to an increase in the energy necessary for localization of electrons to form a simple olefin–metal π complex. *cis*-Stilbene gave typical class II shifts; however *trans*-stilbene gave very slow[11] spectral changes, further evidence for conjugation effects. Maleic and fumaric acids gave results which were analogous to the isomeric stilbenes.

Since Co(II)MPIXDME is coordinately unsaturated, solvation would be expected to affect the oxidation reaction. Oxidation of Co(II)MPIXDME in solvents of high dielectric constants such as methanol occurs very slowly,[12] while Co(II)MPIXDME is stable in solvents of low dielectric constants such as *n*-hexane, *p*-dioxane, and chloroform. This dependence of oxidation on dielectric constant may be related to Jahn–Teller distortion of the square-planar d^7 Co(II)MPIXDME. As solvent dielectric constants increase, Jahn–Teller distortion decreases and the Co(II)MPIXDME approaches octahedral symmetry, thus facilitating oxidation from a d^7 octahedral Co(II) species to the more stable d^6 octahedral Co(III)MPIXDME.[13] It is known that solvent coordination to square-planar Co(II) complexes lowers the oxidation potential of the metal by over 2 V.[5,14] The addition of olefins to the Co(II)MPIXDME in organic solvents results in a replacement of one or both of the solvent molecules[15,16] which are associated along the *z* axis. This π complexation of the olefin along the positive *z* axis would decrease the electron density along the negative *z* axis. Octahedral symmetry would be more closely approximated by "tighter" coordination of the remaining solvent molecule followed by rapid metal oxidation.[17]

In contrast to solvent-caused oxidation in which the Co(III)MPIXDME is stable,[18] olefin addition gives only a transitory Co(III)-type spectrum. The final spectrum, in the latter case, showed a conspicuous absence of the Soret band. This, and the color loss of the solution, indicate that the porphyrin moiety was decomposed.[19]

Three mechanisms may be postulated for the Co(II)-MPIXDME oxidation. The first is that olefin coordination facilitates octahedral symmetry formation and electron transfer; the second, which is analogous to the homogeneous hydrogenation mechanism suggested by Halpern,[20] involves H· abstraction from the solvent; the third is oxidative olefin addition.[21] The first mechanism is preferred in this case due to solvent dielectric considerations, while the other two are not favored since free-radical reactions are generally not solvent dependent.

The mechanisms of metal oxidation and porphyrin decomposition are being investigated by determining the fates of the olefin and porphyrin moiety.

Acknowledgment. This investigation was supported by National Science Foundation Grant NSF GB-5732. Acknowledgment is also made to Mr. Y. Masada who partially assisted in the experimental part of this investigation.

(11) *cis*-Stilbene gave 25% oxidation and *trans*-stilbene gave 5% metal oxidation after 2.5 hr.

(12) Approximately 25 hr are needed for total oxidation.

(13) Crystal-field stabilization energies are higher for d^6 octahedral Co(III) than for d^6 octahedral Co(II) species; see ref 7.

(14) J. E. Falk and J. N. Phillips, "Chelating Agents and Metal Chelates," F. P. Dwyer and D. P. Mellor, Ed., Academic Press Inc., New York, N. Y., 1964, p 470; F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1966, p 867.

(15) C. H. Langford and H. B. Gray, "Ligand Substitution Processes," W. A. Benjamin, Inc., New York, N. Y., 1965.

(16) J. P. Collman, M. Jubota, and J. W. Hosking, *J. Am. Chem. Soc.*, **89**, 4809 (1967).

(17) Cobalt oxidation occurs upon olefin addition if the solvent dielectric constant is higher than the dielectric constant of the olefin.

(18) Only two species, Co(II)- and Co(III)MPIXDME, are present in solution as evidenced by an isosbestic point in the spectra of solvent-caused oxidation. The Co(III)MPIXDME absorption at 411 mμ does not decrease with time.

(19) Loss of electronic conjugation in the porphyrin moiety would also account for the absence of the Soret absorption band.

(20) J. Halpern, *Chem. Eng. News*, 46, 68 (Oct 31, 1966).

(21) J. P. Collman, "The Role of Vacant Coordination Sites in Homogeneous Catalysis," presented at the New York Academy of Sciences, Dec 1967; J. P. Collman, K. W. Kang, W.F. Little, and M. F. Sullivan, Abstracts, 3rd International Symposium on Organometallic Chemistry, Munich, Aug 1967, p 334.

