Alkaloids of the Genus *Erythroxylum*. Part 5.[1] *E. hypericifolium* Lam. Root-bark

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The root-bark of *Erythroxylum hypericifolium* contains the new bases tropan-3α-yl 3-hydroxyphenylacetate, (+)-tropane-3α,6β-diol 3-phenylacetate, tropane-3α,6β-diol 3-phenylacetate 6-acetate, tropane-3α,6β,7β-triol 3-phenylacetate, and nortropan-3-yl phenylacetate; tropan-3α-yl phenylacetate was also isolated. Chemotaxonomic implications are discussed.

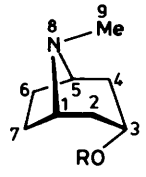
Erythroxylum hypericifolium Lam. is a shrub or small tree, 3—5 m tall and found locally on the volcanic slopes of the islands of Mauritius and Réunion. Together with four other species of Madagascan origin it constitutes the section *Venelia* O. E. Schulz[2] of the genus. None of the species appears to have been recently examined for secondary metabolites; in 1935 the aerial parts of *E. hypericifolium* were reported[3] to be devoid of cocaine and in 1957 roots of the related species *E. previllei* were stated[4] to contain 0.2% of alkaloids. Species of the *E. laurifolium* complex (Section *Pachylobus* O. E. Schulz) are found in the same localities as *E. hypericifolium* and are used medicinally under the name 'Bois de Ronde.' There appears to be some local lack of distinction between the species. As a further contribution to the study of the tropane alkaloids of this genus, authentic material of *E. hypericifolium* collected in Mauritius was examined. The preliminary screening of various morphological parts of the plant indicated, with the exception of the wood, a considerable wealth of alkaloids in all parts; respective alkaloid concentrations on a dry weight basis for the leaves, stem-bark and root-bark were 0.06, 0.58, and 1.63%. We report here on our findings for the root-bark.

Results and Discussion

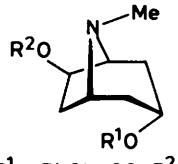
An ethereal solution of the total alkaloids readily gave a crystalline deposit which was examined separately from the mixture afforded by removal of the solvent from the mother-liquor. Repeated fractionation of the bases by p.l.c. on alumina and silica showed both fractions to contain the same four principal bases and a number of minor ones.

The general structures of the tropane moieties were based on the following spectroscopic criteria.[5,6] Esters of tropan-3α-ol give in their mass spectra a prominent ion, often constituting the base peak, at *m/z* 124, and correspondingly for the nor-derivative, *e.g.* compound (**10**) at *m/z* 110. Esters of the diol (**5**) and triol (**8**) fragmented readily by loss of the C(6)–C(7) bridge to give an ion, *m/z* 113 if there were a free hydroxy group at C-3 (*m/z* 99 for a nor-alkaloid); esterification of C-3 caused the corresponding ion to incorporate the acid moiety. By this means the substitution pattern of the tropane nucleus was established. ^{1}H N.m.r. spectroscopy established the substituent at C-3 as being either α-(a triplet at *ca.* δ 5, *J* 5 Hz for an ester, δ 4.0 for the free alcohol) or β-(a quintet, $W_{\frac{1}{2}}$ *ca.* 28 Hz at similar frequencies). In all natural alkaloids when there are substituents at C-6 or C-7, the groups appear to be *exo*, the remaining *endo*-protons showing coupling *J ca.* 0 Hz with the vicinal bridgehead proton; *endo*-substituted tropanes have not been isolated but the *exo*-protons would be expected to show the marked coupling (*J* 3—4 Hz) exhibited in similar fused systems.[7]

The predominant alkaloid (0.57%) which had the lowest R_F value of the characterised bases was shown by mass spectroscopy to have the formula $C_{16}H_{21}NO_3$ and to have the i.r. and



(**1**) R = H
(**2**) R = 3-$HOC_6H_4CH_2CO$
(**3**) R = $PhCH_2CO$



(**4**) R^1 = $PhCH_2CO$, R^2 = H
(**5**) R^1R^2 = H
(**6**) R^1 = R^2 = $PhCH_2CO$
(**7**) R^1 = $PhCH_2CO$, R^2 = MeCO

^{1}H n.m.r. characteristics of a tropan-3α-yl ester. Mass spectroscopy indicated the esterifying acid to be either 3- or 4-hydroxyphenylacetic acid and the former was confirmed by alkaline hydrolysis of the alkaloid and characterisation of 3-hydroxyphenylacetic acid and tropine (**1**). The new base is therefore tropan-3α-yl 3-hydroxyphenylacetate (**2**) and is the first recorded instance of this acid as a moiety of natural tropane alkaloids.

