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Note

A chromatographic comparison of the constituents of nutmeg and mace (*Myristica fragrans* Houtt.) with those of marihuana and hashish (*Cannabis Sativa* L.)*

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In recent years, there has been a marked increase in the illicit use of cannabis products, such as marihuana and hashish, necessitating the development of simple accurate "field tests" for such products. A rapid, sensitive and reasonably specific test of this type has been described by De Faubert Maunder[1] whereby products seized by law enforcement agents and suspected of containing cannabis products can readily be provisionally identified. The test involves a simple extraction of the suspected material with light petroleum (in practice lighter fluid is used). The extract, applied to a filter paper, is treated with a solution of Fast Blue salt B (FBB) after drying. A positive result is indicated by the rapid development of a red to violet colour[1]. Of approximately two hundred plant materials examined by this author[1] only two were found to give strongly false positives to this test, *i.e.* nutmeg and mace, both products of the tree *Myristica fragrans* Houtt. Of these two spices the colour produced by nutmeg is more intense than that produced by mace. Other interfering plant materials include agrimony which also gives a false positive reaction, although the colour produced is of a much paler hue than that obtained with cannabis[2]. Henna also gives a false positive result but only if an excessively large sample is tested[2].

A search for the psychoactive constituents of these spices, which have a long history of abuse (*i.e.* "non-culinary" use) and which have been used as substitutes for cannabis (*cf.* review by Forrest and Heacock[3]) lead us to make a chromatographic comparison of light petroleum extracts of marihuana and hashish with those of nutmeg and mace. Suitable extracts of these materials were subjected to thin-layer chromatography (TLC) in various solvent systems; the developed plates sprayed with the FBB reagent and the nature of the resulting coloured spots which developed, compared for identification purposes.

EXPERIMENTAL

Materials

Freshly ground nutmeg (or mace) (5 g) was shaken with 50 ml of light petroleum

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(b.p. 30–60 °) for 5 min. The resulting suspension was filtered and stored at 4° overnight and any solid that precipitated out was removed by filtration. The filtrate was concentrated to a total volume of *ca.* 10 ml.

Reference samples of Δ^9-tetrahydrocannabinol, Δ^8-tetrahydrocannabinol, cannabidiol, mixed hashish resin reference standard and standardized *Cannabis sativa* L. were kindly supplied for the purposes of this investigation by the Health Protection Branch, Health and Welfare, Canada. The mixed hashish resin reference standard is the purified phenolic fraction isolated from hashish and contained approximately 35% cannabidiol, 39% Δ^9-tetrahydrocannabinol, 18% cannabinol and other cannabinoids which have not been quantitated. The standardized *Cannabis sativa* L. was finely ground and extracted with light petroleum (b.p. 30–60 °), in a similar manner to that used for the spices nutmeg and mace. The extract was evaporated to dryness and the residue redissolved in chloroform to give a similar extract concentration to that which had been used above for the nutmeg and mace.

Chromatography

Thin-layer chromatography. Commercially available silica gel F_{254} (Brinkmann, Westbury, N.Y., U.S.A.) pre-coated thin-layer plates (thickness 0.25 mm) were used. The plates were developed in the solvent systems listed below. Approximately 20 μg of the various spice and plant material extracts were applied to the plates in each case.

Chromogenic reagent. A 0.1% solution of FBB (Matheson, Coleman and Bell, East Rutherford, N.J., U.S.A.) in 70% ethanol was freshly prepared prior to use.

Solvent systems used. (I) light petroleum (60–80 °)–ethyl acetate (5:1); (II) light petroleum (60–80 °)–ethyl acetate–ether (90:5:5); (III) light petroleum (60–80 °)–diethyl ether (4:1); (IV) *n*-hexane–ether–acetic acid (87:12:1); (V) *n*-hexane–acetone–diisopropyl ether (10:1:1). The light petroleum (b.p. 60–80 °) used in solvent systems I–III was AnalaR grade and was obtained from BDH, Poole, Great Britain.

