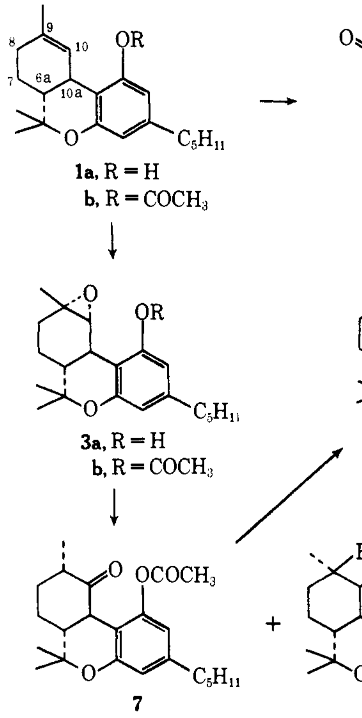
Synthesis and Biological Activity of Five Tetrahydrocannabinol Metabolites

Sir:

In the preceding communication, Gurny, *et al.*,[1] describe the isolation and the elucidation of the structures of five new tetrahydrocannabinol (THC) metabolites produced from *in vitro* incubation with a microsomal supernatant liver fraction of a squirrel monkey. (−)-Δ^9-THC (**1a**)[2,3] was metabolized to Δ^9-THC-8-one (**2a**) and 9,10-epoxyhexahydrocannabinol (**3a**); (−)-Δ^8-THC (**4a**)[5] produced Δ^8-THC-7-one (**5a**) and the C-7 isomers of Δ^8-THC-7-ol (**6a** and **6b**).

We wish to report the syntheses of these metabolites (or their acetates) and the results of their preliminary testing for Cannabis-type activity in rhesus monkeys.

Oxidation of (−)-Δ^9-THC acetate (**1b**) in ethanol with selenium dioxide for 24 hr produced a mixture which on silicic acid chromatography gave (10% yield) Δ^9-THC-8-one acetate (**2b**): an oil; $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ −179°; $\lambda_{\max}^{\mathrm{EtOH}}$ 230 mμ (ϵ 6730), 274 (ϵ 1330), 280 (ϵ 1330); δ (CCl_4) 0.96, 1.18, 1.36, 1.76 (CH_3 groups), 2.22 (acetate CH_3), 3.35 (br, C-10a H), 6.40, 6.50 (aromatic H), 7.22 (C-10 H); $\nu_{\mathrm{CCl_4}}$ 1670 cm^{-1}; mol wt (mass spectrum) 370. These data are in good agreement with those published[1] for the metabolic product.



Treatment of Δ^9-THC acetate (**1b**) with *m*-chloroperbenzoic acid in chloroform gave in 79% yield 9α,10α-epoxyhexahydrocannabinol acetate (**3b**): $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ −121°; $\lambda_{\max}^{\mathrm{EtOH}}$ 225 mμ (shoulder) (ϵ 9050), 276 (ϵ 1860), 282 (ϵ 2035). *Anal.* Found for ($C_{23}H_{32}O_4$): C, 74.08; H, 8.62.

The nmr spectrum is identical with the published one of the metabolic **3b**. The stereochemistry at C-9 and C-10 was established by rearranging **3b** with boron trifluoride etherate to (9*S*,10a*R*,6a*R*)-hexahydrocannabinol-10-one acetate (C-9 methyl group axial) (**7**): mp 93–95°; $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ −140°; $\lambda_{\max}^{\mathrm{EtOH}}$ 275 mμ (shoulder) (ϵ 1770), 281 (ϵ 1900); $\nu_{\mathrm{CCl_4}}$ 1720, 1780 cm^{-1}; δ (CCl_4) 0.91, 1.13, 1.20, 1.35 (CH_3 groups), 2.04 (acetate CH_3), 3.61 (d, J = 12 Hz, C-10a H), 6.40 (2 H, aromatic); mol wt (mass spectrum) 372. Boiling **7** with methanolic sodium hydroxide caused, in addition to hydrolysis of the acetate group, epimerization of the C-9 chiral center giving the thermodynamically more stable (9*R*,10a*R*,6a*R*)-hexahydrocannabinol-10-one (C-9 methyl group equatorial) (**8a**): an oil; $\nu_{\mathrm{CCl_4}}$ 1720 cm^{-1}; δ (CCl_4) 0.97, 1.08, 1.16, 1.34 (CH_3 groups), 3.65 (d, C-10a H), 6.08 (2 H, aromatic). On acetylation **8b** was obtained as an oil: $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ −160°; $\lambda_{\max}^{\mathrm{EtOH}}$ 273 mμ (shoulder) (ϵ 3370) and 281 (ϵ 3630); δ (CCl_4) 0.96, 1.08, 1.14, 1.36 (CH_3 groups), 1.98 (acetate CH_3), 3.42 (d, J = 12 Hz, C-10a H), 6.40 (2 H, aromatic); $\nu_{\mathrm{CCl_4}}$ 1725 and 1775 cm^{-1}.

