

THE ALKALOIDS OF *DUBOISIA LEICHHARDTII*: BUTROPINE AND VALTROPINE

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THE leaves of *Duboisia leichhardtii* contain, in addition to hyoscyne and hyoscyamine, two liquid alkaloids, butropine, (*isobutyryltropine*) and valtropine (*d*- α -methylbutyryl-tropine), which, together, are comparable in quantity with the hyoscyne. The two bases are extracted together, and their hydrobromides are not separable by recrystallisation. The free alkaloids have been isolated by vacuum fractionation. Hydrolysis gives tropine and the respective aliphatic acids, which have been characterised by their *p*-phenylphenacyl esters. The tropine esters of normal and *isobutyric*, and normal and *isovaleric* acids have been prepared. This alkaloid mixture appears to be identical with Base D, described by Mitchell¹, and thought to be a complex of *isovaleryltropine* and *d*- α -methylbutyryltropine.

The mixed bases are obtained by steam distillation of the total alkaloid extract from *D. leichhardtii* leaf, and have been prepared in quantity from mother liquors from the manufacture of hyoscyne and hyoscyamine. Since as much as 0.5 per cent. may be present they can hardly be regarded as minor alkaloids. The percentage is not proportional to total alkaloid content; some figures for individual trees are given in Table I.

TABLE I

Total Alkaloid	Butropine and Valtropine
per cent.	per cent.
2.4	0.40
3.1	0.31
3.1	0.13

The proportion varies from 4 to 16 per cent. of the total alkaloid. The mixture of alkaloids, which we will call Base B, is a thin liquid, distilling between 70° C. and 80° C. at 0.5 mm. It is dextrorotatory, and a stronger base than hyoscyamine, the *pH* of an aqueous solution being approximately 11. Its toxicity is low, and its spasmolytic effect slight. It does not cause mydriasis, and has little effect on salivation, or on the action of the heart.

Base B hydrobromide forms colourless laminæ from ethanol and is very soluble in water, ethanol and chloroform. Its $[\alpha]_D$ and m.pt. vary in proportion to its content of valtropine.

SEPARATION OF BUTROPINE AND VALTROPINE

Butropine is somewhat more volatile than valtropine, and also a slightly stronger base. Attempts at separation by partition chromatography

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were not successful, but the distributions of the two bases between ether and dilute aqueous acid were found to differ appreciably. When a 10 per cent. ethereal solution of Base B of $[\alpha]_D + 2.2^\circ$ was extracted with portions of 3 per cent. acetic acid, each one tenth of a full equivalent, the $[\alpha]_D$ of the first fraction was $+0.8^\circ$, and of the last, $+7.6^\circ$. A more convenient method is vacuum fractionation, a single pass through a small column giving end fractions of $[\alpha]_D + 0.25^\circ$ and $+8.1^\circ$. These were further purified by refractionation, and by conversion to the hydrobromides. Alkaline hydrolysis of the pure bases gave tropine and the corresponding aliphatic acids, which were characterised by their *p*-phenylphenacyl esters.

SYNTHETIC TROPEINES

Normal and *isobutyryl* and normal and *isovaleryl* tropeines were prepared. The melting points of the aurichlorides were found to be more distinctive than those of the picrates, and were preferable for mixed melting points. Mitchell¹ prepared *isovaleryl* tropeine, and gave the m.pt. of its hydrobromide as 225° to 227° C. Barger *et al.*² synthesised *dl*- α -methylbutyryltropine, the hydrobromide melting at 210° C. Since the Base D hydrobromide of these authors had m.pt. 231° C. it is unlikely to be a mixture of these two esters.

TABLE II
MELTING POINTS OF SALTS OF TROPEINES

Tropeine	Hydrobromide	Picrate	Aurichloride
	$^\circ$ C.	$^\circ$ C.	$^\circ$ C.
<i>n</i> -butyryl	188	193	162
<i>isobutyryl</i>	242	224	148
<i>n</i> -valeryl	169.5	193	104.5
<i>isovaleryl</i>	227	231.5	146.5
Base B, $[\alpha]_D$ 1.6°	232	223	140
Butropine	242	224	149
Valtropine	212	227	124

Butropine, which is isomeric with the poroidines, appears to be the first instance of an alkaloid which is a butyl ester. Simple esters of butyric acid occur in a number of essential oils, and the *isobutyl* grouping is found in a number of plant amines, such as affinin, herculin and peltorin, but there it is attached to nitrogen. The presence of these 4- and 5-carbon acids may shed some light on the biogenesis of tropic acid. Trautner,³ has suggested that tropic acid may be a terpene derivative related to limonene or terpinene, and that all the esterifying acids in *Duboisia* may be associated with an isoprene metabolism.

