(−)-Δ^8-Tetrahydrocannabinol: Two Novel in vitro Metabolites

Several groups of investigators have recently reported metabolic studies of both (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC)[1,2] and (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC)[3–8], two of the psychotomimetic constituents of marijuana (*Cannabis sativa* L.).

We wish to report the isolation and identification of two new metabolites, 1'-hydroxy-Δ^8-THC (IIa) and 3'-hydroxy-Δ^8-THC (IIIa).

These were obtained by aerobic incubation of Δ^8-THC-2,4-$^{14}C_2$[9] (I) with the 9000 *g* supernatant fraction of male dog (beagle) liver using the conditions and cofactors of CONNEY et al.[10].

After extraction of the incubate with ethyl acetate, the crude metabolite fraction was acetylated overnight with acetic anhydride: pyridine (1:1). Preliminary silica gel thin-layer chromatography (TLC) in benzene:methanol (98:2), followed by autoradiography, revealed two major radioactive areas plus unmetabolized Δ^8-THC. Approximately 70% of the starting material was recovered. Of the 30% which was metabolized, the less polar area comprised about 13%, the more polar 26%. The remainder was composed of several unidentified radioactive components.

The less polar material (IIb) was further purified by TLC in benzene and analyzed by gas-liquid chromatography (GLC) on 3% OV-225 (6' × 4 mm). Two peaks with relative retention time (RRT) of 1.27 and 3.12 (cholestane = 1.00) were observed. Collection of these peaks during GLC with an effluent splitter showed 70% of the radioactivity of the sample to be in the 3.12 peak. This fraction was further purified by TLC in benzene before mass and NMR spectroscopic analysis.

Compound IIIb, the more polar material, was purified by TLC in benzene:methanol (98:2); when GLC showed a single component, IIIb was submitted directly for NMR and mass spectrometry.

On overnight treatment with dilute methanolic sodium hydroxide, purified IIb and IIIb were deacylated to IIa and IIIa respectively. Mass spectra were obtained for both substances.

High resolution mass spectrometry of IIb showed a molecular ion at *m/e* 414.242 ($C_{25}H_{34}O_5$), with strong fragment ions at *m/e* 372, 354, 312, 297, 289, 271 and 256. Low resolution mass spectrometry of IIa showed a molecular ion at *m/e* 330. These data are consistent with monohydroxylation of the parent molecule.

The high resolution mass spectrum of IIIb showed a molecular ion whose elemental composition was identical to that of IIb. Low resolution mass spectrometry of IIIa showed a molecular ion at *m/e* 330, as in IIa.

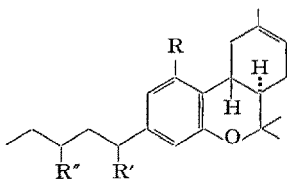
The salient features of the NMR spectrum of IIb (Table) were the absence of peaks due to benzylic protons at δ 2.50 and the appearance of a new methine peak at δ 5.68, indicating acetoxyl substitution on C-1'. Comparison with the model compound 1-(3,5-dihydroxyphenyl-)1-pentanol triacetate (IV) showed a methine proton shift at δ 5.70. Thus, only structure IIa can be assigned to the metabolite.

The NMR spectrum of IIIb (Table) showed a methine proton quintet at δ 4.87 (J = 6Hz), suggesting that the

Chemical shifts of protons[a]

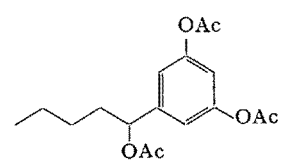
Assignments	IIb	IIIb
5'-CH_3	0.88	0.88
6α-CH_3	1.18	1.09
6β-CH_3	1.42	1.36
9-CH_3	1.70	1.68
1'-OAc	2.05	–
3'-OAc	–	2.01
1-OAc	2.30	2.26
1'-CH_2	–	2.54
8-H	5.45	5.45
2-H	6.47	6.43
4-H	6.57	6.58
1'-H	5.68	–
3'-H	–	4.87

[a] 100 MHz NMR-spectra in $CDCl_3$ solution, using tetramethylsilane as an internal reference.

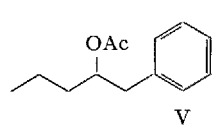


I, R=OH; R'=R''=H
IIa, R=R'=OH; R''=H
IIb, R=R'=OAc; R''=H
IIIa, R=R''=OH; R'=H
IIIb, R=R''=OAc; R'=H

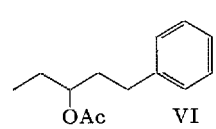
Fig. 1.



IV



V



VI

Fig. 2.

