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ALKALOIDS OF *ERYTHROXYLUM LUCIDUM* STEM-BARK

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Key Word Index—*Erythroxylum lucidum*; Erythroxylaceae; stem-bark; tropane alkaloids; GC; GC-MS.

Abstract—Thirteen alkaloids were identified from the stem-bark of *Erythroxylum lucidum* by GC-MS. Seven of these have not been reported previously in the genus *Erythroxylum*. Most of them are precursors or intermediates in the biosynthetic pathway of tropane alkaloids. Additionally, one alkaloid characterized as 2,1′-dehydrohygrine, a key putative intermediate in the formation of tropinone, has been identified unambiguously. © 1997 Elsevier Science Ltd

INTRODUCTION

The Erythroxylaceae comprises four genera, readily distinguished by leaf and floral characteristics [1]. *Erythroxylum* is the largest genus and comprises some 250 species, which are widely distributed throughout the tropics, but with large areas of diversity in South America, Africa and Madagascar. Only a few *Erythroxylum* species have been examined systematically for alkaloids by modern analytical methods. Except for the cocaine-producing species, the genus has received surprisingly little chemical attention and the constituents of a large number of species used in traditional medicine remain unknown. *Erythroxylum lucidum* ranges from Brazil to Costa Rica and is cultivated in India for ornamental and local medicinal purposes [2]. The name refers to the shiny, slate-hued leaf upper-sides, which contrast with the frequently reddish leaf undersides. It is a shrub or small tree, 3–4 m tall, found locally in the forest areas of Panamá mountains; Schulz placed this species in section II *Macrocalyx* [3]. It was first described in 1821 [4] and two varieties of *E. lucidum* were described in 1975 by Woodson *et al.* [2].

Erythroxylum lucidum was examined for cocaine and cinnamoylcocaine content only, and Plowman and Rivier [5] reported that the leaves contained traces of cocaine. More recently, Japanese scientists suggested that the leaves might have antiherpesviral activity [6]. Moreover, Gupta *et al.* [7] reported that the methanol extract of *E. lucidum* (branches) was non-toxic in the brine shrimp toxicity assay and was also inactive in tumour inhibition and in DNA-intercalating test. The stem-bark does not appear to have been investigated for alkaloids.

As part of a larger investigation of the genus *Erythroxylum* for tropane and related alkaloids having pharmacological and chemotaxonomic properties [8, 9], we now report our findings on the basic constituents of the stem-bark of *E. lucidum*.

RESULTS AND DISCUSSION

Capillary GC has been developed recently for comprehensive and quantitative analyses of tropane alkaloids. The combination of capillary GC with mass spectrometry (GC-MS) offers a sensitive tool which has demonstrated that tropane alkaloid-containing plants generally have a large number of alkaloids which are not detected by other methods [10]. In the present study, a GC-MS procedure was developed for the identification of tropane alkaloids in the aerial parts of *E. lucidum*; 13 compounds were characterized (Table 1). Eleven tropane alkaloids and hygrine derivatives were identified, together with nicotine and pseudopelletierine.

Most of these alkaloids are known precursors in the biosynthesis of tropane alkaloids [11], e.g. hygrine (**1**), tropinone (**2**) and tropine (**3**), whereas cuscohygrine (**12**) and 2,1′-dehydrohygrine (**7**) are products of side-reactions of the biosynthetic pathway. The latter compound had been produced when Leete *et al.* [12] achieved a biomimetic synthesis of tropinone from hygrine. Its mass spectrum exhibits a quite strong $[M]^+$ at m/z 139 and a base peak at m/z 124 $[M-15]^+$,

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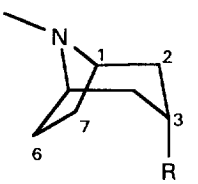
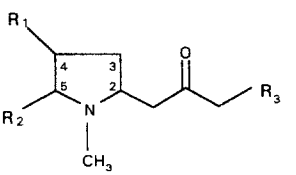
Table 1. Alkaloids identified in stem-bark of *E. lucidum*

