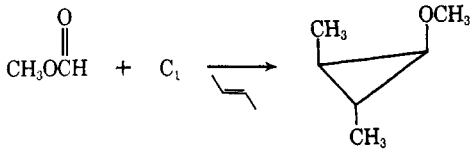
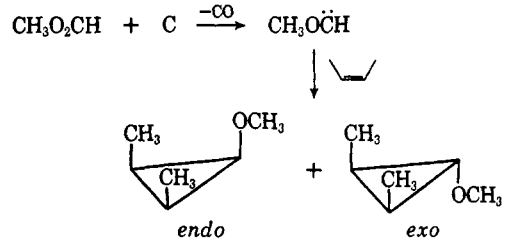
trans-2-butene was used as the reactive matrix, only the 1,1-dichloro-*trans*-2,3-dimethylcyclopropane was formed (20% yield[3]) free of the *cis* isomer. These results indicate that dichlorocarbene formed in the deoxygenation of phosgene is a singlet species. This observation is also consistent with spin conservation considerations presented previously[1] concerning the deoxygenation process.

When a mixture of 78% methyl formate and 22% *trans*-2-butene was used as a matrix for carbon vapor, deoxygenation took place with production of methoxycarbene, which gave only the *trans*-2,3-dimethylmethoxycyclopropane in 28% yield[3] (no more than



1% of the inverted isomer could have been formed). This result again implicates a singlet carbene intermediate.

The use of a reactive matrix containing 56% methyl formate and 44% *cis*-2-butene under deoxygenative conditions gave only the *exo*- and *endo*-*cis*-2,3-dimethylmethoxycyclopropanes[5] in 15% yield[3] with an *endo*:*exo* ratio of 6.2. This is in reasonable agreement with the



endo:*exo* value of 7.0 obtained from the addition of methoxycarbene from lithium chloromethyl methyl ether to *cis*-2-butene.[6] The correspondence of the *endo*:*exo* ratios for these two methoxycarbenes under greatly different conditions suggests that the same intermediate is involved in both reactions.

Recent work comparing the relative reactivity of dichlorocarbene produced from gas-phase pyrolysis of chloroform with dichlorocarbene from lithium trichloromethane[7] has shown that carbenes produced from α-halolithiums are free. The correspondence of the above *endo*:*exo* ratios despite different media and temperatures of generation indicates that the methoxycarbene intermediate is also present in both these reactions.

Acknowledgment. We acknowledge the financial support of the Air Force Office of Scientific Research.

(5) The authors thank Dr. W. H. Atwell of Dow Corning, Midland, Mich., for providing authentic samples of the 2,3-dimethylmethoxycyclopropanes in question to facilitate the product identification.
(6) U. Schollkopf and J. Paust, *Chem. Ber.*, **98**, 2221 (1965).
(7) P. S. Skell and M. S. Cholod, *J. Amer. Chem. Soc.*, **91**, 6035 (1969).
(8) National Institutes of Health Predoctoral Fellow, 1967–1970.

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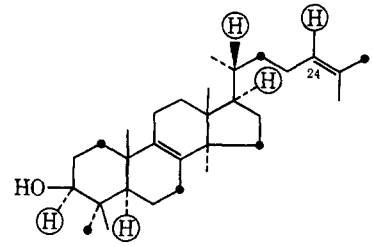
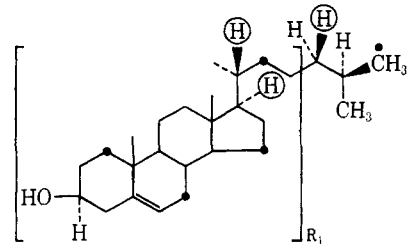
trans Reduction of Δ^{24} of Lanosterol in the Biosynthesis of Cholesterol by Rat Liver Enzymes

Sir:

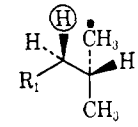
An obligatory step in the sequence of the biosynthetic transformations of lanosterol (**1a**) to cholesterol[1-3] is the reduction of the C-24 double bond. We have proved, with the use of cholesterol biosynthesized from 4*R*-(2-^{14}C,4-^{3}H)-MVA in a rat liver enzyme preparation, that the hydrogenation of lanosterol (**1a**) is stereospecific at C-24 and proceeds by the addition of a 24-*pro*-*S* hydrogen.[4] The available evidence suggests that the addition of a hydrogen at C-25 is also stereospecific.[5,6] In addition, it has been shown that protonation takes place at C-24 and a "hydride ion" from TPNH adds at[7] C-25.