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acetoxyl substituent was at either C-2′ or C-3′. The model compound 1-phenyl-2-pentanol acetate (V) showed a methine shift at δ 5.07. However, 1-phenyl-3-pentanol acetate (VI) showed a methine shift at δ 4.87 (quintet, J = 6Hz), thus establishing IIIa as the structure of the second metabolite.

The configuration of the hydroxyl groups on C-1′ and C-3′ is not known[11].

Zusammenfassung. (−)-Δ^8-Tetrahydrocannabinol ist einer der Aktivstoffe in Marihuana (*Cannabis sativa* L.). Inkubation dieser Verbindung mit der überstehenden Zentrifugationsfraktion (9000 *g*) aus männlicher Hundeleber ergab zwei Hauptmetaboliten, welche durch Massenspektrometrie und Kernresonanzspektroskopie identifiziert wurden. Den beiden Verbindungen werden die Strukturen des 1′-Hydroxy-Δ^8-tetrahydrocannabinols und des 3′-Hydroxy-Δ^8-tetrahydrocannabinols zugeordnet.

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Nutley (New Jersey 07110, USA), 25 June 1971.

[11] We thank Dr. K. E. Fahrenholtz for the preparation of the model compounds IV, V and VI, and Dr. A. Brossi for his continuing encouragement.

Changes in Microsomal Lipids of Rat Liver after Chronic Carbon Tetrachloride Intoxication

Previous studies from our laboratory[1,2] described changes in the fatty acid composition of rat liver microsomal lipids following acute carbon tetrachloride intoxication. The observed decrease in arachidonic acid was interpreted on the basis of CCl_4-induced lipid peroxidation. This interpretation is supported by several lines of evidence which demonstrate the occurrence of such a process in the liver cell structural lipids of carbon tetrachloride poisoned rats[3–7]. It is suggested that this reaction plays an important role in the pathogenesis of the hepatotoxic effect of the poison.

Recently it has been shown[8] that the hepatic triglyceride accumulation resulting from chronic carbon tetrachloride administration to rats is due to an inhibition of the secretion of low density lipoproteins, i.e. to the same mechanism leading to fatty liver following acute CCl_4 poisoning[9,10].

Therefore it seemed to be of interest to investigate whether the alterations of liver microsomal lipids induced by acute CCl_4 intoxication could still be detected (and if so, to what extent) after chronic carbon tetrachloride treatment. Positive evidences for changes, possibly related to a lipoperoxidation process, would support a common origin for both acute and chronic CCl_4-induced fatty liver.

Female Wistar rats maintained on a pellet diet (Piccioni, Brescia, Italy) were used. CCl_4 was mixed with an equal volume of olive oil and the mixture was administered s.c., twice weekly, in a dose of 0.25 ml/100 g body weight. Control rats received an equal volume of olive oil. Since a study of a long term effect of CCl_4 was planned, the treatment was prolonged for 8 months. About 50% of the CCl_4-treated rats died throughout the experimental period.

Eight randomly chosen survivors and as many controls were used for the present study. They did not receive injections 3 days in advance and were starved 15 h before sacrifice. The mean body weight at the start of the CCl_4 treatment was 162 ± 4 g for the control and 166 ± 6 g for the experimental group. The final body weight was 268 ± 6 and 265 ± 16 g for each group, respectively.

The fatty acid composition of microsomal phospholipids was determined by gas-liquid chromatography as previously reported[1,11]. Heptadecanoic acid (GLC grade, Sigma Chem. Co.) was used as an internal standard.

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Fatty acid composition of microsomal phospholipids of rat liver after chronic carbon tetrachloride intoxication

	Group	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:6}$
Composition (%)	Control	0.2 ±0.02	17.4 ±0.7	0.8 ±0.1	28.8 ±0.8	7.0 ±0.4	9.4 ±0.2	30.2 ±0.3	6.4 ±0.3
	CCl_4	0.3 ±0.05	23.4 ±1.2[a]	1.4 ±0.2[b]	23.8 ±0.5[a]	11.0 ±0.3[a]	10.4 ±0.3	24.9 ±0.9[a]	4.7 ±0.9[b]
mg/microsomes/ g liver	Control	0.01 ±0.002	1.16 ±0.08	0.05 ±0.01	1.91 ±0.08	0.46 ±0.05	0.63 ±0.04	2.02 ±0.10	0.41 ±0.02
	CCl_4	0.01 ±0.002	1.13 ±0.09	0.07 ±0.01	1.15 ±0.09[a]	0.54 ±0.05	0.51 ±0.04[c]	1.22 ±0.12[a]	0.23 ±0.02[a]

Results are expressed as mean of 8 rats each group ± S.E. [a] $P < 0.001$. [b] $P < 0.01$. [c] $P < 0.05$.