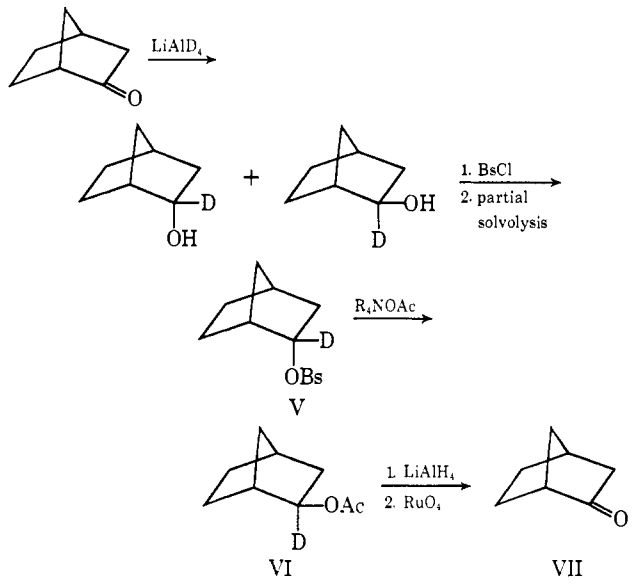
Chart I



placement reaction on V in acetone with tetramethylammonium acetate. In the preparation of *exo*-norbornyl-2-*d* acetate (VI), if the scheme functioned without rearrangement, the norcamphor VII isolated from the cycle in Chart I should contain no deuterium. In practice we found variable amounts of nonexchangeable deuterium in VII (usually 9–13%) by mass spectral analysis. These figures correspond to ionization of 18–26% of the *endo*-brosylate. The method may not always fail, but we were unable to control the reaction.[15] The difficulty was circumvented by use of tetra-*n*-butylammonium acetate in refluxing benzene. This process is much faster than its acetone counterpart (3 hr *vs.* 12 days).

The C_3-labeled compounds employed in our studies were also prepared by use of the new displacement conditions.[10] Another necessary ingredient in the preparation of the C_3-labeled compounds was the ability to oxidize *exo*-norborneol-3-*d* without significant loss of deuterium. This was accomplished with ruthenium tetroxide in Freon-11 at 0°.[16] Immediate work-up afforded ketone with less than 2% loss of deuterium (by mass spectrum). When the reaction mixture was allowed to stand overnight the ketone showed 25% loss of deuterium. *exo*-3-Deuterium was introduced by deuterioboration. *endo*-3-Deuterium was introduced by $LiAlD_4$ reduction of *exo*-norbornene oxide. Norcamphor-3,3-d_2 was prepared by exchange.[11] All deuterium in the C_3-deuterated norborneols was shown to be in that position by oxidation, exchange with trifluoroacetic acid,[11] and mass spectral analysis.

Acknowledgment. We thank Professor A. Nickon for helpful criticism and Professor H. C. Brown for suggesting deuterioboration to introduce *exo*-3-deuterium.

(15) Spectral analysis by nmr suggested that little or no rearrangement accompanied one tetramethylammonium acetate–acetone run. Professor C. C. Lee has informed us that he has also detected the rearrangement.

(16) E. J. Corey, J. Casanova, Jr., P. A. Vatakencherry, and R. Winter, *J. Amer. Chem. Soc.*, **85**, 169 (1963).

(17) National Science Foundation Cooperative Fellow, 1965–1969.

B. L. Murr, J. A. Conkling[17]
Department of Chemistry, The Johns Hopkins University
Baltimore, Maryland 21218

Received February 17, 1970

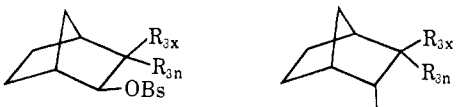
β-Deuterium Isotope Effects on the Solvolyses of Norbornyl Brosylates[1]

Sir:

The small isotope effect on *ionization* (k_α) caused by C_3 dideuteration of *exo*-norbornyl bromide (k_H/k_D = 1.09) was attributed to participation, which imparts SN2 character to C_2 and lowers the demand for hyperconjugative electron release.[2] The even smaller effect on *solvolysis* (k_H/k_D = 1.04) was ascribed to isotopic scrambling to C_7 by internal return.[2] A low isotope effect (k_H/k_D = 1.014) was also reported for *exo*-norbornyl-3,3-d_2 brosylate.[3] The latter isotope effect is extraordinary because secondary bromides invariably have shown smaller isotope effects than corresponding arenesulfonates.

