Cannabis sativa L. (Marijuana) II: Standardized and Reliable Microscopic Method for Detection and Identification of Marijuana

Keyphrases ☐ Marijuana—microscopic identification, differential staining with fast blue B ☐ Cannabinoid plant tissues—microscopic identification, differential staining with fast blue B ☐ Fast blue B chromogenic reagent—used to identify marijuana ☐ Chromogenic reagents, fast blue B—used to identify marijuana

Sir:

This preliminary communication describes a highly reliable and easily performed procedure for the microscopic identification of marijuana[1].

It is considered desirable for an investigator to utilize one or more chemical methods as well as the microscopic examination of suspect material to confirm the presence or absence of marijuana (1). Although several chemical test procedures have been reported for this purpose (2), detailed methods dealing specifically with the microscopic detection of marijuana have been lacking in the scientific literature.

The positive microscopic identification of marijuana in a particular sample is based on observing the presence of certain morphological structures such as the glandular hairs, cystolith hairs, and nonglandular hairs (3) which are characteristic and diagnostic for *Cannabis sativa*. However, these diagnostic criteria may be complicated by the fact that marijuana is frequently admixed prior to use with a variety of nonmarijuana plant substances including tobacco (*Nicotiana tabacum*), lavender (*Lavendula officinalis*), catnip (*Nepeta cataria*), and oregano (*Origanum vulgare*). Indeed, in many instances we have examined plant material presumed to be marijuana but which instead proved to be composed entirely or in part of catnip, oregano, or lavender. The latter three plant species, as do many other members of the Labiatae (mint family), exhibit glandular hairs which, particularly if they are crushed and fragmented, may be confused with the glandular hairs of marijuana (4). Furthermore, Nakamura (1) recently described more than 80 different plant species containing cystolith hairs similar to those found in marijuana.

The present study was undertaken to develop a microscopic method that would serve to distinguish marijuana unequivocally from various other plant species having similar morphological features. This method, outlined in stepwise fashion, is presented below:

1. Thoroughly mix a small amount of suspect material (1 mg. or less) with 2 drops of clearing solution [chloral hydrate (75 g.), propylene glycol (10 ml.), and distilled water (sufficient quantity to make 100 ml.)] on a clean microscope slide.

2. Carefully heat the slide from below, using a microburner, until the mixture boils for a total of 3 sec. Cool momentarily, add 1 additional drop of clearing solution, and heat to boiling as before. Allow the slide to cool for 1 min. before proceeding. The chloral hydrate clearing solution serves to dissolve starch and plant pigments and thus allows the diagnostic elements to be more readily observed.

3. Treat the wet mount with 2 drops of *freshly prepared* chromogenic reagent [fast blue B salt[2] (0.3 g.) and clearing solution (sufficient quantity to make 100 ml.)], mix well, cover the preparation with a glass (not plastic) coverslip, and examine the prepared mount microscopically (100–400× magnification). It has been shown that fast blue B salt couples with cannabinoid substances to yield characteristic red to purple-colored compounds (5). Accordingly, upon microscopic examination, cannabinoid-rich plant tissues will be observed to acquire a red-purple color. This phenomenon is particularly intense in the case of the marijuana glandular hairs. Furthermore, when one is dealing with a marijuana-containing sample, the entire wet mount rapidly acquires the characteristic red-purple color which is easily observed with the naked eye.

4. Finally, place 1 drop of glacial acetic acid along one edge of the coverslip, draw the acid beneath the coverslip by touching the opposite edge of the coverslip with a piece of filter paper, and observe microscopically. The presence of cystolith hairs is evidenced by the liberation of gas (carbon dioxide) bubbles from the bases of these calcium carbonate-containing hairs.

The most significant aspect of this procedure concerns the fast blue B chromogenic reagent. The use of this reagent allows the marijuana glandular hairs as well as other cannabinoid-containing elements to be differentially stained. Thus, for example, lactiferous tissue and fragments of resin both acquire the characteristic red-purple color. To assess the possibility of false-positive fast blue B reactions, we studied a large number of different noncannabis plant species, including many having glandular hairs, using the described procedure. In no case did we observe staining of the glandular hairs, although in certain instances we noted that some plant tissues or the entire wet mounts acquired diffuse pink to red-brown colors following treatment with fast blue B reagent. These colors, probably the result of the reaction of fast blue B with plant phenolic substances, were distinct and could usually be differentiated from the characteristic red-purple colors observed with the marijuana samples. Thus, under the experimental conditions described, it appears that fast blue B selectively stains cannabinoid-containing plant tissues.

On the basis of the consistently reliable results obtained from evaluating more than 300 different plant species, we have found this easily performed method to be highly reliable for confirming the presence or absence of marijuana in suspect material. The method should find wide application in the area of forensic science. Further studies utilizing this method together with TLC will be reported subsequently.

[1] The term marijuana as used herein refers to the ground stems, leaves, and flowering tops of *C. sativa*.

