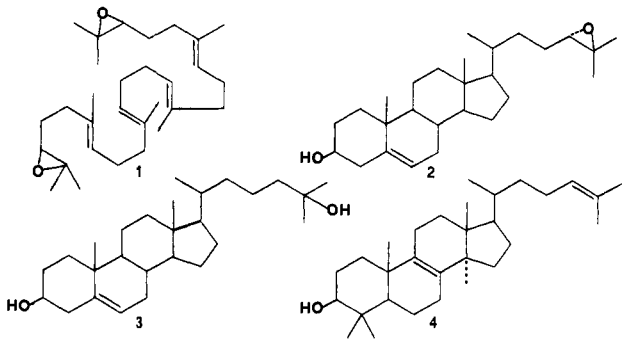
The remainder of the CF was diluted with unlabeled **2**, and an ethereal solution of the resulting mixture was treated with $LiAlH_4$ at room temperature for 4 days. TLC analysis of the product from this reaction showed 10.6% of the radioactivity at the R_f value of **3**. The product was diluted with authentic unlabeled **3**,[15] purified by preparative TLC, further diluted with **3**, and recrystallized to constant specific activity.[16] The amount of [^{14}C]**3** obtained indicated that the CF from the RLH incubation contained 6.3% [^{14}C]**2**.



In order to rule out the possibility that the [^{14}C]**3** was not arising via **1**, but by some other route, such as autoxidation of cholesterol[17] or epoxidation of a steroidal precursor of cholesterol, we conducted an analogous study of the CF obtained from incubation of [^{3}H]lanosterol (**4**)[18] with RLH. Incubations of [^{3}H]**4** with RLH were performed in exactly the same manner as those with [^{14}C]acetate. The nonsaponifiable extracts contained 85% of the initial radioactivity. Preparative TLC was used to separate a CF containing 67% of the isolated ^{3}H. Treatment of this CF with $LiAlH_4$, as before, yielded material which, upon TLC analysis, showed no peak in radioactivity at the R_f of **3**. The entire $LiAlH_4$ reduction product was mixed with unlabeled **3** and purified by preparative TLC to afford a 25-hydroxycholesterol (**3**) fraction which had a specific activity corresponding to 1.2% of the CF. After several recrystallizations, the ^{3}H content was 0.17% of the initial radioactivity in the CF.[19] Although there seems to be a trace of [^{3}H]**3** in the product obtained from [^{3}H]**4**, it is clear that the principal pathway to **3**, and thus **2**, involves introduction of the second oxygen atom at a stage prior to the cyclization of squalene 2,3(*S*)-oxide to lanosterol. The intermediacy of squalene 2,3(*S*);22(*S*),23-dioxide (**1**) seems overwhelmingly reasonable.[20]

Finally, both [^{14}C]acetate and [^{3}H]lanosterol (**4**) were incubated with the same portion of RLH. The procedures used to obtain and purify **3** from this incubation were all exactly the same as those used in the separate incubations. The amount of [^{3}H]**3** obtained indicated that less than 0.68% of the CF could have been [^{3}H]**2**, whereas the amount of purified [^{14}C]**3** obtained indicated that 7.6% of the CF had been [^{14}C]**2**.

The results described above establish unequivocally that a substantial amount of 24(*S*),25-epoxycholesterol (**2**) is formed in the normal course of steroid biosynthesis by rat liver enzymes. This discovery makes further study of the heretofore obscure **2** imperative. Among the things we will be attempting to determine are the metabolic fate of **2**, the biochemical role or roles which **2** plays, and whether the "cholesterol" biosynthesized by humans contains a comparable amount of **2**.

Acknowledgment. This research was supported by NIH Grant HL 23083. Initial support from Merck, Sharp, & Dohme Research Laboratories is also appreciated. We thank Dr. Sandra K. Erickson for valuable discussions.