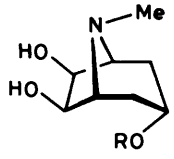
The second major base (0.45%) was characterised as the new alkaloid (+)-tropane-3,6-diol 3-phenylacetate (**4**). It was a dextrorotatory solid with R_F 0.33 (system C, see Experimental section) and by spectroscopy was show to be derived from tropane-3α,6β-diol with a 3α-phenylacetoxy grouping. Its spectroscopic properties were identical with those of a sample of the (±) compound (**4**) prepared by partial synthesis. The new alkaloid was shown to be derived from (+)-tropane-3α,6β-diol (**5**) by comparison of the picrate of its phenylacetate with the picrates of the (−)- and (+)-bisphenylacetates (**6**) prepared respectively from the (+)- and (−)-diols. In contrast to that of the picrate of the (+)-alkaloid, the (−)-bisphenylacetate picrate showed no depression of m.p. on admixture with the natural product derivative. The 6-acetate (**7**) of the natural alkaloid, prepared by standard means, readily furnished a picrate, m.p. 132 °C.

A third base (0.43%) was identified as tropan-3α-yl phenylacetate (**3**), an alkaloid previously reported as a constituent of the roots of *Erythroxylum dekindtii*.[6]

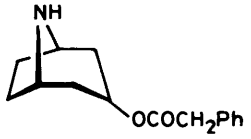
A crystalline alkaloid, $C_{18}H_{23}NO_4$ (0.07%), formed the fastest running chromatographic band and readily gave a crystalline picrate, m.p. 133 °C. The spectral data accorded with those expected for tropane-3α,6β-diol 3-phenylacetate 6-acetate (**7**) and this structure was confirmed by comparison of the picrate with that of the 6-acetate prepared from the natural alkaloid (**4**) above. It follows that this new alkaloid is also biosynthetically derived from (+)-tropane-3α,6β-diol.

A minor crystalline component (0.02%) of the alkaloid mixture had R_F 0.48 (system C) and gave by mass spectroscopy an ion *m/z* 291 corresponding to $C_{16}H_{21}NO_4$, a base peak *m/z* 94, and other ions characteristic of a derivative of a tropane-

3,6,7-triol (**8**). Characteristic ions relating to phenylacetic acid, others in accord with its loss from the substituted triol, and an ion *m/z* 231 ($M^+ - 60$) representing a loss of the C(6)–C(7) moiety of the molecule indicated esterification at the 3-position of the triol. The presence of the ester function and the free hydroxy group were supported by the i.r. spectrum, and the stereochemistry (3α,6β,7β) and confirmation of the phenylacetate group were given by ^{1}H n.m.r. spectroscopy. This new alkaloid was therefore identified as tropane-3α,6β,7β-triol 3-phenylacetate (**9**).



(**8**) R = H
(**9**) R = $PhCH_2CO$



(**10**)

Another minor component (0.01%), R_F 0.37 (system C), gave a mass spectrum consistent with that predictable for nortropan-3-yl phenylacetate (**10**), the base peak at *m/z* 110 being typical of a substituted nortropan-3-ol; the presence of *m/z* 91 (C_7H_7) indicated that the esterifying acid was $C_7H_7CO_2H$, and the absence of *m/z* 119 (ArCO) suggested phenylacetic acid rather than a toluic acid. The former acid was also suggested by the ^{1}H n.m.r. spectrum of the alkaloid which showed singlets at δ 3.63 and 7.27 for the CH_2 and aromatic protons respectively. Lack of material prevented further investigation. A related base may also occur in the roots of *E. cumanense* of Venezualan origin.[1]

Admixtures of small quantities of other bases involving esterification with acetic, benzoic, and phenylacetic acids were not further investigated.

The tropane moieties of the alkaloids of *E. hypericifolium* root-bark isolated above are typical of the range so far commonly found in the roots of the genus and afford, in themselves, little possiblity of use as intrageneric chemotaxonomic characters. Esterifying acids, however, are often distinctive features of certain species.[8] 3-Hydroxyphenylacetic acid has not previously been reported as a component of tropane alkaloids and thus the combination of acetic, phenylacetic acid and 3-hydroxyphenylacetic acids for any one species is, to date, unique. Whether it constitutes a common characteristic for the Section Venelia, requires investigation of the other four species. Species which at the moment contain monospecific acids are *E. vacciniifolium* (pyrrole-2-carboxylic),[9] *E. dekindtii* (2-furoic and also isovaleric),[6] *E. australe* (*E*)-2-methylbutenoic,[10] and now *E. hypericifolium*.

