Soc.*, 1960, 4928.

‡ This molecular formula, based on high-resolution mass spectrometry, contains two more hydrogen atoms than that earlier [1] deduced.

[1] I. R. C. Bick, K. Doebel, W. I. Taylor, and A. R. Todd, *J. Chem. Soc.*, 1953, 692.

[2] I. R. C. Bick, J. H. Bowie, J. Harley-Mason, and D. H. Williams, following Paper.

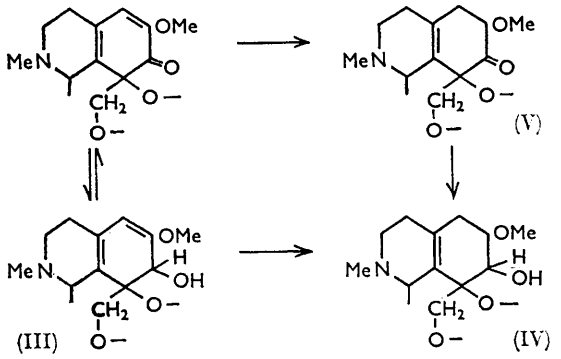
[3] K. Aoki and J. Harley-Mason, *J. Chem. Soc.* (*C*), 1967, 1957.

[4] J. Kunitomo, *J. Chem. Soc. Japan*, 1962, **82**, 1152.

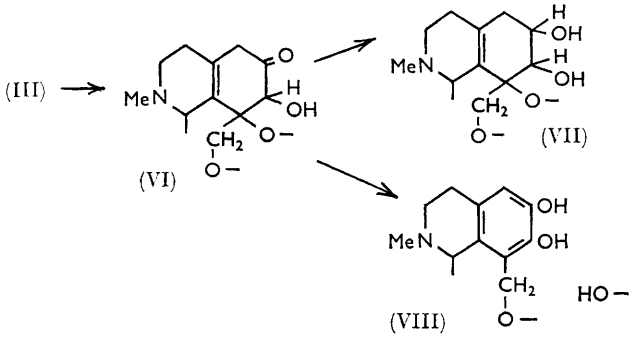
[5] S. M. Kupchan, S. Kubota, E. Fujita, S. Kobayashi, J. H. Black, and S. A. Telang, *J. Amer. Chem. Soc.*, 1966, **88**, 4212.

[6] S. Soloway and S. H. Wilenol, *Analyt. Chem.*, 1952, **24**, 979.

acid hydrolysis of an enol-ether, and the product is de-*O*-methylrepandulinol (VI). On borohydride reduction of the latter, dihydrode-*O*-methylrepandulinol

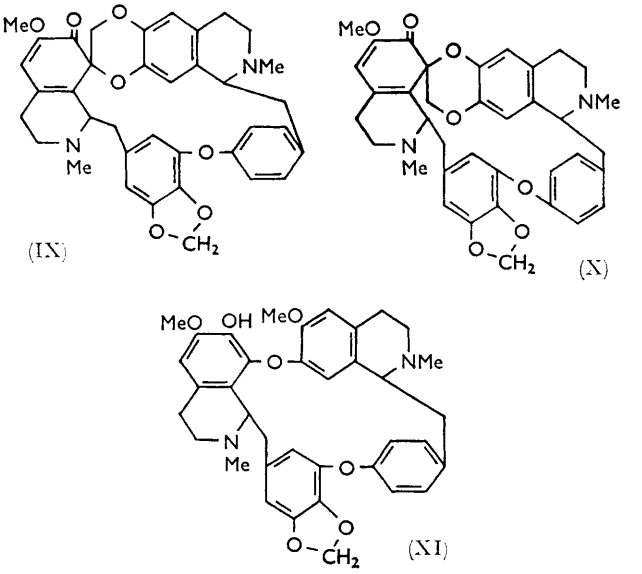


(VII) was obtained, and this gave a positive reaction with periodate–Schiff reagent as a 1,2-diol. On the other



