185. Biosynthesis of the Irregular C_{12}-Terpenoid Dehydrogeosmin in Flower Heads of *Rebutia marsoneri* WERD. (*Cactaceae*)

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Dedicated to Prof. Dr. *L. Jaenicke* on the occasion of his 70th birthday

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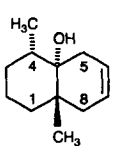
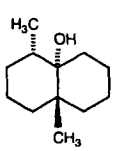
Farnesol (**3a**) is the precursor to the irregular C_{12} terpenoid (4*S*,4a*S*,8a*S*)-1,2,3,4,4a,5,8,8a-octahydro-4,8a-dimethylnaphthalen-4a-ol (= dehydrogeosmin; **1**). The irregular C-backbone originates from the oxidative removal of a C_3 side chain from a C_{15} eudesmane-type intermediate (*Scheme 2*). The bicyclic C-framework is assembled by a formal addition of H_2O across the endocyclic double bonds of a monocyclic germacradiene-type precursor. The biosynthetic pathway follows from administration of the deuteriated farnesols **3b–e** to flower heads of the cactaceae *Rebutia marsoneri* WERD. and mass-spectroscopic analysis of the collected volatiles.

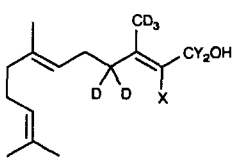
Introduction. – (+)-(4*S*,4a*S*,8a*S*)-1,2,3,4,4a,5,8,8a-Octahydro-4,8a-dimethylnaphthalen-4a-ol (= (+)-dehydrogeosmin; **1**) is the olfactory dominant component of the flower scent of several *Cactaceae* like, *e.g., Rebutia marsoneri* WERD., *Dolichothele longimamma* (DC.) BR. *et* R., and *Sulcorebutia kruegeri* (CARD.) RITT [1]. Natural **1** has the same absolute configuration as (–)-geosmin (**2**) [2] which is produced by certain cyanobacteria, actinomycetes, and fungi that inhabit aquatic and soil environments [3]. (–)-Geosmin (**2**) is the typical off flavor component to be associated with brackish H_2O or freshly ploughed soil. Considering the sometimes extreme dry and hot habitats of cactaceae, it appears reasonable to assume that in such an environment, the strongly earthy-musty odor of **1** might attract pollinators and, hence, **1** may represent an important signal in the reproduction biology of the *Cactaceae* [1].



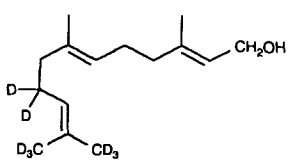
1 (+)-dehydrogeosmin



2 (–)-geosmin



3a X = Y = H, CH_3 and CH_2 instead of CD_3 and CD_2, resp.
b X = Y = H
c X = H, Y = D
d X = D, Y = H



3e

The closely related structures of geosmin (**2**) and dehydrogeosmin (**1**) suggest a common biosynthetic pathway which probably involves an oxidative side-chain degradation of an eudesmane-type sesquiterpene precursor. Some indirect evidences for an isoprenoid pathway starting from, *e.g.*, farnesol and the involvement of a mixed-function oxidase in

the bacterial biosynthesis of geosmin (**2**) were reported [4], but details of the cyclization-oxidation-degradation sequence are not known. Since **1** possesses an endocyclic double bond, the oxidative removal of the C_3 side chain might be considered as another example of a natural oxidative bond cleavage which degrades functionalized precursor molecules into an olefin and a carbonyl compound [5] [6]. The best studied reactions of this type are the side-chain cleavage reactions in the field of steroids [7] and the biosynthesis of acyclic C_{11} and C_{16} homoterpenes in flower scents [8] or volatiles emitted from leaves damaged by herbivores [9].

Here we report of the sesquiterpenoid origin of dehydrogeosmin **1** by successful administration of deuterium-labelled [^2H_n]farnesols **3b–e** to the *Cactaceae Rebutia marsoneri* WERD. and the metabolic conversion by flower heads of this plant.

