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Content and Distribution of *Erythroxylum coca* Leaf Alkaloids

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Fully expanded leaves of *Erythroxylum coca* var. *coca* Lam. (Erythroxylaceae) 35–70-d-old were harvested from 21-month-old plants grown under greenhouse conditions. Each harvested leaf was placed on a plexiglass silhouette according to its dimensions and divided into four primary sections (petiole, base, mid and anterior) and three sub-sections (lamina periphery, false mid-rib, and true mid-rib) to determine the distribution and content of hygrine, cuscohygrine, *trans*-cinnamoylcocaine, *cis*-cinnamoylcocaine, tropacocaine, tropinone, methyl ecgonine and cocaine. The leaf sub-sections and petiole were extracted and analysed for alkaloid content (%) by HPLC (cocaine alkaloid only) gas chromatography and GC/MS. Cocaine, methyl ecgonine and hygrine were highest in the lamina periphery with a content of 0·48, 0·46 and 0·32%, respectively. *Trans*-cinnamoylcocaine was pre-eminent of the cinnamoylcocaines and was most abundant in the petiole at a content of 0·24%. *Cis*-cinnamoylcocaine, tropinone, cuscohygrine and tropacocaine were ubiquitously distributed throughout the leaf where the average contents were 0·14, 0·004, 0·16 and 0·04%, respectively. © 1995 Annals of Botany Company

Key words: Alkaloids, *Erythroxylum coca* var. *coca*, *E. coca*, leaf alkaloids, petiole alkaloids, cocaine, cuscohygrine, methyl ecgonine, hygrine, tropinone, tropacocaine, *trans*-cinnamoylcocaine, *cis*-cinnamoylcocaine.

INTRODUCTION

A previous paper reported the variation of alkaloid content in leaves of *Erythroxylum coca* var. *coca* Lam. (Erythroxylaceae) chronologically similar in age from the appearance of the leaf until leaf drop (Johnson and Emche, 1994). It was observed that many of the alkaloids decreased in content (% dry weight of leaf) 14 d after bud break. Tropacocaine and tropinone contents remained low in comparison to cocaine, methyl ecgonine, cuscohygrine, hygrine and the cinnamoylcocaines, even though these alkaloids varied during leaf duration (Johnson and Emche, 1994). Youssefi, Cooks and McLaughlin (1979) showed the pattern of distribution for cocaine and cinnamoylcocaine in different plant parts of *E. coca* grown in two geographical regions of South America. They concluded that: (*a*) leaves of *E. coca* contained the highest concentration of cocaine, (*b*) individual leaves showed wide variations in cocaine/cinnamoylcocaine ratios, (*c*) leaf margins contained lower amounts of cinnamoylcocaine relative to cocaine, than did the leaf centre, and (*d*) the leaf stem (petiole and veins) contained relatively more cinnamoylcocaine than the leaf margins.

In preliminary studies, the focus of interest was on the distribution of alkaloids in different parts of individual leaves of *E. coca* that were 35–70 d old. These results showed that alkaloid content was not uniform throughout the leaf. Therefore, based on these initial studies a more detailed experiment was undertaken. This report describes the content and distribution of hygrine, tropinone, methyl ecgonine, cuscohygrine, tropacocaine, *cis* and *trans*-cinnamoylcocaine and cocaine (benzoylmethylecgonine) in mature leaves of Bolivian coca (Huanuco coca; Plowman, 1979) *Erythroxylum coca* var. *coca* Lam.

