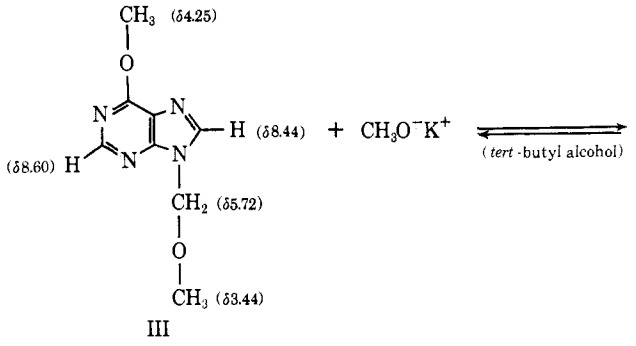
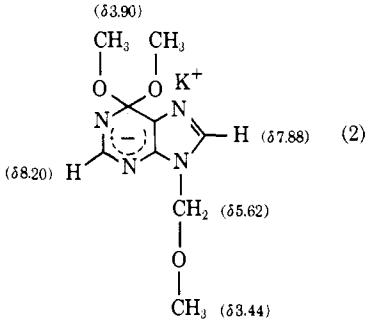
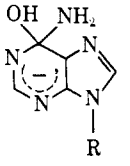


III



(2)

IV



V

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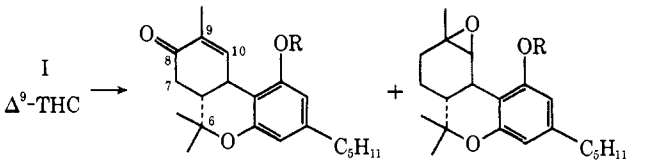
Metabolism of (−)-Δ^9- and (−)-Δ^8-Tetrahydrocannabinol by Monkey Liver[1]

Sir:

A number of investigations on the metabolism of (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychotomimetic constitutent of marijuana (*Cannabis sativa* L.), and (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC), a minor constituent, have been described.[2] We now wish to report the first studies on the metabolism of these two compounds utilizing a liver microsomal fraction of the squirrel monkey, *Saimiri sciureus*.

These investigations on the *in vitro* metabolism of Δ^9-THC (I) have resulted in the identification of two new metabolites IIa and IIIa; the studies on the metabolism of Δ^8-THC (IV) have resulted in the identification of the novel metabolites Va, VIa, and VIIa.



IIa, R = H
b, R = Ac

IIIa, R = H
b, R = Ac

Va, R = H
b, R = Ac

VIa, R = H; R¹ = —OH
b, R = Ac; R¹ = —OAc
VIIa, R = H; R¹ = ····OH
b, R = Ac; R¹ = ····OAc

Aerobic *in vitro* incubation of synthetic[3] (−)-Δ^9-THC-2,4-$^{14}C_2$ (I) and (−)-Δ^8-THC-2,4-$^{14}C_2$ (IV) was carried out using a 9000*g* microsomal supernatant fraction of male squirrel monkey liver, under the same conditions as reported previously.[2] The crude extract was purified by thin-layer chromatography on silica gel. Autoradiography of the thin-layer plates of metabolized I revealed one major (more polar) and several minor radioactive bands plus unchanged parent compound. Unchanged starting material represented 63% of the total radioactivity; of the 37% which was converted, 30% of the radioactivity was found in the major band. Acetylation of the major radioactive band with acetic anhydride–pyridine (1:1) overnight at room temperature followed by tlc separation on silica gel gave two acetylated metabolites IIb and IIIb in an approximate ratio of 60:40, respectively.

Gas–liquid chromatographic analysis[4] of IIb showed a relative retention time (rrt) of 3.40, while the rrt of IIIb was 1.30. Low resolution mass spectrometry of IIb showed a molecular ion at *m/e* 370 indicating the addition of oxygen to and loss of two hydrogens from the acetylated parent compound, suggesting the introduction of a carbonyl group. A fragment ion at *m/e* 231[5] suggested substitution in ring C. The uv spectrum (C_2H_5OH) of IIb suggested the presence of an α,β-unsaturated ketone by absorption (shoulder) at 230 nm, with a high background absorption due to impurities. The ir spectrum (neat) of IIb showed a strong band at 1673 cm^{-1}, supporting the presence of an α,β-unsaturated ketone.

