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Microcrystalloptic tests for lysergic acid diethylamide and other hallucinogens

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Microcrystallopic tests for LSD, *NN*-diethyltryptamine, *NN*-dimethyltryptamine, bufotenine, psilocin, psilocybin and STP are described. The tests are recommended in conjunction with other analytical techniques if applied for the forensic identification of hallucingens.

Many analytical techniques for the separation and identification of hallucinogens have been reported. They are mainly based on thin-layer chromatography (TLC), gas-chromatography, spectrofluorometry or infrared spectroscopy (e.g. Genest & Farmilo, 1964; Dal Cortivo & Broich, 1966; Look, 1968; Genest & Hughes, 1969; Andersen, 1969; Katz, Tadjera & Aufricht, 1969; Lerner & Katsiaficas, 1969; Mesley & Evans, 1969). Clarke (1969), in his recent handbook pointed out the usefulness of microcrystal tests for identification purposes. While this book contains descriptions of microcrystal tests for several hallucinogens, none are listed for psilocybin, *NN*-diethyltryptamine and LSD. Also results of microcrystalloptic measurements are not given in any of the existing test procedures for hallucinogens.

EXPERIMENTAL

The methods and equipment were those described earlier (Genest & Hughes, 1968b, c; 1969a, b; Genest, Lowry & Hughes, 1969, Genest, 1970). Instead of microrods, used previously, disposable microcaps (Drummond Sci. Co., Broomall, Pa.) of 1 and 3 μl capacity were used with pedestal slides for support of the cover glasses. For TLC the system chloroform–acetone (1 : 4) on Silica Gel G (Merck) plates and elution techniques as described by Genest & Hughes (1968a, b) were used. Hallucinogens (1 μg/ml) were dissolved in 1% acetic acid, except in the case of LSD for which 1% hydrochloric acid was the solvent of choice. Blank tests consisting of equal volumes of reagent and solvent were prepared from all reagents. Again, the set of reagents proposed by Clarke & Williams (1955, 1957) were the major source in the search for useful tests.

RESULTS

The most characteristic tests for each hallucinogen are given in this section with the results of micro-optical measurements and photographs of typical crystals. For LSD some lesser tests are also listed. All tests were made on pure compounds in concentrations as noted. The time after which the crystals were formed and photographed (in brackets) is also given. LSD tests 2, 3 and 7 were checked in aliquots of TLC-eluates of 20 μg bands.

LSD (*lysergic acid diethylamide, lysergide*)

1. Sodium carbonate (5% in water). Rods, in clusters, some pointed and prism-like; Class 5 (descriptive class-designation according to Farmilo & Genest, 1961);

5 μg; 15 min (2 h); moderate birefringence, 2nd order; parallel extinction; $\pm$ sign of elongation (Fig. 1A).

2. Trinitrobenzoic acid (saturated solution in water). Needles, radiating, in tufts and sheaves, Class 3; 5 μg; 5 min (15 min); moderate birefringence, second order; parallel extinction; $\pm$ sign of elongation (Fig. 1B).

3. Potassium tri-iodide (2 g iodine and 4 g KI in 100 ml water). Rods, small, single, some crossed, Class 5; 1 μg; 15 min (30 min); dim birefringence, 2nd order; parallel extinction; indifferent sign of elongation (Fig. 2A). This test worked equally well in acetic acid solution.

4. Sodium phosphate (5% Na_2HPO_4 in water). Rods, in sheaves or very dense aggregates, Class 5; 5 μg; 5 min; moderate birefringence, 2nd order; parallel extinction; $\pm$ sign of elongation.

5. Potassium cyanide (5% in water). Rods and blades, in clusters, Class 5 and 6; 5 μg; 10 min; moderate birefringence, 2nd order; parallel extinction; $\pm$ sign of elongation.

6. Potassium ferrocyanide (5% in water). Grains, round, showing centered black crosses in polarized light, Class 2; 5 μg; 1 h; parallel extinction; + sign of elongation.

7. Thallium bromide/HBr (2 g TlBr suspended in water), Br_2 added dropwise until dissolved, excess Br_2 removed on water bath). Grains, in burrs, Class 2; 5 μg; 30 min; dim birefringence, 1st order; parallel extinction; + sign of elongation.

8. Styphnic acid (5% in water). Grains, round, small and medium sized, Class 2; 5 μg; 1 h; dim birefringence, 1st order; parallel extinction; + sign of elongation.

Sensitivities of the LSD tests are shown in Table 1. The tests were carried out in 1 μl portions of test solution of decreasing concentration.

