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Practical Synthesis of [3-($^2H_3$)Methyl]mevalonolactone and Incorporation Experiment of [3-($^2H_3$)Methyl]mevalonolactone and [^{13}C]Labeled Acetate in the Biosynthesis of Isoprenoidal Diether Lipids of Halophilic Archaea

Noriaki Yamauchi,*,# Yoshiaki Natsubori,† and Tatsushi Murae

Department of Earth and Planetary Sciences, Graduate School of Sciences, Kyushu University, Hakozaki, Fukuoka 812-8581

†Laboratory of Chemistry, Faculty of Education, Yamagata University, Yamagata 990-8560

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The biosynthesis of the saturated isoprenoid portion of membrane lipids of the halophilic archaea *Natrinema pallidum* was investigated. At first, a practical method for the synthesis of [3-($^2H_3$)methyl]mevalonate was developed. Then, incorporation experiments of [3-($^2H_3$)methyl]mevalonate into the lipid core indicated that the isoprenoid portion was derived from mevalonate. However, a [2-^{13}C]acetate-incorporation experiment revealed a significant difference in the degree of ^{13}C enrichment between the expected position from the mevalonate pathway, the CH_2 groups (and at one terminal CH_3 group) and the CH_3 groups in the saturated isoprenoid chain. These results suggest that (1) the mevalonate pathway exists and is active, and (2) a part of acetoacetyl-CoA is not derived from an acetate in the biosynthesis of the isoprenoidal lipid-core of the archaebacterium.

Archaea (archaebacteria) live in relatively hostile environments, such as those characterized by low pH (2—3), high temperature (ca. 110 °C), completely anaerobic conditions, or high salt concentration. These bacteria can now be clearly distinguished from eucaryotes and eubacteria on the phylogenetic tree.[1] Many investigators regard the archaea as the evolutionarily oldest among living organisms, based on their molecular biological characteristics and adaptations to their environments. The archaea also have characteristic lipids that consist of saturated isoprenoids bonded with glycerol by an ether-linkage, as distinct from the lipids of eucaryotes or eubacteria, which are composed of straight long- chain fatty acids with an ester-linkage.[2]

The mevalonate pathway had been widely accepted as that for the biosynthesis of all isoprenoidal compounds. Starting from the three molecules of acetate, the first and second acetate are condensed to form acetoacetyl-CoA, and the third acetate attacks the ketone carbonyl carbon of acetoacetyl-CoA to give 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA). The two-step reduction of HMG-CoA with NADPH yields a C-6 compound, mevalonate, the first important intermediate. After the decarboxylation of mevalonate, a C-5 compound (the first isoprene unit), 2-isopentenyl diphosphate (IPP), is formed. IPP and 3,3-dimethylallyl diphosphate (DMAPP), the isomerized compound of IPP, are polymerized to give various lengths of the straight-chain isoprenoid; these are further converted to various types of isoprenoidal compounds. However, Rohmer recently pointed out the unique incorporation pattern of the ^{13}C-labeled carbon source in several hopanoids and proposed an alternative pathway, the "mevalonate-independent pathway". The pathway contains the condensation of glyceraldehyde-3-phosphate and decarboxylated pyruvate to give another C-6 compound, 1-deoxy-xylulose. That is further converted to IPP, a key intermediate in the middle of the mevalonate pathway. A clarification of the mevalonate-independent pathway presents an intriguing problem in the biosynthesis of isoprenoids, and many parts of metabolites (e.g., bacterial secondary metabolites and plant secondary metabolites included in terpenoids and pigments, respectively) have been shown to be biosynthesized from this pathway.[3] Recently, the incorporation of advanced intermediates directed to the mevalonate-independent pathway has been observed, and enzymatic studies on the key reactions of the pathway have been carried out.[4]

The entire outer sphere of archaea consists of isoprenoid-based biomembrane lipids. Therefore, efficient biosynthetic pathway(s) of the isoprenoid must exist. To date, Kates et al. have shown the incorporation of radiolabeled mevalonate in diether lipid,[5] and Kakinuma et al. recently reported the incorporation of [$^2H_9$]mevalonate in halophilic archaea.[6] These studies thus suggest the existence of a "normal" mevalonate pathway. However, Smith et al. pointed out that ^{13}C-labeled acetate was not incorporated at the branched methyl and methine carbon of the isoprenoidal lipid in *Halobacterium salinarium* NRC34001.[7] In order to clarify the precise pathway-

Graduate School of Sciences, Kyusyu University, and Faculty of Education, Yamagata University

(s) of the biosynthesis of the lipid-core in halophilic bacteria and to provide insights into the origin of the branched methyl and methine carbon in halophilic archaea, we conducted two types of experiments. The first was intended to confirm the fate of the branched 3-methyl group of mevalonate in halophilic archaea using [3-($^2H_3$)methyl]mevalonolactone, and the second to quantitatively examine the labeling patterns of the isoprenoid with [1-^{13}C]- and [2-^{13}C]acetates.

