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Cocaine distribution in wild *Erythroxylum* species

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Abstract

Cocaine distribution was studied in leaves of wild *Erythroxylum* species originating from Bolivia, Brazil, Ecuador, Paraguay, Peru, Mexico, USA, Venezuela and Mauritius. Among 51 species, 28 had never been phytochemically investigated before. Cocaine was efficiently and rapidly extracted with methanol, using focused microwaves at atmospheric pressure, and analysed without any further purification by capillary gas chromatography coupled to mass spectrometry. Cocaine was reported for the first time in 14 species. *Erythroxylum laetevirens* was the wild species with the highest cocaine content. Its qualitative chromatographic profile also revealed other characteristic tropane alkaloids. Finally, its cocaine content was compared to those of two cultivated coca plants as well as with a coca tea bag sample.

Keywords: Erythroxylaceae; *Erythroxylum*; *Erythroxylum laetevirens*; Cocaine; Tropane alkaloids; Gas chromatography; Mass spectrometry; Focused microwave-assisted extraction

1. Introduction

The last 30 years have seen an increasing interest in cocaine analysis resulting from its expanding illicit use in Western Europe and North America. Regardless of the importance of cultivated coca plants from an economical point of view, these species always played a key role for South American natives (Grinspoon and Bakalar, 1981; Naranjo, 1981; Schultes, 1981; Plowman, 1984a). Coca chewing in South America has persisted from ancient times, but is still poorly understood from many points of view. This traditional habit is largely considered noxious by many regulatory authorities.

The family Erythroxylaceae is composed of four genera: *Aneulophus*, *Erythroxylum*, *Nectaropetalum* and *Pinacopodium* (Hegnauer, 1981). The genus *Erythroxylum*, by far the most well-known genus of the family comprises roughly 230 species of tropical trees and shrubs, which are widely distributed in South America, Africa and Madagascar (Plowman and Hensold, 2004). In 1907, Schulz divided this genus into 19 sections, providing a useful scheme for comparative phytochemical considerations. *Erythroxylum* and more particularly *Erythroxylum coca* and *Erythroxylum novogranatense*, as well as their varieties, is the only natural source of cocaine (Plowman, 1984b).

Even if some attention has been focused on non-cultivated *Erythroxylum* species for the possible presence of cocaine, systematic investigation of the genus is still incomplete and several species used in traditional medicine remain unknown (Evans, 1981). Aynilian et al. (1974) reported the concentration of cocaine in herbarium specimens of seven *Erythroxylum* species. Holmstedt et al. (1977) analysed 62 samples of 13 tropical South American species by capillary gas chromatography coupled to mass spectrometry (GC-MS). Cocaine was found only in the leaves of two species, *Erythroxylum coca* and *Erythroxylum novogranatense*, but no measurable amount of cocaine was detected in any of the other 11 species. Subsequently, Plowman and Rivier (1983), using more sensitive assays, detected trace amounts of cocaine in 13 neotropical wild *Erythroxylum* species representing five sections of the genus. Besides, they found that two species from Venezuela, namely *Erythroxylum recurrens* and *Erythroxylum steyermarkii*, contained cocaine amounts comparable to those found in cultivated species.

This study is part of a large investigation of the genus *Erythroxylum* for tropane and related alkaloids (Brachet et al., 1997, 2002; Christen et al., 1993, 1995; Brock et al., 2005). We report

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here on the specific investigation of cocaine in 51 wild species. Due to the presence of an appreciable amount of cocaine, *Erythroxylum laetevirens* was qualitatively investigated for the presence of other tropane alkaloids and its chromatographic profile was compared to those of two cultivated species, together with a coca tea bag sample.

2. Materials and methods

2.1. Plant material and chemicals

Most species were collected in South America between 1979 and 1984 by the late T. Plowman and kindly provided by Dr. Laurent Rivier (Lausanne, Switzerland). Collection details are given in Table 1. Two species originating from sites other than South America were also included in this study, namely *Erythroxylum areolatum* from the Bahamas (USA) and *Erythroxylum macrocarpum* from Mauritius. A voucher specimen of all plants is deposited at our Institute.

For each species, only the leaves were analysed. Dry plant material was ground to a fine homogenous powder by a ball-mill (MM 200 RETSCH, Switzerland) and finally sieved to an average particle size of less than 125 μm.