NN-Diethyltryptamine

1. Potassium cyanide. Rhombs, single, Class 7a; 1 μg; 2 h (2 h); dim birefringence, 1st order; parallel extinction; positive sign of elongation (Fig. 2B).

2. Styphnic acid. Plates and rods, single, Class 7a & 5; 1 μg; 5 min (15 min); moderate birefringence, 2nd order; parallel extinction; indifferent sign of elongation (Fig. 3A).

Table 1. *Sensitivity of microcrystal test for LSD*

Reagent	Sensitivity (μg)
Sodium carbonate	0·8
Trinitrobenzoic acid	0·6
Potassium tri-iodide	0·2
Sodium phosphate	1·0
Potassium cyanide	2·0
Potassium ferrocyanide	2·0
Thallium bromide/HBr	0·8
Styphnic acid	2·0

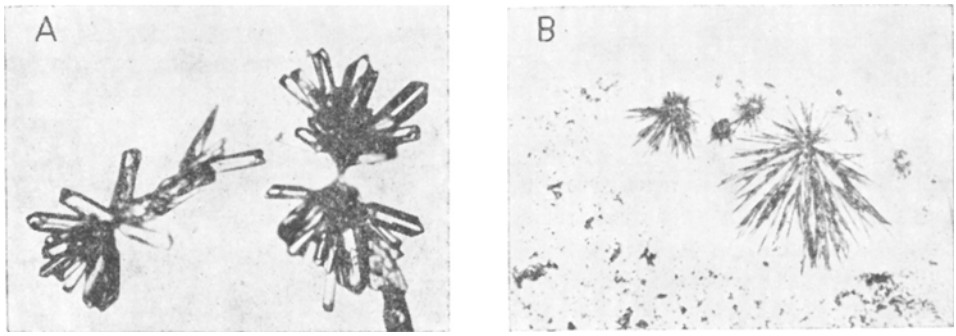

FIG. 1.A. LSD (5 μg) with sodium carbonate after 2 h; 160 ×. B. LSD (5 μg) with trinitrobenzoic acid after 15 min; 160 ×.

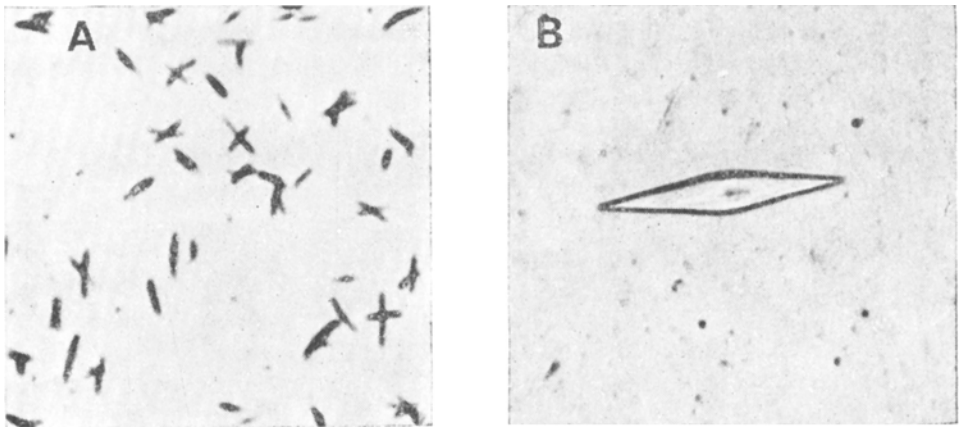

FIG. 2.A. LSD (1 μg) with potassium tri-iodide after 30 min; 400 ×. B. DET (1 μg) with potassium cyanide after 2 h; 160 ×.

