Evidence for the Biosynthesis of Squalene via the Methylerythritol Phosphate Pathway in a *Streptomyces* sp. Obtained from a Marine Sediment

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Squalene (**1**), an all-trans polyene originally isolated from shark liver oil in the 1920s, is an obligatory intermediate in the biosynthesis of triterpenoids and steroids.[1] Its symmetrical C_{30} structure arises from reductive condensation of two molecules of 2*E*,6*E*-farnesyl pyrophosphate in a tail-to-tail fashion. The biochemical transformation that leads to steroids from acetate via isopentenylpyrophosphate (IPP), dimethylallylpyrophosphate (DMAPP), and **1** has been studied in exceptional detail in eukaryotes. Recognition of the role of cholesterol in heart disease has made many of the enzymes involved in this biosynthetic pathway important targets for developing cholesterol-lowering drugs. Squalene and various reduced analogues are also known to be common constituents of the nonpolar lipids produced by many prokaryotes, including both archeabacteria and eubacteria. For example, they have been isolated from cultures of halophiles,[2] methanogens,[3] and thermoacidophiles[3] and, within the eubacteria, from several Actinomycetes of the genus *Streptomyces*.[4] As a result, reduced analogues of squalene are commonly used as markers of microbial contribution to the production of petroleum and marine sediment hydrocarbons.[5]

Until quite recently, it was thought that IPP and DMAPP, the monomeric units in terpenoid biosynthesis, were made exclusively by the mevalonate pathway (MVA).[6] However, a growing body of experimental evidence has shown that there is an alternative nonmevalonate pathway, named the "methylerythritol phosphate" (MEP) pathway,[7] for the formation of IPP and DMAPP. The newly discovered MEP pathway has been observed in eubacteria, algae, and plant chloroplasts[8] but it does not appear to be present in archeabacteria and animals. Interestingly, some Actinomycetes are capable of making terpenoids via either the MVA or MEP pathways. These organisms can switch from one pathway to the other at different stages of the growth cycle, with the MEP route active during log phase and the MVA route active during stationary phase. [9]

Although some steps of the MEP pathway are still unknown (Scheme 1), five enzymes involved in the nonmevalonate biosynthesis of IPP have been discovered and characterized.[8a−c,10a−c] The MEP pathway starts with the formation of 1-deoxy-D-xylulose-5-phosphate (DXP) from glyceraldehyde 3-phosphate (GAP) and pyruvate. The reaction is catalyzed by DXP synthase and is similar to that of transketolases, with a two-carbon transfer of an "activated acetaldehyde" to GAP. The five-carbon intermediate of DXP is then transformed into 2-*C*-methylerythritol 4-phosphate (MEP), the first committed intermediate in the pathway, by DXP reductoisomerase. In two further steps, MEP undergoes phosphorylations catalyzed by the pyrophosphorylase MEP cytidyltransferase and the kinase cytidine-5′-diphospho-MEP kinase.[10a,b] The end product of these transformations is 4-(cytidine 5′-diphospho)-MEP 2-phosphate (CDP-ME2P), which is converted to MEP 2,4-cyclodiphosphate (MECDP) by the action of MECDP synthase.[10c] MECDP accumulates in several bacteria under oxidative stress conditions, and recently it has been reported that ^{14}C-labeled MECDP is incorporated into carotenoids in plant chloroplast preparations.[10d]

As part of an ongoing program to examine the secondary metabolites produced by microorganisms isolated from marine habitats,[11] it was found that laboratory cultures of a *Streptomyces* sp. (isolate QC45B) obtained from a sediment sample collected at a depth of ca. 40 m off the Queen Charlotte Islands in British Columbia produced significant quantities of squalene (SQ, **1**), 2,3-dihydrosqualene (DHSQ, **2**), tetrahydrosqualene (THSQ, **3**), and other more fully reduced analogues. In this paper, we report evidence for the biosynthesis of **1**−**3** in cultures of the isolate QC45B via the MEP pathway. The involvement of the MEP pathway has been supported by feeding experiments with [1-^{13}C]-D-glucose and [4,4-$^{2}H_2$]-2-*C*-