In this note we report identification of a heretofore unrecognized cause for the low C_3 isotope effect on solvolysis of *exo*-norbornyl derivatives. The known geometric dependence of the β-isotope effect[4,5] and the particular geometry of the nonclassical norbornyl cation suggested that low β-isotope effects might result from a low *endo*-3-*d* isotope effect due to its unfavorable geometric orientation in the nonclassical transition state. This would result in a substantial effect for an *exo*-3-*d* and a low effect for an *endo*-3-*d*. Epimeric C_3-deuterated *endo*-norbornyl brosylates that ionize to a classical cation should both show substantial isotope effects.

We have determined isotope effects (Table I) on aqueous ethanolysis and acetolysis of *exo*-norbornyl-3-*exo*-*d* brosylate (I-3-*exo*-*d*), *exo*-norbornyl-3-*endo*-*d* brosylate (I-3-*endo*-*d*), *exo*-norbornyl-3,3-d_2 brosylate (I-3,3-d_2), *endo*-norbornyl-3-*exo*-*d* brosylate (II-3-*exo*-*d*), *endo*-norbornyl-3-*endo*-*d* brosylate (II-3-*endo*-*d*), and *endo*-norbornyl-3,3-d_2 brosylate (II-3,3-d_2).[6]



I
I-3-*exo*-*d*
I-3-*endo*-*d*
I-3,3-d_2

II
II-3-*exo*-*d*
II-3-*endo*-*d*

{$R_{3x} = R_{3n} = H$}
{$R_{3x} = D$; $R_{3n} = H$}
{$R_{3x} = H$; $R_{3n} = D$}
{$R_{3x} = R_{3n} = D$}

Entries 1–4 (Table I, 80% ethanol) show that essentially the entire isotope effect of I-3,3-d_2 is caused by the *exo*-3-*d*. The isotope effects, however, for II-3-*exo*-*d* and II-3-*endo*-*d* are both substantial (entries 5–7) and similar to values recently reported for *trans*- and *cis*-cyclopentyl-2-*d* brosylates (entries 8 and 9).[7]

(1) This work was supported by the National Science Foundation.

(2) J. P. Schaefer, M. J. Dagani, and D. S. Weinberg, *J. Amer. Chem. Soc.*, **89**, 6938 (1967).

(3) J. M. Jerkunica, S. Borčić, and D. E. Sunko, *Chem. Commun.*, 1302 (1967).

(4) V. J. Shiner, Jr., and J. S. Humphrey, Jr., *J. Amer. Chem. Soc.*, **85**, 2416 (1963).

(5) V. J. Shiner, Jr., B. L. Murr, and G. Heinemann, *ibid.*, **85**, 2413 (1963).

(6) Compounds were prepared by methods given in the preceding paper: B. L. Murr and J. A. Conkling, *ibid.*, **92**, 3462 (1970). See also ref 7 of this paper.

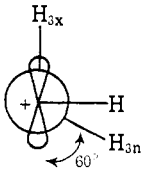
(7) J. O. Stoffer and J. D. Christen, *ibid.*, **92**, 3190 (1970). The au-

Table I. Isotope Effects on the Solvolysis of *exo*-Norbornyl Brosylate, *endo*-Norbornyl Brosylate, and Cyclopentyl Brosylate

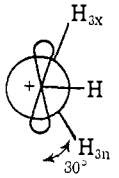
Entry	Compounds compared[a] and position(s) of deuteriation	k_H/k_D 80% ethanol	k_H/k_D per D	k_H/k_D HOAc–KOAc	k_H/k_D per D
		exo-Norbornyl Brosylates, 25°			
1	I-3-*exo*-*d* *vs.* I	1.11 ± 0.01	1.11	1.07 ± 0.02	1.07
2	I-3-*endo*-*d* *vs.* I	1.02 ± 0.01	1.01	1.02 ± 0.01	1.01
3	I-3,3-d_2 *vs.* I	1.11 ± 0.01	1.06	1.07 ± 0.01	1.03
4	I-3-*exo*-*d* *vs.* I-3,3-d_2	1.00 ± 0.01	1.00		
		endo-Norbornyl Brosylates, 55°			
5	II-3-*exo*-*d* *vs.* II	1.19 ± 0.01	1.19		
6	II-3-*endo*-*d* *vs.* II	1.12 ± 0.01	1.12		
7	II-3,3-d_2 *vs.* II	1.31 ± 0.01	1.15		
		Cyclopentyl Brosylates,[b] 25°			
8	*cis*-2-d_1	1.153			
9	*trans*-2-d_1	1.180			
10	2,2,4,4-d_4	1.888			

