ANALYSIS OF ALKALOIDS IN LEAVES OF CULTIVATED ERYTHROXYLUM AND CHARACTERIZATION OF ALKALINE SUBSTANCES USED DURING COCA CHEWING*

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Summary

Several solvents were tested for the extraction of the alkaloids in Erythroxylum coca. The resulting crude extracts were analyzed by gas chromatography–mass spectrometry. Ethanol extraction was found to be the only quantitative method presenting no artifacts. It was established that cocaine and cis- and trans-cinnamoylcoacaine were the endogenous alkaloids in E. coca leaves. From the several breakdown compounds arising during long-term extraction with H₂SO₄ or CHCl₃, ecgonine methyl ester was the only alkaloid fully identified; ecgonine methyl ester was tentatively identified on the basis of its mass spectrum fragmentation pattern.

Quantification by mass fragmentography of the three endogenous compounds was performed using a stable-isotope dilution technique on individual leaves of single branches of E. coca, E. novogranatense and E. novogranatense var. truxillense. The relative amounts of these alkaloids ranged with leaf age as well as between species and varieties. The variation in alkaloid levels between individual leaves was too small to allow the use of the ratio between cocaine and the cinnamoylcoacaine levels as a taxonomic marker.

The initial pH value of 17 different alkaline substances traditionally used during coca leaf chewing was measured after dissolution in H₂O; values ranged between 10.1 and 12.8. Buffer capacity was determined by titration with HCl. Three types of curve shapes were obtained which could correspond to NaOH, Na₂CO₃ and NaHCO₃ titration curves. One sample of alkaline material had no buffer capacity at all. The recovery and breakdown of the cocaine contained in E. coca leaf powder was monitored for one hour at various pHs at 37 °C. The levels of cocaine and benzoylecgonine did not change by more than 17% at any of the pHs tested (6.0, 9.0 and 11.5). It was concluded that the alkaline substances are mainly responsible for the

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transformation of the alkaloids to free bases and not for a major hydrolysis of cocaine.

Introduction

Two closely related species of the genus Erythroxylum, namely E. coca Lam. and E. nooegrantense (Morris) Hieron., have long been cultivated in South America. For thousands of years Indians have used it as a mild stimulant and medicine. The principal active constituent of the leaves of cultivated Erythroxylum species is cocaine which is traditionally absorbed during chewing. When the coca leaf is masticated, an alkaline substance is generally added to the quid. Without such a substance the chewer does not experience fully the desired effect. It has been suggested that when cocaine is ingested, as in the case of coca chewing, a complex metabolic process takes place (Montesinos, 1965). Although it has been demonstrated that cocaine is present in the plasma of coca leaf chewers (Holmstedt et al., 1979), considerable controversy still exists concerning the fate of the alkaloids during chewing.

Variability in the alkaloid content of Erythroxylum species has long been recognized (Morris, 1889; de Jong, 1906). Many chemical analyses have been carried out on cultivated coca leaves of uncertain origin using methods that were at best only semi-quantitative. Consequently there is a real lack of reliable data on the alkaloids of Erythroxylum species.

The principal objectives sought in this study were: (1) to identify by gas chromatography–mass spectrometry (GC–MS) the principal coca alkaloid present in well-preserved Erythroxylum leaf materials collected in Peru and cultivated in North America; (2) to compare the efficacy of extraction of these alkaloids by different organic solvents and to determine the alkaloid levels in leaves by using a stable-isotope dilution technique; and (3) to determine the buffer capacity of alkaline substances commonly used during coca leaf chewing in Peru and Colombia, and to investigate the stability of the alkaloids in leaf material during incubation in aqueous buffers under simulated conditions of mastication.

Materials and methods

Chemicals

Cocaine hydrochloride was obtained from the Karolinska Apoteket, Stockholm, Sweden. Cocaine deuterated on the methyl-ester moiety (cocaine-d$_3$) had been synthesized previously (Holmstedt et al., 1977). Truxcinnamoylcocaine was prepared following the procedure described by Moo (1973) starting with ecpoline hydrochloride. All other chemicals were of the "pro analysis" grade from Merck (Darmstadt, F.R.G.).
Plant materials

Descriptions of the voucher herbarium specimens used in this study are given in Table 1.

Alkaline substances

In general, coca leaf chewers add alkaline substances to the leaves — sometimes lime, sometimes vegetable ashes prepared in the shape of balls or rolls. Descriptions of the voucher specimens used in this study are given in Table 2. The cal is prepared by burning calcium-containing rock fragments or shells and grinding the remains into fine powder. To form illipta, the ashes of the stalks of quinoa (Chenopodium quinoa Wild.) and cahitua (Ch. pallidicaule Nellen) two indigenous crops of the Peruvian Andes, are mixed with water to form a paste which is formed into small grey balls and dried in the sun. A small piece is broken off and taken with each mouthful of coca leaves. Other plant materials — such as plantain roots or cacao pods — may also be used for the same purpose. In the Amazon, leaves of Cecropia spp. are burned and mixed with coca leaf powder (Schultes, 1957; Holmstedt et al., 1979; Plowman, 1981).

Extraction methods

Accurate measurement of the total alkaloid content in plant material should be obtained using an extraction procedure that is as complete as possible. Since the different alkaloids that have so far been found in coca leaves vary in their chemical nature (bases, esters, acids and/or others), their isolation from biological material is difficult. At the present time no single method of extraction for all the coca alkaloids is available. During this study, six extraction methods were tested and compared, using a single collection of dried E. coca leaves (voucher specimen No. 7351) ground to a powder: methods A - D were qualitative and methods E and F were quantitative.

