Microbial Hydroxylation of Precursors of Sinensal

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In order to produce the important flavour compound sinensal microbial ω-hydroxylation of farnesene and its sulfone derivative were investigated. While farnesene proved to be a poor substrate, its sulfone could be hydroxylated to the ω-hydroxyfarnesene sulfones in up to 27% yield. Some strains could discriminate between the *E*- and *Z*-configurated substrate. In low yields products hycroxylated at different positions and two monocyclofarnesane derivatives could also be isolated.

Introduction

α- and β-sinensal are important flavour compounds of Chinese orange oil (*Citrus sinensis* L.) [1]. One of the drawbacks of their chemical syntheses from acyclic sesquiterpenoids is the low regioselectivity of the oxidation of the terminal methyl group. This oxidation step also demands toxic chemicals which are often separated from the product only with difficulties [2]. On the contrary biotransformations have the advantage of proceeding with high regioselectivity without producing toxic wastes. For these reasons we decided to use microorganisms for the ω-oxidation of acyclic sesquiterpenoid precursors of sinensal.

Results

Despite of our experience that biotransformations of sesquiterpene hydrocarbons proceed usually very slowly and in low yields (*e.g.* humulene [3], cedrene [4] or isolongifolene [5]) we started with farnesene as substrate.

Attempts to oxidize β-farnesene with microorganisms led always to a complete consumption of the substrate while no metabolites could be detected. The presumable cause for this result is the sensitivity of the conjugated diene to oxygene and acids.

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Our results with other sesquiterpenes told us that a polar function in the substrate makes the biotransformation to proceed faster and in higher yields [6]. We tried to protect the dienyl moiety of farnesene by a dienophile resulting in a polar substrate which can be cleaved easily after the biotransformation simply by heating. For this purpose we choose sulfur dioxide as dienophile which reacted under pressure with the diene to a sulfolene [7]. This sulfolene was completely stable under fermentation conditions [8].

Fermentation of *Nocardia* sp. DSM 43130 with β-farnesene sulfone (**1**) led after 161 h to the ω-hydroxylated farnesene sulfones **2–5** in 28% yield. *Pseudomonas lapsa* DSM 50274 oxidized the same substrate to **2–5** in 16% yield (Fig. 1).

Since α-farnesene was not available in sufficient amount and high purity we dehydrated *trans*-nerolidol to the three possible farnesenes, *i.e.* β-farnesene and 3*E*- and 3*Z*-α-farnesene. This mixture was reacted with sulfur dioxide to the corresponding farnesene sulfones **1** and **23.** Biotransformation of these sulfones led to a complex mixture of products with the majority of the strains screened. Only few strains possessed sufficient selectivity to select either α- or β-6*E*-farnesene sulfone for omega oxidation. *Pseudomonas lapsa* DSM 50274 transformed this substrate to 12-hydroxy-β-farnesene sulfone (**4**) (17% yield) and 12-hydroxy-α-farnesene sulfone (**24**) (10% yield). This problem could probably be avoided by the synthesis of pure α-farnesene sulfone (**23**), *e.g.* by the method of Tso *et al.* [9].

The stereochemistry at the double bonds could be determined from the NMR spectra. Methyl

