Multigram Synthesis of Mevalonolactone-d_9 and Its Application to Stereochemical Analysis by ^{1}H NMR of the Saturation Reaction in the Biosynthesis of the 2,3-Di-*O*-phytanyl-*sn*-glycerol Core of the Archaeal Membrane Lipid

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Abstract: A synthetic method for (*RS*)-mevalonolactone-d_9 (**8**) was developed starting from deuterated dimethoxyphenylacetone and trimethyl phosphonoacetate. The overall yield of **8** was 33% in seven steps on a multigram scale. Synthesized mevalonolactone-d_9 was applied to biosynthetic studies of the archaeal core membrane lipid 2,3-di-*O*-phytanyl-*sn*-glycerol. Mevalonolactone-d_9 was highly incorporated into the phytanyl chains of the archaeal lipid, and the total enrichment was approximated to be as high as 70%. The mevalonate pathway is clearly responsible for the biosynthesis of the phytanyl chains of the core lipid in the archaeal membrane. Further, saturation of the geranylgeranyl group to the phytanyl group was shown to take place through the addition of hydrogen in a *syn* manner by analyzing the behavior of protium on the heavily deuterated archaeal lipid by ^{1}H NMR spectroscopy.

Introduction

Archaea (archaebacteria) have been attracting considerable attention from both biochemical and evolutionary perspectives. It has been established that these bacteria are distinct from prokaryotes and eukaryotes and are now classified in a third independent domain.[1] We have been studying the biochemical characteristics of archaea on a molecular and mechanistic level, including the similarities to or differences from other categories of living organisms. The most characteristic feature of archaea is found in the chemical structure of its core membrane lipids, the fundamental structure which segregates and identifies the bodies of these living cells from the environment. The lipid structure is the most crucial criterion for the classification of archaebacteria. The basic core structure of the archaeal membrane is 2,3-di-*O*-phytanyl-*sn*-glycerol (Figure 1).[2] We have already looked at the biochemistry of the biosynthesis of the glycerol moiety[3] and now have turned our attention to the hydrophobic group. The hydrophobic aliphatic group is a saturated isoprenoid chain presumably derived from geranylgeranyl diphosphate.[4] The stereochemistry of the sites of methyl branching in the phytanyl group has been rigorously determined on the core lipids of an extreme halophile *Halobacterium halobium* and a methanogen *Methanobacterium thermoautotrophicum*.[5] However, the stereochemistry of hydrogenation (or saturation) has not yet been elucidated. Comparative studies of the biochemistry with other systems such as the saturation of the isoprene moiety of vitamins and steroids in different organisms are thus intriguing. The stereochemistry of saturation in cholesterol biosynthesis[6] and of enoyl reductase in fatty acid biosynthesis[7] has already been elucidated.

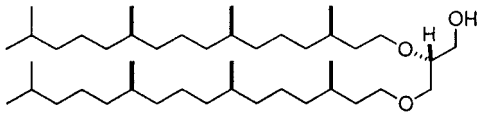

Figure 1. Structure of 2,3-di-*O*-phytanyl-*sn*-glycerol, core lipid of the archaeal membrane.

The hydrophobic moiety of the core lipid molecule is known to contain polyisoprenoids, which may be derived from mevalonate.[8] Interestingly, however, Rohmer et al. recently determined that an alternate non-mevalonate pathway operates in various organisms to biosynthesize isoprenoids, in addition to

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(1) (a) Woese, C. R.; Fox, G. E. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5088. (b) Woese, C. R.; Kandler, O.; Wheelis, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4576. (c) Delong, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5685. (d) Kjems, J.; Larsen, N.; Dalgaard, J. Z.; Garrett, R. A.; Stetter, K. O. *Syst. App. Microbiol.* **1992**, *15*, 203. (e) Benachenhou, L. N.; Forterre, P.; Labedan, B. *J. Mol. Evol.* **1993**, *36*, 335. (f) Klenk, H. P.; Schleper, C.; Schwass, V.; Brudler, R. *Biochim. Biophys. Acta* **1993**, *1174*, 95. (g) Barns, S. M.; Fundyga, R. E.; Jeffries, M. W.; Pace, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1609.

(2) (a) Kates, M. In *The Biochemistry of Archaea (Archaebacteria)*; Kates, M., Kushner, D. J., Matheson, A. T., Eds.; Elsevier: Amsterdam, 1993; p 261. (b) Kates, M. *Experientia* **1993**, *49*, 1027. (c) Gambacorta, A.; Gliozzi, A.; De Rosa, M. *World J. Microbiol. Biotechnol.* **1995**, *11*, 115.

(3) (a) Kakinuma, K.; Yamagishi, M.; Fujimoto, Y.; Ikekawa, N.; Oshima, T. *J. Am. Chem. Soc.* **1990**, *112*, 2740. (b) Kakinuma, K.; Obata, Y.; Matsuzawa, T.; Uzawa, T.; Oshima, T. *J. Chem. Soc., Chem. Commun.* **1990**, 925.

(4) (a) Zhang, D.; Poulter, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 1270. (b) Zhang, D.; Poulter, C. D. *J. Org. Chem.* **1993**, *58*, 3919. (c) Chen, A.; Zhang, D.; Poulter, C. D. *J. Biol. Chem.* **1993**, *268*, 21701.

(5) (a) Kates, M.; Joo, C. N.; Palameta, B.; Shier, T. *Biochemistry* **1967**, *6*, 3329. (b) Heathcock, C. H.; Finkelstein, B. L.; Aoki, T.; Poulter, C. D. *Science* **1985**, *229*, 862. (c) Heathcock, C. H.; Radel, P. A. *J. Org. Chem.* **1986**, *51*, 4322. (d) Heathcock, C. H.; Finkelstein, B. L.; Jarvi, E. T.; Radel, P. A.; Hadley, C. R. *J. Org. Chem.* **1988**, *53*, 1922.

(6) (a) Yagi, T.; Kobayashi, N.; Morisaki, M.; Hara, N.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 680. (b) Fujimoto, Y.; Ikuina, Y.; Nagakari, M.; Ikekawa, N.; Kakinuma, K. *J. Chem. Soc., Chem. Commun.* **1991**, 688.

(7) (a) Frössl C.; Boland, W. *J. Chem. Soc., Chem. Commun.* **1991**, 1731. (b) Saito, K.; Kawaguchi, A.; Seyama, Y.; Yamakawa, T.; Okuda, S. *Eur. J. Biochem.* **1981**, *116*, 581. (c) Saito, K.; Kawaguchi, A.; Seyama, Y.; Yamakawa, T.; Okuda, S. *J. Biochem.* **1981**, *90*, 1697. (d) Sedgwick, B.; Morris, C.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193.

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the well-known mevalonate pathway.[9] It seemed worthwhile to clearly address whether a mevalonate or non-mevalonate pathway operates in the biosynthesis of archaeal membrane lipids.

Although various isotopomers of mevalonolactone have been prepared to date,[10] a new approach was necessary for our study, and an easy and convenient method for the synthesis of (*RS*)-mevalonolactone-d_9 was developed. It was anticipated that, if mevalonate is efficiently incorporated into the archaeal lipids, the phytanyl group would be highly deuterated, since the membrane is essential to the growth and proliferation of the bacterial cells. Using the deuterated lipids, the fate of the hydrogens could later be traced by ^{1}H NMR spectroscopy.

Tritium and/or deuterium as tracers are usually used in this type of stereochemical analysis to follow the incoming hydrogens to a double bond. However, it is also conceivable that protium can be utilized to track hydrogen, provided the background protium can be significantly reduced. We show here an example in which protium can be successfully used to trace the incoming hydrogens. In fact, ^{1}H NMR is used to analyze the tracer *vide infra*. The essential prerequisite for such studies is the availability of a highly deuterated target metabolite.