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MATERIALS AND METHODS

Growth, harvesting and sectioning

Erythroxylum coca var. *coca* Lam (Erythroxylaceae) was germinated and grown from seed as described by Johnson and Emche (1994) under greenhouse conditions at the Beltsville Agricultural Research Center, Beltsville, Maryland, USA. After 21 months of growth, leaves 35–70 d old were harvested from the canopy of the *E. coca* plants and immediately chilled (0 °C). Each leaf (Fig. 1A) was placed on a prepared plexiglass silhouette according to its dimensions and divided into four primary sections: (0) the petiole; (1) the base or proximal portion of the leaf; (2) the mid-section and; (3) the anterior or distal section of the leaf (Fig. 1B). The base, mid and anterior sections were divided so that each section comprised 33% of the expanded leaf's area (Fig. 1B). These three leaf sections were further divided into three sub-sections: 1A, 2A, and 3A, the lamina periphery (a 2 mm strip around the border of the leaf); 1B, 2B, and 3B, the area between the lamina periphery and false mid-rib; and 1C, 2C and 3C, the area between the false and true mid-rib (Fig. 1B). For an anatomical description of the *E. coca* leaf, readers should consult the citations of Blatter (1899) and Schulz (1907).

Preliminary experiments showed that 4 g fresh weight (g f. wt ± 0·30 g f. wt) afforded 1·10 g dry weight (g d.

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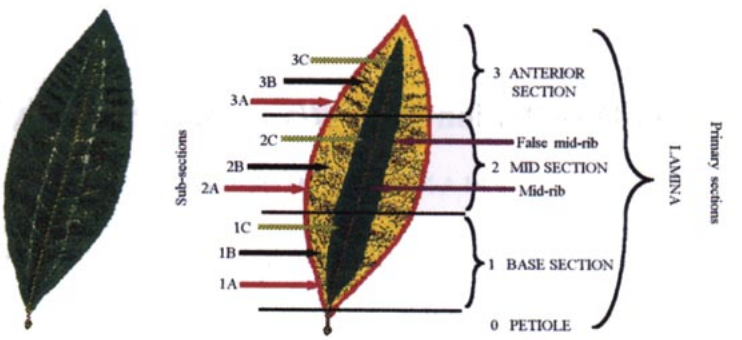
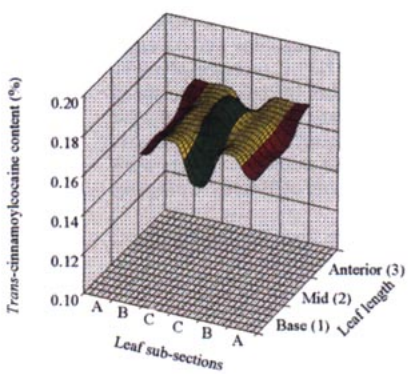
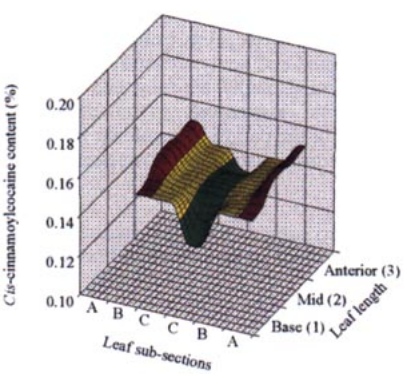

3C
3B
3A
2C
2B
2A
1C
1B
1A
Sub-sections
3 ANTERIOR SECTION
False mid-rib
2 MID SECTION
Mid-rib
1 BASE SECTION
0 PETIOLE
LAMINA
Primary sections

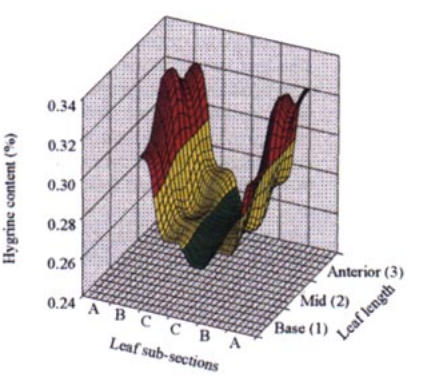
A B


Trans-cinnamoylcocaine content (%)
0.20
0.18
0.16
0.14
0.12
0.10
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

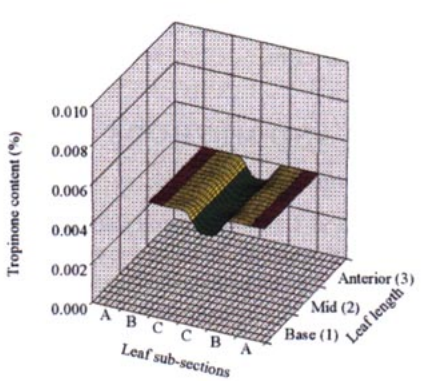
C


Cis-cinnamoylcocaine content (%)
0.20
0.18
0.16
0.14
0.12
0.10
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