Method A ("acid extract")

Four grams of the leaf powder were extracted with 200 ml of 1 N \(\text{H}_2\text{SO}_4\) at room temperature for 20 h. After filtration on a G4 glass filter, the solution was brought to pH 9 by the addition of solid \(\text{Na}_2\text{CO}_3\). The alkaloids were extracted by partitioning the aqueous phase three times with 100 ml of chloroform-diethyl ether \(1:4, v/v\) mixture. The organic layers were dried \(v\) the addition of anhydrous \(\text{Na}_2\text{SO}_4\) and taken to dryness under reduced pressure at 40°C. The residue was taken up in a minimum volume of chloroform or silylated with Supelco (Supelco, Crans, Switzerland) pyridine \(1:3, v/v\) and injected directly into the GC-MS system.

Method B ("volatile alkaloid extract")

Four grams of the leaf powder were mixed with 100 ml of \(\text{H}_2\text{O}, 150 \text{ ml anhydrous glycerin and } 20 \text{ g of NaOH (Fikenscher, 1959); } 100 \text{ ml of the solution were evaporated under mild vacuum in a rotary evaporator. The}
<table>
<thead>
<tr>
<th>Plowman No.*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7351</td>
<td><em>Erythroxylum coca</em> Lam.</td>
</tr>
<tr>
<td>7611</td>
<td><em>E. coca</em></td>
</tr>
<tr>
<td></td>
<td>Branch A: leaf No. 1 = first unrolled leaf, linear. No. 2 = new growth, newly expanded leaf, No. 3 = old growth leaf, Nos. 4 and 5 = idem. No. 6 = old, yellowing leaf.</td>
</tr>
<tr>
<td></td>
<td>Branch B: leaf No. 1 = first unrolled leaf, linear. Nos. 2 - 5 = as in branch A.</td>
</tr>
<tr>
<td>6275</td>
<td><em>E. novogranatense</em> (Morris) Hieron.</td>
</tr>
<tr>
<td>6235</td>
<td><em>E. novogranatense</em> var. <em>truxillense</em> (Rusby) Plowman</td>
</tr>
</tbody>
</table>

*Voucher specimens are deposited in the Herbarium of the Department of Botany, Field Museum of Natural History, Chicago, Illinois, and at the Botanical Museum of Harvard University, Cambridge, Massachusetts, U.S.A.*
collected fraction was then extracted as in Method A with the chloroform–diethyl ether mixture at pH 9.

**Method C**

Four grams of the leaf powder were extracted sequentially at room temperature with 200 ml of n-hexane (20 h), 200 ml of chloroform (20 h) and finally 200 ml of ethanol (20 h). The three fractions obtained were evaporated separately to dryness; the residues were then each redissolved in the corresponding solvent for GC-MS analysis.

**Method D** ("crude ethanolic extract")

Two grams of the leaf powder were extracted with 100 ml of boiling 96% ethanol for 15 min and left to cool for 4 h before filtration. The solvent was evaporated as described previously and the residue was either redissolved in ethanol or silylated.

**Method E**

The isotope dilution technique using deuterated cocaine as an internal standard (Holmstedt et al., 1977, 1979) was applied to small amounts (approx. 100 mg dry weight) of plant material.

**Method F**

In order to extract cocaine and its hydrolysis compounds, it was necessary to adapt a method which has been reported to give 99% recovery for cocaine and 80% recovery for benzoylecgonine from urine (von Minden and D'Amato, 1977). Briefly, 100 mg dry weight of the leaf powder were left for a given time, ranging from 0 to 60 min, in 5 ml of the 0.4 M buffers used for the calibration of the pH-meter electrodes (Radiometer, Copenhagen, Denmark). For extraction, the solution was brought to pH 1 with 2 N HCl. Following centrifugation, the solids were removed and the supernatant was immediately adjusted to pH 9.5 with a mixture of solid NaHCO₃ and Na₂CO₃ (3:1, w/w). One milligram of cocaine-d₃ was added and 10 ml of an ethanol–chloroform (1:4, v/v) mixture was used to extract the bases and the amphetamine substances. The organic mixture was evaporated to dryness at 60 °C under a flow of nitrogen. The residue was treated with 1 ml of an ice-cold solution of diazoethane in diethyl ether, reduced to dryness and redissolved in 50 µl of toluene for quantitative GC-MS analyses.

**Titration of the basic substances**

Weighed amounts (50 - 100 mg) of the alkaline materials (see Table 2) were dissolved as fully as possible by stirring them in 5 ml of freshly double-distilled water for 5 min at room temperature. pH measurements and titrations with 1 N HCl (Titrisol, Merck) were performed automatically by a titration assembly (Model TTA 60, Radiometer) at a flow-rate of 70 µl/min.
Gas chromatography - mass spectrometry

Qualitative analyses
The MS analyses were carried out on a combined GC-MS instrument (Model 5988A, Hewlett-Packard, Palo Alto, U.S.A.).

Typical GC conditions used throughout were: SP-2100 WCOT glass capillary column (10 m x 0.25 mm I.D.), temperature isothermal at 100 °C for 1 min, programmed at 10 °C/min to 240 °C. Split-mode injections of 1 μl were used at a helium pressure of 0.5 kg/cm² giving a carrier gas flow of 1 ml/min through the column and a split ratio of 1:50. The capillary column was connected directly to the MS source. The ion source parameters were set routinely by using the Autotune program providing a 70 eV ionization energy and 300 μA emission current. The temperature of the ion source was maintained at 200 ± 2 °C. The resolution of the mass spectrometer was 2.5 times the mass, when measured at half peak height. Repetitive scanning from m/z 40 to m/z 540 was performed at one scan per 1.3 sec. By using the Batch program, each peak of the reconstructed chromatogram that was bigger than 1% of the base peak in the total run was further processed for a library search. Identifications were based on similarity indices higher than 0.98 (absolute identity had an index of 1.00) and by direct comparison with authentic standards run under the same GC-MS conditions. Both the retention time and the mass spectrum were criteria for the identification of unknown compounds.