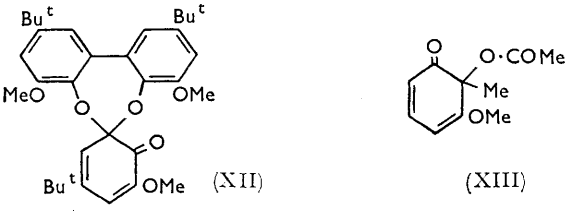
hand, treatment of (VI) with hot methanolic sodium methoxide under nitrogen gave a phenolic alkali-soluble isomer, which, since it gave an intense green colour with ferric chloride characteristic of a catechol derivative, was formulated as isode-*O*-methylrepandulinol (VIII). It will be noted that (VI), unlike any of the compounds earlier described, contains an ether linkage β to a carbonyl group, so ready base-catalysed elimination to give a benzenoid system would be expected. All the above structural assignments are strongly supported by n.m.r. and mass-spectral evidence.[2]

Direct chemical evidence concerning the remainder of the repanduline molecule, *i.e.* the "upper right hand corner," is lacking. Such evidence might be expected to be available from study of the alkali-soluble fractions from the potassium–liquid ammonia cleavage; however, these were intractable tars. The physical evidence (following Paper), however, makes it very difficult to assign any other structure to this portion than a simple *N*-methyltetrahydroisoquinoline, the ether links being joined to the 6- and 7-positions. This leads to the expressions (IX) or (X) for repanduline, containing three asymmetric centres of relative configuration at present unknown. It will be noted that one of these highly unusual structures (IX) is very closely related to that of nortenuipine[7] (XI), a "normal" bisbenzylisoquinoline alkaloid occurring in the same plant and containing two more hydrogen atoms. We suggest that repanduline is indeed formed in the plant by an oxidative



process involving the *O*-methyl group of the "right hand half" of nortenuipine. This process would be similar to that proposed[8] for the formation of a methylenedioxy-group from an *o*-methoxyphenol, except that in this case, an intramolecular carbon–carbon bond is formed instead of a carbon–oxygen bond. Insularine[4] and cissempareine[5] are similar cases, and an analogy could also be drawn with the formation of the "berberine bridge" from a methylimino-group.[9] The alternative structure (X) cannot, however, be excluded on chemical or indeed on spectroscopic grounds,[2] though on the grounds given above we prefer (IX).

Some features of the spectra of repanduline and its degradation products are worthy of comment. The



nearest model for the visible chromophore appears to be the dioxepin[10] (XII). For this bright yellow compound the authors quote only three maxima in the u.v. spectrum below 300 mμ, but on re-examination we find that there is strong absorption in the visible region extending between

[7] I. R. C. Bick, J. Harley-Mason, and M. J. Vernengo, *Anales Assoc. quím. argentina*, 1963, **5**, 135.
[8] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.
[9] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *J. Chem. Soc.*, 1965, 6379.
[10] F. R. Hewgill and B. S. Middleton, *J. Chem. Soc.*, 1965, 2914.

310 and 390 mμ. Another model is the simple cyclohexadienone [11] (XIII), though this has the methoxy-group differently placed. This compound is also yellow and has a broad band at 360 mμ, comparing well with repanduline which has a long wave maximum at 350 mμ. The carbonyl bands in the i.r. spectra of repanduline (1703 cm.$^{-1}$), dihydrorepanduline (1750 cm.$^{-1}$), and de-*O*-methylrepandulinol (1724 cm.$^{-1}$) are all unusually high. In the first two the carbonyl group is flanked on each side by an oxygen substituent. It is well known that equatorial electronegative substituents (*e.g.*, halogens) adjacent to carbonyl groups cause a shift to higher wave-numbers of the carbonyl absorption, and we suggest that this effect is operative here. This effect is plainly apparent in the dioxepin (XII) which in chloroform solution has carbonyl absorption at 1703 cm.$^{-1}$ in exact agreement with repanduline. The last compound, de-*O*-methylrepandulinol, may be compared with cyclohexane-1,2-dione monoethylene acetal [12] which has carbonyl absorption at 1729 cm.$^{-1}$.

