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Feeding of [5,5-$^2H_2$]-1-desoxy-D-xylulose and [4,4,6,6,6-$^2H_5$]-mevalolactone to a geosmin-producing *Streptomyces* sp. and *Fossombronia pusilla*

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Abstract

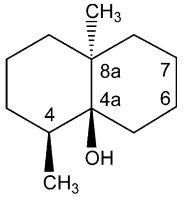
The biosynthesis of the trisnor sesquiterpenoid geosmin (4,8a-dimethyl-octahydro-naphthalen-4a-ol) (**1**) was investigated by feeding labeled [5,5-$^2H_2$]-1-desoxy-D-xylulose (**11**), [4,4,6,6,6-$^2H_5$]-mevalolactone (**7**) and [2,2-$^2H_2$]-mevalolactone (**9**) to *Streptomyces* sp. JP95 and the liverwort *Fossombronia pusilla*. The micro-organism produced geosmin via the 1-desoxy-D-xylulose pathway, whereas the liverwort exclusively utilized mevalolactone for terpenoid biosynthesis. Analysis of the labeling pattern in the resulting isotopomers of geosmin (**1**) by mass spectroscopy (EI/MS) revealed that geosmin is synthesized in both organisms by cyclization of farnesyl diphosphate to a germacradiene-type intermediate **4**. Further transformations en route to geosmin (**1**) involve an oxidative dealkylation of an *i*-propyl substituent, 1,2-reduction of a resulting conjugated diene, and bicyclization of a germacatriene intermediate **13**. The transformations largely resemble the biosynthesis of dehydrogeosmin (**2**) in cactus flowers but differ with respect to the regioselectivity of the side chain dealkylation and 1,2-reduction

Keywords: Geosmin; Biosynthesis; Desoxy-D-xylulose; Mevalolactone; Liverwort; *Fossombronia pusilla*; *Streptomyces* sp. JP95

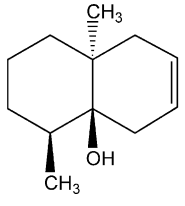
1. Introduction

Geosmin (**1**) (−)-(4*S*,4a*S*,8a*R*)-(4,8a-dimethyl-octahydro-naphthalen-4a-ol, Fig. 1) is a well-known scent compound with a characteristic musty, earthy odor reminiscent of freshly ploughed soil. Geosmin (**1**) was first isolated from a culture broth of actinomycetes (Gerber, 1967, 1968; Gerber and Lecheval, 1965). Geosmin is also produced by cyanobacteria (Kikuchi et al., 1973), fungi, (Bjurman and Kristensson, 1992; Mattheis and Roberts, 1992) and certain mosses (Spörle et al., 1991). Due to its very low odor threshold (ca. 5–7 ng/l), geosmin is an important off-flavor contaminant of drinking water (Dupuy et al., 1986; Izaguirre et al., 1982) and causes problems for fish farmers, since it bioaccumulates in fish and shellfish, giving them a muddy taste. On the other hand the same compound is appreciated as an aroma component in beetroot (*Beta vulgaris*) (Murray et al., 1975) and wine (Darriet et al., 2000).

Although the structure of geosmin was already elucidated in 1969, not much is known about the details of its biosynthesis. Bentley and Meganathan (1981) demonstrated the principal origin of geosmin from terpenoid metabolism in *Streptomyces antibioticus*. Structurally related to geosmin (−)-(**1**) is dehydrogeosmin (+)-(**2**) (Fig. 1). (+)-**2** is emitted from flowers of certain Cactaceae belonging to the genera of *Rebutia*, *Sulcorebutia*, *Mammillaria,* and *Dolichothele* (Kaiser and



(-)-geosmin **1**



(+)-dehydrogeosmin **2**

Fig. 1.

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Nussbaumer, 1990). Sometimes dehydrogeosmin (**2**) is accompanied by small traces of geosmin (**1**), suggesting a related, if not largely identical, biosynthesis. The origin of dehydrogeosmin (**2**) was studied (Feng et al., 1993) using flowers of *Rebutia marsoneri* and deuterium labeled farnesols. According to this study dehydrogeosmin (**2**) has to be considered as a trisnor sesquiterpene and is assembled from farnesyl diphosphate (FDP) (**3**), as outlined in Fig. 2. The labeling pattern of the resulting dehydrogeosmin suggested an initial electrophilic attack of the farnesyl cation **3** onto the terminal double bond yielding a ten-membered monocycle of the germacradiene type **4** followed by oxidative removal of the exocyclic C_3-substituent, introducing a new double bond. Bicyclization is achieved by protonation and subsequent transannular ring closure to the tertiary cation **6a**. Following a suprafacial 1,2-hydride shift of the angular hydrogen atom, addition of water completes the sequence, yielding dehydrogeosmin (**2**). Although the sequence of the reactions, especially dealkylation/reduction preceding bicyclization, needs further proof by isolation or successful conversion of intermediates, the essential aspects of the biosynthesis of dehydrogeosmin are covered by Fig. 2.