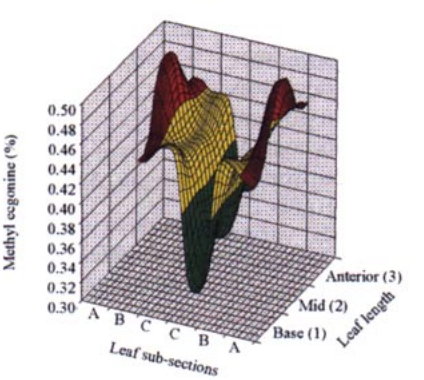
D


Hygrine content (%)
0.34
0.32
0.30
0.28
0.26
0.24
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

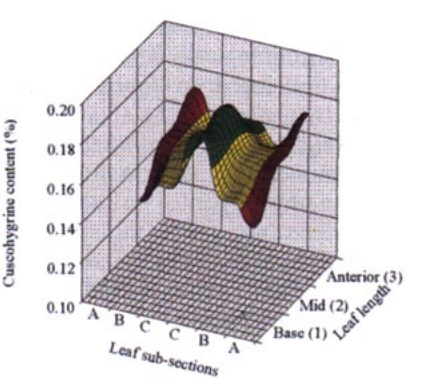
E


Tropinone content (%)
0.010
0.008
0.006
0.004
0.002
0.000
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

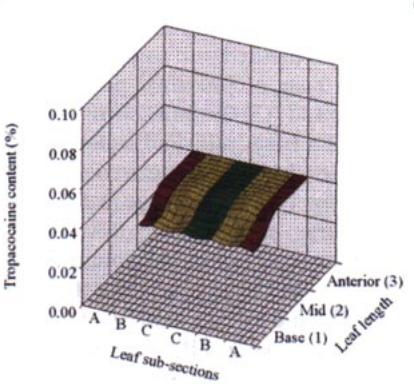
F


Methyl ecgonine (%)
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

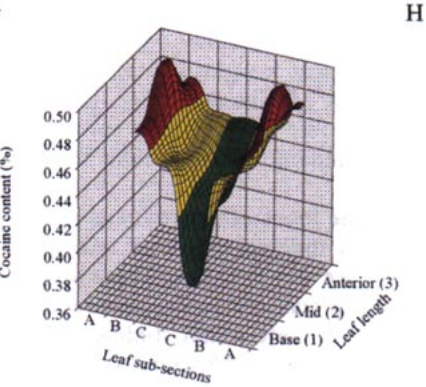
G


Cuscohygrine content (%)
0.20
0.18
0.16
0.14
0.12
0.10
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

H


Truxillocaine content (%)
0.10
0.08
0.06
0.04
0.02
0.00
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

I


Cocaine content (%)
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
A B C C B A
Leaf sub-sections
Anterior (3)
Mid (2)
Base (1)
Leaf length

J

wt ± 0·040 g d. wt) of lamina sub-sectional tissue and petiole. In addition, approx. 1·0 g d. wt samples of lamina sub-sections and petiole were more efficient in resolving the content of cuscohygrine, *trans* and *cis*-cinnamoylcocaine, tropinone and tropacocaine. Using a scalpel, the lamina sub-sections were excised and separately placed into labelled chilled glass beakers until 4 g f. wt (±0·03 g f. wt) of replicated samples (three samples per replication) including the petiole (i.e. 4 g f. wt per three replications) were collected and subsequently placed into a circulating air oven, and dried at 70 °C for 2 h. It was previously determined that oven drying at 70 °C would remove approx. 72% (±3%) of leaf f. wt (Johnson, unpubl. res.). After oven drying, the leaf sub-sections and petiole afforded 1·10 g d. wt (±0·040 g d. wt). Oven dried leaves and petioles were stored over Drierite at −20 °C until analysis. The experiment was repeated three times so that the standard error of the mean for each alkaloid could be determined. Consequently, to allow for maximal visualization, analytical data for each alkaloid has been represented by 3-D surface plots (Fig. 1C–J).