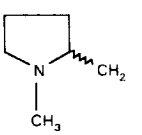
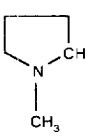
Compound no.	Alkaloid	R_t (min)	$[M]^+$ *m/z*	Reference material	Reference mass spectrum*
1	Hygrine	6.94	141	+	+
2	Tropinone	8.21	139	+	+
3	Tropine	8.32	141	+	+
4	3α-Acetoxytropane	9.91	183	+	+
5	Pseudopelletierine	9.98	153	–	+
6	Nicotine	10.05	162	+	+
7	2,1′-Dehydrohygrine	10.39	139	+	+
8	5-(2-Oxopropyl)-hygrine	11.70	197	–	+
9	5-(2-Hydroxypropyl)-hygrine	11.81	199	–	+
10	*N*-Methylpyrrolidinyl-hygrine A (or B)	13.09	224	–	+
11	*N*-Methylpyrrolidinyl-hygrine B (or A)	13.15	224	–	+
12	Cuscohygrine	13.99	224	+	+
13	Littorine	15.63	289	+	+

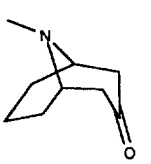
* Indicates spectrum available or in literature.



	R
2	O =
3	HO
4	CH_3COO
13	$PhCH_2CHOHCOO$

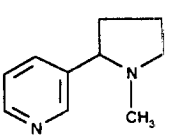


	R_1	R_2	R_3
1	H	H	H
8	H	CH_3COCH_2	H
9	H	$CH_3CHOHCH_2$	H
10, 11		H	H
12	H	H	



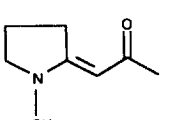
Pseudopelletierine

5



Nicotine

6



2,1′-Dehydrohygrine

7

since it leads to stabilization of the positive charge. Other typical fragments with an alkaloid structure related to hygrine were observed at *m/z* 96, 94 and 68. The identity of compound **7** was confirmed by running under the same conditions the synthesized compound from the original Leete preparation. This vinylogous amide has been detected in root extracts of the Solanaceous shrub, *Vassobia breviflora*, and in two *Erythroxylum* species, *E. coca* and *E. novogranatense* (Bachmann, P., personal communication). This weakly basic alkaloid has been reported to be readily extracted from acidic solutions with CH_2Cl_2 [12], but it is the first time that 2,1′-dehydrohygrine is shown to be a constituent of the basic fraction.

Some of the alkaloids listed are, to our knowledge, hitherto unknown in the genus *Erythroxylum*. Newly detected compounds are hygrine derivatives, presumably isomeric *N*-methylpyrrolidinyl-hygrines (**10** and **11**). The position of ring fusion could not be deduced from the spectral data. They were previously characterized in *Datura innoxia* [10], *D. candida* [13] and *Hyoscyamus albus* [14]. The $[M]^+$ appearing at

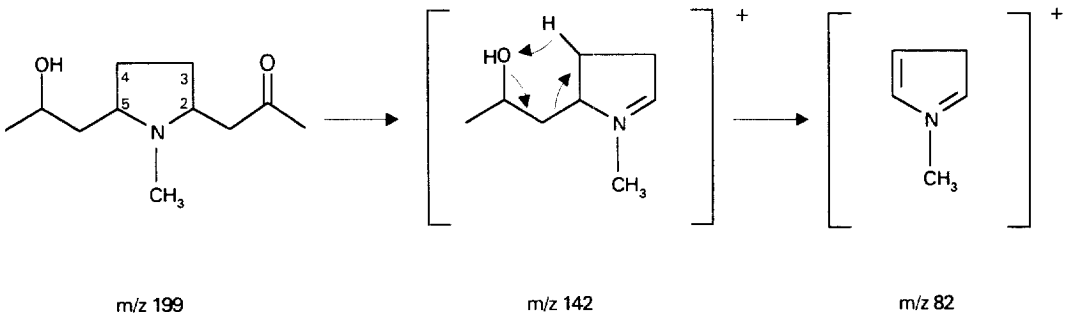


Fig. 1. Fragmentation scheme of 5-(2-hydroxypropyl)-hygrine (**9**).

