Alkaloids of the Genus *Erythroxylum*. Part 2.[1] *E. dekindtii* (Engl.) O. E. Schultz

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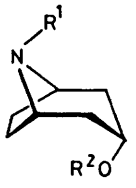
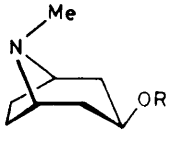
1α*H*,5α*H*-Tropan-3α-yl isovalerate is the principal alkaloid of the root-bark of *Erythroxylum dekindtii*; other new bases characterised are 1α*H*,5α*H*-tropan-3α-yl phenylacetate and 1α*H*,5α*H*-tropan-3α-yl 2-furoate. The root-bark also contains methylecgonidine, valeroidine, poroidine, isoporoidine, and tropine.

Erythroxylum dekindtii (Engl.) O. E. Schulz, one of 15 species of section Lagynocarpus of the Erythroxylaceae,[2] is a small West African shrub found particularly in the mountainous ravines of Angola. Called Olokuto by the natives it is used as a febrifuge in the form of a decotion prepared from the roots and the leaves.[3] The paper chromatographic identification of ecgonine, methylecgonine, ψ-tropine, and tropacocaine in the leaves appears to be the only reported work [3] on the constituents of this plant.

A preliminary t.l.c. investigation of extracts of the root-bark, root-wood, stem-bark, stem-wood, and leaves of this species showed alkaloids to be distributed throughout the plant; the further detailed studies reported here were confined to the root-bark which contained at least five bases representing 0.07% of the dried material.

Column chromatography at pH 6.8 of an ether extract of the root-bark followed by p.l.c. gave a base (R_F 0.7, system C; see Experimental section) which was purified by repeated recrystallisation of the picrate. Spectroscopic analysis indicated an ester of tropine and a saturated C_5 acid. Hydrolysis afforded tropine and isovaleric acid and the structure of the natural alkaloid was confirmed by comparison of the picrate with that of 1α*H*,5α*H*-tropan-3α-yl isovalerate (1) produced by partial





(1) R^1=Me, R^2=$Me_2CH{\cdot}CH_2{\cdot}CO$
(2) R^1=Me, R^2=$PhCH_2{\cdot}CO$
(3) R^1=Me, R^2=o-$MeC_6H_4{\cdot}CO$
(4) R^1=Me, R^2=2-furoyl
(5) R^1=Me, R^2=3-furoyl
(6) R^1=H, R^2=$Me_2CH{\cdot}CH_2{\cdot}CO$
(7) R^1=H, R^2=$MeCH_2{\cdot}CHMe{\cdot}CO$
(8) R^1=Me, R^2=H

(9) R=$Me_2CH{\cdot}CH_2{\cdot}CO$
(10) R=$PhCH_2{\cdot}CO$
(11) R=2-furoyl
(12) R=3-furoyl
(13) R=H

synthesis. This base represented the principal alkaloid (0.02%) of the roots; the 3β-derivative (9) is dissimilar in properties and was not detected in the root.

From the mother-liquors of the above recrystallisations, material separated having as a component a base $C_{16}H_{21}NO_2$. I.r. and mass spectroscopy indicated an ester of tropine and an aromatic C_6–C_2 acid with no free hydroxyls. The mass spectrum also revealed a second tropane ester alkaloid incorporating a C_5 acid. The m.p. of the picrate of the natural product was 19° higher than that of the picrate of 1α*H*,5α*H*-tropan-3α-yl phenylacetate (2) but a mixture of the synthetic picrate and the isovalerate picrate (above) (1 : 1) possessed the same m.p. (undepressed mixed m.p.) and gave similar d.t.a. curves each with a single peak, at 161—197 and 170—206°, respectively. The i.r. spectra of the picrate of the mixture and the natural product were identical. Further support for the identity of the new alkaloid (2) was given by g.l.c. retention data involving the isolated mixture and a crude alkaloid extract of the plant. The synthetic isomeric *o*-toluate (3) and the 3β-phenylacetate (10) were prepared for comparison; they possess dissimilar properties to the natural alkaloid. A base, m.p. 66°, was also eluted from the partition column in ether after the above alkaloids and was purified by p.l.c.; it was characterised as methylecgonidine (0.017%).

