140. *Alkaloids of* Daphnandra *Species. Part V.* An Examination of the Alkaloidal Content of* D. micrantha *and of* D. tenuipes. *Isolation of Some Minor* Daphnandra *Alkaloids.*

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The alkaloidal content of the bark of *D. micrantha* collected in various localities and at different times varies considerably. Samples from Queensland contained mainly micranthine, but daphnoline and daphnandrine were also found in barks from New South Wales. The bark of *D. tenuipes*, a species found in northern New South Wales, yielded repanduline, aromoline, and a new alkaloid tenuipine; the leaves yielded a de-*N*-methyltenuipine. An examination of *D. repandula* for minor alkaloids led to the isolation of *O*-methylrepandine and repandinine, probably identical with (±)-tenuipine; *D. dielsii* similarly examined yielded *O*-methylrepandine, repandinine, and tenuipine. The structure of the new alkaloids is discussed.

In his work on *Daphnandra micrantha*, Pyman (*J.*, 1914, **105**, 1679) isolated from the bark of this Australian tree three alkaloids, daphnandrine, daphnoline, and micranthine. As recorded earlier (Bick, Ewen, and Todd, *J.*, 1949, 2767; Bick and Todd, *J.*, 1950, 1606) we were unable to detect any alkaloid other than micranthine in samples of *D. micrantha* bark collected in Southern Queensland. This might mean that the material examined by Pyman was wrongly identified or that the alkaloid content of the species varied according to the locality in which it was grown, the size of the tree, or the season in which the bark was collected. To clarify the position we have now examined samples of *D. micrantha* collected at various times, not only in Queensland but also in northern New South Wales. Pyman's material was supplied to him by the Director of the Sydney Botanic Gardens, and presumably was collected in New South Wales, although unfortunately there appears to be no record of the transaction either in this country or in Australia. Our results are shown in the annexed Table

* Part IV, preceding paper.

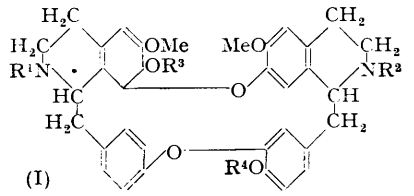
Locality	Size of tree	Date collected	Alkaloids
Mt. Glorious, Queensland (bark)	Stem diam. 3′ at breast height	March, 1945	Micranthine
,, ,, ,, ...	80′ × 2′	April, 1946	Micranthine
Upper Brookfield, Queensland (wood)...	15—20′ × 2—4″	April, 1946	Micranthine
Draper's crossing, near Brisbane (bark)	25—30′ × 3—4″	January, 1948	Micranthine
Upper Brookfield (bark)	35′ × 5″	August, 1949	Micranthine, daphnoline
Wauchope, N.S.W. (bark)	Stem diam. 14″	September, 1949	Micranthine, daphnoline, daphnandrine
Toonumbar State Forest, N.S.W. (bark)	75′ × 16″	December, 1950	Micranthine, daphnandrine

It is noteworthy that bark obtained from Wauchope on the New South Wales coast some 200 miles north of Sydney contained daphnandrine, daphnoline, and micranthine, although the relative proportions differed from those found by Pyman (*loc. cit.*), and that material collected in the Toonumbar State Forest, N.S.W., just 25 miles south of the Queensland border, contained daphnandrine and micranthine. Micranthine seems to be the dominant alkaloid in the more northerly specimens, and was indeed the only one isolated from most of the Queensland material; that even there it may have been accompanied by traces of daphnoline cannot be wholly excluded, since in some of our earliest examinations the technique employed might not have revealed minute amounts of this alkaloid. In all our later work separation of the individual alkaloids has been carried out as far as possible by chromatography on neutral alumina, and their identification by comparison with authentic specimens. From our results it seems probable that the material examined by Pyman (*loc. cit.*) was in fact *D. micrantha*, and that the alkaloidal content of this species varies according to the locality in which the tree is grown; there is no evidence from the admittedly small number of samples we have studied that there is any very marked variation according to age or season.

