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27. Essential Oils from the Queensland Flora

Part XXI.—*Evodia Elleryana*

BY

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Part I.—*Daphnandra repandula.*

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ALKALOIDS OF QUEENSLAND FLORA.

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PART I.—THE ALKALOIDS OF *DAPHNANDRA REPANDULA*.

Daphnandra repandula (F. v. M.) of the family *Monimiaceae* is native to Queensland and is found distributed throughout the tropical scrubs in the north of this State. It is a tree of moderate size with thick bark which is yellowish in colour and bitter to the taste. The bark was first examined by T. L. Bancroft (1, 2, 3), who found it to be remarkably rich in alkaloids, with a total of about 6 per cent. Bancroft claimed to have isolated three different bases from this plant in a colourless crystalline condition. He gave no account of their chemical and physical properties other than noting their solubilities in various solvents, but described the physiological effect on injecting into frogs and other animals. Of the three, a water-soluble alkaloid was found to be the most active physiologically.

No instance of this tree having caused fatalities to stock is known, but it is listed by White (4) as being potentially dangerous to stock, since it sometimes suckers in paddocks.

Bancroft also examined the bark of *Daphnandra micrantha*, which grows in the rain forests of central and southern Queensland and northern New South Wales, and reported that it contained the same three alkaloids as *Daphnandra repandula*.

Pyman (5) carried out further investigations into the alkaloids of *Daphnandra micrantha*, and described the isolation and characterisation of three new alkaloids, daphnandrine, micranthine and daphnoline, but did not discover any water-soluble alkaloid as reported by Bancroft. A third species, *Daphnandra aromatica*, also contains alkaloids (1) and is at present under investigation. Other members of this family known to contain alkaloids include *Boldea frangrans* (6), *Atherosperma moschatum* (6) and *Doryphora Sassafras* (7).

The present paper describes the investigation of the bark of *Daphnandra repandula* and the isolation of two new alkaloids in a pure crystalline state, and their characterisation. Neither of these is water soluble. Indications of the presence of a water-soluble alkaloid have been obtained, but none has so far been isolated in a pure condition. One of the bases described is yellow in colour and thus does not correspond with any of Bancroft's three colourless bases. Neither corresponds with any of the alkaloids isolated by Pyman from *Daphnandra micrantha*, nor with any described in the literature. For these two alkaloids the names repanduline and repandine are suggested.

Repanduline forms bright yellow needle shaped crystals when pure. It has the formula $C_{40}H_{46}O_8N_2$ and is neutralised by two equivalents of acid. It contains two methoxyl groups. two methylimide groups, and at least one methylenedioxy group.

Repandine crystallises in very fine white needles which tend to stick together in soft masses. Its molecular formula is $C_{38}H_{42}O_6N_2$, and it contains three methoxyl groups, and two methylimide groups; unlike repanduline it contains no methylenedioxy group.

EXPERIMENTAL.

Isolation of the Alkaloids.

About 60 lb. of the dried bark were finely ground and percolated in the cold with alcohol until the percolated solvent gave only faint alkaloid tests. The alcoholic solution was concentrated under reduced pressure to a thick syrup, the temperature being kept below 60° C. during the initial part of the distillation, and during the latter stages, below 50° C.

The sticky residue (about 3 litres in all) was taken up in dilute (½ per cent.) hydrochloric acid, and the small amount of material remaining undissolved after allowing to stand, was filtered from the viscous solution and washed with further quantities of dilute hydrochloric acid, the washings being added to the main bulk.

On making this solution slightly alkaline with ammonia, a copious yellow precipitate formed, which became brown on standing. This precipitate was filtered off, leaving a solution which still gave tests for alkaloid indicating the presence of a water-soluble alkaloid. The aqueous solution was concentrated under reduced pressure to a few hundred cubic centimetres, the temperature being kept less than 40° C., whereupon some gummy material was deposited and was filtered off. The solution, which gave strong tests for alkaloids, was extracted in a continuous extractor with immiscible solvents. Chloroform and ether failed to remove significant amounts of alkaloid, but removed a certain amount of impurity. Of the methods so far tried for concentrating and purifying this water-soluble base, the following has yielded the best results. The neutral aqueous solution is extracted in a separating funnel with m. cresol, which removes the base almost quantitatively; after washing with a dilute solution of ammonium carbonate, the m. cresol layer is then diluted with about 5 times its volume of ether and extracted with water. The aqueous solution, purified in this way, may then be carefully evaporated down. Attempts to crystallise the alkaloid or its salts have not yet succeeded.

