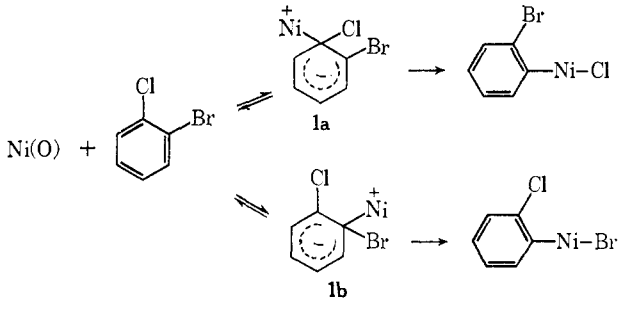
crude product from this reaction was protonated, and the relative yields of chlorobenzene and bromobenzene were determined by glpc. Since product formation in the oxidative addition is irreversible, the relative yields of products are directly proportional to their rates of formation, and therefore k_{Cl}/k_{Br} can be calculated to be 0.0030,[15] a value much lower than the k_{Cl}/k_{Br} = *ca.* 1–0.1 for most organic SNAr reactions.[16] The low



value of k_{Cl}/k_{Br} indicates that the intermediate **1a**, and likely both **1a** and **1b**, revert to reactants much faster than they proceed to products. Interestingly, the low value of k_{Cl}/k_{Br} is within the range 0.0015–0.023 found for k_{Cl}/k_{Br} in the SN2 reactions of alkyl halides with vitamin B_{12s} and cobaloximes(I).[17]

(15) Although the competing halogens are not in identical environments, the activating powers of Cl and Br as *ortho* substituents in SNAr reactions are approximately the same.[16]

(16) J. F. Bunnett and Roland E. Zahler, *Chem. Rev.*, **49**, 273 (1951).

(17) G. N. Schrauzer and E. Deutsch, *J. Amer. Chem. Soc.*, **91**, 3341 (1969).

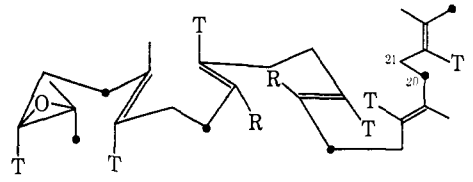
Darryl R. Fahey
Research and Development Division
Phillips Petroleum Company, Bartlesville, Oklahoma 74003
Received November 20, 1969

Mechanism of Squalene Cyclization. Biosynthesis of Fusidic Acid from (4*R*)-[2-^{14}C,4-^{3}H]Mevalonic Acid

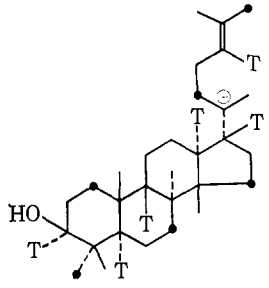
Sir:

Squalene obtained from (4*R*)-[4-^{3}H]MVA contains six tritium atoms,[1] and the derived 2,3-oxidosqualene[2] will therefore be labeled as indicated in **1a** (• denotes carbon atoms originating from C-2 of MVA; T ≡ ^{3}H). Enzymatic cyclization of 2,3-oxidosqualene to sterols and certain triterpenes is thought to proceed through the cation[3] **2** or its stabilized equivalent.[4] Cation **2** should retain six 4-*pro-R* protons (^{3}H) of MVA at C-3, 5, 9, 13, 17, and 24 and have the indicated stereochemistry.

