Structure of an acidic metabolite of Δ^1-tetrahydrocannabinol* isolated from rabbit urine†

During the last few years a number of *in vitro* and *in vivo* metabolites of (−)-Δ^1-tetrahydrocannabinol (Δ^1-THC), the main active constituent of *Cannabis sativa* L., have been isolated and identified. The *in vivo* metabolism leads to highly oxidized structures often containing carboxylic functions and appearing in urine as free acids or their conjugates. So far, Δ^1-THC-7-oic acid (Wall, Brine & Perez-Reyes, 1973) and the corresponding acids carrying additional hydroxyl groups in the 1″- and 2″-positions of the pentyl side-chain, respectively (Burstein, Rosenfeld & Wittstruck, 1972), have been identified with reasonable certainty. Two more monocarboxylic acids have recently been isolated (Ben-Zvi, Bergen & Burstein, unpublished). We wish to report the isolation and structure of a new *in vivo* urinary metabolite containing two carboxylic groups.

(−)-1″-^{3}H-Δ^1-THC (Agurell, Gustafsson & others, 1973) with a chemical and radiochemical purity greater than 95% according to t.l.c. and g.l.c. was administered intravenously in 70% aqueous ethanol to four female rabbits (2 kg). The daily dose was slowly raised from 1 to 4 mg kg^{-1}. The rabbits received a total dose of 175 mg ^{3}H-Δ^1-THC with a specific activity of 0·1 μCi mg^{-1}. The frozen urine samples were thawed, pooled (4100 ml) and filtered through Celite. The recovery in urine of the administered dose was 24%.

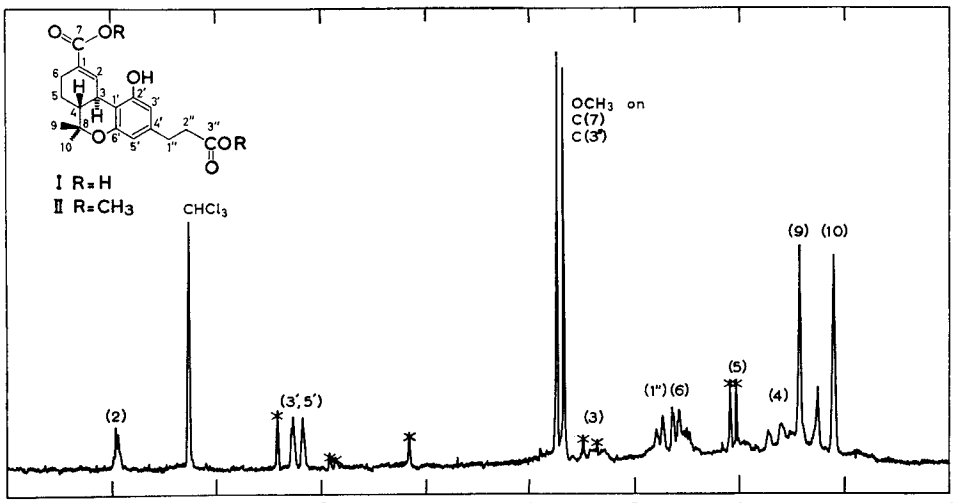
For preparative isolation of non-conjugated acidic metabolites we used the scheme previously described for isolation of urinary metabolites of 7-hydroxy-$\Delta^{1(6)}$-THC (Nilsson, Agurell & others, 1973). This involves an initial purification on an Amberlite XAD-2 column followed by extractions with diethyl ether at pH 3 and subsequent re-extractions to obtain an acidic extract free from neutral compounds. The final ether extract (containing 28% of radioactivity excreted in urine) was dried over sodium sulphate, the solvent evaporated *in vacuo* and the residue dissolved in 5% aqueous bicarbonate. After adjusting to pH 7, metabolites were absorbed on Amberlite XAD-2. A rough separation of the acidic metabolites was obtained by elution with 300 ml 10% aqueous ammonia (ammonia fraction) and followed by 200 ml methanol (methanol fraction).

The ammonia fraction was lyophilized, dissolved in water, acidified to pH 3 and extracted with diethyl ether. The dried ether extract was dissolved in 4 ml 0·1M ammonium bicarbonate (1% butanol to prevent microbial growth) and after adjustment to pH 8 chromatographed on Sephadex G-25 Superfine (1·5 × 180 cm) with 0·1M ammonium bicarbonate as eluant. The elution rate was 4 ml h^{-1} and fractions of 4 ml were collected. Aliquots were subjected to liquid scintillation counting. The elution pattern suggested the presence of three metabolites with retention volumes (V_R): 250, 325 and 385 ml. The major peak (V_R = 325 ml) was isolated, and rechromatographed on the same column.

The main acid fraction was converted to methyl esters using diazomethane and chromatographed on a Sephadex LH-20 column (Nilsson & others, 1973). A single peak (V = 46 ml; void volume 19 ml) containing the methyl ester II was recovered (1·5 mg; 60% pure). The methyl ester II was purified twice by t.l.c. on washed Silica gel F plates (Merck) with benzene–methanol (98·2) and chloroform as eluants respectively. The isolated material (0·6 mg) contained no further impurities according to t.l.c. and g.l.c.