RESULTS AND DISCUSSION

The colours obtained from the light petroleum extracts of nutmeg, mace, marihuana (*i.e. Cannabis sativa* plant material as supplied) and hashish resin, after samples of these extracts had been spotted onto paper, cellulose layers or silica gel layers and sprayed with the FBB reagent were all red to red-violet in nature and were virtually indistinguishable to the naked eye. However, it can readily be seen from Figs. 1–3 that comparison of the TLC behaviour of the extracts of nutmeg (A), mace (B), marihuana (C) and hashish (D) readily distinguishes the substances in question. The chromatograms were obtained using the developing solvents listed above and with triple development in each case. In view of the use of a multiple development technique no R_F values are reported in this paper. Numerous solvent systems have been described in the literature for the TLC of cannabis products (*cf.* refs. 4–6). The first four solvents described above have been previously reported in the literature for this purpose, whilst as far as the authors are aware the final, useful solvent V, has not been used before for the separation

of cannabinoids. Whilst the results of using only the first three solvent systems are given in this paper, those obtained with the other two are basically similar in nature.

The De Faubert Maunder field test procedure for cannabis derivatives elutes essentially the "fat-soluble" phenols, such as the tetrahydrocannabinols from the plant material. Such compounds, which will couple with the stabilised diazonium salt FBB (tetra-azotised di-*o*-anisidine), produce the typical red colour given by a phenol. A brief extraction of ground nutmeg and mace with light petroleum also readily extracted some "fat-soluble" phenols, however this solvent also extracts a vast quantity of unwanted fatty material in both cases. The trimyristin, which makes up about 80% of the total weight of the nutmeg, does however readily precipitate out of solution on cooling and can be removed by filtration, prior to carrying out the TLC.