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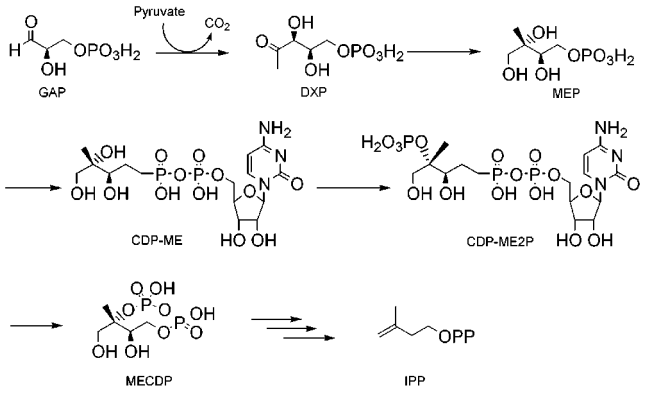
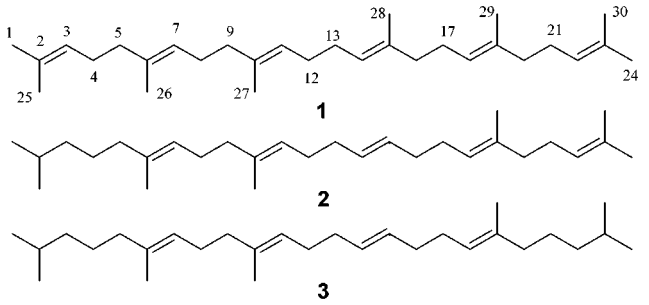
Scheme 1. Biosynthesis of Isopentenylpyrophosphate (IPP) via the Mevalonate-Independent Pathway (MEP)



methylerythritol (**4**). As part of this investigation, optically active **4** has been prepared following the approach recently utilized for the synthesis of 2-*C*-methylerythritol[12a] (ME) and of 2-*C*-methylerythritol 4-phosphate (MEP).[12b] To date, there have been no other direct investigations of the nonmevalonate origin of squalene in bacteria.



Experimental Section

Materials. Chemicals were obtained from Aldrich Chemicals and were used without further purification. All the organic solvents were distilled prior their use. NMR spectra were recorded at room temperature on Bruker 400 and 300 MHz spectrometers. ^{1}H and ^{13}C chemical shifts are reported in ppm from $CHCl_3$ (δ 7.26 and 77.0). Optical rotations were measured on a JASCO DIP370 polarimeter. Column chromatographs were performed on Waters Sep-Pak Vac (2 g) silica cartridges. GC−MS analyses were run on a Hewlett Packard apparatus equipped with a 5989 EI mass spectrometer.

Extraction and Isolation of 1−3. Frozen pellets of bacteria were covered by MeOH and sonicated for 2 min. The MeOH was filtered through paper, and the residue was extracted twice with $CHCl_3$/MeOH 1:1. The solvents were filtered and combined with the MeOH extract. After removal of the organic solvents, the resulting residues were diluted with distilled H_2O (6 mL) and extracted with *n*-hexane (5 mL × 3) and Et_2O (5 mL × 2). The organic layers were combined, dried over Na_2SO_4, and concentrated in vacuo. The residues were redissolved in *n*-hexane, applied to a silica cartridge, and eluted with *n*-hexane and *n*-hexane/Et_2O 90:10. A final purification of **1**−**3** was obtained by normal-phase HPLC with *n*-heptane as eluant. Squalene (**1**): colorless oil; EIMS *m*/*z* 410 (8, $C_{30}H_{50}$), 341 (15), 137 (35), 123 (25), 81 (80), 69 (100); for ^{13}C NMR data see the Supporting Information. Dihydrosqualene (**2**): colorless oil; HR EIMS *m*/*z* 412.40658 (calcd 412.40690 for $C_{30}H_{52}$); EIMS *m*/*z* 412 (25, $C_{30}H_{52}$), 369 (15), 343 (30), 81 (60), 69 (100); for ^{13}C NMR data see the Supporting Information. Tetrahydrosqualene (**3**): colorless oil; EIMS *m*/*z* 414 (15, $C_{30}H_{54}$), 371 (20), 123 (100), 83 (35), 69 (95); for ^{13}C NMR data see the Supporting Information.