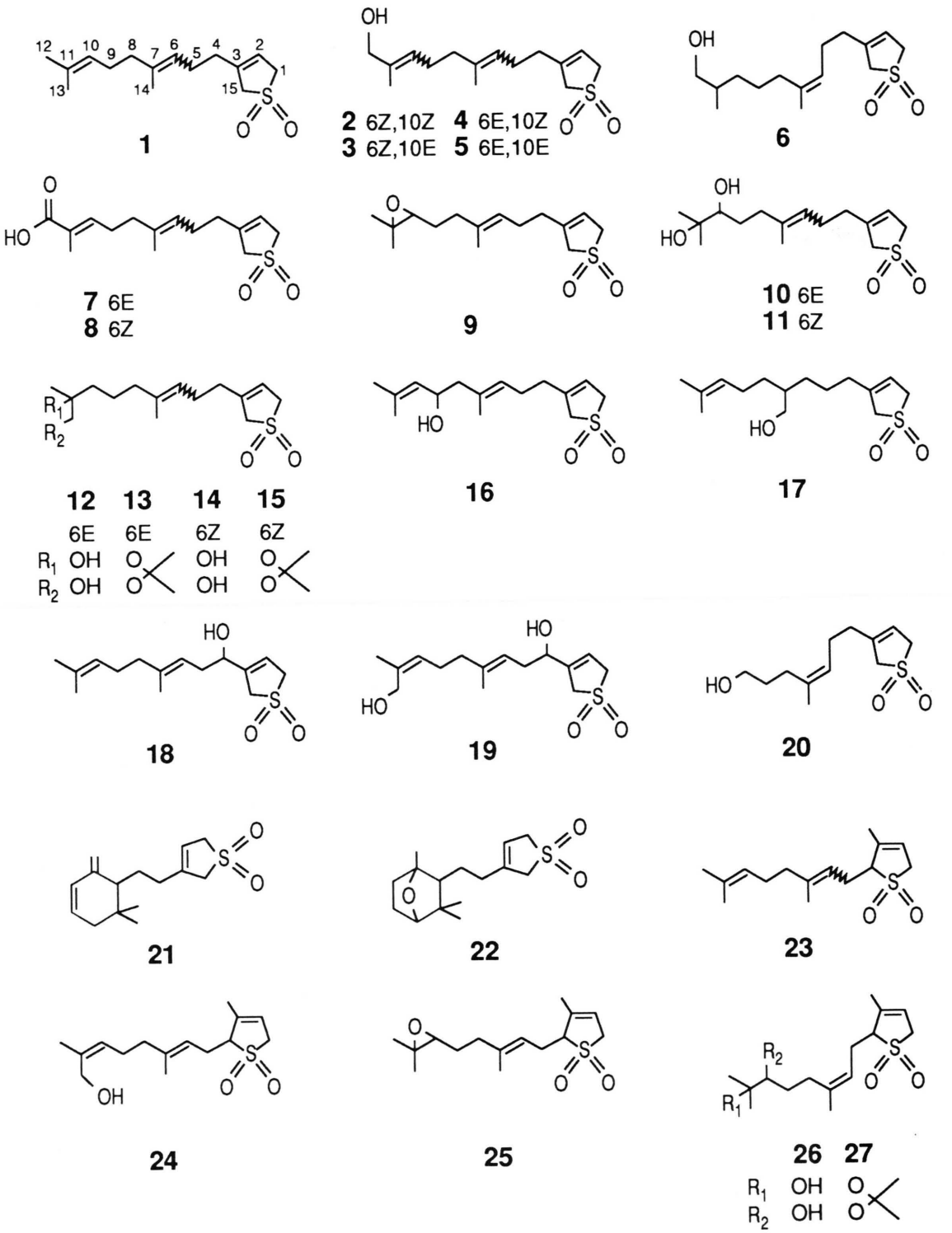

Fig. 1. Farnesene sulfones and their biotransformation products.

groups in *E*-position show their resonances at δ_H = 1.60–1.63 and δ_C = 16–17 while those in *Z*-position usually have resonances at δ_H = 1.69–1.70 and δ_C = 23. In the ω-alcohols these resonances in the ^{13}C NMR spectra were shielded by 5–7 ppm due to the γ-effect of the hydroxy group (Tables I and II).

Beside the hydroxylation at the terminal methyl group some other hydroxylations were detected. *Bacillus megaterium* DSM 510 oxidized **1** in 9-position to **16** and *Nocardia* sp. DSM 43130 hydroxylated **1** to **18** which is further oxidized to **19** (Table I). Oxidation at C-14 and hydrogenation of the 6,7-double bond to the metabolite **17** by *Nocardia gardneri* DSM 43020, *Arthrobacter simplex* DSM 20299 and *Pseudomonas lapsa* DSM 50274 is rather unusual. Hydrogenation of **2/3** to **6** was also observed. As in many other acyclic terpenes epoxi-

Table I. ^1H NMR data of sesquiterpene sulfone derivatives **2–9** ($CDCl_3$, 400 MHz).

