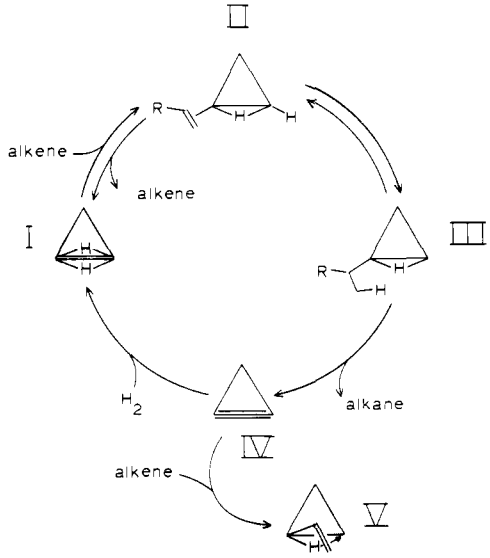


Figure 2. Reaction scheme for the interaction of $H_2Os_3(CO)_{10}$ (I) with alkenes (II, $HOs_3(CO)_{10}$(alkene); III, $HOs_3(CO)_{10}$(alkyl); IV, $Os_3(CO)_{10}$; V, $HOs_3(CO)_{10}$(alkenyl)).

$Os_3(CO)_{10}L$, where L is an adventitious donor, such as an ester or an additional alkene. This question is being examined in ongoing work, but we note at this point the recent reports on the characterization and reactivity of $[(C_5H_5)Mo(CO)_2]_2$,[14] a molecule with the same level of unsaturation as the proposed intermediate IV.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the National Science Foundation (MPS 75-14460) for support of this research. J. B. K. acknowledges a National Science Foundation Predoctoral Fellowship. The authors also thank Engelhard Industries for a generous loan of osmium trichloride.

