Mechanism of Squalene Cyclization: The Chiral Origin of the C-22 Hydrogen Atoms of Fusidic Acid

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Summary The C-2 protons of mevalonic acid are incorporated with retention of configuration into C-22 of fusidic acid; this finding excludes the intermediacy of products with a $\Delta^{20(22)}$ double bond in the formation of a $\Delta^{17(20)}$ double bond in fusidic acid.

THE difficulty[1,2] of rationalizing the collapse of cation (**1**)[3] to protosterol antibiotics like fusidic acid (**2a**) and others[4] all having the *Z* geometry at $\Delta^{17(20)}$ has been pointed out. Stabilization of (**1**) through direct elimination of the 17β-proton (route '*a*') would lead according to Cornforth's hypothesis[3b] to the wrong geometry about the 17(20) double bond.[1,2] Additionally in such a process the cyclase system of F. *coccineum* would be expected to obviate the backbone rearrangement[3] through a kinetically controlled stabilization of (**1**) (route '*a*'). In contrast during the enzymatic transformation of (**1**) into lanosterol, it has been suggested that the cyl se system exerts only a marginal influence.[5]

The interpretational difficulty could be alleviated were the formation of (**2a**) to proceed through a $\Delta^{20(22)}$ intermediate (**3a**) (**1**; route '*b*') as noted by Corey *et al.*[6] for the analogue (**3b**). Enzymatic isomerization of (**3a**) could afford (**2a**) (origin of C-22 hydrogens not assigned). Rotation around C–17(20) of (**3a**) would permit positioning of the side chain and the 17-hydrogen suitable for the formation of a $\Delta^{17(20)}$ double bond in (**2a**).

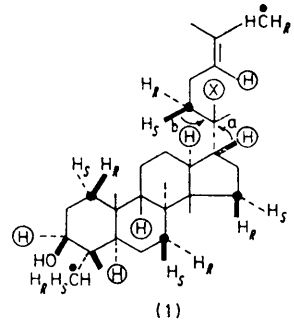
The presence of a $\Delta^{20(22)}$ intermediate was supported by an observation that incubation[1,7] of (3*RS*;2*S*)-[2-^{14}C,2-^{3}H]-mevalonic acid (MVA) (^{3}H : ^{14}C ratio 5·26 : 1 atomic ratio 1 : 1) with F. *coccineum* gave *S*-fusidic acid (**2a**) with a ^{3}H : ^{14}C ratio of 4·65 : 1, corresponding to the incorporation of 5·3 atoms of tritium and 6 atoms of ^{14}C. In the case of *R*-fusidic acid (**2a**) biosynthesized from (3*RS*,2*R*)-[2-^{14}C,2-^{3}H]-MVA (^{3}H : ^{14}C ratio 5·15 : 1) the ^{3}H : ^{14}C ratio of 5·04 : 1 corresponded to an atomic ratio of 6 : 6. The results with (2*S*)-[2-^{14}C,2-^{3}H]-MVA could be interpreted as arising from the

TABLE

Experiment with:		I; (2*R*)-[2-^{3}H,2-^{14}C]MVA			II; (2*S*)-[2-^{3}H,2-^{14}C]MVA		
				^{3}H : ^{14}C ratio			^{3}H : ^{14}C ratio
No.	Product	Derivative counted	^{14}C-Spec. act.[a]	Isotopic	Atomic	^{14}C-Spec. act.[a]	Isotopic	Atomic
1.	Mevalonic acid	*N*-Diphenylmethylamide	8·79	5·15	1·02 : 1	4·75	5·26	1·13 : 1
2.	Methyl fusidate	(**2b**)	86·7	5·04	6·00 : 6	82·4	4·65	6·00 : 6
3.	Methyl dihydrofusidate	(**2c**)	85·6	5·05	6·00 : 6	82·5	4·62	5·96 : 6
4.	6-Methylheptane-1,2-diol	(**4b**)	30·2	4·92	1·95 : 2	29·0	4·58	1·97 : 2
5.	5-Methylhexan-1-al	(**4d**)	30·4	5·04	2·00 : 2	29·0	4·66	2·00 : 2
6.	4-Methylpentan-1-ol	(**5c**)	2·7	5·02	1·99 : 2	4·0	4·60	1·98 : 2
7.	4-Methylpentan-1-al	(**6b**)	1·5	3·02	1·20 : 2	4·9	4·54	1·95 : 2
8.	Methyl 4-methylpentanoate	(**6d**)	—	—	—	—	2·38	1·02 : 2

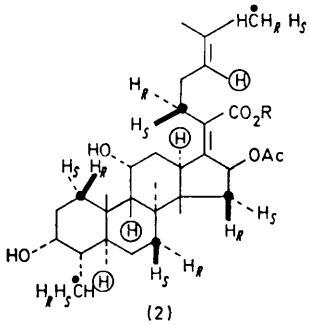
[a] D.p.m. per mmol × 10^4; entries 1, 6 and 7 were counted at higher dilutions.

loss of a 22-*pro*-*S* hydrogen *via* pathway ['*b*' shown in (**1**)] in the biosynthesis of (**2a**). The specimens of *R* and *S*-fusidic acid were thus degraded as shown in the Scheme and the chirality and ^{3}H content at C-22 determined.



