measurement (calcd, 230.1096; found, 230.1086) and nmr spectroscopy were used to characterize **7** and to estimate its isotopic purity (90%). Nmr and ir spectroscopy showed that **4b** and **4a** were present in approximately a 9:1 ratio. The minor products were $Ph_2(CD_3)SiH$ (**8**) and **5**.

These reactions provide the first examples in which photolytically generated intermediates like I have been trapped and the products characterized.[8] Another unique feature of this reaction is that it is formally the reverse process of the well-known addition reaction of Si–H compounds to alkenes.[9]

Acknowledgment. We thank the National Science Foundation for vital support which made this work possible and Mr. Kei Miyano for vital mass spectral studies.

(8) The diradical $H_2\dot{C}-\dot{S}iH_2$ was postulated as an intermediate upon photolysis of CH_3SiH_3: K. Obi, A. Clement, H. E. Gunning, and O. P. Strausz, *J. Amer. Chem. Soc.*, **91**, 1622 (1969).

(9) C. Eaborn, "Organosilicon Compounds," Academic Press, New York, N. Y., 1960.

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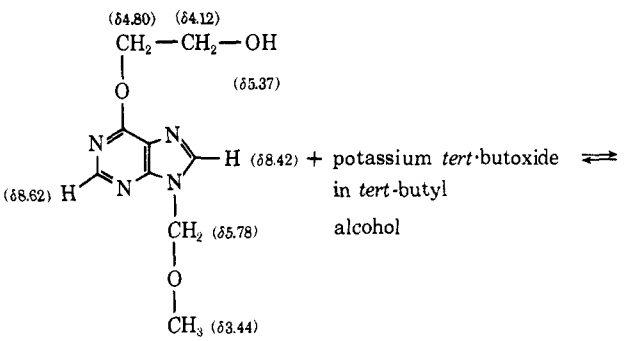
Direct Observation of Reversible Formation of Anionic σ Complexes[1] Related to Transition State Analogs for Adenosine Deaminase

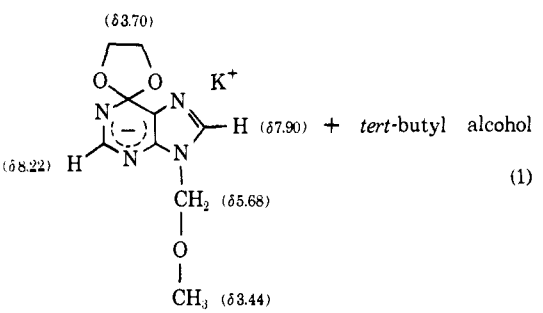
Sir:

Adenosine deaminase has been reported to catalyze the hydrolysis of nitrogen, halogen, oxygen, and sulfur leaving groups located at the 6 position of purine ribonucleosides.[2] Evidence has been presented which indicates that the mechanism involves nucleophilic substitution of water or enzyme-bound water on the purine nucleus proceeding *via* a tetrahedral intermediate.[3-7] Recently, Evans and Wolfenden[8,9] have prepared purine analogs with tetrahedral carbon at C-6 *via* photochemical addition of methyl alcohol to purine ribonucleoside and have shown that these act as powerful enzyme inhibitors. *We now report (1) the first direct observation of the reversible formation of purine analogs with tetahedral carbon at C-6 via a nucleophilic addition mechanism, and (2) the first observable σ anionic complexes not stabilized by nitro groups.*

The reaction of 6-(β-hydroxyethoxy)-9-methoxymethylpurine (I) (0.32 *M*) with potassium *tert*-butoxide (0.32 *M*) in *tert*-butyl alcohol to form anionic σ complex II was followed using nmr spectroscopy (eq 1). The cyclization was essentially complete within 90 min. The peak assignments are illustrated in eq 1. The negative charge introduced in the ring[10] and/or the diminished diamagnetic anisotropy resulting from decreased ring current[11] causes the absorptions of the protons attached to C-2 and C-8 to shift from δ 8.62 and 8.42 to 8.22 and 7.90, respectively. The methylene protons of the hydroxyethoxy group in I show two absorptions (δ 4.80 and 4.12) which become a broad absorption at 3.70 in II.[12,13] The methylene protons of the methoxymethyl group are shifted from δ 5.78 to 5.68 while the methoxy protons remain unchanged.



