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R. B. Hughes, [1] *M.S. and V. J. Warner, Jr.,* [1] *B.S.*

A Study of False Positives in the Chemical Identification of Marihuana

Many authors have reported using the Duquenois, Duquenois-Negm, Duquenois-Levine, or modified Duquenois-Levine color tests on various substances with varying results. Some authors [*1*] apparently consider any resultant color to be a positive response. Recently this diversity of testing methods has been used by defense lawyers in an attempt to confuse judges and juries. Therefore, a search of the literature was conducted for the various substances reported to give a positive response to any of the above methods; all of those that were commercially available were tested with the modified Duquenois-Levine test and thin-layer chromatography (TLC) employed by this laboratory for marihuana identification. In addition, other compounds of similar structures were selected and tested in like manner, as were minor components of *Cannabis* resin [*2*]. Another major objective was to determine if a mixture of materials that would lead to a false positive identification of marihuana could be made.

Of all the materials mentioned in the literature only fresh coffee might be misleading when the modified Duquenois-Levine test is used. Furthermore, there is no compound or mixture of compounds reported that will coincidentally chromatograph and develop the same colors as the cannabinol (CBN), tetrahydrocannabinol (THC), and cannabidiol (CBD) found in a marihuana sample.

Methods

One hundred 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 1 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 a benzene/diethylamine (95:5) system. 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 B salt (3,3′-dimethoxybiphenal-4,4′-bisdiazonium chloride), and the colors were noted. The Fast Blue B was supplied by K and K Chemicals, Inc. Extreme caution should be taken with this compound because it is a suspected carcinogen.

While the plate was developing, the petroleum ether solution was again evaporated. The residue was dissolved in 1 ml of Duquenois [*3*] reagent, and 1 ml of concentrated hydrochloric acid was added. Color changes were observed over 2-min and 10-min time periods.

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[1] Forensic chemist and supervisory forensic chemist, respectively, Drug Enforcement Administration, Mid-Atlantic Regional Laboratory, Washington, D.C. 20537.

After 10 min, 1 ml of chloroform was added and the color transferred to the chloroform layer noted. With the essential oils, sometimes more than 1 ml hydrochloric acid was added. If this was not done, then only one layer was formed when chloroform was added.

Results and Discussion

Pitt et al [*4*] stated that the Duquenois color test is primarily based on the presence of the resorcinol partial structure. That is, the resorcinol partial structure is necessary but is not the sole prerequisite. However, Table 1 shows that numerous resorcinol structures do not give a positive result to the modified Duquenois-Levine test. The colors are not similar to that of a marihuana sample. Yet, guaiazulene (Fig. 1), which does not have the resorcinol structure, passes a 10-min time test (as prescribed by the Association of Official Analytical Chemists) but not the 2-min interval test.

Based on the color changes we observed, the modified Duquenois-Levine test can be made more specific by adding chloroform after 2 to 3 min and noting the color. This has previously been reported by Maunder [*5*]. Illustrations of this are guaiazulene, olivetol (5-pentylresorcinol), and (+)-pulegone. They initially give red or blue colors that gradually darken. After 10 min they yield a purple color in the chloroform layer. However, these substances cannot be mistaken for marihuana if the chloroform is added after 2 min.

As has been pointed out [*6,7*], the modified Duquenois-Levine test is a screening test. While it is not specific for a single compound, as an infrared or mass spectra would be specific, we have found it to be a highly selective test for marihuana. Of the 71 compounds tested, only some brands of fresh coffee gave a color within 2 min that was soluble in chloroform and similar to the color developed for marihuana. Aged coffee is not a problem because the initial color developed is red.