The precipitate which was thrown down with ammonia, and which contains the bulk of the alkaloids, was extracted by stirring with several portions of chloroform. These chloroform extracts were combined and extracted several times with 5 per cent. sodium hydroxide solution and finally washed with water. The sodium hydroxide solution removed a considerable amount of impurity from the chloroform extract, but did not remove any alkaloid; thus apparently this species does not contain any phenolic alkaloid corresponding to daphnoline isolated by Pyman from *Daphnandra micrantha*.

The chloroform extract was then diluted with alcohol and a little ethyl acetate, and on standing for a few hours, yellowish well-shaped crystals were deposited. These crystals proved to consist mainly of repanduline, together with a little repandine.

Further quantities of the mixed alkaloids were obtained by evaporating down the mother liquor. The latter was finally evaporated under reduced pressure almost to dryness, and the residue extracted with benzene. This benzene solution on cooling

deposited white crystals of repandine, which is only sparingly soluble in cold benzene, whereas repanduline is readily soluble, and thus the mixture of alkaloids can be separated. The repanduline, which was obtained in larger amount, was recrystallised first from ethyl acetate, and then several times from ether; the repandine was recrystallised from toluene, and then from alcohol, until the melting point became constant.

A further extraction was applied to the bark which had already been percolated with alcohol, with the object of completely removing any repanduline unextracted by the former process. The extraction was carried out with benzene which had been saturated with ammonia by shaking with concentrated aqueous ammonia. The benzene extract was concentrated to a thick sticky mud by evaporation under reduced pressure at about 200 mm., and a temperature under 45° C. The residue was shaken with dilute hydrochloric acid (½ per cent.) and the resulting liquid was allowed to stand. The insoluble gummy material was filtered off from the solution, which was then made alkaline with ammonia. The resulting thick yellow precipitate was filtered off and dried in sunlight, and the dark brown dried material was powdered and extracted at room temperature with benzene. To the filtered benzene extract, a quantity of ethyl alcohol was added, causing copious precipitation of the yellow alkaloid, repanduline, which was purified by recrystallisation as described before.

The residue remaining after cold extraction with benzene was refluxed with benzene. The filtered solution, on cooling, deposited a quantity of white crystalline repandine, which was purified as before.

The total amount of alkaloid in the bark was determined by mixing a weighed sample with slaked lime, moistening and drying, then extracting in a Soxhlet apparatus with chloroform. After evaporating off the chloroform, the residue was shaken with excess standard acid and back titrated with standard alkali. It amounted to the high figure of 6·3 per cent.

Repanduline.

Repanduline crystallises from ether in bright yellow fine needle-shaped crystals. Slow decomposition takes place when it is kept at 100° C. for any length of time and decomposition is rapid at about 160° C. It is readily soluble in benzene, chloroform, dioxan, acetone and dilute acids in the cold. In the following it is sparingly soluble in the cold, but moderately soluble in the boiling solvent: ethyl and methyl alcohol, ether, ethyl acetate, petroleum ether. It is practically insoluble in water at all temperatures.

Determination of the molecular weight by the Signer method (8) gave a value of 658 in acetone and 671 in chloroform; by Rieche's ebullioscopic method (9) values of 707 and 713 in chloroform were obtained.

A solution of repanduline in 50 per cent. alcohol was titrated electrometrically against hydrochloric acid using a glass electrode, and from the titration curve (Fig. I.), plotted from a large number of readings, the exact equivalent point was calculated by the method of Fenwick (10). The equivalent weight found by this method was 341. Repanduline is thus a diacidic base with a molecular weight of about 682.