References and Notes

(1) (a) Activation of Hydrocarbons by Unsaturated Metal Cluster Complexes. III. (b) Part II: M. Tachikawa, J. R. Shapley, and C. G. Pierpont, *J. Am. Chem. Soc.*, **97**, 7172 (1975).
(2) The possibilities for generation of unsaturated species are richer for metal–metal bonded compounds. One unique mode is via metal–metal bond scission (cf. E. L. Muetterties, B. A. Sosinsky, and K. I. Zamaraev, *J. Am. Chem. Soc.*, **97**, 5299 (1975)). However, our interest here is in the reactions of an intact metal aggregate with empty "surface" coordination sites (cf. ref 1b).
(3) J. B. Keister and J. R. Shapley, *J. Organomet. Chem.*, **85**, C29 (1975).
(4) The $HOs_3(CO)_{10}$(alkenyl) clusters have been characterized by their ir, NMR, and mass spectral data. No hydridoalkenyl species have been derived from 1,2-disubstituted alkenes. Substituents appear in the 2-positions only, a single substituent is trans to the Os–C σ bond. Thus, the compounds formed here from 1-alkenes are identical with those formed by insertion of 1-alkynes.[5]
(5) (a) A. J. Deeming, S. Hasso, and M. Underhill, *J. Chem. Soc., Dalton Trans.*, 1614 (1975); (b) W. G. Jackson, B. F. G. Johnson, J. W. Kelland, J. Lewis, and K. T. Schorpp, *J. Organomet. Chem.*, **87**, C27 (1975); (c) J. R. Shapley, S. I. Richter, M. Tachikawa, and J. B. Keister, *ibid.*, **94**, C43 (1975), and unpublished data.
(6) Analogous reactions of $H_2Os_3(CO)_{10}$ with diazo and azide compounds have been observed and will be reported separately.
(7) Weak molecular ions are observed in the mass spectra of **1** and **2**, but in each case the most abundant ions are $H_xOs_3(CO)_y^+$ (x = 0–2, y = 0–10), due to decomposition and subsequent fragmentation. Solution ir spectra indicate only terminal carbon monoxide ligands. In each case an absorption band due to the coordinated acyl group is observed (**1**, 1615 cm^{-1}; **2**, 1653 cm^{-1}; **3**, 1569 cm^{-1}; **4**, 1589 cm^{-1}); for **1** and **2** a band for the free carbonyl is also found (**1**, 1688 cm^{-1}; **2**, 1855 cm^{-1}). Proton NMR data (exclusive of carboethoxy signals) ($CDCl_3$, τ): **1**, 24.47 (s, 0.3 H), 24.05 (s, 0.7 H), 7.18 (dd, 1 H_c), 6.46 (dd, 1 H_b), 6.26 (dd 1 H_a, J_{bc} = −18.5, J_{ab} = 2.5, J_{ac} = 7.5 Hz); **2**, 23.62 (s, 0.6 H), 22.93 (s, 0.4 H), 6.99 (m, 1 H_c), 6.71 (m, 1 H_b), 6.66 (m, 1 H_a, J_{bc} = −18.6, J_{ab} = 5.2, J_{ac} = 10.9 Hz); **3**, 23.51 (s, 0.2 H), 22.99 (s, 0.8 H), 8.33 (d, 3H_b), 7.32 (q, 1 H_a, J_{ab} = 6.6 Hz); **4**, 23.26 (d, 1 H_c), 8.09 (dd, 1 H_b), 7.87 (d, 1 H_a, J_{ab} = −19.0, J_{bc} = 1.2 Hz).
(8) The data at hand do not unambiguously eliminate a six-membered ring for **1**. However, a five-membered ring is strongly indicated for **2**–**4** (chelation of one osmium center with a four-membered ring is highly unlikely) and assumed for **1**. The distribution of one- and two-electron donors closely resembles that for the adducts $H_2Os_3(CO)_{10}L$.[9]
(9) J. R. Shapley, J. B. Keister, M. R. Churchill, and B. G. DeBoer, *J. Am. Chem. Soc.*, **97**, 4145 (1975).
(10) Only one isomer is obtained for the related compound $HOs_3(CO)_{10}(C(=CH_2)CO_2Et)$ (formed from $H_2Os_3(CO)_{10}$ and ethyl propiolate), since isomerism at the α-carbon is not possible (M. Tachikawa and J. R. Shapley, unpublished results).
(11) Similar conformations have been assumed for $HMoCp_2(CH(CO_2Me)CH_2CO_2Me)$[12a] and for $Co(CN)_5(CH(CO_2^-)CH_2CO_2^-)^{2-}$.[12b]
(12) (a) A. Nakamura and S. Otsuka, *J. Am. Chem. Soc.*, **94**, 1886 (1972); **95**, 7262 (1973); (b) L. M. Jackman, J. A. Hamilton, and J. M. Lawlor, *ibid.*, **90**, 1914 (1968).
(13) The deuterium loss reported recently (S. A. R. Knox, J. W. Koepke, M. A. Andrews, and H. D. Kaesz, *J. Am. Chem. Soc.*, **97**, 3942 (1975)) in the preparation of $D_2Os_3(CO)_{10}$ is due to exchange with silica gel during chromatography and can be avoided by recrystallization from CCl_4 (J. B. Keister and J. R. Shapley, unpublished results, and H. D. Kaesz, private communication).
(14) (a) R. J. Klingler, W. Butler, and M. D. Curtis, *J. Am. Chem. Soc.*, **97**, 3535 (1975); (b) D. S. Ginley and M. S. Wrighton, *ibid.*, **97**, 3533 (1975).

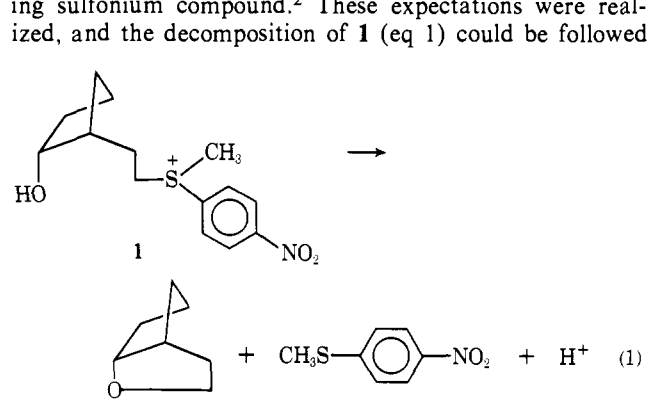
Jerome B. Keister, John R. Shapley*
Department of Chemistry, University of Illinois
Urbana, Illinois 61801
Received November 18, 1975

