Pergamon

0031-9422(94)00777-2

Phytochemistry, Vol. 38, No. 4, pp. 1053-1056, 1995
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0031-9422/95 $9.50 + 0.00

ALKALOIDS OF *ERYTHROXYLUM MONOGYNUM* ROOT-BARK*

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(*Received in revised form* 1 *September* 1994)

Key Word Index—*Erythroxylum monogynum*; Erythroxylaceae; root-bark; tropane alkaloids; 3α-isobutyryloxynortropane; 3α-(4-methylvaleroyloxy)tropane; 3β-phenylacetoxytropane; GC-MS.

Abstract—Twenty-six tropane alkaloids were identified from the root-bark of *Erythroxylum monogynum* by GC and GC-MS and twenty of these alkaloids had not previously been reported as constituents of the root-bark. 3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropane was the main base and in addition, three new alkaloids were identified as 3α-isobutyryloxynortropane, 3α-(4-methylvaleroyloxy)tropane and tentatively, 3β-phenylacetoxytropane.

INTRODUCTION

Erythroxylum monogynum Roxb. is a small tree originating from India and Sri Lanka. This is the only species placed by O. E. Schulz [1] in section *Sethia*. It was previously reported [2] that the leaves contain (−)-ecgonine and cinnamoylcocaine, and that the wood constitutes a rich source of diterpenoids [3]. More recently, Agar and Evans [4] isolated from the root-bark 3α-(3′,4′,5′-trimethoxycinnamoyloxy)tropane, 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane, 3α-(3′,4′,5′-trimethoxycinnamoyloxy)-6β-benzoyloxytropane, 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane-6β,7β-diol, tropine and pseudotropine. During their investigation a number of other bases were detected in small quantity or as mixtures which precluded their identification. Further investigation of *E. monogynum* root-bark has been undertaken in our Laboratories using the techniques of capillary gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS) which have been used successfully for the identification of tropane alkaloids in hairy root cultures of *Datura candida* × *D. candida* cultivar Flintham Hall [5] and in the stem-bark of *Erythroxylum zambesiacum* [6].

RESULTS AND DISCUSSION

The advantages of the simultaneous detection by the use of flame ionization detection (FID) and nitrogen phosphorus detection (NPD) such as the improvement in sensitivity of the NPD and the reliability and constant performance characteristics of FID allowed us to successfully separate 26 tropane alkaloids representing 0.18% of the dried plant material. Most of them could be identified by GC-MS (Table 1), the fragmentation pattern of tropane alkaloids being well documented [7]. Among the alkaloids identified, only six of them have been already reported to occur in the root-bark [4].

Hygrine (**1**), tropinone (**2**), tropine (**3**) and pseudotropine (**4**) are precursors in the biosynthesis of tropane alkaloids [8], whereas cuscohygrine (**9**) and dihydrocuscohygrine (**10**) are products of side-reactions of the biosynthetic pathway. These alkaloids are widely distributed in the genus *Erythroxylum* except tropinone (**2**) which has been identified in *E. zambesiacum* only [6]. Three *nor*-derivatives (**5**, **7** and **14**) have been identified. Their fragmentation pattern and the base peak at *m/z* 110 are typical of monoesters of nortropan-3-ol. The $[M]^+$ of **5** at *m/z* 197, together with ions at *m/z* 126, 110, 82, 80, 69 and 68 suggested a 3-substituted *nor*-tropane nucleus with an esterifying acid $C_4H_8O_2$. Further indication of the M_r came from the CI mass spectrum which displayed a $[M + H]^+$ peak at *m/z* 198. The assignment of alkaloid **5** as an isobutyric rather than a *n*-butyric ester could be deduced from the fragmentation pattern of the acid moiety. The presence of a peak at *m/z* 43 (isopropyl ion) obtained through α-cleavage of the acid moiety and the absence of a peak at *m/z* 169 $[M - 28]^+$ resulting from a McLafferty rearrangement of *n*-butyric moiety, strongly suggested that **5** is 3-isobutyryloxynortropane. Furthermore, the identity of this alkaloid was confirmed by comparison with a sample prepared synthetically and which displayed the same properties (TLC, GC, mass spectrum) as alkaloid **5**. This alkaloid has not been previously recorded and may be considered as a new compound.

*Part 12 in the series 'Alkaloids of the genus *Erythroxylum*'. For part 11 see ref. [6].