1a, Ⓗ = 4-*pro*-*R*,H of MVA; • = C-2 of MVA
b, Ⓗ = ^{3}H; • = ^{14}C



2



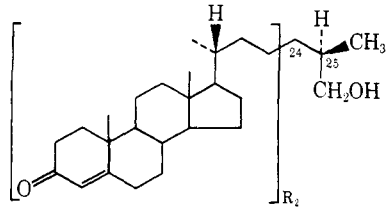
3

A *cis* reduction of Δ^{24} would give cholesterol with the geometry indicated in **2**, while in a *trans* reduction the geometry would be as in **3**. The two methyls at the 25-*pro*-chiral carbon atom differ in that one originates from C-2 and the other from C-3′ of MVA. Hence, knowledge of the configuration at C-25, taken together with the already proven addition of a 24-*pro*-*S*-hydrogen, allows definition of the *overall* mechanism of reduction of the C-24 double bond of **1**. For the determination of the C-25 *pro*-chirality, it was necessary to differentiate between the 26- and 27-methyl groups. Consequently, cholesterol was incubated with *Mycobacterium smegmatis*,[8] and the nonsaponifiable residues from several experiments were pooled and purified by chromatography. The obtained **4a** was crystallized from ethyl acetate (mp 129–131°) (110 mg) and showed $[\alpha]^{23}D$ +87.1°

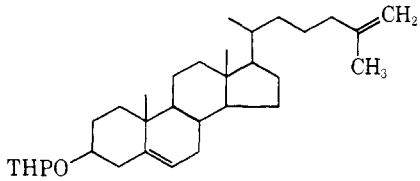
(1) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, K. Shimizu, S. Ichii, E. Forchielli, and E. Caspi, *J. Amer. Chem. Soc.*, **87**, 3224 (1965).
(2) J. W. Cornforth, *Angew. Chem., Intern. Ed. Engl.*, **7**, 903 (1968).
(3) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes, and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964, p 200, 210, and references therein.
(4) E. Caspi, K. R. Varma, and J. B. Greig, *Chem. Commun.*, 45 (1969).
(5) O. Berseus, *Acta Chem. Scand.*, **19**, 325 (1965).
(6) K. A. Mitropoulos and N. B. Myant, *Biochem. J.*, **97**, 26c (1965).
(7) M. Akhtar, K. A. Munday, A. D. Rahimtula, I. A. Watkinson, and D. C. Wilton, *Chem. Commun.*, 1287 (1969).
(8) K. Schubert, G. Kaufmann, and C. Hörhold, *Biochem. Biophys. Acta*, **176**, 163, 170 (1969).

(*c* 2.56, $CHCl_3$) and +86.1° (*c* 1.9, $CHCl_3$). The product was homogeneous when tested on tlc and glc.[9]

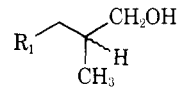
Samples for configurational assignments were synthesized by several routes. Hydroboration of cholesta-5,25-diene-3β-ol-3-tetrahydropyranyl ether (THP) (**5**) with disiamylborane[10] gave **6**, which was hydrolyzed to 25*RS*-26-hydroxycholesterol (**7**). Alternatively, oxi-



4a, microbiological
b, asymmetric hydroboration
c, from kryptogenin



5

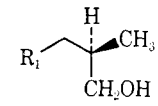


7, 25-*RS*

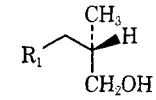
dation of **6** followed by hydrolysis provided 25*RS*-3β-hydroxycholest-5-en-26-oic acid (**8a**), which was partially resolved, *via* crystallization of the (−)-quinine salt, to give the 25*R*-acid **8b** from the crystallized salt and the 25*S*-acid **8c** from the mother liquor. The acids **8b** and **8c** were reduced (LAH) to the 25*R*-26-hydroxycholesterol (**9a**) and the 25*S*-epimer **10a**, respectively, The 3-THP ether **6** was acetylated and hydrolyzed to yield 25*RS*-26-acetoxycholest-5-en-3β-ol (**11**). Oppenauer oxidation of **11** followed by saponification gave 25*RS*-26-hydroxycholest-4-ene-3-one (**12**).