I

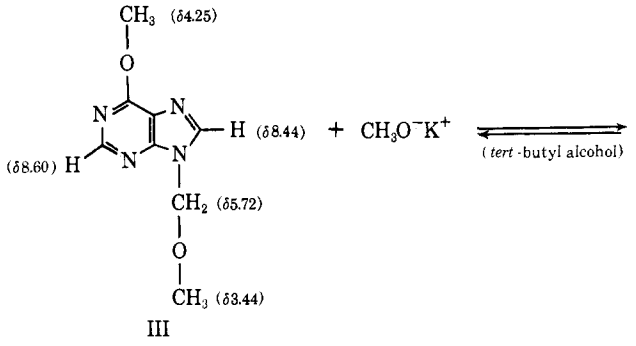


II

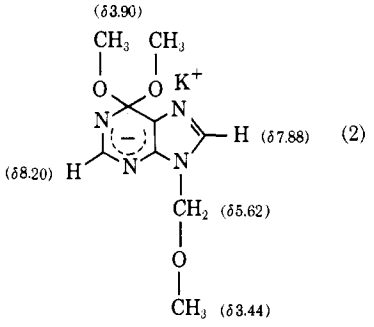
The reaction of 6-methoxy-9-methoxymethylpurine (III) (0.48 *M*) with potassium methoxide (0.48 *M*) in *tert*-butyl alcohol to form anionic σ complex IV was followed using nmr spectroscopy (eq 2). After 60 min the ratio of III/IV was 62:38.[14] The nmr assignments of III and IV are indicated in eq 2. These appear to be consistent with those of eq 1.

Wolfenden[9] has speculated that the protonated form of structure V is a reasonable representation of the intermediate formed in the adenosine deaminase catalyzed nucleophilic attack of water on 6-aminopurine ribonucleoside. The work presented in this communication indicates two important points related to structure V: (1) stable anionic σ complexes with two electronegative atoms attached to the tetrahedral carbon can form at the C-6 position of the purine ring system by an aromatic nucleophilic addition mechanism and (2) the presence of nitro groups on the aromatic ring is not a necessary condition for the formation of such complexes.

(1) For an excellent comprehensive review of anionic σ complexes through Jan 1970, see M. J. Strauss, *Chem. Rev.*, 667 (1970).
(2) R. Wolfenden, *J. Amer. Chem. Soc.*, **88**, 3157 (1966).
(3) R. Wolfenden, T. K. Sharpless, I. S. Ragade, and N. J. Leonard, *ibid.*, **88**, 185 (1966).
(4) R. Wolfenden and J. F. Kirsch, *ibid.*, **90**, 6849 (1968).
(5) B. T. Walsh and R. Wolfenden, *ibid.*, **89**, 6221 (1967).
(6) R. Wolfenden, *Biochemistry*, **8**, 2409 (1969).
(7) R. Wolfenden, J. Kaufman, and J. B. Macon, *ibid.*, **8**, 2412 (1969).
(8) B. Evans and R. Wolfenden, *J. Amer. Chem. Soc.*, **92**, 4751 (1970).
(9) R. Wolfenden, *Accounts Chem. Res.*, **5**, 10 (1972).
(10) M. R. Crampton and V. Gold, *J. Chem. Soc.*, 3293 (1964).
(11) P. Caveng, P. B. Fischer, E. Heilbronner, A. L. Miller, and H. Zollinger, *Helv. Chim. Acta*, **50**, 848 (1967).
(12) It should be emphasized that the four methylene protons in II are not equivalent since two of the protons will be directed toward the imidazole ring and two away from it.
(13) The unsymmetrical spiro complex formed from the reaction of 2-(β-hydroxyethoxy)-3,5-dinitropyridine and sodium methoxide in DMSO also shows a singlet for the methylene protons; C. A. Fyfe, *Tetrahedron Lett.*, 659 (1968).
(14) This ratio does not represent the final equilibrium. It is merely an indication of the rate of formation of IV as compared to II.

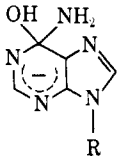


III



(2)

IV



V

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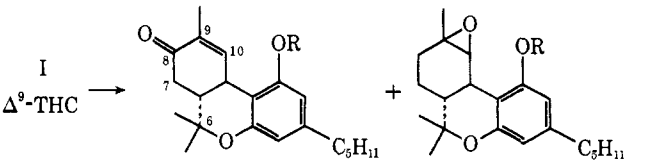
Metabolism of (−)-Δ^9- and (−)-Δ^8-Tetrahydrocannabinol by Monkey Liver[1]

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.