Unlike the highly efficient transformation of farnesol to dehydrogeosmin (**2**) in flowerheads of Cactaceae, attempts to study the mode of cyclization of farnesol en route to geosmin (**1**) in bacteria (*Streptomyces* spp., *Microbispora rosea*) remained, however, unsuccessful (Huber and Boland, unpublished). Since externally

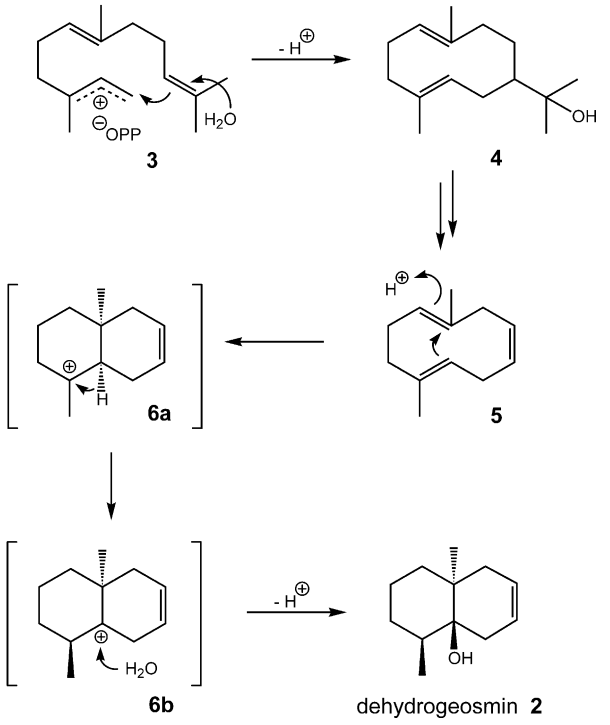

Fig. 2. Postulated pathway from farnesyl diphosphate to dehydrogeosmin (+)-**2** in Cactaceae flowers.

added farnesol inhibited the growth of the tested bacterial strains, we now report the results of administration of early precursors, namely [$^2H_5$]-mevalolactone **7**, [$^2H_2$]-mevalolactone **9**, and [$^2H_2$]-deoxy-D-xylulose **11**, to *Streptomyces* sp. JP95 and the liverwort *Fossombronia pusilla*. The current study demonstrates that the biosynthesis of geosmin in bacteria (via the methylerythritol pathway (MEP pathway)) and the liverwort (via the mevalonic acid pathway (MVA pathway)) follows the same principal sequence described for dehydrogeosmin in Cactaceae. Remarkably, the labeling experiments support an oxidative removal of the C_3-substituent via an unsaturated intermediate analogous to the biosynthesis of dehydrogeosmin.

2. Results

2.1. Feeding of precursors

Streptomyces sp. JP95 and the liverwort *Fossombronia pusilla* (order: Calobriales) were chosen for a comparative biosynthetic study, since the two species utilize alternative pathways to generate the early precursors of terpenoid biosynthesis (for review see Eisenreich et al., 2001). The classical MVA pathway is utilized in bryophytes for the biosynthesis of sesquiterpenoids (Adam et al., 1998; Thiel and Adam, 2002; Warmers and König, 2000), while *Streptomyces* sp., depending on the growth phase, are able to synthesize certain (sesqui)-terpenoids along both routes, namely the MVA and the MEP pathway (Seto et al., 1996, 1998). Cultures of *Streptomyces* sp. JP95 and *F. pusilla* all exhibit the strong, earthy, musty odor of geosmin. As proven by GLC on a chiral stationary phase (Experimental), both species produce enantiomerically pure (−)-geosmin (**1**). Dehydrogeosmin (**2**) was not found. Labeled precursors [$^2H_5$]-MVA **7** (Schwarz, 1994), [$^2H_2$]-MVA **9** and [$^2H_2$]-desoxy-D-xylulose ([$^2H_2$]-DOX) **11** (Piel and Boland, 1997) were added to the culture medium (Fig. 3), and the released volatiles were collected by continuous absorption on charcoal traps (Donath and Boland, 1995) or by SPME (Nilsson et al., 1996; Watson et al., 1999). According to mass spectroscopy of the products, both precursors were efficiently channeled into terpenoid biosynthesis and converted into farnesyl diphosphates [$^2H_{12}$]-FDP **8** or [$^2H_6$]-FDP **10** via the MVA route in the liverwort *F. pusilla* and to [$^2H_6$]-FDP **12** via the MEP route in *S.* sp. JP95. Only at high concentrations of labeled desoxy-D-xylulose (5 mg/ml) in the medium, geosmin from *F. pusilla* showed a low degree of labeling (<1%) while a simultaneously produced monoterpene displayed up to 6% deuterium labeling. Interestingly, both organisms produced either unlabelled or labeled geosmin assembled from farnesyl diphosphate containing three labeled C_5-units. Products