Three bases were obtained in the chloroform eluate, the principal one (0.013%) when irradiated at 254 nm on chromatographic plates had a blue fluorescence; it was purified by preparative paper chromatography. The mass spectrum exhibited the characteristic pattern of an ester of a monohydroxytropane.[4] Fragmentation of the molecular ion ($C_{13}H_{17}NO_3^+$) showed the esterifying acid to be $C_5H_4O_3$. The i.r. spectrum showed $\nu_{max.}$ 1 583 cm^{-1} and no hydroxyl bands; the ^1H n.m.r. spectrum gave the AMX pattern of a 2-furoate [5] [δ 6.47 (dd, *J* 3.6 and 0.8 Hz, 4-H), 7.16 (dd, *J* 3.6 and 0.8 Hz, 3-H), and 7.60 (dd, *J* 1.8 and 0.8 Hz, 5-H)]; the remaining signals were consistent with a 1α*H*,5α*H*-tropan-3α-yl residue.[1] The structure of the new alkaloid was confirmed by partial synthesis; the 3β-isomer (11) and the 3α- (5) and 3β-esters (12) involving 3-furoic acid were synthesised and their properties shown to differ from those of the natural alkaloid. In the rabbit 1α*H*,5α*H*-tropan-3α-yl 2-furoate produced mydriasis of brief duration and on the isolated guinea-pig ileum it had *ca.* 1/200th the activity of atropine. A comparative pharmacological report on the four isomers will be given elsewhere.

A second base isolated from the chloroform eluate of the original partition column had properties consistent

with those of 1α*H*,5α*H*-tropane-3α,6β-diol 3-isovalerate (valeroidine) an alkaloid first isolated [6] as a by-product in the manufacture of cocaine from Peruvian cocoa leaves. The third fraction (0.01%) was characterised as a mixture of 1α*H*,5α*H*-nortropan-3α-yl isovalerate (6) and 2-methylbutyrate (7) and shown to be identical with an original sample of ' Base Z ' of Barger *et al.* (poroidine + isoporoidine) [7] isolated from *Duboisia myoporoides*. Tropine (8) was characterised from later fractions of the chromatogram but no ψ-tropine (13) (*cf.* *Erythroxylum monogynum* [1] and the leaves of *E. dekindtii* [3]) was detected by the methods employed.

The tropane alkaloid composition of *E. dekindtii* is distinct from that reported so far for other *Erythroxylum* species. It apparently lacks the esters of benzoic, trimethoxybenzoic, trimethoxycinnamic, tiglic, and pyrrole-2-carboxylic acids variously reported [1,8,9] for other species. Affinities with the cocaine-producing species are given by the suggested leaf alkaloids [3] and by the occurrence of methylecgonidine and valeroidine. Isovaleric acid is the principal esterifying acid of the species; 1α*H*,5α*H*-tropan-3α-yl isovalerate, the principal alkaloid of the root-bark, has not previously been clearly defined as a natural product, Mitchell [10] considering it a possible component of his ' Base D ' of *Duboisia leichhardtii*, and Loder and Russell [11] reporting the presence of either this alkaloid or the 2-methylbutyrate as a component of *Bruguiera sexangula* (Rhizophoraceae) stem-bark. 2-Furoic acid has hitherto not been reported as a moiety of the tropane alkaloid series; its limited known natural distribution includes it as a component of the essential oil of the leaves of *Nicotiana tabacum*,[12] as a β-L-alanine derivative in the hydrolysed seeds of *Koelreuteria paniculata* (Sapindaceae),[13] and in the seeds of *Fagopyrum esculentum* (Polygonaceae).[14]

Further work on other species of the genus is in progress.