General Base Catalysis in Nucleophilic Attack at sp^3 Carbon of Methylase Model Compounds

Sir:

Despite the apparent simplicity of the overall reaction, there are little data available in the literature on the mechanism of enzyme-catalyzed transmethylation reactions in which the sulfonium compound, *S*-adenosyl-L-methionine (SAM) serves as the alkyl donor.[1,2] In this communication, we report a facile intramolecular transalkylation involving general-base-catalyzed attack of an alcohol on carbon bonded to trivalent sulfur.

Over the past several years, we have studied intermolecular and intramolecular transalkylation reactions as nonenzymic models for SAM-requiring methylases.[2-4] General base catalysis of nucleophilic attack at sp^3 carbon is a rare phenomenon, the only example being the cyclization of 4-chlorobutanol.[5,6] We have synthesized **1**[7] with the expectation that proximity effects would result in a facile intramolecular transalkylation reaction, in spite of the fact that 4-chlorobutanol is ca. 10^3 more reactive than the corresponding sulfonium compound.[2] These expectations were realized, and the decomposition of **1** (eq 1) could be followed



(1)

spectrophotometrically over a wide range of pH in water at 25°. The pH-rate profile shown in Figure 1 fits the general rate law, $k_o = k_{H_2O} + k_{OH}[OH^-]$, where k_o is the k_{obsd} extrapolated to zero buffer concentration. However, the following facts suggest the apparent k_{OH} term is really a term associated with the ionization of the secondary hydroxyl group. We have previously studied the reactions of several

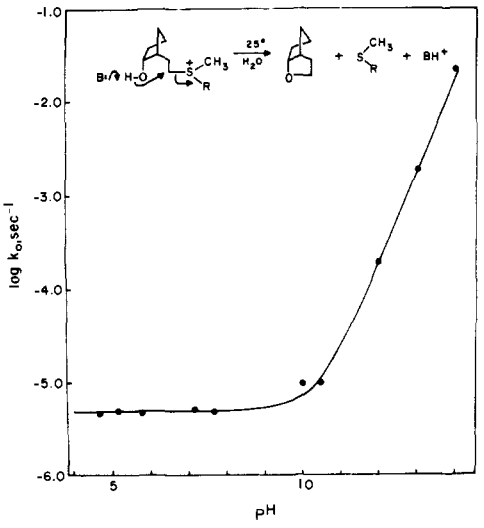


Figure 1. pH-rate profile for the intramolecular alkylation reaction of **1** at 25°. At pH greater than 11, pH = pK_w + antilog [OH$^-$].

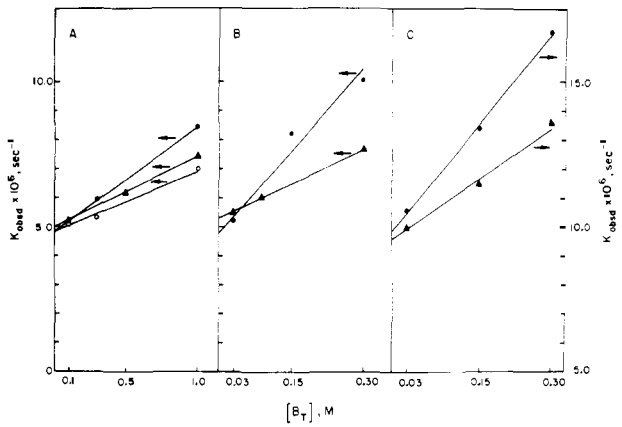
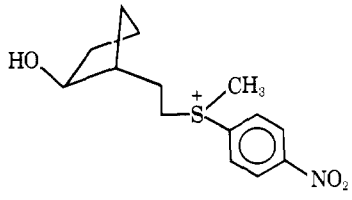


Figure 2. The effect of total buffer concentration on the observed rate of cyclization of **1**: (panel A, acetate buffer) ○, [B]:[BH] = 1:1, pH 4.67; ▲, [B]:[BH] = 3:1, pH 5.15; ●, [B]:[BH] = 9:1, pH 5.75; (panel B, phosphate buffer) ▲, [B]:[BH] = 3:1, pH 7.15; ●, [B]:[BH] = 9:1, pH 7.66; (panel C, carbonate buffer) ▲, [B]:[BH] = 1:1, pH 10.00, ●, [B]:[BH] = 3:1, pH 10.45. T = 25°; μ = 1.0 M with KCl.