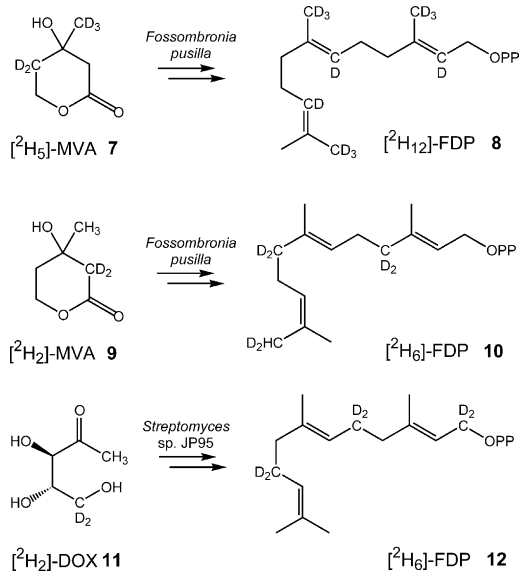

Fig. 3. Conversion of labeled, externally added, precursors into labeled farnesyl diphosphates. Labeling patterns of **8**, **10**, and **12** are in agreement with established pathways and mass spectroscopic analysis of labeled geosmins derived from the respective farnesyl diphosphates.

of farnesyl diphosphate containing only one or two labeled C_5-units were not observed. Owing to the labeling pattern of the three precursors **7**, **9**, and **11**, virtually all hydrogen atoms of the farnesyl diphosphate intermediate could be substituted by deuterium atoms, thus surveying completely the fate of hydrogens during geosmin biosynthesis. For instance, feeding of $[^2H_2]$-DOX **11** to *S.* sp. JP95 at a concentration of 2 mg ml^{-1} resulted in two partly overlapping GLC-peaks of $[^2H_5]$-**1** (27%) and unlabeled, natural **1** (73%). Higher concentrations of the precursor raised the amount of labeled product (>95% $[^2H_5]$-**1**). Administration of labeled mevalolactone to growing *S.* sp. *JP95* (4 mg/ml) did not result in labeled geosmin.

2.2. Mass spectroscopy of geosmin and its isotopomers

The mass spectrum of geosmin (**1**) displays two even-numbered fragments of high diagnostic value (see Fig. 4A). Besides a weak molecular ion at $m/z = 182$ (I), two other well-defined fragments at $m/z = 126$ (III) and $m/z = 112$ (II, base peak) can be used to localize deuterium atoms in the molecule. The fragment at $m/z = 167$ results from loss of a methyl group followed by elimination of water generating $m/z = 149$. The base peak of geosmin, $m/z = 112$, stems from homolytic cleavage of the A-ring, hydrogen transfer from the protonated oxonium ion to the radical site, followed by a McLafferty rearrangement eliminating pentene as a neutral fragment (Fig. 5). Since the degradation of the A-ring involves the more stable secondary radical intermediate, this reaction channel is preferred. Cleavage of the B-ring involves a primary radical and, hence, generates the less

