J. Pharm. Pharmac., 1972, **24**, 1–6 *Received August* 16, 1971

Δ^1-Tetrahydrocannabinolic acid, an important component in the evaluation of cannabis products*

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The occurrence and some analytical properties of Δ^1-tetrahydrocannabinolic acid have been investigated, including its chromatographic behaviour in the presence of other cannabinoids. The acid is inactive but is converted on smoking into the active tetrahydrocannabinol. The acid is present in abundant amounts in various cannabis samples, marihuana in particular, and these will be more active on smoking than when administered by injection or orally. A method for the separate determination of tetrahydrocannabinol and its acid is also described.

Δ^1-Tetrahydrocannabinolic acid (Δ^1-THC acid) is known to be a naturally occurring component of cannabis products like hashish and marihuana. Although inactive as such, it can be converted, e.g. on smoking, into Δ^1-tetrahydrocannabinol (Δ^1-THC†), which is the major psychomimetically active component in cannabis. The isolation of Δ^1-THC acid from hashish and marihuana has been described (Korte, Haag & Claussen, 1965; Yamauchi, Shoyama & others, 1967), but so far, it seems that little or no attention has been paid to the behaviour of this substance in the evaluation of cannabis products. We have therefore investigated some analytical properties of Δ^1-THC acid, the possibilities for its evaluation and its chromatographic behaviour in the presence of other cannabinoids.

MATERIALS AND METHODS

Materials

Δ^1-THC acid and Δ^1-THC were isolated from an authenticated marihuana sample, the properties and origin of which will be described elsewhere. The dried and powdered sample was repeatedly extracted with fresh portions of light petroleum (b.p. 40–60°), followed by extraction with chloroform until extraction was complete. This was checked by thin-layer chromatographic examination of the extracts.

The combined extracts were evaporated to dryness in a flash evaporator (Büchi), the oily residue was weighed and redissolved in a small volume of chloroform. After filtration through a sintered glass filter the solution was applied as a 35 cm band, 1·5 cm from the bottom of a 40 × 20 preparative thin-layer plate by means of an Autoliner (Desaga). The plate was coated with a 1 mm layer of acetone-washed Silica gel PF_{254}(Merck), slurried in the normal way and dried 1 h at 110°, cooled and stored in a box under normal laboratory conditions. Development was done in an un-

* Presented at the 31st International Congress on Pharmaceutical Sciences, September 7–12, 1971, Washington, D.C., U.S.A.

† Numbered Δ^9 according to IUPAC rules.

saturated tank (Shandon), over 17 cm, using light petroleum (40–60°)–ether (80 + 20) as solvent (Machata, 1969). Bands were located by spraying small strips at the side edges with a freshly prepared 0·5% solution of tetrazotized di-*o*-anisidine (Fast Blue salt B, Merck) in water, followed by exposure of the plate to ammonia vapour. The corresponding non-sprayed areas were scraped off, extracted with adequate amounts of acetone and the filtered extracts were evaporated to dryness in a flash evaporator.

The thus isolated Δ^1-THC acid and Δ^1-THC were weighed, immediately redissolved in acetone and stored under nitrogen at −45°. In addition, hashish and marihuana samples from different origins were used. 0·1 g resin or 0·5 g herb was extracted as described above. The combined filtered extracts were concentrated to a volume of about 2 ml before chromatography.

Two batches of Extractum Cannabis C.M.N., a Dutch preparation, and at least ten years old, were also used. 0·1 g was dissolved in about 1 ml of acetone and filtered.

The identity of the cannabinoids was confirmed by combined g.l.c.-mass spectrometry according to Vree, Breimer & others (1971).

Qualitative t.l.c.

Plates were coated with layers of 0·25 mm Silica gel G (Merck), slurried in the normal way, dried for 30 min at 110° and stored in a box under normal laboratory conditions. Starting points were 1·5 cm from the bottom edge of the plate. Solvent systems were: 1) light petroleum–ether (80 : 20) (Machata, 1969); 2) benzene, in combination with a trough with 10 ml 25% ammonia at the bottom of the chamber; 3) cyclohexane on dimethylformamide impregnated plates (Korte & Sieper, 1964), using the procedure described by Merkus (1971). Development was always in unsaturated chambers to a height of 15 cm over the starting points. Visualization of the spots by spraying with Fast Blue salt B was as described above.

