Phytochemistry, Vol. 28, No. 2, pp. 671–673, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00
Pergamon Press plc.

ALKALOIDS OF *ERYTHROXYLUM HYPERICIFOLIUM* STEM BARK*

MANSOUR S. AL-SAID, WILLIAM C. EVANS and RAYMOND J. GROUT

Department of Pharmaceutical Sciences, The University, Nottingham NG7 2RD, U.K.

(*Received* 11 *July* 1988)

Key Word Index—*Erythroxylum hypericifolium*; Erythroxylaceae; tropane alkaloids; 3α-phenylacetoxynortropan-6β-ol; 6β-acetoxy-3α-benzoyloxytropane; 3-acetoxy-6-phenylacetoxytropane; hygrine; chemotaxonomy.

Abstract—Thirteen bases were characterized from the stem bark of *Erythroxylum hypericifolium*; hygrine is the principal component. As in the root bark esters of phenylacetic acid predominate; other alkaloids involve acetic, benzoic and trimethoxycinnamic acids. Alkaloids reported for the first time are 3α-phenylacetoxynortropan-6β-ol, 6β-acetoxy-3α-benzoyloxytropane and 3-acetoxy-6-phenylacetoxytropane.

INTRODUCTION

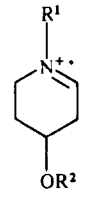
In comparison with the tropane alkaloid mixtures contained in all other species of *Erythroxylum*, that found in the root bark of *E. hypericifolium* Lam. is distinctive in containing a predominance of esters of phenylacetic acid [1, 2]. In continuation of our studies on this socially and medicinally important genus we now report on the findings for the stem bark of the above species.

RESULTS AND DISCUSSION

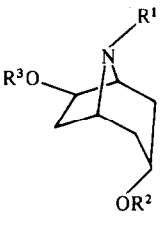
The stem bark was obtained from the same plants studied for the alkaloid composition of the root bark. Concentration of an ether extractive of the dried stem bark gave a mixture of crystalline bases; the latter, and those remaining in the mother-liquor were separated fractionally by TLC. Thirteen bases (Table 1), of which three were new, were identified by the usual spectroscopic criteria [1]. The total basic yield was 0.58%.

A new base R_f 0.07 (system C, see Experimental) of the non-crystalline fraction of the extract, was obtained in 0.01% yield and identified as 3α-phenylacetoxynortropan-6β-ol (**2a**). IR spectroscopy indicated ester and hydroxy functions; mass spectrometry gave a molecular formula $C_{15}H_{19}NO_3$ and a fragmentation pattern consistent with that of a monosubstituted nortropane-3,6-diol ester, with prominent peaks at *m/z* 80, 81 and 126. 3-Substitution in this new ester was demonstrated by the presence of *m/z* 217 (**1a**), resulting from the loss of $[C(7)H_2–C(6)HOH]$ from the $[M]^+$ with the retention of the ester function at C-3 and the absence of an ion at *m/z* 99 $[C(7)H_2–C(6)HOCOCH_2Ph]^+$. Phenylacetic acid involvement was shown by ions at *m/z* 136 and 91 [1]. The structure was confirmed by PFT ^{1}H NMR which also demonstrated the 3α-ester linkage (*t*, δ4.99, $J = 5$ Hz) and a 6α proton (*dd*, $J_{6\alpha,7\alpha}$ and $J_{6\alpha,7\beta}$ 6.6 and 2.0 Hz, respectively); the remaining signals were for phenylacetic acid (*s*, δ3.61 and 7.29), thus confirming the structure of the new alkaloid.

Also from the non-crystalline fraction a new base, 6β-acetoxy-3α-benzoyloxytropane (**2b**), R_f value 0.70 (system C), was isolated as its picrate in 0.004% yield. Mass spectrometry showed it to be a disubstituted tropane-3,6-diol with molecular formula $C_{17}H_{21}NO_4$. Ions at *m/z* 181 and 198 were consistent with the loss of PhCOO and PhCO from M_r 303; similarly ions at *m/z* 244 and 260 were attributed to the loss of MeCOO and MeCO, respectively. The presence of these two acids was further indicated by their acylium ions at *m/z* 105 and 43. The benzoyl moiety was associated with C-3 $\{m/z\ 217\ [M-C(7)H_2–C(6)HOCOMe]^+\}$ (**1b**) and absence (< 1%) of *m/z* 155 (**1c**). The ^{1}H NMR spectrum showed the orientation of the C-3 proton to be β (*t*, δ5.42) and that at