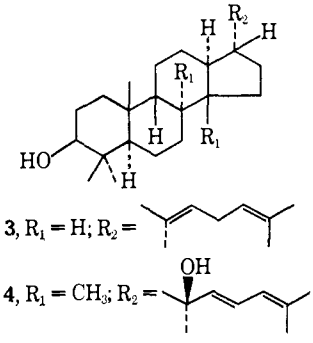
In the sequence leading from **2** to sterols, four 1,2 migrations were postulated,[5] terminating in the elimination of a ^{3}H atom from C-9 to yield lanosterol. The transformation of lanosterol to cholesterol entails the loss of two more ^{3}H atoms[6] from C-3 and C-5. We have proved that cholesterol prepared from (4*R*)-[2-^{14}C,-4-^{3}H]MVA retained only three tritium atoms[6] at the 17α, 20,[7] and 24-*pro-R*[8] positions. The presence of isotopic hydrogens at C-17 and C-20 was taken as evidence in support of the intermediacy of cation **2** and of



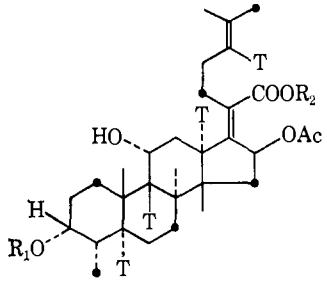
1a, R = CH_3
b, R = H (no tritium atoms)
c, R = CH_3; $\Delta^{20(21)}$ (no tritium atoms)



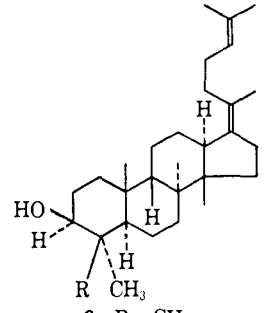
2



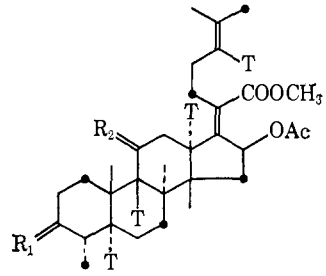
3, R_1 = H; R_2 =
4, R_1 = CH_3; R_2 =



5a, R_1 = H; R_2 = H
b, R_1 = H; R_2 = CH_3
c, R_1 = Ac; R_2 = CH_3
d, R_1 = H; R_2 = H; no Δ^{24}
e, R_1 = H; R_2 = CH_3 no Δ^{24}



6a, R = CH_3
b, R = CH_2OH



7a, R_1 = 3α OAc; R_2 = O
b, R_1 = O; R_2 = O

the rearrangements. Corey, *et al.*,[9,10] have shown that analogs **1b** and **1c** of 2,3-oxidosqualene undergo cyclization with rat liver enzymes to equivalents of cation **2**,

(1) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *Proc. Roy. Soc. (London), Ser. B*, **163**, 492 (1966).

(2) E. J. Corey, W. E. Russey, and P. R. O. de Montellano, *J. Amer. Chem. Soc.*, 88, 4750 (1966); E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *ibid.*, **88**, 4752 (1966).

(3) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(4) J. W. Cornforth, *Angew. Chem.*, **7**, 903 (1968).

(5) R. B. Woodward and K. Bloch, *J. Amer. Chem. Soc.*, **75**, 2023 (1953).

(6) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, Y. Shimizu, S. Ichii, E. Forchielli, and E. Caspi, *ibid.*, **87**, 3224 (1965).

(7) E. Caspi and L. J. Mulheirn, *Chem. Commun.*, 1423 (1969).

(8) E. Caspi, K. R. Varma, and J. B. Greig, *ibid.*, 45 (1969).

(9) E. J. Corey, P. R. O. de Montellano, and H. Yamamoto, *J. Amer. Chem. Soc.*, **90**, 6255 (1968).

(10) E. J. Corey, K. Lin, and H. Yamamoto, *ibid.*, **91**, 2132 (1969).