Alkaloid extraction, identification and quantification

For alkaloid extraction and determination, a 1·10 g d. wt (±0·040 g d. wt) sample of each replicated leaf sub-section and petiole was extracted and analysed for alkaloids by gas chromatography and HPLC (cocaine alkaloid only) as described by Glass and Johnson (1993). Briefly, gas chromatography was performed on a Hewlett-Packard (H-P) 5990A GC including an H-P 7673A auto sample injector and ChemStation (Hewlett-Packard, Avondale, PA, USA). GC conditions: flame ionization detector: carrier gas, He; flow rate, 75 ml^{-1}; column used to separate alkaloid extracts and analytical standards, dimethylsilicone capillary (DB-5, 15 m × 0·25 mm i.d., 0·25 μm film thickness; J.W. Scientific Products, Rancho, Cordova, CA, USA); injection temperature, 275 °C in increments of 25 °C min^{-1} with a 2·5 min hold; run time, 12 min; injection volume, 1 μl. The HPLC chromatography for cocaine was performed by a modified method of LeBelle *et al.* (1988) using a Spectra-Physic Model 8800 ternary pump (San Jose, CA, USA) equipped with a Rheodyne Model 7125 valve fitted with a 5 μl loop. Cocaine was detected with a variable wavelength detector (Spectra-Physic, Model 8440) set at 240 nm. The conditions for HPLC determination of cocaine were: Column, 12·5 cm × 4·6 mm (i.d.) 5 μm particles, C-8 Reverse Phase (R. E. Gourley, Laurel, MD, USA); mobile phase, 1·0% triethylamine, pH 4 (40:60, v/v) isocratically at 1·2 ml min^{-1}. There were no significant differences between cocaine contents of the cocaine standard and sample extracts when quantified by GC or HPLC (Glass and Johnson, 1993). Alkaloid standards and extracts were confirmed by GC/MS as described by Johnson and Elsohly (1991). The alkaloids extracted, identified and quantified from the leaf's primary sub-sections and petiole were: hygrine, cuscohygrine, *trans*-cinnamoylcocaine, *cis*-cinnamoylcocaine, tropacocaine, tropinone, methyl ecgonine and cocaine (benzoylmethylecgonine). Alkaloid standards were obtained from the following sources: hygrine and cuscohygrine, from Dr Marcy Newquist, Natural Products Laboratory, University of Minnesota, St Paul, MN, USA; *trans* and *cis*-cinnamoylcocaines, from DEA Special Testing Laboratory, McLean, VA, USA; tropacocaine, tropinone, methyl ecgonine and cocaine from Sigma Chemical Co., St Louis, MO, USA.

RESULTS AND DISCUSSION

In the petiole of 35–70-d-old *E. coca* leaves (Section 0; Figs 1B and 2) *trans*-cinnamoylcocaine was the most abundant alkaloid (0·24%) and was pre-eminent of the cinnamoylcocaines present. The content (%) of alkaloids in the leaf petiole according to their abundance was: *trans*-cinnamoylcocaine < cuscohygrine < hygrine < methyl ecgonine < cocaine < *cis*-cinnamoylcocaine < tropacocaine < tropinone (Fig. 2). The relatively high content of cinnamoylcocaines in the leaf petiole concords with the findings of Youssefi *et al.* (1979) where higher concentrations of cinnamoylcocaines were reported in the leaf stem, which was considered to be the leaf's petiole. It should be noted that the predominance of *trans*-cinnamoylcocaine in the leaf petiole has not been previously reported (Fig. 2). It has been demonstrated that as the *E. coca* leaves aged, *trans*-