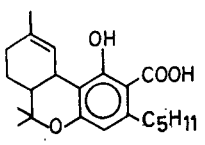
Temperatures ranged from 20–22° and the relative humidity from 22–45%. In these ranges reproducible R_F values could be obtained with the first two solvent systems; with the third system R_F values may vary, depending on the drying conditions after the impregnation, but the separation sequence does not change (Merkus, 1971).

Gas-liquid chromatography

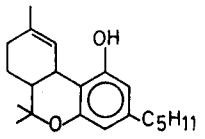
A Becker 409 instrument with flame ionization was used. Stainless steel columns, 2 mm × 2 m, packed with 5% SE 30 on DMCS-treated Chromosorb G-AW, 80–100 mesh. Carrier gas nitrogen, 20 ml/min, inlet pressure 2·5 kg/cm^2; injection block 275°; oven 230°; detector 275°. Trimethylsilyl derivatives of cannabinoids were obtained by evaporating small samples to dryness under a stream of nitrogen, these were then redissolved in 0·5 ml Tri-Sil (Pierce). After shaking and standing for 5 min the solution was ready for injection.

RESULTS AND DISCUSSION

The marihuana sample from which cannabinoids were isolated contained about 0·5% Δ^1-THC acid and 0·1% Δ^1-THC by weight. They were obtained as yellowish oily materials, of which the nmr, ultraviolet, infrared and mass spectra fitted those in the literature. The spectra also indicated the acid to be Δ^1-THC acid *A* (Mechoulam, Ben-Zvi & others, 1969), with the carboxyl group in the 4′ position (monoterpenoid numbering). The mass spectrum of the acid taken at 135° inlet temperature and 70 eV



Δ^1-THC acid



Δ^1-THC

energy is almost identical to that of THC. The only differences are that the acid shows a very small peak (less than 1%) at m/e 358 as the parent peak and a peak at m/e 44 (CO_2) which is about 20 times stronger than in THC (Claussen, Fehlhaber & Korte, 1966). In addition, minor quantities of cannabidiolic acid (CBD acid) and cannabinol (CBN) were found in the marihuana sample in concentrations of about 0·5% each. They did not interfere with the isolation of THC acid and THC. The isolated Δ^1-THC proved to be chromatographically pure, at least 99% by t.l.c. and g.l.c. Its solution in acetone kept under nitrogen at −45° showed no decomposition after two months.

The instability of the isolated Δ^1-THC acid was confirmed (Korte & others, 1965; Yamauchi & others, 1967): decarboxylation takes place rapidly under the influence of light, heat, alkali, and most probably, on active surfaces like thin layers. This makes it very difficult to obtain a sufficiently pure sample of Δ^1-THC acid that can be used for exact determinations of physical constants, or as a reference standard for quantitative analysis. In our opinion, it will always be contaminated by varying amounts of Δ^1-THC. However, in products like hashish, marihuana and pharmaceutical extracts the acid shows a much better stability. This could be shown by stability tests made at room temperature (20°) with the isolated acid and the acid-containing marihuana. Moreover, the C.M.N. extracts, after storage for at least ten years on the shelves of a University pharmacy, still contained large amounts of Δ^1-THC acid, as well as other cannabinoid acids. It seems likely that the complex composition of these products has a stabilizing effect on the acid when kept at room temperature. At elevated temperatures, however, rapid decomposition cannot be prevented. A hashish sample showed about 50% conversion of THC acid into THC when heated for 1 min at 50°; when heated for 1 min at 100° conversion increased to about 80%, and when heated for 1 min at 150° conversion was complete. Isomerization in the terpenoid part of the acid molecule, as described by Claussen & Korte (1968b), was not seen.

The behaviour of Δ^1-THC acid in t.l.c. systems generally in use for the evaluation of cannabis products is erratic. With various alkaline solvents it was retained at the start, possibly with decomposition. Conversion into Δ^1-THC and migration as such was not seen. However, on the reversed phase system cyclohexane on dimethylformamide impregnated plates, the acid moves as such and can be found in the same area as CBD, visualized as a scarlet red elongated spot with the Fast Blue spray reagent. An exact R_F value cannot be given as the migration rate of the acid depends on its loading. With low concentrations of THC acid the streak moves slightly behind the spot of CBD; with higher concentrations of the acid the top of its streak starts to coincide with the CBD spot (see Fig. 1) or may even run higher. The position of the CBD spot itself is not dependent on the concentration. Other major acids like CBD acid and CBN acid remain at or near the starting point in this system.

