addition of Me_2SO to the tetrahydrofuran (THF) solution of Fl^-Na^+. In the mixture of Me_2SO and THF, the ^{13}C splitting has a maximum at 0.005–0.006 mole fraction of Me_2SO and approaches a limiting value with a mole fraction of greater than 0.2 (Figure 2). The existence of the maximum indicates that there are two processes; one is the process which increases the ^{13}C splitting and the other decreases the ^{13}C splitting; first the former takes place. Reaction 2 depicts the two processes

$$\underset{\text{(in THF)}}{RCO^-Na^+(T)_n} \rightleftarrows \underset{D}{RCO^-Na^+(T)_n} \rightleftarrows \underset{D}{RCO^-Na^+(T)_{n-1}(D)} \quad (2)$$

where T is a THF and D a Me_2SO molecule. In the first step the ^{13}C splitting is increased by the solvation of the anion while the second step increases the anion–cation separation to reduce ^{13}C splitting.[5] The solvation of the anion easily takes place since the anion is nearly naked in THF whereas the approach of a Me_2SO molecule to the sodium ion is hindered by the already solvating THF molecules.

In a comparison of the mixtures of toluene and dipolar aprotic solvents, the mixture of toluene and THF exhibits several characteristic features: (1) The decomposition of the clustered ion pairs into monomeric ones occurs only when a considerable amount of THF is added (in less than 0.4 mole fraction of THF, the ESR signal is weak and featureless). (2) In any mole fraction of THF, the ^{13}C splitting does not approach a limiting value but monotonously decreases until the fraction of THF becomes unity (similar features were observed with the mixture of toluene and pyridine). The ^{13}C splitting of the pure THF solution of Fl^-Na^+ is known to be appreciably temperature dependent.[6] These facts suggest that even in pure THF, the solvent shell around the Na^+ forming the ion pair does not get completed, but two different solvation states exist in equilibrium.

Every ESR spectrum of the Fl^-Na^+ ion pair studied exhibits sodium hyperfine lines. This indicated that in any solvation stage the Fl^-Na^+ ion pair cannot exist in the form of a solvent separated ion pair, but the contact Fl^-Na^+ ion pair directly dissociates into the free ions.

(5) K. S. Chen, S. W. Mao, K. Nakamura, and N. Hirota, *J. Am. Chem. Soc.*, **93**, 6004 (1971). In this paper, mixtures of ethereal solvents and DMF were used, and only the second step was assumed.
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24(*S*),25-Epoxycholesterol Is a Natural Product of Mammalian Steroid Biosynthesis

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Received August 21, 1981

We have recently shown[1] that squalene 2,3(*S*);22(*S*),23-dioxide (**1**) is converted, upon incubation with S_{10} rat liver homogenate (RLH),[2] to the previously uncharacterized 24(*S*),25-epoxycholesterol (**2**),[3] with an efficiency comparable to that of the conversion of squalene 2,3(*S*)-oxide to cholesterol.[4] This result was obtained by use of biosynthesized **1**, which accumulates along with squalene oxide when mevalonate is incubated with RLH in the presence of 4,4,10β-trimethyl-*trans*-decal-3β-ol (TMD),[1] an effective inhibitor of oxidosqualene cyclase (EC 5.4.99.7).[5,6] We now report that 24(*S*),25-epoxycholesterol (**2**) is biosynthesized in RLH in the absence of inhibitor.

The search for **2** as a natural product of mammalian steroid biosynthesis was triggered by observation of a small amount of material with the TLC mobility[7] of squalene dioxide **1** in the nonsaponifiable extract[2] from RLH incubations to which no cyclase inhibitor had been added. Because our previous work[1] convinced us that if **1** were indeed present, the more stable, crystalline **2** would inevitably also be formed, we undertook to determine if the steroids produced in RLH contained **2**.

Incubations of [^{14}C]acetate[8] with RLH in the standard manner[2] afforded a nonsaponifiable extract[2] which had incorporated 2.0% of the initial radioactivity. TLC analysis[7] showed that this ^{14}C was divided among three major bands with the R_f values of squalene (18%), lanosterol (15%), and cholesterol (66%). Because we knew that 24(*S*),25-epoxycholesterol (**2**) migrated essentially the same as cholesterol upon TLC,[1] attention was focused on the cholesterol fraction (CF),[9] which contained 65% of the originally isolated ^{14}C after separation by preparative TLC.[7]