Quantitative analyses
GC conditions were similar to those used for the qualitative analyses except that an oven temperature program of 30 °C/min was used. Sample injection was carried out in the split-less mode over 30 sec. By this means all the sample injected was transferred to the capillary column. For the quantitative measurements, the GC-MS was operated in the selected ion monitoring mode, a technique also termed mass fragmentography (MF) (Hammar et al., 1969). For analyzing the extracts obtained using method F, the mass spectrometer filter was set to record the ions at m/z 182.1 and 303.2 for cocaine, 306.2 for cocaine-d₃, and 182.1 and 329.2 for both cis- and trans-cinnamoylcocaine. Each mass was measured over a 150-msec period in each cycle. Calibration curves for the accurate determination of the endogenous substances were obtained routinely by calculation of the response factors of each individual component against cocaine-d₃ as previously described (Holmstedt et al., 1979).

When cocaine and benzoylcegonine ethyl derivative were quantified in the extracts obtained using method F, ions at m/z 182.1 and 303.2 were chosen for cocaine, 306.2 for cocaine-d₃, and 317.2 for benzoylcegonine ethyl ester. Cocaine and benzoylcegonine ethyl ester were quantified from the ratio of ion intensities at m/z 303/306 and 317/306, respectively.
Results and discussion

Alkaloid content of E. coca

The genus *Erythroxylum* is the only natural source of cocaine and related compounds of the ephedrine type. Although minute quantities of cocaine have been detected to date in 17 wild species of the genus, including several Old World species (Aynilian et al., 1974; Holmstedt et al., 1977; Sivler and Plowman, unpublished data), only two main cultivated species — *E. coca* and *E. novogranatense* — have been found to contain large amounts of ephedrine-related substances. As regards the distribution of the minor alkaloids in the genus, 12 such compounds have been reported for the cultivated coca (Raffauf, 1970).

Besides cocaine (methyl-35-benzoyloxy-tropan-2-carboxylate; (1)) (Wöhler, 1862), other alkaloids have been isolated from leaves of cultivated coca. Pseudococaine (C-2a,C-3b) was found in coca leaves by Liebermann and Giesel (1890), but this compound is probably an artifact of the isolation procedure, arising from the action of alkali on cocaine during prolonged work-up (Archer and Hawks, 1976). Cinnamoylcocaine has been isolated from several coca leaf samples (Liebermann, 1889); Moore (1973) was the first to be able to identify the cis (2) and trans (3) isomers by separating

![Chemical structures](image)

them by gas chromatography. Benzoylecgonine has been isolated from Peruvian (de Jong, 1940) and Colombian (Espeinel Ovalle and Guzman Parra, 1971) coca leaves but not from the variety obtained from Java (de Jong, 1940). It may also be an artifact, possibly arising from hydrolysis of cocaine (Archer and Hawks, 1976). De Jong (1940) has found that ephedrine methyl ether (4) occurs naturally in Java coca leaves. Tropacocaine, the benzoate ester of tropan-3-ol, occurs in Java (Willstaetter, 1896) and Peruvian (Hesse, 1902) coca leaves. The hygrine group of coca alkaloids is usually found in an ethereal extract of a slightly alkaline percolate of coca leaves (Henry, 1849). It is evident that the use of a base for extracting the hygrines raises the question of the natural occurrence of these substances as such in living plant material.
In the last 20 years, only two reports have dealt with the minor alkaloids in cultivated coca leaves. The first study was on commercial Java coca (probably *E. novogranatense var. novogranatense*) (Fikenscher, 1959). Mostly cocaine and cinnamoylcocaine were found in the dried leaves with small amounts of methylecgonine, hygrine, tropacocaine and traces of cuscohygrine and nicotine (Fikenscher, 1958, 1959; Hegnauer and Fikenscher, 1960). Unfortunately, all identifications were based on *R* values in thin-layer chromatography (TLC) and colour reactions with specific reagents; quantification was performed on thin-layer spot areas. The second work was done on *E. coca var novogranatensis [sic]* (Espinel Ovalle and Guzmán Parra, 1971). Using coca inc as the single standard, these authors found 62% cocaine, 16% cinnamoylcocaine, 14% benzoylecgonine and 8% tropacocaine in the leaves. Identifications were based on comparison of melting points, odour of hydrolysis components and infrared spectra which were recorded for each substance separated by paper and thin-layer chromatography.

It is impossible to evaluate *a posteriori* the importance of artifacts in the data briefly reported above. It is also impossible to prove that some alkaloids found previously in coca might originate from breakdown of alkaloids during poor drying conditions, long storage and/or analytical work-up procedures.

