Cocaine Content of *Erythroxylum* Species

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Abstract □ Concentrations of cocaine ranging from 0.00008 to 0.00882% were found in herbarium specimens of seven of eight species of the genus *Erythroxylum* that were examined by GLC. A sample of *E. coca* which was at least 44 years old contained 0.03% cocaine.

Keyphrases □ *Erythroxylum* species—cocaine content of herbarium specimens, GLC □ Cocaine—content of herbarium specimens, GLC □ GLC—analysis, cocaine content of herbarium specimens of *Erythroxylum* species

According to Engler (1) there are about 200 known species of the genus *Erythroxylum*. Although *E. coca* is well known as the major source of cocaine, knowledge as to the distribution of this alkaloid in other *Erythroxylum* species is limited to its occurrence in *E. lucidum*, *E. coca* var. *spruceanum (E. truxillense)*, and *E. novogranatense (E. coca* var. *novogranatense)* (2, 3).

The total alkaloid content of *E. coca* leaves varies from 0.5 to 1.5%, but Javan leaves have been reported to contain as much as 1.0–2.5% total bases (4). Bolivian *E. coca* leaves reportedly contain 70–80% of their total alkaloids as cocaine, whereas Peruvian leaves contain only 50% of their total alkaloids as cocaine (4). It has been reported that the alkaloid content of coca leaves rapidly diminishes during storage and is practically lost after about 7 months (4).

This report presents results from the analyses of single leaves of nine *Erythroxylum* species, including a specimen of *E. coca* stored for at least 44 years. With the exception of *E. coca*, all samples were obtained from herbarium specimens. No data were found indicating that the herbarium specimens had been treated with fumigants, such as mercuric chloride or formalin, which might have affected the analytical results. Quantitation of data was by means of GLC.

EXPERIMENTAL

Alkaloid Extraction—Pulverized leaf samples of *Erythroxylum* species weighing from 19 to 200 mg were processed for alkaloids by maceration with 10% ammoniacal chloroform for 24 hr in separate 5-ml erlenmeyer flasks. The solutions thus obtained were filtered through cotton-plugged Pasteur pipets, and the filtrates were dried over anhydrous sodium sulfate and then with a stream of nitrogen. For GLC analyses, suitable solutions of the crude total alkaloids were prepared in acetone so that each 1.0 ml represented the extractive from 0.2 g of air-dried leaf material.

GLC—A gas chromatograph[1] equipped with a flame-ionization detector was employed for the analyses. A 1.8-m, 0.6-cm (6-ft, 0.25-in.) coiled glass column was packed with 5% OV-101 on Gas Chrom Q, 100–120 mesh[2]. The column bath temperature was maintained at 215°, the injector port was heated to 250°, and the detector temperature was set at 235°. Helium was employed as the carrier gas at a flow rate of 50 ml/min. The internal standard was tetraphenylethylene, and samples of 1–2 μl were injected.

For the quantitative analyses of the cocaine in the extracts, a modification of the method employed by Chu and Mika (5) for the analysis of tropane alkaloids was used. Accordingly, separate acetone solutions containing known quantities of cocaine were prepared and the internal standard solution was added. The solutions were chromatographed and the integrated peak areas of cocaine and tetraphenylethylene were obtained. A standard curve for cocaine was established by plotting the peak areas of cocaine *versus* the weight (Fig. 1). The reproducibility of the standard curve was determined by the analysis of freshly prepared solutions of cocaine on alternate days. The quantity of cocaine in the leaf sample extracts was determined by computing the cocaine peak area from the chromatogram and obtaining the corresponding weight of cocaine from the standard curve. The resulting value was then converted to cocaine concentration as a percentage of air-dried powered leaf sample (Table I).

RESULTS AND DISCUSSION

The relative retention time (rR_t) for reference cocaine was 0.46, with the retention time for internal standard being 7.32 min. Under the experimental conditions described, the minimal quantity of cocaine detectable was 0.077 μg.

Table I presents the results of the quantitative analyses of the *Erythroxylum* species examined. The data represent the average of two separate analyses. Additional analyses could not be performed due to the restricted sample sizes.

Trace quantities of cocaine, ranging from 0.00008 to 0.00882%, were found in the leaves of *E. campestre*, *E. deciduum*, *E. novogranatense*, *E. panamense*, *E. pelleterianum*, and *E. pulchrum*. The plant samples had been collected during 1930–1968. Cocaine could not be detected in *E. citrifolium* (1966) or *E. rufum* (1929 and 1950).