[a] For entries 1–7, the compounds named were run simultaneously in the same thermostated spectrophotometer compartment. Each isotope effect is the average of from four to eight determinations by the method of C. G. Swain and C. R. Morgan, *J. Org. Chem.*, **29**, 2097 (1964). [b] The cyclopentyl brosylates were studied in 70% ethanol.[7]

The strikingly different isotope effects of I-*exo*-3-*d* and I-*endo*-3-*d*[8] accord with nonclassical ion theory if allowance is made for geometric dependence of the isotope effect. Newman projections along the C_2–C_3 bond of the nonclassical (III)[9] and classical (IV) norbornyl cations show the relative dispositions of the protons at C_3 and the vacant p orbital at C_2. H_{3x} and H_{3n} are the protons originally *exo* and *endo* at C_3 in the substrate. In III, H_{3x} is ideally aligned for interaction



III



IV

with the vacant p orbital at C_2 whereas H_{3n} is 60° from the orbital. A C–D bond at 60° to the developing p orbital exhibited a small isotope effect on ionization of *t*-butyl chloride (1.01–1.03 per D).[5] Inclusion of the leaving group in a consideration of the transition-state precusor of III would alter the geometry but not the conclusion that H_{3x} should have a larger isotope effect than H_{3n}. In the initial state H_{3x} is eclipsed with the leaving group. The geometric requirement of participation maintains the eclipsed alignment in the transition state.[10] In the initial state H_{3n} is 30° from the plane containing H_{3x}, C_2, C_3, and the leaving group. In the transition state this angle approaches 60°. If I ionized through a classical transition state the isotope effects of H_{3x} and H_{3n} would be similar, as they are for II.

In the classical norbornyl cation (IV), H_{3x} and H_{3n} are equivalently disposed at 30° from the vacant orbital. In the ionization transition state the leaving group would modify this equivalence. A 30° orientation of the C–H is not optimum but it is better than a 60° orientation.[4] In the initial state of II, H_{3n} is eclipsed with the leaving group but at the transition state the C–H_{3n} bond tends toward the 30° orientation of the classical cation. The substantial isotope effects for H_{3n} and H_{3x} on the ionization of II to the classical cation reflect the similarity in the dispositions of these two protons.

The acetolysis isotope effects of I-*exo*-3-*d* and I-3,3-d_2 are lower than for aqueous ethanolysis. The smaller values are readily explained by isotopic scrambling to C_7 by internal return.[2] The lower isotope effects at C_7 than at C_3 may result from a smaller initial-state zero-point energy of the C_7 C–H bonds or the lack of equivalence of C_3 and C_7 in the transition state. The near equality of the C_6 *exo* and *endo* isotope effects does not require the equivalence of C_3 and C_7.[11,12] The C-6 isotope effects are determined by weakening the C_1–C_6 bond which effects both C_6 bonds similarly.[11,13]

The isotope effect for I-*exo*-3-*d* is smaller than that of the *trans* proton of *t*-butyl chloride (1.31).[5] The diminished effect could result from the SN2 effect of participation already noted[2] as well as two additional factors: (1) eclipsing of the leaving group that suppresses hyperconjugative electron release; and (2) steric crowding of the *exo*-3 proton of I that increases zero-point energy in the transition state.

The β-isotope effects of II-*exo*-3-*d* and II-*endo*-3-*d* are only half the maximum effect.[5] The primary cause of the decrease is the unfavorable orientation of the C–H bonds to the developing orbital as has been suggested for the cyclopentyl case.[4] The lower H_{3n} isotope effect in II compared to H_{3x} could result from hindrance of hyperconjugative electron release and/or steric constraints by the leaving group.