(15) Steraloids, Inc., Wilton, NH.
(16) Initial specific activity = 1900 dpm/mg. Recrystallization from acetone yielded **3** with, successively, 1680, 1680, and 1690 dpm/mg.
(17) Smith, L. L.; Matthews, W. S.; Price, J. C.; Bachman, R. C.; Reynolds, B. *J. Chromatogr.* **1967**, *27*, 187–205. van Lier, J. E.; Smith, L. L. *J. Org. Chem.* **1970**, *35*, 2627–2632. Teng, J. I.; Kulig, M. J.; Smith, L. L.; van Lier, J. E. *Ibid.* **1973**, *38*, 119–123.
(18) [2-^{3}H]Lanosterol, specific activity = 7.37 mCi/mmol, was prepared by treatment of the corresponding ketone with acidic tritium oxide in THF by the method of: Nadeau, R. G.; Hanzlik, R. P., ref 2, pp 346–349.
(19) Initial specific activity = 1440 dpm/mg. Recrystallization from acetone yielded **3** with, successively, 410, 240, 200, 190, and 180 dpm/mg.
(20) In a separate study (Steckbeck, S. R.; Nelson, J. A.; Spencer, T. A., unpublished results) we have shown that 24(*R*),25-oxidolanosterol is not converted by RLH to 24(*R*),25-epoxycholesterol, thus ruling out the logical but implausible possibility that some of the **3** obtained was derived from 24(*R*),25-epoxycholesterol.

Coordination and Coupling of Alkylidene Groups on a Triosmium Cluster Framework. Crystal Structure of $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHSiMe_3)$

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Surface-bound methylene groups are key intermediates in recent mechanistic proposals for the metal-catalyzed formation of hydrocarbons from carbon monoxide and hydrogen (Fischer–Tropsch synthesis).[1-4] In particular, Pettit[4] has shown that such species, as generated from diazomethane, can (1) couple to form ethylene in the absence of hydrogen or (2) generate hydrocarbon chains in the presence of hydrogen. The chemistry of discrete polynuclear transition-metal compounds which have methylene ligands may provide mechanistic details relevant to the catalytic process, but rather little is known about the reactivity of such ligands in terms of forming carbon–carbon bonds with similar (alkylidene) or different (alkyl, alkene, alkyne) hydrocarbon moieties.[5] We wish to report the synthesis of a set of alkylidenetriosmium cluster compounds, to establish the molecular structure of one member, and to describe a facile coupling reaction that leads to alkenyltriosmium derivatives.

Treatment of $Os_3(CO)_{12}$ with 1 equiv of sublimed Me_3NO in the presence of acetonitrile produces the labile derivative $Os_3(CO)_{11}(NCMe)$[6] in nearly quantitative yield. Addition of ethereal diazomethane to this complex, optimally in hot cyclohexane, produces $Os_3(CO)_{11}CH_2$ in up to 50% isolated yield. Analogous compounds $Os_3(CO)_{11}CHR$ are prepared in a similar fashion (reaction 1) with isolated yields currently ranging from >50% (R

$$Os_3(CO)_{11}(NCMe) + N_2CHR \rightarrow Os_3(CO)_{11}CHR + N_2 + NCMe \quad (1)$$

= $SiMe_3$) to <10% (R = Me). These air-stable, red compounds display characteristic μ-alkylidene α-CH ^{1}H NMR signals and have closely similar IR (ν_{CO}) spectra.[7] Steinmetz and Geoffroy[8]

(1) For a recent review of mechanistic aspects, see: Muetterties, E. L.; Stein, J. *Chem. Rev.* **1979**, *79*, 479.
(2) Ponec, V.; Van Barnevald, W. A. *Ind. Eng. Chem. Prod. Res. Dev.* **1979**, *18*, 268.
(3) Biloen, P.; Helle, J. N.; Sachtler, W. M. H. *J. Catal.* **1979**, *58*, 95.
(4) Brady, R. C.; Pettit, R. *J. Am. Chem. Soc.* **1980**, *102*, 6181; **1981**, *103*, 1287.
(5) (a) Review: Herrmann, W. A. *Adv. Organomet Chem.*, in press. (b) See also: Theopold, K. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 2489. Dyke, A. F.; Knox, S. A. R.; Naish, P. J.; Taylor, G. E. *J. Chem. Soc., Chem. Commun.* **1980**, 803.
(6) (a) Shapley, J. R.; Pearson, G. A.; Tachikawa, M.; Schmidt, G. E.; Churchill, M. R.; Hollander, F. J. *J. Am. Chem. Soc.* **1977**, *99*, 8064. (b) Johnson, B. F. G.; Knox, J.; Pippard, D. A. *J. Chem. Soc., Dalton Trans.* **1972**, 407 and references therein.