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1-H	3.68 m	3.7 m	3.68 m	3.69 m	3.69 m	3.68 m	3.70 m	3.68 m
2-H	5.69 m	5.71 m	5.68 m	5.69 m	5.68 m	5.68 m	5.70 m	5.68 m
4-H	2.15 m	2.12 m	2.15 m	2.13 m	2.19 m	2.1 m	2.19 m	2.2 m
5-H	2.15 m	2.12 m	2.15 m	2.13 m	2.19 m	2.1 m	2.19 m	2.2 m
6-H	5.10 t	5.11 t	5.08 t	5.09 t	5.06 t	5.23 t	5.13 m	5.12 m
8-H	2.15 m	2.1 m	2.15 m	2.13 m	2.01 t	2.1 m	2.19 m	2.2 m
9-H	2.15 m	2.1 m	2.15 m	2.13 m	1.7–	2.31 td	2.28 t	1.65 m
10-H	5.28 t	5.42 t	5.26 t	5.37 t		6.86 t	6.76 t	2.69 t
11-H	–	–	–	–	1.3 m	–	–	–
12-H	1.81 s	4.01 s	1.81 s	4.01 s	3.51 dd	–	–	1.30 s
12′-H					3.45 dd			
13-H	4.11 s	1.71 s	4.12 s	1.66 s	0.93 d	1.85 s	1.83 s	1.27 s
14-H	1.70 s	1.69 s	1.61 s	1.62 s	1.68 s	1.63 s	1.72 s	1.63 s
15-H	3.80 m	3.82 m	3.79 m	3.81 m	3.80 m	3.80 m	3.81 m	3.79 m

J (Hz): **2–5:** 5, 6 = 9, 10 = 7. **6:** 5, 6 = 8, 9 = 7; 11, 12 = 11, 12′ = 6; 12, 12′ = 11; 12, 13 = 7. **7:** 5, 6 = 8, 9 = 9, 10 = 7. **8:** 8, 9 = 9, 10 = 7. **9:** 9, 10 = 7.

Table I (continued). ^1H NMR data of sesquiterpene sulfone derivatives **10–11, 13** and **15–19** ($CDCl_3$, 400 MHz).

	10	**11**	**13**	**15**	**16**	**17**	**18**	**19**
1-H	3.72 m	3.68 m	3.67 m	3.67 m	3.69 m	3.68 m	3.83 m	3.83 m
2-H	5.69 m	5.69 m	5.68 m	5.68 m	5.70 m	5,70 m	5.93 m	5.93 m
4-H	2.24 m	2.24 m	2.18 m	2.18 m	2.24 m	2.2 m	4.23 t	4.24 t
5-H	2.24 m	2.24 m	1.99 m	1.99 m	2.14 m	2.2 m	2.08 m	2.32 m
6-H	5.14 t	5.11 t	5.07 t	5.07 t	5.18 t	1.5 m	5.09 t	5.10 t
7-H	–	–	–	–	–	2.0 m	–	–
8-H	2.24 m	2.24 m	2.18 m	2.18 m	2.24 m	1.5 m	2.33 m	2.08 m
9-H	2.24 m	2.24 m	1.4 m	1.4 m	4.44 ddd	2.0 m	2.08 m	2.19 m
10-H	3.33 dd	3.30 dd	1.4 m	1.4 m	5.17 d	5.09 t	5.10 t	5.22 t
12-H	1.16 s	1.16 s	3.78 d	3.78 d	1.70 s	1.69 s	1.70 s	1.79 s
12′-H			3.67 d	3.67 d				
13-H	1.21 s	1.21 s	1.27 s	1.27 s	1.73 s	1.61 s	1.64 s	4.10 s
14-H	1.63 s	1.70 s	1.60 s	1.69 s	1.68 s	3.60 dd	1.61 s	1.65 s
14′-H						3.52 dd		
15-H	3.81 m	3.81 m	3.78 m	3.78 m	3.80 m	3.80 m	3.83 m	3.81 d
15′-H								3.74 d
17-H	–	–	1.40 s	1.40 s	–	–	–	–
18-H	–	–	1.38 s	1.38 s	–	–	–	–