The nmr spectrum[6] of this material is in agreement with the preceding spectroscopic evidence for structure IIb. The chemical shift of the C-9 methyl group (δ 1.77) and the C-10 olefinic proton (δ 7.28) as compared

(1) This work was presented in part by D. E. M. at the Fifth International Congress on Pharmacology, San Francisco, Calif., July 27, 1972.

(2) For leading references, see D. E. Maynard, O. Gurny, R. G. Pitcher, and R. W. Kierstead, *Experientia*, 27, 1154 (1971).

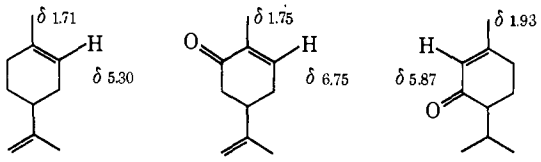
(3) A. A. Liebman, D. H. Malarek, A. M. Dorsky, and H. H. Kaegi, *J. Label. Compounds*, 7, 241 (1971).

(4) Gas–liquid chromatography was conducted on a Hewlett-Packard Model 402 gas chromatograph. The liquid phase used was 3% OV-225 on 80–100 mesh Supelcoport (6 ft × 4 mm). The samples were run isothermally at various temperatures between 190 and 230°; retention times were expressed relative to cholestane = 1.00.

(5) H. Budzikiewicz, R. T. Alpin, D. A. Lightner, C. Djerassi, R. Mechoulam, and Y. Gaoni, *Tetrahedron*, 21, 1881 (1965).

(6) All nmr spectra were determined on an HA-100 spectrometer using $CDCl_3$ as solvent with Me_4Si as internal reference.

to the chemical shift in Δ^9-THC (I) at δ 1.69 and δ 6.33 indicated that substitution had occurred in ring C. Comparison of the chemical shifts of IIb with those of the model compounds *p*-mentha-1,8-diene (VIII) and carvone (IX) permitted assignment of the carbonyl group to C-8. The chemical shift of the C-9 methyl group (δ 1.77) of IIb is in excellent agreement with that of the methyl group of IX at δ 1.75. The olefinic proton in IX shows a downfield shift of 1.45 ppm (relative to VIII) due to the presence of its carbonyl group. The C-10 olefinic proton in Δ^9-THC acetate occurs at δ 6.01,[7] while that of IIb is at δ 7.28. This downfield shift of 1.27 ppm for the C-10 olefinic proton of IIb is in good agreement with that of the model compounds.



VIII IX X

The high resolution mass spectrum of IIIb, which showed a molecular ion at *m/e* 372.2279 ($C_{23}H_{32}O_4$), indicated the addition of a single oxygen atom to the acetylated parent compound. The nmr spectrum of IIIb showed the following peaks: δ 0.89 (5′-CH_3, t), 1.01 (6α-CH_3, s), 1.29 (6β-CH_3, s), 1.37 (9-CH_3, s), 2.35 (1-OAc, s), 2.50 (1′-CH_2, t), 3.32 (10-H, s), 6.47 (2-H, d), and 6.55 (4-H, d). An upfield shift in IIIb of the C-9 methyl group and the absence of the characteristic C-10 olefinic proton, as compared to Δ^9-THC, indicated substitution at C-9 and C-10. The presence of a new peak at δ 3.32 indicated a methine proton at C-10 on a carbon bearing oxygen and strongly suggested the presence of an epoxide. This was confirmed by epoxidation of acetylated I with *m*-chloroperbenzoic acid to give authentic IIIb, whose physical constants (mass spectrum, nmr, and chromatographic behavior on tlc and glpc) were identical with those of the acetylated metabolite IIIb. The configuration of the epoxide group was not established.

The preliminary incubation of Δ^8-THC (IV) and separation of metabolites were carried out as outlined for Δ^9-THC (I). Of the 36% of Δ^8-THC (IV) which was converted, 16% was found as metabolite Va, 19% as VIa, and 16% as VIIa. The separated metabolites were then acetylated and characterized further.