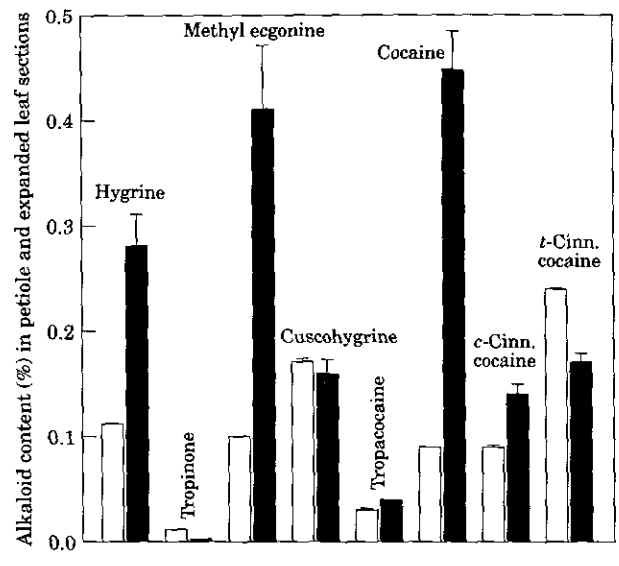


FIG. 2. Alkaloid content (%) in *E. coca* leaf petioles (□) compared to the amount in the expanded leaf section (■). Horizontal bars on the graph represents the mean average of replicated petiole and expanded sectional leaf samples ± s.e.m. Those points without error bars had s.e. smaller than the dimension of the symbol.

FIG. 1. A, A mature *E. coca* leaf: B, primary and sub-sectional divisions of a mature *E. coca* leaf (lamina); sub-sections 1A, 2A, 3A, the lamina periphery; 1B, 2B, 3B, the false mid-rib; and 1C, 2C, 3C, the mid-rib. Sub-sections of the lamina from each side of the mid-rib were used to determine the alkaloid content and distribution after oven drying: C–J, sub-sectional distribution and content (% dry weight) of: *trans*- (C) and *cis*-cinnamoylcocaine (D), hygrine (E), tropinone (F), methyl ecgonine (G), cuscohygrine (H), tropacocaine (I) and cocaine (J), respectively, in mature leaves of *E. coca*.

cinnamoylcocaine increased in the leaf, and by week 36 of leaf duration (at leaf drop) *trans*-cinnamoylcocaine was the predominant leaf alkaloid (Johnson and Emche, 1994). The cinnamoylcocaines (*trans* and *cis*) were equally distributed throughout the respective expanded sections of the *E. coca* leaf (Fig. 1C, D). However, the content of *trans* and *cis*-cinnamoylcocaine in the leaf petiole was 1·7 and 1·5 fold (respectively) higher than in the expanded leaf sections (Figs 2 and 1C, D).

In the expanded sections of the *E. coca* leaf (base, mid and anterior) hygrine content was highest in the lamina periphery (0·32%) and least in sub-sections between the false and true mid-ribs, and petiole, where the content was 0·25 and 0·11%, respectively (Fig. 1E). Hygrine was the third most abundant alkaloid within the *E. coca* leaf (Fig. 2).

The content of tropinone was 0·004% and was almost equally distributed throughout the expanded sections of the leaf (Fig. 1F). The highest amount of tropinone was in the leaf petiole of *E. coca* (0·011%). It was least in content among the alkaloids observed in *E. coca* leaves in this study (Fig. 2) and may suggest rapid interconversion as proposed by Leete *et al.* (1991).

Methyl ecgonine was one of the most abundant alkaloids in the expanded leaf sections of *E. coca*. Its content was highest in the lamina periphery and false mid-rib, where the content was 0·46 and 0·43%, respectively (Fig. 1G). Methyl ecgonine was least in the sub-section between the false and true mid-ribs (Fig. 1G) and was similar to hygrine and cocaine in content in the petiole (Fig. 2). A high content of methyl ecgonine was also observed in *E. coca* leaves from week 1 through week 16 of duration (Johnson and Emche, 1994). Further, Leete *et al.* (1991) demonstrated that more [9-^{14}C]-2-carbomethoxy-3-tropinone was incorporated into methyl ecgonine than cocaine and that younger leaves of *E. coca* had a higher percentage of the label than older leaves. There are no reports that suggest that methyl ecgonine is enzymatically converted directly into cocaine or other leaf alkaloids of *E. coca*. However, Novák, Salemink and Kahn (1984) indicated that all alkaloids including methyl ecgonine, which are capable of yielding ecgonine after hydrolysis, may be converted to cocaine.