EXPERIMENTAL

Instrumentation and methods for the preparation and hydrolysis of esters were as recorded previously [1] except that ^{1}H n.m.r. spectra were run on a Varian EM 360 spectrometer for solutions in deuteriochloroform (tetramethylsilane as internal standard). T.l.c. and p.l.c. alumina plates were developed with (A) ether, (B) ether–ethanol (1 : 1), and (C) chloroform–ethanol (98 : 2); silica plates were developed with (D) acetone–ammonia (*d* 0.88) (4 : 1) and (E) chloroform–diethylamine (9 : 1). Preparative paper chromatography involved Whatman Chromatographic Paper 3MM pretreated with a 0.05M solution of potassium dihydrogen phosphate for 5 min and dried; the developing solvent was the upper layer from n-butanol (80 ml), ethanol (20 ml), orthophosphoric acid 85% (0.2 ml), and water added to saturation point.

Compounds isolated, but previously recorded, were identified by the usual criteria.

Plant Material.—Root-bark and herbarium specimens were collected in 1973 at Huila, Humpata, Angola.

Extraction and Fractionation of Alkaloids.—In typical experiments the alkaloids were extracted from the powdered root-bark (100 g) as previously described.[1] A preliminary fractionation of the bases was effected by chromatography [Kieselguhr (15 g), 0.5M-phosphate buffer solution 7.5 ml] at pH 6.8 by successive development with light petroleum (b.p. 40—60°), ether, chloroform, and ammoniacal chloroform. The eluate fractions (5 ml) were each titrated with 0.005N-sulphuric acid and the separated bases further fractionated by p.l.c. as indicated below.

1α*H*,5α*H*-*Tropan-3α-yl Isovalerate* (1) *and Phenylacetate* (2).—The bases from the titrated ether eluate fractions were recovered and submitted to p.l.c. [system (C)]. Alkaloid bands of R_F 0.92 (methylecgonidine) and 0.70 were obtained and recovery of the lower one in methanol and repeated recrystallisation (× 21) of the picrate gave (1) picrate, plates, from aqueous ethanol; m.p. 230° (Found: C, 50.1; H, 5.7; N, 12.0. $C_{13}H_{23}NO_2 \cdot C_6H_3N_3O_7$ requires C, 50.2; H, 5.8; N, 12.3%. Found, for basic moiety: M^+, 225.173 0. $C_{13}H_{23}NO_2$ requires M^+, 225.179 2), $\nu_{max.}$(KBr disc) 1 724 cm^{-1} (ester C=O), *m/e* 42, 82, 83, 94, 95, 96, and 124 (100%, $M - C_4H_9CO \cdot O$). Hydrolysis of the base in the usual manner afforded isovaleric acid, with i.r., g.l.c. (DEGA, 162°, R_T 5.6 min), and ^{1}H n.m.r. (AX_6 system) identical with those of authentic sample. The alkaline fraction contained tropan-3α-ol (8) [R_F 0.47, system (D), *cf.* R_F 0.64 for tropan-3β-ol (13)] which gave on acetylation tropan-3α-yl acetate (R_F, i.r., picrate). The base (1), prepared from tropine (160 mg) and isovaleryl chloride (160 mg), had identical properties (picrate, R_F values, spectroscopic data) to those of the natural product.

The mother-liquors remaining from the repeated recrystallisation of the picrate of the natural base (1) deposited chunky crystals, m.p. 187°, the mass spectrum of which exhibited signals also given by the synthetic base (2) but showed, in addition, the presence of some residual isovalerate (1). The mass spectrum of the mixed picrate was identical with that of a synthetic mixture (1 : 1), m.p. 186°, of the picrates of (1) and (2), as was the g.l.c. retention data for the liberated bases.

1α*H*,5α*H*-*Tropan-3α-yl 2-Furoate* (4).—The chloroform eluate from the partition column contained three bases which were fractionated by p.l.c. [system (C)], R_F values 0.66, 0.54 (valeroidine), and 0.15 respectively. The fastest running base which exhibited a dark blue fluorescence on irradiation at 254 nm contained base (1) as impurity. Purification was effected by paper chromatography and the new *alkaloid*, m.p. 66°, afforded a *picrate*, plates from aqueous ethanol, m.p. 209° (Found: C, 49.2; H, 4.0; N, 12.35. $C_{13}H_{17}NO_3 \cdot C_6H_3N_3O_7$ requires C, 49.1; H, 4.3; N, 12.1%), *m/e* 42, 82, 83, 94, 95, 96, 124 ($M - C_4H_3O \cdot COO$, 100%), 229 (picric acid), and 235.120 5 (M^+ for base) ($C_{13}H_{17}NO_3$ requires 235.120 8).