Analyses.—

Found in air-dried base :—

C = 70·3, 70·4, 70·4, 70·4, 70·2, 70·3 ;

H = 6·78, 6·65, 6·64, 6·75, 6·81, 6·61 ;

N = 3·96, 4·08, 4·14, 3·97, 4·07, 4·02 ;

OCH_3 = 10·3, 8·7, 9·7, 8·9, 8·9, 8·9 ;

NCH_3 = 7·0, 8·7, 6·8, 7·3, 7·3, 8·1 ;

$C_{40}H_{46}O_8N_2$ (682·8) requires C = 70·4 ; H = 6·79 ; N = 4·10 ;
2 OCH_3 = 9·1 ;

2 NCH_3 = 8·5.

The carbon and hydrogen determinations were performed by the semimicro method of Sucharda and Bobranski (11). Nitrogen was determined by the semimicro Kjeldahl method described by Belcher and Godbert (12), using the indicator described by Ma and Zuazaga (13). The methyoxl determination was carried out by Vieböck and Schwappach's method (14), and the methylimide group by a modification of Friedrich's method (15).

The specific rotation of repanduline was determined in benzene and in chloroform solution.

Benzene :—

$\alpha_D^{25°C} = +34·3°$; $C = 3·233$; $l = 2$ dcm ; $[\alpha]_D^{25°C} = +531°$

$\alpha_D^{19°C} = +8·77°$; $C = 0·8356$; $l = 2$ dcm ; $[\alpha]_D^{19°C} = +525°$

Chloroform :—

$\alpha_D^{18°C} = +3·92°$; $C = 0·4320$; $l = 2$ dcm. ; $[\alpha]_D^{18°C} = +443°$

From an examination of the titration curve for repanduline (Fig. 1) by the method described by Britton (16), it would seem that repanduline is a diacid base with apparent dissociation constants not very different from one another, of the order of 2×10^{-9}. From accounts of similar titrations in aqueous alcohol (17, 18, 19) the dissociation constant is apparently not much affected by the presence of alcohol, and thus repanduline as a base is comparable in strength with pyridine ($K_b = 1·2 \times 10^{-9}$) and isoquinoline ($K_b = 1·1 \times 10^{-9}$).

In contrast to the readiness with which repanduline itself crystallises, its salts are difficult to crystallise and difficult to obtain pure. Certain of them were prepared by exactly neutralising an alcoholic solution of the base with the appropriate acid, carefully evaporating and allowing to crystallise. The hydrochloride crystallises from water in yellow needles which decompose at about 218° C. The hydrobromide crystallises from water in the form of yellow prisms decomposing at about 240° C. The tartrate and perchlorate crystallise from water in yellow radiating needles which on heating decompose without melting.

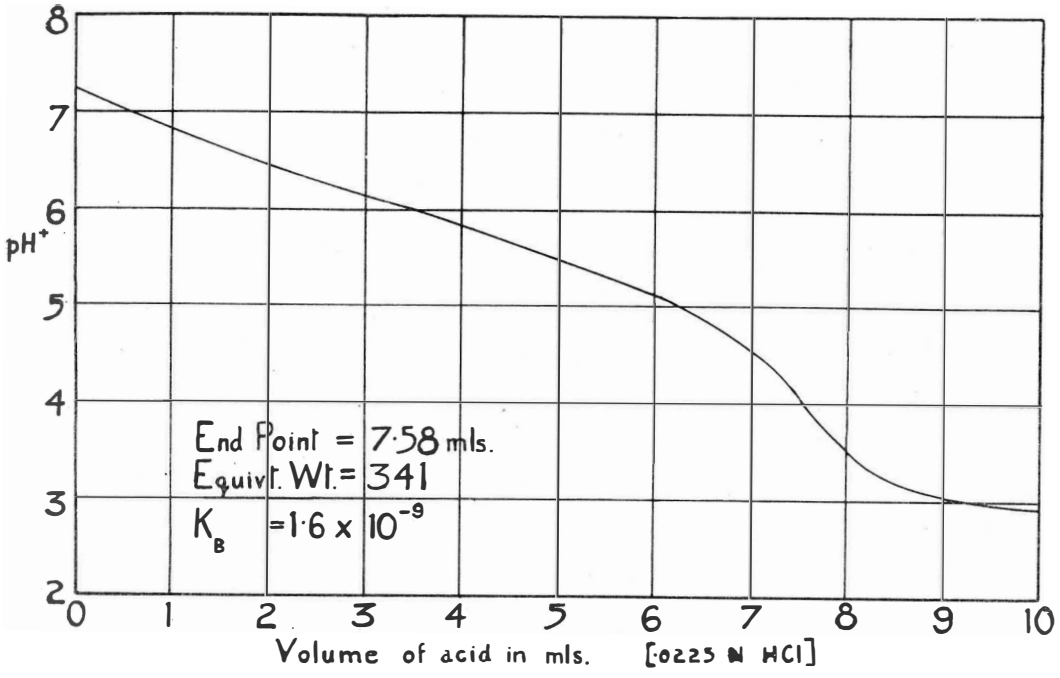

Figure 1.
Titration of 0·0581 g. repanduline with 0·0225 N. acid.

