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DETERMINATION OF COCAINE IN SOME SOUTH AMERICAN SPECIES OF *ERYTHROXYLUM* USING MASS FRAGMENTOGRAPHY

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Key Word Index—*Erythroxylum* species; Erythroxylaceae; alkaloid; cocaine; mass fragmentography.

Abstract—Thirteen South American species of *Erythroxylum* have been analyzed for their cocaine content. Cocaine was found only in *E. coca* Lam., *E. novogranatense* (Morris) Hieron. and *E. novogranatense* var. *truxillense* (Rusby) Machado. The amount of cocaine was determined by mass fragmentography using deuterium labelled cocaine as internal standard

INTRODUCTION

Two closely related species of the genus *Erythroxylum*, *E. coca* Lam. and *E. novogranatense* (Morris) Hieron., have long been cultivated in South America. The leaves are widely employed by the native population for their stimulant, medicinal and nutritional properties. The principal active constituent of the leaves is the alkaloid cocaine, but they also contain several minor alkaloids, terpenes, vitamins and minerals [1].

Both *E. coca* and *E. novogranatense* are known primarily as cultivated plants. *E. novogranatense* is grown principally in drier regions of Colombia and Venezuela. Its variety *truxillense* (Rusby) Machado is cultivated in northern Peru on the desert coast and in the dry Marañón Valley. *E. coca* occurs throughout the wet, tropical valleys of the eastern Andes from Ecuador south to Bolivia, and in many parts of the Amazon Basin. Unlike *E. novogranatense*, individuals of *E. coca* are frequently encountered throughout its range as escapes from cultivation, suggesting that this species may exist in a semi-wild state.

Over 250 species of *Erythroxylum* are now recognized, of which nearly 200 are found in tropical America. Certain South American species such as *E. cataractarum* Spruce, *E. gracilipes* Peyr., *E. hondense* H.B.K. and *E. ulei* O.E. Schulz, are morphologically similar to the cultivated species and often confused with them. In addition, several wild species are known by common names and are employed in folk medicine. It is, therfore, of appreciable phytochemical interest to determine if these or other wild species of the genus also contain cocaine.

RESULTS

In the present study, 62 samples of air-dried leaves or other plant parts of 13 South American species of *Erythroxylum* were analyzed for cocaine content, according to methods described below. These samples are of recent origin and were collected from living plants or purchased in the local markets in Colombia, Peru and Bolivia. Cocaine was detected only in the leaves of the cultivated species: *E. coca* (0.13 to 0.68%), *E. novogranatense* (0.17 to 0.76%) and *E. novogranatense* var. *truxillense* (0.64%). In addition, the twigs of *E. novogranatense* contained 0.13% cocaine, but no cocaine was found in the seeds of *E. coca* (see Table 1).

Significantly, no cocaine was detected in any parts of the other 11 species tested, including those morphologically related to *E. coca* and *E. novogranatense*. These species, however, may contain other alkaloids which were not investigated in this preliminary study.

Small amounts of cocaine (0.00008 to 0.00882%) were recently reported in the leaves of 5 species of *Erythroxylum* from South America and Panama: *E. campestre* A. St. Hil., *E. pelleterianum* A. St. Hil., *E. deciduum* A. St. Hil., *E. pulchrum* A. St. Hil and *E. panamense* Turcz. [2]. None of these is considered closely related to *E. coca* or *E. novogranatense*, and in fact they are placed in separate sections of the genus [3]. Cocaine or related ecgonine derivatives have also been cited from several species of the Old World tropics, such as *E. acuminatum* (Arn.) Walp. (reported as *E. lucidum* Moon) [4]; *E. dekindtii* (Engl.) O.E. Schulx [5]; and *E. monogynum* Roxb. [6]. Unidentified alkaloids have been found in still other species [4]. These reports indicate that cocaine and related compounds may be widespread throughout the genus.