EXPERIMENTAL

All melting points are corrected. Specific rotations of liquid alkaloids and acids are those of the undiluted liquids, and of hydrobromides in 5 per cent. aqueous solution. The ultimate analyses were made by Dr. Zimmermann of the Organic Microanalytical Laboratory, Commonwealth Scientific Industrial and Research Organisation.

Separation of Base B.

The leaves, in No. 20 powder, were extracted with 90 per cent. ethanol, and a chloroform solution of the total alkaloids was prepared by the usual methods. After removing most of the solvent, the residue was placed, together with water, in one flask of an apparatus for simultaneous steam distillation and immiscible-solvent extraction⁴. Chloroform was placed in the second flask, and after the contents of both had boiled for 1½ hours all the volatile alkaloid had been transferred to the chloroform, except for a loss by hydrolysis of about 10 per cent. The bases were purified by vacuum distillation, followed by recrystallisation of their hydrobromides from ethanol-acetone. Larger quantities were recovered from process mother liquors by direct steam distillation, preferably under moderately reduced pressure, followed by extraction of the distillate with chloroform.

Base B is a thin strongly basic liquid, $n^{15^\circ\text{C}}$. 1.4725 to 1.4730, $[\alpha]_{\text{D}}$ varying from $+1.2^\circ$ to $+2.6^\circ$ in various samples. The hydrobromide forms colourless laminae from ethanol, the $[\alpha]_{\text{D}}$ rising and m.pt. falling with increase in valtropine content. Figures for some samples are given in Table III.

TABLE III
SAMPLES OF BASE B HYDROBROMIDE FROM LEAF

$[\alpha]_{\text{D}}$	m.pt. °C.
+1.4°	233
+1.6°	232
+1.8°	231.5
+2.2°	229
+2.6°	226

A sample of Base B hydrobromide of m.pt. 232°C . was estimated from its $[\alpha]_{\text{D}}$ of $+2.0^\circ$ to contain approximately 25 per cent. of valtropine. Found, C, 50.2; H, 7.6; N, 4.6; Br, 26.8; 75 per cent. of $\text{C}_{12}\text{H}_{21}\text{O}_2\text{N}$, HBr to 25 per cent. of $\text{C}_{13}\text{H}_{23}\text{O}_2\text{N}$, HBr require C, 49.8; H, 7.7; N, 4.7; Br, 27.0 per cent. From a sample of Base B of $[\alpha]_{\text{D}}$ $+1.4^\circ$ were obtained the hydrochloride, colourless, deliquescent needles, m.pt. 213°C ., the picrate, yellow needles, m.pt. 224°C ., and the methiodide, fragile laminae, m.pt. 287° to 288°C .. The aurichloride formed golden needles, m.pt. 140°C ., and, unlike the aurichloride of the Base Z of Barger, Martin and Mitchell^{2,5}, could be easily recrystallised from very dilute hydrochloric acid.

Isolation of Butropine and Valtropine.

160 g. of Base B hydrobromide of $[\alpha]_{\text{D}}$ $+1.6^\circ$ and m.pt. 232°C . was dissolved in water, made strongly alkaline, and the free base extracted into ether. After removal of solvent, the weight was 119 g., $[\alpha]_{\text{D}}$ $+2.0^\circ$, and $n^{15^\circ\text{C}}$. 1.4728. 110 g. was fractionated at 0.5 mm. through a column containing a 15-cm. Dufton spiral, the vapour temperature rising gradually from 70°C . to 75°C .. The $[\alpha]_{\text{D}}$ of the first 40 per cent. to distil was $+0.8^\circ$. The hydrobromide of this melted at 237°C .. Mixed with an

equal amount of synthetic *isovaleryl*tropine (m.pt. 227° C.) it showed a depression to 220° C., but with an equal amount of synthetic *isobuteryl*tropine (m.pt. 242° C.), a rise to 238° C.

Refractionation of earlier and later portions of the distillate gave a butropine fraction of $[\alpha]_D + 0.05^\circ$ and a valtropine fraction of $[\alpha]_D + 8.3^\circ$, unchanged on further distillation. These were converted to the hydrobromides and purified by recrystallisation from ethanol.