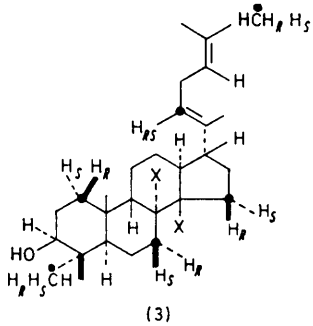
(1)

Ⓧ — prosthetic group or (+)charge
● — C-2 carbon of MVA
H_R and H_S — protons from the 2-*pro*-*R* and 2-*pro*-*S* of MVA
Ⓗ — protons from 4-*pro*-*R* of MVA.



(2)

a ; R = H
b ; R = Me
c ; R = Me ; no Δ^{24}

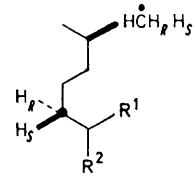


(3)

a ; X = Me
b ; X = H

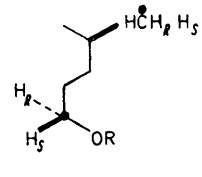
The products† obtained from *R*-fusidic (Table; Experiment I; entries 2—6) exhibited ^{3}H : ^{14}C ratios which corresponded to the predicted[1,8] atomic ratios. One atom of tritium‡ was lost on the NAD$^+$–YADH oxidation of (**5b**) to (**6a**) (Experiment I; entries 6, 7), thus establishing the 1*R* configuration of the alcohol.[11]

The products from the *S*-fusidic acid had constant ^{3}H : ^{14}C ratios (Experiment II; entries 2–7). Clearly NAD$^+$–YADH oxidation of the alcohol (**5b**) to aldehyde (**6a**) proceeded without loss of tritium. Hence if (**5b**) had a tritium atom at C-1 the alcohol must have the 1*S* configuration. Consequently the diene[1,12] (**7**) (from *S*-fusidic acid) was oxidized[13] to (**6c**) which was purified as the methyl ester (**6d**) by g.l.c. The ^{3}H : ^{14}C ratio of (**6d**) indicates that of the two ^{3}H atoms present in (**5b**) only one was retained. Therefore one atom of tritium must be present at C-1 of (**5b**) and (**6a**). It follows that a tritium atom originating from (2*S*)-[2-^{14}C,-2-^{3}H]-MVA is present a the 22-*pro*-*S* position of the derived *S*-fusidic acid.



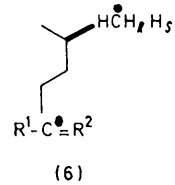
(4)

a ; $R^1 = CH_2OH$; $R^2 = OH$
b ; $R^1 = CH_2O{\cdot}CO{\cdot}NHPh$; $R^2 = O{\cdot}CO{\cdot}NHPh$
c ; $R^1 = H$; $R^2 = O$
d ; $R^1 = H$; R^2 = dimedone derivative



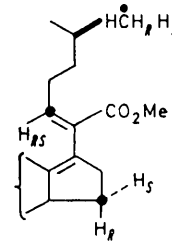
(5)

a ; R = OCHO
b ; R = H
c ; R = OC·NHPh



(6)

a ; $R^1 = H$; $R^2 = O$
b ; $R^1 = H$; R^2 = dimedone derivative
c ; $R^1 = OH$; $R^2 = O$
d ; $R^1 = OMe$; $R^2 = O$



(7)

(**6d**) ← (**7**) ← (**2a**) → (**2c**) → (**4a**) → (**4c**) → (**5b**) → (**6a**)

SCHEME

a; i H_2;[7,9] ii CH_2N_2. *b*; i O_3; ii $LiAlH_4$. *c*; H_5IO_6. *d*; i CF_3COOOH;[10b] ii $LiAlH_4$; iii prep. g.l.c. *e*; NAD$^+$–Yeast alcohol dehydrogenase (YADH). *f*; i LiCl–HCO·NMe_2; [12] ii CH_2N_2. *g*; i RuO_4; [13] ii CH_2N_2; iii prep. g.l.c.

The results demonstrate that the C-2 protons of MVA are incorporated into C-22 of fusidic acid with retention of their stereochemical integrity. The intermediacy of a $\Delta^{20(22)}$

† 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.

‡ The removal of only 0·8 atom of tritium may have been caused by partial air oxidation of the alcohol (see ref. 11).

precursor is thus excluded. Because the C-13 hydrogen of fusidic acid is derived from the 4-*pro R* proton of MVA,[1] Δ^{12} or $\Delta^{13(17)}$ intermediates also cannot be involved in the biosynthesis. Hence it seems that the stabilization of (**1**) in the formation of (**2a**) cannot be rationalized solely on an organic chemical basis[5] without some enzyme participation.[1]

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