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ALKALOID CONTENT IN *ERYTHROXYLUM COCA* TISSUE DURING REPRODUCTIVE DEVELOPMENT

EMANUEL L. JOHNSON

United States Department of Agricultural ARS, PSI, WSL Bldg 050 Rm 132 BARC-W, Beltsville, MD 20705-2350, U.S.A.

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Key Word Index—*Erythroxylum coca*; Erythroxylaceae; alkaloid content; hygrine; tropinone; methyl ecgonine; cuscohygrine; tropacocaine; cocaine; *trans*-cinnamoylcocaine; *cis*-cinnamoylcocaine; reproductive tissue.

Abstract—The content (% dry wt) of eight alkaloids in branch segments with and without flower buds, terminal leaves, and divided flower parts of *Erythroxylum coca* var. *coca*, varied greatly in the different tissues. Cocaine, the principal alkaloid in *E. coca*, was the most abundant alkaloid in all plant parts analysed. Cuscohygrine was least among the alkaloids, and observed only in leaf tissue. Cocaine content in unopened flower buds, pedicels, perianths and stamens was three times that in immature flower buds; and in pistils of opened flowers, two times that in the immature flower buds. Of all the tissue tested during reproductive development, young leaves at the terminal end of branches contained the most alkaloids.

INTRODUCTION

In cultivated fields of *E. coca*, (*Erythroxylum coca* var. *coca* Lam.) leaf harvesting occurs three to six times annually [1]. Leaf harvesting transforms the *E. coca* plant from a vegetative to a reproductive phase of development. This is a unique phenomenon, because: (i) the events that initiate and preface reproductive development in most woody plants are induced by cultural practices [2–6], (ii) regardless of the number of times leaves are harvested per annum, the *E. coca* plant enters the reproductive phase of development after each harvest, yet remains viable for many years [1], and (iii) there is no woody plant under cultivation whose leaves are harvested at such frequency per annum. In many cases, where the plant is completely denuded, leaf yield and alkaloid content of subsequent harvests remains mostly constant.

It appears that the events activating reproductive development, the effects of leaf harvesting, recovery, and survival of *E. coca*, were recognized and understood by the Andean Society before cultivated species of *Erythroxylum* were transplanted from the original habitats. Genetic studies have shown that: (i) the gene pool of cultivated *E. coca* has shown little variation over 5000 years of cultivation, thus, wild and abandoned *E. coca* show no morphological or genetic differences from those persisting under cultivation [1, 7], (ii) crosses between *E. coca* and *E. novogranatense* var. *novogranatense* (Morris) Hieron failed; however, dwarfed and did not survive [7], and (iii) two cultivated varieties (*E. n.* var. *novogranatense* and *E. n.* var. *truxillense*) appear to have originated by long-term cultivation, geographic segregation, a quest for savour, palatability and resistance to aridity [8].

Young rolled leaves of *E. coca* are known to contain a higher level of the major alkaloids than fully expanded leaves [9–12]. However, the alkaloid content of *E. coca* during transformation from vegetative to the reproductive phase of development has not been extensively studied. Published research has described reproductive components of *E. coca*; the development of ovule and seed-coat, comparative morphology and embryology of several *Erythroxylum* species, heterostyly in *E. coca*, isolation of ecgonidine methyl ester from seed and *de novo* synthesis of cocaine in embryos and endosperms of *E. coca* [13–18]. The current research was conducted to determine the content (% dry wt) of hygrine, tropinone, methyl ecgonine, cuscohygrine, tropacocaine, cocaine, *trans* and *cis*-cinnamoylcocaine in various reproductive and vegetative tissues of *E. coca*.

RESULTS AND DISCUSSION

Ontogenetic changes of woody plants from the juvenile to adult phase of development have been defined and described [3–6]. The juvenile phase of growth for *E. coca* grown near 39°01′54″ N (latitude)

ment, if leaves were abscised due to environmental conditions or removed due to human manipulation, the juvenile phase culminated and the adult phase commenced. After *E. coca* began flowering it remained at the reproductive phase of growth and thereafter, cycled between vegetative and reproductive development. Leaf abscission of *E. coca* grown under greenhouse conditions occurred three times annually, transforming *E. coca* from vegetative to the reproductive stage of development on each occurrence. Immature flower buds were initiated along denuded branches 7 to 10 days after leaf drop, succeeded by the first flower bud and flower flushes 14 days, thereafter. After leaf abscission, the terminal leaf buds at the distal end of denuded *E. coca* branches were approaching and/or had reached the final stage of maturation before bud break; on many branches, leaf bud break had occurred and the terminal leaves were expanding.

