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Note

Marihuana metabolites in urine of man

IX. Identification of Δ^9-tetrahydrocannabinol-11-oic acid by thin-layer chromatography

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In 1974, we described a major human metabolite of Δ^9-tetrahydrocannabinol (THC) which we could not positively identify[1]. Its mobility when chromatographed in a thin-layer solvent system of petroleum ether–diethyl ether–glacial acetic acid (50:50:1) (solvent system A), was about the same as that of 8β-hydroxy-THC. It was not the latter, however, as in a solvent system of chloroform–acetone–glacial acetic acid (16:4:1) (solvent system B), the mobilities were different. Then gas–liquid chromatography–mass spectrometry (GLC–MS) specific-ion detection techniques eliminated other metabolites: 11-hydroxy-, 8α-hydroxy-, 8,11-dihydroxy, as well as 8β-hydroxy-THC. It also eliminated the possibility of other compounds of molecular weight 328 (keto-derivatives of THC) and 330 (monohydroxy- or epoxy-derivatives of THC).

At that time Δ^9-THC-11-oic acid (THC-11-oic acid) was not available as a reference standard. Acidic metabolites of THC that were described in the literature had been obtained from rabbits[2,3]. Based upon newer developments in our thin-layer chromatography (TLC) procedures, refinement of our multistep extraction procedure, and the availability of THC-11-oic acid as a reference standard, we can identify this major metabolite as THC-11-oic acid[4,5].

The new development in our TLC procedure, sequential TLC, permits separation of THC compounds into natural neutrals, alcoholic neutrals and acidics. The refinement of our multistep extraction procedure was to extract with hexane at pH 8 instead of 5.5, because it was found that THC-11-oic acid was partially extracted by hexane from an aqueous solution of the latter pH[5]. Using these techniques, we were subsequently able to show THC-11-oic acid as a major THC metabolite. The question arose as to whether or not it was the same major metabolite we had previously found but had not been able to identify.

We used two urine specimens known to contain THC-11-oic acid as determined by our later techniques. These urine specimens were obtained from two separate subjects, one taken 6–12 h after drug ingestion, the other 24–48 h after. These urines were hydrolyzed, concentrated and extracted with hexane at pH 5.5 and the residues of the extracts were separately chromatographed, one in solvent system A, the other in solvent system B[1]. Chromatograms of reference standards of THC-11-oic acid, 8β-

hydroxy-, 11β-hydroxy-, and 8β,11-dihydroxy-THC were also run on each plate. Zones corresponding to the THC-11-oic acid reference standard were eluted with ethanol from untreated chromatograms (Figs. 1A and 1B). These ethanol eluates were then chromatographed in chloroform–acetone–triethylamine (80:20:1) (solvent system C) in which neutral compounds move away from the origin, but in which acidic compounds, such as THC-11-oic acid, remain. Ethanol eluates of the material at the origin of this plate were chromatographed in petroleum ether–ether–glacial acetic acid (50:50:1) (solvent system D) in which acidic compounds move[4]. This procedure is shown in Figs. 2A and 2B.