M. Tsutsui, R. Velapoldi
K. Suzuki, A. Ferrari
New York University
New York, New York 10453

Received January 24, 1968

Studies on the Action of 2,3-Oxidosqualene–Sterol Cyclase on Unnatural Substrates Produced by Alkylidene Transfer from Sulfonium Alkylides to 4,8,13,17,21-Pentamethyldocosa-4,8,12,16,20-pentaenal

Sir:

Studies carried out in these laboratories during the period 1961–1966 established that 2,3-oxidosqualene (**1**) is an intermediate in the biosynthesis of sterols from squalene.[1,2] More recently the enzyme which effects the cyclization of **1** to sterol has been obtained in partially purified, soluble form[3] and has been shown to cyclize certain unnatural analogs of **1**.[4–6] In addition, a powerful inhibitor of 2,3-oxidosqualene–sterol cyclase, the corresponding imine, has been developed.[7]

The ^{14}C-labeled 2,3-oxidosqualene used in most of our work was prepared very conveniently and with high specific activity by the reaction of diphenylsulfonium isopropylide (**3**)[8,9] with the aldehyde **2** which is readily available from 2,3-oxidosqualene[1a] by hydration to the 2,3-diol[1a] followed by periodate oxidation. Specifically, aldehyde **2** (2 mmoles) was allowed to react at -70 to $-30°$ with ^{14}C-labeled diphenylsulfonium isopropylide prepared[8,9] from diphenylsulfonium ethylide (2 mmoles) and ^{14}C-labeled methyl iodide (286 mg, 2.00 mCi) to give, after isolation[8,9] and purification using thin layer chromatography (tlc) on silica gel (buffered to pH 10), 2,3-oxidosqualene (**1**) (30–35%) of specific activity 2.8×10^6 dpm/μmole.

(1) (a) E. J. Corey and W. E. Russey, *J. Am. Chem. Soc.*, **88**, 4751 (1966); (b) E. J. Corey, W. E. Russey, and P. R. Ortiz de Montellano, *ibid.*, **88**, 4750 (1966); (c) W. E. Russey, Ph.D. Thesis, Harvard University, 1966.

(2) The same conclusion has been reached by other workers; see J. D. Willet, K. B. Sharpless, K. E. Lord, E. E. van Tamelen, and R. B. Clayton, *J. Biol. Chem.*, 242, 4182 (1967).

(3) P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch, and E. J. Corey, *ibid.*, 242 3014 (1967).

(4) E. E. van Tamelen, K. B. Sharpless, J. D. Willett, R. B. Clayton, and A. L. Burlingame, *J. Am. Chem. Soc.*, **89**, 3920 (1967).

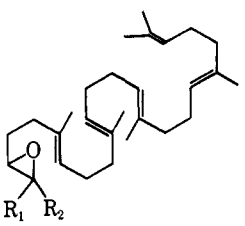
(5) E. J. Corey and S. K. Gross, *ibid.*, **89**, 4561 (1967).

(6) E. E. van Tamelen, K. B. Sharpless, R. Hanzlik, R. B. Clayton, A. L. Burlingame, and P. C. Wszolek, *ibid.*, **89**, 7150 (1967).

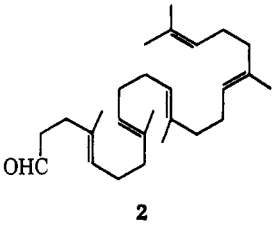
(7) E. J. Corey, P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean, *ibid.*, **89**, 2797 (1967).

(8) E. J. Corey, M. Jautelat, and W. Oppolzer, *Tetrahedron Letters*, 2325 (1967).

(9) E. J. Corey and M. Jautelat, *J. Am. Chem. Soc.*, **89**, 3912 (1967).



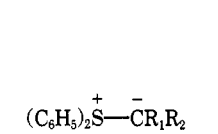
1, $R_1 = R_2 = CH_3$
4, $R_1 = CH_3$; $R_2 = C_2H_5$
5, $R_1 = CH_3$; $R_2 = H$
6, $R_1 = H$; $R_2 = H$
7, $R_1 = C_2H_5$; $R_2 = H$