The excised branch sections contained low levels of alkaloids. Branches with immature flower buds had a higher alkaloid content than branch sections without flower buds (Table 1). Hygrine, methyl ecgonine, cocaine and *trans*-cinnamoylcocaine were reported to be the most abundant alkaloids in seven-day-old and mature leaves of *E. coca* [12]. These alkaloids were also highest in immature flower buds (Table 1). Cuscohygrine, a major alkaloid in young leaves of *E. coca* [12], was not found in branches or reproductive structures (Table 1). The low alkaloid content in branch sections adjacent to the branches containing quiescent flower buds and immature flower buds (Table 1) may indicate transport to the regions of new growth, i.e. flower bud, flower, terminal branch buds and young leaves, as few mature leaves remained on *E. coca* after leaf abscission. Alkaloid content in leaf tissue of *E. coca* has been previously described [11, 12, 19].

The alkaloid content (% dry wt) of unopened flower buds compared to the averaged amount in fully expanded leaves of *E. coca* [12] was: hygrine, 37%; tropinone, 67%; methyl ecgonine, 27%; cuscohygrine, 0%; tropacocaine, 150%; cocaine, 53%; *cis*-cinnamoylcocaine, 4%; and *trans*-cinnamoylcocaine, 49%. It was noteworthy that several alkaloids present in the unopened flower buds were at concentrations that represented ≈50% of the amount present in the mature leaf of *E. coca* [10–12, 19]. Moreover, cocaine content increased by threefold in unopened flower buds when compared to the amount in immature flower buds (Table 1). Further, if cocaine content in flower appendages are considered, the amount in pedicels, perianths, and stamens increased by threefold, and in the pistils by twofold, compared to the quantity in immature flower buds (Table 1). The cocaine alkaloid of *E. coca* has been cited as a natural occurring insecticide in leaves [20] and may provide similar protection for immature flower buds, unopened flower buds, the flower and

Table 1. Concentration of alkaloids (% dry wt) in *E. coca* tissue during reproductive development

	Branch segments w/o flower buds*	Branches with quiescent flower buds	Immature flower buds	Unopened flower buds	Opened flowers				Young Leaves†
					Pistils	Pedicels	Perianths	Stamens	
ight (g)	3.210	3.150	2.320	0.874	0.554	0.250	0.879	0.119	1.170
,	0.006±0.001†	0.03±0.002	0.05±0.004	0.18±0.002	0.17±0.002	0.22±0.023	0.08±0.001	0.16±0.015	0.49±0.001
ine	n.d.§	n.d.	n.d.	0.02±0.001	0.02±0.0004	0.02±0.001	n.d.	0.04±0.003	0.01±0.002
ecgonine	0.005±0.001	0.03±0.002	0.15±0.016	0.13±0.005	0.14±0.007	0.14±0.001	0.05±0.001	0.08±0.140	0.24±0.009
ygrine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03±0.009
ocaine	0.002±0.003	0.005±0.0004	0.02±0.002	0.06±0.001	0.04±0.001	0.03±0.004	0.06±0.003	0.01±0.002	0.02±0.002
,	0.005±0.001	0.03±0.002	0.12±0.007	0.39±0.005	0.27±0.003	0.32±0.002	0.33±0.001	0.32±0.005	0.61±0.016
namoylcocaine	0.002±0.001	0.005±0.002	0.01±0.002	0.02±0.001	0.04±0.001	0.07±0.001	0.02±0.001	0.10±0.006	0.03±0.002
innamoylcocaine	0.003±0.001	0.009±0.001	0.02±0.004	0.17±0.007	0.17±0.002	0.21±0.004	0.06±0.002	0.06±0.001	0.27±0.035

nch segments without (w/o) flower buds, but adjacent to branches with quiescent flower buds.
ves at the end of the branches.
ues are means ±s.e. for three replicate samples.
aloid content was not detected.

flower [21, 22]. In addition, the presence and content of cocaine in ripened ovaries of *E. coca* has been reported [18] and may provide chemical defence as indicated by ref [20].