At the present time, our knowledge of the alkaloidal constituents of *Erythroxylum* species is still rudimentary. Further studies are needed for a better understanding of the distribution and variation of these compounds within the genus and to determine their potential usefulness in systematics.

Voucher specimens for all analyses reported in this study are deposited at the Economic Herbarium of Oakes Ames (ECON), Botanical Museum of Harvard University Cambridge, Massachusetts, U.S.A. All identifications were made by Plowman.

Table 1. Cocaine content of *Erythroxylum species*

Species	Collector(s) and number	Country and Department	Date	Plant part	% Cocaine
E. carthagenense Jacq.	Martin 1420	Colombia: Magdalena	1966	Leaf	0
E. carthagenense Jacq.	Martin 1421	Colombia: Magdalena	1966	Leaf	0
E. carthagenense Jacq.	Plowman and Davis 3512	Colombia: Magdalena	1974	Leaf	0
E. cataractarum Spruce	Plowman, Davis and Jacobs 4256	Colombia; Meta	1974	Leaf	0
E. citrifolium A. St. Hil.	Mass and Plowman 1937	Colombia: El Valle	1974	Leaf	0
E. citrifolium A. St, Hil,	Plowman and Davis 5170	Bolivia La Paz	1975	Leaf	0
E. coca Lam	Plowman and Davis 4107	Colombia; Nariño	1974	Leaf	0.13
E. coca Lam.	Plowman 4627	Peru: Cuzco	1974	Leaf	0.66
E. coca Lam.	Plowman 4628	Peru: Cuzco	1974	Leaf	0.70
E. coca Lam.	Plowman 4648	Peru: Ayacucho	1975	Leaf	0.70
E. coca Lam	Plowman 4711	Peru: Ayacucho	1975	Seeds	0.00
E. coca Lam	Plowman 4821	Peru: Cuzco	1975	Leaf	0.86
E. coca Lam.	Plowman 5203	Peru: Cuzco	1975	Leaf	0.69
E. coca Lam.	Plowman 5204	Peru: Cuzco	1975	Leaf	0.70
E. coca Lam.	Plowman 5205	Peru: Cuzco	1975	Leaf	0.71
E. coca Lam.	Plowman 5206	Peru: Cuzco	1975	Leaf	0.79
E. coca Lam.	Plowman 5207	Bolivia: La Paz	1975	Leaf	0.70
E. coca Lam.	Plowman 5208	Bolivia: La Paz	1975	Leaf	0.74
E. coca Lam.	Kress, Goodell and Sorrenti 75-50-A	Peru: Huánfco	1975	Leaf	0.77
E. coca Lam.	Zarruchi 1147	Colombia: Vaupés	1975	Leaf	0.34
E. coca Lam.	Weil 102	Peru: Huánuco	1975	Leaf	0.67
E. coca Lam.	Weil 202	Peru: Cuzco	1975	Leaf	0.74
E. coca Lam.	Weil 204	Peru: Cuzco	1975	Leaf	0.75
E. densum Rusby	Plowman and Davis 3580	Colombia; Magdalena	1974	Leaf	0
E. floribundum Mart.	Plowman, Davis and Jacobs 4199	Colombia; Meta	1974	Leaf	0
E. floribundum Mart.	Plowman, Davis and Jacobs 4200	Colombia Meta	1974	Leaf	0
E. floribundum Mart.	Plowman, Davis and Jacobs 4216	Colombia: Meta	1974	Leaf	0
E. aff. *gracilipes* Peyr.	Zarruchi 1383	Colombia: Vapués	1975	Leaf	0
E. havanense Jacq.	Martin 1423	Colombia: Magdalena	1966	Leaf	0
E. havanense Jacq,	Plowman and Davis 3563	Colombia: Magdalena	1974	Leaf	0
E. havanense Jacq.	Plowman and Davis 3597	Colombia: Cesar	1974	Leaf	0
E. hondense H.B.K.	Martin 1416	Colombia: Cundinamarca	1966	Leaf	0
E. hondense H.B.K.	Plowman and Jacobs 3759	Colombia: Cundinamarca	1974	Leaf	0
E. hondense H.B.K.	Plowman 4284	Colombia: El Valle	1974	Leaf	0
E. macrophyllum Cav.	Plowman and Davis 4245	Colombia: Putumayo	1974	Leaf	0
E. novogranatense (Morris) Hieron	Martin 1415	Colombia: Cundinamarca	1966	Leaf	0.37
E. novogranatense (Morris) Hieron.	Maas and Plowman 1836	Colombia: El Valle	1974	Leaf	0.42
E. novogranatense (Morris) Hieron.	Plowman and Davis 3628-D	Colombia: Cesar	1974	Leaf	0.76
E. novogranatense (Morris) Hieron.	Plowman and Davis 3734	Colombia: Antióquia	1974	Leaf	0.47
E. novogranatense (Morris) Hieron.	Plowman and Davis 3734	Colombia: Antióquia	1974	Twigs	0.12
E. novogranatense (Morris) Hieron.	Plowman and Davis 4108	Colombia: Cauca	1974	Leaf	0.24
E. novogranatense (Morris) Hieron.	Plowman and Davis 4151	Colombia: Huila	1974	Leaf	0.68
E. novogranatense Morris) Hieron	Plowman and Davis 4151-A	Colombia: Huila	1974	Leaf	0.50
E. novogranatense (Morris) Hieron.	Plowman and Davis 4152	Colombia: Huila	1974	Leaf	0.62
E. novogranatense (Morris) Hieron	Plowman and Vaughan 5272	Colombia: El Valle	1976	Leaf	0.43
E. novogranatense (Morris) Hieron.	Plowman and Vaughan 5385	Colombia: Cauca	1977	Leaf	0.17
E. novogranatense (Morris) Hieron. var. *truxillense* (Rusby) Machado	Weil 301	Peru: Cajamarca	1975	Leaf	0.71
E. orinocense H.B.K.	Plowman and Davis 3600	Colombia: Cesar	1974	Twigs	0
E. orinocense H.B.K.	Plowman and Davis 3600	Colombia; Cesar	1974	Leaf	0
E, orinocense H.K.B.	Plowman 4287	Colombia: El Valle	1974	Leaf	0
E. orinocense H.B.K.	Plowman and Davis 4301	Colombia Cauca	1974	Leaf	0