[1,1-$^2H_2$]-4-Benzyloxy-3-methylbut-2-en-1-ol (7). Under argon at −78 °C, 690 mg (3.14 mmol) of **6**, prepared in agreement with ref 12b, in dry THF (12 mL) was treated with a solution of AlD_3 (18.8 mmol) prepared at 0 °C from $AlCl_3$ (628 mg, 4.71 mmol) and $LiAlD_4$ (592 mg, 14.1 mmol). The reaction mixture was stirred for 6 h at −78 °C, and then the mixture was warmed to 0 °C over 1 h. After addition of MeOH/H_2O 1:1 (12 mL), the white suspension was vigorously stirred for 10 min at room temperature. Then, Et_2O (15 mL) was added to the slurry solution and the resulting mixture was filtered off. The white residues were washed twice with fresh Et_2O and the clear filtrates were combined and evaporated to dryness at reduced pressure. Purification on SiO_2 column (10 g, *n*-hexane/ethyl acetate 80:20) gave the d_2-labeled **7** (oil, 475 mg, 2.45 mmol, 78%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.72 (s, 3H, H_3-5), 3.93 (s, 2H, H_2-4), 4.49 (s, 2H, H_2−Bn), 5.69 (s, 1H, H-2), 7.34−7.36 (bs, 5H, Bn); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.0 (C-5), 72.0 (C-4), 75.3 (Bn), 125.9 (C-3), 127.6 (Bn), 127.7 (Bn), 128.4 (Bn), 135.8 (C-2), 138.3 (Bn); CIMS (NH_3) *m*/*z* 212 (25, M + NH_4^+), 194 (10, M^+), 91 (100).

[1,1-$^2H_2$]-(2*S*,3*S*)-4-Benzyloxy-2,3-epoxy-3-methylbutan-1-ol (8). Under argon atmosphere, 1.0 g (4.89 mmol) of (+)-diethyl tatrate (DET) was dissolved in 4.5 mL of dry CH_2Cl_2 in an oven-dried flask. Through a septum, neat Ti(IV) isopropoxide (1.11 g, 3.92 mmol) was added to the solution at −23 °C. After the resulting mixture was stirred for 10 min at the same temperature, **7** (473 mg, 2.44 mmol) in dry CH_2Cl_2 (4.5 mL) was added. The yellow mixture was stirred for another 10 min at −23 °C prior the addition of *t*-BuOOH in nonane (1367 μL, 7.52 mmol). The resulting mixture was stirred at the same temperature for 30 min and then kept in the freezer (−20 °C) for 16 h without stirring. The reaction was quenched with 3 mL of H_2O. After being stirred at −23 °C for 45 min, the mixture was treated with 15% NaOH (4 mL) and stirred at room temperature for an additional 45 min. The resulting suspension was partitioned between CH_2Cl_2 and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, hexane/EtOAc 80:20) to give **8** (452 mg, 2.15 mmol, 88%) as a colorless oil: $[\alpha]^{20}_D$ −1.7° (*c* 10.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 3H, H_3-5), 3.11 (s, 1H, H-2), 3.46 (d, *J* = 11.0 Hz, 1H, H-4a), 3.51 (d, *J* = 11.0 Hz, 1H, H-4b), 4.52 (d, *J* = 12.0 Hz, 1H, H−Bn), 4.58 (d, *J* = 12.0 Hz, 1H, H−Bn), 7.33−7.35 (bs, 5H, Bn1); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.5 (C-5), 60.0 (C-3), 60.3 (C-2), 73.2 (C-4), 74.1 (Bn), 127.7 (Bn), 128.4 (Bn), 137.8 (Bn); CIMS (NH_3) *m*/*z* 228 (30, M + NH_4^+), 212 (10), 91 (100).

[4,4-$^2H_2$]-(2*S*,3*R*)-1-Benzyloxy-2-*C*-methylerythritol (9). The epoxy alcohol **8** (449 mg, 2.14 mmol) was dissolved in 7 mL of *t*-BuOH. Distilled H_2O (7 mL) and 0.5 M NaOH (7 mL) were added under stirring to the clear solution. The reaction mixture was stirred at 78 °C for 24 h and then extracted with EtOAc. The organic layer was dried over Na_2SO_4, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) to give **9** (345 mg, 1.51 mmol, 70%) as a colorless oil: $[\alpha]^{20}_D$ + 2.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.17 (s, 3H, H_3-5), 3.38 (d, *J* = 9.4 Hz, 1H, H-1a), 3.46 (d, *J* = 9.4 Hz, 1H, H-1a), 3.58 (bs, 1H, −OH), 3.61 (bd, *J* = 3.5 Hz,1H, H-3), 3.80, (bs, 1H, −OH), 4.51 (s, 2H, Bn), 7.28−7.33 (m, 5H, Bn); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 20.9 (C-5), 62.3 (small and broad signal, C-4), 73.4 (C-2), 73.6 (C-1), 74.9 (Bn), 75.4 (C-3), 127.6 (Bn), 127.8 (Bn), 128.4 (Bn), 137.6 (Bn); FAB$^+$ (thioglycerol) *m*/*z* 229 (M + H)$^+$, 103, 91.