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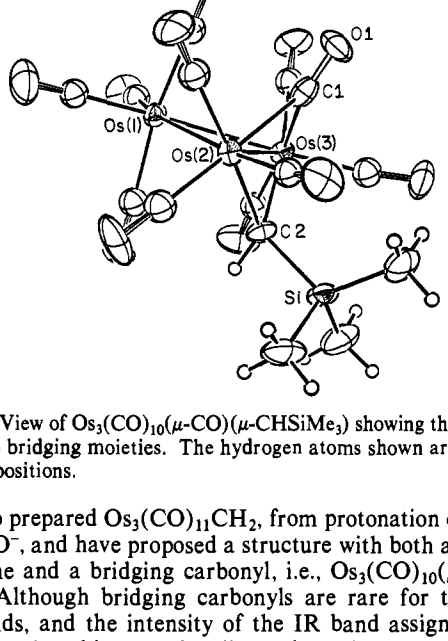
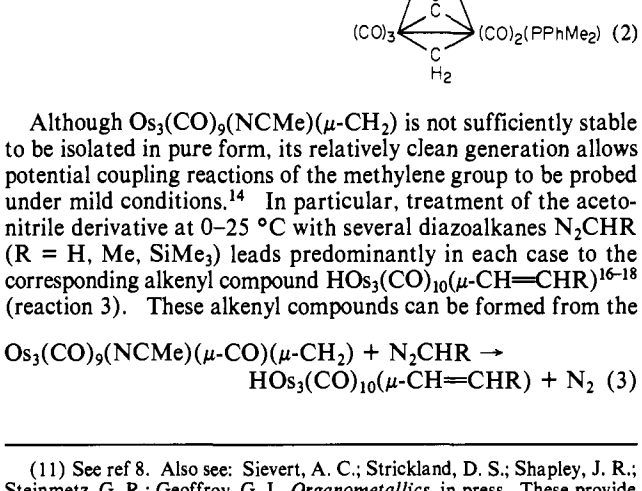


Figure 1. View of $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHSiMe_3)$ showing the similarity of the two bridging moieties. The hydrogen atoms shown are placed in assumed positions.

have also prepared $Os_3(CO)_{11}CH_2$, from protonation of $Os_3(CO)_{11}CHO^-$, and have proposed a structure with both a bridging methylene and a bridging carbonyl, i.e., $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CH_2)$. Although bridging carbonyls are rare for triosmium compounds, and the intensity of the IR band assigned to the bridging carbonyl is exceptionally weak, we have supported formulating this set of compounds as $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHR)$ by a single-crystal X-ray diffraction study of the (trimethylsilyl)methylidene derivative.[9]

The molecular structure of $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHSiMe_3)$ is shown in Figure 1. The triangular cluster has two equivalent osmium atoms, Os(2) and Os(3), each coordinated to three terminal carbonyl ligands, a bridging carbonyl, and a bridging $CHSiMe_3$ moiety. The unique osmium atom Os(1) is bonded to four terminal carbonyl ligands. The two nonbridged Os–Os bond lengths are equal [Os(1)–Os(2) = 2.859 (1) Å; Os(1)–Os(3) = 2.861 (1) Å], whereas the dibridged metal–metal vector is substantially shortened, with Os(2)–Os(3) = 2.765 (1) Å. The (trimethylsilyl)methylidene ligand bridges symmetrically with Os(2)–C(2) = 2.159 (15) and Os(3)–C(2) = 2.188 (16) Å. The bridging carbonyl is also nearly symmetrical, with Os(2)–C(1) = 2.186 (17) and Os(3)–C(1) = 2.101 (17) Å. The dihedral angle with respect to the Os_3 plane is nearly the same for each bridging system: $\angle Os_3/Os_2C(1) = 68.82°$, $\angle Os_3/Os_2C(2) = 66.94°$.