To determine the alkaloid distribution within the opened flower of *E. coca* rather than the total amount present therein, the flower was divided into the stamen, pistil, perianth, and pedicel. The content of cocaine in the stamen, pistil, perianth and pedicel was 0.32%, 0.27%, 0.33%, and 0.32%, respectively, (Table 1) and represented ≈50% of the amount reported for leaves of *E. coca* [11, 12, 19]. Additionally, hygrine, methyl ecgonine, and *trans*-cinnamoylcocaine were also most abundant in the stamen, pistil, perianth and pedicel of *E. coca* (Table 1). Cuscohygrine was not detected in the divided flower parts, however, it was identified in terminal young leaves of branches (Table 1).

Of all the tissue tested in the current study, young leaves at the terminal end of branches contained the most alkaloids (Table 1). Compared with the amounts in mature *E. coca* leaves (between weeks 8 through 16) [12], analyses of terminal branch young leaves during reproductive development showed that (a) cocaine, hygrine, and *trans*-cinnamoylcocaine contents were similar to; (b) tropinone, tropacocaine and methyl ecgonine were ≈50% of; and (c) cuscohygrine was 25% of those mature leaves. Further, the content of *trans*-cinnamoylcocaine in the pedicel was similar to the amount reported for cinnamoylcocaine present in berry stems of *E. coca* [23].

EXPERIMENTAL

Plant material. The living collection of *Erythroxylum coca* var. *coca* Lam. established, 1982–1986, and authenticated by T. Plowman, 1988, P. M. Rury, 1992) were germinated and grown under greenhouse conditions near 39°01′54″ N (latitude) 76°55′63″ W (longitude) similar to conditions previously described [12]. During the week of 22 May 1994, the *E. coca* plants self defoliated, and changed from a vegetative to the reproductive phase of development. Quiescent flower bud (i.e. buds on defoliated branches adjacent to the cortex but more visible under magnification of 10×) development commenced *ca* 7 to 10 days after leaf defoliation along denuded branches of *E. coca*. Sections of *E. coca* branches with quiescent flower buds, and without flower buds not more than 2.5 cm long were excised and collected. The amount of branches collected provided three replicated samples of 3.1 g (±0.2 g dry wt; × three per sample). After harvest, the branches were stored at −20° for alkaloid analyses. Similar amounts of immature flower buds (i.e. clusters of flower buds along branches protruding ≤0.3 mm, crustose in appearance) were excised from branches and stored as above for alkaloid determi-pedicel) were harvested by excising the flower bud at the interface of the branch and the pedicel. The average fr. wt of an unopened flower bud was 12.1 mg (±0.04 mg, $n = 10$). The flower buds were stored as above for alkaloid analyses. Subsequently, the opened flower with exposed perianth, stamen and pistil were excised at the branch and pedicel interface and collected. Care was taken to ensure that minimum damage occurred to flowers during excision and storage at −20°. The flowers were removed from storage and the stamen and pistil (appendages) dissected under magnification (10×). The average fr. wt (mg) of divided flower parts where $n = 10$ were: flower, 14.2 (±0.03); corolla, 6.2 (±0.2); stamen, 0.4 (±0.002); pistil, 2.6 (±0.03); sepal, 3.4 (±0.04); and pedicel, 1.0 (±0.003). Each divided flower part was separately placed into glass beakers, situated in an ice bath, until sufficient amounts were collected to provide three replicated samples. *E. coca* flowers were known to be distylous before floral division [7]; however, long and short styles were not separated. After sufficient floral parts were collected, all excised flower parts, branch sections, immature flower buds, unopened flower buds and terminal (branch) young leaves were separately placed into glass beakers and oven dried [12].

Extraction and identification of alkaloids. After oven drying, replicated samples of all collected *E. coca* parts were weighed for dry wt determination, then extracted and analysed for the content (% dry wt) of eight alkaloids (hygrine, tropinone, methyl ecgonine, cuscohygrine, tropacocaine, cocaine, *cis* and *trans*-cinnamoylcocaine) by GC as previously described [12]. Analytical standards for the alkaloids were obtained from sources indicated in ref. [12]. GC of standards and plant extracts were determined and confirmed by GC/MS [12, 18]. Data represents the average of the three replicated samples.

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