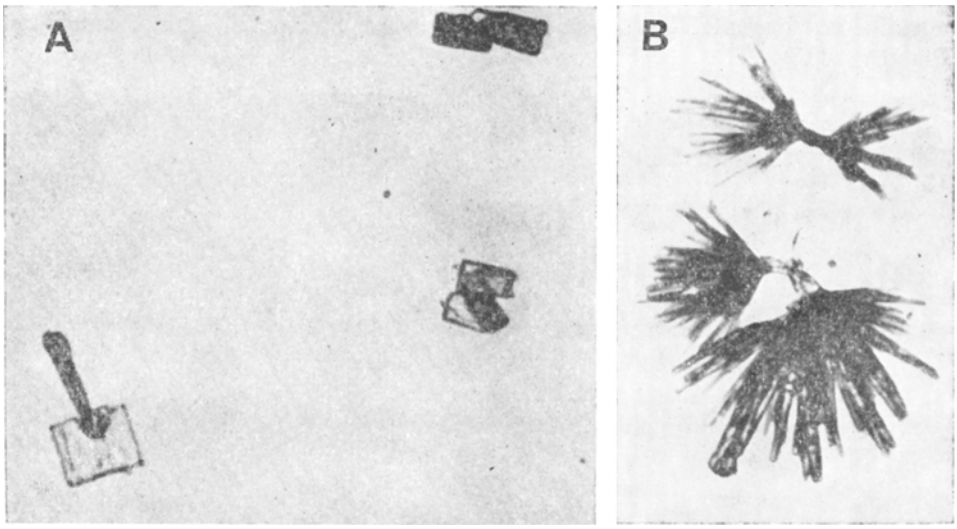

FIG. 3.A. DET (1 μg) with styphnic acid after 15 min; 160 ×. B. DMT (1 μg) with lead iodide after 30 min; 160 ×.

NN-Dimethyltryptamine

1. Lead iodide (30% solution of lead acetate adjusted to pH 6 with acetic acid and saturated with lead iodide). Sheaves of needles, Class 9b; 1 μg; 30 min (30 min); moderate birefringence, 2nd order; parallel extinction; indifferent sign of elongation (Fig. 3B).

2. 5-Nitrobarbituric acid (saturated solution in water). Rods, Class 5; 1 μg; 5 min (15 min); moderate birefringence, 2nd order; parallel extinction; — sign of elongation (Fig. 4A).

Bufotenine

1. Picrolonic acid (saturated solution in water). Tablets; class 7b; 1 μg; 15 min (30 min); moderate birefringence, 1st order; inclined extinction, angle of extinction 28°; indifferent sign of elongation (Fig. 4B).

2. Gold bromide/HCl (5 g $AuCl_3$ and 5 g NaBr in 100 ml conc HCl). Rods and prisms, in irregular aggregates, Class 5; 1 μg; 20 min (30 min); dim birefringence, 2nd order; parallel extinction; indifferent sign of elongation.

Psilocin

1. Picric acid (saturated solution in water). Hairs and needles, Class 4 and 3; 1 μg; 20 min (30 min); moderate birefringence, 2nd order; inclined extinction, angle of extinction 7°; + sign of elongation (Fig. 5A).

Psilocybin

1. Potassium cyanide. Rods and some needles in aggregates, Class 5 and 3; 1 μg; 20 min (30 min); dim birefringence, 1st order; parallel extinction; + sign of elongation (Fig. 5B).

4-*Methyl*-2,5-*dimethoxy*-α-*methylphenethylamine* (*STP*)

1. Picrolonic acid. Needles, branching fine tufts, Class 3; 1 μg; 15 min (30 min); moderate birefringence, 2nd order; parallel extinction; indifferent sign of elongation (Fig. 6A).

2. 2,4,6-Trinito-*m*-cresol (saturated solution in water). Rods in aggregates, Class 5; 1 μg; 10 min (15 min); moderate birefringence, 2nd order, inclined extinction, angle of extinction 150; + sign of elongation (Fig. 6B).

DISCUSSION

The wide-spread misuse of hallucinogens and the concurrent legal implications make unequivocal proof of identity of seized samples containing these compounds a forensic necessity of vital importance. Microcrystal tests play an useful part in identification schemes for confirmatory purposes. While microcrystal tests are mainly used in conjunction with other analytical techniques and after chromatographic purification procedures (Clarke, 1965, 1969; Genest & Hughes 1968b, c; 1969a, b) they apparently can also give valuable results when applied directly to residues obtained by extraction techniques (Moss, 1965). That the evaluation of microcrystal tests based on crystal form is to be done with caution has been noted earlier (Kuhnert-Brandstätter, 1956). Reproducibility is assured only if the experi-

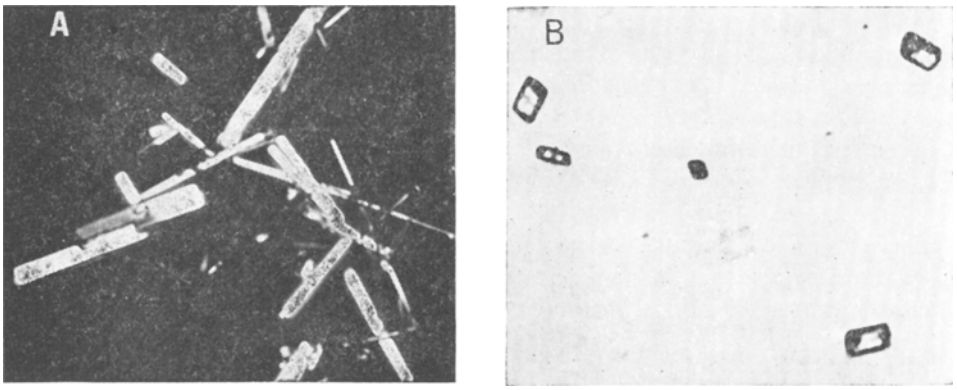

FIG. 4.A. DMT (1 μg) with 5-nitrobarbituric acid after 15 min; 160 ×. B. Bufotenine (1 μg) with picrolonic acid after 30 min; 160 ×.

