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Note

Diazotized dapsone as a reagent for the detection of cannabinoids on thin-layer chromatographic plates

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Cannabis and other forms of cannabis plants (bhang, ganja, charas) are very frequently submitted to forensic laboratories. In routine cases the identification of cannabinoids in marihuana is achieved unequivocally by the 'three-parameter approach' [morphology, colour tests and thin-layer chromatography (TLC)] as suggested by Coutts and Jones[1]. Although the instrumental methods are sensitive they are expensive and there are limitations to their use in routine forensic work owing to the large number of samples to be handled.

A number of chromogenic reagents such as Duquenois reagent[2], Fast Blue Salt B[3], 1-nitroso-2-naphthol[4], Fast Blue Salt 2B[5] and 2-hydrazono-2,3-dihydro-3-methylbenzothiazole hydrochloride (HMBT)[6,7] have been reported for the detection of cannabinoids. Although Fast Blue Salt B as a chromogenic reagent seems to be the most commonly used reagent, its safety is questionable because of its potential carcinogenicity[7,8]. In a search for an alternative chromogenic reagent, diazotized dapsone was found to be suitable for the detection of cannabinoids in marihuana.

EXPERIMENTAL

All the solvents used were of analytical-reagent grade.

Solutions

Recrystallised dapsone tablets (Burroughs Wellcome) and sulphadiazine tablets (May and Baker) and other analytical-reagent grade chemicals such as aniline, *p*-chloroaniline, *p*-toluidine, α-naphthylamine, *o*-aminobenzoic acid and *p*-aminobenzoic acid were dissolved in ethanol to give 1 mg/ml solutions.

For the extraction of cannabis, the cannabis sample (bhang, ganja or charas) was extracted with chloroform, the extract was filtered and evaporated to dryness and the residue was dissolved in chloroform for spotting.

Cannabinol and cannabidiol pure reference standards were dissolved in ethanol.

A solution of α-naphthol in ethanol was prepared.

Spray reagent

A 10-ml volume of dapsone solution was pipetted into a 25-ml volumetric flask

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TABLE I

R_F VALUES OF CANNABINOIDS WITH RESPECT TO α-NAPHTHOL

Cannabinoid	R_F value ± S.D.**		Colour of spot
	I*	II*	
Cannabinol	1.29 ± 0.03	1.62 ± 0.03	Brownish yellow
Cannabidiol	1.45 ± 0.03	2.02 ± 0.02	Yellow
Δ^9Tetrahydrocannabinol	1.62 ± 0.03	1.78 ± 0.02	Yellow
α-Naphthol	1.00	1.00	Red

* Solvent systems: I = *n*-hexane–acetone (85:15) (α-naphthol, R_F = 0.36); II = *n*-hexane–diethyl ether (80:20) (α-naphthol, R_F = 0.30).

** Standard deviations based on 10 measurements.

and 2 ml of 0.1 *M* hydrochloric acid followed by 6 ml of 0.1% sodium nitrite solution were added. The solution was allowed to stand for 10 min and then diluted to 25 ml with distilled water.

Other spray reagents were prepared using the requisite amounts of acid and sodium nitrite.

Thin-layer chromatography

Standard glass plates (10 × 20 cm) were coated with a 0.25-mm layer of silica gel G (ACME, Bombay, India) in water (1:2), allowed to dry at room temperature and then activated at 110°C for 1 h before use. Two solvent systems, *n*-hexane–acetone (85:15) and *n*-hexane–diethyl ether (80:20), were used for developing the plates. The cannabis extract together with α-naphthol and the standard reference solutions of cannabinol (CBN) and cannabidiol (CBD) were spotted on the plate, which was developed by the ascending technique in a presaturated chamber. After a run of about 10 cm the plate was removed and allowed to dry at room temperature. It was sprayed uniformly with freshly prepared diazotized dapsone reagent. The R_F values of cannabioids with respect to α-naphthol (red spot) are given in Table I.