Rearrangement of epoxides to ketones involves a hydride shift, with retention of configuration of the leaving group;[6] hence the C-9 methyl group in **7** has to have the same configuration as the epoxide group in **3**. The equilibration experiment shows that this methyl group is in a thermodynamically unstable configuration. Assuming that the cyclohexanone ring in **7** is in the quasichair conformation, the C-9 methyl group is axial (α configuration). Hence the epoxide group is likewise in the α configuration.

As the metabolite **3b** has been reported[1] to be identical with the synthetic product obtained from Δ^9-THC acetate and *m*-chloroperbenzoic acid, the above correlations establish the stereochemistry of the metabolite.

A second compound formed from **3b** on treatment with boron trifluoride etherate is 9β-fluoro-10α-hydroxyhexahydrocannabinol acetate (**9**): an oil; $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ −89°; $\lambda_{\max}^{\mathrm{EtOH}}$ 283 mμ (ϵ 1828); mass peaks 392 (molecular peak), 350 [M^+ − 42 (ketene from acetate)], 332 (M^+ less ketene and water), 330 (M^+ less ketene and HF), while the rest of the peaks are typical for cannabinoids;[7] δ (CCl_4) 0.88, 0.99, 1.35 (CH_3 groups), 1.25 (d, J = 21 Hz, CH_3CF splitting), 3.12 (t, J = 19 Hz, C-10a H), 4.80 (q, J = 22.5 Hz, C-10 H), 6.10 (2 H, aromatic). *Anal.* Found for ($C_{23}H_{33}FO_4$): C, 69.57; H, 8.48.

The stereochemistry assigned to the C-9 and C-10 chiral centers is based on the nmr spectrum and has analogy to literature[8] precedents: the secondary hydroxyl group formed by opening of the epoxide and the tertiary fluoro group are trans to each other.

Δ^8-THC acetate (**4b**) in carbon tetrachloride was oxidized by boiling for 10 hr with a solution of *tert*-butyl chromate[9] in the same solvent containing acetic acid and acetic anhydride. The neutral fraction ob-

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[1] O. Gurny, D. E. Maynard, R. G. Pitcher, and R. W. Kierstead, *J. Amer. Chem. Soc.*, **94**, 7928 (1972). We thank Dr. Kierstead for informing us of his results prior to publication and for agreeing to simultaneous publication.

[2] Y. Gaoni and R. Mechoulam, *ibid.*, **86**, 1646 (1964); Y. Gaoni and R. Mechoulam, *ibid.*, **93**, 217 (1971).

[3] Our group usually prefers the "terpene nomenclature"[4] for cannabinoids rather than the one used here. However, in order to allow facile interpretation and comparison of the preceding paper and the present one both papers employ the same numbering system.

[4] R. Mechoulam, *Science*, **168**, 1159 (1970).

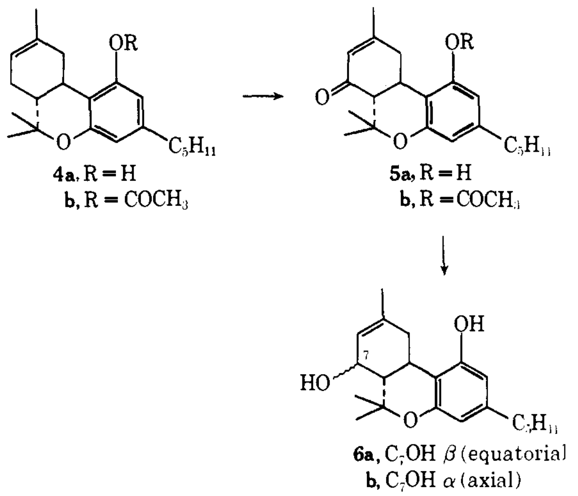
[5] R. L. Hively, W. A. Mosher, and F. W. Hoffman, *J. Amer. Chem. Soc.*, **88**, 1832 (1966).