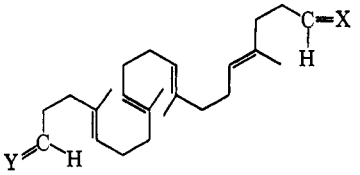
2

The use of alkylidene transfer reactions from sulfonium ylides to the aldehyde **2** has been found to provide an efficient synthetic route not only to [^{14}C]2,3-oxidosqualene but also to various higher and lower homologs differing in terminal substitution. Consequently, an investigation has been made of the possible conversion of the readily available oxides **4–7** to sterol by the action of 2,3-oxidosqualene–sterol cyclase.[10]

Radioactive substrates **4–7** were prepared starting both from 1-^{3}H-labeled aldehyde **2** (**2a**) (1.6×10^6 dpm/μmole) and from 22,22′-^{3}H-labeled **2** (**2b**) (1.7×10^6 dpm/μmole). The former was made by reduction of **2** with sodium borotritide in methanol followed by Pfitzner–Moffat oxidation,[11] and the latter was made by the sequence 2,3:22,23-dioxidosqualene → 2,3,22,23-tetrahydroxysqualene (H_2O–$HClO_4$) → dialdehyde **8** (HIO_4) → hydroxy aldehyde **9** (1 equiv of $NaBH_4$ in CH_3OH) → ^{3}H-labeled hydroxy olefin **10** (^{3}H-labeled isopropylidenetriphenylphosphorane[12]) → **2b** (oxidation[11]). Reaction of the labeled aldehydes **2a** or **2b** with diphenylsulfonium 2-butylide (**11**)[13] led to the labeled homo-2,3-oxidosqualene **4**.[14] Similarly, the labeled oxidosqualene analogs **5**, **6**, and **7** were obtained from labeled **2** and the corresponding diphenylsulfonium alkylides.[15,16] Treatment of the oxides **4–7** with perchloric acid in aqueous glyme produced the corresponding *vic*-glycols, which upon oxidation with periodic acid afforded aldehyde **2**, providing chemical confirmation of structure.

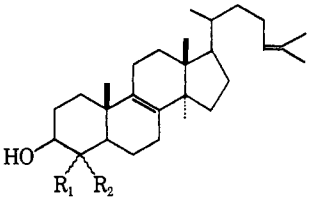


3, $R_1 = R_2 = CH_3$
11, $R_1 = CH_3$; $R_2 = C_2H_5$



8, X = Y = O
9, X = O; Y = H,OH
10, X = $C(CH_3)_2$*; Y = H,OH
2b, X = $C(CH_3)_2$*; Y = O

Anaerobic incubation of labeled **4**, the higher homolog of 2,3-oxidosqualene, with a solution of 2,3-oxidosqualene–sterol cyclase[3,17] afforded a product which contained two major radioactive components by tlc analysis, one of R_f 0.45 corresponding to starting oxide **4** (*ca.* 80% of total counts) and the other of R_f 0.22 (*ca.* 20% of total counts) in the region expected for a sterol (*cf.* R_f 0.21 for lanosterol). Acetylation of the product, R_f 0.22, led to formation of a monoacetate which showed upon tlc analysis with silica gel (CH_2Cl_2) R_f 0.59 and with silica gel–10% $AgNO_3$ (CH_2Cl_2) R_f 0.37, the corresponding values for lanosteryl acetate being 0.56 and 0.34, respectively, indicating that the enzymic transformation of **4** led to a sterol. Confirmation was obtained from the mass spectra of the new sterol and its acetate which revealed molecular ion peaks at *m/e* 440 and 482, respectively, as expected for homolanosterol ($C_{31}H_{51}OH$) and its acetate. Further, the fragmentation patterns of these derivatives were completely analogous to the lanosterol series. In addition, successive recrystallization of a mixture of unlabeled lanosteryl acetate and labeled sterol acetate from **4** led to no change in specific activity over four recrystallizations, indicating a close similarity of molecular shapes. Finally, anaerobic incubation of labeled **4** and a like amount of 2,3-iminosqualene[7] with the cyclizing enzyme led to complete recovery of **4** and no detectable sterol. The enzymic transformation product from **4** is therefore formulated as the homolanosterol structure **12**.