Since the compound $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CH_2)$ is prepared in the presence of excess diazomethane, substitution of a carbonyl by a second methylene is clearly not facile; prolonged heating causes an intramolecular transformation of the methylene group.[11] As with $Os_3(CO)_{12}$, however, a labile derivative is accessible via oxidative decarbonylation. When 1 equiv of sublimed Me_3NO in CD_3CN is added to $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CH_2)$ in CD_2Cl_2 at −78 °C, the ^1H NMR spectrum of the solution at 0 °C shows that the signals due to $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CH_2)$ are nearly gone (>90%), and two new doublets (δ 8.83 and 8.42, J = 6.0 Hz) have appeared,[12] which are attributed to $Os_3(CO)_9(NCMe)(\mu\text{-}CO)(\mu\text{-}CH_2)$. When 1 equiv of $PPhMe_2$ is added to this solution, the above signals disappear, and new signals due to $Os_3(CO)_9(PPhMe_2)(\mu\text{-}CO)(\mu\text{-}CH_2)$ appear. This derivative is a stable, red solid, which is fully characterized by spectroscopic data.[13] Since the NMR signal of one of the methylene protons in this compound is shifted considerably upfield and displays strong coupling to phosphorus, the substitution site appears to be on one of the osmium atoms bridged by the methylene group (reaction 2).



(2)

Although $Os_3(CO)_9(NCMe)(\mu\text{-}CH_2)$ is not sufficiently stable to be isolated in pure form, its relatively clean generation allows potential coupling reactions of the methylene group to be probed under mild conditions.[14] In particular, treatment of the acetonitrile derivative at 0–25 °C with several diazoalkanes N_2CHR (R = H, Me, $SiMe_3$) leads predominantly in each case to the corresponding alkenyl compound $HOs_3(CO)_{10}(\mu\text{-}CH{=}CHR)$[16-18] (reaction 3). These alkenyl compounds can be formed from the

$$Os_3(CO)_9(NCMe)(\mu\text{-}CO)(\mu\text{-}CH_2) + N_2CHR \rightarrow HOs_3(CO)_{10}(\mu\text{-}CH{=}CHR) + N_2 \quad (3)$$

(7) (a) $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CH_2)$ (the data we have obtained for this compound are in essential agreement with those reported in ref 8; we record our data here for comparison with the properties of other members of the set): IR ν(CO) (C_6H_{12}) 2113 (w), 2068 (m), 2060 (vs), 2035 (m), 2027 (s), 2014 (m), 2004 (m) cm^{-1}; (Nujol) 2113 (w), 2058 (vs), 2026 (s), 1993 (m), 1867 (w) cm^{-1}; ^1H NMR (C_2D_6CO, 35 °C) δ 7.83 (d, 1 H, J = 6.9 Hz), 6.68 (d, 1 H); mass spectrum (^{192}Os), m/z 898 (M^+). (b) $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHMe)$: IR ν(CO) (C_6H_{12}) 2110 (vw), 2099 (w), 2068 (w), 2057 (vs), 2033 (s), 2026 (s), 2014 (w), 2003 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 20 °C) δ 9.82 (q, 1 H, J = 7.8 Hz), 2.55 (d, 3 H); mass spectrum (^{192}Os), m/z 912 (M^+). (c) $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHSiMe_3)$: IR ν(CO) (C_6H_{12}) 2101 (w), 2056 (vs), 2028 (s), 2000 (w) cm^{-1}; (Nujol) 2111 (m), 2055 (s), 2040 (m), 2032 (m), 2027 (m), 2004 (m), 1992 (m), 1979 (m), 1862 (w) cm^{-1}; ^1H NMR (C_2D_6CO, 35 °C) δ 8.72 (s, 1 H), 0.06 (s, 9 H); mass spectrum (^{192}Os), m/z 970 (M^+).

(8) Steinmetz, G. R.; Geoffroy, G. L. *J. Am. Chem. Soc.* **1981**, *103*, 1278. Crystals of $Os_3(CO)_{11}CH_2$, provided by Professor Geoffroy, proved to be disordered. Churchill, M. R.; Wasserman, H. J. *Inorg. Chem.*, in press.