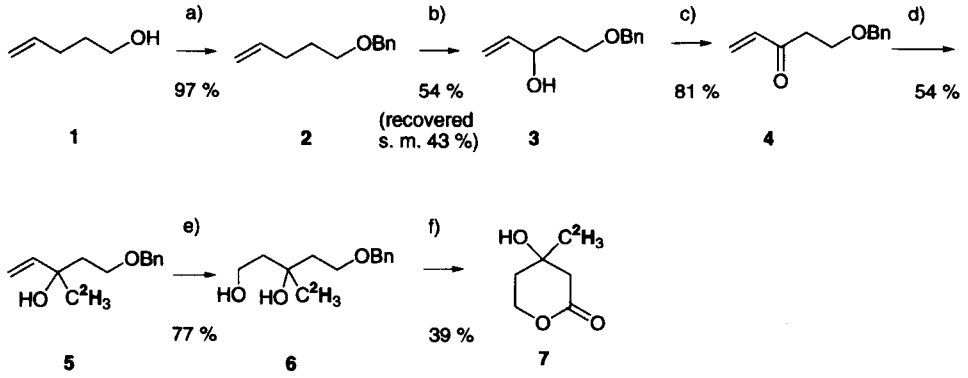
Practical Synthesis of [3-($^2H_3$)Methyl]mevalonolactone

Mevalonate is a ubiquitous biosynthetic precursor of various types of isoprenoid (hormones, pigments, terpenoids and steroids, the archaeal membrane lipid-core, etc.) in a wide range of animals, plants, and microorganisms. As essential tools for tracer experiments in the biosynthetic study of a above-mentioned metabolites, various types of isotopically labeled mevalonate have been prepared chemically. Concerning several types of syntheses of labeled mevalonate at the 3-methyl position, Fetizon et al. reported the synthesis of [3-($^2H_3$)methyl]mevalonolactone and Scott and Shishido reported the synthesis of [3-(^{13}C)methyl]mevalonolactone.[8] Their key reaction is the Grignard reaction of (modified) acetate and allylmagnesium bromide. However, Fertizon's method requires a large excess of expensive silver carbonate. Also, Scott's method uses [2-^{13}C]ethyl acetate, a corresponding deuterium-labeled compound that is not commercially available. In the present study, therefore, the [3-($^2H_3$)methyl]mevalonolactone was synthesized from 3-pentene-1-ol (**1**), and deuterium labeling was performed using one of the most inexpensive deuterium-labeled C-1 compounds commercially available, [C^2H_3]methyl iodide, as shown in Scheme 1. First, **1** was converted to benzyl ether **2**, and the resulting ether was oxidized at the allylic position with a catalytic amount of selenium(IV) oxide and *t*-butylhydroperoxide to afford **3**. The alcohol **3** was converted to ketone **4** by Jones oxidation. **4** was relatively unstable, and was therefore directly treated with the Grignard reagent prepared with [C^2H_3]methyl iodide to yield deuterated alcohol **5**. Then, the hydroboration-oxidation of **5** afforded the primary alcohol **6** exclusively. **6** was oxidized to carboxylate and deprotected to give [3-($^2H_3$)methyl]mevalonolactone **7**. The isotopic enrichment of the product was > 95%, as determined from its ^{1}H NMR spectrum.

Incorporation of [3-($^2H_3$)Methyl]mevalonolactone

The halophilic archea *Natrinema pallidum* IAM 13147[9] was incubated for 6 days at 37 °C in 600-ml of a medium containing 177 mg of [3-($^2H_3$)methyl]mevalonate (**7**), synthesized as described above. Lipids were extracted, and then hydrolyzed with methanolic HCl. Morita et al. identified a lipid from this archaea that contained diether **8** and the corresponding C25—C20 diether **9** as a minor component.[10] In this experiment, diethers **8** and **9** were separated with another organic compound, such as pigment or ubiquinone, by silica-gel column chromatography from the mixture of the hydrolyzate (Fig. 1). Further purification was not performed because of a difficulty to separate **8** and **9** with the small structural difference.