Results and Discussion. – 1. *Feeding Experiments and Collection of Volatiles.* From previous work on the biosynthesis of geosmin (**2**), it is known that the irregular C_{12} framework of **2** is not assembled from a monoterpene by addition of two C_1 units [10]. This, together with the position of the endocyclic double bond of **1** suggests that both molecules may be formed from a common C_{15} terpenoid precursor like, *e.g.*, farnesol (**3a**) or nerolidol, respectively. In the present study, a series of deuterium-labelled farnesols **3b–e**, synthesized according to standard procedures, is administered to disconnected flower heads of *Rebutia marsoneri*. Mass-spectroscopic analysis of the scented volatiles is used to unravel the sequence of reactions between the sesquiterpenoid precursor **3** and dehydrogeosmin **1**.

The labelled precursors are emulsified in tap water (2 mg/ml) by sonication (130 W) for 2 min. The resulting emulsions are stable for the period of the feeding experiment (up to 3 days). Flower heads (2–3) from just blooming specimens of the *Cactaceae Rebutia marsoneri* are disconnected close to the peduncle and immediately placed into the aqueous emulsions of the labelled farnesols **3b–e**. If the experimental setup is exposed to bright sunshine, the blossoms still follow their circadian rhythm and emit a strong earthy musty odor when open between *ca.* 9 a.m. and 5 p.m. The immersed blossoms and the culture-medium are placed in a small desiccator connected to a trapping device consisting of a miniature circulation pump (Fa. *E. Fürgut*, D–88319 Aitrach) and a charcoal 'filter' (1.5 mg; *CLSA-Filter*, CH–8405 Winterthur). Flask, pump, and filter holder are joined together forming a closed system (air volume *ca.* 850 ml). The air circulation is started after the first 24 h of incubation and is maintained over the second 24-h period. During circulation, the produced volatiles are adsorbed onto the charcoal trap [11]. Following desorption [12] from the carbon traps with CH_2Cl_2 (2 × 15 µl), the volatiles are directly analyzed by GC/MS. Alternatively, the tissue of the cactus close to the peduncle of the flower head may be topically infiltrated with aqueous emulsions of **3b–e** over a period of 24 h using an open syringe without a piston. The whole plant is transferred into the closed system, and the emitted volatiles are sampled as described.

2. *Mass-Spectroscopic Analysis of the Labelled Metabolites.* Due to the presence of at least 5 ^2H atoms in the precursors **3b–e** and their metabolites [^2H_n]-**1**, natural (+)-dehydrogeosmin (**1**) and labelled [^2H_n]-**1** exhibit near base-line separation upon gas chromatographic separation, and, hence, the MS of the labelled dehydrogeosmins [^2H_n]-**1** ($n \geq 5$) are not superimposed by **1** or other compounds. In the case of [$^2H_1$]-**1**, the product of the feeding experiments with **3e**, the separation is incomplete and results in a superimposed spectrum out of the front area of the eluting **1**.

The MS of natural **1** and [$^2H_6$]-**1** (*Fig.*), obtained from administration of **3d**, will be discussed in more detail. Fragment **I** results from loss of H_2O from the intact molecule and indicates that all 6 ^2H atoms of the precursor **3d** have been incorporated into **1** (*cf. Fig., Scheme 1*, and *Table*; *m/z* 168 in [$^2H_6$]-**1** and *m/z* 162 in natural **1**). Concerted *retro-Diels-Alder* reaction within [$^2H_6$]-**1** removes the four C-atoms of ring B, yielding **III** as the most

