In vivo metabolites of cannabinol identified as fatty acid conjugates

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Recent *in vivo* metabolic studies of cannabinol (CBN), a major component of *Cannabis sativa*, have shown that CBN has a complex metabolic pattern. Monohydroxylation at the C-7 position as well as at each of the carbons of the pentyl side chain (Burstein & Varanelli 1975; Wall, Brine & Perez-Reyes, 1976; Yisak, Widman & others, 1977), dihydroxylation (Burstein & Varanelli 1975, Yisak & others 1977), oxidation to aldehyde (Yisak & others, 1977), acids and hydroxy acids (Burstein & Varanelli, 1975; Wall & others, 1976; Yisak & others, 1977) and conjugation with β-glucuronic acid (Harvey, Martin & Paton, 1977) have been reported. Furthermore CBN and CBN derivatives have also been isolated (Ben-Zvi, Bergen & Burstein, 1974; Widman, Nordqvist & others, 1974; McCallum, Yagen & others, 1975; Ben-Zvi, Bergen & others, 1976) as metabolites of Δ^1-tetrahydrocannabinol (Δ^1-THC). Recently, Leighty, Fentiman & Foltz (1976) reported long retained novel fatty acid conjugates of 7-hydroxy-Δ^1- and 7-hydroxy-Δ^6-THC in the rat. We now wish to report the isolation and characterization of fatty acid conjugates of 4″-hydroxy-, 5″-hydroxy- and 7-hydroxy-CBN as *in vivo* metabolites of CBN isolated from rat faeces. ^{14}C-CBN with a radiochemical purity greater than 97% according to thin-layer chromatography (t.l.c.) and gas chromatography (g.c.) and a specific activity of 8·0 μCi mmol^{-1} was administered (100 mg kg^{-1}) *via* the tail vein in 70% aqueous ethanol to 12 Sprague-Dawley rats (200–250 g). Urine and faeces were collected for six days and assayed for radioactivity as described earlier (Yisak & others, 1977). The lyophilized and finely ground faeces was extracted with light petroleum (b.p. 40–60°) (1000 ml) using a soxhlet extractor. The extract, which accounted for 19% of the radioactivity in the faeces, was chromatographed on a Florisil column (160 g; 1·5 × 115 cm) and eluted with ether–light petroleum (1 : 9). The eluate afforded two radioactive fractions on the Sephadex LH-20 column (1 × 70 cm) eluted with light petroleum–chloroform (1 : 1). The fraction with the elution volume (Ve) of 30–50 ml contained metabolites less polar than CBN on t.l.c., while the fraction with the Ve of 125–160 ml afforded unchanged CBN. Purification of the metabolite fraction (Ve 30–50 ml) by t.l.c. (Yisak & others, 1977) using ether–light petroleum (1 : 9) as the solvent system yielded two radioactive bands. Gas chromatography at 300° on a Varian 2100 (1·06 m × 2 mm i.d. glass column, 3% SE-30 on 100–120 mesh Gas-Chrom Q of the trimethyl silylated metabolites from each band showed band 1 to contain two metabolites with retention times 3·2 and 4·2 min while band 2 yielded four metabolites with retention times 3·0, 3·9, 4·0 and 5·3 min.

§ Correspondence.

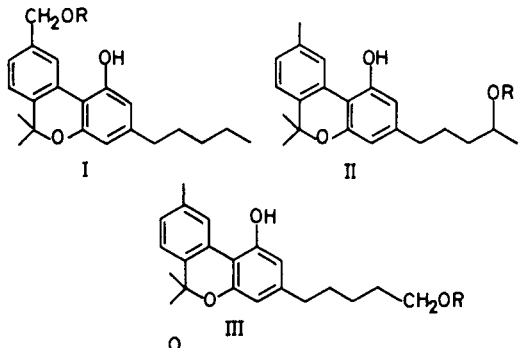
Table 1. *G.c. and ms data of fatty acid esters of CBN metabolites.*

G.c.[a] Rt (min)	M+·	Ms[b] M+·–15	Other diagnostic ions
7-Hydroxy-CBN palmitate (IA)			
3·2	636 (50)	621 (100)	606·4*; 381 (23); 366 (51)
4″-Hydroxy-CBN palmitate (IIA)			
3·0	636 (39)	621 (100)	606·4*; 381 (30); 366 (83)
5″-Hydroxy-CBN palmitate (IIIA)			
3·9	636 (36)	621 (100)	606·4*; 381 (9); 366 (32)
7-Hydroxy-CBN oleate (IB)			
4·2	662 (41)	647 (100)	632·3*; 381 (13); 366 (44)
4″-Hydroxy-CBN oleate (IIB)			
4·0	662 (34)	647 (100)	632·3*; 381 (40); 366 (88)
5″-Hydroxy-CBN oleate (IIIB)			
5·3	662 (33)	647 (100)	632·3*; 381 (8); 366 (30)

[a] Retention time of the silylated metabolite on 3% SE-30 at 300°. [b] Ms of the silylated metabolite at 20 eV. Rel. intensity within parentheses. * = metastable ion.

Mass spectra of the silylated metabolites at 20 eV (Table 1) indicated them to be esters of palmitic and oleic acids due to their molecular ions *m/e* 636 and *m/e* 662 respectively. Furthermore the ms included *m/e* 381 which is in agreement with the loss of the fatty acid moiety and *m/e* 366 which is consistent with the fragment M+·–15–fatty acid moiety. Hydrolysis of the metabolites with methanolic KOH yielded 7-hydroxy-CBN from band 1 and 4″-hydroxy- and 5″-hydroxy-CBN from band 2. The aglycones were identified by comparing their t.l.c., g.c. and ms properties with those of reference synthetic samples (Widman, Dahmén & others, 1975).