Diethers **8** and **9** were converted to their acetates, **10** and **11**, and analyzed by ^{2}H NMR, ^{1}H NMR and MS as a mixture of **8** and **9** or **10** and **11**. In ^{2}H NMR, the deuterium signal



a) BnCl, NaOH / DMSO. b) SeO_2, tBuOOH / CH_2Cl_2. c) Jones Reagent / acetone.
d) [C^2H_3]MgI, ether-CH_2Cl_2 e) BH_3-THF / THF then H_2O_2 / *aq.* NaOH
f) Jones Reagent / acetone, then H_2. Pd-C / AcOH

Scheme 1. Synthesis of [3-$^2H_3$]mevalonate.

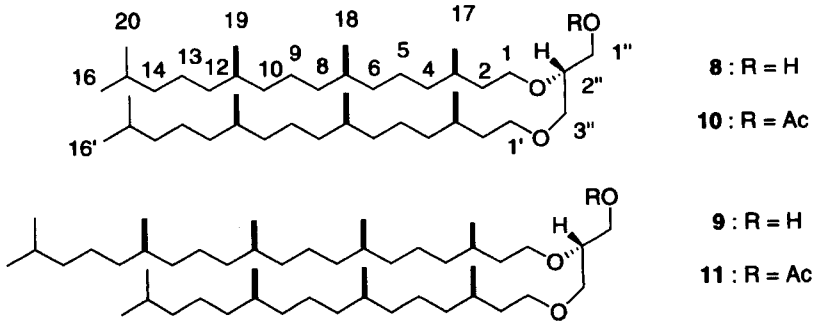


Fig. 1. The Structure of isolated diether lipids.

observed at δ = 0.81 was assigned to the methyl groups of **10** and **11** by a comparison with the chemical shift of the corresponding signal (δ = 0.87) in the ^{1}H NMR spectrum. Furthermore, lowering of the integration intensity of the methyl proton signals was clearly observed in the ^{1}H NMR spectrum. The degree of deuterium incorporation was estimated to be about 25% in comparison with the intensity of the signals of the labeled **10** and **11** at the signals of the hydrocarbon region and those of the non-labeled compound. (The degree of deuterium incorporation was normalized with the signal intensity of acetyl-CH_3 hydrogen that was chemically added after purifying of the labeled material.) The degree of deuterium incorporation was extremely high.

Further, the mass spectra of **8** and **9** clearly show the incorporation of deuterium (Fig. 2). The degree of deuterium incorporation was extremely high. In addition, the mass spectrum of **8** shows a molecular ion for which the value of every 3 mass units (i.e., 3, 6, 9 ... mu) is higher than the corresponding value for the non-labeled lipid. Also, if the degradation of mevalonate to acetate and reincorporating of the labeled acetate occurred, a scrambling of the deuterium was observed and the deuterium was distributed to, possibly, all of the carbon in the isoprenoid randomly. Therefore, for a cluster of higher peaks at each 3 mass units would not be observed in the region of the molecular ion peaks. The results reveal a series of lipid molecules labeled by the 1, 2, 3 ... etc., molecules of mevalonate, and suggest that the degradation of mevalonate to acetate and subsequent reincorporation were negligible.

A high degree of incorporation of deuterium was observed by the ^{13}C NMR spectrum of the labeled **10** and **11**. The isotopic β-effect of the ^{13}C carbon chemical shift around the C-C-$^2H_3$ carbon lowered the resonance frequency of the peaks (27.62 and 32.45 ppm) corresponding to the methine carbon from a non-labeled material at 27.96 and 32.78 ppm. No peaks corresponding to the C-C-$^2H_2$ and C-C-$^2H_1$ carbon were observed; the absence of such peaks was attributed to the loss of deuterium at the methyl group of the 3-position of mevalonate around the incorporation and the conversion to a part of the isoprenoidal lipid molecule.

Therefore, mevalonate was incorporated to the isoprenoidal part of the lipid with high efficiency. These results suggest that the degradation of mevalonate to acetate and the subsequent reincorporation were negligible under these conditions. These findings are consistent with those of the [$^2H_9$]mevalonate experiment by Kakinuma et al., in which the stereochemistry of the reduction of the double bond on the geranylgeranyl precursor of the diether lipid was elucidated.[6] Further, these results suggest that this member of the halophilic archaea biosynthesizes the lipid-core saturated isoprenoid mainly via mevalonate pathways.

Acetate-incorporation Experiment

Next, acetate-labeling experiments were performed as follows. A ^{13}C enriched diether corresponding to **10** was prepared from lipids of halophilic bacteria incubated in the same media as described above in the presence of labeled sodium acetate. The labeling ratios of the carbons in the diether are given in Table 1. At first glance, it appears that the labeling pattern is the usual one predicted from the mevalonate pathway. However, a closer analysis of the ratios of the incorporation of each carbon reveals differing degrees of incorporation among each C_2 unit, probably due to acetate. In the case of the incorporation of [2-^{13}C]acetate, there was significant incorporation of ^{13}C at the CH_2 groups (and at one terminal CH_3 group). The relative intensities of the ^{13}C signal at the CH_2 groups (C-4, C-8, C-12, and C-16) and at one terminal CH_3 group were about 30.[11] On the other hand, those of the ^{13}C signal at the other CH_3 groups (C-17, C-18, C-19, C-20) were 4.9—5.2.