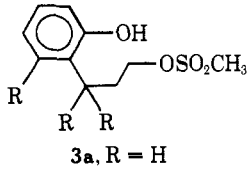
dimethylphenylsulfonium compounds with hydroxide ion,[3] and the apparent k_{OH} term of 1.92×10^{-2} M^{-1} s^{-1} at 25°, obtained from Figure 1, is much larger than k_{OH} values obtained previously with other sulfonium compounds at more elevated temperatures.[3] Also, this value of k_{OH} is higher by ca. 10^2 than that predicted from a Bronsted plot if hydroxide ion were acting as a general base catalyst such as described below for several basic buffer species. In addition, we have synthesized the trans isomer, **2**, in which *no* participation by the ring hydroxyl group is possible but which could undergo the usual intermolecular hydroxide-catalyzed reaction normally associated with the k_{OH} term. Compound **2** is completely inert under conditions where **1** is rapidly decomposed according to eq 1. These data are consistent with a rate law of the general type shown in eq 2.



2

$$k_o = k_{H_2O} + k_{RO^-}[Ka/(a_H + K_a)] \quad (2)$$

Assuming a pK_a of 16 for the hydroxyl group of **1** (pK_a of isopropyl alcohol = 16.2[8]), the experimental data in Figure 1 lead to a value of $k_{H_2O} = 5 \times 10^{-6}$ s^{-1} and $k_{RO^-} = 1.92$ s^{-1}. The latter value may be compared to a k_{PhO^-} value of 2.37×10^{-1} s^{-1} obtained by Borchardt and Cohen[9] in studying the intramolecular displacement by the phenolate ion of **3a**. Of course, the "trimethyl lock"[9] derivative, **3b**, had a much larger $k_{PhO^-} = 5 \times 10^4$ s^{-1} due to its increased rigidity.



3a, R = H
b, R = CH$_3$

If the pH-independent rate, k_{H_2O}, could be compared to similar data for intermolecular reactions, a rate-enhancement value for this intramolecular reaction could be calculated. However, the intermolecular reaction of water with sulfonium compounds is exceedingly slow.[3] We have calculated an approximate k_{H_2O} value for *p*-nitrophenyldimethylsulfonium perchlorate as follows. Hydroxide ion reacts with a series of substituted phenyldimethylsulfonium perchlorates to give methanol and the corresponding thioanisole with $\rho = 1.60$.[3] In contrast, the *p*-nitro compound undergoes ring attack to give *p*-nitrophenol and dimethyl sulfide.[3] By extrapolation of the σ-ρ plot, however, one can calculate a theoretical value of k_{OH} for the demethylation of *p*-nitrophenyldimethylsulfonium perchlorate. This rate constant, derived from data obtained at 78°, can be converted to k_{OH^-} at 25° using activation energies obtained experimentally for similar sulfonium compounds.[3] Finally, assuming a Bronsted β value of 0.3,[3] k_{H_2O} at 25° can be calculated as *ca.* 10^{-11} M^{-1} s^{-1}. When compared with $k_{H_2O} = 5 \times 10^{-6}$ s^{-1} obtained for the decomposition of **1** (Figure 1), an effective molarity (EM) of ca. 5×10^5 M is obtained.