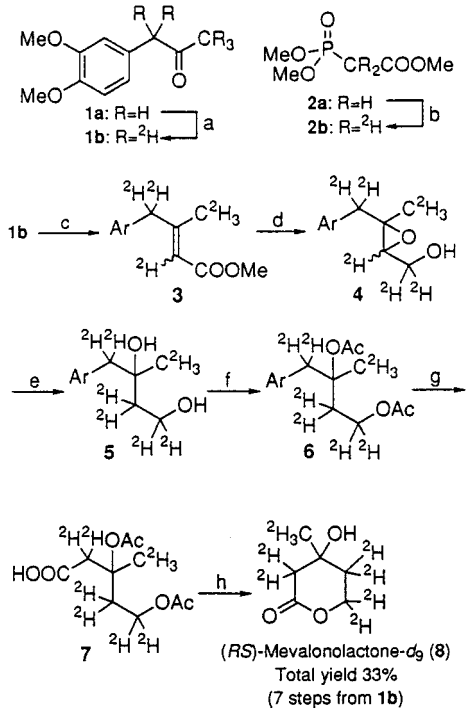
Described here are the synthesis of (*RS*)-mevalonolactone-d_9, its supplementation to the culture of *Haloarcula japonica* JCM7785, and ^{1}H NMR analysis of the biosynthesized lipids. The core lipids of *H. japonica* were proved to be exclusively composed of di-*O*-phytanylglycerol.[11]

Results and Discussion

Synthesis of Mevalonolactone-d_9. We recently described the enantioselective synthesis of (*R*)-mevalonolactone-d_9 using chirality control on a carbohydrate template.[12] Since mavalonate kinases are generally highly enantioselective,[13] the deuterated probe could be a racemate for these biochemical studies. Therefore, a more convenient and easier synthesis was exploited for the preparation of such heavily deuterated molecules on a gram scale.

First, two precursors, (3,4-dimethoxyphenyl)acetone-d_5 (**1b**) and the phosphonate-d_2 **2b**,[14] were prepared by simple treatment of the nondeuterated precursors with ^2H_2O in the presence of K_2CO_3 as shown in Scheme 1. The Wittig−Horner reaction between the two deuterated precursors **1b** and **2b** proceeded smoothly to give ester **3** as a mixture of geometrical isomers (*E*/*Z* = 3) in 80% yield. The resulting ester **3** was subsequently reduced with $LiAl^2H_4$. Under these reduction conditions, the saturated alcoholic product derived from the 1,4-addition of deuteride and reduction of the ester function was formed along with the desired allylic alcohol; however, the product mixture was immediately subjected without purification to the achiral Sharpless epoxidation[15] with *tert*-butyl hydroperoxide and VO(acetylacetonate)$_2$. The resulting epoxide **4** was purified to homogeneity at this stage, and the yield was 76% in two steps. Further reduction of **4** with $LiAl^2H_4$ easily afforded the desired diol **5**. Throughout this method, the donors of deuterium were the rather less expensive ^2H_2O and $LiAl^2H_4$. Two hydroxyl groups were then protected as acetate **6** by standard conditions. Ruthenium oxidation[16] of **6** proceeded smoothly, and the carboxylic acid **7** was obtained in good yield. Deprotection and final extraction under acidic conditions afforded the desired (*RS*)-mevalonolactone-d_9 (**8**) in 33% overall yield in seven steps. The deuterium contents were estimated by ^{1}H NMR to be 93, 96, 99, and 93% at C-2, C-4, C-5, and the methyl group, respectively. The structure of mevalonolactone-d_9 was confirmed by spectroscopic data and by repetition of the synthesis using nondeuterated substrate as well.

Scheme 1



Reagents and conditions: a) ^2H_2O, K_2CO_3, 70°C, quant.; b) ^2H_2O, K_2CO_3, 91%; c) NaH, **2b**, THF, 80%, d) i) $LiAl^2H_4$, ether, ii) TBHP, VO(acac)$_2$, benzene, reflux, 76 %, e) $LiAl^2H_4$, THF, 79%, f) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 93%, g) $RuCl_3$, $NaIO_4$, CCl_4-CH_3CN-H_2O, 87 %, h) K_2CO_3-MeOH, and then H_3O^+, 85%

Feeding to the Culture of *Haloarcula japonica* and Isolation of the Core Lipid. The culture of *H. japonica*

(8) (a) De Rosa, M.; De Rosa, S.; Gambacorta, A. *Phytochemistry* **1977**, *16*, 1909. (b) De Rosa, M.; Gambacorta, A.; Nicolaus, B. *Phytochemistry* **1980**, *19*, 791. (c) Ekiel, I.; Smith, I. C. P.; Sprott, G. D. *J. Bacteriol.* **1983**, *156*, 316. (d) Ekiel, I.; Sprott, G. D.; Patel, G. B. *J. Bacteriol.* **1985**, *162*, 905. (e) Ekiel, I.; Sprott, G. D.; Smith, I. C. P. *J. Bacteriol.* **1986**, *166*, 559. (f) Moldoveanu, N.; Kates, M. *Biochim. Biophys. Acta* **1988**, *960*, 164.

(9) (a) Schwender, J.; Seemann, M.; Lichtenthaler, H. K.; Rohmer, M. *Biochem. J.* **1996**, *316*, 73. (b) Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. *J. Am. Chem. Soc.* **1996**, *118*, 2564.

(10) (a) Isler, O.; Rüegg, R.; Würsch, J.; Gey, K. F.; Pletscher, A. *Helv. Chim. Acta* **1957**, *40*, 2369. (b) Cornforth, J. W.; Conforth, R. H.; Pelter, A.; Horning, M. G.; Poják, G. *Tetrahedron* **1959**, *5*, 311. (c) Gray, W. F.; Deets, G. L.; Cohen, T. *J. Org. Chem.* **1968**, *33*, 4532. (d) Scott, A. I.; Phillips, G. T.; Reichardt, P. B.; Sweeny, J. G. *J. Chem. Soc., Chem. Commun.* **1970**, 1396. (e) Seiler, M.; Acklin, W.; Arigoni, D. *J. Chem. Soc., Chem. Commun.* **1970**, 1394. (f) Cornforth, J. W.; Ross, F. P. *J. Chem. Soc., Chem. Commun.* **1970**, 1395. (g) Floss, H. G.; Tcheng-Lin, M.; Chang, C.; Naidoo, B.; Blair, G. E.; Abou-Chaar, C. I.; Cassady, J. M. *J. Am. Chem. Soc.* **1974**, *96*, 1898. (h) Cane D. E.; Levin, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 1183. (i) Scott A. I.; Shishido, K. *J. Chem. Soc., Chem. Commun.* **1980**, 400. (j) Hawkins, D. R.; Calvin, M. *J. Label. Comput.* **1987**, *24*, 1229.

(11) Morita, M.; Yamauchi, N.; Eguchi, T.; Kakinuma, K. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 596.

(12) Kishida, M.; Yamauchi, N.; Sawada, K.; Ohashi, Y.; Eguchi, T.; Kakinuma, K. *J. Chem. Soc., Perkin Trans. 1*, **1997**, 891.