EXPERIMENTAL

Hemirepanduline (I).—Repanduline (3 g.) in toluene–benzene (3 : 2; 100 ml.) was added to liquid ammonia (250 ml.) cooled in an acetone–solid CO_2 bath. The mixture was stirred under nitrogen during the addition of potassium (2·3 g.) in small pieces during 2 hr., when the solution retained a permanent blue colour. The vessel was removed from the freezing-bath and the ammonia evaporated in a stream of nitrogen. Water (150 ml.) and ether (50 ml.) were added, and the organic layer was separated, washed with sodium hydroxide solution (1%), dried (Na_2SO_4), and the solvent removed. The residue in benzene solution was chromatographed on neutral alumina yielding *hemirepanduline*, prisms, m. p. 149—151° (from ether). Yields in a number of runs varied from 10 to 300 mg. $[\alpha]_D^{20}$ −33·8° (*c* 3·0 in $CHCl_3$). Ultraviolet spectrum (95% ethanol): $\lambda_{max.}$ 284 mμ (ε 4460) (Found: C, 73·2; H, 7·7; *O*-Me, 18·9; *N*-Me, 8·25; *C*-Me, 3·1. $C_{20}H_{25}NO_3$ requires C, 73·4; H, 7·7; 2 *O*-Me, 19·0; *N*-Me, 8·9; *c*-Me, 4·6%).

Repandulinol (III).—To a suspension of repanduline (1 g.) in methanol (30 ml.) and water (1 ml.) sodium borohydride (0·5 g.) was added; the mixture was then refluxed for 15 min. when it became colourless. Water (70 ml.) was added and the crude solid product collected by filtration. Recrystallisation from methanol gave *repandulinol* (0·8 g., 80%) as needles, m. p. 180—182°, $\lambda_{max.}$ (95% ethanol) 287 mμ (ε 10,210) (Found: C, 71·4; H, 6·8; N, 4·3. $C_{37}H_{38}N_2O_7$ requires C, 71·4; H, 6·2; N, 4·4%).

Reconversion of Repandulinol into Repanduline.—To repandulinol (220 mg.) and fluorenone (300 mg.) in dry toluene (20 ml.) potassium t-butoxide (94 mg.) was added and the mixture refluxed with stirring for 6 hr. under nitrogen. After cooling, ether (100 ml.) was added and undissolved material removed by filtration. The ethereal solution was repeatedly extracted with 1% aqueous hydrochloric acid. The yellow acidic solution was basified with ammonia and extracted with chloroform, and the organic layer separated, dried, and evaporated. On recrystallisation from ethanol the residue gave yellow needles (105 mg.) of repanduline, identified by its i.r. spectrum.

Dihydrorepanduline (V).—Repanduline (1 g.) in glacial acetic acid (50 ml.) was added to a suspension of pre-reduced Adams catalyst (1 g.) (this large excess was found necessary) in acetic acid (50 ml.) and hydrogenated at room temperature and pressure with shaking. A rapid uptake of hydrogen occurred (1·2 mol.) and when uptake had ceased the now colourless solution was filtered and most of the solvent removed under vacuum. The residue was basified with aqueous sodium hydroxide and the precipitate taken up in ether. After drying and removal of the ether, recrystallisation from ethanol yielded *dihydrorepanduline* (0·8 g., 80%) as needles, m. p. 260—262° (decomp.) (Found, on material dried at 100°/0·1 mm.: C, 70·4; H, 6·2; N, 4·3. $C_{37}H_{38}N_2O_7,0{\cdot}5H_2O$ requires C, 70·4; H, 6·2; N, 4·4%), $\lambda_{max.}$ (95% ethanol) 285 mμ (ε 5620), $\nu_{max.}$ 1750 cm.$^{-1}$ (no absorption in the 3500 cm.$^{-1}$ region).

Dihydrorepandulinol (IV).—(*a*) Repandulinol (1 g.) was hydrogenated as described above to give *dihydrorepandulinol* (0·6 g.), microcrystalline powder, m. p. 157—160° (Found, on material dried at 90°/0·1 mm.: C, 70·8; H, 6·8. $C_{37}H_{40}N_2O_7$ requires C, 71·2; H, 6·6%), $\lambda_{max.}$ (95% ethanol) 286 mμ (ε 5200), $\nu_{max.}$ 3540 cm.$^{-1}$ (no absorption in the carbonyl region).