J (Hz): **10, 11:** 5, 6 = 7; 9, 10 = 10; 9, 10′ = 2. **13, 15:** 5, 6 = 7; 12, 12′ = 10. **16:** 5, 6 = 7; 8, 9 = 8; 8′, 9 = 5; 9, 10 = 7. **17:** 7, 14 = 7, 14′ = 6; 9, 10 = 7; 14, 14′ = 11. **18:** 4, 5 = 5, 6 = 9, 10 = 7. **19:** 4, 5 = 5, 6 = 9; 10 = 7; 15, 15′ = 15.

Table I (continued). ^{1}H NMR data of sesquiterpene sulfone derivatives **20–22, 24–25** and **27** ($CDCl_3$, **21** in C_6D_6, 400 MHz).

	20	**21**	**22**	**24**	**25**	**27**
1-H	3.70 m	2.95 m	3.68 m	3.72 d	3.70 d	3.71 d
1′-H				3.63 d	3.64 d	3.64 d
2-H	5.69 m	4.72 m	5.69 m	5.68 m	5.69 m	5.69 m
4-H	2.21 m	1.72 m	2.15 m	3.53 m	3.52 m	3.52 t
4′-H		1.44 m				
5-H	2.21 m	1.20 m	1.48 m	2.56 t	2.58 t	2.58 q
5′-H		0.87 m				
6-H	5.10 t	1.45 m	1.19 dd	5.20 t	5.27 t	5.28 t
7-H	–	–	–	–	–	–
8-H	2.11 t	5.98 d	1.48 m	2.05 m	2.18 m	2.24 m
8′-H						2.06 m
9-H	1.65 tt	5.50 m	1.92 ddd	2.16 m	1.64 m	1.6 m
9′-H			1.70 dddd			
10-H	3.64 t	1.84 d	3.75 d	5.21 t	2.72 t	3.66 m
10′-H		1.54 dd				
11-H	–	–	–	–	–	–
12-H		0.88 s	1.06 s	1.80 s	1.30 s	1.25 s
12′-H						
13-H		0.80 s	1.01 s	4.12 s	1.27 s	1.10 s
14-H	1.71 s	4.84 s	1.33 s	1.67 s	1.70 s	1.70 s
14′-H		4.57 s				
15-H	3.80 m	3.08 s	3.80 m	1.86 s	1.85 s	1.86 s
15′-H						
17-H	–	–	–	–	–	1.42 s
18-H	–	–	–	–	–	1.33 s

J (Hz): **20:** 5, 6 = 8, 9 = 9, 10 = 7. **21:** 8, 9 = 9, 10 = 10; 9, 10′ = 5; 10, 10′ = 17. **22:** 5, 6 = 6; 5′, 6 = 7; 8, 9 = 9; 8, 9′ = 8′, 9 = 5; 8′, 9′ = 11; 9, 9′ = 13; 9′, 10 = 6. **24, 25:** 1, 1′ = 14; 4, 5 = 5, 6 = 9, 10 = 7. **27:** 1, 1′ = 14; 4, 5 = 5, 6 = 7.

dation of the 10,11-double bond to **9** is observed. This product is opened by some strains to the diol **10.** The diol **11** with 6*Z*-double bond was also observed although its intermediary epoxide could not be isolated. *Nocardia* sp. DSM 40350 cleaved this diol to the trinorsesquiterpenoid **20** which was only found with *Z*-configurated double bond (Table II). As with the analogous monoterpenes the 10,11-double bond is isomerized to the 11,12-position and oxidized to the compounds **12** and **14.** These diols could only be separated by reacting the mixture with acetone to the corresponding acetonides **13** and **15.**

Pseudomonas lapsa DSM 50274 displayed a rather strange cyclization of the substrate. From the culture broth of this strain the monocyclofarnesanes **21** and **22** were isolated. The cyclic ether **22** resembles farnesiferol C [10]. While **22** can be explained by the cationic cyclization of the epoxide, the cyclization to **21** seems to be more complex.