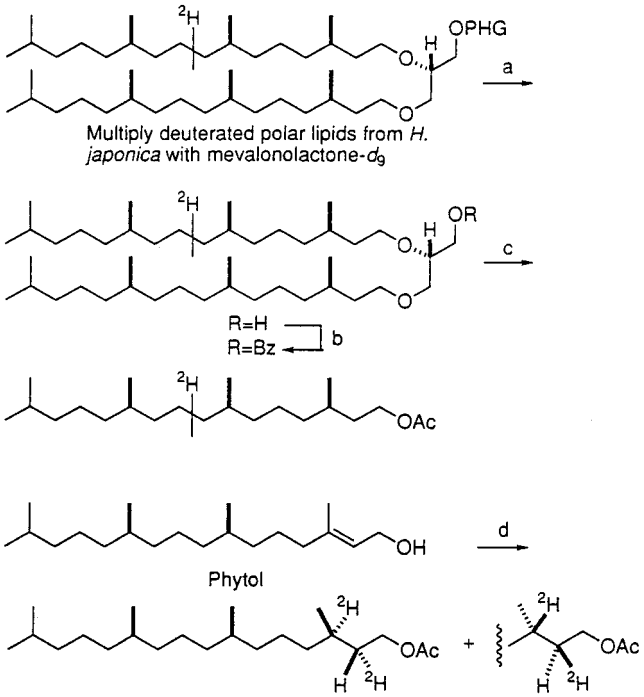
(13) Rétey, J.; Robinson, J. A. In *Stereospecificity in Organic Chemistry and Enzymology*; Verlag Chemie: Weinheim, 1982; p 209.

(14) Iyoda, M.; Ogura, F.; Akiyama, S.; Nakagawa, M. *Chem. Lett.* **1983**, 1883.

(15) (a) Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136. (b) Itoh, T.; Jitsukawa, K.; Kaneda, K.; Teranishi, S. *J. Am. Chem. Soc.* **1979**, *101*, 159.

(16) (a) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936. (b) Nuñez, M. T.; Matín, V. S. *J. Org. Chem.* **1990**, *55*, 1928.

Scheme 2



JCM7785 was carried out as already described.[11,17] The synthesized mevalonolactone-d_9 was aseptically added to the culture to a final concentration of 1.0 g/L. After cultivation for 9 days, the cells were harvested by centrifugation. The lipids were purified according to the standard manner[18] through solvolysis of the polar headgroups. The neutral core lipid was finally isolated by repeat chromatography to afford 18 mg of homogeneous di-*O*-phytanylglycerol. The core lipid was analyzed by ^{1}H NMR and was further converted to the benzoate (Scheme 2).

In general, if the mevalonate pathway is involved in the biosynthesis of the phytanyl moieties, these should be rather good incorporation of deuterium, since eight hydrogens of the mevalonolactone remain in each isoprene unit. If the alternative non-mevalonate pathway were responsible for the biosynthesis of the isoprene units, deuterium incorporation would be poor, if any, through catabolism of mevalonolactone and reconstruction to other small molecules. The benzoate derivative of the core lipid was first analyzed by ^{1}H NMR spectroscopy (Figure 2). One can immediately see extremely reduced signals ascribable to the protons of the phytanyl groups. The signals due to the two oxymethylene groups (3.50 and 3.70 ppm) of the phytanyl groups were reduced significantly compared with those (3.59, 3.78, 4.36, and 4.48 ppm) of the glycerol portion. The deuterium was so highly incorporated that the total enrichment was approximated to be some 70% based on the intensities of the intramolecular reference signals of the nondeuterated benzoyl group. The mass spectrum of the benzoate was also significant as shown in Figure 3. While the molecular

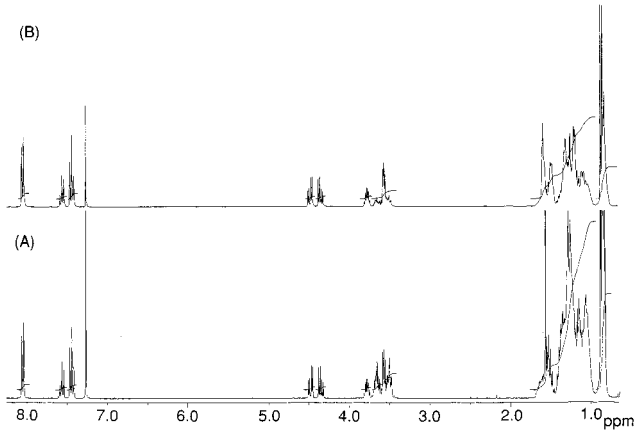


Figure 2. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of (A) authentic 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate[11] and (B) multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate from *H. japonica* with mevalonolactone-d_9.

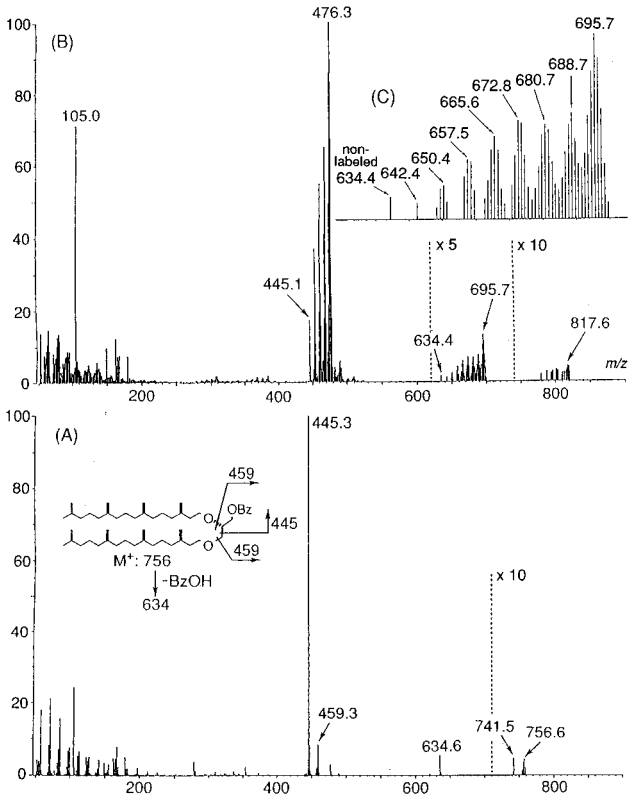


Figure 3. EI-MS spectra of (A) authentic 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate, (B) multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate from *H. japonica* with mevalonolactone-d_9, and (C) expansion of spectrum B.

ion was observed at *m*/*z* 756 in the EI-MS spectrum of the nondeuterated lipid benzoate,[11] the signal intensity was not high enough to analyze the isotopomers. A relevant major fragment ion was observed at *m*/*z* 634 due to the loss of benzoic acid from the molecular ion. In the spectrum taken similarly with the deuterated lipid benzoate, a series of signals was observed at the molecular ion as well as at the fragment ions. As already mentioned above, eight hydrogens out of nine were expected to be incorporated into each isoprene unit. Since eight isoprene units are incorporated into two phytanyl groups, one would expect the most highly deuterated isotopomer to give a parent ion which is 64 amu higher than the nonlabeled, and this is observed (see Figure 3C). Clearly, the ions derived from nonlabeled lipid molecules, one isoprene unit labeled molecules,

(17) (a) Takashina, T.; Hamamoto, T.; Otozai, K.; Grant, W. D.; Horikoshi, K. *System. Appl. Microbiol.* **1990**, *13*, 177. (b) Nakamura, S.; Aono, R.; Mizutani, S.; Takashina, T.; Grant, W. D.; Horikoshi, K. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 996. (c) Horikoshi, K.; Aono, R.; Nakamura, S. *Experientia* **1993**, *49*, 497.

