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Identification of (8*S*,9*S*,10*S*)-8,10-dimethyl-1-octalin, a key intermediate in the biosynthesis of geosmin in bacteria

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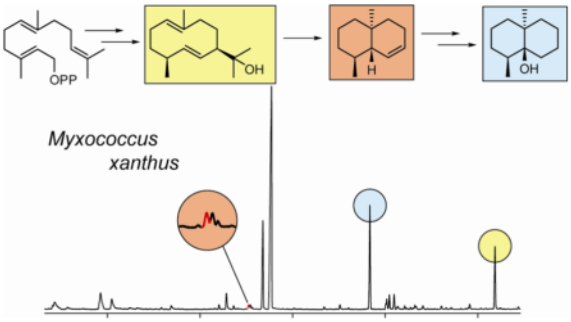
Abstract



(−)-Geosmin (**1**, blue) is an important odor component produced by many bacteria, including actinomycetes, myxobacteria, and cyanobacteria, but has also been reported for eukaryotic organisms such as fungi, liverworts, insects, and plants. Recent research has shown that the biosynthesis of **1** starts with the cyclization of farnesyl pyrophosphate to (1(10)*E*,5*E*)-germacradien-11-ol (**2**, yellow), the first key intermediate en route. In a retro-Prins-reaction acetone is lost, and the second key intermediate, (8*S*,9*S*,10*S*)-8,10-dimethyl-1-octalin (**3**, red), is formed. After reprotonation, a 1,2-H-shift and attack of water, geosmin is finally released. Octalin **3** occurs in the bouquets of volatiles released by myxobacteria as well as in enzyme extracts of incubation experiments of the purified geosmin synthase from *Streptomyces coelicolor*. Here the identification of **3**, a new natural compound, the elucidation of its stereochemistry, and its synthesis are reported.

(−)-Geosmin (**1**) has a strong earthy smell and is an important odor component produced by many bacteria, including actinomycetes, myxobacteria, and cyanobacteria, as well as a number of eukaryotic organisms such as fungi, liverworts, insects, and plants.[1,2] Geosmin was first described by Gerber and Lechevalier, who isolated it from the actinomycete

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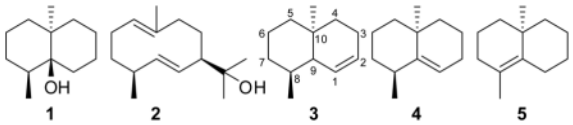
Supporting Information Available: General methods and experimental information, as well as GC-MS data are available free of charge via the Internet at http://pubs.acs.org.

Streptomyces griseus.[2] Humans can detect extremely low levels (parts per trillion) of geosmin, which is also a frequently occurring off-flavor in water treatment and in fishery.[3,4] The biosynthesis of this degraded sesquiterpene **1** has recently received much attention and has been investigated in streptomycetes[5,6] and myxobacteria.[7] The pathway starts with the cyclization of farnesyl pyrophosphate (**6**) to (1(10)*E*,5*E*)-germacradien-11-ol (**2**).[5d,7] A retro-Prins fragmentation results in the loss of acetone[6] with the formation of 8,10-dimethyl-1-octalin (6,10-dimethylbicyclo[4.4.0]dec-2-ene, **3**) of unknown stereochemistry at the ring junction. After reprotonation of **3** and a 1,2-H-shift,[5] final attack of water leads to **1** (Scheme 1).[5d,6,7] Germacradienol **2** frequently occurs together with **1** in extracts or scent bouquets of myxobacteria and streptomycetes.[8] Furthermore, **2** has been detected among the products of incubation of the purified geosmin synthase with FPP, and can also be converted to geosmin by the synthase, thus firmly establishing its role as an intermediate en route to **1**.[5]



The bouquets of volatiles released by the myxobacteria *Stigmatella aurantiaca* and *Myxococcus xanthus* contain several unidentified $C_{12}H_{20}$ (m.w. 164) hydrocarbons whose mass spectra are similar to that of 8,10-dimethyl-1(9)-octalin (6,10-dimethylbicyclo[4.4.0]dec-1-ene, **4**).[9] Here we report on the identification of the latest key intermediate in the biosynthesis of **1**, octalin **3**, and a side product of geosmin biosynthesis, octalin **4**.

During GC-MS analysis of the volatiles produced by the myxobacterium *Myxococcus xanthus* (Figure 1) we observed two compounds **A** and **B** with mass spectra (see supporting information) similar or identical to the published spectrum of **4**,[9] showing the strong M-15 ion expected for dimethyloctalins. Control experiments ruled out formation of either **A** or **B** by decomposition of geosmin during GC-MS analysis.

Both of these octalin isomers are therefore likely intermediates or side products of geosmin biosynthesis. Besides **4**, previously found as a constituent in liverworts and mosses,[10] the only previously described terpenoid dimethyloctalin is the synthetic compound 1,10-dimethyl-1(9)-octalin (2,6-dimethylbicyclo[4.4.0]dec-1-ene, argosmin C, **5**).[11b] Argosmin C has been obtained by treatment of **1** with conc. HCl together with four unknown $C_{12}H_{20}$ (m.w. 164) compounds,[2,11] but unfortunately no mass spectrum has been published.

