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TECHNICAL NOTE

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Increased Safety and Specificity in the Thin-Layer Chromatographic Identification of Marihuana

An in-depth study of alleged false positives in the chemical identification of marihuana has previously been reported [*1*]. Subsequent to publication of that paper, two health and safety considerations have come to our attention. Fast Blue B (3,3'-dimethoxybiphenyl-4,4'-bisdiazonium chloride) is suspected of being carcinogenic [*2*] because of the presence of unreacted amines, which are known carcinogens [*3*]. More importantly, benzene, which is the major component in the thin-layer chromatographic (TLC) system [*1*], has now been conclusively established as a carcinogen. The Occupational Safety and Health Administration (OSHA) has issued an emergency temporary standard because a grave danger exists for workers exposed to benzene. Because of the danger and stringent regulations [*4*] it was decided to discontinue the use of benzene.

Thus, the search for a TLC system without benzene was initiated. It was also deemed prudent to change the detection spray. It was suggested that Fast Blue 2B salt (4-benzoylamino-2,5-diethoxybenzene diazonium chloride) might be an acceptable alternative [*5*].

Methods

A petroleum ether extract of a known marihuana sample was spotted on each of five TLC plates. Each plate was then run in one of the five TLC systems listed in Table 1. The best system was determined based on the data in Table 1. The method described by Hughes and Warner [*1*] was followed in preparing samples for analysis.

Fifty milligrams of material (chemical, plant, or essential oil) was placed into a 50-ml beaker. Twenty-five millilitres of petroleum ether was added and allowed to remain in contact for 1 to 2 min. The petroleum ether was poured off, filtered, and evaporated to dryness. The residue was redissolved in 2 ml of petroleum ether, and 5 to 10 μl of this solution was spotted on a 10-cm, 250-μm thick silica gel thin-layer plate manufactured by Analtech, Inc., Wilmington, Del.

The thin-layer plate was developed in System 1. The solvents were American Chemical Society grade and supplied by J. T. Baker, Phillipsburg, N.J. After the plate was fully developed, at approximately 8 cm running distance, it was removed and sprayed with a saturated aqueous solution of Fast Blue 2B salt, and any colors were noted. The Fast Blue 2B salt was supplied by Pfaltz and Bauer, Inc., Flushing, N.Y.

Results and Discussion

From Table 1, it is apparent that System 1, ether/hexane (1:4), is the system of choice. In Tables 2 to 4 are the lists of compounds previously studied [*1*] with the benzene/

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TABLE 1—*Results of solvent systems on marihuana standard.*

System	Solvents	Separation
1	ether/hexane (1:4)	3 major components
2	hexane/chloroform (4:1)	multicolor streak
3	heptane/chloroform (4:1)	multicolor streak
4	petroleum ether/chloroform (3:2)	multicolor streak
5	hexane/ether (88:12)	2 major components

diethylamine (95:5) system sprayed with Fast Blue B salt and ether/hexane (1:4) system using the Fast Blue 2B salt spray. An analysis of Table 2 shows that 13 chemicals that developed a color with Fast Blue B salt did not develop a color with Fast Blue 2B salt. Further, only one compound (napthoresorcinol) developed a color when Fast Blue 2B salt was used versus no color for Fast Blue B salt. Since napthoresorcinol remains at the origin, it cannot be mistaken for a marihuana component.

Previously, Maunder [*6*] reported two herbal materials (nutmeg and mace) that developed a color with Fast Blue B salt that might be confused with *Cannabis*. It is reported that their TLC results can be readily distinguished from *Cannabis* [*1*]. Forrest and Heacock [*7*] had to resort to triple development, which is not necessary with System 1 and Fast Blue 2B salt. From Table 3 it is clear that none of the plant materials alleged to give a false positive Duquenois-Levine test result developed a color when sprayed with Fast Blue 2B salt. Five of these plant materials developed a color when sprayed with the Fast Blue B salt. A similar comparison using the essential oils listed in Table 4 shows only clove oil developed a color when sprayed with Fast Blue 2B salt compared to five oils that developed a color when sprayed with Fast Blue B. Out of 33 substances previously reported to develop color with Fast Blue B salt, only 12 substances developed colors with Fast Blue 2B salt. We therefore have concluded that Fast Blue 2B salt is more selective than Fast Blue B salt.

All of the chemicals, essential oils, and plant substances listed in Tables 2 to 4 were run on TLC versus a mixture of tetrahydrocannabinol (THC), cannabinol (CBN), and cannabinadiol (CBD) (U.S. Pharmacopeia reference standards). None of these compounds, with their various colors, singly or in combination, was confused with a genuine marihuana sample. The three spots of marihuana that we report as being CBN, THC, and CBD have been confirmed by removing the TLC spot and obtaining a mass spectra on each.

There are inconveniences with System 1. First, ether can form peroxides, so the system should not be made up in large quantities and stored. Second, because of the volatility of ether, the system must be made up daily. The Fast Blue 2B salt is not stable in solution for long periods of time. Initially, the solution is yellow, but as it ages it turns to a dark purple. It is advised to discard the spray reagent containing Fast Blue 2B salt after two or three days.

Summary and Conclusions

We are reporting what we believe to be a safe, rapid TLC system and spray for the identification of marihuana. The use of Fast Blue 2B salt greatly enhances the specificity of the TLC analysis of suspected marihuana samples.