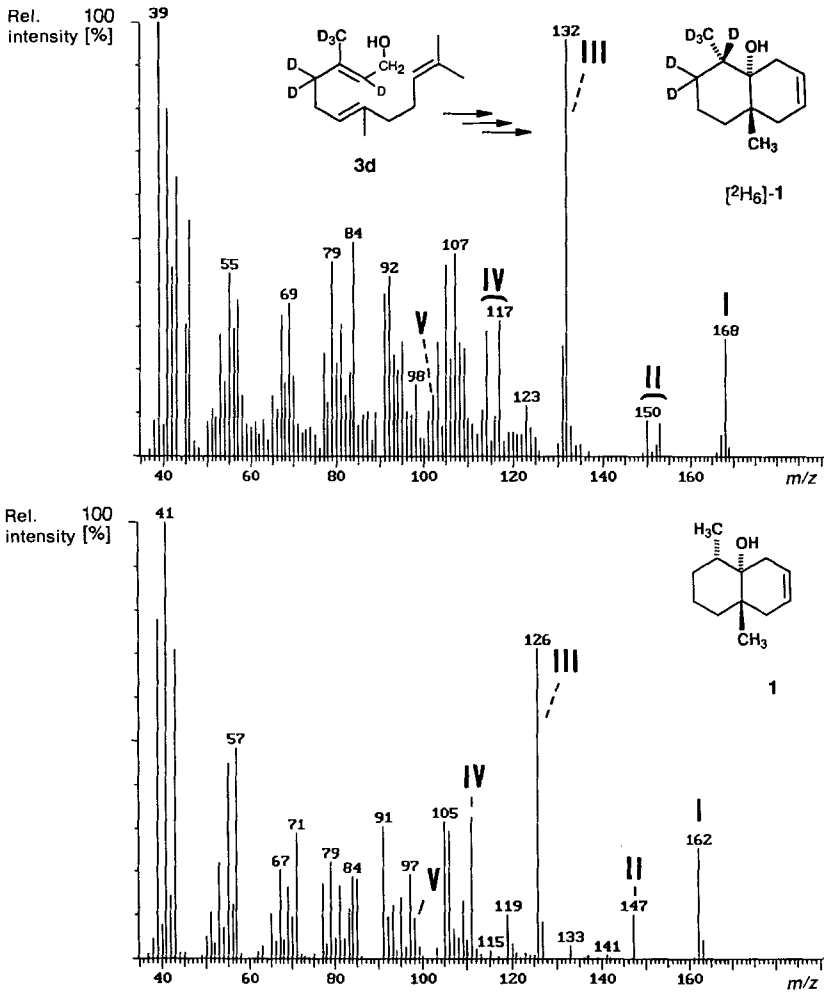


Figure. *EI-MS* (70 eV) *of labelled dehydrogeosmin [$^2H_6$]-***1** *from incubation of flower heads of* Rebutia marsoneri *with [$^2H_6$]farnesol* (**3d**) *and of natural (+)-dehydrogeosmin* **1**. GC Separation: *SE 30* fused-silica capillary column (10 m × 0.31 mm) under programmed conditions (50° for 5 min, then at 10°/min to 250°). MS: *Finnigan Ion Trap ITD 800;* transfer line 270°; scan range 35–249 Da/s. For fragments **I**–**V**, see *Scheme 1* and *Table*.

abundant fragment at *m/z* 132 (*m/z* 126 for natural **1**), indicating that all 6 ^2H atoms of **3d** must reside in ring A of [$^2H_6$]-**1**. Fragment **III** decomposes further into **IVa/b** (*m/z* 114 and 117, resp.) by loss of a CD_3 or CH_3 group, respectively. An intact CD_3 substituent is also evidenced by the fragments **IIa/b** at *m/z* 150 and 152, respectively, derived from **I**. If [$^2H_7$]farnesol (**3c**) is administered, the two ^2H atoms at C(1) of the precursor are obviously incorporated into ring B of [$^2H_7$]-**1**. Fragment **I** is now located at *m/z* 169, but **III** is found at *m/z* 131 which accounts for 5 ^2H atoms in ring A. The fragments **IIa/b** and **IVa/b** confirm this incorporation pattern (*cf. Table*). Significant information is gained from administration of [$^2H_8$]farnesol (**3e**). In this case, out of the 8 ^2H atoms of precursor **3e**, only one is found in ring B of the resulting [$^2H_1$]-**1** (*cf. Table*, **I** and **III**); the other ^2H atoms