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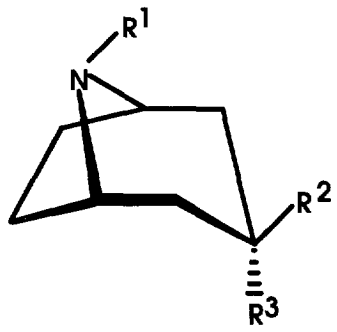

	R^1	R^2	R^3
5	H	H	$(Me)_2CHCOO$
12	Me	H	$(Me)_2CH(CH_2)_2COO$
17	Me	$PhCH_2COO$	H

3α-Isobutyryloxytropane (**6**) is reported to occur in the genus *Erythroxylum* for the first time. This alkaloid was isolated previously from the leaves of *Duboisia leichhardtii* [9] and identified by GC [10] from *Bruguiera sexangula* and *B. exaristata* (Rhizophoraceae).

Alkaloid **7** was identified as isoporoidine, a compound which has been isolated from the root-bark of *E. dekindtii* [11]. The *N*-Me analogue, **8**, has been identified from the stem-bark of *E. zambesiacum* [6]. The assignment of alkaloids **7** and **8** as 2-methylbutyryl esters was deduced from the fragmentation pattern of the acid moieties. The presence of $[M - 15]^+$ and $[M - 29]^+$ ions and the absence of a $[M - 43]^+$ ion are assigned to the loss of a 2-methylbutyryl moiety.

Valeroidine (**11**) was recently identified by GC-MS in the stem-bark of *E. zambesiacum* [6] and isolated from the root-bark of *E. dekindtii* [11].

Alkaloid **12** is a new compound identified as 3α-(4-methylvaleroyloxy)tropane. Its mass spectrum exhibits the fragmentation pattern of a 3-substituted tropane nucleus. The molecular ion peak at *m/z* 239, together with signals at *m/z* 196 $[M - 43]^+$, 183 $[M - 56]^+$, 140, 124 (base peak), 96, 94 and 82 strongly suggests the attachment of the ester function at C-3 and an esterifying acid $C_6H_{12}O_2$. Further support for the identity of the new alkaloid as 3α-(4-methylvaleroyloxy)tropane was confirmed by comparison (TLC, GC, mass spectrum) with the synthetic compound. It is noteworthy that 4-methyl-

Table 1. Alkaloids identified in the stem-bark of *Erythroxylum monogynum*

Compound no.	Alkaloid	R_t (min)	M^+	Reference material	Reference mass spectrum*	Previously identified in the root-bark†
1	Hygrine	2.08	141	+	+	–
2	Tropinone	3.32	139	+	+	–
3	Tropine	3.79	141	+	+	+
4	Pseudotropine	4.10	141	+	+	+
5	3α-Isobutyryloxynortropane‡	12.59	197	+	+	–
6	3α-Isobutyryloxytropane (Butropine)	15.15	211	+	+	–
7	3-(2-Methylbutyryloxy)nortropane (Isoporoidine)	16.32	211	–	–	–
8	3-(2-Methylbutyryloxy)tropane	17.51	225	–	+	–
9	Cuscohygrine	18.43	224	+	+	–
10	Dihydrocuscohygrine	18.72	226	–	+	–
11	3α-Isovaleryloxytropan-6β-ol (Valeroidine)	19.12	241	+	+	–
12	3α-(4-Methylvaleroyloxy)tropane‡	20.38	239	+	+	–
13	3β-Benzoyloxytropane (Tropacocaine)	22.02	245	+	+	–
14	3-Phenylacetoxynortropane	22.19	245	–	+	–
15	3-(2-Methylbutyryloxy)tropan-6,7-diol	22.47	257	–	+	–
16	3α-Phenylacetoxytropane	23.25	259	+	+	–
17	3β-Phenylacetoxytropane‡	23.42	259	+	+	–
18	6β-Benzoyloxytropan-3α-ol	25.09	261	+	+	–
19	3α-Phenylacetoxytropan-6β-ol	25.35	275	+	+	–
20	3α-Cinnamoyloxytropane	26.39	271	+	+	–
21	3α-(3′,4′-Dimethoxybenzoyloxy)tropane (Convolamine)	28.78	305	+	+	–
22	3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropane	31.05	335	+	+	+
23	3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropan-6β-ol	32.28	351	+	+	–
24	3α-(3′,4′,5′-Trimethoxycinnamoyloxy)tropane	34.33	361	+	+	+
25	3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropan-6β,7β-diol	37.61	367	+	+	+
26	3α-(3′,4′,5′-Trimethoxycinnamoyloxy)-6β-benzoyloxytropane	41.92	481	+	+	+

*Indicates MS available or in literature.
†Indicates alkaloids present in root-bark.
‡New alkaloids.

valeric acid is reported for the first time as an esterifying acid of a tropane nucleus.