Butropine, isobutyryltropine, $C_{12}H_{21}O_2N$, is a strongly basic liquid, optically inactive, $n^{15^\circ C.} 1.4726$. Butropine hydrobromide forms colourless prisms, m.pt. 242° C., not depressed by synthetic *isobutyryltropine* hydrobromide. Found, C, 49.7; H, 7.5; N, 4.9; Br, 27.3; $C_{12}H_{21}O_2N$, HBr requires C, 49.4; H, 7.6; N, 4.8; Br, 27.4 per cent.

Butropine picrate forms yellow needles, m.pt. 224° to 225° C. The aurichloride forms golden needles, m.pt. 149° C. A mixture with an equal amount of synthetic *isobutyryltropine* aurichloride (m.pt. 148° C.), melts at 147° C., with synthetic *isovaleryl*tropine aurichloride (m.pt. 146.5° C.) depressed to 134° C. Equivalent weight of butropine base by titration with acid, 212 (calc. 211).

Hydrolysis of Butropine. 10 g. of base refluxed with 20 g. of hydrated barium hydroxide in 150 ml. of water for 3 hours was 97 per cent. hydrolysed. After extracting unhydrolysed base with ether, the solution was acidified, and again extracted with ether. After removing solvent, the acid was distilled. b.pt. 154° to 156° C., $n^{15^\circ C.} 1.3955$, equivalent weight by titration, 89 (calc. for *isobutyric* acid, 88.1). m.pt. of *p*-phenylphenacyl ester, 89° C., not depressed by the *p*-phenylphenacyl ester of authentic *isobutyric* acid (m.pt. 89° C.). The aqueous solution was concentrated, and the base extracted with chloroform, and recrystallised from ether. It melted at 63° C., not depressed by tropine from the hydrolysis of hyoscyamine; the picrate of both melted at 291° to 293° C. with decomposition. The hydrobromide of the acetyl derivative melted at 192° C., not depressed by authentic acetyl tropine hydrobromide, derived from very pure hyoscyamine, (m.pt. 192° C., previously recorded as 188° C.).

Valtropine, d- α -methylbutyryltropine, $C_{13}H_{23}O_2N$, is a thin, strongly basic liquid, dextrorotatory, $[\alpha]_D + 8.3^\circ$, $n^{15^\circ C.} 1.4725$. Valtropine hydrobromide forms small colourless prisms, m.pt. 212° C., $[\alpha]_D + 6.2^\circ$. Found, C, 51.0; H, 8.1; N, 4.4; Br, 26.1; $C_{13}H_{23}O_2N$. HBr requires C, 51.0; H, 7.9; N, 4.6; Br 26.1 per cent. The picrate forms yellow needles, m.pt. 227° C. The aurichloride forms golden needles, m.pt. 124° C. Equivalent weight of base by titration with acid, 223 (calc. 225). Barger, Martin and Mitchell² synthesised *dl*- α -methylbutyryltropine which is described as a thin syrup. Its hydrobromide melted at 210° C. and the picrate at 225° C.

Hydrolysis of Valtropine. When hydrolysed as described for butropine, this base yielded tropine (m.pt. of picrate 293° C. (decomp.), m.pt. of hydrobromide of acetyl derivative 192° C., not depressed by authentic acetyl tropine hydrobromide), and a liquid acid of b.pt. 174° to 176° C., $n^{15^\circ C.} 1.4075$, equivalent weight by titration, 103 (calc. 102.1 for α -methylbutyric

acid) and $[\alpha]_D + 11.5^\circ$. Since the $[\alpha]_D$ calculated stoichiometrically from the $[\alpha]_D + 8.3^\circ$ of the valtropine is $+18.3^\circ$, and the Beilstein figure is $+18^\circ$, partial racemisation appears to have taken place during the alkaline hydrolysis. The *p*-phenylphenacyl ester melted at 71°C . Barger, Martin and Mitchell² found 71°C . for the ester of *dl*- α -methylbutyric acid, and it is given as 70.6°C . by Drake and Veitch⁶; m.pt. of anilide, 109°C .

Determination of pK_b Values.

The basicities of butropine and valtropine were compared with those of hyoscine, hyoscyamine and homatropine. 0.2 g. of each base was dissolved in carbon dioxide-free distilled water containing 20 per cent. of ethanol, and titrated with 0.05 N hydrochloric acid from a standardised burette. pH readings were taken by a Jones meter, and the *pK_b* values read from the half-neutralisation point of the titration graph to within 0.1 pH unit. *pK_b* values:—butropine, 3.9; valtropine, 4.2; hyoscine, 6.5; hyoscyamine, 4.8; homatropine, 4.3.