In the present work, the GC-MS results show clearly that the crude ethanolic extract obtained using method D (see Materials and methods) can be resolved on a capillary GC column without any further purification (Fig. 1). The reconstructed total ion chromatogram displays only a few peaks. Ethanol extracts many compounds from the plant material: sugars, steroids, as well as alkaloids. Sugars do not appear in the chromatogram as they are not sufficiently volatile. When the extract was silylated, it was possible to find additional peaks in the chromatogram which were identified as trimethylsilylated (TMS) derivatives of monosaccharides such as glucose, mannose, galactose, xylose, etc. As far as eggonine alkaloids are concerned all esters are volatile enough for GC analysis. Acids such as benzoylecgonine and eggonine have to be derivatized; when the crude ethanolic extract was silylated neither of these two substances could be detected as their TMS derivative. Cocaine (the main peak in the chromatogram) and both cis- and trans-cinnamoylcocaine were positively identified (Fig. 3A-C). None of the other volatile alkaloids previously reported in the literature could be detected in the material used.

In contrast, the chromatograms of the intact or silylated acid extract obtained from the same material using method A (Fig. 2) were less complex than the crude ethanolic extract. This is due mainly to the additional partitioning step included in this procedure, which eliminated most of the watersoluble compounds, in particular the sugars. In addition to the three substances found in the ethanolic extracts, eggonine methyl ester (Fig. 3D) was also identified. In the acid extract, the quantity of eggonine methyl ester was

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*Most probably *E. novogranatense var. novogranatense* (vide Plowman, 1979a and b.*)
Fig. 1. Total ion chromatogram of the GC-MS analysis of the crude ethanolic extract of *E. coca* leaves (voucher specimen No. 7351). No peak appeared before 13.5 min retention time under the GC conditions used (see text). Peak 1 was identified as cocaine. Peaks 2 and 3 were found to be cis- and trans-cinnamoylcocaine, respectively (see Fig. 3). No other substance of the ecgonine type has been detected. The other peaks could not be fully identified when using the MS library search. The peak emerging at 25.3 min was found to be of the alcohol type.

Fig. 2. Total ion chromatogram of the acid extract of *E. coca* (voucher specimen No. 7351) run under the same GC-MS conditions as in Fig. 1. The first peak appeared at 0.2 min retention time. Identities of peaks 1 - 3 were the same as in Fig. 1. Peak 4 was identified as ecgonine methyl ester (see Fig. 3). Peak 5 could not be fully identified, but is postulated to be related with ergonidine methyl ester (see text).
estimated to be half that of cocaine. Ecgonidine methyl ester was tentatively identified from its mass fragmentation pattern (Jindal et al., 1978)*. However, lack of a reference substance meant that this identification could not be verified. Ecgonidine methyl ester, reported to occur in Java and Peruvian coca seeds (Henry, 1949) but not detected in the crude ethanolic extract, was probably formed by the action of 1 N H₂SO₄ during extraction using method A. Similarly, ecgonine methyl ester appeared during acid treatment and after storage of the leaves under certain conditions (Turner et al., 1981).

When organic solvents were used sequentially for extraction as in method C, complete extraction of the alkaloids was difficult to achieve. With n-pentane, for example, cocaine and cinnamoyl cocaine were only

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*The mass spectrum at 70 eV of peak 5 of Fig. 2 was: m/z 181 (M⁺, 32%), 166 (M⁺ - CH₃, 10%), 152 (M⁺ - N - CH₃, 100%), 122 (M⁺ - COOCH₃, 14%), 82 (22%) and 42 (20%).
partially extracted since subsequent extraction with chloroform revealed additional amounts of these compounds. Prolonged extraction with chloroform resulted in some breakdown of cocaine since 15% of the alkaloids could be found as ergonine methyl ester. This amount, however, was smaller than the quantity found in the acid extract (see Fig. 2).

As shown previously (Holmstedt et al., 1977; Turner et al., 1979, 1981), and confirmed here, short extraction in boiling ethanol is the most efficient technique for minimizing the breakdown of cocaine and ensuring quantitative extraction of all alkaloids of the ergonine type, as they are all soluble in this solvent.

In an attempt to detect nicotine in E. coca leaves, the procedure used for Java coca (E. novogranatense var. novogranatense) by Fikenscher (1958) was repeated. No trace of nicotine was found in the plant material used in this study even using MF. Although a different species of Erythroxylum was used, unambiguous proof of the occurrence of this alkaloid in the genus has still to be provided, since the single previous identification of nicotine was based on TLC and colour reactions only.

**Quantitative analysis of alkaloids in Erythroxylum**

In this study, deuterated cocaine, labeled in the methyl ester moiety, was used as an internal standard as previously reported (Holmstedt et al., 1977, 1979). With ethanol as the extraction solvent (method E) no isotope exchange was observed to take place and a simple linear relationship between the appropriate ion intensity ratios and concentrations of cocaine, cis- and trans-cinnamoylcocaine and benzoylecggonine was obtained within the range of concentrations used throughout this study.

Analysis by MF allowed the determination of all components in a single evaporation of the crude ethanolic extract without further purification due to the specificity of the ions chosen. Since previous GC runs of extracts showed that cocaine and the cinnamoylcocaines do occur naturally (see above), MF analysis was carried out during the retention times corresponding to these compounds (Fig. 4). Identification of cocaine in the plant extracts was based on the ratio of ion intensities m/z 303/182 occurring at a retention time identical to that of the internal standard (R₁ = 6.2 min). cis-cinnamoylcocaine (R₁ = 7.3 min) was identified from its mass spectrum which is almost identical to that of the trans isomer which emerged subsequently from the C-cocaine column (R₁ = 8.4 min). Consequently both isomers could be quantified using the same ions m/z 329 and 182. A mass fragmentogram from a single coca leaf extract is shown in Fig. 5.