Of primary interest, however, is the fact that the reaction is catalyzed by buffer species. Effects of added buffer species were investigated using acetate (pH 4.67-5.78), phosphate (pH 6.51-7.67), and carbonate (pH 10.00-10.48) buffers at concentrations from 0.03 to 1.0 M (Figure 2). In all cases studied, the basic form of the buffer was found to catalyze the reaction with second-order rate constants (k_B) of 4.1×10^{-6}, 1.56×10^{-5}, and 2.87×10^{-5} M^{-1} s^{-1} for acetate, phosphate, and carbonate, respectively. A Bronsted plot of these data, together with k_{H_2O} (Figure 1) converted to a second-order rate constant by dividing the concentration of water (55.5 M), yields a β value of 0.21. The fact that **2** is inert in these buffered media strongly suggests that the buffer effects observed with **1** are associated with the cyclization reaction of eq 1, and are not simply the result of intermolecular nucleophilic attack of the buffer on carbon. A solvent isotope effect was determined for the reaction of **1** and found to be close to unity, $k_{H_2O}/k_{D_2O} = 1.37$, as expected for a nucleophilic reaction catalyzed by general bases with a transition state early on the reaction coordinate.[3]

The data presented in this communication provide evidence for catalysis of nucleophilic attack at sp^3 carbon by general bases over a wide range of pH in aqueous media. These findings suggest a role for basic residues of proteins in promoting enzyme-catalyzed alkylation reactions, such as in the methylation of catecholamines by SAM.[2,10]

Acknowledgment. This research was supported by United States Public Health Service Grants No. MH-18,038 and CA-10,748.

References and Notes

(1) W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, p 283.
(2) J. K. Coward in "The Biochemistry of S-Adenosylmethionine", E. Borek et al., Ed., Columbia University Press, New York, N.Y., in press.
(3) J. K. Coward and W. D. Sweet, *J. Org. Chem.*, **36**, 2337 (1971).
(4) R. Lok and J. K. Coward, *Bioorg. Chem.*, in press.
(5) C. G. Swain, D. A. Kuhn, and R. L. Schowen, *J. Am. Chem. Soc.*, **87**, 1553 (1965).
(6) T. H. Cromartie and C. G. Swain, *J. Am. Chem. Soc.*, **97**, 232 (1975).
(7) The sulfonium compounds, **1** and **2**, were prepared as previously described for related hydroxy sulfonium compounds.[4] The thioether precursors were prepared by alkylation of an appropriate ketal tosylate, followed by ketal hydrolysis and reduction of the resulting ketone with aluminum isopropoxide. Separation of the cis and trans isomers (precursors of **1** and **2**, respectively) was accomplished by preparative TLC on silica gel in *n*-hexane:ethyl acetate, 3:1. The ketal tosylate was prepared in three steps from ethyl (2-oxocyclopentyl)acetate. (R. P. Linstead and E. M. Meade, *J. Chem. Soc.*, 935 (1934)).
(8) C. K. Sauers, W. P. Jencks, and S. Groh, *J. Am. Chem. Soc.*, **97**, 5546 (1975).
(9) R. T. Borchardt and L. Cohen, *J. Am. Chem. Soc.*, **94**, 9166 (1972).
(10) J. K. Coward, E. P. Slisz, and F. Y.-H. Wu, *Biochemistry*, **12**, 2291 (1973).
(11) Postdoctoral Fellow supported by U.S. Public Health Service Training Grant GM-59.
(12) Coop Student from Antioch College, Yellow Springs, Ohio, April–June 1974.

James K. Coward,* Roger Lok,[11] Orinne Takagi[12]
Department of Pharmacology
Yale University School of Medicine
New Haven, Connecticut 06510
Received October 29, 1975

Dinosterol, the Major Sterol with a Unique Side Chain in the Toxic Dinoflagellate, *Gonyaulax tamarensis*[1]

Sir:

Phytoplankton constitute the basis of the food chain in the marine life, and their chemical constituents are of particular interest in regard to peculiar compounds often found in marine organisms.[2]

In search for possible sources of unusual marine sterols, the toxic dinoflagellate, *Gonyaulax tamarensis*, which is causing serious problems on the North Atlantic coasts, was investigated.

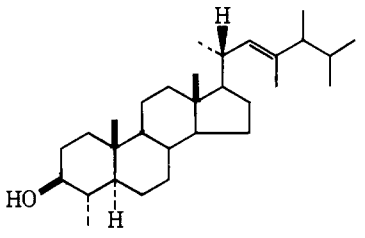
The chloroform extract of unialgal cultured *G. tamarensis* cells[3] afforded a sterol fraction which essentially consisted of cholesterol and a new C_{30} sterol (named dinosterol) in a ratio of 2:3 (GLC analysis).[4] Dinosterol (I) was purified by high speed liquid chromatography and recrystallized from $CHCl_3$–MeOH to needles (yield ca. 6 mg from 370 × 10^6 cells), mp 220–222°, $[\alpha]_D$ ±5° (*c* 0.6, $CHCl_3$), $C_{30}H_{52}O$ (calcd *m/e* 428.4041; found *m/e* 428.4054).