(18) Minnikin, D. E.; Collins, M. D.; Goodfellow, M. *J. Appl. Bacteriol.* **1979**, *47*, 87.

and a series of lipid molecules with up to eight labeled isoprene units were observed. The isotopomer content seemed to follow a statistical distribution since the supplemented deuterated mevalonolactone was not labeled evenly at each labeled site. As already described, the enrichment at each site was estimated to be 93−99%. Therefore, the molecular masses of the multiply labeled lipid molecules are in the statistical distribution, which is shown in the mass spectrum in Figure 3. Interestingly, the isotopomer composed of eight labeled isoprene units is the most abundant and the relative abundance decreases according to the number of deuterated isoprene units incorporated. It now appears that the phytanyl groups of the core membrane lipid of *H. japonica* JCM7785 are, in fact, biosynthesized from mevalonate in an extremely efficient manner. While the possibility of involvement of the so-called non-mevalonate pathway (or Rohmer pathway) cannot be excluded,[9] the mavalonate pathway is the most likely for the isoprenoid anabolism in this organism. The possible effects of culture conditions upon the incorporation of mevalonate are currently under investigation.

Since the incorporation of mevalonolactone-d_9 was so efficient, we turned our attention to the incorporation of protium into the phytanyl groups. As geranylgeranyl diphosphate has been believed to be the biosynthetic precursor to the phytanyl group,[4] it would be expected that two hydrogens must be incorporated into each isoprene unit. The stereochemistry of the saturation step in fatty acid biosynthesis (enoyl reductase reaction) was known to vary depending on the species,[7] while the saturation reaction at $C_{24}-C_{25}$ of desmosterol in cholesterol biosynthesis was reported to take place by *syn* (*cis*) addition.[6] We anticipated that, by looking more closely at the ^{1}H NMR spectrum of the deuterated lipid or its derivatives, the mode of saturation of the geranylgeranyl group to produce the phytanol group in archaeal lipid biosynthesis could be elucidated. It should be emphasized here that archaea have biochemical similarities to both bacteria and eukarya. Therefore, the stereochemistry of the saturase reaction of the hydrophobic chain biosynthesis has evolutionary significance as well.

To address this issue, the highly deuterated core lipid obtained above was subjected to degradation with aqueous hydroiodic acid to phytanyl iodide, which was subsequently converted into deuterated phytanyl acetate in a standard manner (Scheme 2).[19] The ^{1}H NMR spectrum of authentic phytanyl acetate[20] clearly showed the C-1 signal at 4.10 ppm as a multiplet, but the geminal protons of the C-2 methylene group were well separated at 1.43 and 1.66 ppm by the effect of the nearby stereogenic center at C-3. The spin-coupling network of the C-1, -2, and -3 regions correlated well with each other by the ^{1}H−^{1}H COSY technique. Unfortunately, the C-3 methine signal overlapped with another signal, the C-15 methine proton at 1.52 ppm. Most crucial in this study was the unequivocal stereospecific assignment of the C-2 methylene signal since the stereochemistry at C-3 was assigned to be *R* in the literature.[5] Phytol was treated with deuterium gas ($^2H_2$) in CH_3O^2H in the presence of PtO_2 under standard hydrogenation conditions to afford a mixture of diastereoisomeric phytanols deuterated at the C-2 and C-3 positions, and the phytanol mixture was subsequently acetylated (Scheme 2). While the product was a mixture, the crucial ^{1}H NMR signals were affected only by the nearest stereogenic center, so it was suitable for the assignment of each methylene

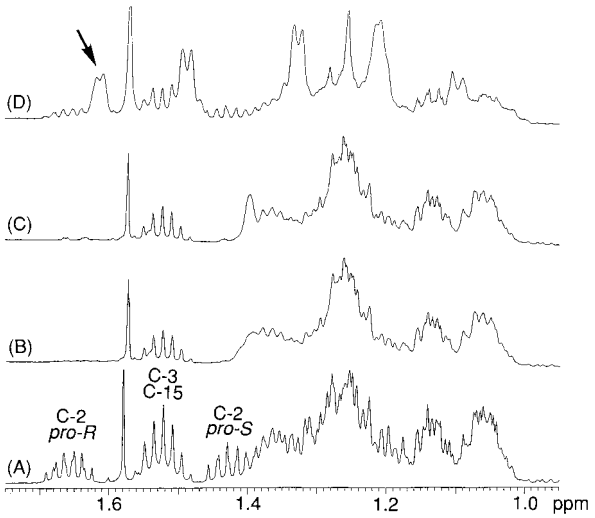


Figure 4. Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of (A) authentic phytanyl acetate, (B) synthesized [2,3-$^2H_2$]phytanyl acetate, (C) synthesized [2,3-$^2H_2$]phytanyl acetate decoupled at the C-1 signal (4.10 ppm), and (D) multiply deuterated phytanyl acetate from *H. japonica* with mevalonolactone-d_9.

signal at C-2, since hydrogenation generally proceeds by *syn* addition. As shown in the ^{1}H NMR spectrum of the chemically dideuterated phytanyl acetate (Figure 4), no signal was observed at 1.66 ppm and a broad signal was observed at 1.39 ppm. The latter was apparently shifted from 1.43 ppm by the isotope shift caused by the introduction of deuterium into the geminal position. Similarly, the ^{2}H NMR spectrum of the chemically deuterated phytanyl acetate clearly showed two peaks at 1.51 and 1.64 ppm (data not shown). Therefore, it appears that the signal at 1.66 ppm was ascribable to the *pro-R* hydrogen at C-2 in the natural diastereoisomer and the signal at 1.43 ppm to *pro-S*. In the ^{1}H NMR spectrum of the multiply deuterated phytanyl acetate prepared from the above-mentioned deuterated lipid by supplementation culture with mevalonolactone-d_9, a broad doublet (J = 7 Hz) signal was observed at 1.61 ppm, which was of course shifted from 1.66 ppm by the isotope effect of the geminal deuterium. The shifted C-3 proton was also observed at 1.49 ppm as a broad doublet (J = 7 Hz). No proton signal was observed at 1.39 ppm.

It now appears that the *pro-S* position of C-2 was deuterated and the *pro-R* position was protonated. Since the stereochemistry at C-3 is *R* configuration in the natural phytanol structure, the hydrogenation or saturase reaction upon C-2 and C-3 of the geranylgeranyl group occurred in *syn* (or *cis*) fashion.

The stereochemical course of the saturation reaction for the remaining double bonds was also analyzed. The ^{1}H−^{13}C COSY spectrum of the authentic phytanyl acetate showed the one-bond ^{1}H−^{13}C connectivities at C-6 and C-10 as shown in Figure 5. The methine protons at C-7 and C-11 appeared as an overlapped signal at 1.37 ppm. Although the carbon signals at C-4, -6, -8, -10, and -12 were not well resolved in the ^{13}C NMR spectrum, the geminal protons of the C-6 and C-10 methylene groups were well separated at 1.05 and 1.27 ppm due to the effect of the nearest stereogenic centers. In the catalytic deuteration of phytol to phytanol, the far remote stereogenic centers had essentially no effect on the proton chemical shifts. Therefore, the chemical shifts of each proton at C-6 and C-10 could be similar to those of the C-2 methylene group. Thus, the signals at 1.05 ppm of C-6 and C-10 were assigned to the *pro-R* protons and the signals at 1.27 ppm of C-6 and C-10 to the *pro-S* protons.[21] These assignments were confirmed by manipulation of the above-

(19) Kates, M.; Yengoyan, L. S.; Sastry, P. S. *Biochim. Biophys. Acta* **1965**, *98*, 252.