[2] *o*-Dianisidine diazotate, K & K Laboratories, Plainview, NY 11803

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(2) R. Mechoulam, *Science*, **168**, 1159(1970).
(3) B. P. Jackson and D. W. Snowdon, "Powdered Vegetable Drugs: An Atlas of Microscopy for Use in the Identification and Authentication of Some Plant Materials Employed as Medicinal Agents," American Elsevier, New York, N. Y., 1968, pp. 62–65.
(4) H. W. Youngken, Sr., "Textbook of Pharmacognosy," Blakiston, Philadelphia, Pa., 1950, pp. 706–749.
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We dedicate this paper to Professor Maynard W. Quimby, University of Mississippi, on the occasion of his appointment to the position of Senior Scientist and Emeritus Professor of Pharmacognosy.

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BOOKS

REVIEWS

Biochemical Applications of Mass Spectrometry. Edited by G. R. WALLER. Wiley, 605 Third Ave., New York, NY 10016, 1972. xiv + 872 pp. 21 × 28 cm. Price $49.95.

The editor and authors of this encyclopedic work have succeeded admirably in assembling into one volume a large amount of theoretical and practical material.

The book is divided into five parts. Part I is an introduction and historical survey. Part II covers the different types of spectrometers and contains an exhaustive section on data acquisition and processing by most of the world's principal mass spectrometric laboratories.

About 80% of the book is devoted to the interpretation of mass spectra. These parts, consisting of 28 chapters, provide both the novice and experienced investigator with a wealth of information on mass spectrometric applications to the entire range of biochemicals and compounds commonly classified as natural products. In addition, there are excellent chapters on the "Origin of Mass Spectra," how to interpret metastable ions, and field and chemical ionization mass spectrometry. Interdisciplinary areas such as those relating to drug metabolism and clinical applications are also covered.

This well-edited volume is about 60% tables and illustrations. The mass spectra are uniformly and clearly presented. Each chapter is adequately, but not excessively, referenced. A good working index is present so that subjects not discussed under a separate chapter, *e.g.*, mass fragmentometry and GC–MS systems, can be quickly found. If anything, the book suffers somewhat from covering the literature *only* through 1969, since it was that long in preparation. Attempts have been made to update, in proof, some of the newer areas like chemical ionization mass spectrometry by the citation of more recent references.

In summary, the book should be useful to investigators working in any of the areas allied with natural products and as a reference work for others using mass spectrometry as an analytical tool. Insofar as it is not out of date, it represents a how-to manual on the subject of data aquisition. As a final point, one would be remiss in not mentioning the efforts of the editor and various contributors in presenting the history and background of the entire field.

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Methods and Techniques in Clinical Chemistry. By P. L WOLF, D. WILLIAMS, T. TSUDAKA, and L. ACOSTA. Wiley, 605 Third Ave., New York, NY 10016, 1972. 417 pp. 14.5 × 23 cm. Price $11.50.

This publication compiles all of the currently significant chemical tests being utilized by the Stanford Hospital Clinical Laboratory. The tests are given in alphabetical order; each test monograph includes a discussion of the principle involved, reagents and equipment, procedure, normal values, references, and clinical interpretation. Throughout the book, the authors have emphasized the practical aspects of clinical chemistry.

Staff Review ■

NOTICES

Proceedings of the International Symposium on Gas Chromatography–Mass Spectrometry. Organized by the Istituto di Richerche Farmacologiche "Mario Negri" of Milan. Edited by ALBERTO FRIGERIO. Tamburini Editore s.p.a. Milano, Italy, 1972. 505 pp. 17 × 24 cm.

Biological Oxidation of Nitrogen in Organic Molecules, Proceedings of the Symposium held at Chelsea College, London, December 1971. Edited by J. W. BRIDGES, J. W. GORROD, and D. V. PARKE. Halsted Press, Wiley, 605 Third Ave., New York, NY 10016, 1972. 269 pp. 17 × 25 cm. Price $21.00.

International Directory of Investigators in Psychopharmacology. U. S. Department of Health, Education, and Welfare, National Institute of Mental Health, 5600 Fishers Lane, Rockville, MD 20852, 1972. 439 pp. 20 × 26 cm.

Die Starke in der Medizin. By JOHANNES SEIDEMANN. Paul Parey, Berlin 61, Lindenstrasse 44–47, Germany, 1972. 92 pp. 16 × 24.5 cm. Price DM 38. (*German*)

Organic-Chemical Drugs and Their Synonyms, Volumes I and II. By MARTIN NEGWER. Akademie-Verlag, DDR 108 Berlin, Leipziger Str. 3–4, Germany, 1971. 1216 pp. 16 × 24 cm. (*German*)

Terpenoids and Steroids, Volume 2, A Specialist Periodical Report. Senior Reporter, K. H. OVERTON. Chemical Society, Blackhorse Road, Letchworth, Herts, SG6 1HN, England, 1972. 450 pp. 13 × 21.5 cm. Price £9.00.