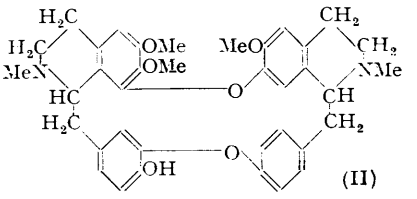
In previous papers degradative evidence has been given for the structures of repandine (Bick and Todd, *J.*, 1948, 2170), daphnandrine, daphnoline, aromoline (Bick, Ewen, and Todd, *loc. cit.*) and micranthine (Bick and Todd, *J.*, 1950, 1606). The first four all belong to the oxyacanthine series of bisbenzyl*iso*quinoline alkaloids, but it was not known which of the structures (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$) and (II) represented oxyacanthine and which berbamine. This ambiguity has now been removed by the elegant work of Tomita, Fujita, and Murai (*J. Pharm. Soc. Japan*, 1951, **71**, 226) who by degradation of *iso*tetrandrine with metallic sodium in liquid ammonia have established that berbamine is (II) and hence that oxyacanthine is (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$). Repandine, a diastereoisomer of oxyacanthine, is therefore (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$), daphnandrine is (I; $R^1 = Me$ and $R^2 = H$ or *vice versa*, $R^3 = H$, $R^4 = Me$), daphnoline (I; $R^1 = H$, $R^2 = Me$ or *vice versa*, $R^3 = R^4 = H$), and aromoline (I; $R^1 = R^2 = Me$; $R^3 = R^4 = H$). The structure assigned to micranthine (III; R^1 or R^2, and R^3 or $R^4 = Me$; others = H) (*J.*, 1950, 1606) remains as before uncertain only as regards the precise distribution of methyl groups. Since our earlier report (Bick, Ewen, and Todd, *loc. cit.*) we have been able, through the kindness of Dr. H. Kondo, to compare purified specimens of trilobamine directly with daphnoline by m. p., mixed m. p., and *X*-ray powder photography, and have fully confirmed our view that these two substances are identical.

Following our examination of *D. micrantha* barks from various sources we re-examined the barks of *D. repandula* and *D. dielsii* for alkaloid content, using our improved methods of extraction and chromatographic analysis to supplement the original studies of Bick and Whalley (*Univ. Queensland Papers, Dept. Chem.*, 1946, **1**, No. 28; 1947, **1**, No. 30; 1948, **1**, No. 33) and to extend our studies to another as yet unexamined *Daphnandra* species, *D. tenuipes*. From *D. repandula* bark we have now isolated, in addition to the repandine and repanduline reported by Bick and Whalley (*loc. cit*, No. 28), two new minor alkaloids in very small amount. One of these, a colourless base $C_{38}H_{42}O_6N_2$, has been identified as *O*-methylrepandine by showing that its dimethiodide is identical with *O*-methylrepandine dimethiodide (Bick and Todd, *J.*, 1948, 2170). The second alkaloid, for which we propose the name repandinine, gives analytical values corresponding to $C_{38}H_{40}O_7N_2$. It gives a positive reaction for methylenedioxy-groups, is colourless, contains 3 methoxy- and 2 methylimino-groups, and is non-phenolic. Repandinine has zero optical rotation

in chloroform and in 0·1N-hydrochloric acid for either the sodium or the mercury line, which suggests that it is a racemic compound or that it is internally compensated. The infra-red spectrum of repandinine in chloroform solution is identical with that of tenuipine (see below) so that it is highly probable that it is the racemic form of tenuipine. Because of the small



(I)



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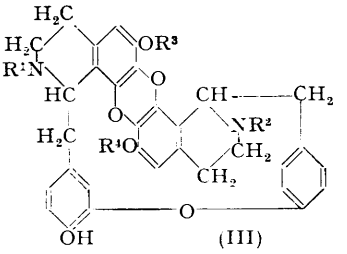

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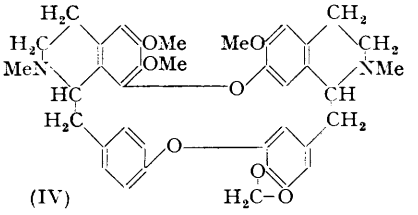

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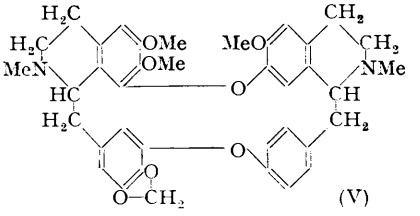

(V)

amount of material available we have not been able to confirm the identity by exhaustive methylation.