Table I

	^{14}C specific activity[a] (dpm/mmol × 10^{-5})	$^{3}H:^{14}C$ (dpm)	$^{3}H:^{14}C$ (atomic) Experimental	Theory
Methyl fusidate (**5b**)	3.95	18.0		4.0:6
Methyl fusidate-3α-acetate (**5c**)	3.87	17.6	3.9:6	4.0:6
11-Keto-3α-acetoxy ester (**7a**)	3.88	18.1	4.0:6	4.0:6
Methyl fusidate-3,11-diketone (**7b**)	4.17	18.1	4.0:6	4.0:6
9(11),17(20),24-Triene (**8**)	4.20	14.9	3.3:6	3.0:6
Methyl dihydrofusidate (**5e**)	4.00	16.8	3.7:6	4.0:6
13(17),20(22)-Diene (**9**)	3.75	13.7	3.0:6	3.0:6
p-Bromophenacyl ester (**10b**)	1.29	9.8	0.7:2	1.0:2
9β,13β-Triketone (**11**)	2.60	14.2	2.1:4	2.0:4
9α,13β-Triketone (**12**)	2.78	7.0	1.0:4	1.0:4
Lactone (**13**)		16.9	3.7:6	4.0:6
Diketo ester (**14**)		16.5	3.0:5	3.0:5

[a] Some degradations were performed on samples of higher dilution. All results are computed for the specific activity of methyl fusidate (**5b**).

which are capable of stabilization, without rearrangement, to **3** and **4**, respectively. Structures of helvolic acid,[11] cephalosporin P_1,[12] fusidic acid[13a] (**5a**), and related compounds[14-16] (**6**) suggest their formation by stabilization of **2** without rearrangement.

We reasoned that, if the intermediate cation yields protosterols *without rearrangement*, then the 4-*pro-R* protons of MVA retained in these compounds, particularly those at C-9 and C-13, must be located at their original positions, as shown in **2**. We therefore examined the biosynthesis of the protosterol fusidic acid (**5a**). This antibiotic was shown to retain six C-2 carbon atoms[16,17] of MVA and was obtained from 2,3-oxidosqualene.[18] Hence, fusidic acid biosynthesized from (4-*R*)-[2-^{14}C,4-^{3}H]MVA should have isotopic hydrogen atoms at C-5, 9, 13, 24, and possibly at C-3. The determination of location of the tritium atoms is now reported.

Fusidium coccineum was grown (7 days; 26°) in a medium[19] containing (4*R*)-[2-^{14}C,4-^{3}H]MVA (50 μ Ci of ^{14}C) to yield **5a** (1.8×10^5 dpm ^{14}C; 0.2% incorporation). Methyl fusidate (**5b**) (3.95×10^5 dpm/mmol ^{14}C; $^{3}H:^{14}C$ ratio 18.0; atomic ratio (ar) 4.0:6) (Table I) was acetylated and the diacetate[13] **5c** oxidized to the monoketone **7a** without loss of tritium ($^{3}H:^{14}C$ ratio 18.1; ar 4.0:6). Oxidation of methyl fusidate (**5b**) gave the diketone **7b** with an unchanged $^{3}H:^{14}C$ ratio ($^{3}H:^{14}C$ ratio 18.1; ar 4.0:6), demonstrating the absence of tritium from C-3 of fusidic acid. Dehydration[13] of **5c** proceeded with loss of tritium from C-9 to yield the triene **8** ($^{3}H:^{14}C$ ratio 14.9; ar 3.3:6).

Hydrogenation of **5a** gave dihydrofusidic acid[13] (**5d**), characterized as the ester **5e** ($^{3}H:^{14}C$ ratio 16.8; ar 3.7:6). The reaction was accompanied by partial exchange of isotopic hydrogen from C-13 and mainly from C-24 (*vide infra* **10b**). Transformation of the acid **5d** to the diene[20] **9** ($^{3}H:^{14}C$ ratio 13.7; ar 3.0:6) proceeded with the loss of a tritium atom from C-13.