	R^1	R^2	*m/z*
1a	H	$PhCH_2CO$	217
1b	Me	PhCO	217
1c	Me	MeCO	155



	R^1	R^2	R^3
2a	H	$PhCH_2CO$	H
2b	Me	PhCO	MeCO
2c	Me	MeCO	$PhCH_2CO$

*Part 9 in the series 'Alkaloids of the Genus *Erythroxylum*'. For part 8 see El-Imam Y. M. A., Evans, W. C. and Grout R. J. (1988) *Phytochemistry* **27**, 2181.

Table 1. Alkaloids of *Erythroxylum hypericifolium* stem bark

Alkaloids identified	Other sources in *Erythroxylum*
3α-Phenylacetoxynortropan-6β-ol	New alkaloid (**2a**), (preliminary report for *E. cumanense* roots [3])
6β-Acetoxy-3α-benzoyloxytropane	New alkaloid (**2b**)
3-Acetoxy-6-phenylacetoxytropane	New alkaloid (**2c**)
3α-Phenylacetoxytropane	Root barks of *E. hypericifolium* [1] and *E. dekindtii* [4]
3α-Phenylacetoxytropan-6β-ol	Root barks of *E. hypericifolium* [1] and *E. zambesiacum* [2]
6β-Acetoxy-3α-phenylacetoxytropane	*E. hypericifolium* root bark [1]
3α-Phenylacetoxytropane-6β,7β-diol	ditto
3α-(3-Hydroxyphenylacetoxy) tropane	ditto
3α-Benzoyloxynortropane	Leaves and barks of *E. sideroxyloides* and *E. macrocarpum* [5]
3-Benzoyloxytropan-6-ol	*E. sideroxyloides* leaves [5]
3α-Trimethoxycinnamoyloxytropane	*E. ellipticum* leaves [6], root barks of *E. monogynum* [7] and *E. zambesiacum* [1]
Hygrine (principal base)	Leaves and roots of *E. coca* and *E. novogranatense* [8, 9], *E. australe* bark [10], *E. argentinum* leaves [3]
Cuscohygrine	Leaves and roots of *E. coca* and *E. novogranatense* [8, 9], leaves of *E. argentinum* and *E. cataractarum* [3]

C-6 to be α (*dd*, δ5.89, $J = 7.5$ and 3.0 Hz, respectively); the remaining diagnostic signals confirmed NMe and showed aromatic protons characteristic of a benzoate, thus confirming the structure of the new diacyloxytropane. This alkaloid, prior to final purification, contained two other minor components, the mass spectrum showing both to be diesters of tropane-3,6-diol, one M_r 317, seemingly involving phenylacetic acid (*m/z* 136 and 91) and acetic acid and the other M_r 329, involving cinnamic acid (*m/z* 148 and 131) [δ_H 6.67 (*d*, $J = 15.4$ Hz) and 7.78 (*d*, $J = 15.4$ Hz, *trans* CH=CH)] and acetic acid. The former probably arises from incomplete separation from a new diol ester of similar R_f value isolated from the crystalline fraction, and described below. Cinnamate esters feature prominently in the tropanes found in the leaves of this species (unpublished data).

A new base, 3-acetoxy-6-phenylacetoxytropane (**2c**) was isolated from the crystalline fraction in low yield (0.0004%) and was tentatively identified by mass spectrometry. The molecular formula was shown as $C_{18}H_{23}NO_4$ and the fragmentation pattern was characteristic of a diesterified tropane-3,6-diol. Phenylacetic and acetic acid moieties were established by the usual criteria, with the acetate being located at C-3 [*m/z* 155 (**1c**)]. The base was slightly contaminated with **2b**.

As with the alkaloid mixture of the root bark, that of the stem bark has phenylacetic acid as the principal esterifying acid; six such esters were identified. With the exception of the new alkaloid **2b** the remaining bases have been variously recorded as constituents of other *Erythroxylum* species (Table 1). The occurrence of hygrine as a principal base is unusual.

The barks of indigenous Mauritius *Erythroxylum* species are much valued locally as a treatment for various kidney disorders and it would appear that the *E. laurifolium* complex, under the name 'bois de ronde' (for alkaloids, see reference [5]) is mainly employed. Compared with *E. hypercifolium*, these barks have significantly lower alkaloid content (0.003–0.2%) and differ in the chemical nature (simple benzoyl esters) of their alkaloids. Although the medicinally important constituents of 'bois de ronde' have not been established, it might be expected that the pharmacological activities of the two groups of barks in relation to their alkaloid content, would differ considerably.