Asymmetric hydroboration of the 5,25-dien-3-THP ether **5** with (−)-diisopinocampheylborane and (+)-diisopinocampheylborane[11] as previously described[12] gave, after hydrolysis, authentic 25*S*-26-hydroxycholesterol (**10b**) and 25*R*-26-hydroxycholesterol (**9b**), respectively. The diols **9b** and **10b** were converted to 26-monotrityl ethers[13] and oxidized (Oppenauer) to yield, after acid hydrolysis, authentic 25*R*-26-hydroxycholestenone (**4b**) and the 25*S*-epimer **13**. Another specimen of 26-hydroxycholesterol (**9c**) was obtained from kryptogenin diacetate[14] and similarly converted to the Δ⁴-3-keto-26-ol (**4c**).

Comparison of the rotation of the microbially prepared 26-hydroxycholest-4-en-3-one (**4a**) with those of the authentic samples unequivocally proves the 25*R* configuration of **4a** (Table I). The microbially prepared 26-hydroxycholest-4-en-3-one, $[\alpha]_D$ +95°



9a, from resolution of 25-*RS* acid
b, asymmetric synthesis
c, from kryptogenin



10a, from resolution of 25-*RS* acid
b, asymmetric synthesis

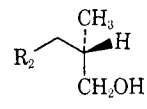
(CHCl₃),[15] obtained by Kogan, *et al.*,[16] is also shown by our results to have the 25*R* configuration. The inferred 25*R* configuration of kryptogenin[17] is confirmed.

Table I. Specific Rotation $[\alpha]_D$ of 26-Hydroxycholesterols and Their Derivatives[a]

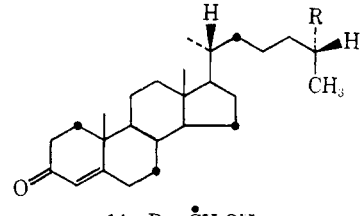
Configuration at C-25	26-Hydroxycholesterol ($CHCl_3$)	26-Hydroxycholestenone ($CHCl_3$)	26-Cholestenoic acid (MeOH)
	(**9b**)[b] −35.0	(**4b**)[b] +87.4	
		+86.0	
R	(**9a**)[d] −33.7		
		(**4c**)[e] +84.45	(**8b**)[d] −25.3
	(**9c**)[e] −33.5	+85.6	−27.2
		(**4a**)[f] +87.1	
		+86.1	
RS	(**7**)[g] −36.0	(**12**)[g] +80.35	(**8a**)[g] −23.9
	−35.9		−23.0
S	(**10b**)[c] −38.0	(**13**)[c] +74.8	(**8c**)[d] −19.1
	(**10a**)[d] −37.8		−21.9

[a] The numbers in parentheses refer to compounds (see text). The superscripts indicate the method of preparation. Rotations (in degrees) were measured at concentrations of *ca.* 2–3% at 23 ± 2°. [b] *Via* asymmetric hydroboration of **5** with (+)-diisopinocampheylborane. [c] *Via* asymmetric hydroboration of **5** with (−)-diisopinocampheylborane. [d] *Via* resolution of 25*RS*-26 acid. [e] From kryptogenin. [f] Microbiological. [g] *Via* hydroboration of **5** with disiamylborane.

It was now necessary to determine the origin, with respect to MVA, of the C-26 microbially oxygenated methyl group. A sample of $^{14}C_5$-cholesterol[1] biosynthesized from 2-^{14}C-MVA[1,4] in a rat liver enzyme prep-



13



14a, R = $\overset{\bullet}{C}H_2OH$
b, R = $\overset{\bullet}{C}HO$
c, R = H

aration was mixed with 25*RS*-25-^{3}H-cholesterol[10] (^{3}H:^{14}C ratio, 10.8) and incubated with *M. smegmatis*.[8] Unreacted $^{14}C_5$-25-^{3}H-cholesterol (^{3}H:^{14}C ratio, 10.3) and $^{14}C_5$-25-^{3}H-26-hydroxycholest-4-en-3-one (**14a**) (specific activity 5.24 × 10⁵ dpm/mmol of ^{14}C; ^{3}H:^{14}C