MF is a very sensitive method of detection; small pieces of plant material can be analyzed successfully and nanogram quantities of cocaine can be quantified at a 5% precision level. When working at higher concentrations (in the present assay, 100 - 500 ng were routinely injected) a 1.5% precision was obtained for within sample variation.

It is known that enzymatic conversion of cis-cinnamic acid to its trans isomer occurs in plants but no attempt to correlate this reaction to cis-cin-
Fig. 4. Mass fragmentogram of authentic standards mixture consisting of 50 ng of cocaine, 100 ng of deuterated cocaine and 20 ng of trans-cinnamoylcocaine. The ions at m/z 182.1 and 303.2 were used to monitor cocaine, the ion at m/z 306.2 cocaine-d₃, and the ions at m/z 182.1 + 339.2 cinnamoylcocaine. Cocaine and cocaine-d₃ emerged simultaneously at Rᵥ = 6.2 min and trans-cinnamoylcocaine at Rᵥ = 8.45 (see abscissa). Ion intensities are given as normalized for each channel individually (see ordinate) and area counts in arbitrary units as calculated by the computer, were obtained for each peak.

Fig. 5. Mass fragmentogram of a 3-µl injection of a 5-ml ethanolic extract of the first unrolled leaf of E. coca (voucher specimen No. 6235) obtained by using method E to which 500 µg of cocaine-d₃ had been added as internal standard before extraction. Each channel was normalized to the highest peak of the run. Cis-cinnamoylcocaine emerged at Rᵥ = 7.3 min. Correct retention times and m/z 303/182 + 329/182 ratios are additional proof of the identity of cocaine and the cinnamoylcocaines, respectively. Quantification was based on calibration curves of the m/z 303/306 and 329/306 ratios (see text).
namoylokcocaine has ever been tempted. No isomerization of trans-cinnamoyl-
ococaine was observed during extraction of the plant material by the present
procedure. This has been confirmed by the results obtained by Turner et al.
(1981) using similar conditions. However, the stability of cis-cinnamoyl-
ococaine has not been studied in vitro and this is due probably to the diffi-
culties in obtaining cis-cinnamoyl anhydride for synthesizing the correspond-
ing cinnamoylokcocaine (Moore, 1973). When considering all the results for
whole leaves obtained in the present study (Figs. 6 - 8), systematic conver-
sion of the cis-cinnamoyl cocaine to the trans isomer seemed not to occur
since variable amounts of both compounds were found in the different plant
specimens. In fact, it can be assumed that if any isomerization did occur
before or during work-up, both isomers would be present in all samples. If
the trans isomer was more stable than the cis isomer, it would have been
present in all samples in which cinnamoylokcocaine was detected. In fact,
yses of individual leaves of E. coca (Fig. 6B) indicate that only the cis
isomer is present in leaves 4 and 5. Consequently, it can be assumed that no
isomerization occurred here.

It has long been known that the quantities of alkaloids in coca leaves
can vary depending on the environmental conditions and the age of the leaves
(Reena, 1919 a and b). Quantities of the total alkaloids in Java coca leaves
gathered from branches according to their age remained almost constant for
eight successive leaves with the exception of the youngest leaf; however,
younger leaves contained more cinnamoylokcocaine than the older ones (de
jong, 1906). De Jong concluded that a continuous metabolism of cocaine
occurs in the leaf with age. In the present investigation, it was possible to
obtain more precise measurements of these changes in the leaves of two
different species and one variety of cultivated Erythroxylum [for the recent
botanical classification of the genus, the reader is referred to Plowman
(1979a, 1979b)]. The whole leaf was used for analysis (even though much
smaller portions could have been used) because the alkaloids are not uni-
f ormly distributed within the leaf itself (Yousef et al., 1979; Cooke et al.,
1981). In order to avoid the influences of ecological factors (temperature,
light, nature of the soil, humidity, etc.) plants were grown in greenhouses.
Leaves were collected the same day, numbered, weighed and air-dried in the
shade. All plants were grown from seeds and were two years of age. The
leaves had the morphological characteristics indicated in Table 2. The endo-
ogenous content of cocaine and of both cis- and trans-cinnamoylokcocaine
were calculated from the MF analyses.

In E. coca var. coca, two branches originating from the same plant were
analyzed (Fig. 6), one with five leaves and the other with six. The dry weight
of the leaves followed a sharp increase between leaf 3 and leaf 4, correspond-
ing to the opening of the young leaf and concomitant with an increase in leaf
surface area. The alkaloid content followed a similar pattern. Cinnamoylo-
kocaines represented about 20% of the total alkaloids measured in leaves 1
and 2. This proportion dropped to 2 - 10% in the next oldest leaves.

In E. noxogranatense var. noxogranatense (Fig. 7) the dry weight of the
leaves of the same branch followed the same distribution as in E. coca. Leaf 1
Fig. 6. Dry weight and alkaloid content of individual leaves of two branches (A and B) of E. coca (voucher specimen No. 7611). The hatched areas represented the cocaine levels, the white and black areas cis- and trans cinnamoylcocaine, respectively.

was unrolled and leaf 10 was yellowed. The alkaloids were at their highest level in leaves 4 and 8. The relative amounts of cocaine and cinnamoylcocaines were very different to those found in the respective leaves of E. coca. Levels of cinnamoylcocaines were higher in leaves 1 and 2 and decreased gradually with leaf age, in contrast to cocaine. The highest level of cocaine was found in leaf 8.