Furthermore, in the case of the incorporation of [1-^{13}C]-acetate, the relative intensities of the ^{13}C signals corresponding to CH_2 groups (C-1, C-5, C-9, and C-13) of the saturated isoprenoidal chain were 4.5—5.4 in comparison to those of non-labeled lipids. On the other hand, the relative intensity of the ^{13}C signals of the CH groups (C-3, C-7, C-11, and C-15) were 3.2—3.6. These results show that the C_2 unit at C-1/C-2 and C-3/C-17 (branched CH_3) was possibly different on the labeling ratio. However, the result of the [1-^{13}C]acetate-incorporation would be within the experimental error.

The unique labeling pattern obtained by the experiment is shown in Fig. 3. In previous biosynthetic studies of secondary metabolites, such as antibiotics or microbial products,

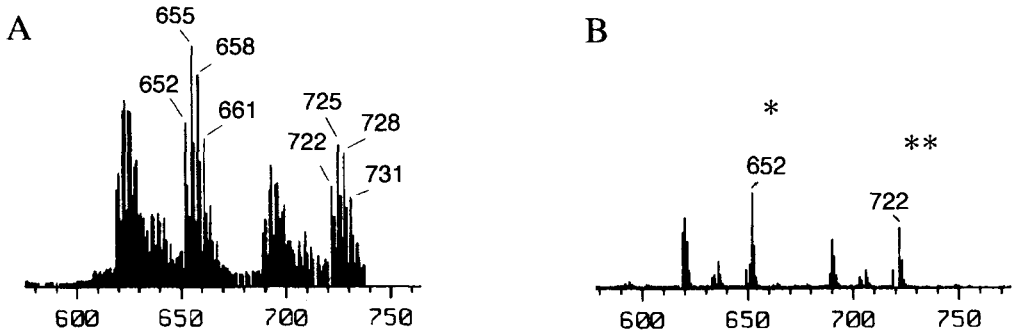


Fig. 2. Mass spectra (molecular ion region) of diether **8**; A : Deuterium-labeled **8**, B : non labeled standard. The ion peak at *m/z* 652(*) in B is due to the molecular ion peak of C20-C20 diether (**8**) and the ion peak at *m/z* 722 (**) is due to those of coexistig C20–C25 lipid **9**.

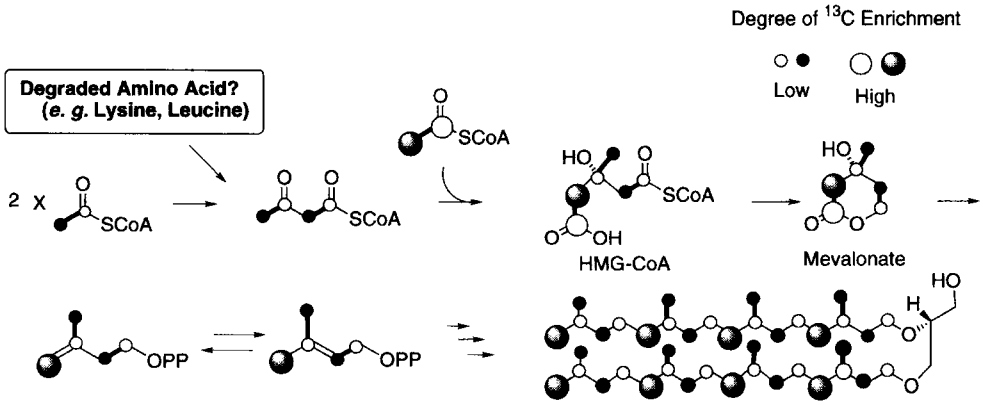


Fig. 3. Incorporation of [^{13}C]acetates in *Natrinema pallidum*.