(9) $Os_3(CO)_{10}(\mu\text{-}CO)(\mu\text{-}CHSiMe_3)$ crystallizes in the centrosymmetric monoclinic space group $P2_1/n$ with a = 15.463 (9) Å, b = 9.352 (3) Å, c = 16.757 (7) Å, β = 107.38 (3)°, V = 2312.6 (17) Å^3, and ρ_{calcd} = 2.77 g cm^{-3} for Z = 4. Diffraction data [T = 23 (1) °C] were collected in the range 3.5 < 2θ < 40° by the coupled θ(counter)–2θ(crystal) scan technique[10a] by using Mo Kα radiation on a Syntex $P2_1$ diffractometer. The structure was solved by a combination of direct methods (MULTAN)[10b,c] and difference-Fourier techniques and was refined via full-matrix least squares to R_F = 4.2%, R_{wF} = 4.8% and GOF = 1.03 for all 2170 unique data (none rejected). Anisotropic thermal parameters were used for all nonhydrogen atoms except those of the ten terminal carbonyl carbon atoms for which isotropic thermal parameters were used.

(10) (a) Churchill, M. R.; Lashewycz, R. A.; Rotella, F. J. *Inorg. Chem.* **1977**, *16*, 265. (b) Syntex XTL interactive program package: "Syntex XTL Operations Manual", 2nd ed.; Syntex Analytical Instruments: Cupertino, CA, 1976. (c) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* **1971**, *A27*, 368.

(11) See ref 8. Also see: Sievert, A. C.; Strickland, D. S.; Shapley, J. R.; Steinmetz, G. R.; Geoffroy, G. L. *Organometallics*, in press. These provide evidence that heating $Os_3(CO)_{11}(CH_2)$ gives $H_2Os_3(CO)_9(C{=}C{=}O)$.

(12) Another new signal also appears, a singlet at δ 9.05, which has an intensity of ca. 15% of the two doublets. This minor component is under investigation.

(13) $Os_3(CO)_9(PMe_2Ph)(\mu\text{-}CO)(\mu\text{-}CH_2)$: IR ν(CO) (C_6H_{12} 2093 (m), 2067 (vw), 2053 (w), 2039 (s), 2022 (m), 2011 (vs), 1998 (w), 1986 (m), 1973 (m), 1969 (m) cm^{-1}; (Nujol) 2092 (w), 2031 (s), 2021 (vs), 1998 (s), 1958 (m), 1932 (w), 1839 (vw) cm^{-1}; ^1H NMR ($CDCl_3$, 0 °C) δ 7.39–7.53 (m, 5 H), 7.00 (d, 1 H_a, $J(H_a\text{–}H_b)$ = 7.0 Hz), 5.25 (dd, 1 H_b, $J(H_b\text{–}P)$ = 15.3 Hz), 2.06 (d, 3 H, J(P–H) = 9.5 Hz), 2.04 (d, 3 H, J(P–H) = 9.5 Hz); mass spectrum (^{192}Os), m/z 1008 (M^+).

(14) Hydrogenation of $Os_3(CO)_{10}(NCMe)(\mu\text{-}CH_2)$ affords the tautomeric mixture $H_2Os_3(CO)_{10}(\mu\text{-}CH_2)/HOs_3(CO)_{10}(\mu\text{-}CH_3)$[15] together with some $H_2Os_3(CO)_{10}$. Since the methyl/methylene mixture forms $H_2Os_3(CO)_{10}$ when hydrogenated,[15c] the tautomers are likely intermediates in the formation of methane from hydrogenation of $Os_3(CO)_{11}(\mu\text{-}CH_2)$ in boiling benzene.[8]

(15) (a) Calvert, R. B.; Shapley, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 5225. (b) Calvert, R. B.; Shapley, J. R. *Ibid.* **1978**, *100*, 7726. (c) Calvert, R. B. Ph.D. Thesis, University of Illinois at Urbana-Champaign, 1979.

(16) Deeming, A. J.; Hasso, S.; Underhill, M. *J. Chem. Soc., Dalton Trans.* **1975**, 1614.

(17) Keister, J. B.; Shapley, J. R. *J. Am. Chem. Soc.* **1976**, *98*, 1056.