(20) Authentic phytanyl acetate was prepared by acetylation under standard conditions (Ac_2O/py) from synthetic stereochemically defined phytanol. See: Eguchi, T.; Arakawa, K.; Terachi, T.; Kakinuma, K. *J. Org. Chem.* **1997**, *62*, 1924.

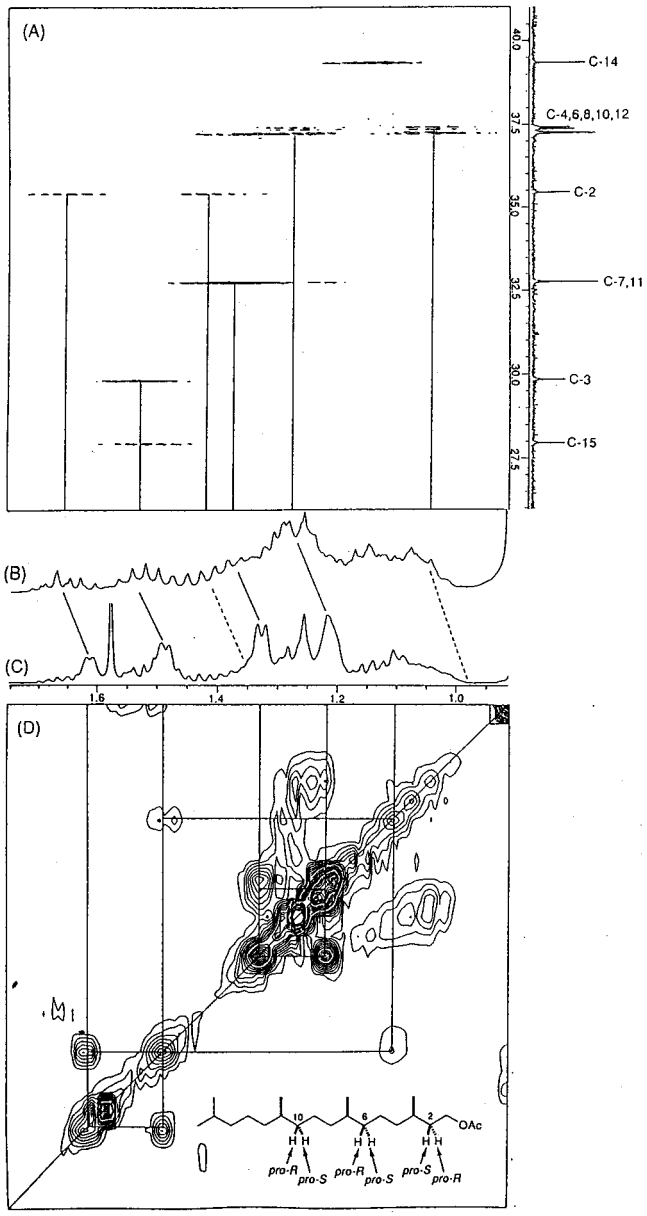


Figure 5. (A) $^1H-^{13}C$ COSY spectrum of authentic phytanyl acetate, (B) partial ^1H NMR spectrum of authentic phytanyl acetate, (C) partial ^1H NMR spectrum of multiply deuterated phytanyl acetate from *H. japonica* with mevalonolactone-d_9, and (D) $^1H-^1H$ COSY spectrum of multiply deuterated phytanyl acetate.

mentioned chemically dideuterated phytanol to sesterterpenyl acetate.[22] Although the latter was again a mixture of epimers at C-6 and C-7, its ^2H NMR spectrum only showed signals at 1.26 (C-6, *pro-S*) and 1.33 ppm (C-7). No signal was observed at 1.05 ppm. In the ^1H NMR spectrum of the multiply deuterated phytanyl acetate, a broad doublet ($J = 7$ Hz) signal due to the methine protons at C-7 and C-11 was clearly observed at 1.33 ppm, which was apparently shifted by an isotope effect of the neighboring deuterium. The $^1H-^1H$ COSY spectrum of

(21) Definition of the C-6 and C-10 prochirality by the *RS* convention is different from that of C-2.

(22) Synthesis of [6,7-$^2H_2$]sesterterpenyl acetate from [2,3-$^2H_2$]phytanol was carried out as follows. [2,3-$^2H_2$]Phytanol (**i**) was treated with I_2-Ph_3P-imidazole in benzene to give iodide **ii** in 93% yield. The reaction between the anion derived from **iii**[20] with butyllithium and iodide **ii** in THF−HMPA at −78 °C smoothly provided the coupling product (**iv**) in 37% yield. Reduction with Na(Hg) in methanol, catalytic hydrogenation, followed by acetylation afforded [6, 7-$^2H_2$]sesterterpenyl acetate **v** in 73% yield. Details are described in the Experimental Section.

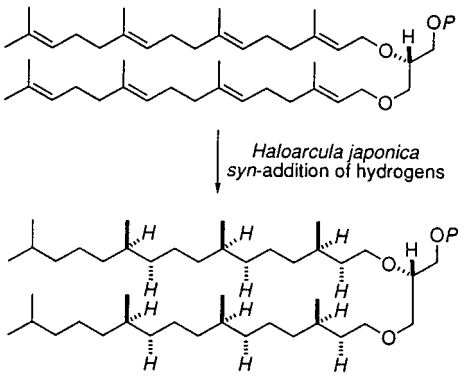


Figure 6. Saturation reaction in the lipid biosynthesis of *H. japonica*.

the multiply deuterated phytanyl acetate, as also shown in Figure 5, clearly showed a cross-peak between 1.33 (H-7 and H-11) and 1.22 ppm (*pro-S* H-6 and *pro-S* H-11). The latter was shifted from 1.27 ppm by the isotope effect. It now appears that the *pro-S* positions of C-6 and C-10 were protonated and the *pro-R* positions were deuterated. Since the stereochemistries at C-7 and C-11 are also *R* configuration in the natural phytanol moieties,[5] the saturation reaction at C-6,7 and C-10,11 of the geranylgeranyl groups also proceeds in a *syn* (or *cis*) fashion as in the case of the C-2,3 position as illustrated in Figure 6. Unfortunately, this present study cannot prove the saturation reaction at C-14,15. It is reasonable to assume the involvement of the same reaction to the double bond.

It seems relevant to point out here that the saturation of the geranylgeranyl group described above differs from the 1,4-addition catalyzed by enoyl reductases in fatty acid biosynthesis.[7] Rather, it is quite similar to the saturation reaction of sterol biosynthesis.[6] Enzymological, genetic, and evolutionary relationships between these reactions are quite intriguing. It should also be pointed out that a more precise mechanism which might include the involvement of any cofactors must await studies on the enzyme level.

In conclusion, the present study now clearly shows that the mevalonate pathway is responsible for the biosynthesis of the phytanyl chains of the core lipid in the archaeal membrane. Saturation of a geranylgeranyl group to a phytanyl group was shown to take place through the addition of hydrogens in a *syn* manner by closely observing the fate of protium by ^1H NMR spectroscopy on the heavily deuterated archaeal lipid. The present study also demonstrates that mevalonolactone-d_9 is a useful tool for studying isoprene metabolism.