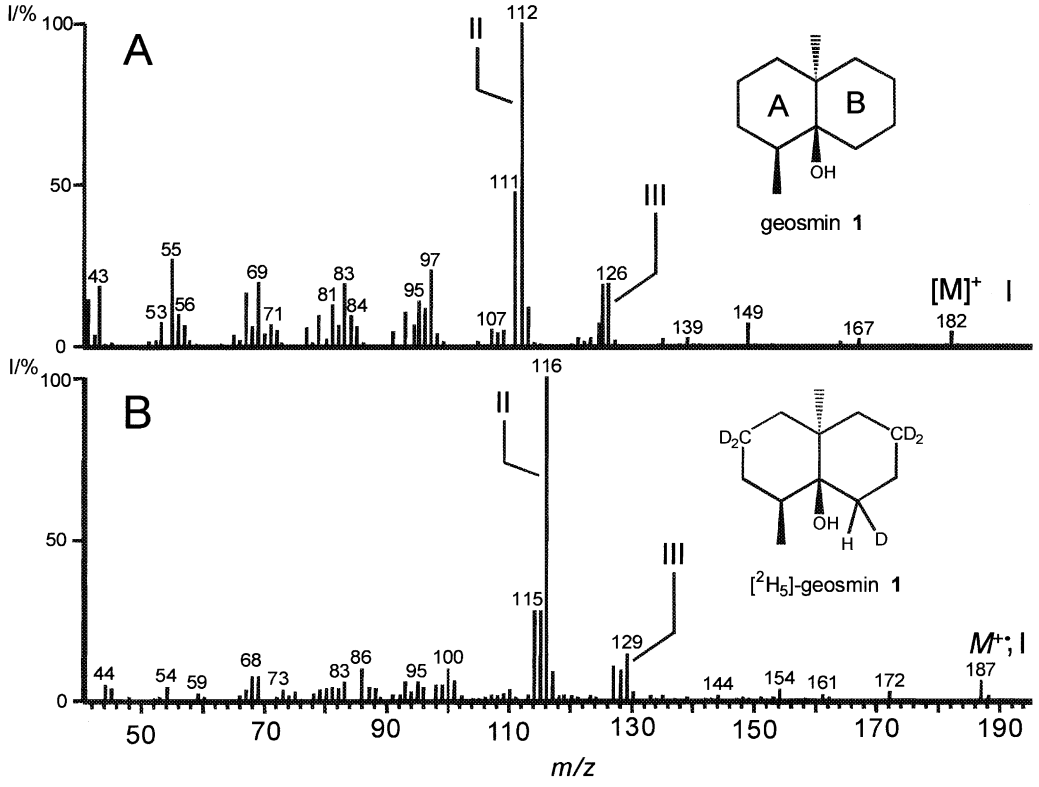

Fig. 4. Mass spectra of labeled and natural geosmin. **4A**: natural geosmin. **4B**: $[^2H_5]$-geosmin obtained from *S.* sp. JP95 after administration of $[^2H_2]$-**11**. The relevant fragments I, II, and III are indicated in the spectra.

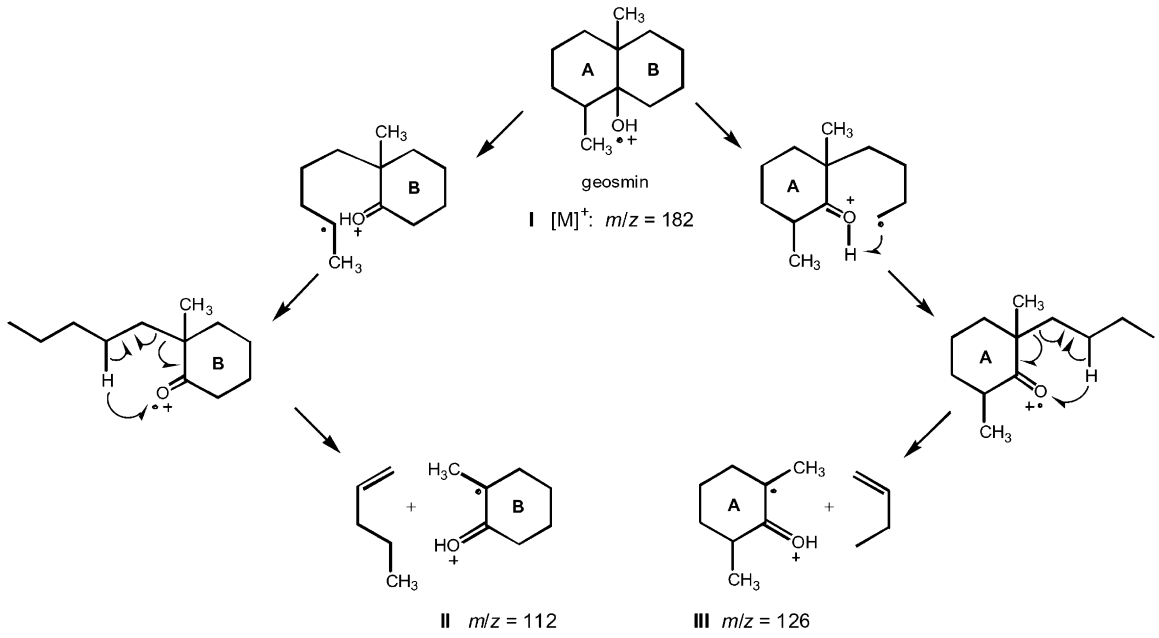

Fig. 5. Mass spectroscopic fragmentation pattern of geosmin. Shown is the origin of the most relevant fragments I, II, and III.

abundant fragment at $m/z = 126$. This cleavage process is supported by H/D-exchange experiments. Although the molecular ion rises to $m/z = 183$ after treatment with CD_3OD, the fragment II at $m/z = 112$ remains the major peak in the labeled compound, because the deuterium atom of the –OD group is transferred to the side chain and eliminated along with the McLafferty product pentene (see Fig. 5 and Table 1). Analogous findings hold for an initial B-ring cleavage.

Owing to this well-defined fragmentation pattern, the number of deuterium atoms in the two ring moieties of the metabolites can be reliably determined. This is exemplified by [$^2H_5$]-**1** produced by *S.* sp. JP95 after administration of [$^2H_2$]-DOX **11**. The molecular ion is shifted from *m/z* 182 to *m/z* 187 indicating the presence of five deuterium atoms in the intact molecule (Fig. 4B). Two of them reside in the A-ring and three in the B-ring, each of them at the assigned positions. This arrangement of deuterium atoms follows (i) from transformation of [$^2H_6$]-FDP **12** to geosmin [$^2H_5$]-**1** according to Fig. 6 and (ii) from analysis of the mass fragmentation pattern of [$^2H_5$]-**1** (Table 1). After homolytic cleavage of the A-ring and hydrogen transfer, the McLafferty rearrangement shuttles one of the two deuterium atoms from the side chain (former A-ring) to the oxonium ion, raising the number of deuterium atoms in the B-ring fragment II to four. A complementary initial homolytic cleavage of the B-ring, followed by a transfer of a single deuterium atom from the original C(7) of the metabolite to the carbonyl group of the A-ring generates the signal at $m/z = 129$. Since the intensity of $m/z = 128$ is not enhanced (compare the intensity of $m/z = 126$ and 125 of the unlabeled compound), two deuterium atoms must reside on C(7). The third deuterium atom of the B-ring should be located at C(5) in agreement with the labeling pattern of the precursor and the overall folding process of [$^2H_6$]-FDP **12** *en route* to dehydrogeosmin (**2**) (Fig. 6).

