Journal of Steroid Biochemistry, 1973, Vol. 4, pp. 433–437. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

MECHANISM OF SQUALENE CYCLIZATION: THE CHIRAL ORIGIN OF THE C-7 AND C-15 HYDROGEN ATOMS OF FUSIDIC ACID

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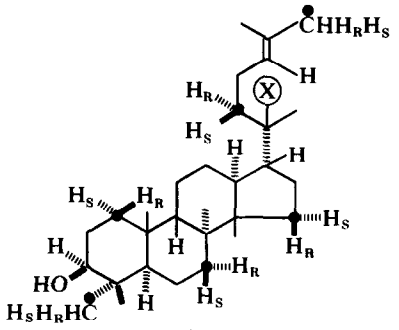
(*Received* 24 *March* 1973)

SUMMARY

The C-2 protons of mevalonic acid are incorporated into C-7 and C-15 of fusidic acid with retention of their stereochemical integrity; this finding is in agreement with the accepted mode of squalene-oxide cyclization to the C-20 protosterol cation, and supports the proposed mechanisms for the enzymatic events at C-7 and C-15 in the biosynthesis of cholesterol in rat liver.

CHOLESTEROL biosynthesized in the S10 fraction of rat livers is devoid of hydrogens derived from 2-pro S of mevalonic acid (MVA) at C-7 and C-15. These hydrogens are lost in the course of formation of precursors having a 7(8) double bond[2] and a 14(15) double bond[3]. The retained hydrogens originating from 2-pro R of MVA have the 7β and 15α stereochemistry in cholesterol[1]. Based on the mode of incorporation of the 2-pro R and 2-pro S hydrogens of MVA into squalene[4], they should assume the indicated stereochemistry in the hypothetical protosterol cation[5] (1) and hence in lanosterol (2). It is apparent that the 7β and 15α stereochemistry of the 2-pro R hydrogens of MVA in cholesterol is inverted with respect to their 7α and 15β orientation in (1) and (2).

The results for cholesterol are the basis of the interpretation of the stereochemistry of formation of 7-ene and 14-ene and of the mechanism of their subse-



(1)

⊗ = prosthetic group or ⊕ charge
● = C-2 carbon of MVA.
H_R and H_S = protons from 2-pro R and 2-pro S hydrogens of MVA.

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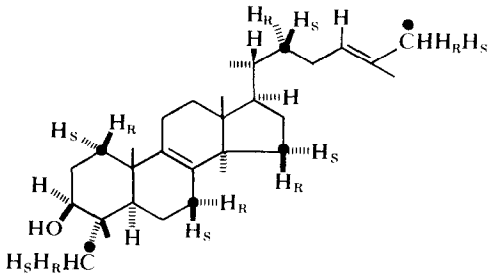
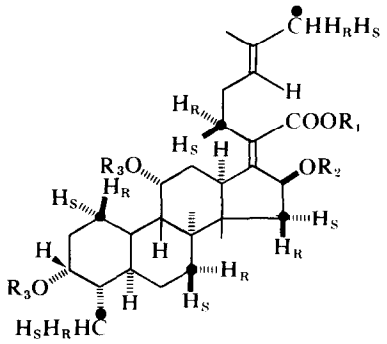


(2)

quent *trans* reduction[1]. Because of the mechanistic importance of these observations, we considered advisable to determine the stereochemistry of the protons derived from C-2 of MVA at C-7 and C-15 in a protosterol. As a model we used fusidic acid (3a) whose protosterol skeleton is thought to be formed via elimination of the 17β-hydrogen[6] in (1).

Specimens of "R" and "S" fusidic acid (3a) were biosynthesized by incubating (3R,2R) (2^{14}C,2^{3}H)-MVA and (3R,2S) (2^{14}C,2^{3}H)-MVA with *F. Coccineum*[6]



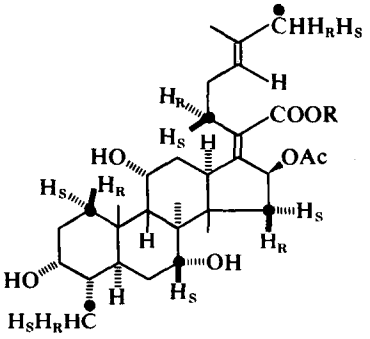
(3)

(a) R_1 = H; R_2 = Ac; R_3 = H; (b) R_1 = CH_3; R_2 = Ac; R_3 = H; (c) No−24-ene; R_1 = H; R_2 = Ac; R_3 = H; (d) No−24-ene; R_1 = CH_3; R_2 = Ac; R_3 = H; (e) No−24-ene; R_1 = H; R_2 = Ac; R_3 = THP (tetrahydropyranyl); (f) No−24-ene; R_1 = CH_3; R_2 = H; R_3 = THP; (g) No−24-ene; R_1 = CH_3; R_2 = Ac; R_3 = THP (from 3f); (h) No−24-ene; R_1 = CH_3; R_2 = $CH_3{\cdot}SO_2$; R = THP.

H_R = ^{3}H in "R"-Fusidic acid; H_S = ^{3}H in "S"-Fusidic acid.