Re-examination of the bark of *D. dielsii*, reported by Bick and Whalley (*loc. cit.*, No. 30) to contain only repanduline, has shown that it contains in addition small amounts of *O*-methylrepandine, repandinine, and tenuipine identical with a new alkaloid obtained from *D. tenuipes*.

D. tenuipes is a distinct *Daphnandra* species which occurs in the Tweed River and Doyle's River districts of northern New South Wales, where *D. micrantha* apparently is not found; it is a common tree in the Whian Whian State Forest near Lismore, New South Wales. We are indebted for this information to Dr. L. J. Webb who also provided a sample of the bark for investigation. From it we isolated repanduline as the main alkaloidal constituent together with a very small amount of aromoline (I; $R^1 = R^2 = Me$, $R^3 = R^4 = H$), and somewhat larger amounts of a colourless alkaloid which we name tenuipine. Tenuipine has a formula $C_{38}H_{40}O_7N_2$ and contains 3 methoxy- and 2 methylimino-groups. It has no phenolic properties, but gives positive reactions for a methylenedioxy-group. Its molecular formula and occurrence in a *Daphnandra* species make it almost certain that tenuipine is a bisbenzyl*iso*quinoline alkaloid and if, as is a reasonable assumption, it bears the methylenedioxy-group in the same position as in the accompanying repanduline (Bick, Doebel, Taylor, and Todd, *loc. cit.*) it has a probable structure (IV) or (V). From the leaves of *D. tenuipes* we have isolated in small yield a single colourless base, $C_{37}H_{38}O_7N_2$, which has no phenolic properties but gives a positive reaction for a secondary amino-group, the presence of which can also be inferred from its infra-red absorption spectrum. Methylation with methyl iodide in methanol affords tenuipine dimethiodide, identified by m. p., mixed m. p., optical rotation, *X*-ray powder photographs, and infra-red absorption spectrum. We therefore conclude that this new alkaloid is a de-*N*-methyltenuipine. De-*N*-methyltenuipine shows only two methoxy-groups in the Zeisel estimation, the third appearing as an apparent

methylimino-group in the *N*-methyl determination. Determination of methoxy- and methylimino-groups in the *Daphnandra* alkaloids by standard methods is difficult; the results frequently vary and are in general rather unreliable.

Experimental

Alkaloids of D. micrantha.—The following example illustrates the general procedure employed : the finely ground bark (250 g.) from Toonumbar State Forest was shaken for 4 days with 5% hydrochloric acid (2 l.). The extract was filtered and made alkaline with ammonia, and the crude alkaloids were centrifuged off, washed well with water, dried, and extracted with chloroform. Shaking the chloroform extract with aqueous sodium hydroxide (5%) yielded only a trace of phenolic material, too small to be worked up. The crude alkaloids (8 g.), dissolved in methanol–ethyl acetate, slowly deposited crude micranthine (4·5 g.), m. p. 188° (decomp.). This (1·5 g.) was chromatographed in chloroform on neutral alumina (*ca.* 100 g.). The chloroform eluate (1·5 l.) gave daphnandrine (330 mg.), which after recrystallisation from methanol had m. p. 270° (decomp.), $[\alpha]_D^{16}$ +480° (*c*, 1·2 in $CHCl_3$, on a dried sample). Further elution with chloroform containing methanol (2%) yielded micranthine; recrystallised from methanol it had m. p. 190—194° (decomp.), $[\alpha]_D^{16}$ −221° (*c*, 1·3 in $CHCl_3$, on a dried sample) (Found : C, 72·0; H, 6·0; N, 5·3. Calc. for $C_{34}H_{32}O_6N_2$: C, 72·3; H, 5·7; N, 5·0%).