(9) A Perkin-Elmer instrument, Model 811, was used with an XE-60 glass column at 260° with helium gas elution.
(10) K. R. Varma, J. A. F. Wickramasinghe, and E. Caspi, *J. Biol. Chem.*, **244**, 3951 (1969).
(11) H. C. Brown, N. R. Ayyangar, and G. Zweifel, *J. Amer. Chem. Soc.*, **86**, 397, 1071 (1964); H. C. Brown and G. Zweifel, *ibid.*, **83**, 2544 (1961).
(12) K. R. Varma and E. Caspi, *Tetrahedron*, **24**, 6365 (1968); *J. Org. Chem.*, **34**, 2489 (1969); *ibid.*, **33**, 2181 (1968).
(13) That no epimerization or kinetic resolution occurred was demonstrated by hydrolysis to the alcohols which showed an unchanged rotation.
(14) I. Sheer, M. J. Thompson, and E. Mossettig, *J. Amer. Chem. Soc.*, **78**, 4733 (1956).
(15) No concentration or temperature reported.
(16) I. I. Zaretskaya, L. M. Kogan, O. B. Tikhomirova, J. D. Sis, N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin, G. R. Skryabin, and I. V. Torgov, *Tetrahedron*, **24**, 1595 (1968).
(17) For pertinent references see L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 838.

ratio, 10.0) were recovered. The keto alcohol **14a** was oxidized[18] to the 26-aldehyde **14b** and decarbonylated[19] to $^{14}C_4$-25-^{3}H-26-norcholestenone (**14c**) (specific activity 4.37 × 10^5 dpm/mmol of ^{14}C; ^{3}H:^{14}C ratio, 11.9).

The decreased (16.6%) specific activity and parallel increase (16.0%) in the ^{3}H:^{14}C ratio correspond to the loss of nearly one ^{14}C atom. It follows that *the methyl originating from C-2 of MVA* was hydroxylated. Since **4a** has the 25*R* configuration, the $^{14}C_5$-26-hydroxycholest-4-en-3-one must have the configuration **14a**. Consequently, cholesterol has the configuration as in **3**. The geometry at the C-24 double bond of lanosterol[1-3] is that shown in **1**. Therefore, the reduction of this double bond in rat livers is equivalent to a *trans* addition of two hydrogens, and the methyl originating from C-2 of MVA has the 25-*pro*-*S* configuration.

It is noteworthy that hydroxylation of the 25-*pro*-*S*-methyl of cholesterol by *M. smegmatis* contrasts with that in rat livers where the oxygenation of the 25-*pro*-*R*-methyl (originating from 3′ of MVA) is indicated.[5,6] Also, evidence suggests that the reduction of the Δ^{24} intermediate in the biosynthesis of tigogenin in *D. lanata*[20] differs from that in rat livers. In tigogenin, which has the 25*R* configuration, the methyl originating from 3′ of MVA bears the oxygen function. Consequently, the addition of the C-25 proton in *D. lanata* occurs on the opposite side to that in rat liver enzyme systems.

Acknowledgment. This work was supported by Grants AM12156, HE10566, and CA-K3-16614 from the National Institutes of Health, Grant No. P-500H from the American Cancer Society, and Grant No. GB-8277 from the National Science Foundation. We are indebted to Professor Kurt Schubert of the Institute for Microbiology and Experimental Therapy of the Academy of Science of Berlin, Jena, D.D.R., for the specimen of *M. smegmatis*.

(18) M. Fetizon and M. Golfier, *C. R. Acad. Sci., Paris, C*, **276**, 900 (1968).
(19) Y. Shimizu, H. Mitsuhashi, and E. Caspi, *Tetrahedron Lett.*, 4113 (1966).
(20) R. Joly and Ch. Tamm, *ibid.*, 3535 (1967).

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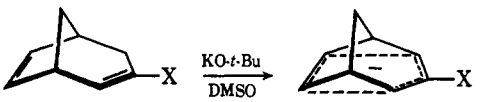
Base-Catalyzed Rearrangement of 3-Bromobicyclo[3.2.1]octa-2,6-diene to *endo*-6-Ethynylbicyclo[3.1.0]hex-2-ene. Possible Intermediacy of a Homoconjugated Carbene

Sir:

Bicycloheptadienes such as **1a** undergo rapid proton exchange in strongly basic media *via* the "bishomoaromatic"[1] anion **2a**. We now wish to report that **1b**,[2] the 3-bromo analog of **1a**, under conditions which

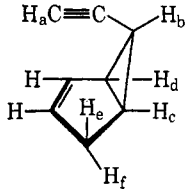


1a, X = H
b, X = Br
c, X = Cl

2a, X = H
b, X = Br
c, X = Cl

should form the bromoanion **2b** (potassium *t*-butoxide in DMSO at room temperature), is immediately transformed into a new product (29% isolated yield; >99% pure) in an unusual and deep-seated rearrangement.

