

THE EFFECTS OF FIELD PRESERVATION ON ALKALOID CONTENT OF FRESH COCA LEAVES (*ERYTHROXYLUM* SPP.)

MICHAEL J. BALICK

The New York Botanical Garden, Bronx, New York 10458 (U.S.A.)

LAURENT RIVIER

Institute of Plant Biology and Physiology of the University, 6 Place de la Riponne, 1005 Lausanne (Switzerland)

TIMOTHY PLOWMAN

Botany Department, Field Museum of Natural History, Chicago, Illinois 60605 (U.S.A.)

(Received November 1, 1981; accepted January 29, 1982)

Summary

In order to test the effects of commonly used preservation agents on the alkaloid content of herbarium specimens, fresh leaves of *Erythroxylum coca*, *E. novogranatense*, and *E. novogranatense* var. *truxillense* were air- or heat-dried or treated with six different liquid preservatives. The leaves were then extracted and analyzed quantitatively for cocaine content. Leaves which were soaked in preservatives showed appreciable pre-extraction of cocaine and probably of other alkaloids. The results compare well with a similar experiment conducted on flavonoid content of the leaves of a palm *Jessenia bataua*. If portions of herbarium specimens are to be useful for phytochemical screening using microtechniques, at least part of the collection must be air- or heat-dried to retain the chemical constituents.

It is common practice to screen plant materials for certain chemical constituents. In addition to shedding light on taxonomic relationships among plants, new compounds from plants are of great potential value as medicines, insecticides, poisons, or as industrial raw materials.

Phytochemical screening programs employing microanalytical methods now may make use of small fragments of herbarium specimens as the primary source of plant material. Herbarium specimens offer the advantages of being readily available, for the most part are correctly identified, and have a cited locality which would facilitate recollection of additional material should the plant prove to be of interest. And, most importantly, the original specimen serves at once as a voucher for future corroboration of the identity of the plant.

Herbarium specimens can retain their chemical constituents virtually unchanged for several hundred years (Holmstedt and Lindgren 1972; Bruhn *et al.*, 1976, 1978). However, it has been found that some specimens, collected during the last four decades or so, upon analysis yield few or none of the expected chemical compounds (Schultes *et al.*, 1969, 1977). Often the explanation for the negative results in species which should be positive lies in the method of field collection of the plant specimen and its subsequent handling.

In recent years, there has been a great increase in the use of chemical preservatives during the collection of herbarium specimens, especially in the tropics where collecting activities are now most intensive. Such preservation eliminates the need for immediate, on-site drying during valuable field time, and frees the botanist from carrying bulky driers, blotters, corrugates and heaters which would otherwise be necessary. In addition, insect and fungal damage are minimized.

Various preservation regimes have been suggested in the literature. Hodge (1947) recommended treating fresh specimens with an ethanol mixture. Schultes (1947) considered formaldehyde as a useful preservative with similar properties; Moore (1950) suggested hydroxyquinoline sulfate for the same purpose. At present, individual botanists employ a variety of preservation agents during fieldwork, depending on what is available and considered effective under particular conditions. Most frequently, preservatives now contain some percentage of formaldehyde, or ethanol, or both.

Cooper-Driver and Balick (1978) noted the absence of flavonoid profiles in certain herbarium specimens of palms and suspected the prior treatment of the specimens with chemical agents. An experiment was undertaken using leaves of the palm *Jessenia bataua* (Mart.) Burret which subsequently demonstrated that treatment with dilute alcohol and/or formaldehyde effectively pre-extracted the phenolic compounds from the leaves. Preservative-treated material contained only about 10 - 20% of the total phenolics known to be present in the plant compared with untreated controls. These results compare favorably with a similar study of the flavonoids in seventeen species of plants which had been previously chemically preserved (Coradin and Giannasi, 1980).

The implications of these results for the study of other important plant constituents were obvious: it appeared of immediate interest to us to determine the effects of field preservation of alkaloid-containing plants.

As with the previous experiment mentioned above, plants of a single genus were selected for study. We chose two species of the known alkaloid-bearing genus *Erythroxylum*, the sole source of coca leaves and the medicinal alkaloid cocaine. Fresh leaf material was collected from greenhouse-grown plants of three of the cultivated varieties of coca [*Erythroxylum coca* Lam., *E. novogranatense* (Morris) Hieron. var. *novogranatense*, and *E. novogranatense* var. *truxillense* (Rusby) Plowman]. The leaves were subjected to the following treatments in order to simulate the procedures which have been most often used in botanical collecting: (1) sun-dried; (2) dried in an

herbarium drier at 105 °F (40 °C); (3) soaked in water; (4) soaked in 95% ethanol; (5) soaked in white rum (86 proof, approx. 44% ethanol, (6) soaked in FAA (ethanol–glacial acetic acid–formalin, 90:5:5); (7) soaked in formalin, approx. 40% formaldehyde) and water in a 1:1 mixture; (8) soaked in pure formalin.

The material for treatments 3 - 8 was stored in the dark for four weeks at room temperature, and the leaves were then removed from the solutions, air-dried at room temperature, and submitted for analysis for the alkaloid cocaine.

