139. *Alkaloids of* Daphnandra *Species. Part IV.* Observations on Repanduline.*

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Repanduline, a yellow alkaloid isolated from the bark of several Australian *Daphnandra* species, yields, on oxidation with potassium permanganate, 5 : 4′-dicarboxy-2 : 3-methylenedioxydiphenyl ether (I ; R = R′ = CO_2H). It is concluded that the alkaloid belongs to the bisbenzyl*iso*quinoline group.

REPANDULINE was first described by Bick and Whalley (*Univ. Queensland Papers, Dept. Chem.*, 1946, **1**, No. 28) who obtained it, together with repandine, from the bark of *Daphnandra repandula.* Later the same authors isolated it from the bark of *D. Dielsii* (*ibid.*, 1947, **1**, No. 30) and more recently it has also been obtained in this laboratory from the bark of *D. tenuipes* (Part V, following paper). Repanduline is unique among the known *Daphnandra* alkaloids in being yellow, the others being colourless. On the basis of their analytical results Bick and Whalley (*loc. cit.*) suggested for the alkaloid a formula $C_{40}H_{46}O_8N_2$ and considered it to contain two methoxy- and two methylimino-groups. We found considerable difficulty in obtaining consistent analytical data for the alkaloid, owing partly to the tenacious retention of solvent of crystallisation (see Experimental Section) and partly to the overlapping of methoxy- and methylimino-determinations. There is no doubt but that repanduline is a diacid base with a molecular weight in the range 600—650 but, as is frequently the case with alkaloids of the bisbenzyl*iso*quinoline group, it is well-nigh impossible to assign to it a molecular formula on analysis and molecular-weight determinations alone. It would seem that only after some degradation products of lower molecular weight have been isolated and characterised will it be possible to reach finality on the molecular formula of the alkaloid. We quote a formula $C_{37}H_{34}O_7N_2$ with one methoxy- and two methylimino-groups, in reasonable agreement with most of the analytical values, but we emphasise that it is purely provisional. The tendency of repanduline to hold solvent of crystallisation tenaciously may well account for the spread in earlier analytical values; the alkaloid can best be obtained free of solvent by crystallisation from light petroleum or tetrahydrofuran.

Repanduline contains at least one methylenedioxy-group since it gives strong positive reactions in the Labat and Gaebel tests. It is non-phenolic, being insoluble in the Claisen cryptophenol reagent, and, since it is unaffected by cold chromic acid in acetic acid and cannot be acetylated, it contains neither primary nor secondary hydroxyl groups; it does not appear to react with the usual carbonyl reagents. The ultra-violet absorption spectrum (see Fig.) shows a maximum at 2830 Å corresponding in position to that shown by the other *Daphnandra* bases (Part II, *J.*, 1949, 2767; Part III, *J.*, 1950, 1606) and a second broad maximum centred at 3460 Å which in acid solution is displaced to 3260 Å.

Since all the *Daphnandra* alkaloids so far studied, repandine (Part I, *J.*, 1948, 2170) daphnandrine, daphnoline, aromoline (Part II, *loc. cit.*), and micranthine (Part III, *loc. cit.*), are members of the bisbenzyl*iso*quinoline group of alkaloids it seemed *a priori* likely that repanduline would belong to the same group. However, all attempts to carry out Hofmann degradations on repanduline metho-salts yielded only resins or small amounts of repanduline itself, and Emde degradations were equally unsuccessful. Next, it was found that repanduline with alkaline potassium permanganate gave, in moderate yield, a dicarboxylic acid, repandulinic acid $C_{15}H_{10}O_7$, whose absorption spectrum resembled those of diphenyl ether derivatives (cf. Tomita and Uyeo, *J. Chem. Soc., Japan*, 1943, **64**, 70). On alkali fusion repandulinic acid gave *p*-hydroxybenzoic acid, and decarboxylation furnished a crystalline neutral product which was evidently a diphenyl ether derivative and, like the parent acid, gave a positive reaction for methylenedioxy-groups and contained neither hydroxyl nor methoxyl. From these facts and on biogenetic considerations it seemed probable that repandulinic acid was 5 : 4′-dicarboxy-2 : 3-methylenedioxydiphenyl ether (I ; R = R′ = CO_2H) and it was decided to synthesise this compound. Methyl 5-bromo-

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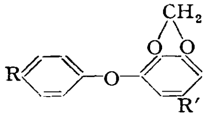
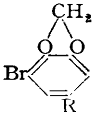
* Part III, *J.*, 1950, 1606.