The reaction product exhibits acetylenic carbon–carbon and carbon–hydrogen absorptions in the infrared (2130 and 3320 cm^{-1}). After purification by vpc, its mass spectrum shows a parent peak at *m/e* 104, corresponding to overall loss of HBr, with abundant peaks at *m/e* 103, 91, 78, and 63. That the structure of this new material is *endo*-6-ethynylbicyclo[3.1.0]hex-2-ene (**3**) is strongly suggested by its proton nmr spectrum.[3] At 220 MHz, signals for eight nonequivalent hydrogens are observed. The acetylenic hydrogen (H_a) is a sharp doublet (J = 2 Hz) at 347 Hz downfield from tetramethylsilane (TMS). Cyclopropyl proton H_b appears at 335 Hz as a doubled triplet, coupled to H_c and H_d ($J \cong 6$ Hz) as well as H_a. The resonance at 390 Hz for H_c is a broadened quartet due to coupling of similar magnitude ($J \cong 6$ Hz) with H_b, H_d, and H_f and that at 480 Hz (H_d) a slightly doubled (J = 2 Hz) triplet. H_e (510 Hz) and H_f (560 Hz) are coupled to one another (J = 18 Hz); the latter doubled again by coupling (J = 6.5 Hz) to H_c. The two vinyl hydrogens appear as complex and overlapping signals at 1222 and 1228 Hz downfield from TMS.



3

Assignment of structure **3** to the rearrangement product is confirmed by an independent synthesis of the material. Irradiation (λ >3000 nm) of diazopropyne[4] in the presence of cyclopentadiene gives two major products (ratio ~1:1) which are separable by vapor phase chromatography on a 10 ft × 3/8 in. column packed with 10% UCC-W98 on 60–80 Chromosorb P operated at 120°. On the basis of spectral and analytical data and by analogy with other propargylene additions[4] these materials are assigned the 6-ethynylbicyclo[3.1.0]hex-2-ene structure. One product has nmr and ir spectra identical with **3**. The other has *exo* stereochemistry (**4**), an assignment made on the basis of the lower coupling constant between H_b and H_c or H_d (J = 2.5 Hz).[5]

(1) (a) J. M. Brown and J. L. Occolowitz, *Chem. Commun.*, 376 (1965); (b) J. M. Brown, *ibid.*, 639 (1967); (c) J. M. Brown and J. L. Occolowitz, *J. Chem. Soc., B*, 411 (1968); (d) S. Winstein, M. Ogliaruso, M. Sakai, and J. M. Nicholson, *J. Amer. Chem. Soc.*, **89**, 3656 (1967). For a review of the concept of homoaromaticity, see (e) S. Winstein, *Quart. Rev. Chem. Soc.*, **23**, 141 (1969).
(2) W. R. Moore, W. R. Moser, and J. E. LaPrade, *J. Org. Chem.*, **28**, 2209 (1963).
(3) Nmr spectra were determined on a Varian Model HR-220 spectrometer.
(4) (a) P. S. Skell and J. Klebe, *J. Amer. Chem. Soc.*, **82**, 247 (1960); (b) J. V. Gramas, Ph.D. Dissertation, Pennsylvania State University, 1965.
(5) (a) P. S. Wharton and T. I. Bair, *J. Org. Chem.*, **30**, 1681 (1965); (b) D. J. Patel, M. E. H. Howden, and J. D. Roberts, *J. Amer. Chem. Soc.*, **85**, 3218 (1963); (c) J. D. Graham and M. T. Rogers, *ibid.*, **84**, 2249 (1962); (d) H. M. Hutton and T. Schaefer, *Can. J. Chem.*, **40**, 875 (1962); (e) A. A. Bothner-By, *Advan. Nucl. Magn. Reson.*, **1**, 195 (1965); (f) M. Rey and A. S. Dreiding, *Helv. Chim. Acta*, **48**, 1985 (1965); (g) R. S. Cooke, private communication.