[4,4-$^2H_2$]-2-*C*-Methylerythritol (4). A solution of **9** (340 mg, 1.49 mmol) in 2.6 mL of EtOH was hydrogenated by H_2 and catalytic 10% Pd/C at room temperature for 5 h. The catalyst was filtered off, and the filtrate was evaporated to dryness to give 202 mg (1.48 mmol, quantitative) of **4** as an amorphous white solid: $[\alpha]^{20}_D$ +7.5° (*c* 3.3 in MeOH); ^{1}H NMR (CD_3OD) δ 1.01 (s, 3H, H_3-5), 3.34 (d, *J* = 10.8 Hz, 1H, H-1a), 3.43 (d, *J* = 10.8 Hz, 1H, H-1b), 3.50 (s, 1H, H-3); ^{13}C NMR (CD_3OD) δ 19.7 (C-5), 68.4 (C-1), 74.9 (C-2), 76.0 (C-3); ^{2}H NMR (CH_3OH) δ 3.53 (bs, ^{2}H-4a), 3.74 (bs, ^{2}H-4b); FAB$^+$ (thioglycerol) *m*/*z* 137 (M + H)$^+$, 91.

Feeding Experiements. Stable isotope feeding experiments with QC45B were carried out in liquid culture using a medium

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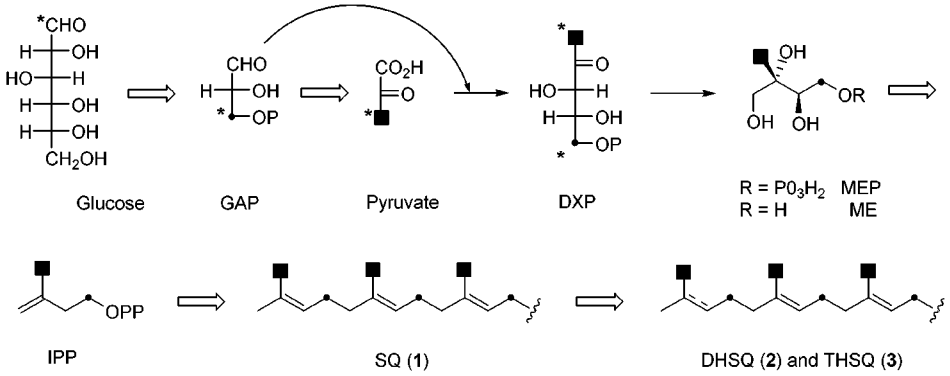
Scheme 2. Labeling Pattern of 1−3 in Feeding Experiments with [1-^{13}C]-Glucose



of tryptic soy broth with added sodium chloride (5 g/L). Typically, labeled precursors were dissolved in sterile water and added to 500 mL of the culture medium containing a fresh inoculum of QC45B in 1 L flasks. The flasks were placed on a shaker table for 8 days at room temperature and then harvested by centrifugation. The *n*-hexane-soluble material from the EtOAc extracts of the cell pellet was fractionated on a silica gel Sep-Pak column (eluent: *n*-hexane) to give a pool of C-30 linear terpenoids (approximately 5−6 mg per liter of broth) from which squalene (**1**), dihydrosqualene (**2**) and tetrahydrosqualene (**3**) were further purified by normal-phase HPLC and identified by NMR and GC−MS analysis.

GC−MS Analysis of Labeled Isoprenoids from Feeding Experiment with [4,4-^{2}H$_2$]-2-*C*-Methylerythritol. Squalene (**1**) (t_R 15.5 min), dihydrosqualene (**2**) (t_R 14.9 min), and tetrahydrosqualene (**3**) (t_R 14.6 min) were analyzed on GC−MS by a linear gradient of 3 °C/min from 220 to 300 °C. Isotopically abundant natural SQ (**1**): *m/z* 410.3 (100, M_0), 411.4 (35.8, M_1), 412.3 (7.2, M_2). Labeled SQ: *m/z* 410.3 (100, M_0), 411.4 (35.7, M_1), 412.3 (10.3, M_2). Isotopically abundance natural DHSQ (**2**): EIMS *m/z* 412.4 (100, M_0), 413.3 (36.0, M_1), 414.4 (6.4, M_2). Labeled DHSQ: *m/z* 412.4 (100, M_0), 413.3 (37.0, M_1), 414.4 (14.3, M_2). Isotopically abundance natural THSQ (**3**): EIMS *m/z* 414.4 (100, M_0), 415.4 (35.8, M_1), 416.4 (6.9, M_2). Labeled THSQ: *m/z* 414.4 (100, M_0), 415.4 (35.8, M_1), 416.4 (15.6, M_2).