3 : 4-methylenedioxybenzoate (II; R = CO_2Me) was synthesised, by a route essentially similar to that employed independently by Kondo and his co-workers (*Ann. Rep. ITSUU hab. Japan,* 1950, **54**; Jap. P. 142,596), from 3 : 4-dihydroxybenzaldehyde by bromination, methylenation with methylene sulphate, and oxidation to the corresponding acid (II; R = CO_2H). Erne and Ramirez (*Helv. Chim. Acta,* 1950, **33**, 912) have used a similar method for the preparation of (II; R = CHO). Fusion of (II; R = CO_2Me) with the potassium salt of methyl *p*-hydroxybenzoate yielded the ester (I; R = R′ = CO_2Me) which was identical with dimethyl repandulinate in m. p., mixed m. p., and infra-red spectrum (Nujol mulls).

(I)

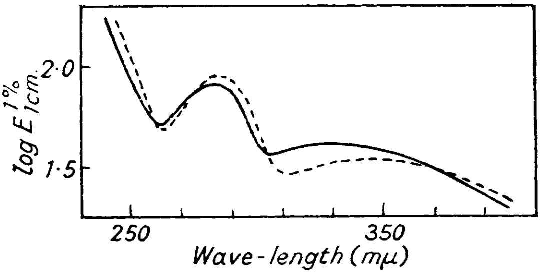




(II)

The isolation of (I; R = R′ = CO_2H) as an oxidation product coupled with the molecular size of repanduline makes it virtually certain that the latter is an alkaloid of the bisbenzyl*iso*quinoline group. The presence in it of a methylenedioxy-group situated in the diphenyl ether portion of the molecule is, however, unique among the known alkaloids of this group. The only bisbenzyl*iso*quinoline alkaloids known to contain a methylenedioxy-group are cepharanthine (Kondo and Keimatsu, *Ber.*, 1938, **71**, 2553), stephanine (Kondo and Sanada, *J. Pharm. Soc Japan,* 1927, **47**, 31; 1928, **48**, 163), and some of the minor *Daphnandra* bases (Part V, following paper). Of these, the only one of known structure (cepharanthine) has its methylenedioxy-group in one of the tetrahydro*iso*quinoline residues. As far as we are aware the only related alkaloid in which two hydroxyl (or potential hydroxyl) groups are found in one of the benzyl residues is magnolamine (Proskurnina, *J. Gen. Chem. U.S.S.R.*, 1946, **16**, 129; Tomita, Fujita, and Nakamura, *J. Pharm. Soc. Japan,* 1951, **71**, 1075). Interesting as this feature is, there is nothing in the structure of repandulinic acid which would account for the yellow colour of the parent alkaloid.

Absorption spectrum of repanduline in (– – – –) 95% *ethanol and* (——) 0·1N-*hydrochloric acid.*



Repanduline has not the properties of a betaine and its colour is probably associated with some unusual modification of either or both of the tetrahydro*iso*quinoline residues in its molecule; such a view would be in accord with the failure to date of Hofmann and Emde degradation procedures. The number of methylenedioxy-groups in repanduline is not, of course, known, but it seems unlikely that there is more than one. On the assumption that the two (perhaps modified) tetrahydro*iso*quinoline residues are linked by an ethereal oxygen (and there cannot be two such links since repanduline does not give the diphenodioxin colour reactions), five of the seven oxygen atoms in the provisional formula are accounted for in two ether linkages and one methoxy- and one methylenedioxy-group. It seems hardly likely that both the remaining oxygens are present in a second methylenedioxy-group; in view of the yellow colour of the alkaloid some other mode of linkage of at least one of the remaining oxygen atoms is probable.

EXPERIMENTAL

Repanduline.—The isolation procedure used by Bick and Whalley (*loc. cit.*) was modified in that extraction of *D. repandula* bark (4 kg.) was carried out exhaustively with boiling methanol. The extract was evaporated *in vacuo* and the crude alkaloids were dissolved in dilute hydrochloric acid, filtered, and reprecipitated at pH 8 with sodium carbonate. The resultant solid was extracted with chloroform (Soxhlet). The chloroform extract was concentrated and diluted with ethyl acetate, whereupon crude repanduline (60 g.) crystallised. Final purification could be achieved by recrystallisation or by chromatography in benzene on neutral alumina.