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TABLE 2—*Results of TLC test on several chemical substances.*

Chemical	Benzene/Diethylamine (95:5) Fast Blue B Salt[a]		Ether/Hexane (1:4) Fast Blue 2B Salt	
	R_f to THC	Color	R_f to THC	Color
Resorcinol	origin	purple	...	...
5-Methylresorcinol	origin	purple	origin	burgundy
4-Hexylresorcinol	0.05	purple	origin	burgundy
Citral dimethylacetal	1.1-1.3	black	...	...
Citral	0.9-1.4	black	...	...
α-Terpineol	...	...	...	...
1-2-Pinene	0.6-0.7	yellow	...	...
dl-Catechin	...	...	...	...
8-Benzoflavone	...	...	...	...
Flavone	...	...	...	...
Naphthoresorcinol	...	...	origin	burgundy
d-Catechin	...	...	...	...
Phloroglucinol	...	...	...	...
Thymol	0.6	olive	0.8-1.4	yellow
2-Methylresorcinol	...	...	...	...
Carvacrol	0.6	yellow	0.6-1.1	brown-yellow
α-Phellandrene	0.5-1.0	purple	...	...
Isoeugenol	0.0-0.9	yellow	0.67	yellow
Eugenol	0.4-0.5/0.5-0.9	tan/brown	0.7	rust-orange
β-Caryophyllene	0.4	tan	...	...
Citronellal	1.2	green	...	...
O-Eugenol	0.4-0.5/0.5-1.0	tan/brown	0.7	orange
Linalool	1.0	yellow	...	...
Geraniol	1.1	gray	...	...
Citronellol	1.1	gray	...	...
Nerol	1.1	gray	...	...
1,2-Dimethoxy-4-propenylbenzene	...	...	...	...
Guaiazulene	1.2	purple	1.7-2.0	red
Farnesol	1.1	gray	...	...
Olivetol	0.4/origin	red/purple	0.2	brown
4,4′-Dihydroxystilbene	...	...	...	...
4-Hydroxystilbene	0.3	green	...	...
(+)-Pulegone	...	...	...	...
4-Methylumbelliferone	...	...	...	...
d-α-Tocopherol	...	...	...	...
1′,-3′,3′-Trimethyl-6-hydroxyspiro 2H-1-benzopyran-2,2′-indoline[b]	0.5	purple	0.3	dark brown
Beetle Bait® containing 2.6% eugenol, 6.0% phenylethyl propionate	...	...	...	...
Cannabinol	0.85	purple	0.89	purple
Cannabinolic acid	1.2	yellow	...[c]	...[c]
Cannabinol acetate	0.77	purple	...[c]	...[c]
Cannabidiol	1.12	brown-yellow	1.22	orange
Cannabigerol	0.72	yellow-red	0.82	brown/red
Δ^9-THC	1.0	red	1.0	red
Cannabichromene	0.38	purple	0.45	red/brown
Δ^8-THC	0.94	red	1.07	red
Cineole	...	...	...	...

[a] Values and colors from Ref *1* presented for comparison.
[b] Eastman Chemical Products Co. No. 11418.
[c] Not tested.

TABLE 3—*Results of TLC test on several plant substances.*

Plant Substance	Benzene/Diethylamine (95:5) Fast Blue B Salt[a]		Ether/Hexane (1:4) Fast Blue 2B Salt	
	R_f to THC	Color	R_f to THC	Color
Mace	0.2	black	...	...
	0.5	black	...	...
	0.6	yellow	...	...
Nutmeg	0.3	purple	...	...
8 O'Clock coffee	...	...	...	...
Red Circle coffee	...	...	...	...
Bohar coffee	...	...	...	...
Maxwell House coffee	...[b]	...[b]	...	...
Caraway	...	...	...	...
Cardamom	...	...	...	...
Ginger	origin	red	...	...
Cloves	0.5/1.1	black/black	...	...
Thyme	0.6	yellow	...	...
Agrimony	...	...	...	...
Henna	...	...	...	...
Currant	...	...	...	...
Sandalwood	...	...	...	...
Betony	...	...	...	...
Eucalyptus	...	...	...	...
A & P tea	...	...	...	...
Marihuana (sample)	0.85	purple	0.89	purple-brown
	1.0	red	1.0	red
	1.12	brown/yellow	1.22	orange
Sage	...[b]	...[b]	...	...
Savory	...[b]	...[b]	...	...
Oregano	...[b]	...[b]	...	...
Marjoram	...[b]	...[b]	...	...
Basil	...[b]	...[b]	...	...
Hops (*Humulus japonica*)	...	...	...	...

[a] Values and colors from Ref *1* presented for comparison.
[b] Not tested.

TABLE 4—*Results of TLC test on several essential oils.*

Essential Oil	Benzene/Diethylamine (95:5) Fast Blue B Salt[a]		Ether/Hexane (1:4) Fast Blue 2B Salt	
	R_f to THC	Color	R_f to THC	Color
Cardamom	...	...	...	...
Anise	...	...	...	...
Patchouli	...	...	...	...
Camphor	...	...	...	...
Caraway	...	...	...	...
Clove	0.7	yellow	0.69	brown
Fennel	...	...	...	...
Nutmeg	0.5	brown	...	...
Peppermint	...	...	...	...
Sandalwood	0.8	faint red	...	...
Peruvian balsam	0.2	red	...	...
Parsley	0.8	yellow	...	...
Cumin	...	...	...	...
Spearmint	...	...	...	...
Coriander	...	...	...	...

[a] Values and colors from Ref *1* presented for comparison.

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