Synthesis of butyryl- and valeryl-tropeines.

Acid chlorides were prepared by refluxing 0.1 mole of the acid with 0.2 mole of thionyl chloride for $1\frac{1}{2}$ hours. After removing excess of thionyl chloride, the product was fractionated, yielding about 70 per cent. of the pure acid chloride. Equimolecular quantities of this and tropine hydrochloride were heated in an oil bath at 100°C . for 2 hours. The reaction mixture was dissolved in water, and the base extracted at pH 10 with ether, and distilled at 0.5 mm. after removing solvent. The product was converted to the hydrobromide, which was recrystallised from an ethanol-ether mixture. Yields were from 50 per cent. to 60 per cent. of the theoretical. Picrates were recrystallised from water, and aurichlorides from very dilute hydrochloric acid.

n-Butyryltropeine is a thin liquid base, $n^{15^\circ\text{C}}$. 1.4762. The hydrobromide forms glistening plates, very soluble in water, ethanol and chloroform, m.pt. 188°C . Found, Br, 27.4; $\text{C}_{12}\text{H}_{21}\text{O}_2\text{N}$, HBr requires Br 27.4 per cent. The picrate forms yellow needles, m.pt. 193°C . The aurichloride forms golden needles, m.pt. 162°C .

*iso*Butyryltropeine is a thin liquid base, $n^{15^\circ\text{C}}$. 1.4722. The hydrobromide forms fine needles and prisms, very soluble in water, ethanol and chloroform, m.pt. 242°C . Found, Br 27.3; $\text{C}_{12}\text{H}_{21}\text{O}_2\text{N}$, HBr requires Br, 27.4 per cent. The picrate forms yellow needles, m.pt. 224°C . The aurichloride forms golden needles, m.pt. 148°C .

n-Valeryl-tropeine is a thin liquid base, $n^{15^\circ\text{C}}$. 1.4747. The hydrobromide forms colourless prisms, very soluble in water, ethanol and chloroform, m.pt. 169.5°C . Found, Br, 26.2; $\text{C}_{13}\text{H}_{23}\text{O}_2\text{N}$, HBr requires 26.1 per cent. The picrate forms yellow needles, m.pt. 193°C . The aurichloride forms golden needles, m.pt. 104.5°C .

*iso*Valeryl-tropeine is a thin liquid base, $n^{15^\circ\text{C}}$. 1.4715. The hydrobromide forms colourless needles and prisms, very soluble in water, ethanol and chloroform, m.pt. 227°C . Found, Br, 26.2; $\text{C}_{13}\text{H}_{23}\text{O}_2\text{N}$, HBr requires

Br 26.1 per cent. The picrate forms yellow needles, m.pt. 231.5° C. The aurichloride forms golden scales, m.pt. 146.5° C.

SUMMARY

1. Two new alkaloids, butropine (*iso*-butyryltropeine) and valtropine (*d*- α -methylbutyryltropeine) have been found in the leaves of *Duboisia leichhardtii*. They may occur in quantity comparable with that of the hyoscyne.

2. The two bases have been separated by vacuum fractionation, and by fractional extraction of an ether solution with aqueous acid. They form well defined crystalline salts.

3. *n*-Butyryl, *isobutyryl* and *isovaleryltropeines* have been synthesised, and the m.pt. of a number of their salts are recorded.

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Supplementary Note by the Authors

After this paper had been submitted for publication, the "Chemische Berichte" for November 1953 became available in Melbourne. This contained a paper¹ giving an account of the identification of *isobutyryltropeine* and *d*- α -methylbutyryltropeine in *D. leichhardtii*. This paper was received by the Editor on September 11, 1953. The results given by the authors are mainly similar to our own, and we attribute some differences in melting points to the greater purity of our derivatives. We had the advantage of working with much greater quantities of material, and our bases were vacuum-distilled, eliminating all traces of hyoscyne and hyoscyamine.

The isolation and identification of butropine and valtropine was mentioned briefly, and a method for their estimation in *D. leichhardtii* leaf described, in a paper by one of us in the *Australian Journal of Applied Science*². This paper was received by the Editor on February 18, 1953, but was delayed in publication.

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