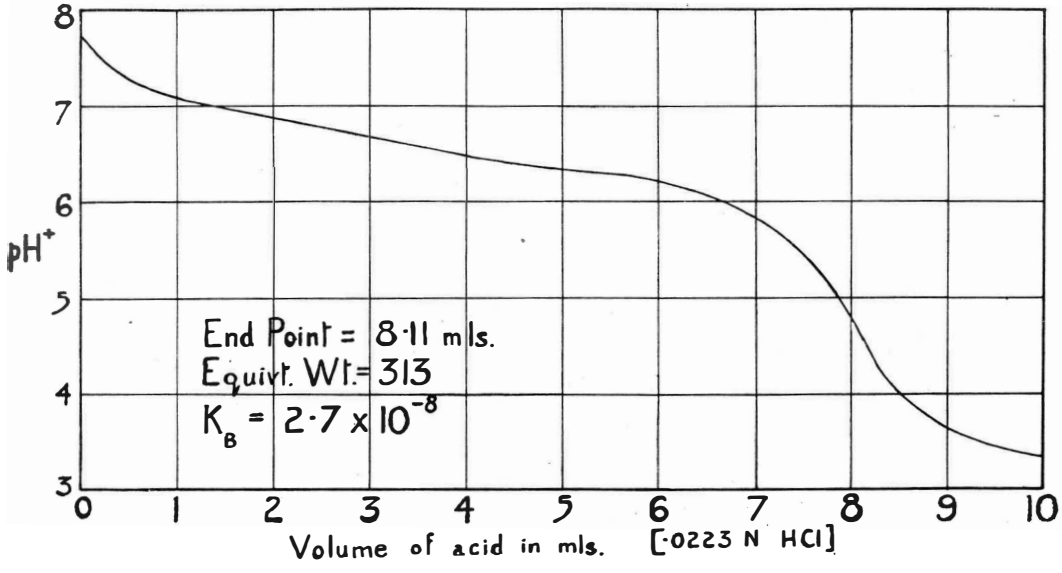

Figure 2.
Titration of 0·0567 g. repandine with 0·0223 N. acid.

Repanduline forms sparingly soluble amorphous complexes with salts of heavy metals; gold, platinum, mercury and zinc yield compounds of this nature, which decompose on heating.

The specific rotation of the hydrochloride was determined in aqueous solution at two temperatures:—

$$\alpha_D^{18°C} = +3{\cdot}44°;\quad C = 0{\cdot}4188;\quad l = 2 \text{ dcm};$$

$$[\alpha]_D^{18°C} = +411° \text{ for basic ion};\ = +372° \text{ for salt.}$$

$$\alpha_D^{26°C} = +3{\cdot}71°;\quad C = 0{\cdot}2240;\quad l = 4 \text{ dcm};$$

$$[\alpha]_D^{26°C} = +428° \text{ for basic ion};\ = +386° \text{ for salt.}$$

Repandine.

This base crystallises in very fine white microscopic needles which melt at 255° C. with slight decomposition. It is readily soluble in cold chloroform and dioxan, and moderately soluble in cold methyl alcohol. In the following solvents repandine dissolves sparingly at ordinary temperatures but readily on heating; benzene, toluene, acetone, ethyl and isopropyl alcohol, carbon tetrachloride. It is very sparingly soluble in ether and petroleum ether, and insoluble in ethyl acetate and water, although it dissolves readily in dilute acids.

The molecular weight as determined by Signer's method in chloroform was 699; the values obtained by the ebullioscopic method in the same solvent were 609 and 649. The equivalent weight, calculated from the titration curve of repandine (Fig. II.), was found to be 313. Thus repandine like repanduline is a diacidic base, with a molecular weight of about 626.