Alkaloids of D. repandula.—Finely powdered bark (4 kg.) was exhaustively extracted with boiling methanol until the extract gave a negative reaction with Meyer's reagent. The dark brown extract was evaporated to dryness *in vacuo* at 40°. The bases were taken up in hydrochloric acid (1·5%; 5 l.), filtered, and reprecipitated at pH 8 with sodium carbonate. The crude product so obtained (370 g.) was extracted (Soxhlet) with chloroform in 60-g. lots, the extracts were washed with 3% aqueous sodium hydroxide, then water, dried (Na_2SO_4), and evaporated *in vacuo*. The resulting solid was dissolved in ethanol (500 c.c.), and ethyl acetate (300 c.c.) added, whereupon crude repanduline crystallised. The crude base (60 g.) in benzene was chromatographed over neutral alumina (1800 g.), to yield from the benzene and benzene–chloroform (1 : 1) eluates repanduline (34·9 g.), decomp. 180°, $[\alpha]_D^{17·5}$ +473° (*c*, 0·98 in $CHCl_3$), $[\alpha]_D^{16}$ +434° (*c*, 0·19 in MeOH). Crude repanduline (24·9 g.) from the mother-liquors of the extraction above was taken up in 0·1% hydrochloric acid and a solution of ammonium reineckate added until there was no further precipitate. The dried precipitate in acetone (2 l.) was poured on activated alumina which by elution with acetone gave a colourless crystalline solid (0·8 g.), followed by a yellow solution which yielded repanduline (0·9 g.), $[\alpha]_D^{14}$ +469° (*c*, 1·2 in $CHCl_3$), and finally a reineckate which contained repanduline (12 g.). The crystalline solid was chromatographed in benzene–chloroform (1 : 1) on neutral alumina; the benzene–chloroform (1 : 1) eluate afforded repandinine (0·4 g.) and on further elution with chloroform–methanol (1 : 1) *O*-methylrepandine (*ca.* 0·2 g.).

Repandinine.—Crystallised from acetone–methanol *repandinine* formed fine needles, m. p. 243°, $[\alpha]_D^{13}$ ±0·0° (*c*, 0·25 in $CHCl_3$ or 1·0 in 0·1N-hydrochloric acid) [Found : C, 71·6, 71·6; H, 6·5, 6·3; N, 4·4; MeO, 13·1; MeN, 7·5, 10·0; *M* (Rast), 533. $C_{38}H_{40}O_7N_2$ requires C, 71·7; H, 6·3; N, 4·4; 3MeO, 14·6; 2MeN, 9·1%; *M*, 637]. It gave a pink colour with concentrated sulphuric acid and a positive test for a methylenedioxy-group with Gaebel's and Labat's reagents. There was no reaction with either ferric chloride or Millon's reagent.

Repandinine (100 mg.) was refluxed in methanol for 20 minutes with excess of methyl iodide, the solution evaporated to dryness, and the *dimethiodide* recrystallised from water as colourless needles, decomp. 275°, $[\alpha]_D^{15}$ ±0·0° (*c*, 0·4 in 50% aqueous EtOH) (Found, in air-dried material : C, 50·5; H, 5·8. $C_{40}H_{46}O_7N_2I_2,2H_2O$ requires C, 50·3; H, 5·3%).

O-Methylrepandine crystallised in needles (from methanol), m. p. 211°, $[\alpha]_D^{13}$ −73° (*c*, 0·3 in $CHCl_3$), $[\alpha]_D^{20}$ −108° (*c*, 1·7 in 0·1N-hydrochloric acid) (Found : C, 73·7; H, 7·0; N, 4·3; MeO, 18·1; MeN, 9·7. $C_{38}H_{42}O_6N_2$ requires C, 73·4; H, 6·8; N, 4·5; 4MeO, 19·9; 2MeN, 9·3%).

O-Methylrepandine (50 mg.) was refluxed in methanol with methyl iodide to yield the dimethiodide, colourless needles (from water), decomp. 255—260°, $[\alpha]_D^{20}$ −93° (*c*, 1·6 in 50% aqueous EtOH) (Found : C, 50·5; H, 5·8; I, 26·6; loss at 110°/0·1 mm., 4·8. Calc. for $C_{40}H_{48}O_6N_2I_2,2·5H_2O$: C, 50·5; H, 5·6; I, 26·6; H_2O, 4·7%). It gave a Debye–Scherrer diagram identical with *O*-methylrepandine dimethiodide ($[\alpha]_D^{15}$ −95°) described by Bick and Todd (*J.*, 1948, 2170).

