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Microscopic Morphology of Marijuana Ash

The common methods for the identification of marijuana depend on the chemical reactions of certain of the resin components with various color reagents or upon an examination of the leaf fragments and other parts of the plant under the stereoscopic-binocular microscope. Comprehensive studies of both chemical [*1,2*] and morphological [*3*] methods have led investigators to conclude that the two approaches are complementary. A combination of the two provides experienced analysts with a very reliable means for identifying *cannabis* fragments [*4*].

Certain advantages are obtained by studying the plant's microscopic morphology using a scanning electron microscope (SEM). The increased depth of field and the high resolving power attainable with this instrument greatly facilitate the examination [*5,6*]. The resin can be further characterized by obtaining a chromatographic separation [*7,8*] of the resin components before the chemical color reagents are employed. These refinements are not often required in routine analyses, although there seems to be a recent trend on the part of some drug chemists to substitute thin-layer chromatography for a rigorous morphological examination.

When cigarette butts (roaches) or pipe bowls are encountered as evidence, the usual procedure is to attempt to recover enough unburned material so that the conventional testing methods can be applied. These methods are excellent with unburned material but cannot be applied to samples which have been burned completely. It would seem that another approach which could be used to complement existing methods (or tried where these methods fail) would be desirable [*9*]. The development of a method for identifying the ash itself should be useful in a number of circumstances. Such a procedure could, for example, be used to demonstrate the presence of marijuana where the organic portion has been destroyed by burning.

Methods and Materials

The ash is readied for examination by spreading or sprinkling it over a layer of Canada balsam or other similar mountant which has been placed on the sample area of a conventional microscope slide. A cover glass is then applied. The cover glass should be carefully selected so that it is the same thickness as that for which the objective is corrected (normally 0.17 mm), especially if examination at higher powers with dry objectives is anticipated. The sample prepared in this manner is then ready for microscopic study.

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If it is necessary to ash some known plant material for comparison studies, this can easily be accomplished by placing a small fragment of the substance in a micro-crucible or on a slighly concave piece of platinum foil. This preparation is then placed over a small flame or in a muffle furnace. A stainless steel scoop or aluminum foil can be substituted for platinum foil. However, aluminum foil is less satisfactory when used with a flame, because it melts and oxidizes rather easily. During the early phase of the heating, smoke will be evolved. With further heating the sample will glow red until the oxidation is complete. At this point a whitish ash is all that will remain and the preparation can be cooled.

If the sample received as evidence is only partially ashed, a portion of it can easily be subjected to the above procedure to completely remove the remaining organic material. In such a case the odor of the smoke evolved should be noted. If the sample is quite small, it should be tested using the conventional methods prior to ashing.

Results

Marijuana retains a remarkable amount of structure when it is ashed. The investigator gains the initial impression that the more characteristic features are retained at the expense of those that are less useful. The bear-claw shaped hairs survive with little alteration and are the most apparent features of the preparation, although other details are seen, some of which are not readily apparent in the normal examination of the dried plant material. These may be derived from structures present in the interior of the leaf and, thus, would not be seen during an examination of the surface features under the stereoscopic-binocular microscope.

Over fifty botanical materials have been ashed and compared with marijuana by this method. The vast majority of these bear no resemblance to marijuana ash, although some of these in their native states could easily be confused with dried *cannabis* fragments by inexperienced examiners. Table 1 is a list of hair bearing plant materials whose ashes retained no residual hair structure. Table 2 lists species of plants whose hairs survived the ashing process but whose ash morphology was grossly different from that of *cannabis*. A cursory inspection was sufficient to make the distinction in each of these cases. The plant materials listed in Table 3 yielded ashes which presented a little more difficulty. These ashes were quite different from marijuana ash, but a more detailed examination was necessary to demonstrate this. Even here, however, distinctions could be made once some experience in studying the ashes was gained. Approximate size ranges of the various types of hairs seen in each of these preparations are also presented in this table. These are included for illustrative purposes only. It is not intended that they be used as identification criteria, as the measurements were obtained from a limited number of samples of each

TABLE 1—*Samples of hair bearing plants in which the hairs did not survive ashing.*

Acanthus mollis	*Thymus vulgaries*
Acer japonica	*Ulumus crassifolia*
Citharexylum	*Vites agnus-castus*
Eucalyptus globulus	
Ficus elastica	Begonia
Nepeta cataria	Buckthorn flowers
Ocimum basilicum	Geranium
Origanum marjoram	Oregano
Origanum vulgare	Peppermint
Salvia officinalis	Summer savory
Satureia montana	

