ETNOLOGISKA STUDIER

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BY

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CONTRIBUTORS

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GÖTEBORGS ETNOGRAFISKA MUSEUM

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ALKALOID ANALYSES OF BOTANICAL MATERIAL MORE THAN A THOUSAND YEARS OLD

Bo Holmstedt *and* Jan-Erik Lindgren

Department of Toxicology, Swedish Medical Research Council, Karolinska Institutet, Stockholm, Sweden

^{14}C-Determinations were made on the material submitted to us by Henry Wassén of the Ethnographical Museum, Gothenburg, before the leaves and powders were worked up for alkaloid analysis according to the procedure given below. The results are given in table 1. A critical appreciation of the ^{14}C-dating is given by Eskil Hultin (this volume).

TABLE 1

PLANT	FAMILY	CAFFEINE CONTENT	REMARKS
Ilex guayusa No. 70.19.20b		0.1%	
Ilex guayusa No. 70.19.42–47		1.0%	^{14}C-Dated to A.D. 375
Ilex guayusa No. 70.19.52b	Aquifoliaceae	0.2%	
Ilex guayusa No. 70.19.58b		0.4%	
Ilex guayusa Collected at Baños, Ecuador		1.8%	Obtained from Mr. H. V. Pinkley, Botanical Mus., Harvard University, Mass., U.S.A. No. HVP and F.S. 24
Ilex paraguariensis		1.1–1.9%	
Camellia thea	Theaceae	1.5–3.5%	
Coffea arabica	Rubiaceae	1.2%	Ref. 3
Paulinia cupana	Sapindaceae	3–5%	
Theobroma cacao	Sterculiaceae	0.1–0.4%	

MATERIAL AND METHODS

Specimens

Four samples of leaves of *Ilex guayusa* Loes. (Collection numbers of the Ethnographical Museum, Gothenburg, Sweden: 70.19.20b, 70.19.42–47, 70.19.52b, and 70.19.58b.) The leaves were identified by Dr. R. E. Schultes, Botanical Museum, Harvard University, Cambridge, Mass., U.S.A.

Eight samples of powders (Collection numbers of the Ethnographical Museum, Gothenburg, Sweden): *70.19.*2b, 8b, 12d, 13b, 14c, 15d, 16c, and 31b.

^{14}C-Determinations

The determinations were performed in the Laboratory for Radioactive Dating, S-104 05 Stockholm 50 on 7 g of leaves, sample numbers 70.19.42–47 and 70.19.20 b. Numbers of analysis: St 3439 and St 3440.

Isolation of alkaloids

Method 1. The powdered leaves (2–5 g) were extracted with methanol. The dried extract was treated according to a procedure used by Fish *et al.* (1) for the isolation of tryptamine derivatives.

Method 2. The powdered leaves (2–5 g) and 25 ml. of water were boiled gently for 15 min. while stirring. After filtration, the residue was washed with 25 ml. of boiling water. A solution of lead subacetate in water was carefully added to the combined water extract until complete precipitation whereafter the mixture was heated to boiling and filtered. The filter was washed twice with 10 ml. of boiling water. Excess Pb^{+2} was precipitated by the addition of 2N sulphuric acid and the water extract was purified with activated charcoal. The mixture was evaporated under reduced pressure to about 10 ml. and filtered while still hot. The water extract was cooled and extracted 3 times with 10 ml. of chloroform. The combined chloroform extracts were dried with anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure.

Gas chromatography

Gas chromatographic analyses were performed with an F and M Model 400 equipped with a hydrogen flame ionization detection system. A 2 m $\times$ 2.6 mm (i.d.) glass column was silanized, packed with 5% OV-17 (Applied Science Laboratories, State College, Pa.), coated on Varaport-30, 100/120 mesh (Varian Aerograph, Walnut Creek, Cal.). Analyses were made at a column temperature of 190° with a nitrogen carrier gas flow rate of 25 ml.

per min. The vaporizer and detector temperatures were 250°. The amounts of alkaloids were determined by planimetry using caffeine as a standard.

Gas chromatography—mass spectrometry

An LKB model 9000 gas chromatograph—mass spectrometer (LKB Produkter, Bromma, Sweden) was used to confirm the structure of the alkaloid that was found. The separations were made on a 1.6 m×2 mm (i.d.) silanized glass column, packed with 3% OV-17 on Varaport -30 100/120 mesh maintained at a temperature of 200°. The flow rate of helium carrier gas was 20 ml. per min. The ionizing potential and trap current were 70 eV and 60 μA, respectively, and the ion source was kept at 250°.

Thin-layer chromatography

Alkaloidal constituents were separated by thin-layer chromatography on precoated Silica Gel G plastic sheets (E. Merck AG, Darmstadt) with methanol-glacial acetic acid—water (75:10:15) as solvent. Alkaloids were located with iodoplatinate reagent.