Table 1. ^{13}C NMR Results from the Incorporation of ^{13}C-Labeled Acetates

Cemical Shift	Relative ^{13}C intensities[a]		Carbon	Carbon No.[b]
	[1-^{13}C]Acetate	[2-^{13}C]Acetate		
19.58	1.0	5.2	CH_3	17
19.67	0.7	2.8	CH_3	17′
19.74	1.1	5.4	CH_3	18(′), 19(′)
20.92	1[d]	1[d]	CH_3	$OCOCH_3$
22.61	1.2	32.1	CH_3	16(′)
22.71	1.0	6.0	CH_3	20(′)
24.34	4.6	1.7	CH_2	13(′)
24.46	5.4	1.7	CH_2	9(′)
24.79	4.8	1.7	CH_2	5(′)
27.96	3.2	0.8	CH	15(′)
29.71	3.6	2.1	CH	3
29.85	3.4	1.1	CH	3′
32.78	3.4	0.8	CH	7(′), 11(′)
36.58	0.9	4.9	CH_2	2
36.95	0.8	5.2	CH_2	2′
37.27[c]	1.2	35.9	CH_2	c)
37.36[c]	1.6	39.9	CH_2	c)
37.39[c]	1.0	31.2	CH_2	c)
37.44[c]	1.2	8.7	CH_2	c)
37.50[c]	1.3	35.8	CH_2	c)
39.36	0.9	5.2	CH_2	14(′)
64.14	1.0	11.7	CH_2	1″
68.91	4.5	2.1	CH_2	1
70.08	5.4	1.5	CH_2	1’
70.25	4.5	4.6	CH_2	3″
76.50	1.3	7.6	CH	2″

a) Peak intensities were normalized to the carbon signal of Acetyl-CH_3 (20.92 ppm). b) Assignments were according to Ref. 11. Carbon No. were shown in Fig. 1. c) These signals were assingned to carbon no. 4(′), 6(′), 8(′), 10(′), and 12(′). Precise assignments were impossible. d) Standard.

unequal incorporations of the different "acetate units" have often been observed. Such differences were especially pronounced in two previous studies. (1) The incorporation of a "starter unit" (i.e., a longer carboxylic acid such as butyric acid, amino acid, or glycerol) was observed at the first unit of the polyketide.[12] (2) The incorporation of a succinate residue was observed in the middle of the polyketide chain.[13] The case shown in Fig. 3 of the present study is unique in that the incorporation ratios differ among the acetate units.

In the mevalonate pathway, two acetate-units are directed to acetoacetyl-CoA via Claisen-type condensation by thiolase, and then the third acetate unit is condensed via aldol-type coupling by HMG-CoA synthase to give HMG-CoA. The formation of HMG-CoA does not require the malonate-type activation of the acetate unit, as in the case of the straightforward elongation of acetate toward the biosynthesis of fatty acid. Although the mechanistic similarity of HMG-CoA synthase in the mevalonate pathway and ketothiolase, ketoacyl-ACP synthase in the fatty acid biosynthetic pathway were suggested recently,[14] the isotope incorporation ratio may be different between the first and second acetate-condensation reactions because the starting material, reaction type and enzymes are different.

Thus, our results indicate the existence of a "normal" pathway from acetate to mevalonate. Kakinuma et al. reported a high degree of incorporation of the totally labeled mevalonate by deuterium in their elucidation of the stereochemistry of the reduction of the double bond on the geranylgeranyl precursor of the diether lipid.[6] They suggested that the mevalonate pathway is the main pathway in these organisms. Our result is consistent with their results. Also, a precise observation of the degree of incorporation of [2-^{13}C]acetate suggests that a part of acetoacetyl-CoA is not derived from an acetate in the biosynthesis of isoprenoid chains of lipid in halophilic archaea. In the microbial metabolism of lysine, the intermediate, crotonoyl-CoA, is easily directed to acetoacetyl-CoA (or acetyl-CoA). Also, the metabolism of leucine and valine to mevalonate via HMG-CoA through the intermediate, 3-methylcrotonoyl-CoA, would also act as a carbon source other than acetate. The significant involvement of these carbon sources dilutes the labeled acetate at the step of acetoacetyl-CoA.

Concerning the labeling pattern of [2-^{13}C]acetate and the carbon sources of the isoprenoid, Smith et al. reported no incorporation of ^{13}C-labeled acetate at the branched methyl and methine carbon in the diether from the lipid of *Halobacterium salinalium*. They claimed that the C_2 unit does not originate from an acetate, but rather from other carbon sources, such as degraded lysine or one of various amino

acids;[7] they concluded that the new biosynthetic pathway, in which the carbon source at the 3 and 3-methyl position of the mevalonate originates from an amino acid, is operative in the organism. Our results suggest the involvement of another carbon source other than acetate at the carbon of the 3 and 3-methyl position, they also pointed out. However, a low level of incorporation was observed at the corresponding 3 and 3-methyl carbon and the low incorporation of acetate was observed at the acetoacetyl-CoA (C_4 carbon) unit in our experiments. Therefore, no new pathway to mevalonate is operative, and the known metabolic pathway of amino acid to a C_4 carbon compound would cause a "dilution" of the labeled acetate. In their experiment, Smith et al. used a mixture of polar lipids as the "lipid fraction", but did not quantitatively analyze the labeling. They therefore probably overlooked the relatively small incorporation of ^{13}C-acetate, particularly the incorporation of [2-^{13}C]acetate (5 times vs. about 30 times).