UV spectra of THC

The cannabis extract was spotted on a TLC plate, the plate was developed with either of the above solvent systems and one of the resolved spots was made visible by spraying the diazotized dapsone reagent. An equal area of silica gel layer was scraped off from a distance equal to the R_F value of Δ^9-tetrahydrocannabinol (Δ^9-THC), treated with 5 ml of ethanol and the solution mixed throughly. The solution was centrifuged and the UV spectrum of the supernatant liquid was recorded.

RESULTS AND DISCUSSION

Most of the cannabinoids have a phenolic group with the *ortho* and *para* positions free. As phenols couple in the *para* position with diazonium salts[9] the cannabinoids also undergo similar reactions with diazotized dapsone to yield coloured products.

The cannabis extract gave three spots, a brownish yellow spot corresponding to

TABLE II

MINIMUM DETECTABLE AMOUNTS OF CANNABINOIDS USING DIFFERENT SPRAY REAGENTS

Diazotized reagent	*Minimum amount detectable (μg)*	
	Cannabinol	*Cannabidiol*
Dapsone	0.6	0.8
Aniline	1	1
p-Chloroaniline	2	2.5
p-Toluidine	2	2.5
Sulphadiazine	2	2
α-Naphthylamine	1	1
o-Aminobenzoic acid	1.5	1.5
p-Aminobenzoic acid	1	1
Fast Blue Salt B[3]	0.01	0.01
HMBT[7]	2.5	2.5

CBN, a yellow spot corresponding to CBD and another yellow spot of Δ^9-THC, which was confirmed by recording its UV spectra. The UV absorpion maximum for Δ^9-THC was in good agreement with reported values (277 and 282 nm)[10]. This also shows that cannabinoids are well separated using either of the solvent systems. The R_F values of Δ^9-THC were confirmed by mixed spotting with control CBN and CBD samples. The colour of the spots was stable for several days. The reagent can be used in routine analysis owing to its high sensitivity (0.6 μg for CBN and 0.8 μg for CBD).

Other diazotized arylamines such as aniline, *p*-chloroaniline, *p*-toluidine, α-naphthylamine, amino-substituted benzoic acids and sulphadiazine were also tried as detection reagents. All diazotized arylamines except α-naphthylamine gave the coloured spots for cannabinoids as given with dapsone. Diazotized α-naphthylamine followed by a 0.1% aqueous sodium carbonate spray gave a reddish brown colour with CBN and a yellow colour with CBD and Δ^9-THC. To reveal and intensify the coloured spots it is essential to spray the reagent followed by 0.1% aqueous sodium carbonate for completion of coupling, as described by Feigl[11]. However, with dapsone, *p*-aminobenzoic acid and sulphadiazine the 0.1% sodium carbonate spray does not increase the sensivitivity of reagent.

Table II shows that the minimum detectable amounts of CBN and CBD for all the reagents used and that reported for HMBT[7] are higher than those obtained with dapsone. Some of the arylamines used are carcinogenic and/or expensive. Dapsone is a cheap, readily available pharmaceutical preparation used as an antibacterial agent (leprostatic)[12].

It was observed that the colour of the CBN and CBD spots after spraying diazotized reagents such as aniline, *p*-chloroaniline and α-naphthylamine faded rapidly. No fading of the colours was observed for the other reagents studied. Diazotized dapsone was stable for a sufficient time. Among the arylamines used in this study, diazotized aniline has been used for the detection of sulpha drugs and phenols by TLC[13]. For the colorimetric determination of aniline, *p*-chloroaniline and dapsone they are diazotized and coupled with tetrahydrobenzoquinolinol, β-naphthol and *p*-aminophenol, respectively[14–16]. However, none of the reagents studied has been reported previously for the detection of cannabinoids.

Diazotized dapsone was also used to detect the alkali hydrolysis products of carbamate insecticides, such as baygon, carbaryl and carbofuran, at a level of 0.1 μg of each as red, violet and red spots, respectively. These insecticides are of interest in forensic toxicology.

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