(18) $HOs_3(CO)_{10}(\mu\text{-}CH{=}CHSiMe_3)$: IR ν(CO) (C_6H_{12}) 2105 (w), 2060 (vs), 2053 (m), 2022 (s), 2011 (m), 2007 (m), 1996 (m), 1988 (w), 1983 (w) cm^{-1}; ^1H NMR ($CDCl_3$, 35 °C) δ 7.75 (d, 1 H_a, $J(H_a\text{–}H_b$ = 16.5 Hz, $CH_a{=}CH_bSiMe_3$), 3.67 (d, 1 H_b), 0.10 (s, 9 H), −18.68 (s, 1 H); mass spectrum (^{192}Os), m/z 956 (M^+). The same compound is prepared in good yield from the interaction of $H_2Os_3(CO)_{10}$ and $H_2C{=}CHSiMe_3$.

corresponding alkene,[17,18] which suggests a possible intermediate. Since alternative pathways are possible, however, we have sought to probe the mechanism of the coupling reaction in the potentially most symmetrical case. Treatment of $Os_3(CO)_{10}(NCMe)(^{13}CH_2)$, prepared from >90% ^{13}C-enriched diazomethane, with an excess of unenriched diazomethane, results in an equimolar mixture of $HOs_3(CO)_{10}(^{13}CH{=}CH_2)$ and $HOs_3(CO)_{10}(CH{=}^{13}CH_2)$, as judged by ^{13}C NMR spectroscopy.[19] Equilibration of the carbon atoms in the vinyl complex itself is not facile,[20] so formation of the carbon–carbon bond and equilibration of the two carbon atoms must *precede* scission of the carbon–hydrogen bond.[21] The most straightforward inference is that two bridging methylenes are coupled into either a di-σ- or a π-C_2H_4 complex; subsequent hydrogen transfer in the unsaturated intermediate would generate the observed vinyl derivative.[22] The remaining reactions expressed in reaction 3 presumably proceed analogously, with formation of the β-substituted alkenyl product preferred for steric reasons.[23]

We conclude that the interaction of diazomethane (diazoalkanes) with reactive triosmium compounds can lead not only to transfer of a methylene (alkylidene) group but also, as found for metal surfaces,[4] to the facile formation of a dicarbon moiety. Ethylene is not readily observed in the triosmium case due to formation of the stable vinyl complex; however, addition of a strong ligand (such as carbon monoxide[16]) can reverse the conversion and force release of the alkene. Further coupling reactions of $Os_3(CO)_{10}(NCMe)(\mu\text{-}CH_2)$ and related compounds are under active investigation.

Acknowledgment. This work was supported by NSF Grants CHE 80-23448 (to M.R.C.), DMR 80-20250, and CHE 81-00140 (to J.R.S.) and in part by the University of Illinois NSF Regional Instrumentation Facility (Grant CHE 79-16100). We thank Professor Geoffroy for valuable discussions regarding the work described in ref 8.

(19) With complete proton decoupling two singlets, equally intense, are seen at δ 69.0 and 101.0. The absence of ^{13}C satellites indicates that essentially no $HOs_3(CO)_{10}(^{13}CH{=}^{13}CH_2)$ is formed; qualitatively, the signal intensity indicates that little label is lost. Off-resonance decoupling shows that the downfield signal corresponds to $CH(C_\alpha)$ and the upfield signal to $CH_2(C_\beta)$.

(20) Potentially, equilibration of the C_α and C_β sites of the vinyl ligand could occur by reductive elimination of the Os–C_α and Os–H bonds to give a π-ethylene complex which could then rotate about the Os–C_2H_4 bond and reform the vinyl complex with C_α and C_β interchanged. In order to test this possibility, we prepared $HOs_3(CO)_{10}(CD{=}CDH)$ by treating $H_2Os_3(CO)_{10}$ with C_2D_2. The ^1H NMR spectrum of the product verified that H was essentially absent at C_α and that the deuterium atoms were cis as expected. The labeled vinyl complex was then treated with ethereal CH_2N_2 under the same conditions as the alkylidene coupling reaction (0 °C in CH_2Cl_2 followed by warming to ambient temperature). The ^1H NMR spectrum of the recovered vinyl complex indicated that exchange between the bridging hydride and deuterium at C_α had not occurred, whereas complete H/D exchange at C_β had taken place. (The latter process occurs in the presence of a variety of bases; cf. $HOs_3(CO)_{10}(CH{=}CH_2)$ + $PPhMe_2$: Churchill, M. R.; DeBoer, B. G.; Shapley, J. R.; Keister, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 2356.) We conclude that C_α and C_β are not equilibrated under these conditions.