Cuscohygrine and tropacocaine contents were similar in their respective leaf sub-sections [0·16 (±0·01%) and 0·04%, respectively] and were ubiquitously distributed throughout the expanded leaf sections and petiole (Fig. 1H, I). The biosynthesis of cuscohygrine in *E. coca* leaves was described by Leete (1983) and Leete *et al.* (1991).

Cocaine, the principal alkaloid of *E. coca*, was highest in the lamina periphery (0·48%) and the sub-section between the lamina periphery and false mid-rib (0·45%) and least in the sub-sections between the false and the true mid-ribs which may be considered as the centre of the leaf (0·41%; Fig. 1J). It may be noted that the contents of methyl ecgonine and cocaine were similar in their respective expanded sections of the leaf but differed in the petiole, where their content was four-fold lower (Figs 2 and 1G, J).

It was noteworthy that the patterns of distribution for cocaine, hygrine, and methyl ecgonine in *E. coca* leaves were similar to the pattern of nicotine distribution in *Nicotiana tabacum* L., (Solanaceae), where the lowest content of nicotine was present in the mid-vein tissue (leaf centre) and was highest in the outer part of the lamina (Burton, Bush and Hamilton, 1983; Djordjevic *et al.*, 1989). Cocaine, hygrine, methyl ecgonine and nicotine are reported to share a common precursor, L-ornithine (Geissman and Crout, 1969; Leete and Yu, 1980; Evans, 1981; Leete, 1983; Leete *et al.*, 1991). Further, Evans (1981) concluded that the nitrogen containing moieties of *Erythroxylum* alkaloids arise by a biosynthetic route similar to that demonstrated for *Datura* (Solanaceae). However, there are other members of Solanaceae (*Hyoscyamus*, *Atropa* and *Datura*) whose route to producing the nitrogenous compounds is *via* L-arginine rather than L-ornithine (Robins and Walton, 1993). In the tested species of the above genera, L-arginine has been shown to feed directly into the biosynthetic pathway without being converted to L-ornithine (Hashimoto, Ykimune and Yamada, 1989; Walton, Robins and Peerless, 1990; Robins, Parr and Walton, 1991).

Dividing the *E. coca* leaf into sections has established an area within the leaf where the distribution patterns for leaf alkaloids can be examined. Cocaine, methyl ecgonine, and hygrine were highest in content in the lamina periphery, and least in the mid-section of the leaf, whereas *trans*-cinnamoylcocaine was most abundant in the leaf petiole, while *cis*-cinnamoylcocaine, cuscohygrine, tropinone and tropacocaine were ubiquitously distributed throughout each leaf section. The values reported for cocaine content in sub-sections of the leaf lamina concords with those reported by Holmstedt *et al.* (1977) and Plowman and Rivier (1983). The leaf petiole section was analysed as an independent unit for alkaloid content, whereas, in previous studies, values reported may have included the petiole with the lamina resulting in higher alkaloid content for the cinnamoylcocaines. The differences in alkaloid content were established by separating the petiole from the lamina. Therefore, values for the content of the cinnamoylcocaines may be less than those previously reported. These data could indicate differential regulation of alkaloid metabolism of cells located in different parts of the *E. coca* leaf. Therefore, future research should define the regulatory mechanism for alkaloid accumulation in leaves of *E. coca*.

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LITERATURE CITED

Blatter G. 1899. Cocae folia. *Pharmaceutical Journal* **62**: 484–485.

Burton HR, Bush LP, Hamilton JL. 1983. Effect of curing on the chemical composition of burley tobacco. *Recent Advances in Tobacco Science* **9**: 91–153.

Djordjevic MV, Gay SL, Bush LP, Chaplin JF. 1989. Tobacco-specific nitrosamine accumulation and distribution in flu-cured tobacco isoline. *Journal of Agricultural and Food Chemistry* **37**: 752–756.