The side products of **23** are very similar to that of **1.** Epoxidation of **23** to **25** and its hydrolysis to **26** was found and also the hydroxylation to **24** was catalyzed by some strains.

A general experience in biotransformation is the fact that most strains show a high substrate specificity. This is also observed with the sulfolenes discussed in this context. *Diplodia gossypina* ATCC 10936 formed the myrcene-6,7-diol from myrcene in 20% yield but with myrcene sulfone no reaction was observed [11]. *Aspergillus niger* ATCC 9142 oxidized *trans*-nerolidol to 12-hydroxy-*trans*-nerolidol in 20% yield but again no reaction was observed with this strain and farnesene sulfone [12].

Discussion

Biotransformation of farnesene were unsuccessful and led to no products. Protection and activation of the dienyl moiety of farnesene by sulfur dioxide resulting in farnesene sulfone produced

Table II. ^{13}C NMR of farnesene sulfone derivatives **2–6, 8–11** and **16–18** ($CDCl_3$, 75.5 MHz).

	2	**3**	**4**	**5**	**6**	**8**	**9**	**10**
C-1	57.1 –[a]	57.0 –	57.1 –	57.1 –	57.1 –	57.1 –	57.1 –	57.1 –
C-2	117.3 +	117.1 +	117.3 +	117.2 +	117.2 +	117.2 +	117.2 +	117.4 +
C-3	136.7 0	138.4 0	138.6 0	138.6 0	138.4 0	138.3 0	138.4 0	138.3 0
C-4	33.3 –	33.3 –	33.1 –	33.0 –	33.0 –	33.1 –	33.0 –	32.9 –
C-5	25.4 –	25.2 –	25.5 –	25.5 –	25.2 –	22.7 +	25.4 –	25.2 –
C-6	123.7 +	123.4 +	122.9 +	122.7 +	123.0 +	124.4 +	122.9 +	123.0 +
C-7	135.1 0	n.d.	136.5 0	136.6 0	137.2 0	135.4 0	136.1 0	136.6 0
C-8	32.3 –	31.6 –	39.8 –	39.2 –	32.0 –	30.4 –	36.4 –	36.5 –
C-9	26.0 –	25.2 –	26.2 +	26.1 –	25.2 –	27.0 –	27.4 –	29.4 –
C-10	127.8 +	125.2 +	127.9 +	125.5 +	33.3 –	144.2 +	64.1 +	78.0 +
C-11	138.5 0	135.0 0	134.7 0	135.0 0	35.7 +	127.3 0	58.3 0	73.1 0
C-12	21.4 +	68.7 –	21.2 +	68.8 –	68.3 –	172.4 0	24.9 0	26.6 +
C-13	61.6 –	13.7 +	61.6 –	13.7 +	16.6 +	12.1 +	18.8 +	23.2 +
C-14	23.4 +	23.3 +	16.2 +	16.1 +	23.3 –	23.1 +	16.1 +	16.0 +
C-15	57.9 –	57.8 –	57.9 –	57.9 –	57.9 –	57.9 –	57.9 –	57.8 –