Analyses.—

Found in air-dried base:—

C = 73·3, 73·6, 73·9, 73·4, 73·7, 73·2;
H = 6·83, 6·73, 6·86, 6·85, 6·84, 6·92;
N = 4·41, 4·45, 4·49, 4·47, 4·39, 4·38;
OCH_3 = 15·3, 15·5, 15·2, 14·8, 15·0, 15·2;
NCH_3 = 8·6, 6·9, 6·6, 9·8, 6·6, 6·4;
$C_{38}H_{42}O_6N_2$ (622·8) requires C = 73·3; H = 6·80; N = 4·50;
3 OCH_3 = 14·9;
2 NCH_3 = 9·3.

The specific rotation was determined in benzene and in chloroform solution.

Benzene:—

$$\alpha_D^{20°C} = -2{\cdot}04°;\quad C = 0{\cdot}6504;\quad l = 4 \text{ dcm};\quad [\alpha]_D^{20°C} = -78{\cdot}4°$$

Chloroform:—

$$\alpha_D^{19°C} = -0{\cdot}84°;\quad C = 0{\cdot}5628;\quad l = 2 \text{ dcm};\quad [\alpha]_D^{19°C} = -74{\cdot}6°$$

The apparent dissociation constants of repandine in 50 per cent. alcohol at 20° C., calculated from the titration curve (Fig. II.), were of the order of $3 \times 10-^{8}$. Thus repandine is a slightly stronger base than repanduline.

When neutralised with acids, repandine yields a number of salts which crystallise from aqueous solution. The hydrobromide forms white needle-shaped crystals which decompose above 200° C.; the tartrate forms white radiating needles which sinter at 199° C. and melt at 210° C.; the phosphate gives thick masses of colourless columnar crystals which decompose gradually above 200° C.; the small white needles of the sulphate decompose gradually at temperatures above 200° C. the oxalate produces flat blade-like white crystals; the perchlorate forms fine white needles aggregated in the form of sheaves.

Repandine forms complexes with salts of heavy metals, including gold, platinum, mercury, and zinc. They are slightly soluble in cold water, and readily in hot. They are white or cream coloured amorphous solids which on heating decompose without melting, with the exception of the mercury compound, which melts at 180° C.

The specific rotation of the hydrochloride was determined in aqueous solution :—

$$\alpha_{D}^{20°C} = -1{\cdot}36° ; \quad C = 0{\cdot}4304 ; \quad l = 2 \text{ dcm.}$$

$$[\alpha]_{D}^{20°C} = -158° \text{ for basic ion} ; = -141° \text{ for salt.}$$

Colour Reactions of the Alkaloids.

The colour test with gallic acid (20, 21) for the presence of methylene-dioxy groups was found to give good results when applied to compounds known to contain this group. Repanduline gave a positive reaction, and the presence of a methylene-dioxy group was confirmed by the test described by Gadamer (22). The tests were negative in the case of repandine.

Other reagents gave tests as shown in the table :—

Reagent.	Repanduline.	Repandine.
Meyer's Reagent	Thick curdy yellowish precipitate	Thick curdy white precipitate
Dragendorff's Reagent	Thick reddish-brown precipitate	Thick reddish-brown precipitate
Wagner's Reagent	Thick brown precipitate	Thick brown precipitate
Picric Acid	Yellow amorphous precipitate	Yellow amorphous precipitate
Concentrated sulphuric acid	Deep yellow changing to dark reddish-brown on heating	No colour change
Concentrated nitric acid	Brownish-red	Yellow-brown
Sulphuric acid containing a trace of nitric acid	Deep pink	Yellow-brown
Fröhde's Reagent	Black changing to reddish-brown	Purple changing to yellow
Mandelin's Reagent	Reddish-brown	Black changing to brown
Potassium Ferricyanide in concentrated sulphuric acid	Orange, changing on dilution to pink	No colour change
Potassium permanganate in concentrated sulphuric acid	Brown	No colour change

These alkaloids are being further investigated with a view to determining their structure. From preliminary physiological tests carried out in the Department of Physiology, University of Melbourne, and the Department of Physiology, University of Queensland, it would appear that repanduline is non-toxic to animals up to comparatively large doses; a slight lowering of blood pressure is produced, but little else. Repandine is considerably more active, a dose of about 150–200 mgm. per kgm. being toxic for mice. A comparatively small dose causes a fall of blood pressure; larger amounts cause a stoppage of the ventricle, but have no effect on the auricles in the case of frogs.

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