TABLE 2—*Samples of hair bearing plants in which the hairs survive the ashing but are grossly different from those of cannabis.*

Aloysia virgata	*Ficus repens*
Anchusa officinalis	*Lippia citriodora*
Blumenbachia insignis	*Lithospermum officinale*
Boehmeria longis pica	*Lithospermum purpureo-coeruleum*
Celtis caucasica	*Thunbergia alata*
Dorstenia yambagaensis	*Verbascum thapsus*

species. Other structures present in these ashes (some of which appear to have no counterpart in the normal examination of the dried plant material) were as helpful as the differences in hair morphology in discriminating between *cannabis* and the other plants listed in Table 3. Comparison of the photomicrographs (Figs. 1–6) illustrates the ease with which many of these differentiations can be accomplished. However, it should be obvious that reference to photomicrographs is not a substitute for actual microscopic observations.

TABLE 3—*Samples of hair bearing plants in which the hairs survive and are somewhat similar to those of* cannabis.

Cannabis small clothing hairs, 70–90 μm large clothing hairs, 150–300 μm cystolith hairs, 100–200 μm	*Ficus pumica* short hairs, 50–200 μm long hairs, 200–400 μm
Boehmeria nivea small hooked hairs, 20–50 μm large hairs, 250–450 μm and larger	*Humulus japonica* short hairs, 20–100 μm long hairs, 200–400 μm
Broussonetia papyrifera small hooked hairs, approximately 10 μm small hairs, 30–70 μm long hairs, 300–450 μm	*Humulus lupulus* 50–200 μm
Celtis occidentalis 180–320 μm	*Lantana camara* 100–250 μm
Cordia superba 50–110 μm	*Lantana montevidensis* short hairs, 140–200 μm long hairs, 300–600 μm
Ficus garcia 100–250 μm	*Symphytum officinale* 90–250 μm
	Urtica dioica 80–200–450 μm
	Urtica urens 170–300–900 μm

The use of the Latin names for the species in the tables indicates that these samples have been authenticated by a qualified botanist or other expert. The samples that are listed with only their common names have been obtained from reasonably reliable sources but they have not been rigorously authenticated.

Examination of the mounted ash preparations between crossed polarizers was found to be useful in a number of instances. A rudimentary polarizing microscope, fashioned by adding a simple polarizer and analyzer to a conventional microscope, sufficed. The ashes of some botanicals contained birefringent structures. For example, many of the hairs of *Lantana montevidensis* were highly birefringent whereas most *cannabis* hairs exhibited little or no birefringence. Where birefringence was observed with *cannabis* hairs it was usually confined to an area near the tip and appeared to arise from inclusions at this location. *Ficus pumica* ash was observed to contain a particularly characteristic gridlike pattern of birefringent granules but the hairs were isotropic. Interesting birefringent features were also noted in a number of species in addition to the two examples cited here.

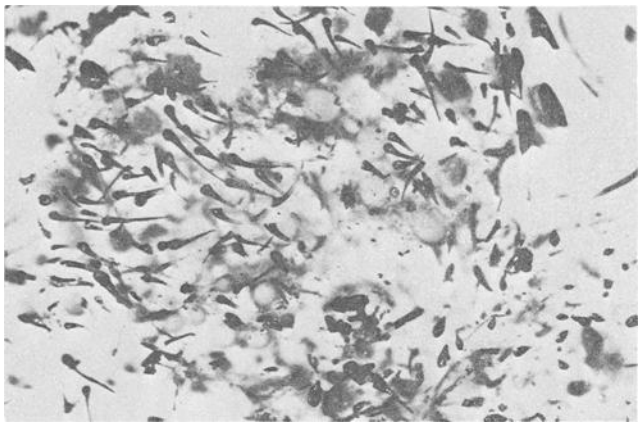

FIG. 1—Cannabis, *original magnification* ×*125*.

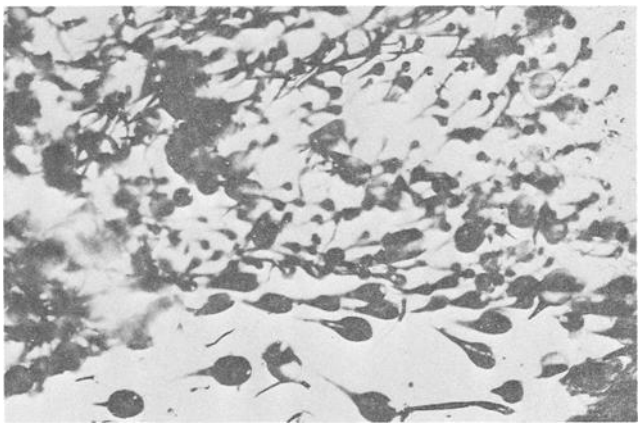

FIG. 2—Cannabis, *original magnification* ×*125*.