Results and Discussion

In a first set of experiments, cultures of *Streptomyces* sp. QC45B were grown in the presence of [6,6-^{2}H$_2$]glucose (approximately 250 mg in 4 L of broth). Purified samples of SQ (**1**, 1.0 mg) and THSQ (**3**, 1.0 mg) obtained from this experiment were analyzed by GC−MS. The M + 2 ions for both compounds (412 and 416 for **1** and **3**, respectively) showed a 7% enrichment indicating significant labeling. Further evidence for the incorporation of deuterium atoms was indicated by the observation of enhanced M + 2 isotopic peaks corresponding to the major MS fragment ions of both **1** [*m/* 341 ($C_{25}H_{41}^+$), 137 ($C_{10}H_{17}^+$), 123 ($C_9H_{15}^+$), 81 ($C_6H_{11}^+$), and 69 ($C_5H_9^+$)] and **3** [*m/z* 329 ($C_{24}H_{41}^+$), 123 ($C_9H_{15}^+$), 83 ($C_6H_{11}^+$), and 69 ($C_5H_9^+$)], thus proving the capacity of isolate QC45B to transform exogenous glucose into SQ and THSQ.

A second feeding experiment utilized [1-^{13}C]glucose (510 mg in 4 L of broth). ^{13}C NMR spectra of purified **1**−**3** obtained from this feeding experiment were recorded in $CDCl_3$, and each spectrum was fully assigned by 2D-NMR (COSY, HMBC, and HMQC) or comparison with published data. MS analysis of the isotopic ions (M + 1) of **1** [unlabeled *m/z* 410.3 (M_0, 100), 411.4 (M_1, 35.8); labeled *m/z* 410.3 (M_0, 100), 411.4 (M_1, 42.6)], **2** [unlabeled *m/z* 412.4 (M_0, 100), 413.3 (M_1, 36.0); labeled *m/z* 412.4 (M_0, 100), 43.3 (M_1, 45.4)], and **3** [unlabeled *m/z* 414.4 (M_0, 100), 415.4 (M_1, 35.8); labeled *m/z* 414.4 (M_0, 100), 415.4 (M_1, 47)] suggested a nonequivalent incorporation in the isoprenoids, THSQ (**3**) being more enriched (approximately 12%). ^{13}C signal enhancements were very clear by comparing the peak height of corresponding signals (normalized to C-6/C-19 and C2/C23 for **1** and **2/3**, respectively) in the spectra of isotopically labeled and natural abundance compounds (see the Supporting Information).[13] The spectrum of labeled THSQ (**3**) showed enrichment in the C-1/C-24, C-4/C-21, C-8/C-17, C-12/C-13, C-25/C-30, C-26/C-29, and C-27/C-28 resonances in agreement with the predicted labeling pattern shown in Scheme 2. A similar enrichment pattern was observed in the SQ (**1**) and DHSQ (**2**) spectra. Of particular significance was the difference in intensity of the carbons derived from C-1 and C-2 of the isoprene unit (Figure 1), which suggested a marginal contribution from the classical acetate/mevalonate pathway to the biosynthesis of **1**−**3** in isolate QC45B. This was confirmed by the

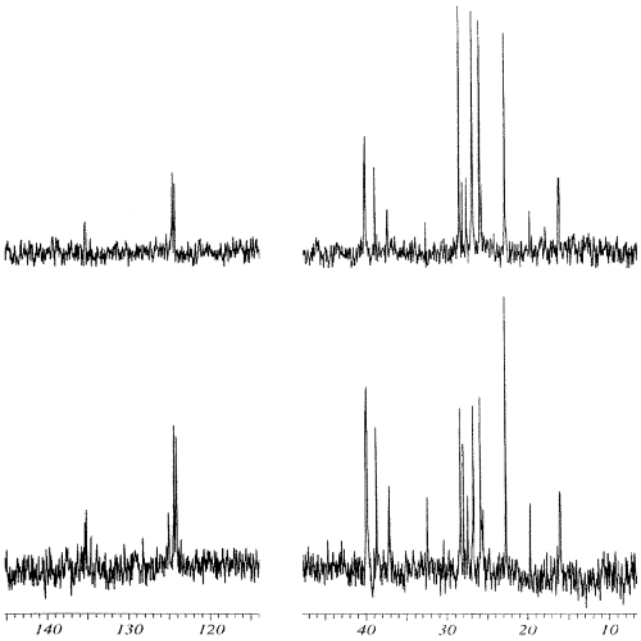


Figure 1. ^{13}C NMR spectra of tetrahydrosqualene (**3**) from isotopically natural (on the bottom) and ^{13}C-enriched (on the top) samples.