Further details on the mode of cyclization are derived from mass spectroscopic analysis of [$^2H_9$]-**1** obtained from feeding of [$^2H_5$]-MVA **7** to the liverwort *F. pusilla*. As outlined in Fig. 2 [$^2H_5$]-MVA **7** is first converted to

Table 1
Mass fragments I, II, and III in different isotopomers of geosmin. Precursors, intermediates and products are listed

Organism	Precursor	Farnesyl diphosphate	Geosmin	Fragment I $[M]^+$ *m/z*	Fragment II *m/z*	Fragment III *m/z*
S. sp. JP95		–	Geosmin	182	112	126
S. sp. JP95	H/^{2}H-exch.	–	[$^2H_1$]-Geosmin	183	112	126
S. sp. JP95	[$^2H_2$]-DOX **11**	[$^2H_6$]-FDP **12**	[$^2H_7$]-Geosmin	187	116	129
S. sp. JP95	H/^{2}H-exch., **11**	[$^2H_6$]-FDP **12**	[$^2H_8$]-Geosmin	188	116	129
F. pusilla	[$^2H_5$]-MVA **7**	[$^2H_{12}$]-FDP **8**	[$^2H_{12}$]-Geosmin	191	116	134
F. pusilla	[$^2H_2$]-MVA **9**	[$^2H_6$]-FDP **10**	[$^2H_4$]-Geosmin	186	114	128

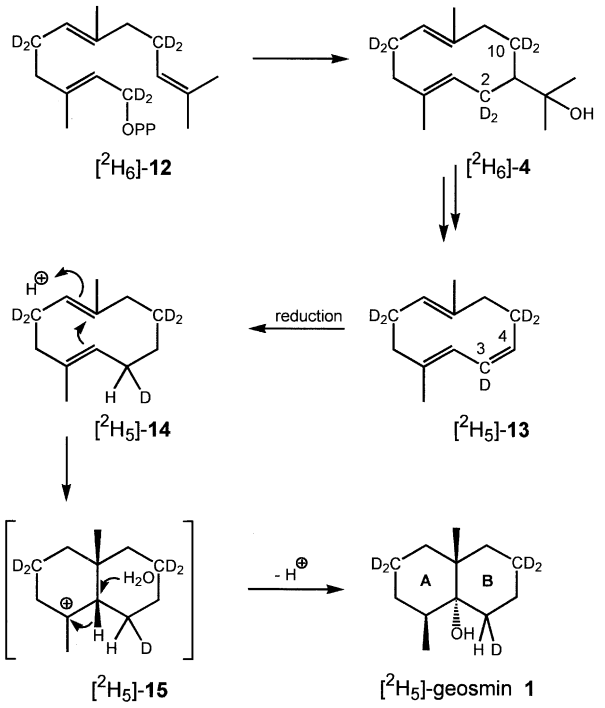

Fig. 6. Postulated pathway from farnesyl diphosphate to geosmin (−)-**1** in *S.* sp. JP95. [$^2H_6$]-**12** is assembled from [$^2H_2$]-**11** via the MEP pathway. The sequence of (i) oxidative dealkylation, (ii) reduction, and (iii) bicyclization is arbitrary.