The pmr spectrum of II (100 mHz, $CDCl_3$, Fourier-Transform) shows a doublet at

* Δ^9-Tetrahydrocannabinol (I.U.P.A.C. nomenclature).
† Metabolism of cannabis xxvi.



100 MHz ^{1}H-nmr spectrum of II in $CDCl_3$
× = impurity

δ7·95 ($J = 2$Hz) corresponding to the vinylic proton on C-2. The two doublets ($J \sim 1$Hz) centred at δ6·27 and δ6·17 arise from H-3′ and H-5′. Two sharp three proton singlets at δ3·75 and δ3·69 correspond to two carboxylic groups in I. The signal of H-3 appears as a broad doublet at δ3·36 ($J = 10$Hz), while the benzylic protons on C-1″ give rise to a multiplet centered at δ2·7. Two further 2-H multiplets at δ2·5 and δ2·0 may represent the protons on C-6 and C-5 or C-2″ respectively. The signals of the geminal methyls C-9 and C-10 occur at δ1·44 and δ1·12. Irradiation on H-4 (~δ1·68) led to a collapse of the doublet at δ3·36 (H-3), while irradiation on H-3 resulted in a sharp singlet for H-2 at δ7·95.

Due to the absence of the signals corresponding to C-7 and C-5″ of Δ^1-THC we conclude the two carboxylic groups of I to be located at C-7 and in the side-chain. The failure to decouple the protons on C-1″ indicates that the side-chain carboxylic group is either located on C-2″ or on C-3″ in which case the protons on C-2″ would appear at a higher chemical shift than in similar compounds with intact side-chains (H-2″ at δ1·7; Binder, Agurell & others, unpublished), too close to the 1″-protons to allow decoupling.

Structure II is supported by the high resolution mass spectrum that gave the M^+ ion at *m/e* 374·1746 ($C_{21}H_{26}O_6$: 374·1729) and the M^+-CH_3 ion (base peak) at *m/e* 359·1515 ($C_{20}H_{23}O_6$: 359·1495), proving a three carbon side-chain. Further prominent peaks (low resolution mass spectrum) were present at *m/e* (rel. intensity): 343 (8, M^+-OCH_3), 331 (4, M^+-C_3H_7), 315 (55, M^+-CO_2CH_3), 301 (4, M^+-$CH_2CO_2CH_3$), 299 (35), 247 (7, M^+-CH , *retro*-Diels-Alder), 209 (7) and 174 (8). These fragmentations are in agreement with those of Δ^1-THC (Budzikiewicz, Alpin & others, 1965).

On the basis of the pmr and mass spectral data we assign the structure 4″, 5″-bisnor-Δ^1-*trans*-tetrahydrocannabinol-7,3″-dioic acid to the new metabolite I.

The authors wish to thank Dr. K. Leander for the supply of Δ^1-tetrahydrocannabinol. We are also grateful to Dr. T. Drakenberg for recording the pmr spectra and to Dr. R. Ryhage and Dr. J.-E. Lindgren for measuring the mass spectra. This work was supported by the Swedish Medical Research Council and Försvarsmedicinska forskningsdelegationen. One of us (M.B.) was supported by the "Schweizerischer Nationalfonds".

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February 6, 1974

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Characteristics of the mass spectra of some aliphatic amine *N*-oxides

The mass spectra of eight amine oxides have been studied because their parent amines are common structural features of drugs. The eight compounds have been prepared by standard methods and their behaviour in an LKB 9000S mass spectrometer has been studied at different temperatures. Compounds were introduced into the mass spectrometer using the insertion probe unheated, heated to 50° or heated to 100°. The temperature of the ion source was maintained at 250° and this resulted in the quartz tip of the probe being heated as soon as it was inserted into the source. Consequently, when it was desired to obtain spectra at the lowest possible temperature and when the probe was unheated, spectra were scanned immediately after probe insertion. At other probe temperatures spectra were scanned when the temperature of the probe heater indicated the appropriate value.

Features of the spectra of the amine oxides, together with limited data on the spectra of the corresponding amines, are listed in Table 1. Six of the eight amine oxides gave a molecular ion when the probe was unheated and the abundance of this ion was markedly reduced when the probe was heated to 50°. At a probe temperature of 100°, no molecular ion was observed for any of the compounds, due, presumably, to thermal degradation to the parent amine. Using an unheated probe, those compounds giving a molecular ion were the cyclic amine oxides (1 to 5) and *NN*-dimethylaniline-*N*-oxide (8). In the former case losses of 1, 16 and 17 mass units resulted in abundant ions and such losses appear to be characteristic. The loss of unit mass is probably associated with the formation of the imonium ion of the amine oxide, the loss of 16 mass units to the loss of oxygen whilst the M-17 ion is likely to be the imonium ion of the amine. Support for these proposals may be found by comparing the relative abundances of the M-16 and M-17 peaks of the oxides (1–5) with the abundances of the M and M-1 peaks of the corresponding amines.

NN-Dimethyl-2-phenylethylamine-*N*-oxide (6) gave no molecular ion, even with the probe unheated, but had abundant ions at *m/e* 104 (100%) and 61 (76%), ions which correspond to styrene and dimethylhydroxylamine respectively, and which are the expected products of Cope elimination (Cope & Le Bel, 1960) *NN*-Dimethyl-