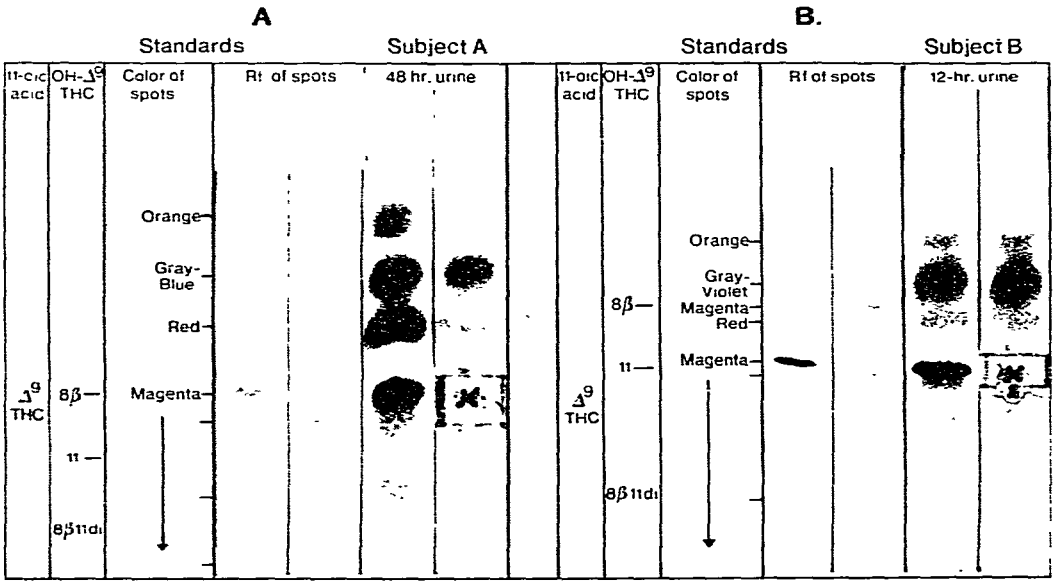

Fig. 1. Chromatograms of hexane extracts prepared at pH 5.5. (A) 48-h post-THC urine of subject A chromatographed in petroleum ether–ether–glacial acetic acid (50:50:1). (B) 12-h post-THC urine of subject B chromatographed in chloroform–acetone–glacial acetic acid (16:4:1). ×, Zones of silica gel, suspected to contain THC-11-oic acid, eluted with ethanol and chromatographed sequentially, Fig. 2. Color of spots due to reaction with Fast Blue Salt B. Spots colored orange, gray-blue, gray-violet and yellow probably not due to cannabinoids. *Correction to Fig. 1B:* The R_F value of Δ^9-THC-11-oic acid should be the same as that of 11-hydroxy-Δ^9-THC.

The silica gel zones corresponding to THC-11-oic acid that were eluted from the chromatograms prepared in solvent systems A and B respectively, had the same mobility as THC-11-oic acid when chromatographed sequentially in solvent systems C and D. This confirmed that the previously unidentified metabolite that was extracted in hexane at pH 5.5 was THC-11-oic acid[1].

Since the publication in 1974, others have demonstrated the presence of THC-11-oic acid in the urine of humans after ingestion of THC and we have also confirmed the presence of this metabolite in extracts of equivalent urines prepared by our multi-step extraction procedure and analyzed by high-performance liquid chromatography and GLC–MS[6–10].

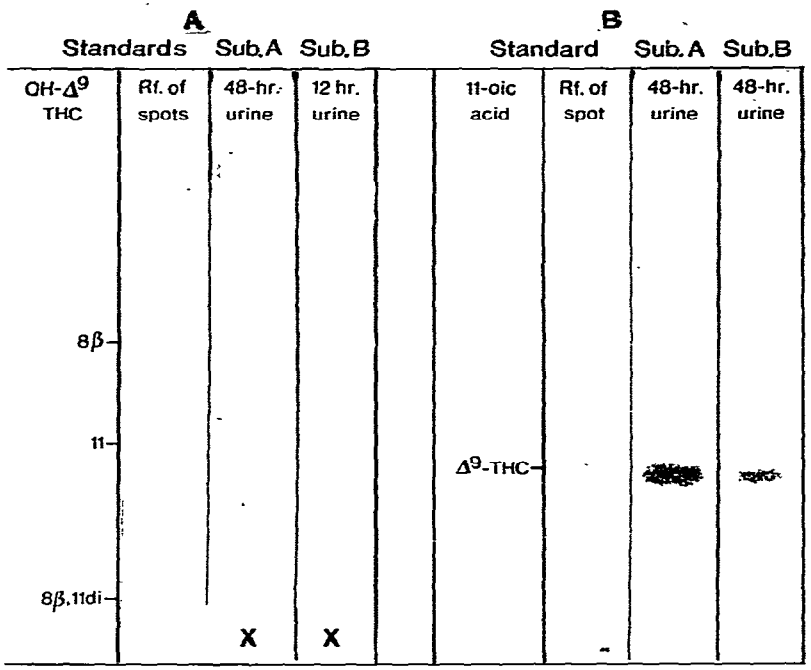

Fig. 2. Sequential chromatography of ethanol eluates of silica gel zones eluted from chromatograms described by Fig. 1. (A) Chromatographed in chloroform–acetone–triethylamine (80:20:1), in which acidic compounds remain at the origin. (B) Chromatograms of the ethanol eluates of the zones described by ×, Fig. 2A, in petroleum ether–ether–glacial acetic acid (50:50:1), in which acids are mobilized[4]. Color of spots due to reaction with Fast Blue Salt B. All spots are magenta colored.

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