RESULTS

Since snuffing tubes and other parafernalia similar to those used by modern Indians were present in the collection, our first thought was that the leaves and powders might contain psychotomimetic phenolic amines. Therefore, the general work-up procedure according to Fish *et al.* (1) was employed (Method 1). All of the powders proved to be void of alkaloids. The extracts of the leaves, however, gave a single iodoplatinate-positive spot on thin-layer chromatogram (Rf=0.6). First programmed and finally isothermal gas chromatography of these extracts gave one main component (Fig. 1). A mass spectrum recorded of this peak showed the fragmentation pattern in fig. 2, upper panel, which could immediately be identified as that of caffeine (2). Subsequently, the extraction procedure was therefore changed to suit material containing caffeine better (Method 2).

The amount of caffeine in the leaves found has been tabulated together with a newly collected specimen of *Ilex guayusa* and some other well-known caffeine containing plants in table 1. By comparison, the caffeine content in the old sample of leaves from *Ilex guayusa* has stood up well against time.

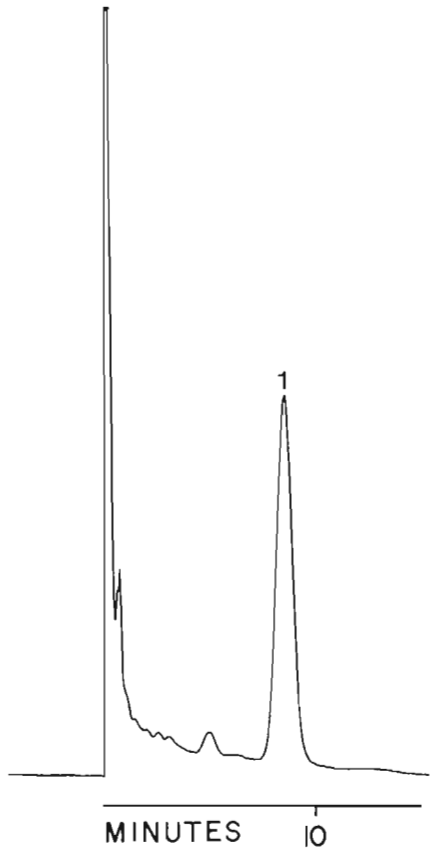

Fig. 1. Gas chromatographic analysis of extract from leaves of *Ilex guayusa* (Collection no. 70.19.42–47).

Conditions: see Materials and Methods.

We do not know the origin of the various powders analyzed. Regardless of their origin it is not surprising that the greater surface area of the powders after such a long time should have caused the destruction of any alkaloids present.

To our knowledge, the finding and quantitation of caffeine in the leaves from *Ilex guayusa* ^{14}C-dated to the fourth century represents results from the oldest material ever submitted to an alkaloid analysis.

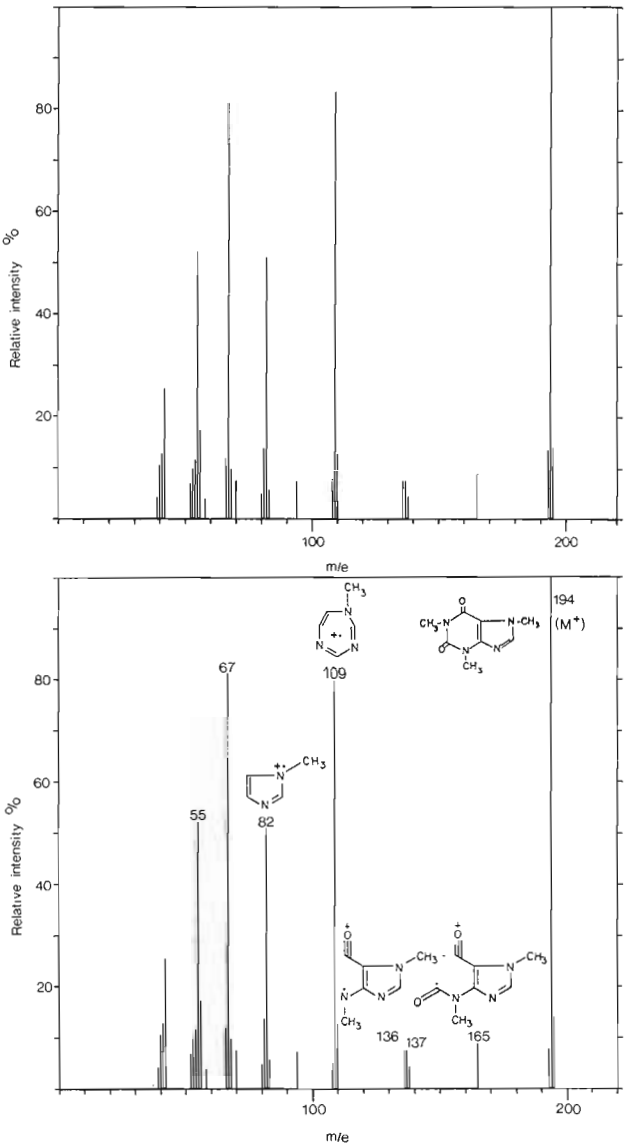

Fig. 2. Mass spectra and fragmentation pattern for (upper panel) major peak in extract from leaves of *Ilex guayusa* (Collection no. 70.19.42–47) and (lower panel) reference compound.

Conditions: see Materials and Methods.

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