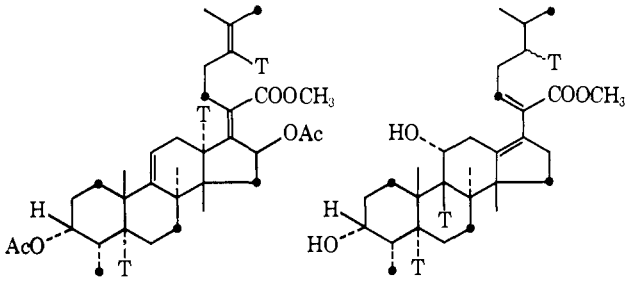
Ozonolysis of methyl dihydrofusidate (**5e**) (Zn–acetic acid work-up[13]) gave **10a** characterized as the *p*-bromophenacyl ester **10b** ($^{3}H:^{14}C$ ratio 9.8; ar 0.7:2), which retained one tritium and two ^{14}C atoms. The tetracyclic fragment from the ozonolysis was oxidized with Jones reagent and the resulting 16β-acetoxytriketone was converted[21] to **11** ($^{3}H:^{14}C$ ratio 14.2; ar 2.1:4). The deacetylation is accompanied by epimerization at C-13 and loss of tritium from this position. Equilibration of **11** proceeded with loss of tritium from C-9 to give the isomeric triketone[21] **12** ($^{3}H:^{14}C$ ratio 7.0; ar 1.0:4).

Transformation of fusidic acid to the 16,21-lactone followed by reduction gave the lactone[22] **13**. Exposure of **13** to osmium tetroxide led to the 24,25-glycol which was cleaved (Jones reagent) to the diketo acid **14a**. The ester **14b** showed an atomic ratio consistent with the loss of one ^{3}H atom from C-24 and the C-26 ^{14}C atom[17] as expected ($^{3}H:^{14}C$ ratio 16.5; ar 3.0:5).

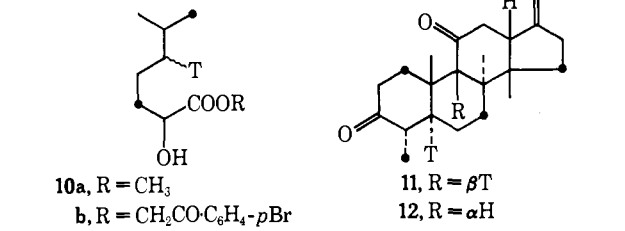
The decrease in $^{3}H:^{14}C$ ratio on formation of the triene **8**, the diene **9**, and the triketones **11** and **12** (Table I) clearly demonstrates the presence of tritium atoms at C-9 and C-13 in fusidic acid derived from (4*R*)-[2-^{14}C,4-^{3}H]MVA. A third tritium atom is apparently located at C-24 (*cf.* **10b** and **14b**) and the fourth is most probably at C-5. These observations are fully consistent with the hypothesis of protosterol formation *via* **2** and constitute the first demonstration that the crucial C-9 and C-13 hydrogen atoms indeed *originate* from the 4-*pro-R* position of MVA.

The reported derivation of the 4α-methyl group of fusidic acid from C-2 of MVA[16,17] is confirmed by our results. The protosterols **6a** and **6b** isolated from cultures of *F. coccineum*[15,16] are probably precursors of fusidic acid formed by cyclization of (3*S*)-2,3-oxidosqualene[18] (*cf.* lanosterol[23]). Hence, the 4α methyl of **6a** will orginate from C-2 of mevalonic acid.[23] Con-