Finally, our results are inconsistent with the hypothesis that the β-isotope effect results solely from relief of steric strain.[14] If the larger H_{3x} isotope effect on I

thors are indebted to Professor Stoffer for communicating his results prior to publication.

(8) The difference in isotope effect corresponds to a zero-point energy (ZPE) difference of 120 cm^{-1} that is far greater than any possible initial-state ZPE difference.

(9) III also describes the C_1–C_7 bond of the nonclassical cation.

(10) The eclipsed alignment is not optimum for maximum isotope effect but it is clearly preferable to 60°. The optimum is *trans*-coplanar.[4]

(11) J. M. Jerkunica, S. Borčić, and D. E. Sunko, *J. Amer. Chem. Soc.*, **89**, 1732 (1967).

(12) Using methods previously described,[13] we can calculate from the acetolysis and ethanolysis isotope effects only a range for k_H/k_D of 1.03 to 1.08 for *exo*-norbornyl-7,7-d_2 brosylate. The *syn*-7-*d* should show a larger isotope effect than the *anti*-7-*d*. We are checking this prediction.

(13) B. L. Murr, A. Nickon, T. Swartz, and N. H. Werstiuk, *J. Amer. Chem. Soc.*, **89**, 1730 (1967).

(14) H. C. Brown, M. E. Azzaro, J. G. Koelling, and G. J. McDonald, *ibid.*, **88**, 2520 (1966).

is rationalized in this way, II-*endo*-3-*d* and *cis*-cyclopentyl-2-*d* brosylate should show larger isotope effects than II-*exo*-3-*d* and *trans*-cyclopentyl-2-*d* brosylate.

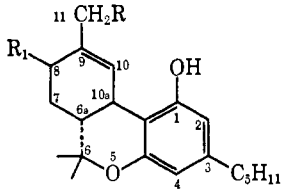
(15) National Science Foundation Cooperative Fellow, 1965–1969.

B. L. Murr, J. A. Conkling[15]
Department of Chemistry, The Johns Hopkins University
Baltimore, Maryland 21218
Received February 17, 1970

Isolation, Structure, and Biological Activity of Several Metabolites of Δ^9-Tetrahydrocannabinol

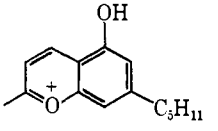
Sir:

Although there has been considerable recent interest in the metabolism of Δ^9-tetrahydrocannabinol,[1a,b] **1a**, none of the metabolites has been isolated or characterized. Since **1a** is the constituent largely responsible for the psychotomimetic properties of cannabis[2] (hashish, marihuana), the properties of its metabolites are of great importance in understanding the physiological disposition of this drug. We wish to report for the first time the *structure* and *biological activity* of several metabolites of **1a** produced by a rat liver microsomal fraction.



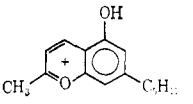
1a, R = R_1 = H
b, R = OH; R_1 = H
c, R = R_1 = OH
d, R = OAc; R_1 = H

Aerobic incubation of synthetic **1a**,[3] containing tritium-labeled **1a**[4] as a marker, with the 10,000*g* supernatant prepared from male rat liver homogenate to which was added appropriate cofactors,[5,6] followed by ethyl acetate extraction and chromatography on silica gel resulted in the isolation of unreacted **1a**, yield 25%,[7] and three new compounds, **1b**, mp 136.5–138°, yield 30%; **1c**, mp 139–140.5°, yield 30%; and **1d**, an oil, yield 15%. Tables I and II compare, respectively, the mass spectral and nmr data of **1a–d**. The structure of **1b**, the major metabolite, was obtained as follows: the uv spectrum [ν_{max}^{EtOH} 283 nm (1280), 276 nm (1250)] of **1b** was the same as that of **1a**,[2] indicating that the basic cannabinoid chromophore is retained in **1b**. High resolution mass spectrometry of **1b** gave a parent ion at *m/e* 330.2203, consistent only with the structure $C_{21}H_{30}O_3$ and showing that one hydroxyl moiety had been substituted for hydrogen in **1a**, $C_{21}H_{30}O_2$. A very strong base peak was found at *m/e* 299 (M − CH_2OH). In addition a very useful diagnostic peak at *m/e* 231 was noted. It has been reported[8] that this peak is due to the fragment