Alkaloids of D. tenuipes.—Finely ground bark (770 g.) was shaken at room temperature with successive quantities of aqueous tartaric acid (0·25%) until Meyer's reagent gave only a faint reaction. The combined extracts (25 l.) were concentrated *in vacuo* at 40° to 1·5 l. and made

alkaline with ammonia, and the precipitate was dried *in vacuo* (P_2O_5). The dry powdered solid (53 g.) was extracted with chloroform and the extract shaken with aqueous sodium hydroxide The aqueous layer containing aromoline was separated and the chloroform layer washed, dried, and evaporated. The residue was redissolved in benzene and chromatographed on neutral alumina. On elution with benzene–chloroform (1 : 1) a colourless fraction was obtained which on crystallisation from acetone–methanol yielded *tenuipine* (0·2 g.). Further elution with benzene–chloroform (1 : 1) gave repanduline (5 g.), $[\alpha]_D^{20}$ +459° (*c*, 1·2 in $CHCl_3$).

Tenuipine. Recrystallised from acetone–methanol or methanol the alkaloid formed colourless prisms, m. p. 140—145° $[\alpha]_D^{20}$ −258° (*c*, 2·8 in $CHCl_3$) [Found : C, 71·6, 71·0; H, 6·7, 6·2; N, 4·7; MeO, 12·9; MeN, 7·7; *M* (Rast), 545. $C_{38}H_{40}O_7N_2$ requires C, 71·7; H, 6·3; N, 4·4; 3MeO, 14·6; 2MeN, 9·1%; *M*, 637]. It gave a positive reaction for a methylenedioxy-group with Gaebel's and Labat's reagents. Tests for a phenolic group with ferric chloride, Millon's reagent, and Claisen's cryptophenol reagent were negative.

Tenuipine (100 mg.) was refluxed in methanol with methyl iodide for 20 minutes. The solvent was removed *in vacuo* and the *dimethiodide* recrystallised from 50% aqueous ethanol as colourless prisms, m. p. 267—272° (decomp.), $[\alpha]_D^{15}$ −165° (*c*, 1·0 in 50% aqueous EtOH) (Found, in material dried *in vacuo* at room temperature : C, 49·1; H, 5·7. $C_{40}H_{46}O_7N_2I_2,3H_2O$ requires C, 49·3; H, 5·4%).

Aromoline. The sodium hydroxide extract (*ca.* 1 l.) obtained above was diluted with water (3 l.) and acidified with dilute hydrochloric acid. A flocculent precipitate was removed, the solution neutralised wtih sodium carbonate, and the precipitated base (1·5 g.) filtered off, dried, and extracted with chloroform. After concentration crystals of aromoline (50 mg.) separated during some days; they had m. p. 175° undepressed in admixture with an authentic sample of aromoline isolated from *D. aromatica* (Bick and Whalley, *loc. cit.*, No. 33).

Alkaloid from the Leaves of D. tenuipes (*De-N-methyltenuipine*).—The finely ground leaves (2 kg.) were stirred with hydrochloric acid (5%; 3·5 l.) for 24 hours, the mixture was filtered, and the residue triturated with water for a further 24 hours. This washing with water was repeated four times in all, and the combined extracts were concentrated *in vacuo* to *ca.* 1 l. and brought to pH 7·5 with ammonia, and the resulting alkaloid was filtered off, washed well with water, and dried. The crude base (1 g.), obtained as a faintly yellow powder after extraction with chloroform (Soxhlet), crystallised from 95% ethanol or chloroform–methanol in needles, m. p. 211° (decomp.), $[\alpha]_D^{12}$ −218° (*c*, 0·8 in $CHCl_3$). *De-N-methyltenuipine* was insoluble in the Claisen cryptophenol reagent, gave a positive Labat test for a methylenedioxy-group, and afforded a red colour followed by a green precipitate on treatment with sodium nitrite in dilute acetic acid solution (Found : C, 71·1; H, 6·1; MeO, 9·6, 10·3; MeN, 7·3, 7·9. $C_{37}H_{38}O_7N_2$ requires C, 71·3; H, 6·2; 2MeO, 10·0; 2MeN, 9·4%). Light absorption in 95% ethanol; max. at 2820 (ε 7200) and 2110 Å (ε 84,800).