	11	**16**	**17**	**18**	**20**	**22**	**24**	**25**
C-1	57.0 –	57.0 –	57.0 –	56.7 –	57.1 –	57.1 –	55.8 –	55.7 –
C-2	117.3 +	117.5 +	117.7 +	117.5 +	117.3 +	117.1 +	117.2 +	118.8 +
C-3	138.3 0	138.4 0	138.6 0	140.9 0	138.4 0	138.7 0	138.5 0	n.d.
C-4	33.3 –	32.9 –	33.4 –	70.9 +	33.3 –	33.3 –	67.4 +	67.2 +
C-5	25.2 –	25.5 –	21.4 –	34.1 –	25.2 –	25.1 –	25.8 –	25.7 –
C-6	124.0 +	126.0 +	30.4 –	118.1 +	123.6 +	55.6 +	118.7 +	123.8 +
C-7	136.6 0	134.9 0	39.8 +	140.1 0	136.6 0	86.5 0	127.9 0	136.0 0
C-8	28.7 –	48.1 –	31.0 –	39.7 –	28.1 –	39.0 –	39.7 –	36.4 –
C-9	29.4 –	66.1 +	25.4 –	26.4 –	30.9 –	25.8 –	26.2 +	27.2 –
C-10	77.8 +	127.6 +	124.4 +	123.9 +	62.6 –	86.2 +	127.7 +	63.8 +
C-11	73.1 0	133.7 0	131.8 0	131.8 0	–	45.4 0	134.7 0	58.3 0
C-12	26.5 +	25.7 +	25.7 +	25.6 +	–	26.2 +	21.3 +	24.8 +
C-13	23.2 +	18.2 +	17.7 +	17.6 +	–	18.9 +	61.6 –	18.7 +
C-14	16.0 +	16.4 +	65.2 –	16.3 +	23.3 +	23.5 +	16.4 +	16.3 +
C-15	57.7 –	57.7 –	57.7 –	55.1 –	57.9 –	57.9 –	18.2 +	18.2 +

[a] Amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = –; quat. C = 0); n.d. = not detected.

stable and reactive substrates. After an extended screen of strains some microorganisms were found to oxidize this substrate in omega position in good yield. Almost all strains could discriminate between the 6*E*- and the 6*Z*-compounds preferring the substrates with a 6*Z*-double bond in most cases. Only *Aspergillus niger* AC 3, which we isolated from garden soil, produced exclusively the ω-hydroxylated all-*E*-β-farnesene sulfone **5.** The variation of the fermentation media influenced the overall yield of the fermentation products but hardly the ratio of products formed.

It seems that the sulfone moiety of the molecule added sufficient polarity to the substrates to attach it to the active sites of the enzymes and it also seems to fit well into it. It is a challenging idea whether this method can also be applied to higher terpenes, *e.g.* carotenoids.

Experimental

The microorganisms were obtained from international collections (DSM, ATCC) and maintained in our department as agar slants at 4 °C or frozen in liquid nitrogen. They were cultivated at 27 °C and 140 rpm in 100 ml conical flasks containing 20 ml of the following medium: 0.5% of glucose, 0.2% of universalpeptone (Merck), 0.5% of malt extract and 0.1% of yeast extract. After 48 h 10 µl of substrate in 100 µl of EtOH was added to the cultures. 24 h after the substrate addition, samples were taken each day and analyzed as

follows: To 1 ml of culture broth 0.2 ml of EtOAc was added and shaken for 2 min prior to centrifugation. 10 µl of the extract was developed on HPTLC plates with CH_2Cl_2–Me_2CO 9:1. The spots were made visible by spraying with anisaldehyde–H_2SO_4 in HOAc and heating to 110 °C for 1 min. For biotransformation on a preparative scale, the microorganisms were grown in five 100 ml flasks, transferred after 48 h into 11 flasks containing 200 ml of the medium and incubated for another 24 h period. The substrate (100 mg/flask dissolved in 1 ml of EtOH) was then added aseptically.

Extraction and purification: The whole broth (bacteria) or culture medium and mycelia were separated by filtration (fungi) and both were extracted three times with EtOAc. The solvent was evaporated and the crude extract separated on Si-60 columns with a *n*-hexane/EtOAc gradient (changing from 19:1 to 1:1). When necessary the collected fractions were further purified by prep. TLC. HPTLC: *n*-hexane/EtOAc 1:2.