In E. novogranatense var. truxillense, smaller leaf size is a consequence of human selection under differing climatic conditions in the dry areas of northern Peru (Plowman, 1979b). The alkaloid pattern in this variety followed the increase in leaf dry weight (Fig. 8). The proportion of cinnamoylcocaines was greater in young leaves than in old ones when compared to cocaine levels.
In conclusion, a clear difference in the alkaloid distribution between leaves of various ages can be observed when comparing the three varieties analyzed here. This difference could be attributed mainly to genetic factors since all three varieties were grown under identical conditions in the same vicinity. However, variations within one species are of importance as shown, for example, in Fig. 6 for E. coca. Consequently, it is not possible to use the relative quantities of cocaine and cinnamoyloccaine as chemotaxonomic markers at the present stage of our knowledge, although tentative moves in this direction have been made recently (Youssefi et al., 1979; Turner et al., 1981). From the point of view of biosynthesis, the present data indicate that coca leaf is certainly the site of a very active metabolism of the alkaloids. Additional studies will be necessary for a better understanding of their metabolism before any further conclusions can be drawn.
Characterization of the alkaline substances

During chewing of the leaves of *Erythroxylum* species in the Andes or eating the powder preparation made from *E. coca var. ipadu* (Plowman, 1979a, 1981) by the natives of the Amazon, alkaline substances are frequently added. The purpose of this practice is not completely understood. It has been suggested that the base aids salivation in extracting the alkaloids in the mouth from the leaf material and also that it could be strong enough to hydrolyse cocaine into eegonine derivatives of less potent psychoactivity (Zapata Ortiz, 1970).

As a first step in the understanding of the action of these alkaline substances, their initial pH in solution and their buffer capacity were measured. Seventeen different alkaline materials of various origin were tested (Table 2). When starting with 50-mg amounts of material and dissolving them in 5 ml of distilled H₂O, the initial pH values ranged from 10 to 11, except for the cal samples which gave a pH > 12.6. Reports on the chemical composition of these materials are scarce and imprecise. Gosse (1861) reported that *illipta* contains CaCO₃, MgCO₃, KHCO₃ and sulfate, chloride and phosphate. Cruz Sánchez and Guillén (1949) found various tocra samples to contain “mainly potassium and calcium (30%) besides carbon, silica and earth (60%)”. Such mixtures in a 1% solution gave pH values of 10.5 to 11.5. Baker and Mazess (1963) showed that *illipta* contains 12% of calcium and a lot of magnesium salts; one sample of *cal de piedra* was found to have 36% of calcium and another 42%, probably in the form of almost pure lime. From all the curves obtained by titration with 1 N HCl of the various alkaline substances dis-
Characteristics of the alkaline substances used with coca leaf chewing

<table>
<thead>
<tr>
<th>Voucher No.</th>
<th>Name</th>
<th>Origin</th>
<th>Starting pH</th>
<th>Buffer capacity</th>
<th>Curve shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>7602</td>
<td>Tocra* de Quinua (Chenopodium quinoa Willd.)</td>
<td>Lima, Peru</td>
<td>10.5</td>
<td>0.075</td>
<td>A</td>
</tr>
<tr>
<td>7603</td>
<td>Tocra de Platano (Musa sp. roots)</td>
<td>Lima, Peru</td>
<td>10.6</td>
<td>0.35</td>
<td>B</td>
</tr>
<tr>
<td>7605</td>
<td>Cal de piedras</td>
<td>Tocache, Huánuco, Peru</td>
<td>12.7</td>
<td>1.52</td>
<td>C</td>
</tr>
<tr>
<td>7936</td>
<td>Liplt a de haba (Vicia faba L.)</td>
<td>Puno, Peru</td>
<td>11.0</td>
<td>0.50</td>
<td>B</td>
</tr>
<tr>
<td>7937</td>
<td>Liplt a de Quinua</td>
<td>Puno, Peru</td>
<td>10.9</td>
<td>0.43</td>
<td>B</td>
</tr>
<tr>
<td>7938</td>
<td>Tocra de Quinua</td>
<td>Andahuaylas, Peru</td>
<td>10.1</td>
<td>0.90</td>
<td>B</td>
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<tr>
<td>7939</td>
<td>Tocra de Tocna</td>
<td>Machuente, Dept. Ayacucho, Peru</td>
<td>10.3</td>
<td>0.35</td>
<td>B</td>
</tr>
<tr>
<td>7942</td>
<td>Tocra</td>
<td>Cerro de Pasco, Peru</td>
<td>10.4</td>
<td>0.015</td>
<td>A</td>
</tr>
<tr>
<td>7943</td>
<td>Mambe (from limestone)</td>
<td>San Agustín, Colombia</td>
<td>11.6</td>
<td>0.84</td>
<td>B</td>
</tr>
<tr>
<td>7944</td>
<td>Liplt a de Quinua</td>
<td>Cusco, Peru</td>
<td>10.6</td>
<td>0.59</td>
<td>B</td>
</tr>
<tr>
<td>7945</td>
<td>Liplt a de Quinua</td>
<td>Cusco, Peru</td>
<td>10.9</td>
<td>0.40</td>
<td>B</td>
</tr>
<tr>
<td>7946</td>
<td>Liplt a de Cahihua</td>
<td>Pucarátambo, Cusco, Peru</td>
<td>10.2</td>
<td>0.56</td>
<td>B</td>
</tr>
<tr>
<td>7947</td>
<td>Liplt a de Cacao (Theobroma cacao L.)</td>
<td>Quillabamba, Cusco, Peru</td>
<td>10.4</td>
<td>0.83</td>
<td>B</td>
</tr>
<tr>
<td>7950</td>
<td>Liplt a de Quinua and Cacao (Theobroma cacao L.)</td>
<td>Espinar, Cusco, Peru</td>
<td>10.6</td>
<td>0.34</td>
<td>B</td>
</tr>
<tr>
<td>7951</td>
<td>Leaf ashes of Cecropia (cf. aleiophylla Mart.)</td>
<td>Mitú, Vaupés, Colombia</td>
<td>11.0</td>
<td>0.15</td>
<td>A (B)</td>
</tr>
<tr>
<td>7953</td>
<td>Cal de Trujillo</td>
<td>Trujillo, Peru</td>
<td>12.6</td>
<td>1.30</td>
<td>C + B</td>
</tr>
<tr>
<td>7954</td>
<td>Cal de Maraiscon</td>
<td>Trujillo, Peru</td>
<td>12.8</td>
<td>1.12</td>
<td>C + B</td>
</tr>
</tbody>
</table>