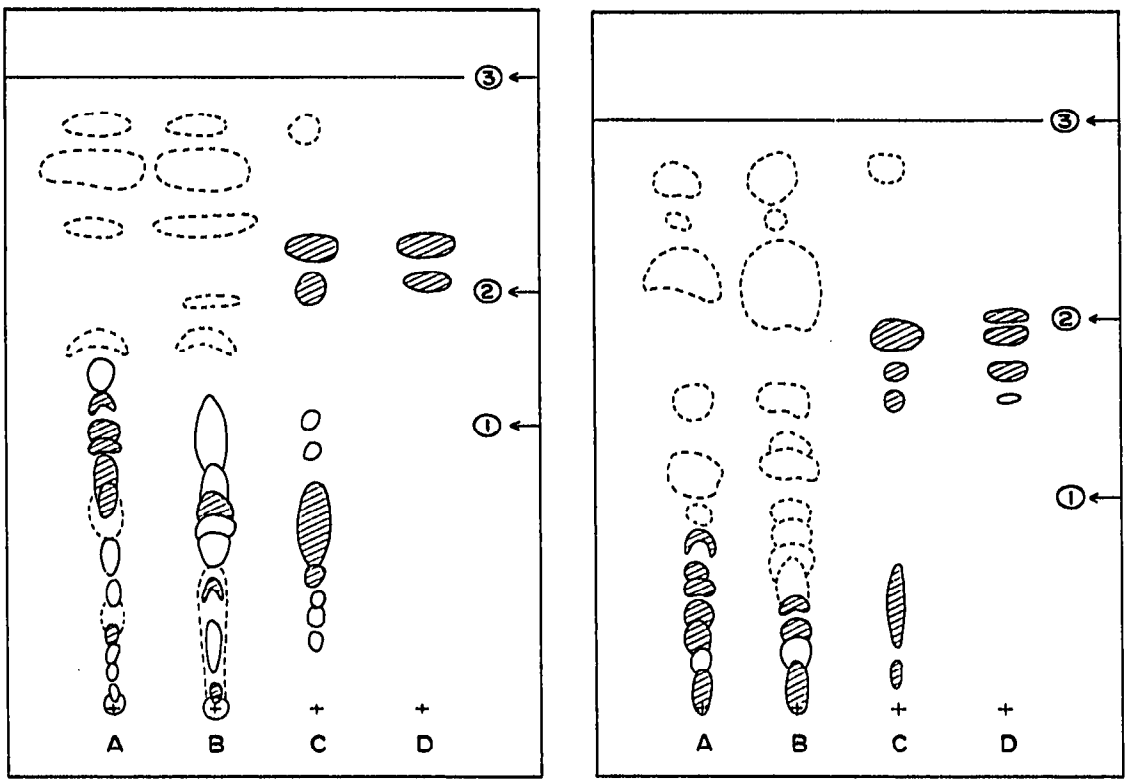

Fig. 1. TLC on silica gel F_{254} of light petroleum extracts of: (A) nutmeg; (B) mace; (C) marihuana and (D) hashish. Solvent I, multiple development was used, the solvent being allowed to ascend to position 1 in the first instance; the plate was then removed from the tank, dried and re-run to position 2; this procedure was repeated and the final development was allowed to proceed to position 3. Visualising reagent: FBB (freshly prepared); spots indicated by dotted lines did not give red colours with FBB and were located either by observing the developed plate in UV light or by dull yellow-brown colours given with FBB; spots indicated by solid line gave weak red colours with FBB and spots indicated by solid line with cross-hatching gave a strongly positive reaction with FBB.

Fig. 2. As for Fig. 1, except that solvent II was used.

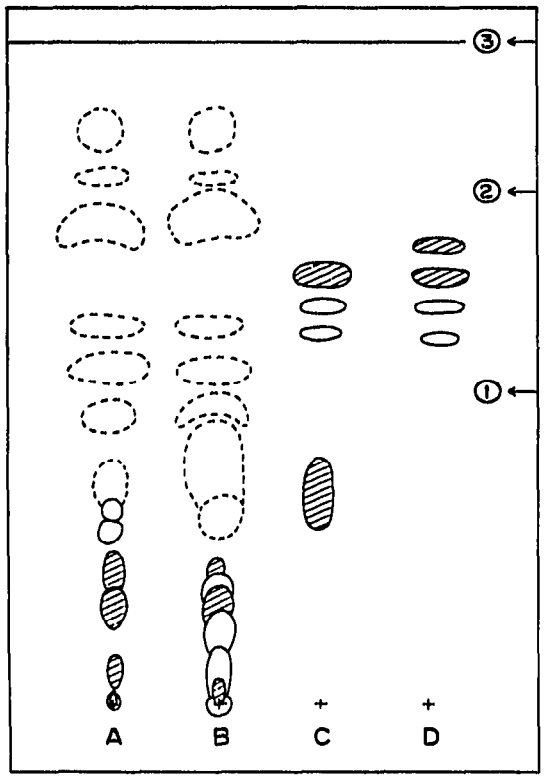

Fig. 3. As for Fig. 1, except that solvent III was used.

It is interesting to speculate on the chemical structures of the FBB positive compounds in nutmeg and mace. The presence of some relatively simple phenolic phenylpropanoids, such as eugenol, isoeugenol and methoxyeugenol has been known for some time (*cf.* ref. 3). However these products do not give red colours with the FBB reagent. This is not too surprising, since they do not have positions *para* to the phenolic —OH group available for the *para*-coupling reaction with the diazonium salt to occur. Recently, a number of dimeric phenylpropanoids has been isolated from nutmeg and mace[8–12]. However, it is doubtful if these products are responsible for the production of the red colours since in the vast majority of cases they do not have the structural requirements that would be expected to give a strongly positive test with the diazo reagent.

In all the solvent systems utilized, the cannabinoids can readily be distinguished from the nutmeg and mace phenolics (see Figs. 1–3), which cannot be demonstrated by the preliminary field test. The cannabidiol, cannabinol and tetrahydrocannabinol have high R_F values in the systems used, whilst the cannabinoids having an acid function in the molecule tend to migrate more slowly[4]. These studies have shown that with the chromatographic systems used for the FBB positive substances extractable from nutmeg and mace with cold light petroleum tend to be in the lower half of the chromatogram in the cannabinoid acid region.

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