Experimental Section

General. IR spectra were taken on a Horiba FT-710 Fourier transform infrared spectrometer. ^1H, ^2H, and ^{13}C NMR spectra were recorded on JEOL LA-300, LA-400, and/or GSX-500 spectrometers. Deuteriochloroform (99.8% atom ^2H, Merck) was used as the solvent for the ^1H and ^{13}C NMR spectra. ^1H, ^2H, and ^{13}C NMR chemical shifts were reported in δ values based on internal TMS ($\delta_H = 0$) or a solvent signal ($CDCl_3$ $\delta^2_H = 7.26$, $CDCl_3$ $\delta_C = 77.0$) as the reference. *J* values are given in hertz. Mass spectra were obtained by using a JEOL AX-505HA mass spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 apparatus. Column chromatography was carried out with Kieselgel 60 (70−230 mesh, Merck). All reactions, except catalytic hydrogenation, were carried out in an inert (Ar or N_2) atmosphere. THF and ether were distilled from sodium/benzophenone ketyl prior to use. Pyridine and CH_2Cl_2 were distilled from potassium hydroxide and calcium hydride, respectively. Deuterium oxide (99.8% atom ^2H) and $LiAl^2H_4$ (99% atom ^2H) were purchased from Merck and Isotec Inc., respectively.

(3,4-Dimethoxyphenyl)[1,1,3,3,3-$^2H_5$]acetone (1b). To a biphasic solution of 3,4-dimethoxyphenylacetone (**1a**, 8.12 g, 41.8 mmol) and

deuterium oxide (6.3 g, 314 mol), K_2CO_3 (144 mg, 1.04 mmol) was added at room temperature. The mixture was stirred for 16 h at 70 °C. After cooling to room temperature, the mixture was extracted twice with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. This procedure was repeated five times to give **1b** (8.29 g, quant.). ^{1}H NMR (300 MHz): δ 3.87 (s, 6H), 6.71−6.85 (m, 3H). ^{13}C NMR (75 MHz): δ 28.04 (septet, J = 19.1), 49.62 (quintet, J = 19.1), 55.59, 111.17, 112.10, 121.29, 126.42, 147.87, 148.83. IR (neat): 1028, 1141, 1161, 1246, 1326, 1415, 1465, 1515, 1589, 1716, 2837, 2910, 2937, 2956, 3001 cm^{-1}. Anal. Calcd for $C_{11}H_9{}^2H_5O_3$: C, 66.31; H + ^{2}H, 7.08. Found: C, 66.26; H + ^{2}H, 7.13.

Trimethyl Phosphono[2,2-$^2H_2$]acetate (2b).[14] To a mixture of trimethyl phosphonoacetate (**2a**, 15.4 g, 84.5 mmol) and deuterium oxide (8.0 g, 40 mmol), K_2CO_3 (150 mg, 1.09 mmol) was added. The solution was stirred for 1.5 h at room temperature and was then extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated to dryness. This procedure was repeated twice to give **2b** (14.1 g, 76.6 mol, 91%). ^{1}H NMR (300 MHz): δ 3.76 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz): δ 32.58 (double quintet, J = 134.5, 19.1), 52.57, 53.08 (d, J = 6.8), 165.99 (d, J = 6.2). IR (neat): 1259, 1736, 2139, 2187, 2243, 2854, 2958, 3001 cm^{-1}.

Methyl 3-[$^2H_3$]Methyl-4-(3,4-dimethoxyphenyl)[2,4,4-$^2H_3$]but-2-enoate (3). Sodium hydride (4.22 g, 60% in mineral oil, 105 mmol) was washed with hexane under Ar, and then THF (250 mL) was added. A solution of **2b** (19.4 g, 105 mmol) in THF (100 mL) was added dropwise at 0 °C, and the mixture was stirred by a mechanical stirrer for 2.5 h at room temperature. The mixture was recooled to 0 °C, and a solution of **1b** (21.0 g, 105 mmol) in THF (100 mL) was added. The resulting mixture was stirred for 1 h at 0 °C and for 25 h at room temperature. Water was then added, and the mixture was extracted five times with ether. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (6:1−3:1) to give oily **3** (21.4 g, 80%) as a geometrical mixture (E/Z = 3). ^{1}H NMR (300 MHz): δ 3.68 (s, *E* isomer), 3.73 (s, *Z* isomer), 3.85 (s, 3H), 3.86 (s, 3H), 6.65−6.82 (m, 3H). IR (neat): 1029, 1058, 1101, 1141, 1163, 1196, 1225, 1327, 1361, 1411, 1463, 1516, 1589, 1630, 1716, 2834, 2908, 2948, 2999 cm^{-1}. Anal. Calcd for $C_{14}H_{12}{}^2H_6O_4$: C, 65.60; H + ^{2}H, 7.08. Found: C, 65.45; H + ^{2}H, 7.08.

2,3-Epoxy-5-(3,4-dimethoxyphenyl)-3-[$^2H_3$]methyl[1,1,2,4,4-$^2H_5$]-pentan-1-ol (4). To a solution of lithium aluminum deuteride (4.30 g, 103 mmol) in ether (250 mL), a solution of **3** (21.0 g, 82.0 mmol) in ether (50 mL) was added at 0 °C. The mixture was stirred at 0−10 °C for 10 h. Saturated aqueous NH_4Cl solution was carefully added at 0 °C, followed by EtOAc. The insoluble matter was filtered and washed with EtOAc. The filtrate and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (4:1−1.5:1) to give a crude product (16.2 g), which was used for the next step without further purification. A mixture of the crude product (15.9 g) and VO(acac)$_2$ (366 mg, 1.38 mmol) in benzene (300 mL) was stirred under reflux. A solution of *tert*-butyl hydroperoxide (5−6 M solution in decane, 13.8 mL) was carefully added. Refluxing was continued for 2.5 h. After the mixture was cooled to room temperature, aqueous NaS_2O_3 was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (2.5:1−1:1) to give **4** as an oil (15.3 g, 76%). ^{1}H NMR (300 MHz): δ 2.27 (br, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 6.72−6.82 (m, 3H). IR (neat): 1025, 1250, 1589, 2102, 2220, 2588, 2837, 2937, 3465 cm^{-1}. Anal. Calcd for $C_{13}H_{10}{}^2H_8O_4$: C, 63.39; H + ^{2}H, 7.37. Found: C, 63.11; H + ^{2}H, 7.43.

5-(3,4-Dimethoxyphenyl)-3-[$^2H_3$]methyl[1,1,2,2,4,4-$^2H_6$]pentane-1,3-diol (5). To a mixture of lithium aluminum deuteride (2.26 g, 46.3 mmol) in THF (200 mL), a solution of **4** (15.2 g, 61.7 mmol) in THF (50 mL) was added. The mixture was stirred at room temperature for 2.5 h and then at 50 °C for 3 h. After the mixture was cooled to room temperature, saturated aqueous NH_4Cl solution was carefully added, followed by EtOAc. The insoluble matter was filtered and washed with EtOAc. The filtrate and washings were combined and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (1:1) and then EtOAc to give **5** as a colorless powder (12.1 g, 79%). Mp 73.5−75 °C. ^{1}H NMR (300 MHz): δ 2.40 (br, 1H), 2.90 (br, 1H), 3.87 (s, 3H), 3.88 (s, 3H), 6.73−6.84 (m, 3H). ^{13}C NMR (75 MHz): δ 25.46 (septet, J = 18.5), 40.69 (quintet, J = 19.1), 47.48 (quintet, J = 18.5), 55.83, 58.87 (quintet, J = 21.6), 73.23, 110.98, 113.76, 122.52, 129.39, 147.76, 148.56. IR ($CHCl_3$): 1029, 1049, 1064, 1141, 1167, 1209, 1220, 1244, 1263, 1324, 1413, 1442, 1465, 1515, 1587, 1606, 2107, 2225, 2911, 2937, 2960, 3010, 3023, 3492 cm^{-1}. Anal. Calcd for $C_{13}H_{11}{}^2H_9O_4$: C, 62.62; H + ^{2}H, 8.08. Found: C, 62.43; H + ^{2}H, 8.08.