However, the difference of our result and Smith's result may mean a change in the isoprenoid biosynthesis mechanism with the evolution of the organism. The difference of strain (*Natrinema pallidum* vs. *Halobacterium salinalium*) and lipid composition (C20-C20 vs. C20-C25) may have caused a difference in the ^{13}C-labeling, thereby leading to a slight change in the isoprenoid biosynthetic pathway in the case of a genus more highly homologous to the halophilic archea.

Further investigations of the carbon source leading to acetoacetyl-CoA, particularly with respect to lysine metabolism, and of the relation between the genetic stock tree (e.g., investigation for the other species of archea) and changes of the biosynthetic pathway are underway.

Experimental

Infrared spectra were obtained with a Hitachi Model 260-10 (at Yamagata) grating spectrometer. ^{1}H NMR spectra were recorded with a JEOL EX-90 (at Fukuoka) or a Lambda-300 spectrometer (at Tokyo). ^{13}C NMR spectra were recorded with a JEOL EX-90 or a Lambda-300 spectrometer. ^{2}H NMR spectra were recorded with a JEOL Lambda-300 spectrometer. For the ^{1}H and ^{13}C NMR spectra, tetramethylsilane (0 ppm) was used as an internal standard at $CDCl_3$. For the ^{2}H NMR spectra, the natural abundance of the deuterium signal of $CDCl_3$ solvent (7.26 ppm) was used as an internal standard. EI- and FAB-MS spectra were recorded with a JEOL-AX505HA spectrometer (FAB gun, Xe) (at Tokyo). Chromatographic separations were carried out with Merck Kieselgel 60, 70-230 mesh columns.

Synthesis of [3-(^{2}H$_3$)Methyl]mevalonolactone. Benzyl 4-pentenyl ether (2). To a solution of 4-penten-1-ol (**1**) (2.20 g, 28 mmol) in dimethyl sulfoxide (20 ml) was added finely powdered sodium hydroxyde (2.20 g, 56 mmol); and the mixture was stirred for 30 min. Benzyl chloride (6.4 ml, 7.04 g, 56 mmol) was then added and the whole mixture was stirred for an additional 3 h. After the addition of a small amount of water, the mixture was then extracted with EtOAc 3 times (50 ml×3). The combined organic layer was washed with 1 M HCl (1 M = 1 mol dm^{-3}), sat. $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. After filtration, the organic solution was evaporated to dryness. The residue was purified by silica-gel column chromatography (50 g, hexane : EtOAc = 10 : 1) to give 4.09 g of **2** (97.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.73 (2H, m), 2.06 (2H, dddd, J = 1.4, 3.3, 6.3, 8.1 Hz), 3.46 (2H, t, J = 8.2 Hz), 4.57 (2H, s), 4.91 (1H, m), 5.06 (2H, m), 5.77 (1H, dddd, J = 3.3, 6.3, 9.6, 13.2 Hz), 7.30 (5H, aromatic). IR ($CHCl_3$) 3160, 2930, 2860, 1640, 1095, 915 cm^{-1}. HRMS Calcd for $C_{12}H_{16}O$: 176.1200. Found: 176.1229.

1-Benzyloxy-4-penten-3-ol (3). To a suspension of SeO_2 (3.7 g, 34 mmol) in CH_2Cl_2 (50 ml) was added an 80% hexane solution of *t*-Butylhydroperoxide (50 ml, 435 mmol); the mixture was stirred for 1 h. Then, the solution of **2** (26.1 g, 145 mmol) in CH_2Cl_2 (20 ml) was added all at once and stirred for 72 h. The whole mixture was evaporated to remove CH_2Cl_2, ether (100 ml) and 2 M NaOH (100 ml) were added to the evaporated mixture, and the organic phase was separated. The water layer was extracted with ether (100 ml×2) and the combined organic layer was washed with 1 M-HCl, sat. $NaHCO_3$ and brine, and then dried over anhydrous $NaSO_4$. After filtration, the organic solution was evaporated to dryness. The residue was purified by silica-gel column chromatography (100 g, hexane : EtOAc = 4 : 1) to give 15.9 g of **3** (54.4%) along with 11.1 g (42.5%) of recovered starting material **2**. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.81 (2H, m), 2.60 (1H, broad), 3.67 (2H, td, J = 5.6, 7.8 Hz), 4.40 (1H, m), 4.51 (2H, t, J = 6.7 Hz), 5.13 (1H, dddd, J = 1.5, 1.9, 3.4, 9.6 Hz), 5.29 (1H, dddd, J = 1.5, 2.3, 4.0, 13.5 Hz), 5.78 (1H, dddd, J = 4.0, 5.3, 9.6, 13.5 Hz), 7.30 (5H, aromatic). IR ($CHCl_3$) 3160, 2930, 2860, 1640, 1095, 915 cm^{-1}. Anal. Found: C, 74.97; H, 8.39%. Calcd for $C_{12}H_{16}O_2$: C, 74.53 ; H, 8.39%.