Its presence is reasonable evidence that the new hydroxyl group in **1b** cannot be located on the two rings shown, or on the amyl side chain. The strong base peak at *m/e* 299 is best accounted for by elimination of the C-9 vinylic hydroxymethyl group in **1b**. The structure assigned was completely confirmed by nmr analysis (Table II). Comparing **1a** and **1b**, it will be noted that the three-proton singlet at 1.62 ppm due to the C-9 methyl[2] of **1a** is *absent* in **1b** and replaced by a new two-proton signal at 3.92 ppm. The chemical shift of this signal is in good agreement for that expected for –C=C—CH_2OH. With these exceptions the nmr signals for **1a** and **1b** are virtually identical. The structure of the closely related compound **1d** was established along similar lines, the presence of the acetoxymethyl group at C-9 being readily established by mass spectral and nmr data, *cf.* Tables I and II, and ir analyses ($\nu_{max}^{CCl_4}$ 1740 cm^{-1}). Furthermore, careful alkaline hydrolysis converted **1d** to **1b**. Compound **1d**

Table I. Low Resolution Mass Spectra of Δ^9-THC and Metabolites

Assignment	Mass no. of ion from compd 1a	1b	1c	1d
M	314	330	346	372
M − CH_3	299	315		
M − H_2O		312	328	
M − CH_2OH		299	315	
M − H_2O − CH_3		297	313	
M − CH_2OH − H_2O			297	
M − CH_3COOH				312
M − CH_3COOH − CH_3				297
M − C_5H_7	271			
M − H_2O − C_5H_7			285	
	231	231	231	231

(1) (a) G. Joachimogliu, J. Kibaris, and C. Miras, *Propt. Acad. Athenon*, 70, 161 (1967); (b) S. Agurell, I. M. Nelsson, A. Ohlsson, and F. Sandberg, *Biochem. Pharmacol.*, 18, 1195 (1969).

(2) An excellent review of the literature on the chemistry and biological activities of the various cannabinoids is presented by R. Mechoulam and Y. Gaoni, *Fortschr. Chem. Org. Naturst.*, 25, 175 (1967).

(3) This material was obtained through Dr. John Scigliano, Center for Drug Abuse, NIMH, NIH, Bethesda, Md.

(4) M. L. Timmons, C. G. Pitt, and M. E. Wall, *Tetrahedron Lett.*, 3129 (1969).

(5) For recent reviews of microsomal hydroxylations and drug transformations, see: (a) "Microsomes and Drug Oxidations," J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, Ed., Academic Press, New York, N. Y., 1969; (b) D. V. Parke, "The Biochemistry of Foreign Compounds," Pergamon Press, Elmsford, N. Y., 1968.

(6) Summary of experimental conditions: 500 g of liver was obtained from 350-g male rats, pretreated with phenobarbital prior to sacrifice. A homogenate was prepared using 0.1 *M* potassium phosphate buffer (5 l., pH 7.4), containing 0.013 *M* magnesium chloride. The ice-cold homogenate was centrifuged at 10,000 G. To the supernatant thus obtained was added 1.0 g of **1a** plus 394 μCi of tritium-labeled **1a** and cofactors, NADP (6.55 g, 1.6×10^{-3} *M*), G6P (14.3 g, 8.0×10^{-3} *M*), and G6P-dehydrogenase, 1000 units. The mixture was incubated aerobically, shaking for 2 hr at 37°, using 10 3-l. Fernbach flasks. The reaction was quenched by extraction with ethyl acetate.

(7) (a) The yields were based on thin layer chromatography of the ethyl acetate extract of the incubation mixture using a radioscanner to reveal the individual labeled peaks. Zones were scraped and counted in a liquid scintillation counter to determine the yields. (b) The same compounds, **1a–d**, were found, but with lower yields of **1b**, **1c**, and **1d**, in the case of rats which were not treated with phenobarbital.

(8) H. Budzikiewicz, R. T. Alpin, D. A. Lightner, C. Djerassi, R. Mechoulam, and Y. Gaoni, *Tetrahedron*, 21, 1881 (1965); U. Claussen, H. W. Fehlhaber, and F. Korte, *ibid.*, 22, 3535 (1966).