Evans WC. 1981. The comparative phytochemistry of the genus *Erythroxylon*. *Journal of Ethnopharmacology* **3**: 265–277.

Geissman TA, Crout DHG. 1969. *Organic chemistry of secondary plant metabolism.* San Francisco: Freeman, Cooper and Company.

Glass RL, Johnson EL. 1993. Comparison of high performance liquid chromatographic and gas chromatographic analyses of cocaine in coca leaves. *Journal of Liquid Chromatography* **16**: 3543–3555.

Hashimoto T, Ykimune Y, Yamada Y. 1989. Putrescine and putrescine N-methyltransferase in the biosynthesis of tropane alkaloids in cultured roots of *Hyoscyamus albus*. I. Biochemical studies. *Planta* **178**: 123–130.

Holmstedt B, Jaatmaa E, Leander K, Plowman T. 1977. Determination of cocaine in some South American species of *Erythroxylum* using mass fragmentography. *Phytochemistry* **16**: 1753–1755.

Johnson EL, Elsohly MA. 1991. Content and *de novo* synthesis of cocaine in embryos and endosperms from fruit of *Erythroxylum coca* Lam. *Annals of Botany* **68**: 451–453.

Johnson EL, Emche SD. 1994. Variation of alkaloid content in *Erythroxylum coca* leaves from leaf bud to leaf drop. *Annals of Botany* **73**: 645–650.

LeBelle MJ, Callahan SA, Latham DJ, Laurialt G. 1988. Identification and determination of norcocaine in illicit cocaine and coca leaves by gas chromatography–mass spectrometry and high-performance liquid chromatography. *Analyst* **113**: 1213–1215.

Leete E. 1983. Biosynthesis of cocaine and cuscohygrine from [1-^{14}C]acetate and [4-^{3}H]phenylalanine in *Erythroxylon coca*. *Phytochemistry* **22**: 699–704.

Leete E, Bjorklund J, Couladis MM, Kim SH. 1991. Late intermediates in the biosynthesis of cocaine: 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate and methyl ecgonine. *Journal of the American Chemical Society* **113**: 9286–9292.

Leete E, Yu ML. 1980. The incorporation of ornithine-[2,3-$^{13}C_2$] into nicotine and nornicotine established by NMR. *Phytochemistry* **19**: 1093–1097.

Novák M, Salemink CA, Kahn I. 1984. Biological activity of the alkaloids of *Erythroxylum coca* and *Erythroxylum novogranatense*. *Journal of Ethnopharmacology* **10**: 261–274.

Plowman T. 1979. The identity of Amazonian and Trujillo coca. *Botanical Museum Leaflets* **27**: 45–68.

Plowman T, Rivier L. 1983. Cocaine and cinnamoylcocaine content of *Erythroxylum* species. *Annals of Botany* **51**: 641–659.

Robins RJ, Walton NJ. 1993. The biosynthesis of the tropane alkaloids. In: Cordell GA, ed. *The alkaloids*, Vol. 44. New York: Academic Press, 115–187.

Robins RJ, Parr AJ, Walton NJ. 1991. Studies on the biosynthesis of tropan alkaloids in *Datura stramonium* L. transformed root cultures. 2. On the relative contributions of L-arginine and L-ornithine to the formation of the tropane ring. *Planta* **183**: 196–201.

Schulz OE. 1907. *Das Pflanzenreich IV. 134 Erythroxylaceae*. Engler A, ed. Leipzig, 1–176.

Walton NJ, Robins RJ, Peerless ACJ. 1990. Enzymes of N-methylputrescine biosynthesis in relation to hyoscyamine formation in transformed root cultures of *Datura stramonium* and *Atropa belladonna*. *Planta* **182**: 136–141.

Youssefi M, Cooks RG, McLaughlin JL. 1979. Mapping of cocaine and cinnamoylcocaine in whole coca plant tissues by MIKES. *Journal of the American Chemical Society* **101**: 3400–3402.