1-Benzyloxy-4-penten-3-one (4). To a solution of **3** (125 mg, 0.64 mmol) in acetone (2 ml) was added Jones Reagent (0.3 ml, 0.81 mmol) in a dropwise manner; the solution was stirred for 30 min. Excess reagent was quenched by the addition of *i*PrOH, and the mixture was stirred several minutes. The mixture was filtered and water (10 ml) and EtOAc (10 ml) were added. The organic phase was separated and the water phase was reextracted with EtOAc (10 ml×2). The combined organic phase was washed with brine and then dried over anhydrous $NaSO_4$. After filtration, the organic solution was evaporated to dryness. The residue was purified by short silica-gel column chromatography (5 g, hexane : EtOAc = 10 : 1) to give 100 mg of **4** (81.3%). ^{1}H NMR (90 MHz, $CDCl_3$) δ = 2.81 (1H, dd, J = 6.5, 13.1 Hz), 2.98 (1H, dd, J = 6.5, 13.1 Hz), 3.69 (2H, t, J = 6.5 Hz), 4.53 (2H, s), 5.86 (1H, dd, J = 8.9, 12.2 Hz), 6.27 (1H, d, J = 8.9 Hz), 6.34 (1H, d, J = 12.2 Hz), 7.30 (5H, aromatic). IR ($CHCl_3$) 3025, 3010, 2870, 1685, 1615, 1280, 1100 cm^{-1}. **4** was relatively unstable in air or in a trace amount of acid or base. Then, further purification of **4** was impossible.

1-Benzyloxy-3-[(^{2}H$_3$)methyl]-4-penten-3-ol (5). To the Grignard reagent solution prepared from [C^2H_3]I (500 ml, 1149 mg, 8.0 mmol) and magnesium (200 mg, 8.0 mmol) in ether (20 ml), 1.11 g of **4** in CH_2Cl_2 (10 ml) was added in a dropwise manner for 10 min; the mixture was then stirred for an additional 20 min at room temperature. The mixture was diluted with ether (20 ml), and sat. NH_4Cl (10 ml) was added. The organic phase was separated, the water phase was reextracted with ether (10 ml×2), and the combined organic phase was washed with brine and then dried over anhydrous $NaSO_4$. After filtration, the organic solution was evaporated to dryness. The residue was purified by silica-gel column chromatography (10 g, hexane : EtOAc = 2 : 1) to give 0.65g of **5** (53.3%). ^{1}H NMR (90 MHz, $CDCl_3$) δ = 1.75 (1H, dt, J = 5.6, 10.8 Hz), 1.91 (1H, ddd, J = 6.3, 8.1, 10.8 Hz), 3.69 (1H, dt, J = 5.6, 6.3 Hz), 3.72 (1H, dt, J = 6.3, 8.1 Hz), 4.49 (2H, s), 5.07 (1H, dd, J = 2.2, 11.1 Hz), 5.27 (1H, dd, J = 2.2, 18.7 Hz), 5.81 (1H, dd, J = 11.1, 18.7 Hz), 7.30 (5H, aromatic). ^{13}H NMR (22 MHz, $CDCl_3$) δ = 40.24, 67.81, 73.09, 73.42, 112.20, 127.75,

128.43, 144.49. IR ($CHCl_3$) 3160, 2930, 2860, 1640, 1095, 915 cm^{-1}. Anal. Found: C, 74.19; H, 8.64%. Calcd for $C_{13}H_{15}{}^2H_3O_2$: C, 74.60 ; H, 8.66%.