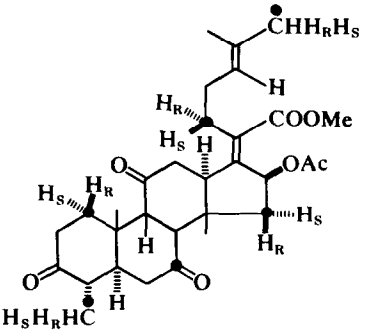
respectively (Table 1; (Experiment I and II, No. 1)). For determination of the stereochemistry of the incorporated tritium atoms at C-7, samples of the "R"-fusidic acid and the "S"-fusidic acid were then incubated with a helvolic acid producing strain of *Acremonium Persicinum* to yield 7α-hydroxy fusidic acids† (4a). The formation of (4a) should occur with the stereospecific removal of the 7α hydrogen, since on enzymatic hydroxylations of secondary nonactivated carbon atoms the incoming hydroxy groups are known to assume the stereochemistry of the displaced hydrogens[7, 1]. Further, oxidation of (4b) gave trione (5).

†All the compounds were homogenous by g.l.c. or t.l.c. Derivatives were identified by n.m.r., mass, and i.r. spectroscopy, etc., and by comparison with authentic samples.



(4)

(a) R = H; (b) R = CH_3.



(5)

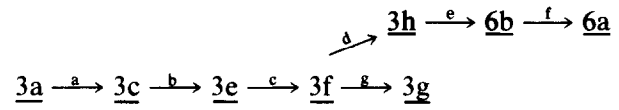
Table 1.

Experiment with:		I – "R"-Fusidic acid from (3R,2R) [2¹⁴C,2³H]-MVA		II – "S"-Fusidic acid from (3R,2S) [2¹⁴C,2³H]-MVA	
		³H : ¹⁴C ratio		³H : ¹⁴C ratio	
No.	Product	Isotopic	Atomic	Isotopic	Atomic
1	3b	5·04	6·00:6	4·65	6·00:6
2	4b	4·27	5·08:6	4·77	6·20:6
3	5	4·20	5·00:6	3·91	5·09:6
4	3d	5·03	6·00:6	4·62	5·95:6
5	3e	4·98	5·92:6	4·61	5·95:6
6	3g	4·96	5·90:6	4·63	5·97:6
7	6a	4·97	5·91:6	3·97	5·12:6
8	6b	4·97	5·91:6	–	–

It is evident (Experiment I, No. 2) that introduction of the 7α-hydroxyl into "R"-fusidic acid involved the loss of a tritium atom. The subsequent oxidation of this "R"-triol (4b) to the "R"-trione (5) proceeded without a change in the ³H : ¹⁴C ratio (Experiment I, No. 3). On the other hand 7α-hydroxylation of "S"-fusidic acid to the "S"-triol (4b) did not involve a loss of tritium (Experiment II, No. 2). However oxidation of the "S"-triol (4b) to the "S"-trione (5) entailed the loss of a

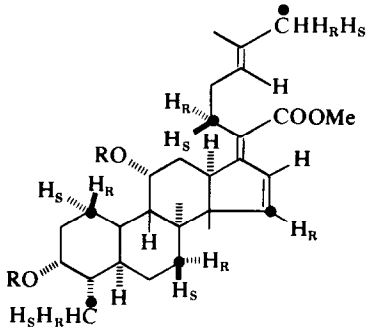
tritium atom from C-7 (Experiment II, No. 3). It is apparent that the 2-pro R and 2-pro S hydrogens of MVA have the 7α and 7β stereochemistry in fusidic acid (3a) respectively.

The stereochemistry of the tritium atoms at C-15 of "R" and "S"-fusidic acid was determined as outlined in the flow sheet.



a, H_2-Pd($BaCO_3$); b, dihydropyran-*p*-$CH_3 \cdot C_6H_4SO_3H$; c, i. $LiAlH_4$-THF., ii. CH_2N_2; d, $CH_3 \cdot SO_2Cl$-Py.; e, collidine; f, $(CH_3)_2CO$-HCl; g, Ac_2O-Py.

The base catalized displacement of a mesylate, resulting in a double bond is a *trans* elimination process[8]. It is apparent that formation of 6a from "R" fusidic acid (Experiment I, No. 7) proceeded without loss of tritium, revealing the 15β-



(6)

(a) R = H; (b) R = THP.

stereochemistry for the isotopic hydrogen. In contrast formation of 6a from "S"-fusidic acid (Experiment II, No. 7) involved the loss of the 15α-tritium atom. It is also clear that no changes in the tritium content occurred in the processing of the fusidic acids prior to the introduction of the 15-double bonds (Experiment I and II, No. 5 and 8). It can therefore be concluded that hydrogens derived from 2-pro R and 2-pro S of MVA have the 15β and 15α stereochemistry in fusidic acid respectively.

Hence the stereochemistry of protons derived from C-2 of MVA at C-7 and C-15 in (3a) is as predicted from the mode of squalene formation and cyclization to (1). This therefore corroborates the deductions on the stereochemistry of formation[2, 3] of 7-ene and 14-ene and the inversion[1] of configuration of the retained 2-pro R of MVA at C-7 and C-15 of cholesterol.

ACKNOWLEDGEMENTS

This work was supported by Grants from the National Institutes of Health and National Science Foundation.

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