Cocaine hydrochloride (COC) and Methadone hydrochloride (MET) were obtained from Siegfried Handel (Zofingen, Switzerland) and Hänsler (Herisau, Switzerland), respectively.

2.2. Extraction procedure

Extractions were performed using focused microwaves at atmospheric pressure at a frequency of 2450 MHz using a Soxwave 3.6 apparatus (Prolabo, France) with a programmable heating power. Typically, 100 mg of powdered plant material was placed into a 20 mL quartz extraction vessel and hydrated with 10 μL of water prior to the addition of 5 mL methanol. The extraction was carried out at 125 W for 30 s. Each extract solution was filtered on a 0.45 μm PTFE filter (Brachet et al., 2002). Solutions obtained from wild species were evaporated to dryness and taken up in 1 mL methanol containing 10 ppm internal standard (methadone), while solutions from cultivated species were diluted four times with methanol. All samples were analysed by GC-MS without any further purification.

2.3. Gas chromatography

GC-MS analyses were carried out using a Hewlett-Packard 5890 series II chromatograph coupled to a HP 5972 mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The mass detector operated in the electron impact ionisation mode at 70 eV. Injections were performed in the splitless mode at 250 °C with a splitless period of 60 s and with purge and septum purge flow rates of 30 and 3 mL/min, respectively. Injections of 1 μL were carried out with a HP 6890 series fast automatic liquid sampler (Agilent Technologies). A laminar liner (Restek, Bellefonte, PA, USA) was used as well as a standard syringe with a 42 mm long needle and a cone tip. Helium was used as carrier gas and operated in the constant flow mode (1 mL/min). For qualitative analysis, a HP5-MS column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness was used with an initial oven temperature of 70 °C (1 min hold) and a linear temperature program from 70 to 285 °C at 5 °C/min and hold at the final temperature for 15 min. Spectra were recorded in the mass range 30–500 Th with 1.3 scan/s and the MS transfer line was set at 280 °C. For quantitative cocaine analysis, the oven was initially set at 70 °C (1 min hold) and linearly increased to 285 °C (5 min hold) at 30 °C/min. GC-MS (SIM mode) was performed using the selective ion 303 Th (molecular ion of cocaine), the qualifier ion 272 Th and the target ion 182 Th (base peak of cocaine). Methadone (MET) was used as internal standard with target ion 294 Th (molecular ion) and qualifier ion 72 Th (base peak of methadone). In order to enhance sensitivity, the potential of the electron multiplier was increased by a 400 V increment for a period of time of 2 min which included elution of the internal standard and cocaine.

2.4. Quantification

Standard calibration curve was obtained with cocaine solutions at seven concentration levels between 0.1 and 100 ppm (0.1, 0.5, 1, 5, 25, 50 and 100 ppm) containing a fixed concentration (10 ppm) of methadone. Quantitative determination was based on the peak area ratio of the target ions of cocaine over methadone. A correlation coefficient of 0.9992 was obtained. The relative standard deviations (R.S.D.) for six consecutive injections with a cocaine standard solution at 5 ppm was inferior to 5%, and inferior to 10% at 0.1 ppm, corresponding to the limit of quantification (LOQ). For any concentration level, the three cocaine ions were detected at the corresponding elution time (between 9.55 and 9.57 min).

Cocaine was considered to be present in a species, but not quantified (NQ) when ions 182 and 303 Th were detected with a signal to noise ratio of at least 2. When the target ion 182 Th was not detected, the symbol ND was used, meaning that no cocaine was present.

3. Results and discussion

Before any discussion of the results, it is important to emphasize that the time elapsed between plant harvesting and analysis is between 20 and 25 years. Since it is believed that a cocaine leaf content may vary with time, the quantitative results reported should be viewed from that perspective despite the fact that the preservation of cocaine in *Erythroxylum coca* leaves has been shown in 44 year-old herbarium samples (Aynilian et al., 1974).

A straightforward sample preparation method involving focused microwave-assisted extraction (FMAE) was used as already described by Brachet et al. (2002). This procedure was particularly well suited for mass limited samples, as it required no more than 100 mg of fine powdered plant material. Indeed, sample amounts of the various examined species at our disposal varied between a few hundred milligrams and a hundred and fifty grams. Furthermore, this method was extremely rapid (30 s), required low amount of organic solvent (5 mL) and thus allowed the extraction of numerous samples in a short period of time. In addition, it is environmentally friendly and does not necessi-