5-benzyloxy-3-[($^2H_3$)methyl]-4-pentan-1,3-diol (6). To a solution of **5** (2.138 g, 10.2 mmol) in 50 ml of tetrahydrofuran (THF) was added 10 ml of BH_3–THF via syringe for 15 min at room temperature. The mixture was stirred for 30 min, then cooled over an ice-water bath, and a small amount of water was added. 6 ml of 3 M NaOH and 6 ml of 30% H_2O_2 were added to the mixture and the whole was stirred for 1 h. The mixture was evaporated to ca. 10 ml, then extracted with ether (50 ml×3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration and evaporation, the product was purified by column chromatography (hexane : EtOAc = 1 : 1 to 1 : 3) to give 1.79 g of **6** (77.2%). ^1H NMR (90 MHz, $CDCl_3$) δ = 1.76 (4H, m), 2.80 (2H, broad), 3.71 (2H, t, J = 7.2 Hz), 3.91 (2H, t, J = 8.0 Hz), 4.48 (1H, d, J = 8.1 Hz), 4.71 (2H, s), 7.30 (5H, aromatic). ^{13}C NMR (22 MHz, $CDCl_3$) δ = 40.16, 41.36, 42.18, 59.49, 66.17, 67.17, 68.56, 72.66, 73.02, 127.72, 128.32, 137.46. IR ($CHCl_3$) 3600, 3380 (broad), 3010, 2940, 1720, 1220, 1050 cm^{-1}. Anal. Found: C, 68.89; H, 8.64%. Calcd for $C_{13}H_{17}{}^2H_3O_3$: C, 68.69; H+^{2}H, 8.86%.

[3-($^2H_3$)Methyl]mevalonolactone. To a solution of **6** (1.64 g, 7.42 mmol) in acetone (40 ml) was added Jones Reagent (5 ml, 13.5 mmol) in a dropwise manner with stirring over an ice-water bath for 2 h. Excess reagent was quenched by the addition of *i*PrOH, and the mixture was stirred for several minutes. The mixture was then filtered and evaporated to 20 ml. After the whole mixture was transferred to a separatory funnel, ether (50 ml) was added. The acid fraction was extracted with 1 M NaOH (50 ml×3). The water layer was acidified with concd. HCl, and then acid was extracted with ether (50 ml×3). The combined organic layer was washed with water and then dried over anhydrous $NaSO_4$. After filtration, the organic solution was evaporated to dryness to give the acid (940 mg). The acid was dissolved into 10 ml of AcOH, and 10% Pd–C (50 mg) was added. The mixture was stirred at H_2 atmosphere for 3 h. The mixture was filtered and evaporated to dryness. The residue was purified by silica-gel column chromatography (hexane : EtOAc = 1 : 1 to 1 : 2) to give [3-($^2H_3$)methyl]mevalonolactone **7** (371 mg, 38.9% for 2 steps). IR ($CHCl_3$) 3160, 2930, 2860, 1640, 1095, 915 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ = 1.90 (2H, m), 2.49 (1H, d, J = 17.3 Hz), 2.64 (1H, td, J = 1.2, 17.3 Hz), 4.34 (1H, dt, J = 4.6, 11.2 Hz), 4.59 (1H, ddd, J = 7.3, 11.2 Hz). ^{13}H NMR (22 MHz, $CDCl_3$) δ = 28.23 (septet, J = 19.8 Hz), 35.26, 44.17, 66.19, 67.38, 171.53. HRMS. Found: 133.0810. Calcd for $C_6H_7{}^2H_3O_3$: 133.0815.

Bacterial Culture and Lipid Extraction. *Natrinema pallidum* (formerly *Halobacterium halobium*) IAM 13147 was obtained from the Institute of Applied Microbiology, University of Tokyo, and the cultivation was carried out for 7 days at 37 °C with shaking in a medium containing 3 g of yeast extract, 3.5 g of casamino acid, 150 g of NaCl, 6 g of $MgSO_4 \cdot 7H_2O$, 1.8 g of trisodium citrate $\cdot 2H_2O$, 0.6 g of KCl, and 170 mg of [3-($^2H_3$)methyl]mevalonolactone or 250 mg of [^{13}C]labeled-sodium acetate. Cells were harvested by centrifugation (300 rpm, 15 min) and washed with water (wet cells, ca. 5 g). Lipids were extracted from wet organism by the procedure of Tornabene et al.[15] The polar lipids were separated from the non-polar lipids by acetone-precipitation. After methanolysis of the polar lipid fraction (3% methanolic HCl, reflux, 12 h), the hydrolyzed fraction was extracted with hexane with 3 times. The combined hexane layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica–gel with hexane–diethyl ether (4 : 1) to give, typically, about 10 mg of lipid (**8** and **9**, as a mixture). The lipids (**8** and **9**) were dissolved into 2 ml of pyridine, and 1 ml of acetic anhydride was added to the mixture. The mixture was heated to 60 °C and stirred for 2 h, then cooled to room temperature. A small amount of water was added and the whole was evaporated to dryness. The residue was chromatographed over silica-gel with hexane–diethyl ether (10 : 1) to give, typically, about 10 mg of lipid (**10** and **11**, as a mixture).

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