(11) S. Okuda, S. Iwasaki, M. I. Sair, Y. Machida, A. Inoue, K. Tsuda, and Y. Nakayama, *Tetrahedron Lett.*, 2295 (1967).
(12) T. G. Halsall, E. H. Jones, G. Lowe, and C. E. Newall, *Chem. Commun.*, 685 (1966); P. Oxley, *ibid.*, 729 (1966).
(13) (a) W. O. Godtfredsen and S. Vangedal, *Tetrahedron*, **18**, 1029 (1962); W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *ibid.*, 21, 3505 (1965). (b) In our case **8** could have been accompanied by some 11-chloro and/or $\Delta^{11(12)}$ analogs; this could explain the observed discrepancy in $^{3}H:^{14}C$ ratio.
(14) S. Okuda, Y. Sato, T. Hattori, H. Igarashi, T. Tsuchiya, and N. Wasada, *Tetrahedron Lett.*, 4769 (1968); T. Hattori, H. Igarashi, S. Iwasaki, and S. Okuda, *ibid.*, 1023 (1969).
(15) Personal communication from Dr. W. O. Godtfredsen.
(16) G. diModrone, Ph.D. Thesis, E. T. H., Zurich, 1968.
(17) D. Arigoni, Conference on the Biogenesis of Natural Products, Academia Nazionale dei Lincei, Rome, 1964.
(18) W. O. Godtfredsen, H. Lorch, E. E. van Tamelen, J. D. Willett, and R. B. Clayton, *J. Amer. Chem. Soc.*, 90, 208 (1968).
(19) W. O. Godtfredsen, H. Lorch, and S. Jahnsen, U. S. Patent No. 3,072,531 (1963).
(20) W. O. Godtfredsen, W. von Daehne, and S. Vangedal, *Chem. Commun.*, 638 (1966).
(21) P. A. Diassi, G. W. Krakower, I. Basco, and H. Ann van Dine, *Tetrahedron*, **22**, 3443 (1966).
(22) W. O. Godtfredsen, W. von Daehne, L. Tybring, and S. Vangedal, *J. Med. Chem.*, **9**, 15 (1966).
(23) K. J. Stone, W. R. Roeske, R. B. Clayton, and E. E. van Tamelen,

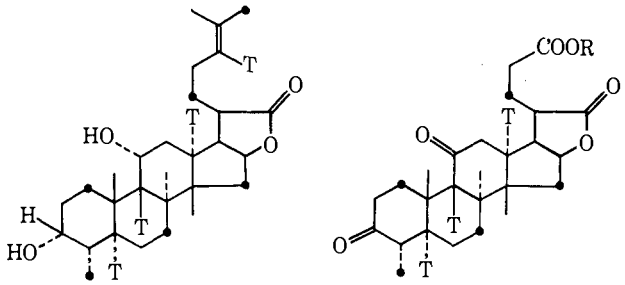


8 9



10a, R = CH_3
b, R = $CH_2CO\text{-}C_6H_4\text{-}p\text{Br}$

11, R = βT
12, R = αH



13

14a, R = H
b, R = CH_3

version to fusidic acid then involves the loss of the 4β-methyl group, presumably *via* a 3-ketone analog (no tritium at C-3 in **5**). This contrasts with recent reports[24] that the demethylation of 4,4-dimethylcholestanol by a rat liver enzyme system and of cycloartanol by *Polypodium vulgare* Linn.[25] involves initial loss of the 4α-methyl group.

Acknowledgments. This work was supported by Grants No. AM12156, HE10566, and CA-K3-16614 from the National Institutes of Health. The authors are grateful to Dr. P. Diassi of the Squibb Institute for Medical Research, New Brunswick, N. J., and to Dr. W. O. Godtfredsen of Leo Pharmaceutical Products, Ballerup, Denmark, for specimens of *Fusidium coccineum* and for generous supplies of fusidic acid.

Chem. Commun., 530 (1969); G. P. Moss and S. A. Nickolaidis, *ibid.*, 1072 (1969).

(24) K. B. Sharpless, T. E. Snyder, T. A. Spencer, K. K. Maheshwari, J. A. Nelson, and R. B. Clayton, *J. Amer. Chem. Soc.*, **91**, 3394 (1969).

(25) E. L. Ghisalberti, N. J. de Souza, H. H. Rees, L. J. Goad, and T. W. Goodwin, *Chem. Commun.*, 1403 (1969).