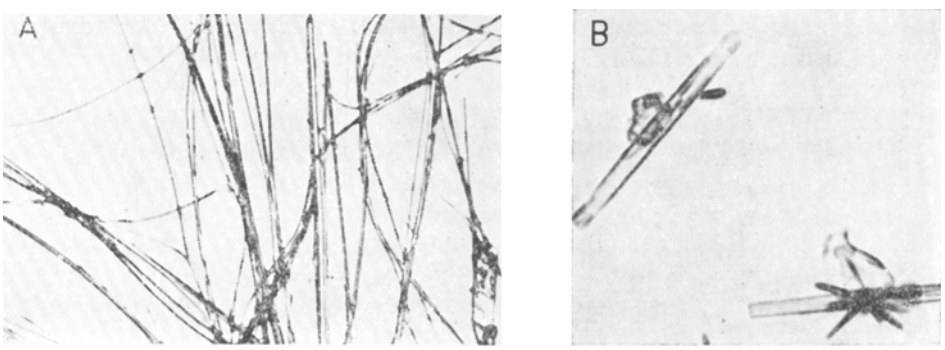

FIG. 5.A. Psilocin (1 μg) with picric acid after 30 min; 110 ×. B. Psilocybin (1 μg) with potassium cyanide after 30 min; 160 ×.

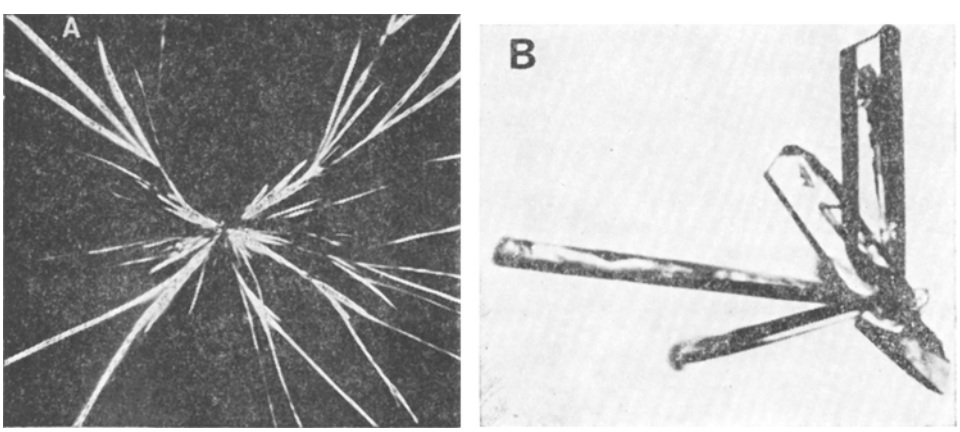

FIG. 6.A. STP (1 μg) with picrolonic acid after 30 min; 110 ×. B. STP (1 μg) with 2,4,6-trinitro-*m*-cresol after 15 min; 160 ×.

mental conditions of solvent, volume and concentration of reagent and reactant, evaporation rate of solvent and time of observation are rigorously controlled. Pedestal slides were preferred in our experiments mainly because of more rapid crystal development. With some reagents, however, especially those consisting of saturated solutions such as the nitrated organic acids, one has to be alert to avoid fallacies due to reagent crystals. For this reason an atlas of blank tests was prepared and consulted regularly to avoid false-positive tests. Thus, when testing LSD in acetic acid with thallium bromide/HBr quite "characteristic" crystals were obtained. These turned out to be probably due to a thallium acetate. The reliability of microcrystal tests can be greatly improved by optical measurements. Where microthermal methods or full crystallographic characterization of crystals cannot be performed, either due to lack of material or experience, measurements of some simple micro-optical properties of the formed crystals in polarized light enhance the value of the tests. In addition to increasing the specificity of microcrystal tests many times (Pozdnyakova & Rozovskii, 1963), these data are sometimes the only means to distinguish closely related alkaloids which give similar crystals with the same reagent (Genest & Hughes, 1968b). In the test for STP with 2,4,6-trinitro-*m*-cresol, crystals of similar form, albeit after a longer time, were obtained in a blank test with the reagent. Micro-optical measurements, however, lead to a clear distinction between blank and test crystals.

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