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Table 1. *continued*

Species	Collector(s) and numbers	Country and Department	Date	Plant part	%Cocaine
E. orinocense H.B.K.	Plowman and Vaughan 5254	Colombia: El Valle	1976	Leaf	0
E. orinocense H.B.K.	Plowman and Vaughan 536.	Colombia: Cauca	1976	Leaf	0
E. raimondii O.E. Schulz	Martin 1399	Peru: Cuzco	1966	Leaf	0
E. raimondii O.E. Schulz	Plowman and Davis 4767	Peru: Cuzco	1975	Leaf	0
E. raimondii O.E. Schulz	Plowman and Davis 4767	Peru: Cuzco	1975	Twigs	0
E. raimondii O.E. Schulz	Plowman and Davis 4768	Peru: Cuzco	1975	Leaf	0
E. ulei. O.E. Schulz	Plowman, Jacobs and Jaramillo 3754	Colombia: Cundinamarca	1974	Leaf	0
E. ulei O.E. Schulz	Plowman, Jacobs and Jaramillo 3754	Colombia: Cundinamarca	1974	Twigs	0
E. ulei O.E. Schulz	Plowman and Davis 4846	Peru; Cuzco	1975	Leaf	0
E. ulei O.E. Schulz	Plowman and Davis 4846	Peru: Cuzco	1975	Twigs	0
E. ulei O.E. Schulz	Plowman and Davis 4846	Peru; Cuzco	1975	Stem	0

EXPERIMENTAL

Mp are corr. Evaporations were carried out under red. pres. at a temp. not exceeding 30°

Ecgonine Me ester-d_3. A soln of ecgonine (3g) in tetradeuteriomethanol (40 ml), containing HCl (4 g), was stirred at room temp. for 18 hr. The soln was evap to dryness and the residue treated with an aq. soln of $NaCO_3$. The alkaline soln was extracted with Et_2O (5 × 30 ml), which was dried and evapd to dryness giving ecgonine Me ester-d_3 (1.48 g).