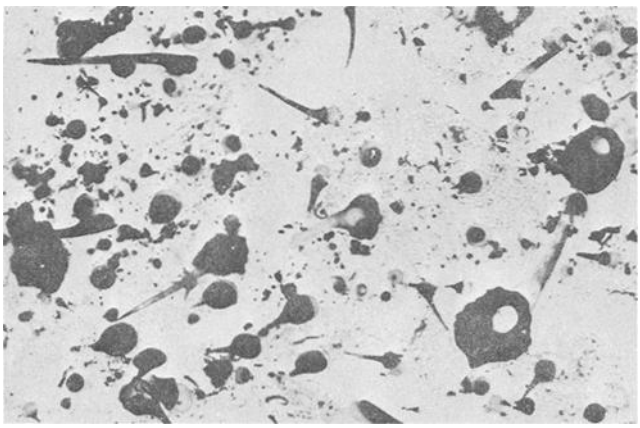

FIG. 3—Humulus japonica, *original magnification* ×*125*.

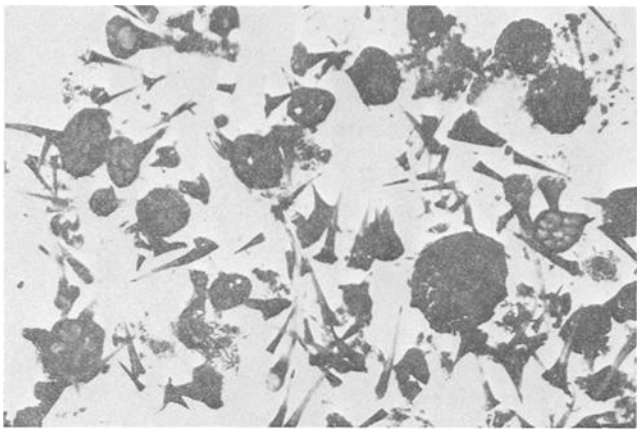

FIG. 4—Lantana camara, *original magnification* ×*125*.

FIG. 5—Broussonetia papyrifera, *original magnification* ×*125*.

FIG. 6—Urtica dioica (*between slightly uncrossed polarizers*), *original magnification* ×*125*.

Discussion and Conclusions

The results have shown that the microscopic morphology of marijuana ash is quite distinctive. Few other botanical materials studied here yield ashes with appearances that would be confused with *cannabis* by a reasonably experienced analyst. Most of these ashes are in fact grossly different from marijuana ash.

One potential advantage of examining the mounted ash preparation is that all the features to be examined can be constrained to essentially a single plane, which makes examination at higher magnifications feasible. This would allow smaller features to be studied more readily than is possible with the conventional methods of examination. The use of high power vertical or "epi" illumination suggests itself as a means of gleaning additional information from the same preparation. Commercial "epi-illuminators" are available which allow alternate or simultaneous use of vertical and transmitted illumination. Although this approach might be useful, it is possible that the expense associated with such an illuminator is not warranted by the limited amount of additional information gained. A preliminary attempt to answer this question using a Nikon "epi-illuminator" led to equivocal results. The Nikon unit was one of the so-called "bright field" types. Reflections from the cover glass resulting in glare made the assessment more difficult. A "dark-field epi-illuminator" such as the Leitz "Ultropak" might yield better results. Where equipment of this type is available it should be evaluated by the analyst. For low-power examination of certain samples a conventional stereo microscope illuminator aimed obliquely at the upper side of the preparation has been found to be generally useful.

Examination of ash morphology could be utilized to identify marijuana ash *per se* or used as an adjunct to the other more conventional means of identification. The need to identify marijuana ash, as such, might arise in connection with the examination of items of evidence such as pipe bowls, ash trays, and clothing. In some jurisdictions the presence of marijuana ash in conjunction with the dried plant material could be construed to indicate possession or use of marijuana or both. Other jurisdictions require the demonstration of "useable" or "saleable" quantities of *cannabis* in order to prove possession. In this latter event the method described here would have little to offer except possibly as a confirmatory procedure. The particular plant components and derivatives proscribed by the law vary widely. An interesting example of this is given by Clarke and Robinson [*10*].

