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Note

Thin-layer chromatographic–mass spectrometric identification of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid*

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Kaistha and Tadrus[1] previously described a procedure for thin-layer chromatographic (TLC) detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (Δ^9-THC-COOH), a major metabolite of tetrahydrocannabinol (THC). An enzyme multiplied immunoassay technique (EMIT) was employed to confirm the presence of Δ^9-THC-COOH. For forensic purposes, we adapted this TLC method for the clean-up of urinary extracts as an important step in isolating the metabolite. The elution of the TLC spot corresponding in R_F and Fast Blue B stain color to Δ^9-THC-COOH provided an extract for gas chromatographic (GC)–mass spectrometric (MS) determination with a low background reading.

EXPERIMENTAL

The hydrolysis and extraction procedures for Δ^9-THC-COOH are essentially those of Kaistha and Tadrus[1]. The cyclohexane–ethyl acetate extract of hydrolyzed urine was passed through a bed of anhydrous sodium sulfate in the cone of Whatman I filter-paper to provide a cleaner residue upon evaporation.

TLC was performed on a silica gel G plate, Analtech Uniplate, 250 μm thick, irrigated with chloroform–methanol–ammonia (70:30:2). The R_F for 11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid (Δ^8-THC-COOH) and Δ^9-THC-COOH is approximately 0.25 under these conditions. The spot for these compounds, after staining with Fast Blue B, was scraped off the plate onto a weighing paper and transferred to a small tube, approximately 12 × 75 mm, to which *ca.* 2 ml methanol were added. The tube was agitated on a Vortex mixer and then centrifuged to clear. The supernatant was transferred by means of a Pasteur pipet to another tube, approximately 10 × 75 mm, and evaporated to dryness under nitrogen.

The resulting residue was methylated according to Whiting and Manders[2] by treating it with 70 μl aqueous 25% tetramethylammonium hydroxide–dimethylsulfoxide (1:20). The tube was agitated and allowed to stand for 2 min. Approximately 5 μl of 1-iodomethane were added to the mixture, agitated and allowed to stand for 5 min. The mixture was acidified with 0.2 ml 0.1 *M* hydrochloric acid and then agitated with 1.0 ml hexane for 1 min. The upper layer was passed through *ca.* 1 cm

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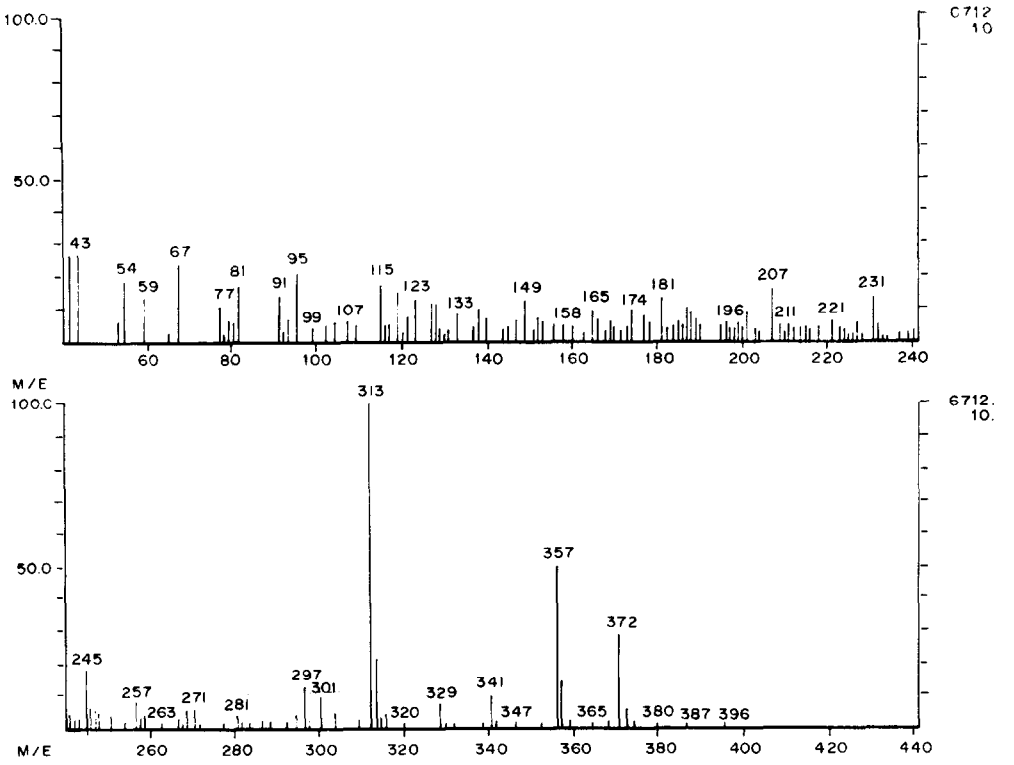