Experimental

Chemical methods for the synthesis and hydrolysis of esters and the preparation of alkaloid derivatives were as previously described;[1,6] mass spectra were determined on VG 16 F (with VG 2035 Data system) and AEI MS 902 (70 eV) spectrometers. ^{1}H N.m.r. spectra were run in $CDCl_3$ solution at 60 and 250 MHz on Varian EM 360 and Bruker WM instruments respectively. T.l.c. and p.l.c. alumina plates were developed with (A) chloroform, (B) chloroform–ethanol (1:1), and silica plates with (C) chloroform–diethylamine (9:1) and (D) acetone–ammonia (*d* 0.88) (4:1).

Plant Material.—Samples were collected in December 1978 from the wooded hilly slopes of Magenta Valley, above Tamarin Bay, Mauritius, and the root-bark removed and air-dried. A voucher specimen F.316, lodged in the Pharmaceutical Society Herbarium, University of Bradford, has been transferred to the Kew Herbarium, Richmond, Surrey.

Extraction and Fractionation of Alkaloids.—A total alkaloid mixture (0.98 g) obtained from the root-bark (60 g) by the method described previously[5] gave, on treatment with ether (4 ml), a crystalline deposit (0.50 g). The latter, and the alkaloids contained in the mother-liquor, were examined separately and fractionated first on alumina (System B) to give two principal zones each of which was then refractionated on silica (system C). Repeated fractionation was sometimes necessary and for bases of high and low R_F value, systems A and D respectively were employed.

Tropan-3α-yl 3-Hydroxyphenylacetate (**2**).—The principal base of both the crystalline and non-crystalline fractions had R_F 0.13 (system C) and, before spraying t.l.c. plates with a visualising reagent, was visible as a brown area. The alkaloid was purified by the preparation of a *picrate*, m.p. 157 °C (rosettes from aqueous ethanol) (Found: C, 52.1; H, 4.9; N, 10.6. $C_{16}H_{21}NO_3{\cdot}C_6H_3N_3O_7$ requires C, 52.4; H, 4.8; N, 11.1%); $\nu_{max.}$(KBr) 3 450(OH) and 1 722 cm^{-1} (ester C=O); *m/z* 275.1527 (41%, M^+, $C_{16}H_{21}NO_3$ requires 275.1521), 229 (picric acid), 124.1122 (100, $C_8H_{14}N$ requires 124.1126), 107.0510 (11, $HOC_6H_4CH_2$ requires 107.0496), 96, 94, 83, and 82; δ_H 1.66, 1.87, and 2.16 (8H, 3 × m, 2-H_2, 4-H_2, 6-H_2, and 7-H_2), 2.33 (3H, s, NMe), 3.10 (2 H, br s, 1-H, 5-H), 3.52 (2 H, s, $ArCH_2CO$), 4.9 (1 H, *J* 5 Hz, 3α-H), 6.71 (3 H, m, ArH), and 7.14 (2 H, after D_2O, 1 H, t, *J* 7.7 Hz, 5-ArH, OH). Alkaline [$Ba(OH)_2$] hydrolysis of the base (0.02 g) afforded on work-up, an acid (0.01 g), white crystals, m.p. 127 °C (Found: C, 62.75; H, 5.1. Calc. for $C_8H_8O_3$: C, 63.1; H, 5.3%), mixed m.p. with authentic 3-hydroxyphenylacetic acid (129 °C) 127 °C; green colour with Fe^{III} reagent; R_F 0.23 (system D) identical with authentic acid [2-hydroxyphenylacetic acid has m.p. 149 °C; gives a violet colour with the above reagent; and has R_F 0.53 (system D)]. The basic moiety of the hydrolysate had an R_F value of 0.38 (system B), identical with that of authentic tropan-3α-ol; on treatment with (*E*)-2-methylbutenoyl chloride it gave (*E*)-tropan-3α-yl 2-methylbutenoate, identical (m.p., i.r. spectrum, and t.l.c.) with authentic material.

(+)-*Tropane-3α,6β-diol 3-Phenylacetate* (**4**).—The second major base was characterised as the title compound and had R_F values of 0.81 and 0.33 (systems B and C respectively), m.p. 85 °C when crystallised from ethanol, $[\alpha]_D^{20}$ + 4.3° (*c*, 1.5 in ethanol); $\nu_{max.}$ 1 727 cm^{-1} (ester CO); *m/z* 275 (M^+), 231 (M^+ – [C(6)HOH–C(7)H_2]), 156, 140, 122, 95, 94 (100%), 91 (C_9H_7), and 82; δ_H 2.47 (3 H, s, NMe), 2.9 (1 H, br s, 1-H), 3.15 (1 H, br s, 5-H), 3.58 (2 H, s, $PhCH_2CO_2$), 4.15 (1 H, dd, *J* 6 and 3 Hz, 6-H), 4.90 (1 H, t, *J* 5 Hz, 3α-H), and 7.33 (5 H, s, ArH). For purposes of comparison the alkaloid (**4**) (0.05 g) was treated with acetyl chloride (0.02 g) and after 6 h at 120 °C, the product was extracted and the purified base (0.02 g) was treated with sodium picrate solution to give *tropane-3α,6β-diol 3-phenylacetate 6-acetate picrate*, as rosettes, m.p. 132 °C (from aqueous ethanol) (Found: C, 52.6; H, 5.1; N, 10.1. $C_{18}H_{23}NO_4{\cdot}C_6H_3N_3O_7$ requires C, 52.7; H, 4.8; N, 10.3%).