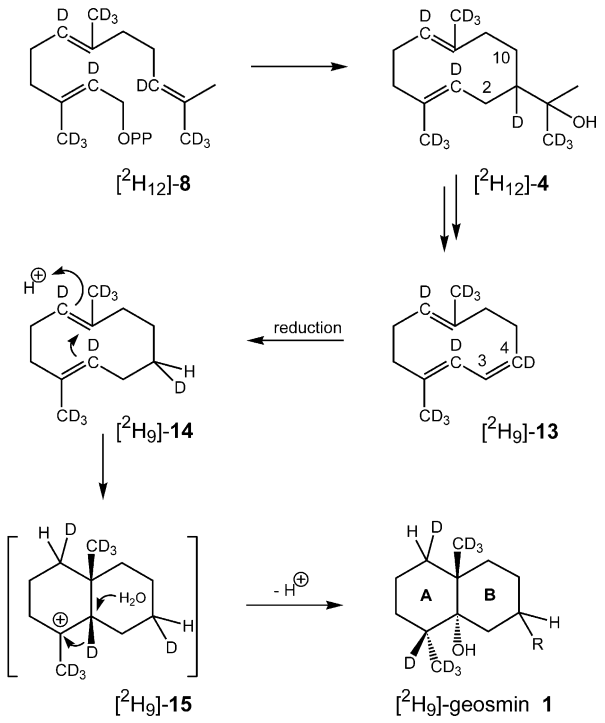

Fig. 7. Postulated pathway from farnesyl diphosphate to geosmin (−)-(**1**) in the liverwort *F. pusilla*. [$^2H_{12}$]-**8** is assembled from [$^2H_5$]-**7** via the MVA pathway. The sequence of (i) oxidative dealkylation, (ii) reduction, and (iii) bicyclization is arbitrary.

[$^2H_{12}$]-FDP **8**. Intramolecular cyclization generates the cyclodecadiene intermediate [$^2H_{12}$]-**4** (Fig. 7), which next loses the *i*-propyloxy group containing three deuterium atoms. All other deuterium atoms are retained in the final product [$^2H_9$]-geosmin. The 1,2-hydrogen shift, originally established for the biosynthesis of dehydrogeosmin (**2**) (Feng et al., 1993), moves the angular deuterium atom of [$^2H_9$]-**15** into the A-ring, resulting in a product with five deuterium atoms in this ring (including the CD_3) and four deuterium atoms in ring B (including the angular–CD_3). This peculiar arrangement of deuterium atoms within the two ring moieties agrees perfectly with the mass numbers of the three relevant fragments I, II, and III compiled in Table 1. For example, the less dominant cleavage of the B-ring of [$^2H_9$]-**1** proceeds with elimination of butene substituted with a single deuterium atom. This is reflected in fragment III that carries the other eight deuterium atoms ($m/z = 134$). Fragment II, located at $m/z = 116$, indicates the presence of four deuterium atoms; five others are removed along with the neutral fragment pentene. The same fragmentation analysis can be carried out for all types of labeled geosmins and gives fully consistent results.

3. Discussion

Based on administration of ^{13}C labeled glucose to callus cultures of the liverwort *Conocephalum conicum* and subsequent ^{13}C NMR analysis of the isotopic pattern of the terpenoid metabolites, Adam et al. (1998) established for this liverwort the classical precursor-product relation, namely mono- and diterpenes as products of the MEP pathway and sesquiterpenes as products of the MVA pathway (Warmers and König, 2000). This principal association of pathways is confirmed by the present work demonstrating that *F. pusilla* produces geosmin almost exclusively via the MVA pathway. On the other hand, the micro-organism *Streptomyces* sp. JP95 utilized only the MEP pathway. This result was unexpected since several *Streptomyces* strains are known to possess both the MVA and the MEP pathway (Seto et al., 1996, 1998; Hamano et al., 2002). In the exponential growth phase the MEP pathway provides precursors for the prenyl side chain of menaquinone, while the MVA pathway generates the building blocks for the prenyl moieties of antibiotics during the stationary phase. Since even high concentrations of labeled mevalolactone (4 mg/ml) did not result in labeled geosmin, at least in *Streptomyces* sp. JP95 this pathway is not utilized for geosmin biosynthesis.

As demonstrated in this work, the biosynthetic sequence to geosmin (**1**) is principally identical with the previously described biosynthesis of dehydrogeosmin (**2**) in flower heads of Cactaceae (Feng et al., 1993). Analogous to the biosynthesis of dehydrogeosmin, also en

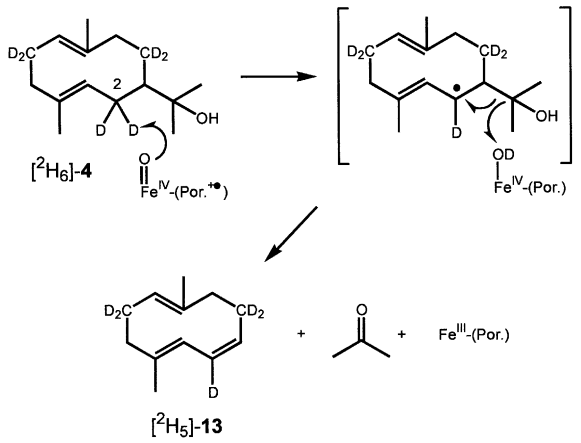

Fig. 8. Oxidative dealkylation of the side chain. The mechanism is modeled after the psoralene synthase (Stanjek et al., 1999) a member of the cytochrome P450 family.

route to geosmin an *i*-propyloxy substituent is removed by an oxidative dealkylation generating an endocyclic double bond (see Fig. 8). This reaction strongly resembles the key step of the oxidative dealkylation of (+)-marmesin to psoralene (Stanjek et al., 1999) and, hence, might also be catalyzed by a cytochrome P450. Considering the mechanistic findings of the psoralene synthase, we assume an initial attack by a reactive (Por.$^{+\bullet}$)-Fe(IV)=O species of the enzyme onto a deuterium atom at C(2) of the precursor [$^2H_6$]-**4**. The resulting allylic radical may stabilize by means of β-cleavage to yield the triene [$^2H_5$]-**13** along with an *i*-propyloxy radical. The latter will immediately recombine with the neighboring (Por.$^{+\bullet}$)–Fe(IV)–OH species and yield acetone, along with water and the reduced catalyst. In the case of the psoralene synthase the reaction proceeds via *syn*-elimination of the attacked hydrogen atom and the *i*-propyloxy substituent. The same stereochemistry has to be expected for the oxidative dealkylation of [$^2H_6$]-**4**.