An identification of an unknown ash as marijuana could yield important data in the nature of an investigative aid as part of an overall inquiry. If a cache of marijuana were burned to avoid discovery, the demonstration of a quantity of marijuana ash might be an important element of the investigation. Similarly, finding a concentration of marijuana ash in the residue resulting from a structural fire would suggest a possible motive for arson. More generally, an inquiry where marijuana ash assumed importance might be unrelated to the question of drug use.

The relative success experienced here in characterizing marijuana ash suggests that other ashes found as evidence could yield information concerning their origins. The need for further research in this area is indicated. The scanning electron microscope could be profitably employed in such studies. It is reasonable to expect that the SEM could reveal surface details of marijuana and other ashes that are not seen in the method described here.

Pyrolysis-gas chromatography suggests itself as a useful adjunct to the examination of ash morphology when partially burned *cannabis* fragments are encountered. With limited sample sizes the residue remaining after pyrolysis could be ashed and examined. Further study would seem to be warranted to ascertain whether or not this technique would be useful with samples of this type.

Although the appearance of *cannabis* ash is quite distinctive, we urge caution in making positive identifications until an examiner has gained a good deal of experience with other similar ashes. Care is also necessary to make certain that the comparison samples are representative of the different anatomical regions of each species. Until the analyst has attained the requisite depth of experience these identifications should be used as investigative aids, in the absence of other corroborating evidence. Extensive experience is not necessary to take advantage of the equally important eliminative aspect of this method as many ashes are quite unlike *cannabis* ash, even on a cursory inspection.

The study reported here is limited with regard to the number of related species which were ashed and examined. For this reason a more comprehensive investigation of ash morphology analogous to that carried out on dried botanical material by Nakamura [*3*] is needed to augment and extend the work reported here.

Summary

The components present in marijuana ash have distinctive morphologies. These can be studied in considerable detail by a simple microscopic method. It would appear that this technique for the identification of marijuana ash would be useful in certain forensic investigations.

Acknowledgments

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References

[*1*] Maunder, M. J. de F., "Two Simple Colour Tests for *Cannabis*," *Bulletin on Narcotics*, BNUNA, Vol. 21, 1969, pp. 37-43.

[*2*] Pitt, C. G., Hendron, R. W., and Hsia, R. S., "The Specificity of the Duquenois Color Test for Marijuana and Hashish," *Journal of Forensic Sciences*, JFSCA, Vol. 17, 1972, pp. 693–700.

[*3*] Nakamura, G. R., "Forensic Aspects of Cystolith Hairs of *Cannabis* and Other Plants," *Journal of the Association of Official Analytical Chemists*, JANCA, *Vol.* 52, 1969, pp. 5–16.

[*4*] Nakamura, G. R. and Thornton, J. I., "The Identification of Marijuana," *Journal of the Forensic Science Society*, FSSAA, Vol. 12, 1972, pp. 461–519.

[*5*] Bradford, L. W. and Devaney, J. R., "Scanning Electron Microscope Applications in Criminalistics," *Journal of Forensic Sciences*, JFSCA, Vol. 15, 1970, pp. 110–119.

[*6*] Mitosinka, G. T., Thornton, J. I., and Hayes, T. L., "The Examination of Cystolithic Hairs of *Cannabis* and Other Plants by Means of the Scanning Electron Microscope," *Journal of the Forensic Science Society*, FSSAA, Vol. 12, 1972, pp. 521–529.

[*7*] Kingston, C. R. and Kirk, P. L., "Separation of Components of Marijuana by Gas-Liquid Chromatography," *Analytical Chemistry*, ANCHA, Vol. 33, 1961, pp. 1794–1795.

[*8*] Davis, T. W., Farmilo, C. G., and Osadchuk, M., "Identification and Origin Determinations of *Cannabis* by Gas and Paper Chromatography," *Analytical Chemistry*, ANCHA, Vol. 35, 1963, pp. 751–755.

[*9*] Kempe, C. R., Tannert, W. K., and Sterngast, A. "Application of Thin Layer Chromatography to the Identification of Charred Marihuana," *Journal of Criminal Law, Criminology, and Police Science*, JCLPA, Vol. 63, 1972, pp. 593–594.

[*10*] Clarke, E. G. C. and Robinson, A. E., "When is *Cannabis* Resin?" *Medicine, Science and the Law*, MDSLA, Vol. 10, 1970, pp. 139–148.

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