Repanduline crystallises from most of the common organic solvents in slender yellow needles, $[\alpha]_D^{17}$ +473° (*c*, 0·98 in $CHCl_3$), $[\alpha]_D^{16}$ +434° (*c*, 0·2 in MeOH). It shrinks to a dark gum at 180—185° and on further heating decomposes with frothing between 215° and 232°. The following are representative analytical figures obtained on different samples: (i) Recrystallised from methanol. Found, in material dried at room temp./0·1 mm.: C, 69·2, 68·8; H, 5·8, 5·7%; equiv., 324. Found, in material dried at 110°/0·1 mm.: C, 69·6, 69·3; H, 6·0, 5·9; N, 4·4, 4·9; OMe, 5·8, 5·1; NMe, 7·3, 6·0; active H, 0·43%; equiv., 322; *M* (Signer method), 640. Found, in material dried at 140°/0·1 mm.: C, 69·9; H, 6·0; loss on drying, 2·9%. (ii) Recrystallised from light petroleum (b. p. 80—100°). Found, in material dried for 12 hours at 100°/0·1 mm.: C, 72·0; H, 5·9; OMe, 5·6, 5·2; NMe, 9·2, 8·0; active H, 0·07%. (iii) Recrystallised from tetrahydrofuran. Found, in material dried at room temp./0·1 mm.: C, 71·2, 71·2; H, 5·7, 5·8%. (iv) Recrystallised from aqueous 2-ethoxyethanol. Found, in material dried for 12 hours at 120°/0·1 mm.: C, 71·6; H, 5·5%. (v) Recrystallised from *cyclo*hexane. Found, in material dried either at room temp. or at 144°/0·1 mm.: C, 73·0, 73·4; H, 6·9, 6·9%; equiv., 361 [Calc. for $C_{37}H_{34}O_7N_2$ (*M*, 618): C, 71·8; H, 5·5. For $C_{37}H_{34}O_7N_2,H_2O$ (*M*, 636): C, 69·8; H, 5·7. For $C_{37}H_{34}O_7N_2,1{\cdot}5H_2O$ (*M*, 645): C, 68·8; H, 5·8; N, 4·3; OMe 4·8; 2NMe, 9·0; active H, 0·46%. For $C_{37}H_{34}O_7N_2,C_6H_{12}$ (*M*, 702): C, 73·5; H, 6·6%)].

Repanduline was dissolved in a slight excess of warm 5N-hydrochloric acid and set aside. The *dihydrochloride* which separated was recrystallised from water containing a little hydrochloric acid and formed yellow prisms, decomp. slowly at >100° (Found, in material dried for 1 hour at 60°/0·1 mm.: C, 57·9, 57·6; H, 6·2, 6·3; N, 4·0, 3·7; Cl, 10·1%; equiv., 350. $C_{37}H_{34}O_7N_2,2HCl,4H_2O$ requires C, 58·0; H, 5·9; N, 3·7; Cl, 9·5%; equiv., 382. Found, in material dried for 12 hours at 100°/0·1 mm.: C, 61·1, H, 6·2. $C_{37}H_{34}O_7N_2,2HCl,2H_2O$ requires C, 61·1; H, 5·4%). *Repanduline dioxalate* separated from a warm solution of the base in concentrated aqueous oxalic acid and crystallised from aqueous methanol as bright yellow tetrahedra, decomp. 214° (Found, in material dried for 1 hour at 60°/0·1 mm.: C, 56·5; H, 5·2; N, 3·3. $C_{37}H_{34}O_7N_2,2C_2H_2O_4,4H_2O$ requires C, 56·6; H, 5·3; N, 3·2%).

Repanduline was refluxed for 6 hours in benzene with an excess of methyl iodide. The precipitated *dimethiodide* was washed with benzene and recrystallised from methanol in which it was only slightly soluble, forming yellow needles, decomp. 240° (Found, in material dried for 12 hours at 100°/0·1 mm.: C, 48·9; H, 4·8. $C_{39}H_{40}O_7N_2I_2,3H_2O$ requires C, 48·9; H, 4·8%). Light absorption in 95% ethanol: max. at 2850 ($E_{1\,\text{cm.}}^{1\%}$, 55) and 3260 Å ($E_{1\,\text{cm.}}^{1\%}$, 29·8).