The mass spectrum pattern of I, *m/e* 316 (88%), 287 (100%), 271 (64%) was reminiscent of that of gorgosterol,[5,6] but the absence of a cyclopropane structure was obvious from the 100-MHz ^1H NMR spectrum which showed seven alkyl linked methyl signals (δ 0.70 (3 H, s), 0.80 (3 H, d, *J* = 7 Hz), 0.84 (3 H, s), 0.85 (3 H, d, *J* = 7 Hz), 0.94 (6 H, d, *J* = 6.5 Hz, isopropyl), 0.95 (3 H, d, *J* = 6 Hz)), an olefinic proton signal (δ 4.87 (1 H, q, *J* = 1.2, 10 Hz)), and a proton signal due to a secondary alcohol (δ 3.10 (1 H, m)). Decoupling study showed the presence of a partial structure -CHCH=C(CH_3)—which seemed to be located in the side chain leaving a few possibilities.

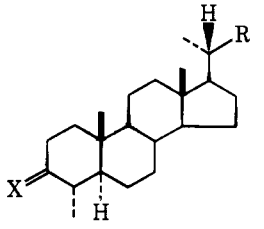
Jones oxidation of I afforded a ketone (V), mp 193–195°, whose positive CD curve, nm (Δε) in dioxane, 309 (+0.52), 298 (+0.97), 290 (+1.07), implicated the structure of 4α-methyl-5α-3-one.[7] Indeed 4α-methyl-5α-stigmast-22-en-3-one (III), mp 164–166°, which was synthesized for comparison by methylation and Birch reduction of stigmast-4,22-dien-3-one, showed a superimposable CD curve, nm (Δε) in dioxane: 310 (0.47), 297 (0.97), 291 (1.05). Moreover, the mass spectra of both II and III were found to show almost an identical fragmentation pattern, *m/e* 426 (M^+, 100%), 383, 328, 314, 287, and 285, strongly suggesting that the double bond in the side chain is located at the 22 position (consequently two methyl groups at the 23 and 24 position). The final proof of the structure was accomplished by the ozonolysis of I followed by $NaBH_4$ reduction of the ozonide to the diol (IV), mp 203–205°, *m/e* 348 (M^+, 100%), 333, 330, 262, 248, 247, and 229, which was unequivocally prepared by the ozonolysis and $NaBH_4$ reduction of III.

Since $NaBH_4$ reduction of 4α-methyl-5α-3-one is known to give the corresponding 3β-ol preferentially,[8] the structure of dinosterol is established as 4α, 23,24ξ-trimethyl-5α-cholest-22-en-3β-ol.

The existence of an unusual methyl group at the C-23 in I seems to be very significant, since the analogy can be only found in gorgosterol, acanthasterol,[9] and demethylgorgosterol[10] whose origins have been the subject of discussion.[11]



I



II, X = O; R = -CH=C(CH_3)CH(CH_3)CH(CH_3)$_2$
III, X = O; R = -CH=CHCH(C_2H_5)CH(CH_3)$_2$
IV, X =  ; R = -CH_2OH

Although the configuration at C-24 and the geometry with the 22-double bond in I are still unknown, I seems to be closely related to the above mentioned sterols. 4α-Methylsterols are intermedicates in sterol biosynthesis and known to be accumulated under anaerobic conditions.[7a,12] It might be significant to note that the mass spectroscopic analysis of sterols from anaerobically kept gorgonian associated zoxanthella was reported to give a molecular ion *m/e* 428 assigned to "dihydrogorgosterol."[11]

Investigation of the sterols in other dinoflagellates is now under way.

Acknowledgment. The authors thank Professor Koji Nakanishi, Dr. W. L. Tan of Columbia University for spectroscopic data, and Dr. M. Koreeda of Johns Hopkins University for CD data.