Similarly, (–)-tropane-3α,6β-diol 3,6-bisphenylacetate prepared from the alkaloid (**4**) and phenylacetyl chloride formed feathery needles, m.p. 150 °C (from aqueous ethanol) (Found: C, 57.5; H, 5.1; N, 9.0. $C_{24}H_{27}NO_4{\cdot}C_6H_3N_3O_7$ requires C, 57.9; H, 4.8; N, 9.0%). There was no depression of m.p. (149—152 °C) with the (–)-synthetic compound, but with the (+)-compound, the m.p. was depressed to 136 °C. The (±)-synthetic derivative had m.p. 130 °C.

Optical Isomers of Tropane-3α,6β-diol 3,6-*Bisphenylacetate* (**6**).—(+)-, (−)-, and (±)-tropane-3,6-diol were treated separately with phenylacetyl chloride (2 equiv.) for 6 h at 120 °C, and the resultant bases were extracted and characterised as picrates. The (+)-diol gave (−)-*tropane-3α,6β-diol* 3,6-*bisphenylacetate picrate*, as needles from ethanol, m.p. 151 °C (Found: C, 57.8; H, 5.0; N, 9.0%); the base had $[\alpha]_D^{20}$ −22.1° (*c*, 0.7 in ethanol); the (−)-diol gave the (+)-*bisphenylacetate picrate*, as needles m.p. 153 °C (ethanol) (Found: C, 57.4; H, 5.1; N, 9.0%), $[\alpha]_D^{20}$ + 19.2° (*c*, 0.4 in ethanol); the (±)-diol gave the (±) *bisphenylacetate picrate*, rods, m.p. 130 °C (from ethanol) (Found: C, 58.0; H, 5.0; N, 8.8%).

Tropan-3α-yl Phenylacetate (**3**).—This base and the corresponding picrate were identified by their spectroscopic properties which were identical with those of the authentic compound.[6]

Tropane-3α,6β-diol 3-*Phenylacetate* 6-*Acetate* (**7**).—The base R_F 0.73 (system B) furnished a *picrate* m.p. 133 °C (clusters from aqueous ethanol), not depressed on admixture with the picrate of the acetate prepared from the natural alkaloid (**4**) (Found: C, 53.0; H, 4.9; N, 10.4. $C_{18}H_{23}NO_4 \cdot C_6N_3H_3O_7$ requires C, 52.7; H, 4.8; N, 10.3%); $\nu_{max.}$(KBr) 1 740 and 1 730 cm^{-1} (C=O); *m/z* 317 (9%, M^+, $C_{18}H_{23}NO_4$), 258 (1, $M^+ - MeCO_2$), 231 (6, $M^+ - [C(6)HOAc-C(7)HOH]$), 229, 182 (21, $M^+ - PhCH_2CO_2$), 138 (77), 136 (2, $PhCH_2CO_2H$), 122 (36), 95, 94(100), and 60 (2, $MeCO_2H$). The semisynthetic alkaloid had identical characteristics.

Tropane-3α,6β,7β-triol 3-*Phenylacetate* (**9**).—The crystalline base (0.02%) showed R_F 0.48 (system C); $\nu_{max.}$ 3 420(OH) and 1 730 cm^{-1} (ester C=O); *m/z* 291 (M^+), 231 (36%, $M^+ - [C(6)HOH-C(7)HOH]$), 156 (8, $M^+ - PhCH_2CO_2$), 136 (4, $PhCH_2CO_2H$), 94 (100), 91 (33, C_7H_7); δ_H 1.65, 2.3 (4 H, 2 × m, 2-H_2 and 4-H_2), 2.65 (3 H, s, NMe), 3.28 (2 H, s, 1-H, 5-H), 3.62 (2 H, s, $PhCH_2CO_2$), 4.1 (2 H, s, 6- and 7-C*H*OH), 5.01 (1 H, t, *J* 4.9 Hz, 3α-H), and 7.24—7.43 (5 H, m, ArH).

Nortropan-3-yl Phenylacetate (**10**).—A base of R_F 0.37 (system C) gave $\nu_{max.}$ 1 730 cm^{-1} (ester C=O); *m/z* 245.1418 (5%, M^+, $C_{15}H_{19}NO_2$ requires 245.1416), 136 (1, $PhCH_2CO_2H$), 110 (100), and 91.

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