3β-Benzoyloxytropane **13** occurs widely in the genus but is identified in *E. monogynum* for the first time. Alkaloid **14** was unequivocally identified as 3-phenylacetoxynortropane but lack of material precluded further investigation to assign the stereochemical orientation of the substituent attached to C-3. This compound has not been reported in *E. monogynum* but has been previously isolated from *E. hypericifolium* [12] and identified in *E. zambesiacum* [6].

The mass spectrum of alkaloid **15** is consistent with that of a monoester of 3,6,7-trihydroxytropane with the ester function attached to C-3. Its structure was assigned as 3-(2-methylbutyryloxy)tropan-6,7-diol, an alkaloid recently identified in the stem-bark of *E. zambesiacum* by GC-MS [6].

The mass spectra of **16** and **17** show identical fragmentation patterns reported for 3-phenylacetoxytropanes. It is reasonable to assume that they represent the 3α- and the 3β-phenylacetoxy-isomers. Their identity was confirmed by comparison (TLC, GC, mass spectrum) with synthetic reference. The 3α-isomer **16** has been previously isolated from *E. dekindtii* [11] and *E. hypericifolium* [12] and identified in *E. zambesiacum* [6] and *Atropa belladonna* [13]. The corresponding 3β-isomer **17** has not previously been reported in nature and may be considered as a new compound.

6β-Benzoyloxytropan-3α-ol **18** was identified by comparison with an authentic sample isolated from the leaves of *E. cuneatum* [14]. This compound has also been isolated from the root-bark [15] and identified in the stem-bark [6] of *E. zambesiacum*.

Alkaloid **19** was identified as 3α-phenylacetoxytropan-6β-ol: *m/z* 275 $[M]^+$, 231 $[M - C(7)H_2C(6)HOH]^+$, 156 $[M - PhCH_2CO]^+$, 140 $[M - PhCH_2CO_2]^+$. The base peak at *m/z* 94 indicated that the free hydroxyl group is at the C-6 position. The presence of *m/z* 91 ($PhCH_2$) suggested phenylacetic acid as esterifying acid. 3α-Phenylacetoxytropan-6β-ol was already isolated from *E. hypericifolium* [12] and from *E. zambesiacum* [15].

The alkaloid **20** gave by mass spectroscopy a molecular ion peak at *m/z* 271, a base peak at *m/z* 124 and other ions corresponding to a 3-substituted tropane derivative with the molecular formula $C_{17}H_{21}NO_2$. The presence of ions relating to cinnamic acid at *m/z* 148 $[PhCH{=}CHCO_2H]^+$, 140 $[M - PhCH{=}CHCO]^+$, 131 $[PhCH{=}CHCO]^+$, 103 $[PhCH{=}CH]^+$ and 77 $[C_6H_5]^+$ was consistent with 3-cinnamoyloxytropane. Comparison with reference material allowed assignment of the stereochemical orientation of the C-3 substituent as 3α. 3α-Cinnamoyloxytropane has not previously been identified in *E. monogynum* but was reported as a component of *Crossostylis seberti* (Rhizophoraceae) [16].

Compound **21** was identified as 3α-(3′,4′-dimethoxybenzoyloxy)tropane, an alkaloid previously reported as a constituent of *E. zambesiacum* [6] and of the genus *Convolvulus* [17]. As with *E. zambesiacum*, 3′,4′,5′-trimethoxybenzoic and 3′,4′,5′-trimethoxycinnamic acids are common esterifying acids in *E. monogynum*. Three alkaloids (**22**, **23**, **25**) involving 3′,4′,5′-trimethoxybenzoic acid and two alkaloids (**24**, **26**) involving 3′,4′,5′-trimethoxycinnamic acid have been identified in *E. monogynum* by comparison with authentic samples described previously in this series. Among them, only one compound, 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropan-6β-ol (**23**) had not previously been detected in the root-bark of this species.

The principal alkaloid identified in the mixture is 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane (**22**). The presence of 3α-(3′,4′,5′-trimethoxycinnamoyloxy)-6β-benzoyloxytropan-7β-ol, previously suspected but not confirmed to occur in *E. monogynum* [18], has not been confirmed.