(13) The results obtained by the comparison of peak heights were further supported by a similar analysis based on the manual integration of area peaks. In the discussion we have preferred to report the comparison of the peak heights because they have been obtained by an automated procedure and, therefore, are less affected by interpretation mistakes.

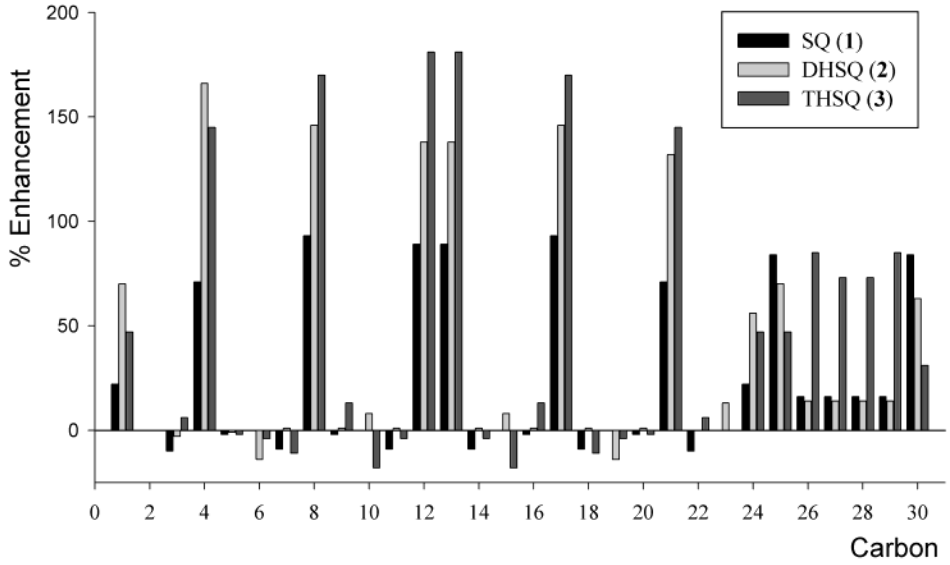


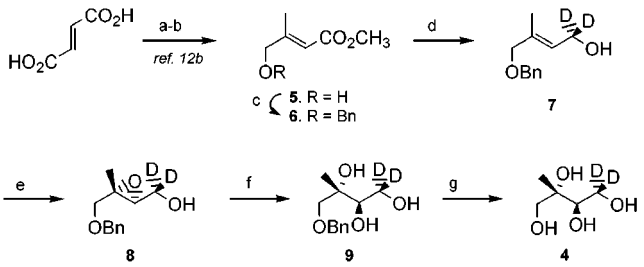
Figure 2. ^{13}C NMR data for [1-^{13}C]-labeled squalene (**1**), dihydrosqualene (**2**), and tetrahydrosqualene (**3**). Signal intensities were compared with those of unlabeled **1**−**3**. Spectra were normalized to C6/19 for squalene (**1**) and C2/23 for dihydrosqualene (**2**) and tetrahydrosqualene (**3**). Experiments were acquired in $CDCl_3$ (75 MHz) with 16K data points and 4.0 Hz line broadening. Assignments are based on DEPT, COSY, and HMQC data.

observation that no incorporation was detectable when [1,2-$^{13}C_2$]-acetate (300 mg in 3L of broth) was supplied to the *Streptomyces* under the conditions described above.[7c] The incorporation data summarized in Figure 2 also showed that the carbons (labeled with a dot in Scheme 2) in THSQ and DHSQ derived from glucose via GAP were enriched to a greater extent (ca. 1.5−2 times more enrichment) than the carbons (labeled with a box in Scheme 2) derived from glucose via pyruvate. A similar trend was present in the SQ enrichment values.