The pmr ($CDCl_3$) of band 1 yielded signals at δ ppm: 8·40 (S, 1H, C-2) 7·26 (1H, C-6); 7·22 (1H, C-5); 6·44 (1H, C-3′); 6·30 (1H, C-5′); 5·34 (m, 2H, olefinic protons); 5·10 (S, 2H, C-7), 2·30 (m, 2H, C-1″); 1·61 (6H, C-9, C-10). The pmr, thus, showed signals similar to that of 7-hydroxy-CBN (Widman, Nilsson & others, 1971) which confirmed that 7-hydroxy-CBN was the aglycone. Furthermore, the multiplet signal at δ = 5·34 ppm due to olefinic protons supported the presence of an unsaturated fatty acid. Synthetic 7-hydroxy-CBN esters of stearic, oleic and palmitic acids were prepared by warming the corresponding fatty acid chlorides with 7-hydroxy-CBN in benzene followed by hydrolysis with $NaHCO_3$ and compared by g.c. and g.c.-ms with the metabolites in band 1. Thus, the metabolites present in band 1 were proved to be identical to 7-hydroxy-CBN palmitate (IA) and 7-hydroxy-CBN oleate (IB).



I II III

IA, IIA, IIIA R= $-\overset{O}{\overset{\|}{C}}-(CH_2)_{14}-CH_3$

IB, IIB, IIIB R= $-\overset{O}{\overset{\|}{C}}-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$

The pmr of the mixture of the metabolites in band 2 exhibited signals at δ ppm: 8·40 (1H, C-2); 7·26 (1H, C-6); 7·12 (1H, C-5); 6·42 (1H, C-3′); 6·30 (1H, C-5′); 5·36 (m, olefinic protons); 3·5–3·8 (m, protons at C-4″, C-5″); 2·18–2·60 (m, 2H, C-1″); 2·38 (S, 3H, C-7); 1·60 (6H, C-9, C-10). The pmr afforded a singlet at δ 2·38 ppm showing that the C-7 methyl group was not functionalized. Instead there was a complex multiplet at δ = 3·5–3·8 ppm showing functionalization of the pentyl side chain had occurred (cf. Binder, Agurell & others, 1974) which agrees with previous finding of 4″-hydroxy- and 5″-hydroxy-CBN being aglycones. Furthermore the multiplet at δ = 5·36 ppm due to olefinic protons supported the presence of oleic acid also in band 2. Thus by pmr, g.c. and g.c.-ms data the metabolites present in band 2 were identified as 4″-hydroxy-CBN palmitate (IIA), 4″-hydroxy-CBN oleate (IIB), 5″-hydroxy-CBN palmitate (IIIA) and 5″-hyroxy-CBN oleate (IIIB). The esterification of the alcohol is supported by the pmr and the immediate colouring of the metabolites with Fast Blue B salt (phenolic reagent).

About 3% of excreted radioactivity in the faeces was due to these conjugated metabolites. G.c. showed the palmitate esters to be minor components compared with the oleates with a ratio of 1:5.

Leighty & others (1976) have shown that fatty acid conjugates can be formed *in vitro* in the liver. Considering the high molecular weight of the fatty acid esters of cannabinol, a biliary excretion of these compounds seems possible in the rat.

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REFERENCES

Ben-Zvi, Z., Bergen, J. R. & Burstein, S. (1974). *Res. Comm. Chem. Path. Pharmac.*, **9**, 201–204.

Ben-Zvi, Z., Bergen, J. R., Burstein, S., Sehgal, P. K. & Varanelli, C. (1976). In: *Pharmacology of Marihuana*, Vol. 1, pp. 63–75. Editors: Braude, M. C. & Szara, S. New York: Raven Press.

Binder, M., Agurell, S., Leander, K. & Lindgren, J.-E. (1974). *Helv. chim. Acta*, **57**, 1626–1641.

Burstein, S. & Varanelli, C. (1975). *Res. Comm. Chem. Path. Pharmac.*, **11**, 343–354.

Harvey, J. D., Martin, B. R. & Paton, W. D. M. (1977). *Ibid.*, **16**, 265–279.

Leighty, E. G., Fentiman, A. F. & Foltz, R. L. (1976). *Ibid.*, **14**, 13–28.

McCallum, N. K., Yagen, B., Levy, S. & Mechoulam, R. (1975). *Experientia*, **31**, 520–521.

Wall, M. E., Brine, D. R. & Perez-Reyes, M. (1976). In: *Pharmacology of Marihuana*, Vol. 1, pp. 93–113. Editors: Braude, M. C. & Szara, S. New York: Raven Press.

Widman, M., Nilsson, I. M., Nilsson, J. L. G., Agurell, S. & Leander, K. (1971). *Life Sci.*, **10**, 157–162.

Widman, M., Dahmén, J., Leander, K. & Petersson, K. (1975). *Acta pharm. suecica*, **12**, 385–392.

Widman, M., Nordqvist, M., Agurell, S., Lindgren, J.-E. & Sandberg, F. (1974). *Biochem. Pharmac.*, **23**, 1163–1172.

Yisak, W., Widman, M., Lindgren, J.-E. & Agurell, S. (1977). *J. Pharm. Pharmac.*, **29**, 487–490.