are lost together with C(11), C(12), and the Me group at C(11). Accordingly, the C-atoms of the polar head of farnesol **3a–e**, except C(1), contribute to ring A of (+)-dehydrogeosmin (**1**), while the aliphatic terminus of **3a–e**, except C(11), C(12), and the Me group at C(11), is incorporated into ring B. Interestingly, both [$^2H_5$]- and [$^2H_7$]-**1**, derived from **3b** ([$^2H_5$]) and **3c** ([$^2H_7$]), respectively, yield the isobaric fragments **III** (*m/z* 131), **V** (*m/z* 101), and **IVa/b** (*m/z* 113 (116)), while feeding of **3d** ([$^2H_6$]) gives rise to fragment ions **III** at *m/z* 132 and **IVa/b** at *m/z* 114 (117) of the metabolite. This clearly indicates that the ^2H atom at C(2) of **3d** finally appears in the A-ring of [$^2H_6$]-**1**, increasing the corresponding fragments by one mass unit. The elemental composition of the above fragments is additionally supported by high-resolution MS using **1**.

Scheme 1

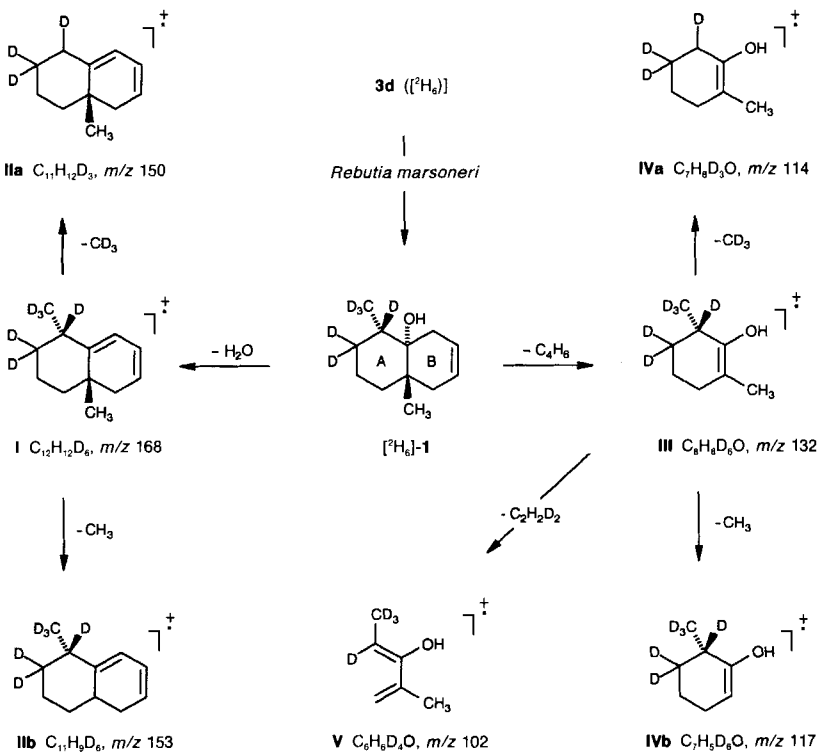


Table. *Mass Fragments (m/z) of Labelled Dehydrogeosmins [^2H_n]-***1** *from* Rebutia marsoneri[a])

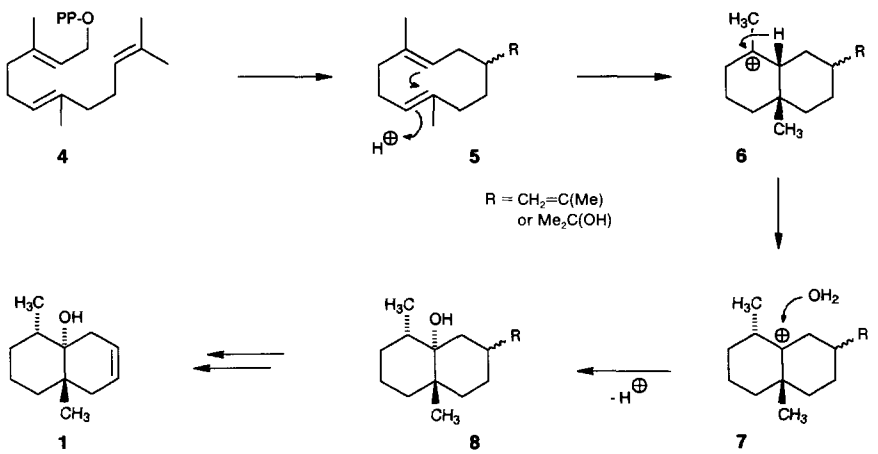
[^2H_n]Farnesol precursor	I	IIa (IIb)[b])	III	IVa (IVb)[b])	V
3a	162	– (147)	126	– (111)	98
b	167	149 (152)	131	113 (116)	101
c	169	151 (154)	131	113 (116)	101
d	168	150 (153)	132	114 (117)	102
e	163	– (148)	126	– (111)	98