m/z 224 corresponds with the elemental composition $C_{13}H_{24}N_2O$. The base peak at *m/z* 84 is typical of the *N*-methylpyrrolidinyl ion. The occurrence of low intensity mass fragments at *m/z* 167, 166 and 152 has been explained previously [10] by assuming fragmentation and McLafferty-type rearrangements of an acetonyl group in an α-position, as in hygrine. A plausible biosynthesis of alkaloids **10** and **11** was suggested by Leete [11].

5-(2-Oxopropyl)-hygrine (**8**) and 5-(2-hydroxypropyl)-hygrine (**9**) exhibit similar fragmentation patterns, with ions at *m/z* 140, 96, 82 and 43. The presence of $[M-15]^+$, $[M-43]^+$ and $[M-57]^+$ ions was ascribed to the loss of an acetonyl group.

Compound **9** with a $[M]^+$ at *m/z* 199, shows a base peak at *m/z* 140 and a quite high intensity mass fragment at *m/z* 142, which is not common in tropane alkaloid fragmentation patterns. The occurrence of fragments at *m/z* 142 and 82 can be explained by the loss of the 2-oxopropyl group at C-2 and by the rearrangement between the hydroxyl group and the γ-hydrogen (C-4), through the generally accepted six-membered intermediate (Fig. 1). In compound **8**, the presence of a $[M]^+$ at *m/z* 197, an important peak at *m/z* 140 $[M-CH_2COCH_3]^+$ and the absence of ion at *m/z* 142, give quite good evidence of a symmetrical structure of the compound. Substituents attached to C-2 and C-5 in pyrrolidinyl ring can therefore only be the 2-oxopropyl group. Alkaloids **8** and **9** have not been reported in any species of the Erythroxylaceae, although they have been identified as constituents of the roots of some *Merremia* species in the Convolvulaceae [15]. Alkaloid **8** has been previously reported by Basey *et al.* as a degradation product of phygrine in the roots and aerial parts of *Physalis alkekengi* [16]. However, phygrine, with a $[M]^+$ at *m/z* 280, was not identified as a constituent of *E. lucidum* stem-bark. Therefore, compound **8** could be an artefact from another unknown dimer or could be produced by the plant itself. Biosynthetically, it is suggested that 5-acetonyl-1-methyl-Δ^1-pyrrolinium condenses with an acetoacetyl unit, with the release of CO_2, to form 5-(2-oxopropyl)-hygrine.

Pseudopelletierine (**5**) and nicotine (**6**) were also identified. The latter compound is widely distributed in the genus *Erythroxylum* [17]. The structure of alkaloid **5** with a $[M]^+$ at *m/z* 153 and a base peak at *m/z* 110 was assigned to a piperidine alkaloid and not to a *N*-methylpyrrolidine alkaloid. Although these two classes of alkaloids are difficult to distinguish, the assignment of alkaloid **5** as a piperidine alkaloid was deduced from the high intensity mass fragments at *m/z* 110 and 96 and the low intensity mass fragment at *m/z* 82. The identity of this compound was confirmed by comparison of its mass spectrum with those in a data base. Pseudopelletierine has never been recorded in *E. lucidum* but has been identified previously in the bark of pomegranate (*Punica granatum*) [18].