12, $R_1 = CH_3$; $R_2 = C_2H_5$
13, $R_1 = CH_3$; $R_2 = H$

The lower homolog **5** of 2,3-oxidosqualene upon anaerobic incubation with a solution of oxidosqualene cyclase[17] gave, in addition to recovered **5** (*ca.* 65%), the corresponding *vic*-glycol (*ca.* 35%) (identified chromatographically and by periodate oxidation to **2**) and variable amounts, 0–1%, of a sterol fraction. Higher conversion to the sterol-like product (4–5%) could be obtained using a suspension of hog liver microsomes.[17] This product and its acetate derivative compared closely to lanosterol and its acetate, respectively, by tlc analysis and in cocrystallization experiments such as described above for the products from **4**, again indicating the sterol formulation, specifically **13**. The orientation of the 4-methyl substituent in the product **13** is considered to be α, since Jones oxidation produced a ketone which was not epimerizable by treatment with sodium methoxide in methanol (using tlc analysis). The formation with either liver microsomes or enzyme

(10) A recent communication describes a related study involving substrate **5**; see R. B. Clayton, E. E. van Tamelen, and R. G. Nadeau, *J. Am. Chem. Soc.*, **90**, 820 (1968). Our experimental data complement those reported and lead to similar conclusions in the case of this particular analog.

(11) K. E. Pfitzner and J. G. Moffat, *ibid.*, 87, 5661 (1965).

(12) The phosphorane [G. Wittig and D. Wittenberg, *Ann.*, **606**, 1 (1957)] was generated in tetrahydrofuran by the action of phenyllithium on isopropyltriphenylphosphonium iodide (from triphenylphosphine and ^{3}H-labeled isopropyl iodide).

(13) This ylide was obtained by a process involving methylation of diphenylsulfonium *n*-propylide in the way previously described.[8]

(14) This substance, as well as **5**, and **7** were obtained as a mixture of *cis*- and *trans*-substituted oxiranes in comparable amounts; the *cis*–*trans* mixtures were used in the biological experiments. The oxidosqualene analogs were characterized by mass spectroscopy, nmr, and infrared analysis after purification and isolation by tlc. The yields of **4–7** were in the range 60–80%.

(15) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **84**, 3782 (1962); **87**, 1353 (1965).

(16) E. J. Corey and W. Oppolzer, *ibid.*, **86**, 1899 (1964).

(17) Incubations were carried out with 12 mμmoles of substrates **4–7** in 1 ml of enzyme solution[3] or 1 ml of hog liver microsome (0.2 g) suspension at 35° and pH 7.4 (0.1 *M* phosphate buffer) for a period of 1 hr. Products were isolated after saponification with 1 *N* methanolic potassium hydroxide by extraction with 1:1 ether–pentane and tlc separation using silica gel with methylene chloride for development.

solution of **13** from **5** was inhibited by 2,3-iminosqualene; however, the hydration reaction of **5** to the *vic*-glycol was not inhibited, indicating that this is not enzymically produced at the same site as the sterol **13**.

The racemic bisnor analog **6** was transformed rapidly in >95% yield by solutions of oxidosqualene–sterol cyclase[17] or microsomal suspensions to the corresponding *vic*-glycol; little, if any, sterol was produced. Analog **7** also yielded little, if any, sterol; some glycol (*ca.* 10% conversion)[17] was formed in this case, but the rate of hydration of **7** was distinctly slower than that for **5** or **6**.

Of special interest in the above results are (1) the formation of lanosterol analogs from **4** and **5**, and the much more efficient conversion with the former, (2) the formation of *vic*-glycols by catalyzed hydration of the oxides **5–7**,[18] and especially the lack of stereospecificity and efficiency of conversion with **6**, and (3) the greater measure of cyclization with **5** using microsomal rather than soluble enzyme preparations.

Additional study of these and related analog systems is planned. One interesting possibility is that there may be a nonspecific, "detoxifying" or scavenging enzyme in liver microsomes which catalyzes hydration of oxirane derivatives, especially if the ring is not highly substituted (*cf.* ref 10).

Acknowledgment. We are grateful to Bruce Ganem, Dennis Crouse, and Paul Ortiz de Montellano for valuable experimental assistance and to the National Science Foundation and National Institutes of Health for financial support.

(18) The oxides **4–7** were found to be completely stable in 0.1 *M* phosphate buffer at pH 7.4 under incubation conditions. Boiled enzyme solutions at pH 7.4 were without effect on **4**, **5**, **6**, and **7**.