Poroidine (6) *and Isoporoidine* (7) *Mixture.*—The chromatographic band of R_F 0.15 [see (4) above] gave a base, picrate, m.p. 162° (× 4 recrystallisations) and undepressed by admixture with the picrate of the original ' Base Z ' of Barger *et al.*[7] Both materials gave by g.l.c. [OV-101 on Gas Chrom (Cl) silanised with HMDS; 160°] R_T 0.82 and 1.07 min. I.r. and ^{1}H n.m.r. spectra for the two samples were identical (Found, for basic unit of the *Erythroxylum* derivative: M^+, 211.156 8. Calc. for $C_{12}H_{21}NO_2$: *M*, 211.157 2).

Tropine {R_F 0.4 [system (D)], 0.47 [system (B)]} occurred in the ammoniacal chloroform fraction of the original chromatographic separation and in the later chloroform fractions of other chromatograms; it was characterised as its acetate.

Tropane Esters.—The following esters were prepared from the appropriate tropanol and acyl chloride and characterised as their *picrates* (which crystallised from aqueous ethanol): 3α-yl 2-furoate (4), m.p. 209° [*hydrochloride*, m.p. 276° (Found: C, 57.3; H, 6.8; N, 5.0. $C_{13}H_{17}N_3O_7$·HCl requires C, 57.5; H, 6.7; N, 5.2%)]; 3β-*yl* 2-*furoate* (11), m.p. 235° (Found: C, 48.8; H, 4.4; N, 11.6. $C_{13}H_{17}NO_3$·$C_6H_3N_3O_7$ requires C, 49.1; H, 4.3; N, 12.1%) [*hydrochloride*, m.p. 284° (Found: C, 57.3; H, 7.0; N, 5.0%)]; 3α-*yl* 3-*furoate* (5), m.p. 214° (Found: C, 49.0; H, 4.4; N, 11.7%) [*hydrochloride*, m.p. 291° (Found: C, 57.8; H, 7.0; N, 5.1%)], δ (base), AMX pattern of acid, signals centred at 6.69, 7.40, 7.94 (all dd) ($J_{2,4}$ 0.8, $J_{2,5}$ 1.8, $J_{4,5}$ 2.0 Hz); 3β-*yl* 3-*furoate* (12), m.p. 286° (Found: C, 48.7; H, 4.5; N, 11.7%) [*hydrochloride*, m.p. 270° (sublimed) (Found: C, 57.1; H, 7.1; N, 5.1%)]; 3α-*yl phenylacetate* (2), m.p. 168° (Found: C, 54.3; H, 4.8; N, 11.3. $C_{16}H_{21}NO_2$,$C_6H_3N_3O_7$ requires C, 54.1; H, 4.9; N, 11.5%), $\nu_{max.}$ (KBr) 1 720 cm^{-1} (ester C=O), *m/e* 42, 77, 82, 91 (C_7H_7), 94, 95, 124 (100%, M − $PhCH_2$·CO·O), 140 (M − $PhCH_2$·CO), and 259 (M^+) [R_F (base) 0.65, system (C)]; 3β-*yl phenylacetate* (10), m.p. 139° (Found: C, 54.0; H, 5.2; N, 11.1%) [R_F (base) 0.91, system (C)]; 3α-*yl* o-*toluate* (3), m.p. 221° (Found: C, 53.8; H, 4.9; N, 11.3%) [R_F (base) 0.74, system (C)]; 3β-*yl isovalerate* (9), m.p. 159° (Found: C, 50.0; H, 6.1; N, 12.2. $C_{13}H_{23}NO_2$,$C_6H_3N_3O_7$ requires C, 50.2; H, 5.8; N, 12.3%) [R_F (base) 0.90, system (C)].

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