1,3-Diacetoxy-5-(3,4-dimethoxyphenyl)-3-[$^2H_3$]methyl[1,1,2,2,4,4-$^2H_6$]pentane (6). A solution of **5** (12.0 g, 48.1 mmol), Et_3N (20.1 mL, 144 mmol), Ac_2O (18.1 mL, 192 mmol), and DMAP (1.50 g, 12.3 mmol) in CH_2Cl_2 (100 mL) was stirred for 44 h at room temperature. Water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (3:1) to give **6** as an oil (14.9 g, 93%). ^{1}H NMR (300 MHz): δ 2.00 (s, 3H), 2.05 (s, 3H), 3.87 (s, 6H), 6.71−6.82 (m, 3H). ^{13}C NMR (100 MHz): δ 20.84, 22.32, 22.87 (septet, J = 19.7), 35.53 (quintet, J = 18.3), 43.17 (quintet, J = 17.5), 55.68, 59.72 (quintet, J = 16.6), 82.41, 110.74, 113.58, 122.53, 128.79, 147.69, 148.33, 170.41, 170.86. IR (neat): 1030, 1246, 1265, 1517, 1587, 1606, 1734, 2131, 2237, 2835, 2937 cm^{-1}. Anal. Calcd for $C_{17}H_{15}{}^2H_9O_6$: C, 61.24; H + ^{2}H, 7.26. Found: C, 61.44; H + ^{2}H, 7.43.

3,5-Diacetoxy-3-[$^2H_3$]methyl[1,1,2,2,4,4-$^2H_6$]pentanoic Acid (7). To a mixture of **6** (8.8 g, 26.5 mmol), CCl_4 (100 mL), CH_3CN (100 mL), and phosphate buffer (pH 7.0, 120 mL), $NaIO_4$ (90.5 g, 423 mmol) and $RuCl_3 \cdot 3H_2O$ (500 mg) were added at 0 °C. The mixture was stirred at 0−20 °C for 2 h, and then ether (100 mL) was added. The mixture was filtered and washed with CH_2Cl_2. The organic layer of the filtrate and washings were combined and concentrated to dryness. The residue was chromatographed over silica gel with $CHCl_3$−methanol (10:1) to give **7** as an oil (5.55 g, 87%). ^{1}H NMR (300 MHz): δ 2.02 (s, 3H), 2.05 (s, 3H), 10.25 (br, 1H). ^{13}C NMR (75 MHz): δ 20.86, 22.10, 23.38 (septet, J = 19.1), 35.93 (quintet, J = 19.7), 41.87 (quintet, J = 19.4), 59.44 (quintet, J = 22.2), 79.47, 170.55, 171.11, 175.51. IR (neat): 1248, 1373, 1734, 2135, 2241, 3170 cm^{-1}. Anal. Calcd for $C_{10}H_7{}^2H_9O_6$: C, 49.78; H + ^{2}H, 6.68. Found: C, 49.57; H + ^{2}H, 6.87.

Mevalonolactone-d_9 (8). To a solution of **7** (1.07 g, 4.42 mmol) in methanol (20 mL), K_2CO_3 (1.20 g, 8.68 mmol) was added. The mixture was stirred at room temperature for 5 h. Water was added, and then 2 N HCl was added until pH 2. The mixture was extracted several times with $CHCl_3$. The combined organic layer was concentrated to dryness. The residue was chromatographed over silica gel with ether to give **8** as an oil (520 mg, 85%). ^{1}H NMR (300 MHz): δ 3.1 (br). ^{13}C NMR (75 MHz): δ 28.38 (septet, J = 19.7), 34.52 (quintet, J = 19.8), 43.71 (quintet, J = 19.8), 65.49 (quintet, J = 19.8), 67.39, 171.38. ^{2}H NMR (61 MHz, $CHCl_3$): 4.59 (1 ^{2}H), 4.30 (1 ^{2}H), 2.60 (1 ^{2}H), 2.48 (1 ^{2}H), 1.82 (2 ^{2}H), 1.30 (3 ^{2}H). IR (neat): 1064, 1300, 1714, 2121, 2227, 3419 cm^{-1}. Anal. Calcd for $C_6H_1{}^2H_9O_3$: C, 51.77; H + ^{2}H, 7.24. Found: C, 51.66; H + ^{2}H, 7.47.

Bacterial Culture and Lipid Extraction. *Haloarcula japonica* JCM7785 was obtained from RIKEN, and the cultivation was carried out for 9 days at 37 °C in a medium containing 10 g of yeast extract, 7.5 g of casamino acid, 200 g of NaCl, 20 g of $MgSO_4 \cdot 7H_2O$, 3 g of trisodium citrate$\cdot 2H_2O$, 2 g of KCl, 50 mg of $FeSO_4 \cdot 7H_2O$, and 1.0 g of mevalonolactone-d_9 in 1 L of water (pH 7.4). Cells were harvested by centrifugation (6000−9000 rpm, 30 min) and were washed with water (wet cells, 6.0 g). Lipids were extracted from the wet organism by the procedure of Collins et al.[18] The polar lipids were separated from nonpolar lipids by precipitation in cold acetone to give 32 mg of a polar lipids fraction.

Methanolysis of the Polar Lipids Fraction. Methanolic HCl (3%, 10 mL) was added to the polar lipids fraction (32 mg), and the mixture was refluxed for 13 h. After cooling to room temperature, the solution was evaporated and the residue was extracted with hexane (five 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−EtOAc (10:1) to give multiply deuterated *sn*-2,3-di-*O*-phytanylglycerol (18 mg).

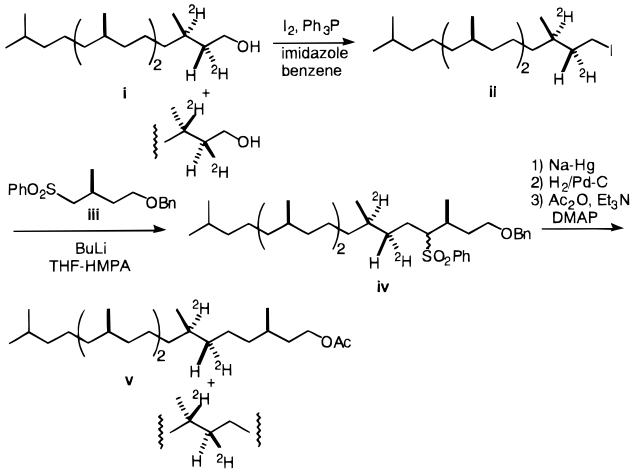
Multiply Deuterated 2,3-Di-*O*-phytanyl-*sn*-glycerol Benzoate. To a solution of multiply deuterated *sn*-2,3-di-*O*-phytanylglycerol (18 mg) in pyridine (5 mL), benzoyl chloride (8 drops) was added at 0 °C. The solution was stirred at 0 °C for 1 h. Water was then added, and the aqueous layer was extracted with hexane (four times). The combined organic layer was successively washed with 2 N HCl, saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed over silica gel with hexane−EtOAc (50:1) to give multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate (15 mg).