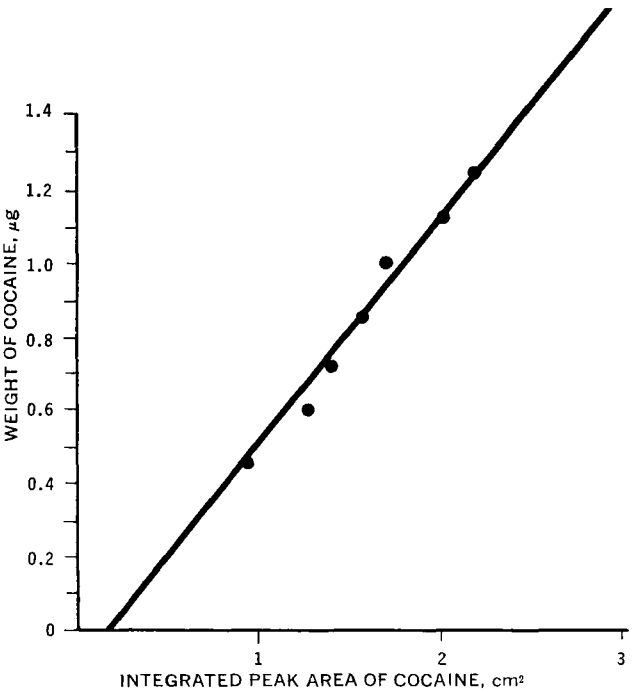

Figure 1—*Standard curve for cocaine.*

[1] Perkin-Elmer model 881.
[2] Applied Science Laboratories, State College, Pa.

Table I—Concentration of Cocaine in Nine Species of *Erythroxylum* as Determined by GLC

Species	Collector	Specimen Number	Locality	Herbarium[a]	Collection Date	Cocaine[b], %
E. campestre A. St. Hil.	P. Dusen	16421	Brazil	MO	?	0.00014
E. citrifolium A. St. Hil.	W. H. Lewis *et al.*	254	Panama	MO	1966	n.d.
E. deciduum A. St. Hil.	L. O. Williams	7424	Brazil	MO	1945	n.d.
E. deciduum A. St. Hil.	L. B. Smith & Klein	7452	Brazil	NY	1956	0.00080
E. novogranatense (Morris) Hieron	R. E. Schultes	—	Cuba	NY	1955	0.00882
E. novogranatense (Morris) Hieron	Br. Daniel	15584	Colombia	OA	1961	0.00216
E. panamense Turcz.	T. B. Croat	5519	Canal Zone	MO	1968	0.00121
E. panamense Turcz.	T. B. Croat	4879	Canal Zone	MO	1968	0.00141
E. pelleterianum A. St. Hil.	Y. Mexia	4364	Brazil	MO	1930	0.00123
E. pulchrum A. St. Hil.	Riedel	390	Brazil	MO	?	0.00008
E. rufum Cav.	E. L. Ekman	11609	Santo Domingo	AA	1929	n.d.
E. rufum Cav.	R. A. Howard	12247	Dominican Republic	AA	1950	n.d.
E. coca Lam.[c]	J. A. Duke	—	—	—	1974	0.53
E. coca Lam.	—	—	—	—	<1930	0.03

[a] MO = Missouri Botanical Gardens, NY = New York Botanical Gardens, OA = Oaks Ames Economic Herbarium, Harvard University; and AA = Arnold Arboretum Herbarium, Harvard University. [b] Single leaves were analyzed, with data being expressed as the mean percent of two determinations; n.d. = cocaine not detected. [c] Leaf material from a 2-year-old living plant was collected and analyzed within 48 hr.

A sample of *E. coca* leaf analyzed within 2 days from the time of removal from the living plant contained 0.53% of cocaine, whereas a dried and stored sample at least 44 years old still contained 0.03% cocaine.

Studies are currently in progress to determine the decomposition rate of cocaine in stored *E. coca* leaves, as well as the natural variability of this alkaloid in various populations of *E. coca*.

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Synthesis and Anti-Inflammatory Evaluation of 2-(Substituted Amino)quinolizinium Bromides

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Abstract □ The synthesis and anti-inflammatory evaluation of a number of 2-(substituted amino)quinolizinium bromides, including some previously unreported analogs, are described. The more active compounds in inhibiting carrageenin-induced edema formation are those containing a *p*-alkoxyanilino group in the 2-position of the quinolizinium ring.

Keyphrases □ Quinolizinium bromides, 2-(substituted amino)—synthesis and anti-inflammatory evaluation □ Aminoquinolizinium bromides (2-substituted)—synthesis and anti-inflammatory evaluation □ Anti-inflammatory activity—evaluation of 38 2-(substituted amino)quinolizinium bromides

Previously, the synthesis and antiparasitic screening of a series of 2-(substituted amino)quinolizinium bromides were reported (1). Many of these compounds (III–XL) exhibited a significant degree of anthelmintic activity. The primary anti-inflammatory evaluation of this series of compounds, including some previously unpublished analogs, is reported herein. Compounds III–XL, when examined in the carrageenin-induced rat paw edema assay, inhibited edema formation as much as 76%.

The previously reported (1) synthesis involves the reaction of an appropriately substituted amine with either of the two intermediates, 2-bromoquinolizinium bromide (I) or 2-bromo-6-methylquinolizinium