Following conversion of [$^2H_6$]-**4** into the triene [$^2H_5$]-**13** (Fig. 6), the newly introduced double bond has to be selectively reduced by a reductase. A comparable reduction, catalyzed be the Δ7-sterol reductase, is well known from steroid metabolism (Moebius et al., 1998). Consistent with this assumption is the observation that the isolated double bond of deuterium labeled dehydrogeosmin is not reduced by growing cultures of *Streptomyces* sp. JP95. This excludes **2** as an intermediate in the biosynthesis of **1**. The cyclization of the ten-membered intermediate [$^2H_5$]-**14** to the bicylic skeleton of geosmin proceeds analogous to the biosynthesis of dehydrogeosmin and also involves a suprafacial-1,2 hydrogen shift prior to the addition of water to the angular carbon atom yielding [$^2H_5$]-**1** (Fig. 6).

In summary, geosmin (**1**) and dehydrogeosmin (**2**) are produced along the same principal pathway in different organisms, but differ in the regioselectivity of the dealkylating enzyme. Production of geosmin [$^2H_5$]-**1** involves an oxidative dealkylation (see Figs. 6 and 8) leading to a conjugated diene, whereas the comparable enzymatic activity in case of dehydrogeosmin (**2**) generates an isolated double bond (Fig. 2). This close analogy may also explain the presence of trace amounts of geosmin in the volatile blend of certain Cactaceae (Kaiser and Nussbaumer, 1990). Phylogenetic studies of the enzymes involved in the biosyntheses of geosmin (**1**) and dehydrogeosmin (**2**) in different organisms are required and may help to clarify whether Cactaceae use enzymes that are derived from an ancestral ensemble of enzymes for geosmin biosynthesis or have independently evolved a novel set of biocatalysts to produce dehydrogeosmin.

4. Experimental

4.1. Labeled precursors

[5,5-$^2H_2$]-1-Desoxy-D-xylulose **11** (Piel and Boland, 1997), [2,2-$^2H_2$]-mevalolactone **9** and [4,4,6,6,6-$^2H_5$]-mevalolactone **7** (Schwarz, 1994) were synthesized as previously described.

4.2. Cultivation of Fossombronia pusilla

Callus of *Fossombronia pusilla* was obtained from Professor H. Becker (University of Saarbrücken, Germany). The liverwort was grown on B5-agar (Sigma, 82024 Taufkirchen, Germany) in 100 ml Erlenmeyer flasks at 21 °C under a regime of 14 h illumination at 200 Lux. B5 medium: 3.1 g B5 salt, 0.112 g B5 vitamin powder, 8 g agar and 20 g saccharose were added to 1 l deionized water and autoclaved.

4.3. Cultivation of Streptomyces sp. JP95

Streptomyces sp. JP95 was isolated from the ascidian *Aplidium lenticulum* found at the Great Barrier Reef. The bacteria were grown at 28 °C in 50 ml A1 liquid medium on a shaker at 100 rpm. *Streptomyces* sp. JP95 was chosen for biosynthetic experiments, since a color change of the culture to purple indicated the onset of secondary metabolite production and concomitant geosmin biosynthesis. A1 medium: 10 g potato starch, 4 g yeast extract, 2 g peptone, 28 g instant ocean (Aquarium Systems) and 10 ml 1 M Tris/HCl buffer (pH 8) were added to 1 l of deionized water and autoclaved.

4.4. Incubation with labeled precursors and volatile collection

Aqueous solutions (1 ml) of the labeled precursors (labeled mevalolactones or [5,5-$^2H_2$]-1-desoxy-D-xylulose **11** at 1–4 mg/ml) were applied to either the

micro-organism or the liverwort in 100 ml Erlenmeyer flasks. The flasks were sealed airtight with a septum. In order to collect volatiles the air volume was continuously circled through a charcoal filter (1.5 mg charcoal, CLSA-Filter, Le Ruisseau de Montbrun, Daumazan sur Arize, France) as described (Donath and Boland, 1995). Absorbed volatiles were eluted from the carbon traps after 1, 2, or 3 days sampling using 3×10 μl CH_2Cl_2 . The combined solutions were used for GC/MS.