EXPERIMENTAL

Plant material. Dried root-bark of *E. monogynum* Roxb., collected in the region of Madras and Coimbatore, India was the same material as examined in part 1 [4] of this series.

Extraction of alkaloids and TLC. Powdered root-bark (50 g) was extracted following the general method previously described [6]. The petrol and ether extracts afforded 2 residues of 64 mg and 24 mg, respectively. TLC was carried out on silica gel F_{254} using the same developing systems as reported in ref. [6].

GC and GC-MS. Alkaloids were identified by GC and GC-MS by comparison of their R_ts and fragmentation patterns with those of authentic samples using the same instrumentation as already reported [6]. A 15 m × 0.252 mm i.d. fused-silica capillary column coated with the methylsilicone phase DB-1 (film thickness 0.25 μm) and with He as carrier gas at 1 bar pressure was used. Operating conditions for GC were isothermal 80° for 1 min, 80–100° at 2° min^{-1}, 100–310° at 7° min^{-1}, isothermal 310° for 5 min. Both injection and detection temps were maintained at 360°. GC-MS was performed in the EI mode at 70 eV. The oven was programmed as follows: isothermal 35° for 2 min, 35–300° at 30° min^{-1}, isothermal 300° for 5 min. CI mass spectra were recorded on the same instrument using NH_3 as reagent gas.

Identification of alkaloids. Retention data and mass spectra of known bases were compared with those of authentic samples or from lit. values. To confirm the identity of alkaloids **5** and **12**, the synthesis of the corresponding compounds was undertaken as described below. The assignments of ^1H and ^{13}C NMR values were confirmed by use of ^{13}C DEPT and ^1H–^{13}C correlation experiments (HETCOR).

Synthesis of 3α-isobutyryloxynortropane (**5**). To a stirred soln of tropine (0.5 g) in 1,4-dioxane (2.0 ml) was added isobutyryl chloride (0.75 g). After 6 hr at 100°, the mixt. was evapd to dryness, redissolved in 5 M NH_3 (3 ml) and extracted with CH_2Cl_2 (3 × 3 ml). Evapn gave an oily liquid which, on treatment of the hydrochloride with a saturated picric acid soln, furnished 3α-isobutyryloxytropane picrate, m.p. 213–215°. The base (0.38 g) recovered from the picrate was dissolved in H_2O (5 ml) and the soln was adjusted to pH 7.0 with $NaHCO_3$. After the addition of $KMnO_4$ (0.86 g), the pH was kept constant by the

addition of 0.5 M H_2SO_4 over 1 hr at 30°. Pptd MnO_2 was removed and 3α-isobutyryloxynortropane recovered in CH_2Cl_2 from the alkalinized (Na_2CO_3) soln. The base afforded a picrate (yellow needles), m.p. 188–190°. The structure of the base, liberated from the picrate was confirmed by ^1H and ^{13}C NMR and had the same R_t (15.60 min), R_f values and fragmentation characteristics as the natural alkaloid **5**. EI-MS (probe) 70 eV, *m/z* (rel. int.): 197.1423 $[M]^+$ ($C_{11}H_{19}NO_2$ requires M_r 197.1410) (6), 126 $[M - C_4H_7O]^+$ (2), 110.0981 (calc. for $C_7H_{12}N$: 110.0966) (100), 82 (18), 81 (10), 80 (33), 68 (28). ^1H NMR (400 MHz, $CDCl_3$): base recovered from picrate (TMS as int. standard), δ1.18–1.20 (6H, *d*, ββ methyls), 1.6–2.1 (8H, *m*, H_2-2, H_2-4, H_2-6, H_2-7), 2.52 (1H, *m*, $Me_2C\underline{H}$), 3.51 (2H, *m*, H-1, H-5), 5.05 (1H, *t*, J = 5.0 Hz, H-3β). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ18.8 (*q*, 2 Me), 29.3 (*t*, C-6 and C-7), 34.4 (*d*, C-2′), 37.6 (*t*, C-2 and C-4), 53.4 (*d*, C-1 and C-5), 67.8 (*d*, C-3), 176.1 (*s*, C=O).