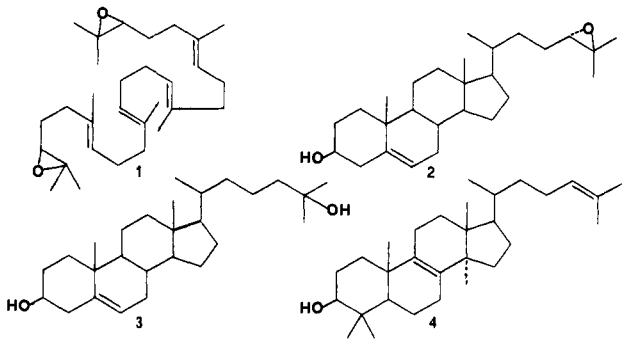
The first indication that the CF might contain **2** was obtained by treatment of a portion of it with $HClO_4$ in H_2O/THF.[10] Analysis by TLC of the product from this procedure showed 74% of the ^{14}C with unchanged R_f and 10% of the ^{14}C as much more polar material. When authentic **2**[3] was treated with $HClO_4$ in the same manner, essentially all the product had the same R_f as the more polar product. These observations suggested that there was [^{14}C]**2** in the CF, which, like the authentic unlabeled **2**, had undergone conversion, probably to a triol,[11] upon treatment with aqueous acid.

Confirmation that **2** was indeed present was obtained by benzoylation of a portion of the CF,[12] dilution of the product with authentic 24(*S*),25-epoxycholesterol benzoate,[1,10] purification by preparative TLC, and recrystallization to constant specific activity.[13] The final radioactivity obtained in the purified benzoate of **2** was 1.2% of that in the CF. However, the earlier results from the $HClO_4$ treatment had suggested that there was a substantially greater amount of **2** present. Since it was plausible that we were not isolating all of the **2** as its benzoate derivative,[14] we decided also to employ $LiAlH_4$ reduction of the CF in order to convert **2** to 25-hydroxycholesterol (**3**), which is stable and easily separable from cholesterol.[1]

(1) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Biol. Chem.* **1981**, *256*, 1067–1068.
(2) Popjak, G. *Methods Enzymol.* **1969**, *15*, 438–440.
(3) We have obtained **2**, mp 160–162 °C, and its 24(*R*) epimer, mp 166.5–168 °C, from their known[10] benzoate derivatives by saponification and have fully characterized both compounds.
(4) Corey, E. J.; Russey, W. E.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1966**, *88*, 4750–4751. van Tamelen, E. E.; Willett, J. D.; Clayton, R. B.; Lord, K. E. *Ibid.* **1966**, *88*, 4752–4754.
(5) Nelson, J. A.; Czarny, M. R.; Spencer, T. A.; Limanek, J. S.; McCrae, K. R.; Chang, T. Y. *J. Am. Chem. Soc.* **1978**, *100*, 4900–4902.
(6) Chang, T. Y.; Schiavoni, E. S., Jr.; McCrae, K. R.; Nelson, J. A.; Spencer, T. A. *J. Biol. Chem.* **1979**, *254*, 11258–11263.
(7) TLC analyses were performed on LK5D silica gel plates (Whatman, Inc., Clifton, NJ); preparative TLC plates were prepared with Silica Gel 60 PF-254+366 (EM Laboratories, Inc., Elmsford, NY). Various ratios of ether–hexane were employed as eluent.
(8) Sodium [1-^{14}C]acetate, specific activity = 56.7 mCi/mmol, was purchased from Amersham Corp, Arlington Heights, IL.
(9) A study of the composition of the CF obtained from an incubation of [^3H]lanosterol (4) with RLH by GLC (Varian Aerograph Model 2100, using 3% OV-17 on Gas-Chrom Q, Applied Sciences Laboratories, Inc., State College, PA, on a 6-ft. × 1/8-in. glass column at 240 °C) showed 80% of the ^3H as cholesterol and 15% of the ^3H as a yet unidentified component. 24(*S*),25-epoxycholesterol (**2**) decomposes under these GLC conditions. We are continuing to try to develop a GLC or HPLC method for the direct detection of **2** in the CF.
(10) According to the conditions of Seki et al. (Seki, M.; Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1975**, 15–18) for the conversion of 24(*R*)- and 24(*S*),25-epoxycholesterol benzoates to 24,25-dihydroxycholesterol benzoates.
(11) Both 24,25-dihydroxycholesterols have been prepared by: Partridge, J. J.; Toome, V.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1976**, *98*, 3739–3741. We did not attempt to characterize the polar product from the CF or authentic **2**.
(12) Typical benzoylation procedure: To 100 μg of alcohol in 100 μL of PhH was added 3 μL of pyridine and 2 μL of benzoyl chloride; the mixture was stirred at room temperature for 4 h and evaporated to dryness under vacuum.
(13) Initial specific activity = 480 dpm/mg. Recrystallization from acetone yielded **2**-benzoate with, successively, 250, 200, 160, 130, 130, and 120 dpm/mg.
(14) Possible reasons why the yield of pure **2**-benzoate may have been low include: incomplete benzoylation, decomposition of **2**-benzoate during purification, and difficulty in separation from cholesterol benzoate.

0002-7863/81/1503-6974$01.25/0

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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Received June 29, 1981

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]

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(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.
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0002-7863/81/1503-6975$01.25/0