*These are different local names for the same preparation.

b0.1 M solutions of NaOH, Na₂CO₃, NaHCO₃ and pure H₂O gave pHs of 12.9, 11.5, 8.2 and 6.3, respectively. The starting pH corresponds to the pH of a 5 ml solution from 50 mg of alkaline material.

cBuffer capacity represents the quantities (expressed in ml) of 1 N HCl required to bring 100 mg of alkaline material dissolved in 5 ml of H₂O to a pH of 5.0.

dCurve shapes correspond to: (A) H₂O titration; (B) NaHCO₃ + Na₂CO₃ titration; (C) NaOH titration by 1 N HCl (see Fig. 9).
solved in water, three distinct shapes were observed (Fig. 9). Clearly, *cal de piedra* had the largest buffer capacity. Its titration curve is similar to that obtained with NaOH. The *llipta* sample was characteristic of a mixture of Na₂CO₃ and NaHCO₃. One other sample of ash ball (voucher No. 7942) had no buffer capacity at all, as illustrated in Fig. 9.

Two differing opinions are held for the action of the alkaline substances during coca chewing. On the one hand, it is thought that the usual addition of basic material facilitates the liberation of cocaine base from its conjugates with plant acids or other constituents. The soluble hydrochloride is only formed in the stomach where it is absorbed (Salomon, 1949). On the other hand, a group of Peruvian workers hold the view that cocaine is broken down to a considerable extent into benzoylecgonine and ecgonine before absorption (see Montesinos, 1965).

The latter view seemed to be strengthened by the experiments of Nieschulz and Schmersahl (1969). Using pure cocaine base — which is almost insoluble in water — and adding it to a solution of saturated Ca(OH)₂ for a period of 30 min at 37°C, these authors found on the basis of TLC analyses that only 15% of the cocaine was hydrolyzed. The final product obtained was ecgonine only. In the same study, when coca leaves were used instead of pure cocaine, the apparent breakdown of the endogenous alkaloid was more rapid. Such results lead some workers to believe that cocaine is degraded to ecgonine prior to uptake in the bloodstream and that this latter compound is the central alkaloid involved in coca chewing pharmacology (Burchard, 1975; 1976). However, it is evident that Nieschulz and Schmersahl used extreme
values have been chosen: pH 11.5, corresponding to the pH of a mixture of coca leaf powder, saliva and 5% \textit{cal de piedra}; pH 9, corresponding to coca leaf powder, saliva and 50% \textit{Cecropia} ashes; and pH 6, given by coca leaves and saliva alone. Instead of saliva and alkaline substances, standard buffer solutions were used to ensure reproducibility of the assays.

In this experiment, the method for extracting cocaine and benzoyl-ecgonine had to be different from the one used for the total alkaloid analyses because of the presence of water in the incubation medium, preventing the use of ethanol as extracting solvent. Another solvent mixture was found to be satisfactory (Von Minden and D'Amato, 1977) and the ethyl ester of benzoyl-ecgonine was synthesized for MP analysis. Cocaine and benzoyl-ecgonine ethyl ester did not separate well under the GC conditions used but their respective molecular ions could be monitored for quantification without mutual interference. Egonine and ecygonine methyl ester, breakdown products of cocaine hydrolysis, were not quantified here because of the lack of extraction methods satisfactory for the simultaneous quantification of all amphoteric metabolites of cocaine in aqueous solution. Consequently, the results of the analysis of cocaine and only its first metabolite, benzoyl-ecgonine, are reported in Table 3.

Evidently, cocaine is present throughout the duration of the assay which was limited to 1 h — when chewed the leaves are usually not kept longer in the cheeks (Holmstedt \textit{et al.}, 1979). A certain breakdown of cocaine occurs during incubation at all the pH values tested. Hydrolysis at the highest pH (11.5) is limited, however, and does not exceed 17%. It is noteworthy that 0.7% more cocaine is found by incubation at pH 9 for 30 min compared to the control. This indicates that an alkaline pH does not significantly increase the extractability of cocaine from the plant powder. This is in agreement

<table>
<thead>
<tr>
<th>Material(^a)</th>
<th>Incubation time (min)</th>
<th>Cocaine content(^b) (%)</th>
<th>Benzylolecggonine(^b) content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6.0</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>coca powder</td>
<td>30</td>
<td>99.3</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>92.9</td>
<td>100.8</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>30</td>
<td>100.5</td>
<td>99.2</td>
</tr>
<tr>
<td>coca powder</td>
<td>60</td>
<td>96.0</td>
<td>97.2</td>
</tr>
<tr>
<td>pH 11.5</td>
<td>30</td>
<td>86.9</td>
<td>103.3</td>
</tr>
<tr>
<td>coca powder</td>
<td>60</td>
<td>83.0</td>
<td>102.6</td>
</tr>
</tbody>
</table>