4.5. Incubation with dehydrogeosmin-d_3

When the medium (5 ml A1 medium) of a growing culture of *Streptomyces* sp. JP95 turned purple (see 4.3), an aqueous solution of (4*S*,4a*S*,8a*S*)-1,2,3,4,4a,5,8,8a-octahydro-4-methyl-8a-[$^2H_3$]methylnaphthalen-4a-ol (100 μg in 1 ml H_2O) was added (synthesis was achieved according to Huber and Boland (1995) using an excess of CD_3-MgBr). Volatile samples were taken by SPME (Nilsson et al., 1996; Watson et al., 1999) after 3, 4 and 6 h and 1 day and subjected to GC–MS analysis (ca. 5 min sampling from the gas phase). No conversion of the labeled dehydrogeosmin-d_3 by *Streptomyces* sp. JP95 to the corresponding geosmin-d_3 was observed.

Dehydrogeosmin-d_3: EI–MS 70 eV, *m*/*z* (rel. int.): 165 (43), 150 (5), 147 (6), 129 (100), 122 (9), 114 (17), 111 (21), 109 (31), 108 (30), 91 (15).

4.6. Gaschromatographic separation and mass spectroscopy

The volatiles were analyzed on a Finnigan TraceMS (70 eV) equipped with an Alltech EC 5 column (15 m×0.25 mm, 0.25 μm), using temperature programed conditions for elution of compounds. Temperature program: 50 °C (2 min) at 10 °C/min to 200 °C followed by 30 °C/min to 280 °C. Carrier gas: helium at 3 ml/min. Injection volume: 1–2 μl. For the deuterium exchange experiments, the geosmin sample was dissolved in CD_3OD. Prior to injection, 3×1 μl CD_3OD were injected into the GC/MS to prevent exchange reactions in the instrument.

4.7. Mass spectra of geosmin isotopomers

Natural geosmin: EI–MS 70 eV, *m*/*z* (rel. int.): 182 $[M]^+$ (5), 167 (2), 149 (6), 126 (16), 125 (19), 112 (100), 97 (18), 95 (12), 83 (11), 81 (9), 69 (15), 67 (14), 55 (18), 43 (18).

Natural geosmin after H/^{2}H-exchange: EI-MS 70 eV, *m*/*z* (rel. int.): 183 $[M]^+$ (4), 168 (2), 149 (4), 127 (6), 126 (27), 113 (77), 112 (100), 111 (12), 98 (13), 96 (12), 95 (9), 84 (10), 83 (10), 67 (13), 55 (25), 43 (18).

[$^2H_5$]-Geosmin from *Streptomyces* sp. JP95 fed with [$^2H_2$]-DOX **11**. EI-MS 70 eV, *m*/*z* (rel. int.): 187 $[M]^+$ (4), 172 (2), 154 (4), 129 (13), 128 (10), 127 (15), 117 (9), 116 (100), 115 (31), 114 (29), 100 (10), 99 (13), 86 (12), 84 (10), 72 (5), 71 (6), 57 (15), 56 (12), 43 (16).

[$^2H_6$]-Geosmin from *Streptomyces* sp. JP95 fed with [$^2H_2$]-DOX **11** and subsequent H/^{2}H-exchange. EI–MS 70 eV, *m*/*z* (rel. int.): 188 $[M]^+$ (6), 173 (3), 154 (8), 130 (7), 129 (20), 128 (17), 117 (43), 116 (100), 115 (49), 114 (20), 99 (18), 86 (13), 70 (13), 69 (11), 57 (15), 56 (14), 43 (16).

[$^2H_9$]-Geosmin from *F. pusilla* fed with [$^2H_5$]-MVA **7**. EI-MS 70 eV, *m*/*z* (rel. int.): 191 $[M]^+$ (59), 173 (3), 155 (5), 134 (10), 117 (19), 116 (100), 115 (37), 55 (12), 43 (9).

[$^2H_4$]-Geosmin from *F. pusilla* fed with [$^2H_2$]-MVA **9**. EI–MS 70 eV, *m*/*z* (rel. int.): 186 $[M]^+$ (4), 171 (2), 153 (4), 128 (13), 127 (19), 115 (15), 114 (100), 113 (25), 99 (18), 83 (9), 69 (15), 57 (14), 43 (20).

4.8. Stereochemistry of geosmin

The enantiomers of geosmin (**1**) were separated by GLC using a fused silica capillary coated with 20% heptakis-2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin, Hydrodex β-3 P 25 m×0.25 mm (Macherey & Nagel, D-52313 Düren, Germany). Compounds were separated under temperature programmed conditions (90 °C for 20 min followed by 5 °C/min to 160 °C maintained for 2 min). Carrier gas: helium at 3 ml/min. The elution order was established by synthetic references [(+)-geosmin: 18.22 min, (−)-geosmin: 20.17 min)]. Geosmin from *Streptomyces* sp. JP 95 and *F. pusilla* coeluted with synthetic (−)-geosmin and proved to be enantiomerically pure (Ayer et al., 1976; König et al., 1992).

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