Cocaine-d_3 A soln of ecgonine Me ester-d_3 (1 g) in Py (10 ml) was treated with benzoyl chloride (0.76 g) at 4° for 18 hr. The mixture was diluted with an aq. soln of $NaCO_3$ and extracted with Et_2O (5 × 30 ml). The organic phase was evap to dryness and the residue recrystallised from EtOH giving cocaine-d_3 (0.8 g), mp 95–96°, $[\alpha]_D^{22}$ − 16.4°). (*c* 4.0, $CHCl_3$) (lit. [7] mp 97–98°, $[\alpha]_D^{22}$ − 16.15°)

Extraction of cocaine. Dry leaves or other parts (~0.2 g) of various *Erythroxylum* species were minced with EtOH (20 ml) containing cocaine-d_3 (2 mg). The mixture was heated at 75° for 1.5 hr and was then allowed to stand at room temp for 18 hr. The ppt. was filtered off and washed with EtOH (15 ml). The combined EtOH sols were diluted with H_2O (50 ml), acidified with HCl (0,5 ml, 1M) and washed with Et_2O (3 × 20 ml). The aq. phase was made alkaline with $NaHCO_3$ and extracted with Et_2O (2 × 10 ml) [8]. The organic layer was dried (Na_2SO_4) and then analysed. Using these conditions cocaine was extracted quantitatively.

Mass fragmentography. An LKB Model 2091 GC–MS was used. The separations were made on a 1.8 m × 2 mm i.d. silanized glass column packed with 3% SE-52 on Gas Chrom Q, maintained at a temp. of 240°. The temp. of the flash heater was 250° and the ion source was kept at 200°. The He flow rate was 25 ml/min. The ionizing potential and trap current were 70 eV and 60μA, respectively. The instrument was used to obtain conventional MS of cocaine-d_0 and cocaine-d_3, (R_t 1.5 min). The instument conditions described were also used for computer controlled mass fragmentography [9]. The mass spectrometer was set to record the intensity of *m/e* 182 and 185 on two different channels. The standard curves were prepared by adding known amounts of cocaine and cocaine-d_3 and carrying out the procedure described. Peak height of cocaine (182) versus peak height of cocaine-d_3 (185) was plotted against known amounts of cocaine. The correctness of the standard curve was checked periodically during analysis.

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REFERENCES

1. Duke, J.A., Aulik, D. and Plowman, T, (1975) *Bot. Mus. Leafl. Harvard Univ.* **24**, 113.
2. Aynilian, G.H., Duke, J.A., Gentner, W.A. and Farnsworth, N.R.(1974) *J. Pharm. Sci.* **63**, 1938.
3. Schulz,O.E.(1907) in *Pflanzenreich* (Engler, A. ed) **4**, 18.
4. Willaman, J.J. and Schubert, B.G. (1961) *Alkaloid-bearing plants and their contained alkaloids*. Technical Bulletin No. 1234 Agricultural Research Service. U.S. Dept. of Agriculture. Washington, DC.
5. Campos Neves, M.T. and Campos Neves, A. (1966) *Carcia Orta* **14**, 97.
6. Agar, J.T.H., Evans, W.C. and Treagust, P.G. (1974) *J, Pharm. Pharmac.* **26**, Suppl. 111.
7. Willstätter, R., Wolfes, O. and Mäder, H. (1923) *Ann. Chem.* **434**, 111.
8. Makisumi, S. and Ota, H. (1959) *Yonago Acta Med.* **4**, 31
9. Elkin, K., Lundberg, J.-E. and Holmstedt, B. unpublished results.