EXPERIMENTAL

Plant material, instrumentation and chromatographic systems are recorded in ref. [1]. Chromatographic system C, specifically mentioned below, involved silica gel plates with $CHCl_3$–Et_2NH (9:1) as developing solvent.

Extraction and fractionation of alkaloids. Bases from stem bark (200 g) were extracted in the usual manner [7]. Total bases, determined by titration (0.58%, calc. as phenylacetoxytropane, dry wt) were recovered in $CHCl_3$ which on removal, cooling and treating with cold Et_2O gave a crystalline deposit (0.15%). The crystalline and non-crystalline residues were fractionated by the methods used previously for the root bark [1], with the addition of a preliminary sepn of the non-crystalline bases using kieselguhr (15 g) loaded with Pi buffer soln (7.5 ml, 0.5 M, pH 6.8) and petrol (bp 40–60°), Et_2O and $CHCl_3$ as successive eluants. Known compounds were identified by comparison with authentic alkaloids using R_f values, IR, MS and, where appropriate, picrate characteristics.

3α-Phenylacetoxynortropan-6β-ol (**2a**). The slowest running component (system C, R_f 0.07) of the non-crystalline fraction gave a base, IR ν_{max}^{KBr} cm^{-1}: 3450, 1728 (OH and ester CO, respectively); EIMS (probe) 70 eV, *m/z* (rel. int.): 261.1368 $[M]^+$ ($C_{15}H_{19}NO_3$ requires M_r 261.1365) (4), 217.1099 $[\mathbf{1a}]^+$ (calc. for $C_{13}H_{15}NO_2$: 217.1103) (7), 136 (2), 126.0908 (calc. for $C_7H_{12}NO$: 126.0918) (20), 108.0818 (calc. for $C_7H_{10}N$: 108.0813) (2), 91.0552 (calc. for C_7H_7: 91.0547) (23), 81.0586 (calc. for C_5H_7N: 81.0578) (100), 80 (76); ^1H NMR (250 MHz, $CDCl_3$) δ2.21 (1H, *m*, NH), 3.16 (H, *br s*, H-1), 3.53 (1H, *m*, H-5), 3.61 (2H, *s*, $PhCH_2CO$), 4.20 (1H, *dd*, $J_{6\alpha,7\alpha} = 6.6$ Hz, $J_{6\beta,7\beta} = 2.0$ Hz, H-6), 4.99 (1H, *t*, $J = 5.0$ Hz, H-3α), 7.29 (5H, *m*, Ar-H_5).

6β-Acetoxy-3α-benzoyloxytropane (**2b**). The fastest running base (R_f 0.70, system C) of the non-crystalline fraction formed a picrate, mp 132° (crude): EIMS *m/z* (rel. int.): 303 $[M]^+$ ($C_{17}H_{21}NO_4$) (7), 229 (picric acid), 198 $[M-PhCO]^+$ (3), 182 $[M-PhCOO]^+$ (12), 138 (7), 122 $[PhCO_2H]^+$ (35), 105 $[PhCO]^+$ (16), 94 (100), 81, 77 (21), 43 (23); ^1H NMR [250 MHz, picrate in $(CD_3)_2CO$]: δ2.05 (3H, *s*, COMe), 3.20 (1H, *s*, NMe), 4.22 (1H, *br s*, H-1), 4.38 (1H, *m*, H-5), 5.42 (1H, *t*, $J = 4.8$ Hz, H-3β), 5.89 (1H, *dd*, $J_{6\alpha,7\alpha} = 7.5$ Hz, $J_{6\alpha,7\beta} = 3.0$ Hz, H-6), 7.65 (3H, *m*, Ar-H_3), 8.12 (2H, *m*, Ar-H_2), 8.85 (2H, Ar-H_2 of picrate).