[6] H. B. Henbest and T. I. Wrigley, *J. Chem. Soc.*, 4596 (1957); C. W. Shoppee, M. E. H. Howden, R. W. Killick, and G. H. R. Summers, *ibid.*, 630 (1959).

[7] H. Budzikiewicz, R. T. Aplin, D. A. Lightner, C. Djerassi, R. Mechoulam, and Y. Gaoni, *Tetrahedron*, **21**, 1881 (1965).

[8] *Cf.* B. N. Blackett, J. M. Coxon, M. P. Hartshorn, and K. E. Richards, *ibid.*, **25**, 4999 (1969).

[9] K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).



4a, R = H
b, R = COCH$_3$

5a, R = H
b, R = COCH$_3$

6a, C$_7$OH β (equatorial
b, C$_7$OH α (axial)

tained was chromatographed on Florisil. Δ^8-THC-7-one acetate (**5b**) was obtained in 20% yield as an oil: $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ $-301°$; $\delta_{\max}^{\mathrm{EtOH}}$ 223 mμ (ϵ 12,700), 281 (shoulder) (ϵ 2100), 285 (ϵ 2140); δ (CDCl$_3$) 1.16, 1.42, 1.65, 1.95 (CH$_3$ groups), 2.16 (acetate methyl), 3.05 (C-10α H), 5.80 (C-8 H), 6.36, 6.51 (aromatic H); $\nu_{\mathrm{CCl_4}}$ 1680, 1780 cm^{-1}.

Anal. Found for (C$_{23}$H$_{30}$O$_4$): C, 74.05; H, 8.11.

These data are in agreement with those presented for the metabolite and support the suggested structure.

Lithium aluminum hydride reduction of 630 mg of **5b** gave a mixture from which on preparative tlc two products were obtained. One of these was shown to be (10a*R*,6a*S*,7*S*)-Δ^8-THC-7β-ol (**6a**): 116 mg; mp 117°; $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ $-240°$; $\lambda_{\max}^{\mathrm{EtOH}}$ 274 (ϵ 1250), 286 (ϵ 1300); δ (CD$_3$COCD$_3$) 0.91, 1.16, 1.57, 1.71 (CH$_3$ groups), 3.52 (C-10α H), 4.13 (br d, J = 9 Hz, C-7 H, axial), 5.35 (s, C-8 H), 6.11, 6.27 (aromatic H); mol wt (mass spectrum) 330. *Anal.* Found for (C$_{21}$H$_{30}$O$_3$): C, 76.48; H, 9.10.

The second compound was identified as (10a*R*,6a*S*,7*R*)-Δ^8-THC-7α-ol (**6b**): 104 mg; mp 154°; $[\alpha]^{\mathrm{EtOH}}\mathrm{D}$ $-345°$; $\lambda_{\max}^{\mathrm{EtOH}}$ 274 mμ (ϵ 1215), 281 (ϵ 1260); δ (CD$_3$COCD$_3$) 0.91, 1.27, 1.46, 1.70 (CH$_3$ groups), 3.25 (C-10α H), 4.30 (br, C-7 H, equatorial), 5.60 (d, J = 4.5 Hz, C-8 H), 6.10, 6.25 (aromatic H); mol wt (mass spectrum) 330. *Anal.* Found for (C$_{21}$H$_{30}$O$_3$): C, 75.72; H, 8.75.

The above assignments of stereochemistry at C-7 in **6a** and **6b** are based on the differences of chemical shifts and splitting patterns of the C-7 and C-8 protons.[10]

The psychopharmacological activity of the above synthetic metabolites (or their acetates[11]) was assessed by the behavior and somatic changes elicited in adult rhesus monkeys when administered by intravenous injection as described previously.[12,13] The epoxy acetate **3b** showed no activity at 0.1 mg/kg; however, at 0.5 mg/kg it caused stupor, ataxia, suppression of motor activity, full ptosis, and a typical crouched posture kept for up to 3 hr. The second Δ^9-THC metabolite, Δ^9-THC-8-one acetate (**2b**), showed no activity up to 5 mg/kg. The three Δ^8-THC metabolites showed activity in the above test, though at different dose levels. Δ^8-THC-7-one acetate (**5b**) at 0.5 mg/kg and Δ^8-THC-7α-ol (**6b**), mp 154°, at 0.25 mg/kg caused significant tranquility; **5b** at 1 mg/kg and **6b** at 0.5–1 mg/kg caused the same symptoms as described above for the epoxy acetate (**3b**) (at 0.5 mg/kg). The 7β-hydroxy isomer **6a** (mp 117°) was six-eight times less active than the 7α-hydroxy isomer **6b**: at 1 mg/kg it caused no observable changes in the monkey; at 2 mg/kg it caused drowsiness, decreased motor activity, occasional partial ptosis, and an occasional head drop.