Incorporation rate of SQ (**1**) and DHSQ (**2**) also showed a clear difference in the isoprene units derived from IPP or DMAPP, which served as starter for the prenyl chain. In fact, the DMAPP-derived carbons (C-25 and C-30) were found to be 4-fold more enhanced than the signals derived from IPP (C-26/C-29 and C-27/C-28). ^{13}C data (Figure 2) also revealed discrimination in the incorporation between the *Z* (C-25/C-30) and *E* (C-21/C-24) methyl groups in SQ, although the low but significant enrichment of C1/24 seemed to imply scrambling between the C-4 and C-5 positions of the DMAPP units. This was even more evident in DHSQ where C-24 and C-30 showed very similar incorporation rate, 56% and 63% of signal enhancement respectively (Supporting Information). On the other hand, the carbon atoms from IPP and DMAPP of THSQ (**3**) had roughly the same enrichment levels (Figure 2).

To provide direct evidence for the involvement of MEP in the biosynthesis of squalene by QC45B, a feeding experiment was carried out with a labeled derivative of ME. Following the synthetic method recently developed for the synthesis of ME and MEP,[12] [4,4-$^2H_2$]-*C*-2-methylerythritol (**4**) was prepared (70% ee) via an enantioselective synthesis starting from fumaric acid (Scheme 3). Introduction of deuterium atoms was achieved by AlD_3 that was generated in situ from $LiAlD_4$ and $AlCl_3$. The reagent provided a very high (>98%) degree of isotopic labeling and minimized the side reactions that rendered the direct use of $LiAlD_4$ impractical. The synthetic plan furthermore led to a C-4-labeled derivative (**4**) that had not been previously tested in feeding experiments.

Scheme 3. Synthesis of [4,4-$^2H_2$]-Methylerythritol[a]



[a] Key: (a) HCl−MeOH at reflux; (b) O_3, CH_2Cl_2, −78 °C; 2-(triphenylphospharylidene)propionaldehyde, −78 °C; $NaBH_4$, MeOH; (c) benzyl trichloroacetamidate, TFMSA, rt; (d) $LiAlD_4$/$AlCl_3$, THF, −78 °C; (e) (+)-DET, Ti(O-*i*-Pr)$_4$, *t*-BuOOH, −23 °C; (f) 0.5 M NaOH, *t*-BuOH/H_2O 1:2; (g) H_2, 10% Pd/C.

The d_2-labeled methylerythritol (**4**) was supplied to liquid cultures of QC45B (190 mg in 2 L of broth), and isoprenoids were isolated as described above. An increase in the intensity of the M + 2 peak was observed in the GC−MS of pure squalene (**1**), dihydrosqualene (**2**), and tetrahydrosqualene (**3**). The isotope cluster (M_{d0}, M_{d1}, M_{d2}) of both molecular and main fragment ions indicated an incorporation rate of approximately 8% in both THSQ and DHSQ, but lower in SQ (see the Experimental Section).

Conclusion

The results of the feeding experiments with QC45B demonstrate **1**−**3** are produced by this strain of marine *Streptomyces* via the nonmevalonate MEP pathway. Growth curves showed that QB45B was in log phase growth during the entire 8 days of the feeding experiments. The failure of QC45B to incorporate labeled acetate into SQ and THSQ during the 8 day feeding experiments indicates, that as reported for other Strep-

tomyces sp.,[9] QC45B apparently utilizes only the MEP to make **1**−**3** during log phase growth. Additional experiments are needed to determine if the acetate/mevalonate pathway is operational in QC45B during the stationary phase.

Feeding [1-^{13}C]glucose (Figure 2) led to greater enrichment of GAP-derived (C-4/C-21, C-8/C-17, and C-12/C-13) carbons than pyruvate-derived (C-1/C-24, C-26/C-29, C-27/C-28) carbons in the isoprenoids **1**−**3**. Since GAP and pyruvate are not directly formed from a common committed intermediate in the MEP pathway, there is no a priori reason to expect the two sets of carbons to be equally enriched. For example, the bacterium may have a larger pool of unlabeled pyruvate that would lead to greater dilution of the enrichment of carbons derived from labeled glucose via pyruvate.

The MS results and ^{13}C NMR data (Figure 2 and Experimental Section) also generally showed highest incorporation in THSQ (**3**), intermediate incorporation in DHSQ (**2**), and lowest incorporation in SQ (**1**) in the GAP-derived carbons.