Tentative identification of 3-acetoxy-6-phenylacetoxytropane (**2c**). A base with the highest R_f value (0.90 system C) of the crystalline fraction was isolated in low yield (0.8 mg, 0.0004%). EIMS *m/z* (rel. int.): 317 $[M]^+$ ($C_{18}H_{23}NO_4$) (4), 182 [M

$-C_8H_7O_2]^+$ (12), 155 $\{M-[C(7)H_2-C(6)HOCOCH_2Ph]\}^+$ (3), 138 (7), 135 (2), 122 (27), 97 (19), 95 (60), 94 (52), 91 (16), 60 (6), 43 (100). Weak signals at *m/z* 303 and 105 suggested contamination with (**2b**).

Acknowledgements—We are greatly indebted to Mr R. Antoine, Director, Sugar Industry Research Institute, Mauritius for plant collection facilities, to Mr J. Guého, for location and identification of specimens and to Dr J. G. Woolley (Department of Pharmacy, Leicester Polytechnic) for supervising some of the MS determinations. We gratefully acknowledge financial support from the King Saud University, Riyadh, Saudi Arabia.

REFERENCES

1. Al-Said, M. S., Evans, W. C. and Grout, R. J. (1986) *J. Chem. Soc. Perkin Trans. I* 957.
2. El-Imam, Y. M. A., Evans, W. C., Grout, R. J. and Ramsey, K. P. A. (1987) *Phytochemistry* **26**, 2385.
3. El-Imam, Y. M. A., Evans, W. C. and Plowman, T. (1985) *Phytochemistry* **24**, 2285.
4. Al-Yahya, M. A. I., Evans, W. C. and Grout, R. J. (1979) *J. Chem. Soc. Perkin Trans. I* 2130.
5. Al-Said, M. S., Evans, W. C. and Grout, R. J. (1986) *Phytochemistry* **25**, 851.
6. Johns, S. R., Lamberton, J. A. and Sioumis, A. A. (1970) *Aust. J. Chem.* **23**, 421.
7. Agar, J. T. H. and Evans, W. C. (1976) *J. Chem. Soc. Perkin Trans. I* 1550.
8. Henry, T. A. (1949) *The Plant Alkaloids* 4th Edn., p. 101. Churchill, London.
9. Hegnauer, R. and Fikenscher, L. H. (1960) *Pharm. Acta Helv.* **35**, 43.
10. Klein, G. and Soos, G. (1929) *Österreich. Botan. Z.* **78**, 157.

Phytochemistry, Vol. 28, No. 2, pp. 673–675, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

HYDROXYNORCYTISINE, A QUINOLIZIDONE ALKALOID FROM *LABURNUM ANAGYROIDES*

ALISON R. HAYMAN and DAVID O. GRAY*

School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS, U.K.

(*Received in revised form* 3 *August* 1988)

Key Word Index—*Laburnum anagyroides*; Leguminosae; pods; 3-hydroxy-11-norcytisine; cytisine; quinolizidone alkaloids; dansyl derivatives.

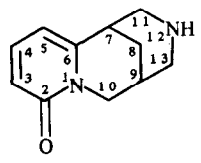
Abstract—A new type of higher plant alkaloid, 3-hydroxy-11-norcytisine, was isolated from the green pods of *Laburnum anagyroides* and characterized spectroscopically as its dansyl derivative.

INTRODUCTION

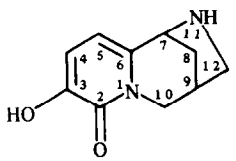
Laburnum anagyroides Med., formerly *L. vulgare* or *Cytisus Laburnum*, a small tree, native to central and southern Europe, is commonly grown in the U.K. for its long racemes of ornamental golden yellow flowers. All parts of the plant are toxic, especially the seeds [1–4]. Although not always fatal to cattle, sublethal doses can be excreted in the milk and indirectly poison humans. Symptoms of poisoning are irregular, weak heart action, vomiting and unconsciousness, followed by death [5]. The major toxin present is considered to be the quinolizidone alkaloid, cytisine (**1**) (synonyms baptitoxine, sophorine and ulexine) [6–8], first isolated from the species in 1862 [9]. We have now established the presence of a related compound, 5-hydroxy-7,11-diazatricyclo[7,2,1,$0^{2,7}$] dodeca-2,4-dien-6-one or 3-hydroxy-11-norcytisine (**2**), the first recorded example of a quinolizidine alkaloid having a five-membered C ring.

RESULTS AND DISCUSSION

During this work the alkaloids and amines present in *L. anagyroides* extracts were initially recovered by ion-exchange on carboxymethylcellulose and then made easier to detect and isolate by conversion to their fluorescent



1



2

*Author to whom correspondence should be addressed.