All of the chemicals, essential oils, and plant substances listed in Tables 1, 2 and 3 were run on TLC versus a mixture of THC, CBN, and CBD (U.S. Pharmacopeia reference standards). None of these compounds, with their various yellow, brown, black, red, green, tan, or gray colors, singly or in combination, was confused with a genuine marihuana sample. This is not surprising since Maunder [*8*] developed a simple and specific test for *Cannabis* based on Fast Blue B salt. Of the 236 herbal materials tested, only 2 (nutmeg and mace) gave a color that might be confused with *Cannabis*. Both of these substances were tested with our modified Duquenois-Levine test and were negative. In addition, their TLC can be readily distinguished from *Cannabis*. This result has been previously reported by Forrest [*9*], who used a triple development which is not necessary with our system. The highly selective and distinctive colors of the cannabinoids with Fast Blue B salt has been confirmed by Nakamura and Thornton [*10*] and Lowry and Garriott [*11*]. The three spots of marihuana which we report as being CBN, THC, and CBD have been confirmed by removing the TLC spot and obtaining a mass spectra on each.

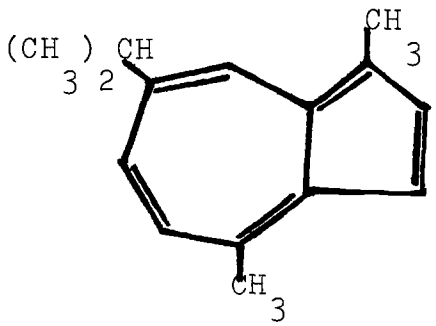

FIG. 1—*Structure of guaiazulene.*

TABLE 1—*Results of color and TLC tests on several chemicals.*

Chemical	Color in Aqueous Layer in 2 min	Color in Aqueous Layer in 10 min	Color in Chloroform in 10 min	TLC R_f to THC
Resorcinol	pale blue	blue	...	purple origin
5-Methylresorcinol	pale blue	blue	...	purple origin
4-Hexylresorcinol	red	violet	red orange	0.05 purple
Citral dimethylacetal	yellow	yellow	yellow	1.1–1.3 black
Citral	yellow	yellow	yellow	0.9–1.4 black
α-Terpineol	...	...	...	...
1-2-Pinene	faint blue	faint blue	...	0.6–0.7 yellow
dl-Catechin	pink	purple	...	...
8-Benzoflavone	yellow	yellow	...	...
Flavone	pale yellow	yellow	...	...
Naphthoresorcinol	pink	purple	pale purple	...
d-Catechin	...	...	...	...
Phloroglucinol	...	...	...	...
Thymol	pink	...	...	0.6 olive
2-Methylresorcinol	...	...	...	...
Carvacrol	blue	blue	pale blue	0.6 yellow
α-Phellandrene	pink	brown	...	0.5–1.0 purple
Isoeugenol	white	blue	...	0.0–0.9 yellow
Eugenol	blue	navy blue	blue	0.4–0.5 tan/0.5–0.9 brown
β-Caryophyllene	brown	brown	...	0.4 tan
Citronella	orange	blue	faint purple	1.2 green
O-Eugenol	blue	navy blue	pale blue	0.4–0.5 tan/0.5–0.1 brown
Linalool	green	green	...	1.0 yellow
Geraniol	green	green	...	1.1 gray
Citronellol	blue	blue	...	1.1 gray
Nerol	green	green	...	1.1 gray
1,2-Dimethoxy-4-propenylbenzene	white	white	...	...
Guaiazulene	red	purple	purple	1.2 purple
Farnesol	green	green	...	1.1 gray
Cineole	...	pink	...	...
Olivetol	blue	purple	purple	0.4 red purple origin
4,4′-Dihydroxystilbene	green	green	...	...

4-Hydroxystilbene	green	green	...	0.3 green
(+)-Pulegone	red	purple	purple	...
4-Methylumbelliferone	...	...	...	...
d-α-Tocopherol	white	blue	...	...
1′, 3′, 3′-Trimethyl-6-hydroxyspiro 2H-1-benzopyran-2,2′-indoline[a]	gold	brown	...	0.5 purple
Bettle Bait® containing 2.6% Eugenol, 6.0% 2-phenylethylpropionate	...	...	...	...
Cannabinol	light blue	light blue	purple	0.85 purple
Cannabinolic acid	purple	purple	purple	1.2 yellow
Cannabinol acetate	light blue	light blue	purple	0.77 purple
Cannabidiol	purple	purple	purple	1.12 brown-yellow
Cannabigerol	purple	purple	purple	0.72 yellow-red
Δ^9-THC	purple	purple	purple	1.0 red
Cannabichromene	blue	blue	blue	0.38 purple
Δ^6-THC	dark blue	dark blue	blue-purple	0.94 red

[a] Eastman Chemical Products Co. No. 11418.