[a]) For fragments **I**–**V** of [$^2H_6$]-**1** (from **3d**), see *Scheme 1*.

[b]) **IIa** and **IVa** arise from loss of CD_3, **IIb** and **IVb** from loss of CH_3.

3. *From Farnesol to Dehydrogeosmin: a Putative Sequence.* The above mass-spectroscopic findings suggest that the terminal double bond and the polar head of **3** are tethered together in ring B of **1**. This is readily accomplished, if C(1) of farnesyl pyrophosphate (**4**), the universal precursor of sesquiterpenes [13], is enzymatically connected to C(10), yielding the germacradiene-type C-framework **5** (*Scheme 2*), *i.e.* germacrene A (R = CH_2=C(Me)) by ensuing loss of H^+ or hedycaryol (R = $Me_2C(OH)$) by addition of H_2O [14].

Scheme 2



Since the C_3-substituent is lost during the biosynthetic sequence, and since no other labelled compound is found besides [^2H_n]-**1**, this question and the absolute configuration at the side-chain-substituted center have to be left open. The plausible biosynthetic pathway outlined in *Scheme 2*, in agreement with the structural and spectroscopic features, exhibits striking parallels to the previously reported biosynthesis of capsidiol, a well known phytoalexin of the pepper plant *Capsicum frutescens* [15]. Thus, after the cyclization of **4**, formal addition of H^+ to the double bond of **5** may induce the formation of the bicyclic framework. The resulting carbenium ion **6** is prone to a suprafacial [1,2]-H shift with concomitant attack of H_2O at the bridgehead carbenium ion **7** (→**8**). Accordingly, the absolute configuration at C(4) and C(4a) of **1** is established. The whole sequence corresponds to an enzyme-catalyzed, formal *cis*-addition of H_2O across the double bonds of **5**. Note, that the required [1,2]-H shift is sufficiently evidenced by the fragments **I** and **III** of the metabolites derived from **3d** and **3c**, respectively. To generate the C-framework of the irregular terpenoid **1**, oxidative cleavage [5] of the C_3 substituent R with simultaneous introduction of the double bond has to occur. It must be emphasized that the oxidative removal of the side chain proceeds with loss of only 1 of the 2 H-atoms from C(7) (see results with precursor **3e**) and, hence, the biosynthesis of **1** in *Cactaceae* may be regarded as another example of the general route towards olefins *via* oxidative bond cleavage as discussed previously [5] [6].

Some of the above administration experiments have already been performed with other *Cactaceae* like, *e.g., Rebutia kupperiana* and a non-defined *Gymnocalicium* sp. In each case, the labelled [^2H_n]farnesol **3** is readily incorporated into the emitted dehydro-

geosmin **1** and, thereby, proves the above pathway to be operative in other *Cactaceae* as well. Whether or not the removal of the C_3 substituent is an early or terminating event in the above pathway is not known and remains to be clarified.

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Experimental Part

General. See [8].

Labelled Precursors. [^2H_n]Farnesols **3b–d** are prepared from [1,1,1,3,3-$^2H_5$]geranylacetone [8] by reaction with the anion derived from ethyl (diethoxyphosphoryl)acetate using NaH as base according to standard procedures ((*E*)/(*Z*) 4:1). Separation of the isomers is achieved by CC (silica gel), and reduction of the ester moiety with $LiAlH_4$ yields farnesol **3b**; reduction with $LiAl^2H_4$ affords **3c**. Isomer **3d** is obtained by the same route using the labelled [^{2}H]phosphonate anion and $LiAlH_4$ for reduction, and **3e** is synthesized as described in [16].

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