With neutral solvents Δ^1-THC acid gives a characteristic streak, again showing a scarlet red colour on spraying with the Fast Blue reagent. Its position is distinctly

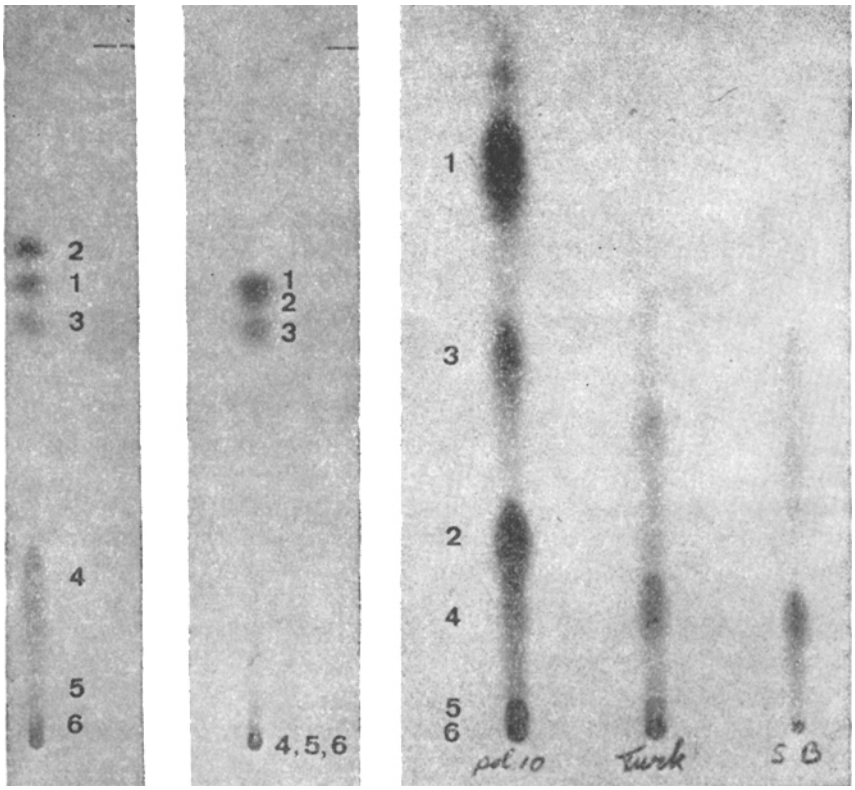


FIG. 1. Thin layer chromatographic behaviour of various cannabinoids. Left: Separation on silica gel with light petroleum–ether (80 : 20) as solvent. Centre: Separation on silica gel with benzene as solvent, with a trough containing 10 ml 25% ammonia at the bottom of the chamber. No migration of cannabinoid acids. Right: Separation on silica gel, impregnated with dimethylformamide, and with cyclohexane as solvent. Code: 1, Δ^1-THC; 2, CBD; 3, CBN; 4, Δ^1-THC acid; 5, CBD acid; 6, CBN acid. Visualization by spraying with Fast Blue salt B.

lower than that of the neutral cannabinoids, but its R_F value changes with the concentration, as in the dimethylformamide-impregnated system. Fig. 1 also shows a chromatogram from the neutral solvent system. Other cannabinoids like CBD and CBN and their respective acids behave similarly. When applied in equal concentrations the acids migrate in the order THC acid > CBD acid > CBN acid, with the latter remaining at or near the starting point with the neutral solvent system used. The colours of the acids upon spraying with Fast Blue salt B are scarlet red for THC acid, orange for CBD acid and violet red for CBN acid, respectively.

It is thus meaningless to give R_F values for the acids. On the other hand, it should be noted, that higher concentrations of CBD acid may well give the impression that Δ^1-THC acid is present. With higher concentrations of CBD acid its streak moves upward and its colour upon spraying deepens to red. That we could easily isolate Δ^1-THC acid by preparative t.l.c. with a neutral solvent was because only a small amount of CBD acid was present, but with other samples the two acids might overlap.

Two-dimensional t.l.c. made with the neutral and the alkaline solvent system gives some more information but still lacks adequate resolution of more complex mixtures. A neutral solvent has to be used in the first direction to let the acids migrate. If an

alkaline solvent is used first, the acids remain at the start, even after running with the neutral solvent in the second direction. This indicates decomposition during the alkaline treatment. Conversion of the acids into their respective neutral analogues on the plate by heat treatment (10 min at 110°) was unsuccessful. No migration of converted acids nor of authentic neutral cannabinoids could be observed with the second solvent. All acids and neutral cannabinoids probably decompose under these conditions.