TABLE 2—*Results of color and TLC tests on several plant substances.*

Plant Substance	Color in Aqueous Layer in 2 min	Color in Aqueous Layer in 10 min	Color in Chloroform in 10 min	TLC R_f to THC
Mace	...	...	...	0.2 black 0.5 black 0.6 yellow
Nutmeg	faint blue	faint blue	purple	0.3 purple
8 O'Clock coffee	purple	purple	purple	...
Red Circle coffee	purple	purple	purple	...
Bohar coffee	purple	purple	purple	...
Caraway	...	...	...	...
Cardamom	...	...	...	...
Ginger	light brown	brown	amber	red origin
Cloves	...	...	...	0.5 black/1.1 black
Thyme	...	...	...	0.6 yellow
Agrimony	light green	light green	...	...
Henna	light green	light green	green-yellow	...
Currant	light green	light green	...	...
Sandalwood	gray	gray	...	...
Betony	light green	light green	...	...
Eucalyptus	light green	light green	...	...
A & P tea	yellow-green	yellow-green	yellow-green	...
Marihuana (sample)	purple	purple	purple	0.85 purple 1.0 red 1.12 brown-yellow
8 O'Clock coffee (exposed to air 1 year)	red	red	red	...
Red Circle coffee (exposed to air 1 year)	red	red	red	...
Bohar coffee (exposed to air 1 year)	red	red	red	...
Hops (*Humulus japonica*)	...	light green	...	...

TABLE 3—*Results of color and TLC tests on several essential oils.*

Essential oil	Color in Aqueous Layer in 2 min	Color in Aqueous Layer in 10 min	Color in Chloroform in 10 min	TLC R_f to THC
Cardamom	brown	brown	...	...
Anise	white	blue	...	...
Patchouli	purple	purple	blue	...
Camphor	...	...	...	...
Caraway	...	...	...	...
Clove	pale blue	blue	...	0.7 yellow
Fennel	...	...	...	...
Nutmeg	red	red	...	0.5 brown
Peppermint	red-purple	red-purple	red-purple	...
Sandalwood	blue	blue	...	0.8 faint red
Peruvian balsam	...	...	...	0.2 red
Parsley	...	yellow	yellow	0.8 yellow
Cumin	yellow	yellow	yellow	...
Spearmint	...	...	...	...
Coriander	...	...	...	...

Summary and Conclusions

If the modified Duquenois-Levine test is performed and only 2 or 3 min are allowed to pass before the addition of the chloroform, the selectivity is greatly increased. The possibility of a false positive [5] becomes negligible.

Of all the materials mentioned in the literature, only a limited number of fresh coffees might be misleading with the modified Duquenois-Levine test. However, neither fresh nor aged coffee developed colors when sprayed with Fast Blue B salt after TLC analysis. Furthermore, there is no compound or mixture of compounds reported that will coincidentally chromatograph and develop the same colors with Fast Blue B salt as the CBN, THC, and CBD found in a marihuana sample.

In conclusion, where morphological structures are not readily observable, both a modified Duquenois-Levine test and suitable TLC must be obtained to identify a substance as having originated from the *Cannabis* plant. However, if the glandular, clothing, and unicellular cystolithic hairs are present then either a modified Duquenois-Levine test or TLC when sprayed with Fast Blue B salt are positive evidence that *Cannabis* is present in the sample.

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Address requests for reprints or additional information to
R. B. Hughes
Drug Enforcement Administration
Mid-Atlantic Regional Laboratory
460 New York Ave., N.W.
Washington, D.C. 20537