Synthesis of 3α-(4-methylvaleroyloxy)tropane (**12**). 4-Methylvaleryl chloride was prepd by refluxing 4-methylvaleric acid (20 g) with thionyl chloride (26 g) for 30 min. Esterification of tropine (0.5 g) with 4-methylvaleryl chloride (1 ml) was accomplished in 1,4-dioxane (2 ml) as described above. The oily base was converted to the picrate which forms yellow needles, m.p. 189–191°. The synthetic base had the R_t (20.41 min), R_f values and fragmentation pattern similar to the natural alkaloid **12**. EI-MS (probe) 70 eV, *m/z* (rel. int.): 239.1882 $[M]^+$ ($C_{14}H_{25}NO_2$ requires M_r 239.1878) (8), 196 $[M - 43]^+$ (3), 183 $[M - C_4H_8]^+$ (2), 140.1079 (calc. for $C_8H_{14}NO$: 140.1071) (11), 124.1120 (calc. for $C_8H_{14}N$: 124.1122) (100), 96 (19), 94 (27), 83 (43), 82 (48), 67 (19). ^1H NMR (400 MHz, $CDCl_3$): base recovered from picrate (TMS as int. standard), δ0.91 (6H, *d*, 2Me), 1.48–2.18 (11H, *m*, H_2-2, H_2-4, H_2-6, H_2-7, H_2-3′, H-4′), 2.28 (3H, *s*, *N*-Me), 2.29 (2H, *t*, H_2-2′), 3.10 (2H, *m*, H-1, H-5), 5.0 (1H, *t*, J = 5.5 Hz, H-3β). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ22.2 (*q*, 2 Me), 25.6 (*t*, C-6 and C-7), 27.7 (*d*, C-4′), 33.0 (*t*, C-2′), 33.8 (*t*, C-3′), 36.5 (*t*, C-2 and C-4), 40.3 (*q*, *N*-Me), 59.8 (*d*, C-1 and C-5), 67.2 (*d*, C-3), 173.2 (*s*, C=O).

Acknowledgements—The authors are indebted to Dr G. J. Langley and Dr M. E. Harrison (ULIRS Mass Spectrometry Laboratory at The School of Pharmacy) for performing the GC-MS analysis. This work was supported by a grant from the Royal Society of Great Britain under the European Science Exchange Programme to P.C.

REFERENCES

1. Schulz, O. E. (1907) in *Das Pflanzenreich* (Engler, A., ed.), Vol. IV, sect. 134. Engelmann, Leipzig.
2. Chopra, R. N. and Ghosh, N. N. (1938) *Arch. Pharm. (Weinheim. Ger.)* **276**, 340.
3. Fairlie, J. C., McCrindle, R. and Murray, R. D. H. (1969) *J. Chem. Soc. (C)* 2115.
4. Agar, J. T. H. and Evans W. C. (1976) *J. Chem. Soc. Perkin Trans. I* 1550.
5. Christen, P., Roberts, M. F., Phillipson, J. D. and Evans, W. C. (1990) *Plant Cell Reports* **9**, 101.
6. Christen, P., Roberts, M. F., Phillipson, J. D. and Evans, W. C. (1993) *Phytochemistry* **34**, 1147.
7. Lounasmaa, M. (1988) in *The Alkaloids* (Brossi, A., ed.), Vol. 33, pp. 1–81. Academic Press, New York.
8. Leete, E. (1990) *Planta Med.* **56**, 339.
9. Rosenblum, E. I. and Taylor, W. S. (1954) *J. Pharm. Pharmacol.* **6**, 410.
10. Loder, J. W. and Russell, G. B. (1969) *Aust. J. Chem.* **22**, 1271.
11. Al-Yahya, M. A. I., Evans, W. C. and Grout, R. J. (1979) *J. Chem. Soc. Perkin Trans. I* 2130.
12. Al-Said, M. S., Evans, W. C. and Grout, R. J. (1986) *J. Chem. Soc. Perkin Trans. I* 957.
13. Hartmann, T., Witte, L., Oprach, F. and Toppel, G. (1986) *Planta Med.* **52**, 390.
14. El-Imam, Y. M. A., Evans, W. C. and Grout, R. J. (1988) *Phytochemistry* **27**, 2181.
15. El-Imam, Y. M. A., Evans, W. C., Grout, R. J. and Ramsey, K. P. A. (1987) *Phytochemistry* **26**, 2385.
16. Gnecco Medina, D. H., Pusset, M., Pusset, J. and Husson, H.-P. (1983) *J. Nat. Prod.* **46**, 398.
17. Yagudaev, M. R. and Aripova, S. F. (1986) *Khim. Prirod. Soedin.* **22**, 80.
18. Agar, J. T. H., (1975) Ph.D. Thesis, University of Nottingham, U.K.