We have now synthesized the proposed intermediate **3** in order to confirm its structure and stereochemistry. (*R*)-2,6-dimethylcyclohexyl-1-phenylethylimine (**9**)[12] was transformed into **10** following a route described by Pfau et al.[13] The enantiomerically enriched octalone **10** was treated with tosylhydrazine to yield the hydrazone **12**, which upon exposure to $NaBH_4$[14] gave a 4:1 mixture of two diastereomers of (1*S*,10*S*)-**3**, containing either a *cis*- or *trans*-fused octalin ring system. In accord with the mechanism of reduction of unsaturated hydrazones,[15] the axial bridgehead methyl group at C-10 in the hydrazone **12** directs the initial attack of the borohydride to the β-face of the ring. The resulting α-diimide therefore transfers its H-atom to the α-face of C-9. This retro-ene reaction expels the N-substituent from the molecule, resulting in the *cis*-fused ring arrangement for the major reaction product, *cis*-**3**. The minor diastereomer is therefore *trans*-**3**. We chose this moderately stereoselective process to have an easy access to a defined mixture of both diastereomers of **3**, enriched each in one enantiomer, for comparison with the natural product.

These stereochemical assignments are further corroborated by NMR experiments because of the known ^{1}H-NMR shifts of bridgehead methyl groups in *cis*- and *trans*-octalin derivatives.[16] For *cis*-compounds a value of 0.98 ppm was reported, while the signal in the *trans*-isomers appeared around 0.85 ppm.[16] The observed methyl chemical shifts of 0.81 ppm for *trans*-**3** and 0.98 ppm for *cis*-**3** therefore support our configurational assignments. In addition, NOESY-experiments showed strong interactions between both methyl groups and the bridgehead hydrogen in case of *cis*-**3**, while no such interaction was found between the C-10 methyl group and the bridgehead hydrogen in *trans*-**3**. Additional proof was obtained by synthesis of pure *trans*-**3** by 1,4-reduction of octalone **10** with lithium, and trapping of the resulting enolate with diethyl chloro phosphate. This procedure is known to selectively furnish trans-fused octalins.[17] The resulting enol phosphonate was then reduced with Li to furnish pure *trans*-**3**, albeit in low yield.[17] Comparison with the naturally occurring compounds proved that compound **A** is identical to *trans*-**3**. The ring junction in *trans*-**3** is therefore identical to the *trans* ring fusion of geosmin (**1**). Gas chromatographic analysis on a chiral phase showed that only the synthetic enantiomer (8*S*,9*S*,10*S*)-8,10-dimethyl-1-octalin (*trans*-**3**) occurs naturally.

Compound **4** was synthesized by transformation of octalone **10** into the dithioketal **13**, followed by desulfurization with tributyltin hydride.[18] Comparison of mass spectra, retention index, and co-injection confirmed the identity of **B** and **4**. The formation of **4** during geosmin biosynthesis can be rationalized by loss of a proton from either cation **7** or **8** (Scheme 1). Both compounds **3** and **4** were also identified in several other myxobacteria such as *Nannocystis exedens* or in *Streptomyces* strains.

Both compounds **3** and **4** were also observed among the products resulting from incubation of farnesyl diphosphate (**6**) with the purified geosmin synthase from *Streptomyces coelicolor*,[3d] along with **1**, **2**, and germacrene D as main products. This experiment unambiguously proves that both **3** and **4** are formed by the geosmin synthase in streptomycetes, with **3** likely an intermediate and **4** being a shunt metabolite. The co-ocurrence of **3** and **4** in head-space extracts of geosmin-producing myxobacteria supports the same conclusion for these species. Incubation of deuterated octalin **3** with *S. coelicolor* geosmin synthase did not give rise to labeled geosmin, most likely because enzymatically generated **3** is normally processed by a transiently activated form of the geosmin synthase in which the distribution of charged residues resulting from the retro-Prins fragmentation of germacradienol **2** is distinct from the resting state of the enzyme. Similar lack of conversion of exogenously added sesquiterpenes by other terpene synthases has previously been observed.[19] Nevertheless, the occurrence of (8*S*,9*S*,10*S*)-**3** both under natural conditions and in enzyme preparations suggests its key role in the biosynthesis of **1**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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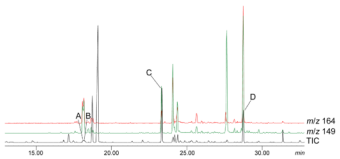
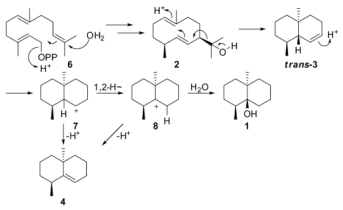
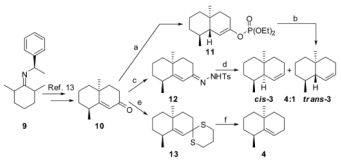


Figure 1.

Total ion chromatogram of a headspace extract of *M. xanthus*. **A**: (8*S*,9*S*,10*S*)-8,10-dimethyl-1-octalin (*trans*-**3**); **B**: (8*S*,10*S*)-8,10-dimethyl-1(9)-octalin (**4**); **C**: geosmin (**1**); **D**: (1(10)*E*,5*E*)-germacradien-11-ol (**2**).



Scheme 1.
Biosynthesis of geosmin (**1**) in *M. xanthus*



Scheme 2.

Synthesis of 8,10-dimethyl-1-octalin (**3**) and 8,10-dimethyl-1(9)-octalin (**4**). a) i. Li, NH_3, ii. $ClPO(OEt)_2$ (84%); b) Li, $EtNH_2$ (10%); c) $TsNHNH_2$, $BF_3{\cdot}Et_2O$ (27 %); d) i. conc. AcOH, ii. $NaBH_4$ (52 %, ee 56 %); e) 1,3-$Pr(SH)_2$, $BF_3{\cdot}Et_2O$ (97 %); f) Bu_3SnH, AIBN (50 %)