Few tropane esters were found in *E. lucidum*. 3α-Acetoxytropane (**4**) was identified as a constituent of the stem-bark. Its EI mass spectrum exhibits the typical fragmentation pattern of a 3-substituted tropane nucleus. The $[M]^+$ at *m/z* 183, together with peaks at *m/z* 155 $[M-C_2H_2]^+$, 140 $[M-COCH_3]^-$, 124 $[M-OCOCH_3]^+$ (base peak), 96, 94 and 82, strongly suggest the attachment of the ester function at C-3 and an esterifying acid, $C_2H_4O_2$. The acetate moiety was supported by the ion at *m/z* 43. The identity of this compound was confirmed by comparison with a reference sample run under the same conditions. 3α-Acetoxytropane is reported to occur in the Solanaceae and Rhizophoraceae [17] but not in the Erythroxylaceae. Although leaves of *E. lucidum* contained traces of cocaine [5], this alkaloid was not identified as a constituent of the stem-bark.

Compound **13** was identified as littorine, with a $[M]^+$ at *m/z* 289, an alkaloid previously reported to occur in the Solanaceae [17]. This alkaloid is a well-known positional isomer of (−)-hyoscyamine. These two alkaloids were difficult to distinguish by GC-MS. Comparison of R_ts and mass spectral data of authentic compounds was not sufficient to assign the structure of alkaloid **13** unambiguously. GC analysis of hyoscyamine is often accompanied by partial dehydration in the heated injection port, yielding apoatropine. When chromatographing pure littorine, there is no similar artefact due to the loss of water. The absence of a peak at *m/z* 271 $[M-H_2O]^+$ in the extract of *E. lucidum* ruled out hyoscyamine. Littorine is reported to occur in the Erythroxylaceae for the first time. It has been recently demonstrated that hyoscyamine is biosynthesized from littorine in a process involving

the intramolecular rearrangement of the phenyl lactate moiety of the alkaloid [19, 20].

EXPERIMENTAL

Plant material. Stem-bark of *E. lucidum* H.B.K. was collected in Cerro Campana, Panama, in July 1990 and identified by Dr M. Correa. A voucher specimen (ciflorpan 368) is deposited in the Herbarium of the University of Panama, Panama.

Extraction of alkaloids and TLC. Powdered stem-bark (208 g) was extracted following the general method previously described [8]. The petrol and Et_2O extracts afforded two residues of 20.4 mg and 32.2 mg, respectively. TLC was carried out on silica gel F_{254} with Me_2CO–conc. NH_3 (13:0.3) and on aluminium oxide 60 F_{254} with Et_2O–EtOH (90:1).

GC-MS. Alkaloids were identified by GC-MS comparison of their R_ts and fragmentation patterns with those of authentic samples. GC-MS was performed in the EI mode at 70 eV. He was used as carrier gas at a flow rate of 1 ml min^{-1}. Injection temp. was maintained at 260° and detection temp. at 280°. The injection was performed in the splitless mode and the injected vol. was 1 μl. Different operating conditions were used: (i) a 30 m × 0.25 mm i.d. fused-silica capillary column coated with the phenyl-methyl silicone phase HP5-MS (film thickness 0.25 μm). The temp. programme was, isothermal 40° for 2 min, 40–100° at 30° min^{-1}, 100–200° at 10° min^{-1}, 200–300° at 5° min^{-1}, isothermal 300° for 5 min. (ii) A 15 m × 0.25 mm i.d. fused-silica capillary column coated with the methyl silicone phase DB1 (film thickness 0.25 μm). The temp. programme was, isothermal 45° for 2 min, 45–100° at 30° min^{-1}, 100–300° at 5° min^{-1}, isothermal 300° for 5 min.

2,1′-*Dehydrohygrine* (**7**): EIMS, *m/z* (rel. int.): 139 $[M]^+$ (ascribable to $C_8H_{13}NO$) (32), 125 (9), 124 $[M-Me]^+$ (100), 96 $[M-CO-Me]^+$ (8), 94 (4), 68 (12), 67 (2), 55 (6).

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