Short Communication

Biosynthetic Studies of Marine Lipids. 40.[1] Generation of the Cyclopropane Ring of Sormosterol

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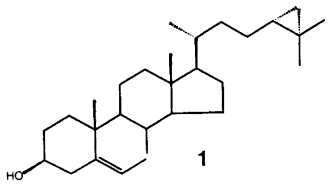
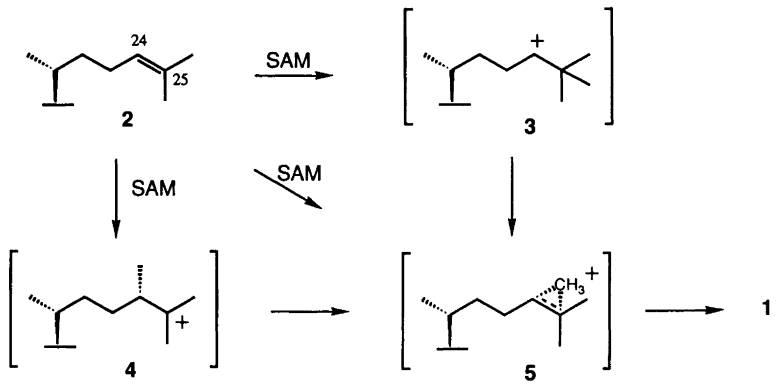
Dedicated to Professor Lars Skattebøl on the occasion of his 65th birthday.

The presence of cyclopropane rings in the sterol side chains of certain sponges and marine algae has long been of great biosynthetic interest.[2] We have recently shown that a group of cyclopropyl sterols isolated from Haplosclerid sponges arise via the enzymatic desaturation of saturated sterol side chains.[3] However, a more common mechanism for biosynthetic cyclopropanation involves the enzyme-catalyzed alkylation of an isolated double bond by the biological sulfonium salt *S*-adenosylmethionine (SAM). Lederer, in early studies of sterol biomethylation, proposed that the ubiquitous 24-methyl sterols arise via the ring-opening of a cyclopropyl intermediate.[4] Although this theory has been disproved, the erstwhile cyclopropyl intermediate (sormosterol, **1**) has recently been isolated from a California sponge, *Lissodendoryx topsenti*.[5] Sormosterol (**1**) was shown both in feeding experiments[5] and in experiments with sponge microsomes[6] to arise via the SAM alkylation of desmosterol (**2**), a process which usually leads directly to 24-methyl or 24-methylene sterols.



Sormosterol

In many cases the formation of cyclopropane rings via SAM bioalkylation is believed to involve the intermediacy of secondary carbonium ions. The cyclopropane-containing bacterial fatty acids arise via such intermediates,[7] and the biosynthesis of gorgosterol in marine dinoflagellates[8] and 24,28-methylenestigmasterol in a marine Chrysophyte[9] are also thought to involve secondary carbonium ions. If the alkylation of the $\Delta^{24,25}$ double bond occurs at the 25 rather than the usual 24-terminus (Scheme 1) a secondary carbonium ion could also be an intermediate in sormosterol biosynthesis. Such a course would initially produce a *tert*-butyl group (**3**) that could conceivably lead to the cyclopropane via ring-closure by any one of the three methyl groups. We found it of interest, therefore, to investigate the origin of the cyclopropane methylene group via degradation of biosynthetically labeled sormosterol (Scheme 2).



Scheme 1. Possible biosynthetic routes to sormosterol.

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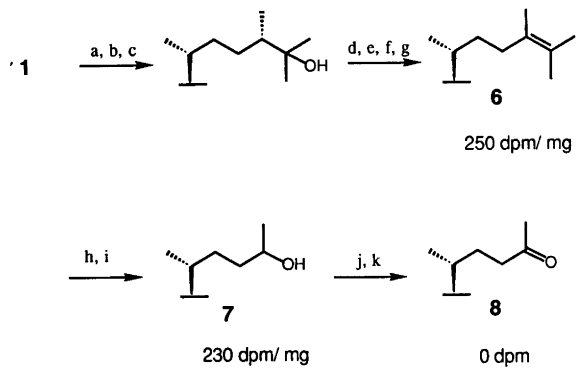

Scheme 2. Degradation of biosynthetically labeled sormosterol. a, Ac_2O–pyr.; b, TFA; c, NaOH; d, $POCl_3$–pyr.; e, LAH; f, TsCl–pyr.; g, MeOH–pyr.; h, O_3; i, $NaBH_4$; j, PCC; k, KOH–MeOH.

Experimental

Labeled sormosterol (**1**) was prepared enzymatically from desmosterol (**2**) and ^{3}H-SAM (70 μCi) using *Lissodendoryx topsenti* microsomes as previously described.[6] After addition of cold carrier the product was purified by reversed-phase HPLC purification using a Waters Associates HPLC system (M 6000 pump, R403 differential defractometer) equipped with two Ultrasphere ODS 5 μm columns [10 mm (i.d.) × 25 cm] in series using methanol (MeOH) as the mobile phase (4 ml min^{-1}). The sterol **1** (25 000 dpm) was converted into the acetate and isomerized with trifluoroacetic acid as previously reported.[5] The resulting mixture was treated with phosphorus oxychloride[10] and deacetylated. 24-Methyldesmosterol (**6**) (58 %) was separated by HPLC from the other sterol products [codisterol (21 %) and ergosta-5,23-dienol (20 %)]. The isomerized sterol **6** was converted into the *i*-methyl ether as described,[9] purified by argentic TLC (hexane–ether 39:1), and the specific activity was determined (250 dpm mg^{-1}). Ozonolysis followed by sodium borohydride as described,[9] gave the alcohol **7**, which was purified by silica gel TLC (hexane–ether 1:1). Measurement of the specific activity (230 dpm mg^{-1}) showed negligible loss of label. To confirm the location of the radiolabel, the alcohol **7** was oxidized to the ketone **8** with pyridinium chlorochromate. Base exchange of the acidic protons as previously described[9] resulted in the loss of all radioactivity. When the same degradation procedure was applied to the 24-methylenecholesterol (250 000 dpm) obtained biosynthetically from the same experiment,[6] the radiolabel was found in the same location. The identity of all intermediates in the degradation was confirmed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy with a Varian XL-400 spectrometer.

Discussion

This experiment indicates that the methylene group of the sormosterol cyclopropane arises from the methyl group of *S*-adenosylmethionine. This is consistent with a mechanism involving initial alkylation at C-24 to give, initially, a tertiary carbonium ion (Scheme 1, **4**) or direct production of the protonated cyclopropane intermediate **5** without discrete classical carbonium ion intermediates. However, initial alkylation at C-25 to give the *tert*-butyl secondary carbonium ion **3** cannot be ruled out because restricted rotation of the *tert*-butyl group is expected in the active site of the enzyme. Since the SAM alkylation is believed to occur from the β-face,[11] the methyl group originating from SAM would be in the best orientation for ring-closure. In contrast with the known instances of normal SAM methylation,[11] in the cyclopropanation reaction the removal of the proton occurs from the same face as the introduction of the methyl group.

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