Fig. 1. Electron impact mass spectrum of the methylated derivative of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid extracted from urine of a suspected marihuana user.

of sodium sulfate packed over glass wool in a Pasteur pipet and collected in a tube approximately 75 × 10 mm. The acidified mixture was extracted again with hexane, the upper layer was filtered and the extracts were combined. The residue was dissolved in 20 μl of methanol and 5 μl of the resulting solution were injected into a Model 1020 Finnegan GC–MS instrument. A 6 ft. × 1/4 in. column packed with OV-17 on Chromosorb W HP (80–1100 mesh) was used in GC and the oven temperature was programmed from 220 to 270°C at 10°C/min. The retention time for Δ^9-THC-COOH was in the range of 5–6 min under these conditions.

RESULTS AND DISCUSSION

In our hands, the clean-up procedure using TLC afforded an extractive residue devoid of much of the impurities normally transferred through a solvent double extraction system[2] to the final residue. The mass spectrum of Δ^9-THC-COOH, shown in Fig. 1, was obtained from the extract of a urine specimen of a suspected user, and is to be compared with that of standard Δ^9-THC-COOH shown in Fig. 2. The spectrum of Fig. 1 exhibits a fragmentation pattern which is consistent with that of Fig. 2, particularly in respect of the peaks at *m/z* 313, 357 and 372 and in the order of relative intensity. Although the TLC R_F values of Δ^8-THC-COOH and Δ^9-THC-COOH are similar, the mass spectra of the two compounds are decidedly different. The spectrum of Δ^8-THC-COOH shows a base peak at *m/z* 372 while Δ^9-

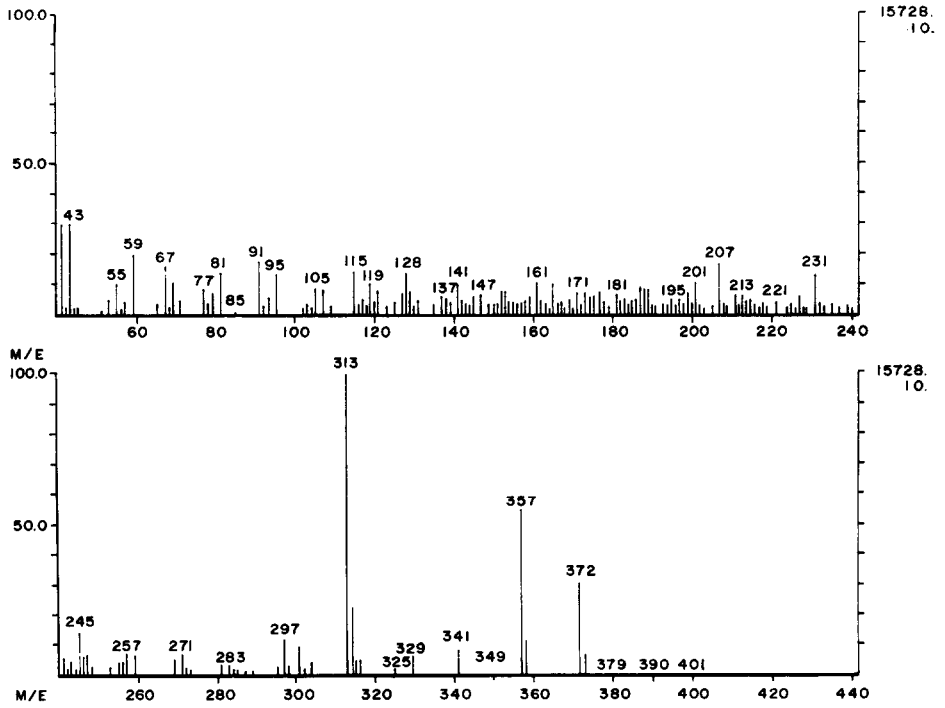

Fig. 2. Electron impact mass spectrum of the methylated derivative of standard 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid.

THC-COOH exhibits a base peak at m/z 313. This difference indicates the need to obtain a mass spectrum of the sample to differentiate the two isomers.

As indicated by Whiting and Manders[2], once the dry extract containing Δ^9-THC-COOH is methylated, the rest of the analysis should be completed on the same day to preclude a possible degradation of the derivatized compound. An inert gas should be used to evaporate to dryness solvent extracts containing the methylated cannabinoid since air tends to degrade the derivative. The TLC procedure *per se* provides a screening procedure for excluding negative specimens, especially when multiple samples are being processed. The "positive" spot can be eluted with methanol and the extract is analyzed by mass spectrometry. For forensic purposes, an MS determination affords an unequivocal identification of Δ^9-THC-COOH.

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