The plethora of metabolites[14] of Δ^9- and Δ^8-THC isolated from *in vivo* or *in vitro* studies with different animal species or animal organ homogenates makes it imperative to determine the *human* metabolic pathways *in vivo* before any conclusions as regards the molecular basis of marijuana activity in humans can be made. However, the fact that many of the THC metabolites so far isolated are active in psychobiological tests in animals (including monkeys)[14d,e,h] and in humans[14b] supports the tentative suggestions[4,13,15] that the effects of Cannabis are caused (in part at least) by metabolites.

Acknowledgment. The research in Jerusalem was supported by a grant (MH-13180) from NIMH.

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[10] *Cf.* S. H. Burstein and H. J. Ringold, *J. Amer. Chem. Soc.*, **86**, 4952 (1964); S. H. Burstein and H. J. Ringold, *ibid.*, **89**, 4722 (1967).

[11] The acetates of active cannabinoids show the same activity as the parent cannabinoid but the onset of activity may be delayed.[12]

[12] H. Edery, Y. Grunfeld, Z. Ben-Zvi, and R. Mechoulam, *Ann. N. Y. Acad. Sci.*, **191**, 40 (1971).

[13] Y. Grunfeld and H. Edery, *Psychopharmacologia*, **14**, 200 (1969).

[14] (a) Review: S. H. Burstein in "Marihuana. Chemistry, Metabolism, Pharmacology and Clinical Effects," R. Mechoulam, Ed., Academic Press, New York, N. Y., in press; (b) L. Lemberger, R. E. Crabtree, and H. M. Rowe, *Science*, **177**, 62 (1972); (c) S. H. Burstein, J. Rosenfeld, and T. Wittstruck *ibid.*, **176**, 422 (1972); (d) Z. Ben-Zvi, R. Mechoulam, H. Edery, and G. Porath, *ibid.*, **174**, 951 (1971); (e) M. E. Wall, *Ann. N. Y. Acad. Sci.*, **191**, 23 (1971); (f) K. Nakazawa and E. Costa, *Nature (London)*, **234**, 48 (1971); (g) J. L. G. Nilsson, S. Agurell, B. Akermark, and I. Lagerlund, *Acta Chem. Scand.*, **25**, 768 (1971); (h) Z. Ben-Zvi, R. Mechoulam, and S. Burstein, *J. Amer. Chem. Soc.*, **92**, 3468 (1970).

[15] L. Lemberger, J. L. Weiss, A. M. Watanabe, I. M. Galanter, R. J. Wyatt, and P. V. Cardon, *N. Engl. J. Med.*, **286**, 685 (1972).

R. Mechoulam,* H. Varconi, Z. Ben-ZVi
Laboratory of Natural Products
Hebrew University Pharmacy School
Jerusalem, Israel

H. Edery, Y. Grunfeld
Israel Institute for Biological Research
Medical School, Tel-Aviv University
Ness-Ziona, Israel

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Evidence Against the Involvement of 0,0-Diradical Intermediates in the Pyrolysis of Five-Membered Ring Azo Compounds

Sir:

We wish to report a study which provides strong evidence against the intervention of "π-cyclopropanes" (or "0,0 diradicals") in the thermal decomposition of five-membered ring azo compounds.[1,2]

exo-4-Methyl-2,3-diazabicyclo[3.2.0]hept-2-ene (**1e**) and its endo isomer (**1n**) were prepared[3] by the addition

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[1] R. Hoffmann, *J. Amer. Chem. Soc.*, **90**, 1475 (1968).

[2] (a) R. J. Crawford and A. Mishra, *ibid.*, **88**, 3963 (1966); (b) A. Mishra and R. J. Crawford, *Can. J. Chem.*, **47**, 1515 (1969).

[3] Stereochemistries of **1e** and **1n** were assigned (as in related systems, see ref 8 and references therein) by proton nmr coupling constants. Compound **1e**, the major isomer, displayed a 1.5-Hz coupling between protons on carbons 4 and 5, indicating a trans relationship, while the coupling for **1n** was 7.0 Hz, indicating a cis eclipsed configuration.