Experimental

Approximately 100 mg dry weight of powdered leaf were extracted with ethanol, and the cocaine content quantified using deuterated cocaine as internal standard (Rivier, 1981). Each plant specimen was analysed three times; sample variation was not higher than 2%.

Results

Table 1 lists the results of the various treatments on the three main varieties of cultivated cocas in which the cocaine content of the leaves was measured.

TABLE 1

Cocaine content (mg/g dry weight) — determined by mass fragmentography — remaining in the leaf of material following the various treatments of field preservation

Treatment	<i>E. coca</i> Bolivia Plowman 6288*	<i>E. novogranatense</i> var. <i>novogranatense</i> Colombia Plowman 6179*	<i>E. novogranatense</i> var. <i>truxillense</i> Peru Plowman 6222*
1. Sun-dried	2.78	1.83	3.24
2. Herbarium-dried	2.40	2.15	3.43
3. Water	0.63	0.40	0.49
4. 95% Ethanol	0.32	0.31	0.39
5. White rum	0.37	0.47	0.47
6. FAA (see text)	0.40	0.48	0.41
7. Formalin–water (1:1)	0.45	0.81	0.44
8. Formalin	0.38	0.49	0.45

*Voucher specimens of the plant collections are deposited at the Economic Herbarium of Oakes Ames, Harvard University, Cambridge, Massachusetts, and at the Field Museum of Natural History, Chicago, Illinois.

Discussion

In a manner comparable to the earlier study on flavonoid content cited above, the alkaloid cocaine was found to be pre-extracted from the leaf specimens by ethanol, formaldehyde and water-based preservatives. In general, sun or herbarium drying is necessary to ensure that cocaine and presumably other non-volatile alkaloids are retained within leaf material of *Erythroxylum*. We would expect the same to hold true for most other alkaloid-containing plant material.

Pretreatment with any of the commonly employed field preservatives retained only a fraction of the original cocaine content of each of the three varieties of coca leaves. Thus, if herbarium specimens of these and of other medicinal plants are to play a useful role in the discovery of new chemical compounds, the recommendations of Cooper-Driver and Balick (1978) should be followed. These suggest that herbarium material be plainly marked on the label as to whether it has been chemically treated in the field, for what length of time the treatment has been made, and with what materials. In this way, the utility of specimens for chemical study can be established. In addition, it is recommended that 10 - 50 grams of leaf material (and/or other relevant plant parts such as flowers, bark or roots) be sun- or blotter-dried separately, and later affixed in a packet or bag to the original specimen for future chemical analysis.

In view of the alarming rate of destruction of tropical forests, which are known to be rich and dynamic chemical storehouses, it is important when collecting potentially valuable plants that the entire collection not be rendered useless for future chemical work by destructive preservation treatments. A small extra effort in the field to collect specimens properly might result in the future discovery of new compounds of immense value.

References

- Bruhn, J. G., Holmstedt, B., Lindgren, J. E., and Wassén, S. H., The tobacco from Niño Korin: identification of nicotine in a Bolivian archeological collection. *Goteborgs Etnografiska Museum Arstryck*, (1976) 45 - 48.
- Bruhn, J. G., Lindgren, J. E., Holmstedt, B. and Adovasio, J. M., Peyote alkaloids: identification in a prehistoric specimen of *Lophophora* from Coahuila, Mexico. *Science*, 199 (1978) 1437 - 1438.
- Cooper-Driver, G. A. and Balick, M. J., Effects of field preservation on the flavonoid content of *Jessenia bataua*. *Botanical Museum Leaflets, Harvard University*, 26 (1978) 257 - 265.
- Coradin, L. and Giannasi, D. E., The effects of chemical preservation on plant collections to be used in chemotaxonomic surveys. *Taxon*, 29 (1980) 15 - 26.
- Hodge, W. H., The use of alcohol in plant collecting. *Rhodora*, 49 (1947) 207 - 210.
- Holmstedt, B. and Lindgren, J. E., Alkaloid analyses of botanical material more than a thousand years old. *Etnologiska Studier*, 32 (1972) 139 - 144.

- Moore, H. E., Jr., A substitute for formaldehyde and alcohol in plant collecting. *Rhodora*, 52 (1950) 123 - 124.
- Rivier, L., Analysis of alkaloids in leaves of cultivated *Erythroxylum* and characterization of alkaline substances used during coca chewing. *Journal of Ethnopharmacology*, 3 (1981) 313 - 335.
- Schultes, R. E., The use of formaldehyde in plant collecting. *Rhodora*, 49 (1947) 54 - 60.
- Schultes, R. E., Holmstedt, B. and Lindgren, J. E., De plantis toxicariis e mundo novo tropicale commentationes III: Phytochemical examination of Spruce's original collection of *Banisteriopsis caapi*. *Botanical Museum Leaflets, Harvard University*, 22 (1969) 121 - 132.
- Schultes, R. E., Holmstedt, B., Lindgren, J. E. and Rivier, L., De plantis toxicariis e mundo novo tropicale commentationes XVIII: Phytochemical examination of Spruce's ethnobotanical collection of *Anadenanthera peregrina*. *Botanical Museum Leaflets, Harvard University*, 25 (1977) 273 - 287.