High resolution mass spectra of Vb exhibited a molecular ion at *m/e* 370.2184 ($C_{23}H_{30}O_4$) which indicated the addition of an oxygen to and the loss of two hydrogens from the parent compound. A strong fragment ion at *m/e* 231 supported substitution of a carbonyl group on ring C. The ir spectrum (neat) of Vb showed a strong band at 1678 cm^{-1}, suggesting the presence of an α,β-unsaturated ketone. Although the background absorption was quite strong, the uv spectrum (C_2H_5OH) of the metabolite is in agreement with the postulated structure, allowing the recognition of an α,β-unsaturated ketone (shoulder at 230 nm). The nmr spectrum confirmed the structure of Vb. The chemical shifts of the C-9 methyl group (δ 1.98) and the C-8 olefinic proton (δ 5.83) were compared with those of the two α,β-unsaturated keto model compounds carvone (IX) and piperitone (X). The shifts of X (δ 1.93 and 5.87) very closely approximate those of the metabolite, while those of IX (δ 1.75 and 6.75) are drastically different.

Metabolite VIa was shown to have a strong molecular ion at *m/e* 330 by low resolution mass spectra, and strong fragment ions at *m/e* 312 ($M^+ - H_2O$), 297 ($M^+ - H_2O$, CH_3), 247, and 231, while the acetylated product VIb gave a molecular ion at *m/e* 414.2480 ($C_{25}H_{34}O_5$) on high resolution mass spectra, indicating that two hydroxyl groups were acetylated. These data confirm monohydroxylation of the parent compound IV. Low resolution mass spectra of VIIa showed a molecular ion at *m/e* 330, and the high resolution mass spectra of VIIb exhibited a molecular ion at *m/e* 414.2312 ($C_{25}H_{34}O_5$), an elemental composition identical with VIb.

Reduction of the ketone Vb with sodium borohydride led to a mixture which was resolved by tlc into two radioactive bands (ratio 40:60) which, as outlined below, were identified as VIa and VIIa, respectively. Low resolution mass spectra of both reduction products showed molecular ions at *m/e* 330. Both compounds were deacetylated during the reduction procedure and the net gain of 2 mass units in the products supported the reduction of a ketone to the epimeric alcohols.

The *metabolites* VIa and VIIa were shown to be identical with the two corresponding borohydride *reduction products* of the ketone Vb, by glpc on three different columns,[8] both as the free alcohols and their corresponding acetates, establishing that both metabolites contained a hydroxyl group in the 7 position. It has been established[9] that the introduction of a 7β-hyroxyl group induces a large downfield shift in the 6β-methyl group (0.28 ppm) and a small downfield shift in the 6α-methyl group (0.09 ppm) compared to the corresponding 6-methyl chemical shifts in Δ^8-THC (δ 1.37 and 1.09),[10] whereas the introduction of a 7α-hydroxyl group only induces a large downfield shift (0.42 ppm) in the 6α-methyl group. The chemical shifts of the 6β- and 6α-methyl groups in metabolite VIa are δ 1.60 and 1.17, respectively, in close agreement with the predicted values (δ 1.65 and 1.18) for the 7β-hydroxy compound and they allow the epimeric alcohol VIIa to be assigned the 7α configuration. The olefinic proton of acetylated metabolite VIb appears in the nmr spectrum at δ 5.30 and in acetylated metabolite VIIb the corresponding signal appears at δ 5.42.

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(7) R. L. Foltz, A. F. Fentiman, Jr., E. G. Leighty, J. L. Walter, H. R. Drewes, W. E. Schwartz, T. F. Page, Jr., and E. B. Truitt, Jr., *Science*, **168**, 844 (1970).

(8) The columns used were: (a) 3.8% UC-W98, 4 ft, 200°; (b) 3% OV-225, 2 ft, 210°; (c) 1% OV-1, 4 ft, 190°.

(9) M. E. Wall, *Ann. N. Y. Acad. Sci.*, **191**, 23 (1971).

(10) R. A. Archer, D. B. Boyd, P. V. Demarco, I. J. Tyminski, and N. L. Allinger, *J. Amer. Chem. Soc.*, **92**, 5200 (1970).

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