(*b*) Dihydrorepanduline (1 g.) was reduced with sodium borohydride (0·5 g.) as described above for repanduline. Dihydrorepandulinol (0·65 g.), identified with the material above by i.r. and n.m.r. spectra, was obtained.

De-O-methylrepandulinol.—Repandulinol (5·5 g.) was boiled under reflux with deaerated hydrochloric acid (300 ml.; 0·1N) in a current of nitrogen for 20 min. The cooled solution was neutralised with sodium hydrogen carbonate and thoroughly extracted with chloroform, then the extract was evaporated to dryness *in vacuo*. The residue (5 g.) formed very pale yellow needles, m. p. 220—230° (decomp.) (from cold acetone), which turned deep yellow on exposure to air and light. *De-O-methylrepandulinol* was also obtained crystalline from ether and from ethanol; it was insoluble in aqueous alkali and did not give a ferric chloride test. $\lambda_{max.}$ 278 mμ (ε 5500), $\nu_{max.}$ (Nujol) 1724, 3425, and 3575 cm.$^{-1}$ (Found, in material dried at 40°/0·1 mm.: C, 71·0; H, 6·2; N, 4·5; OMe, 0. $C_{36}H_{36}N_2O_7$ requires C, 71·1; H, 5·9; N, 4·6; OMe, 0%). De-*O*-methylrepandulinol formed an *oxime*, m. p. 180° (decomp.) (from chloroform) (Found, in material dried at 40°/0·1 mm.: C, 67·3; H, 6·1; N, 6·4. $C_{36}H_{37}N_3O_7,H_2O$ requires C, 67·4; H, 6·1; N, 6·6%).

Dihydrode-O-methylrepandulinol.— De-*O*-methylrepandulinol (1 g.) was dissolved in deaerated aqueous methanol (25 ml.; 95%). Sodium borohydride (*ca.* 1 g.) was added slowly and after the spontaneous reaction had subsided, the solution was boiled for 15 min. under reflux, cooled, and diluted with water (50 ml.), then extracted three times with ether. The extract was dried (Na_2SO_4) and evaporated to leave a white solid which crystallised from carbon tetrachloride and was recrystallised from ether. It gradually darkened and decomposed without melting at temperatures above 200°; $\lambda_{max.}$ 285 mμ (ε 4710), $\nu_{max.}$ (Nujol) 3310 cm.$^{-1}$ (no carbonyl absorption). *Dihydrode-O-methylrepandulinol* gave a positive reaction with periodate–Schiff reagent for a 1,2-diol (Found, in material dried at 40°/0·1 mm.: C, 69·7; H, 6·5; N, 4·4. $C_{36}H_{38}N_2O_7,0{\cdot}5H_2O$ requires C, 69·7; H, 6·3; N, 4·5%).

[11] F. Wessely, J. Swoboda, and V. Gath, *Monatsh.*, 1964, **95**, 649.

[12] R. H. Jaeger and H. Smith, *J. Chem. Soc.*, 1955, 160.

Isode-O-methylrepandulinol.— De-*O*-methylrepandulinol (1 g.) was added to a deaerated methanolic solution of sodium methoxide (100 ml.; 0·4N) which was boiled under reflux with a current of nitrogen passing through it. Boiling was continued for 0·5 hr., then the solution was allowed to cool in a stream of carbon dioxide, the flow being continued until the solution was saturated. Water (150 ml.) and chloroform were added to dissolve the precipitate, and the aqueous layer was thoroughly extracted with chloroform. The chloroform layers were combined, washed with water, dried, and evaporated *in vacuo*. The residue, m. p. 190—200° (decomp.), could not be obtained crystalline; it was sparingly soluble or insoluble in most common solvents including chloroform and methanol, but it dissolved readily in a mixture of these two, also in aqueous alkali, and it gave an intense dark green ferric chloride test; $\nu_{max.}$ 3300—3400 and 3150 cm.$^{-1}$ (no carbonyl absorption) (Found, on material dried at 40°/0·1 mm.: C, 67·9; H, 6·1; N, 4·2. $C_{36}H_{36}N_2O_7,1{\cdot}5H_2O$ requires C, 68·0; H, 6·1; N, 4·4%).

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