Marijuana Identification: A Test for Calcium in Cystolithic Hairs

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The identification of marijuana (Cannabis sativa) is a routine analysis in many forensic laboratories. An important part of this analysis is the identification of trichomes, commonly called cystolithic hairs[1], on plant particles. These hairs contain calcium carbonate. The very word cystolithic derives from this fact, coming from the Greek *kustis* and *lithos* meaning, "bag of stones"[2].

Trichomes are not unique to marijuana but are common on many species of plants. Thus, the mere presence of trichomes on a leaf surface is not sufficient. A required step in the identification of marijuana is the microscopical characterization of the cystolithic hairs present on the leaf fragments.

Two major types of cystolithic hairs are found on the marijuana leaf. The larger warty protuberances commonly called "bear claws" that appear on the upper surface of the leaf, and the longer non-glandular hairs, that appear on the under side of the leaf[2]. Both of these trichome types contain calcium carbonate. The arrangement of calcium carbonate in the cystolithic hairs of marijuana is consonant. It appears as a grain in the base of the hair[3].

Very little attention is paid to the identification and no attention to the mapping of the calcium carbonate in the analytical methods employed in crime laboratories. The forensic method typically used to check for the presence of calcium carbonate in cystolithic hairs is to add dilute HCl to the leaf fragments and observe effervescence[2]. Aside from the fact that even this simple test is not commonly done, it is not specific for either calcium or carbonate, nor does it enable mapping of its placement.

Below we present a simple method that gives a red color reaction for the calcium ion. It allows for a more specific identification of the calcium in the calcium carbonate and also the concurrent mapping of the calcium deposits in the cystolithic hairs. The method is a modification of a test for soluble calcium salt detailed by Feigl[4].

Materials and Method

Leaf fragments of marijuana were pulverized on the surface of a microscope slide with a flat clean object. We commonly use the back of a pair of forceps, a scalpel blade, or a spatula. The reagent, below, is added to the pulverized leaf fragments. The material on the slide is then examined by transmitted light microscopy at a magnification of 200 diameters. No mounting or coverslip is used, and the slide is normally observed while wet.

The test reagent is prepared by dissolving 10 mg. of Alizarin Red S Monohydrate in 2 ml. of ethanol (the Alizarin only partially dissolves) and then adding 1 ml. of distilled water. We have found the reagent to be stable, and that we can approximate the 10 mg. "by eye".

Results and Discussion

A red color forms in the calcium deposits in a few seconds. This color is stable and remains even after the test solution has evaporated. Examples of the reaction and the placement of calcium carbonate in the major types of cystolithic hairs of marijuana are seen in Figures 1 and 2.

In our experiments we discovered that the calcium is generally found as a grain in the base of the cystolithic hairs. This is in agreement with the literature[3]. We also found tip fragments of cystolithic hairs that contain a small bit of calcium at their base. This may be evidence of a fracture line going through the wall of the hair at the tip of the calcium carbonate grain.

In many hairs, we found calcium as a sporad[illegible]ng the shaft of the cystolithic hairs. It also appeared occasionally at the tip, (generally a broken tip), of some of the hairs. While this may indicate that calcium carbonate is a structural part of cystolithic hairs from marijuana in areas other than the base, we feel that it is more likely the result of the softness of calcium carbonate. Like chalk, once it is exposed and subject to mechanical stress, it "powders" and spreads profusely. ■

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3. Azoury, M. Hashish; Division of Identification and Forensic Science, Jerusalem, 1989 (Hebrew).

4. Feigl, F., V. Anger and R.E. Oesper *Spot Tests in Inorganic Analysis*; Elsevier Publishing Co.: London, 1972.

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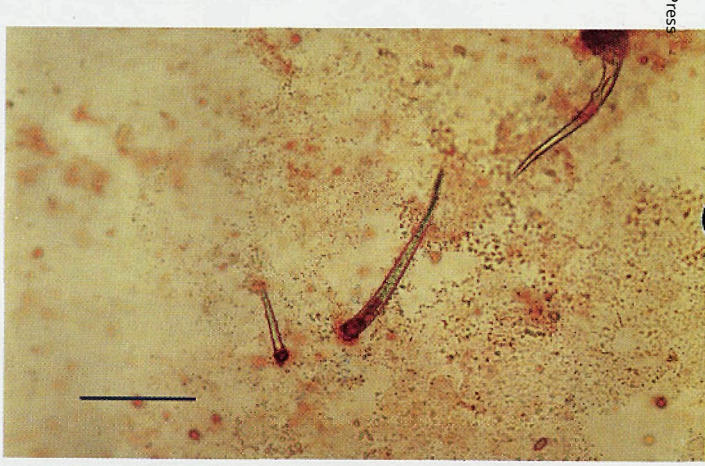

Figure 1: A "bear claw" from marijuana, Magnification at the microscope is 200 diameters. (Scale bar = 100 microns)

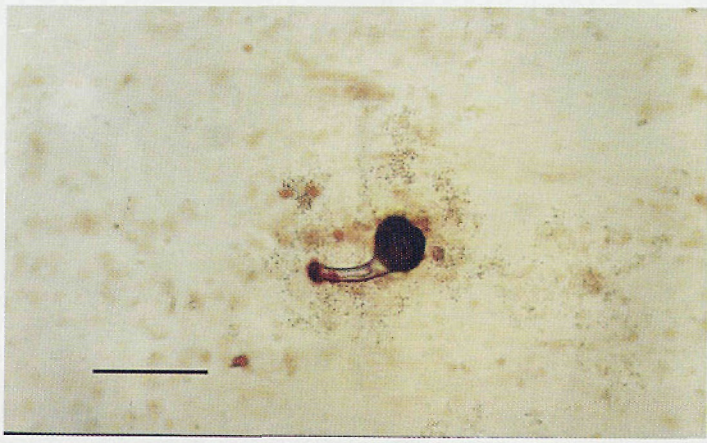

Figure 2: Central hair is a non-"bear claw" cystolith from marifuana. Magnification at the microscope is 200 diamters. (Scale bar = 100 microns