Instruments used: NMR: The ^1H NMR spectra were obtained at 400 MHz and the ^{13}C NMR spectra at 75.5 MHz, $CDCl_3$ was the solvent and TMS the internal standard. Mass spectra were recorded with 70 eV. IR spectra were measured in $CHCl_3$.

Fermentation of *Nocardia* sp. DSM 43130 with β-farnesene sulfone **1** (4.7 g) led, after 97 h to **1** (590 mg), **2** and **3** (860 mg), **4** and **5** (520 mg), **8** (166 mg), **12** (45 mg), **14** (30 mg).

Fermentation of the same strain, but in a different medium (4 g/l glucose, 4 g/l yeast extract, 10 g/l malt extract, 2 g/l calcium carbonate) with **1** and **23** (2:1, 4.7 g) led, after 144 h, to **1** (1858 mg), **2, 3, 4** and **5** (252 mg), **18** (30 mg) and **24** (633 mg).

Reaction of **1** and **23** (2:1, 200 mg) with *Nocardia* sp. DSM 43130 resulted, after 166 h, in **1** (10 mg), **2** (11 mg), **9** (7 mg), **19** (4 mg), **24** (8 mg) and **25** (2 mg).

Biotransformation of **1** (200 mg) with *Mycobacterium smegmatis* DSM 43277 yielded after 23 h **6** (12 mg).

Fermentation of **1** (200 mg) with *Cunninghamella elegans* DSM 63299 resulted, after 41 h, in **1** (33 mg), **8** (17 mg), **10** (3 mg) and **11** (3 mg).

Biotransformation of *E*-**1** and *E*-**23** (2:1, 200 mg) with *Bacillus megaterium* DSM 510 in potato dextrose medium (Difco) yielded, after 144 h, **1** (40 mg), **9** (24 mg), **16** (23 mg) and **25** (12 mg).

Reaction of **1** (200 mg) with *Nocardia gardneri* DSM 43020 for 143 h yielded **17** (20 mg).

Fermentation of **1** (200 mg) with *Nocardia* sp. DSM 40350 produced, after 161 h, **6** (18 mg) and **20** (8 mg).

Biotransformation of **1** and **23** (2:1, 4.7 g) with *Pseudomonas lapsa* DSM 50274 resulted, after 49 h, in **1** and **23** (644 mg), **4** (850 mg), **24** (500 mg) and 200 mg 9,10-dihydro-**24.**

Fermentation of **1** (4.7 g) with *Pseudomonas lapsa* DSM 50274 led, after 50 h, to **1** (40 mg), **2, 3, 4,** and **5** (820 mg), **21** (3 mg) and **22** (6 mg).

Aspergillus niger AC 3 (isolated from garden soil) transformed, after 23 h, **1** (200 mg) to **1** (15 mg), **5** (25 mg), **10** and **11** (11 mg) and **26** (13 mg).

3-(3′*Z*,7′*Z*-4′,8′-Dimethyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*,10*Z*-12-hydroxy-β-farnesene sulfone) **2:** R_f 0.47. MS *(m/z):* 284.1426 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (5%), 266 (53), 220 (12), 202 (24), 135 (90), 93 (82), 41 (100).

3-(3′*Z*,7′*E*-4′,8′-Dimethyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*,10*E*-12-hydroxy-β-farnesene sulfone) **3:** R_f 0.41. MS *(m/z):* 284.1431 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (1%), 266 (45), 220 (8), 135 (79), 107 (60), 91 (100).

3-(3′*E*,7′*Z*-4′,8′-Dimethyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*,10*Z*-12-hydroxy-β-farnesene sulfone) **4:** R_f 0.47. MS *(m/z):* 284.1455 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (3%), 135 (44), 121 (20), 107 (38), 93 (67), 69 (83), 43 (100).

3-(3′*E*, 7′*E*-4′,8′-Dimethyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= *E*,*E*-12-hydroxy-β-farnesene sulfone) **5:** R_f 0.41. MS *(m/z):* 284.1446 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (3%), 266 (12), 201 (21), 135 (59), 107 (58), 93 (72), 43 (100).