E. Caspi, L. J. Mulheirn
The Worcester Foundation for Experimental Biology, Inc.
Shrewsbury, Massachusetts 01545

Received November 19, 1969

On the Probable Intermediacy of Tetrahedrane

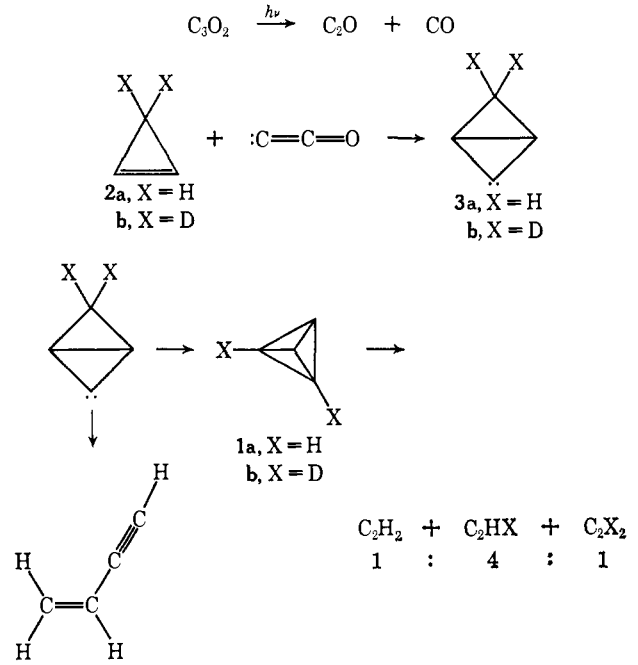
Sir:

We wish to present evidence for the formation of tricyclo[1.1.0.$0^{2,4}$]butane (tetrahedrane, **1a**) as an intermediate in the gas-phase photolysis of carbon suboxide in the presence of cyclopropene. Attempts to prepare tetrahedrane or its derivatives have thus far been unsuccessful.[1] However, recent mass spectral studies suggest structures of tetrahedral symmetry in the fragmentation of substituted cyclopentadienols.[2]

Carbon suboxide was generated by dehydration of malonic acid with phosphorus pentoxide and purified by gas chromatography. The cyclopropene, prepared from allyl chloride by the method of Closs and Krantz,[3] was purified by gas chromatography on a dimethyl sulfolane column at −40° prior to use. A mixture of cyclopropene (2.18×10^{-2} mmol) and carbon suboxide (4.72×10^{-2} mmol) was placed in a 442-ml Pyrex photolysis flask. This mixture was photolyzed for 80 min at room temperature with a 200-W Hanovia medium-pressure lamp placed in a water-cooled immersion well in the center of the flask. The products condensable in liquid nitrogen were analyzed by gas chromatography.

Acetylene (24%)[4] and vinylacetylene (33%) were the products. Vinylacetylene is an expected product, since the photolysis of carbon suboxide with 1,2-dimethylcyclopropene gives 2-methyl-1-penten-3-yne as the major product.[1d]

The appreciable yield of acetylene suggests the presence of tetrahedrane (**1**) as an intermediate. Photolysis



of carbon suboxide produces ketocarbene (*cf.* ref 5 for a discussion of this intermediate) which may react with cyclopropene, to give an adduct leading to the bicyclic carbene intermediate **3**. This carbene may either rearrange to give vinylacetylene or it may undergo intramolecular carbon–hydrogen insertion to give tetrahedrane (**1**). In order to determine if the acetylene

(1) (a) M. P. Cava and M. J. Mitchell, "Cyclobutadiene and Related Compounds," Academic Press, New York, N. Y., 1967, p 69; (b) S. Masamune and M. Kato, *J. Amer. Chem. Soc.*, **87**, 4190 (1965); *cf.*, however, **88**, 610 (1966); (c) E. H. White, G. E. Maier, R. Graeve, U. Zirngibl, and E. W. Friend, *ibid.*, 88, 611 (1966); (d) H. W. Chang, A. Lautzenheiser, and A. P. Wolf, *Tetrahedron Lett.*, 6295 (1966).

(2) M. M. Bursey and T. A. Elwood, *J. Amer. Chem. Soc.*, **91**, 3812 (1969).

(3) G. L. Closs and K. D. Krantz, *J. Org. Chem.*, **31**, 638 (1966).

(4) All product yields are based on the amount of cyclopropene consumed.

(5) D. G. Williamson and K. D. Bayes, *J. Amer. Chem. Soc.*, **90**, 1957 (1968).