This simple trend was not clearly apparent in the pyruvate derived carbons. A potential explanation for the GAP derived incorporations might reside in the sequence of transformations that leads to **1**−**3**. *Streptomyces* isolate QC45B produces a series of squalene-derived compounds, including DHSQ (**2**) and THSQ (**3**), that differ in the number and positions of the double bonds. These partially and fully reduced compounds represent the end-products of the biosynthesis (Scheme 2). Labeled glucose was provided to QC45B cultures as a single pulse at the beginning of the experiment. It seems reasonable to postulate that the SQ (**1**) formed in the early part of the experiment, when the concentration of labeled glucose was at a maximum, would have the highest specific incorporation. This initially produced SQ would then be converted to DHSQ, THSQ, and other more highly reduced analogues, which would in turn reflect the high specific incorporation of the initially formed SQ. Using this line of reasoning, the SQ extracted from the cultures at the end of the 8 day feeding experiment would be biased toward material formed at the end of the experiment when the concentration of labeled glucose would be at its lowest value and this SQ would be expected to have a lower specific enrichment as observed.

An interesting feature in the ^{13}C data of SQ and DHSQ is the significant enrichment of the signals (C-1/C-24 for **1** and C-24 for **2**) derived from C-4 of DMAPP units. This information, which is lost in THSQ (**3**) because of the symmetry of the terminal methyl groups, suggests some scrambling between C-4 and C-5 of DMAPP. A comparable lack of stereospecificity is not observed in the IPP-derived isoprene units, where the *Z* methyl carbons (C-26/C-29 and C-27/C-28) are labeled to a significantly higher extent than the *E* methylene signals (C-5/C-20 and C-9/C-16). These observations are consistent with an irreversible isomerization of IPP to DMAPP that lacks stereochemical fidelity. In SQ (**1**) and DHSQ (**2**), the pyruvate derived carbons from DMAPP isoprene units (C-25 and C-30) are much more highly labeled than the pyruvate derived carbons from IPP (C-26/C-29 and C-27/C-28). This is not seen in the THSQ (**3**) data. The enhanced labeling in the pyruvate/DMAPP derived carbons in DHSQ and SQ is difficult to explain by a single pathway from GAP and pyruvate to IPP and subsequently DMAPP. Under the experimental condition above illustrated (e.g., 8 days of incorporation), these findings might result from the simultaneous operation of two independent routes to DMAPP from a common intermediate along the MEP pathway.[14,15] Differences in the carbon enrichment would be dependent on the gradual depletion of the reservoir of labeled DMAPP.

The feeding experiment with deuterium-labeled methylerythritol **4** provides further support for the involvement of this polyol in the nonmevalonate pathway. In fact, *Streptomyces* isolate QC45B and *E. coli* are the only organisms known to date to incorporate nonphosphorylated methylerythritol into terpenoids. The low incorporation of **4** into SQ (**1**) (ca. 3%), as well as in DHSQ (**2**) and THSQ (**3**) (ca. 8%) might be related to the absence of a specific kinase needed to convert methylerythritol into methylerythritol 4-phosphate, as suggested by Rohmer.[16] Despite the modest enrichment of deuterated labeling, the MS data confirm the result of feeding experiments with [1,1,4,4-^{2}H$_4$]-2-methylerythritol in *E. coli*.[16] The preparation of **4** summarized in Scheme 3 provides a readily accessible source of this biosynthetic probe and complements other synthetic strategies for preparing labeled precursors suitable for MEP pathway feeding experiments (e.g., the C_5 compounds that Lichtentthaler has recently proposed as missing intermediates of the nonmevalonate pathway[17]).

Linear triterpenoid production by the marine *Streptomyces* isolate QC45B may be a useful alternative model system for investigating the MEP pathway. The bacterium grows well in liquid culture and produces good yields of linear triterpenes (ca. 5−6 mg in 1L of broth), including SQ (**1**), DHSQ (**2**), and THSQ (**3**), which are extremely easy to purify from the cell-pellet extract. Known and postulated intermediates in the MEP pathway are incorporated into **1**−**3** by QC45B and the analysis of labeled **1**−**3** can be easily carried out either by GC−MS or ^{13}C NMR. In addition, the scrambling of label between C-4 and C-5 of DMAPP units and the differential incorporation of pyruvate into DMAPP and IPP units in SQ and DHSQ by QC45B are interesting features of the MEP pathway in this organism that deserve further study.

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Supporting Information Available: Assignment and glucose-derived ^{13}C incorporation of **1**−**3** (Table 1), MS and NMR spectra of labeled and unlabeled **1**−**3**, and ^{1}H and ^{13}C NMR spectra of **5**. This material is available free of charge via the Internet at http://pubs.acs.org

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