Oxidation of Repanduline.—A solution of repanduline (9·6 g.) in 2N-sulphuric acid (900 c.c.) was neutralised with aqueous potassium hydroxide (4%), then 2% aqueous potassium permanganate (21 c.c. ≡ *ca.* 27 O) was added during 2 hours with vigorous stirring. Manganese dioxide was filtered off and washed with hot water (1 l.) and the combined filtrate and washings were concentrated to 150 c.c. at 40° under reduced pressure. The solution was made acid to Congo-paper and the precipitate filtered off, dissolved in methanol, and treated with excess of ethereal diazomethane. The product (2·41 g.) was chromatographed in benzene on neutral alumina (60 g.), the benzene eluate yielding *dimethyl repandulinate* (444 mg.) as colourless needles. Recrystallised from methanol this had m. p. 116—118°, and gave a positive test for methylenedioxy-groups with the Labat reagent (Found, in sublimed material: C, 61·9; H, 4·3; MeO, 20·4, 19·5. $C_{17}H_{14}O_7$ requires C, 61·8; H, 4·3; 2MeO, 19·0%). Light absorption in 95% ethanol: max. at 2540—2560 Å (log ε 4·42). Hydrolysis of the diester with methanolic potassium hydroxide (10%) yielded *repandulinic acid*, colourless needles (from methanol), m. p. 290° (decomp.) (Found: C, 60·1; H, 3·5. $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%).

Potash Fusion of Repandulinic Acid.—Repandulinic acid (50 mg.) and zinc dust (25 mg.) were added to potassium hydroxide (1 g.) at 240° and the temperature of the melt raised to 325° during 10 minutes. After a further 12 minutes at this temperature the melt was cooled, triturated with dilute hydrochloric acid, then extracted with ether, leaving a crystalline acidic residue (30 mg.). The latter on methylation with ethereal diazomethane afforded an ester, m. p. 44—45° undepressed in admixture with an authentic sample of methyl *p*-methoxybenzoate (m. p. 44—45°).

Methyl 5-Bromo-3 : 4-methylenedioxybenzoate.—This was prepared by essentially the method used by Kondo and his co-workers (*loc. cit.*); recrystallised from methanol it had m. p. 96° (lit., m. p. 96°).

5 : 4′-*Dicarbomethoxy*-2 : 3-*methylenedioxydiphenyl Ether* (*Dimethyl Repandulinate*).—Methyl

p-hydroxybenzoate (1·54 g.) was added to a solution of potassium (0·39 g.) in methanol (10 c.c.) and evaporated to dryness. The residue was then mixed with methyl 5-bromo-3 : 4-methylenedioxybenzoate (5·21 g.) and copper powder (0·3 g.) and heated at 200° during 5 hours. The cooled melt was extracted with ether, and the extract washed with dilute sodium hydroxide, then water, and dried (Na_2SO_4). The solution was evaporated to dryness and the methyl piperonylate and methyl 5-bromopiperonylate were sublimed off at 100°/10 mm. The residue was dissolved in benzene and filtered through activated alumina (20 g.) which was washed with benzene. The crude ester obtained by evaporation of the filtrate was purified by recrystallisation from methanol, giving 5 : *4′-dicarbomethoxy-*2 : *3-methylenedioxydiphenyl ether* (240 mg.), m. p. 119—120° (Found : C, 61·6; H, 3·9%).

Hydrolysis of the ester afforded synthetic 5 : 4′-dicarboxy-2 : 3-methylenedioxydiphenyl ether, m. p. 290° (decomp.) (from methanol) alone or mixed with repandulinic acid.

2 : 3-*Methylenedioxydiphenyl Ether*.—A mixture of 5 : 4′-dicarboxy-2 : 3-methylenedioxydiphenyl ether (78 mg.) and copper powder (160 mg.) in quinoline (3 c.c.) was refluxed for 30 minutes. The cooled mixture was diluted with ether and filtered, and the solution washed thoroughly with hydrochloric acid (2N), then sodium hydrogen carbonate, and finally water, dried, and evaporated. The residue of crude 2 : *3-methylenedioxydiphenyl ether* (54 mg.) was sublimed at 80°/12 mm. and then recrystallised from ethanol, giving colourless plates, m. p. 51—52° (Found : C, 73·1, 72·9, 73·3; H, 5·0, 5·1, 5·2. $C_{13}H_{10}O_3$ requires C, 72·9; H, 4·7%). Light absorption in 95% ethanol : max. at 2760 (log ε 3·46) and 2700 Å (log ε 3·44). The substance gave no depression in m. p. when mixed with the decarboxylation product (m. p. 51—52°) of repandulinic acid.

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