(21) The requirement of equivalence eliminates two alternative mechanisms which would preserve a distinction, e.g.,

$$\text{(i)}\ Os_3(\mu\text{-}C^*H_2) \rightarrow HOs_3(\mu\text{-}C^*H) \xrightarrow{+CH_2} HOs_3(\mu\text{-}C^*HCH_2)$$

$$\text{(ii)}\ Os_3(\mu\text{-}C^*H_2) \xrightarrow{+CH_2} Os_3(\mu\text{-}C^*HCH_3) \rightarrow HOs_3(\mu\text{-}C^*HCH_2)$$

(22) The possibility of terminal alkylidenes cannot be eliminated in the triosmium coupling reaction, but considering the pronounced preference for bridging over terminal configurations in the observed structures, the intermediacy of even one terminal form seems unlikely for such a facile reaction. A different possibility is direct formation of a C_2 intermediate by intramolecular attack of diazomethane at the methylene center. However, since $Os_3(CO)_{11}(CH_2)$, under the conditions of its synthesis, does not react with diazomethane to form $HOs_3(CO)_{10}(CH{=}CH_2)$, the coordination site provided by the labile NCMe ligand is necessary for the coupling reaction to proceed. On the other hand, the presently available data do not eliminate the possibility, suggested by a referee, that coupling occurs intramolecularly after formation of a diazomethane complex $Os_3(CO)_{10}(CH_2)(CH_2N_2)$.

(23) Substitution at C_α leads to significant destabilization. See: Clauss, A. D.; Tachikawa, M.; Shapley, J. R.; Pierpont, C. G. *Inorg. Chem.* **1981**, *20*, 1528.

Strutures of Two Novel Toxins from *Protogonyaulax*

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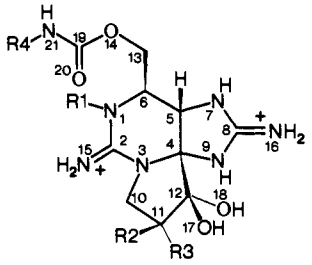
Saxitoxin (**1**) and several other potent neurotoxins (**2**, **4**, **6**–**8**) have been isolated from shellfish and dinoflagellates of the genus *Protogonyaulax*.[2–5] We have recently described a novel class of



	R1	R2	R3	R4
1	H	H	H	H
2	H	H	OSO_3^-	H
3	H	H	OSO_3^-	SO_3^-
4	H	OSO_3^-	H	H
5	H	OSO_3^-	H	SO_3^-
6	OH	H	H	H
7	OH	H	OSO_3^-	H
8	OH	OSO_3^-	H	H

related substances,[6] significant in that they have relatively low in vivo toxicity until they are hydrolyzed to the previously known toxins, and have now established the structures of two of these as carbamoyl-*N*-sulfo-11α-hydroxysaxitoxin sulfate (**3**) and the 11β-epimer **5**.[7] The structure determinations of these new substances are of particular interest since (a) compounds **3** and **5** are the most widespread and abundant toxins produced by dinoflagellates found along the Alaskan coast from Southeast Alaska to the Aleutians, (b) this is the first report of the *N*-sulfocarbamoyl group in a natural product, (c) the attenuation of toxicity associated with sulfonation of the carbamoyl group introduces a new aspect of structure–activity relationships in this group of neurotoxins, and (d) establishment of structure **5** by X-ray crystallography coupled with the chemical interconversions described herein confirms the structural assignments for **2** and **4** previously made principally from spectroscopic data.[3,5]

Compounds **3** and **5** were obtained as white solids by chromatography[8] of extracts from several batch cultures of *Proto-*

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(8) (a) BioGel P2/0.1 M acetic acid; toxins elute as described in ref 6. (b) IRP64 (H^+ form/water; toxins **3** and **5** elute unretained; the other toxins are bound and subsequently eluted with 0.1 M acetic acid. (c) BioGel P2/water; **3** and **5** elute at ca. 135 and 145% bed volume, respectively; the other toxins are bound and are eluted with 0.1 M acetic acid. (d) The data of ref 6 show **3** and **5** to be the most abundant toxins in the mixture produced by this clone, accounting for over a third of the total toxins on a molar basis.

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