Multiply Deuterated Phytanyl Acetate. Hydroiodic acid (3.0 mL, 57%) was added to multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate (15 mg), and the mixture was refluxed for 24 h. After the mixture was cooled to room temperature, water was added and aqueous solution was extracted with hexane (four times). The combined organic layer was successively washed with saturated aqueous $NaHCO_3$ solution, aqueous NaS_2O_3 solution, and brine, dried (Na_2SO_4), filtered, and concentrated to give a crude iodide (17 mg). Silver acetate (130 mg) was added to a solution of the iodide (17 mg) in acetic anhydride, and the mixture was refluxed for 20 h. After the mixture was cooled to room temperature, water was added and the mixture was extracted with ether. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed over silica gel with hexane−ether (200:3) to give multiply deuterated phytanyl acetate (9 mg).

[2,3-$^2H_2$]Phytanyl Acetate. A mixture of phytol (990 mg, 3.34 mmol) and PtO_2 (15 mg, 0.07 mmol) in CH_3O^2H (3 mL) was stirred at room temperature for 13 h under an atmospheric pressure of deuterium gas. The catalyst was filtered and washed with methanol. The filtrate and washings were combined and concentrated to give dideuterated phytanol (1.0 g), which was used for the next step without further purification. Acetic anhydride (0.2 mL) was added to a solution of dideuterated phytanol (100 mg) in pyridine (3 mL) at 0 °C. The mixture was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with hexane (three times). The combined organic layer was successively washed with 2 N HCl, saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed over silica gel with hexane−ether (200:3) to give [2,3-$^2H_2$]phytanyl acetate (105 mg, 92%). ^{1}H NMR (400 MHz): δ 0.84−0.89 (m, 15H), 1.0−1.4 (m, 21H), 1.52 (m, J = 6.7, 1H), 2.05 (s, 3H), 4.09 (m, 2H). ^{13}C NMR (75 MHz): δ 19.31, 19.38, 19.68, 19.73, 21.01, 22.60, 22.70, 24.24, 24.46, 24.78, 27.96, 29.25 (t, J = 19.1), 32.77, 34.97 (t, J = 19.1), 35.03 (t, J = 19.1), 37.08, 37.11, 37.22, 37.27, 37.37, 37.42, 37.46, 39.36, 63.02, 171.19. ^{2}H NMR (61 MHz, $CHCl_3$): δ 1.51 (br), 1.64 (br). Anal. Calcd for $C_{22}H_{42}{}^2H_2O_2$: C, 77.13; H + ^{2}H, 12.94. Found: C, 77.35; H + ^{2}H, 13.14.

[2,3-$^2H_2$]Phytanyl Iodide (ii). To a solution of [2,3-$^2H_2$]phytanol (200 mg, 0.67 mmol), PPh_3 (436 mg, 1.7 mmol), and imidazole (113 mg, 1.7 mmol) in benzene (25 mL) was added I_2 (338 mg, 1.3 mmol). The mixture was stirred for 30 min at room temperature. Aqueous $Na_2S_2O_3$ was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (50:1) to give [2,3-$^2H_2$]phytanyl iodide **ii** as an oil (255 mg, 93%). ^{1}H NMR (300 MHz): δ 0.83−0.88 (m, 15H), 1.0−1.4 (m, 20H), 1.52 (nonet, J = 6.5Hz, 1H), 1.61 (t, J = 6.3, 1H), 3.16 (t, J = 8.9, 1H), 3.25 (dd, J = 5.9, 9.5, 1H). ^{13}C NMR (75 MHz): δ 5.40, 18.55, 18.60, 19.70, 19.76, 22.63, 22.73, 24.16, 24.45, 24.47, 24.79, 27.96, 32.73, 32.74, 32.77, 33.25 (t, J = 17.2), 36.39, 36.46, 37.16, 37.21, 37.26, 37.34, 37.41, 37.45, 39.35, 40.39 (t, J = 19.4), 40.46 (t, J = 19.7). IR (neat): 1190, 1282, 1365, 1377, 1462, 2156, 2854, 2868, 2925, 2954 cm^{-1}. Anal. Calcd for $C_{25}H_{49}{}^2H_2I$: C, 58.52; H + ^{2}H, 10.07. Found: C, 58.82; H + ^{2}H, 10.16.

Phenyl Sulfone (iv). To a solution of sulfone **iii**[20] (94.7 mg, 0.30 mmol) in degassed THF (1.5 mL) was added BuLi (1.61 M hexane solution, 0.37 mL, 0.59 mmol) at −78 °C. The mixture was stirred for 30 min at −78 °C and then at −20 °C for 30 min. The solution was recooled to −78 °C, and HMPA (0.3 mL) and a solution of [2,3-$^2H_2$]phytanyl iodide (99.6 mg, 0.24 mmol) in THF (1.5 mL) were added. After 30 min, saturated aqueous NH_4Cl solution was carefully added and the mixture was extracted four times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−EtOAc (20:1−3:1) to give **iv** as an oil (54.3 mg, 37%). ^{1}H NMR (300 MHz): δ 0.7−1.1 (m, 18H), 0.9−2.0 (m, 26H), 1.5−2.4 (m, 2H), 2.2−2.5 (m, 1H), 2.87−3.07 (m, 1H), 3.36−3.62 (m, 2H), 4.40 (dd, J = 11.9, 13.6, 1H), 4.49 (dd, J = 11.3, 20.7, 1H), 7.23−7.38 (m, 5H), 7.44−7.65 (m, 3H), 7.84−7.86 (m, 2H). IR (neat): 1028, 1086, 1101, 1146, 1205, 1304, 1365, 1377, 1446, 1462, 1585, 2143, 2866, 2925, 2952 cm^{-1}.



[6,7-$^2H_2$]Sesterterpenyl Acetate (v). To a solution of **iv** (54.3 mg, 0.90 mmol) in THF−MeOH (1:1, 15 mL), 5% sodium amalgam (4.1 g) was added. After the solution was stirred for 2.5 h at room temperature, saturated aqueous NH_4Cl solution was carefully added and the insoluble matter was filtered off and washed with EtOAc. The filtrate and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was dissolved in EtOAc (5 mL), and 10% Pd−C (95 mg) was added. The mixture was stirred under an atmosphere of hydrogen. After 1.5 h, the catalyst was filtered off and washed with EtOAc. The filtrate and washings were combined and concentrated to dryness. The residue was dissolved in CH_2Cl_2 (3 mL). Acetic anhydride (20 drops), Et_3N (20 drops), and DMAP (5 mg) were added. The mixture was stirred for 2 h at room temprature. Water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane−ether (200:3) to give [6,7-$^2H_2$]sesterterpenyl acetate (**v**) as an oil (27 mg, 73%). ^{1}H NMR (300 MHz): δ 0.83−0.91 (m, 18H), 0.95−1.72 (m, 29H), 2.05 (s, 3H), 4.03−4.16 (m, 2H). ^{13}C NMR (75 MHz): δ 19.46, 19.53, 19.60, 19.73, 19.78, 21.06, 22.62, 22.72, 24.15, 24.44, 24.79, 27.96, 29.69, 29.80, 29.81, 32.14 (t, J = 18.5), 32.78, 35.42, 35.51, 36.69 (t, J = 19.1), 36.75 (t, J = 18.5), 37.18, 37.21, 37.27, 37.30, 37.33, 37.38, 37.43, 37.48, 39.38, 63.09, 171.25. ^{2}H NMR (61 MHz): δ 1.26, 1.33. IR (neat): 1036, 1053, 1238, 1365, 1377, 1462, 1743, 2139, 2867, 2925, 2954 cm^{-1}. Anal. Calcd for $C_{27}H_{52}{}^2H_2O_2$: C, 78.57; H + ^{2}H, 13.19. Found: C, 78.47; H + ^{2}H, 13.27.

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Supporting Information Available: ^{1}H, ^{2}H, ^{13}C NMR, and IR spectra of **8** (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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