Shoyama, Yamauchi & Nishioka (1970) described a special solvent for t.l.c. of cannabinoid acids, namely hexane–ethyl acetate (50 : 50) on silica gel. In this the acids run higher but at the same time the neutral cannabinoids run into the solvent front, making it unsuitable for evaluating both neutral and acidic cannabinoids. We were unable to achieve a separation between Δ^1-THC and CBD acids with this system.

Thus, if acids are present, t.l.c. cannot be used satisfactorily in the evaluation of cannabis products, except for some of simple composition.

In g.l.c. it is well known that Δ^1-THC acid, like other cannabinoid acids, is instantaneously decarboxylated on the column at the temperatures used. As decarboxylation also occurs on smoking, evaluation of cannabis by g.l.c. includes all the THC that will become available to the smoker. However, variable amounts of this THC become lost by smoke escaping into the air, exhalation from the respiratory dead space, or pyrolysis. Estimations on amounts actually inhaled range from 1–2% (Claussen & Korte, 1968a) to 20–80% (Hollister, 1971). When cannabis is not smoked and administered otherwise, e.g. orally or by injection, Δ^1-THC acid is inactive (Mechoulam Shani & others, 1970). Hence, for those cases normal g.l.c. cannot be used for the evaluation of active components and has to be replaced by g.l.c. of esterified derivatives of the acid, which are stable under the conditions used. This can be done easily by silylation. The trimethylsilyl derivatives were stable in solution for at least 1 h after preparation when protected against water. This was tested by g.l.c. of the silyl derivatives immediately and 1 h after preparation. Peak areas varied less than 1%.

The silyl derivatives of Δ^1-THC acid and Δ^1-THC separate well. The relative retention times, together with those of other major cannabis constituents are in Table 1.

Table 1. *G.l.c. retention times of major cannabis constituents, relative to CBD.*

Compound	Normal g.l.c.	Silylation g.l.c.
CBD	1·00[1]	1·00[2]
Δ^1-THC	1·28	1·31
CBN	1·54	1·71
CBD acid	(1·00)	2·16
Δ^1-THC acid	(1·28)	2·99
CBN acid	(1·54)	3·55

[1] Retention time 16 min 5 s
[2] Retention time 12 min 0 s

However, when exact quantitation of the acid is required, silylation g.l.c. still presents a difficulty. A stable and sufficiently pure sample of Δ^1-THC acid is unavailable as a reference standard so that the surface under the peak cannot be related to the exact amount of acid present. Therefore, in our opinion, the best way of quantitating both Δ^1-THC acid and Δ^1-THC is by determining their sum as Δ^1-THC by normal g.l.c., followed by determining Δ^1-THC alone by silylation g.l.c. From the difference between these determinations the amount of Δ^1-THC acid can be derived.

When acid-containing cannabis products are consumed in tea, conversion of Δ^1-THC acid into Δ^1-THC will occur rapidly if heat is used after cannabis has been added. Exact evaluation can only be obtained from an analysis just before the tea is drunk.

Quantitation by normal g.l.c. after a t.l.c. separation of the acids and the neutral cannabinoids will give too low values for the acid due to decomposition on the plate.

When cannabis products were extracted with light petroleum or hexane the recovery of Δ^1-THC acid usually remained far from complete even on repeated extraction.

A sample of 0·1 g hashish still contained Δ^1-THC acid after 5 subsequent extractions with fresh 10 ml portions of light petroleum. Therefore, if quantitation is required, it is recommended that more polar liquids like chloroform, ether or acetone be used. With each of these liquids extraction of 0·1 g hashish was complete with two fresh portions each of 10 ml. This procedure extracts plant products as well, but these do not interfere with the t.l.c. and g.l.c. procedures.

Although Δ^1-THC acid is considered to be a genuine plant substance, surprisingly little information exists on its occurrence in nature. Much attention has been paid so far to the occurrence of Δ^1-THC, with Δ^1-THC acid being considered a minor component. Our experience, however, is contrary. In various hashish and marihuana samples, selected at random, large amounts of Δ^1-THC acid could be detected, the quantities being similar to that of Δ^1-THC in the sample or even higher. The latter was especially so in several marihuana samples. The sample from which Δ^1-THC acid was isolated had a ratio THC acid/THC of at least 5:1. In further studies on the occurrence of Δ^1-THC acid, we have been unable to detect Δ^1-THC acid *B*, or the Δ^6-THC acids.

Acknowledgements

We are indebted to Dr. A. Segelman, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa., U.S.A. for a sample of authentic Δ^1-THC. Thanks are also due to J. Wijsbeek and S. Batterman for their comments and assistance.

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