\(\text{Material: \textit{Erythroxylum coca} Lam. from Pisac Market, Cusco, Peru; voucher No. Flomostedt \textit{et al.}, 7133; air-dried leaves (see Holmstedt \textit{et al.}, 1978).}

\(\text{Initial cocaine content was 0.48 g per 100 g dry weight; initial benzoyl-ecgonine content as 0.067 g per 100 g dry weight; cinnamylcocodeine was not detected.}

\(\text{pH of saliva = 5.87 ± 0.07; pH of coca leaf powder = 5.8; pH of coca and \textit{Cecropia} ashes = 8.55.}\)
conditions which are not similar to the chewing practices used in the Andes or in the Amazon. More realistically, lower pH values should be used, as indicated by the basic substances analyzed here. In the present study, three pH with the optimum pH value of 7 for the extraction of pure cocaine from water into diethyl ether (Makisumi and Ota, 1959). Benzoylecgonine levels changed significantly between pH 9 and 11.5, suggesting that its turnover is different at these pH values. Consequently, the pH value is decisive in these studies and completely different results can be obtained depending on the basicity of the medium used. Such a view corresponds to the unpublished data obtained recently with pure cocaine (Vitti, 1979): isku and lipta were used for incubating synthetic cocaine, providing a pH of 11.7 and 10.5, respectively. With isku, Vitti found that 90% of the cocaine was destroyed in 20 min via ecgonine methyl ester which was present in a five-fold higher amount than the other metabolites. In contrast, he also reported that with lipta 50% of the incubated cocaine was hydrolyzed after 1 h, the main metabolite being benzoylecgonine. A GC method was used for quantification (Vitti, 1979). In the same kind of assays, Peruvian chemists found earlier that incubation with toca (5% solutions) at 37 ºC resulted in 2.5% and 5.5% breakdown over 30 and 60 min. (Cruz Sánchez and Guillén, 1949). Finally, similar experiments with acidic medium (saliva, simulated gastric fluids, etc., in the pH range 6 - 1.2) produced no breakdown of cocaine (Montesinos, 1965; Vitti, 1979).

The difficulty in these experiments is to simulate natural conditions as realistically as possible. Nieschulz and Schmersahl (1969) used an excessive amount of lime which could not be tolerated in the mouth of coca chewers. Cruz Sánchez and Guillén (1949) and Vitti (1979) used pure cocaine as starting material which does not correspond to the complex plant material. In the present study artificial buffer solutions were used. All of these experimental settings only approximate coca leaf chewing as practised in South America. It is difficult, indeed almost impossible, to extend conclusions drawn from synthetic mixtures to in vivo systems. It should be kept in mind that Peru and Bolivia are the only countries where coca chewing is still a legal practice. Consequently, it is suggested that the work done in 1948 by Ciuффardi, a Peruvian scientist, should be repeated in Peru using modern analytical methods.

In conclusion, the present data, although incomplete, indicate the importance of not overestimating the hydrolysis of cocaine in an alkaline medium since more than 80% of the initial amount of cocaine remains intact after 1 h of incubation of coca leaf powder at pH 11.5. Consequently, the role of lipta or toca consists of providing an alkaline medium in which the cocaine conjugates in leaf material are liberated as the free base, which is absorbed into the bloodstream more readily than the hydrochloride (Beckett and Triggs, 1967) through the lipophilic mucous membrane of the mouth before cocaine is hydrolyzed to benzoylecgonine and ecgonine. Absorption can, however, also occur from the gastrointestinal tract as the saliva containing coca juice is swallowed. Consequently, in the course of ingestion, it is likely that more than one type of absorption is involved.
General conclusions

When using boiling 95% ethanol for extracting the total alkaloids from 
E. coca, no artifacts were observed. By the use of capillary column GC-MS, 
such extracts were resolved without further purification. Cocaine was found 
to be the main alkaloid together with cis- and trans-cinnamoylcocaine. No 
other minor alkaloid previously reported could be detected in the material 
used.

Alkaloid distribution among leaves of various ages in E. coca, E. novogranatense and E. novogranatense var. truxillense could not be used as a 
chemotaxonomic marker since variations within leaves of one species 
exceeded those between leaves of different species.

The buffer capacity of 17 alkaline substances used during coca leaf 
chewing was determined. When incubating coca leaf powder at pH values 
corresponding to the alkaline substances, cocaine hydrolysis did not exceed 
17% after 1 h at 37°C. The role of these alkaline substances during coca leaf 
chewing might be to provide an alkaline medium in the mouth to liberate 
cocaine from the plant material as the free base.

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received from Professor R. Hagnauer and Dr. L. H. Fikenscher.

References

ed.), Cocaine: Chemical, Biological, Social and Treatment Aspects, CRC Press, 
Cleveland, 1976, pp. 15 - 34.
- ishman, G. H., Duke, J. A., Geiszler, W. A. and Farnsworth, N. R., Cocaine content of 
- ter, P. T. and Maseno, R. B., Calcium: unusual sources in the highland Peruvian diet. 
Science, 142 (1963) 1466 - 1467.
- erett, A. H. and Trigg, E. J., Buccal absorption of basic drugs and its application as an 
in vivo model of passive drug transfer through lipid membrane. Journal of Pharmacy 
- rich, R. E., Coca chewing: a new perspective. In V. Rubin (ed.), Cannabis and 

Ciaffi, R., Dosis de alcaloides que ingieren los habitantes a la coca. *Revista de Farmacología y Medicina Experimental*, 1 (1948) 216 - 231.