The base (90 mg.) was refluxed in methanol with methyl iodide for 2 hours. Addition of water to the resulting solution yielded flat plates of the dimethiodide, m. p. 261—265° (decomp.), $[\alpha]_D^{15}$ −154° (*c*, 0·4 in 50% aqueous EtOH), identical in infra-red absorption spectrum (Nujol mull) and *X*-ray powder photograph, with tenuipine dimethiodide in admixture with which it gave no depression in m. p. (Found : C, 49·3; H, 5·4%).

Alkaloids of D. dielsii.—A portion (*ca.* 250 c.c.) of a stock methanolic extract of *D. dielsii* bark remaining from earlier work was used in seeking for minor alkaloids. The extract was evaporated to dryness under reduced pressure, and the residue (*ca.* 120 g.) was then extracted with cold dilute hydrochloric acid, which left behind much non-basic material. The extract was made alkaline with ammonia, the precipitate collected, dried and extracted with chloroform, and the chloroform extract concentrated to small bulk. On addition of methanol the crude alkaloid (8 g.), consisting largely of repanduline, separated; it was dissolved in benzene and put on a column of neutral alumina. Elution with benzene gave pure repanduline, m. p. 183° (decomp.), $[\alpha]_D^{15}$ +490° (*c*, 1·01 in $CHCl_3$). After elution of the repanduline, the column was washed with methanol, and the washings were combined with the mother-liquor from the separation of the above crude alkaloid and evaporated. The residue so obtained was dissolved in benzene, the solution filtered through neutral alumina (1·5 kg.), and the filter washed thoroughly with benzene; the filtrate and washings yielded a further amount of repanduline (2 g.). The alumina was then washed with benzene–methanol (50 : 1), which eluted the remainder of the alkaloids, leaving behind highly coloured impurities. The eluted product was separated roughly into three fractions according to its solubility in methanol. (*a*) Soluble fraction. This material was separated into two fractions of greater and lesser solubility in light petroleum (b. p. 80—100°). From the former small amounts of repanduline, repandinine,

and tenuipine were obtained by fractional crystallisation from methanol, and from the less soluble fraction, tenuipine, repanduline, and *O*-methylrepandine were obtained by chromatography on neutral alumina in benzene. (*b*) Moderately soluble fraction, almost wholly crude repanduline ($[\alpha]_D^{15} = +360°$). (*c*) Insoluble fraction. This gave repandinine and *O*-methyl repandinine on chromatography in benzene.

Details of the minor alkaloids isolated are :

Repandinine. Total yield *ca.* 150 mg.; m. p. 240—242°, $[\alpha]_D^{14} \pm 0°$. A mixed m. p. with repandinine isolated from *D. repandula* showed no depression.

O-*Methylrepandine*. Total yield *ca.* 100 mg.; m. p. and mixed m. p. 212°, $[\alpha]_D^{15} -70·5°$, (*c*, 1·02 in $CHCl_3$) (Found : C, 73·3; H, 7·1. Calc. for $C_{38}H_{42}O_6N_2$: C, 73·4; H, 6·8%).

Tenuipine. Total yield *ca.* 300 mg. Tenuipine is dimorphous and can be obtained in two interconvertible forms, prisms, m. p. 140°, and needles, m. p. 170°, both from methanol. The alkaloid had $[\alpha]_D^{16} -250°$ (*c*, 0·75 in $CHCl_3$) (Found, in material dried at 100°/0·1 mm. for 12 hours : C, 71·2; H, 6·3; N, 4·3; MeO, 13·0; MeN, 8·1%). Light absorption in 95% ethanol : max. at 2820 Å (ε 6560).

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