E. J. Corey, Kang Lin, Manfred Jautelat
Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received February 27, 1968

Chemistry in Super Acids. I. Hydrogen Exchange and Polycondensation of Methane and Alkanes in FSO_3H–SbF_5 ("Magic Acid") Solution. Protonation of Alkanes and the Intermediacy of CH_5^+ and Related Hydrocarbon Ions. The High Chemical Reactivity of "Paraffins" in Ionic Solution Reactions[1]

Sir:

In previous studies[2] we reported that the extremely strong acid FSO_3H–SbF_5 ("magic acid") is capable of forming alkylcarbonium ions from alkanes *via* hydride (alkide) ion abstraction. It was observed that even neopentane is basic enough to undergo reaction in the neat acid. At room temperature, neopentane gave the *t*-butyl cation and methane, and, when reacted at low temperatures in acid diluted with SO_2ClF, the dimethylethylcarbonium ion is formed through the rearrangement of the intermediate neopentyl cation. Hogeveen and Bickel[3] subsequently made similar observations on the protolytic cleavage of neopentane in the related acid system, HF–SbF_5.

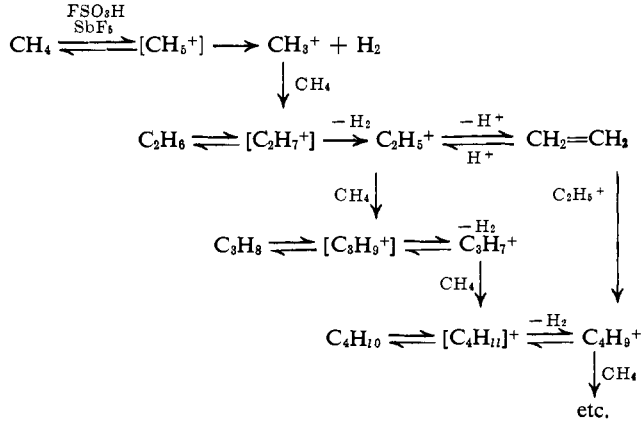
(1) Presented in part at an Organic Colloquim, Harvard University, Cambridge, Mass., Nov 28, 1967.
(2) G. A. Olah and J. Lukas, *J. Amer. Chem. Soc.*, **89**, 2227, 4743 (1967).
(3) H. Hogeveen and A. F. Bickel, *Chem. Commun.*, 635 (1967).

We reported[2] that "Methane reacts only at +140° with FSO_3H–SbF_5 to give, as yet, unidentified products. Ethane gives a mixture of 90% $(CH_3)_3C^+$ and 10% $(CH_3)_2C^+CH(CH_3)_2$ through some dimerization and trimerization pathways."

We wish now to report our studies relating to the behavior of methane, as well as ethane, neopentane, and related saturated hydrocarbons, in super acid solution.

Methane, when allowed to react either in a pressure bomb with tenfold excess of 1:1 FSO_3H–SbF_5 solution or at 140° under atmospheric pressure, gives primarily the trimethylcarbonium ion with some higher molecular weight hydrocarbon ions also formed. If the temperature is kept around +80°, a mixture of the trimethylcarbonium ion and the dimethylisopropylcarbonium ion is formed. Depending on the temperature, reaction time, and acid concentration, higher molecular weight hydrocarbon ions can also be formed in various amounts. Hydrogen gas is liberated in the reactions but, as reported previously,[2] it at least partially reduces the acid if not swept ont continuously and therefore the reaction cannot be monitored by measuring the amount of hydrogen formed. When methane was allowed to react with FSO_3D–SbF_5 (prepared also from DF–SbF_5 with SO_3) or CD_4 (obtained from Merck Sharp and Dohme of Canada) with FSO_3H–SbF_5, recovered "unreacted" methane showed extensive hydrogen scrambling as analyzed by mass spectroscopy. HD was also detected in the mass spectroscopic analysis of the gaseous reaction mixture, along with higher molecular weight ion products. All these observations can be explained only by suggesting that, in the super acid solutions, methane indeed is behaving as a base. It is protonated in solution to the CH_5^+ ion which then either undergoes reversible deprotonation accounting for the hydrogen exchange or loses hydrogen to form the extremely reactive carbonium ion $(CH_3)^+$ which then reacts with excess methane to start a "growth reaction" or "polycondensation" giving, eventually, the trimethylcarbonium ion, dimethylisopropylcarbonium ion, or higher molecular weight hydrocarbon ions (Scheme I). The over-all process is, of course, more

Scheme I



complicated as the intermediate ethyl cation, isopropyl cation, and related carbonium ions themselves can undergo various di-, tri-, and polymerization processes, and the higher molecular weight hydrocarbon ions undergo, in turn, fragmentation. The hydrogen-exchange reaction of methane seems to be much faster than the polycondensation and can be observed even