3-(3′*Z*-4′,8′-Dimethyl-9′-hydroxy-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= *Z*-12-hydroxy-10,11-dihydro-β-farnesene sulfone) **6:** R_f 0.47. MS *(m/z):* 286.1604 ($[M]^+$, 286.1603 calc. f. $C_{15}H_{26}O_3S$) (16%), 222 (2), 137 (40), 135 (31), 95 (75), 81 (91), 43 (100).

$$[\alpha]^{27} = \frac{\begin{matrix}589\text{ nm} & 578\text{ nm} & 546\text{ nm} & 436\text{ nm}\end{matrix}}{\begin{matrix}-3.8° & -3.9° & -4.5° & -7.5°\end{matrix}} \quad (c = 1.00).$$

3-(3′*E*,7′*E*-8′-Carboxy-4′-methyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen

(= *E,E*-β-farnesene-sulfone-12-acid) **7:** R_f 0.13. MS *(m/z):* 298.1239 ($[M]^+$, 298.1240 calc. f. $C_{15}H_{22}O_4S$) (1%), 280 (5), 234 (23), 135 (11), 93 (100).

3-(3′*Z*,7′*E*-8′-Carboxy-4′-methyl-9′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*,10*E*-β-farnesene-sulfone-12-acid) **8:** R_f 0.15. MS *(m/z):* 298 ($[M]^+$) (1%), 280.1145 ($[M-H_2O]^+$, 280.1133 calc. f. $C_{15}H_{20}O_3S$) (7), 269 (9), 264 (1), 234 (18), 135 (13), 133 (43), 93 (100).

3-(3′*E*-4′,8′-Dimethyl-7′,8′-epoxy-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= *E*-10,11-epoxy-10,11-dihydro-β-farnesene sulfone) **9:** R_f 0.55. MS *(m/z):* 284.1448 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (3%), 220 (1), 135 (18), 85 (62), 81 (59), 43 (100).

$[\alpha]^{27}$ = 589 nm +1.7° | 578 nm +1.9° | 546 nm +2.0° | 436 nm +3.5° (*c* = 1.00).

3-(3′*E*-7′,8′-Dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= *E*-10,11-dihydroxy-10,11-dihydro-β-farnesene sulfone) **10:** R_f 0.18. MS *(m/z):* 284 ($[M-H_2O]^+$) (7%), 243 (10), 226 (13), 135 (50), 59 (64), 43 (100).

$[\alpha]^{27}$ = 589 nm +2.8° | 578 nm +2.8° | 546 nm +2.8° | 436 nm +12.0° (*c* = 1.00).

3-(3′*Z*-7′,8′-Dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= *Z*-10,11-dihydroxy-10,11-dihydro-β-farnesene sulfone) **11:** R_f 0.18. MS *(m/z):* 284 ($[M-H_2O]^+$) (5%), 243 (12), 226 (16), 135 (53), 59 (61), 43 (100).

$[\alpha]^{27}$ = 589 nm +2.8° | 578 nm +2.8° | 546 nm +2.8° | 436 nm +12.0° (*c* = 1.00).

3-(3′*E*-8′,9′-Dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*-11,12-dihydroxy-10,11-dihydro-β-farnesene sulfone) **12** and 3-(3′*Z*-8′,9′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*-11,12-dihydroxy-10,11-dihydro-β-farnesene sulfone) **14:** only isolated as 2,2-dimethyl-1,3-dioxolane derivatives (acetonides) **13** and **15. 13** and **15:** R_f 0.66. MS *(m/z):* 342.1858 ($[M]^+$, 342.1865 calc. f. $C_{18}H_{30}O_4S$) (3%), 327 (17), 267 (70), 203 (25), 135 (29), 79 (97), 43 (100).

$[\alpha]^{27}$ =

589 nm 0° | 578 nm 0° | 546 nm 0° | 436 nm −1.1° | 365 nm −3.4° (*c* = 1.00).

3-(3′*E*,7′*E*-4′,8′-Dimethyl-6′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*,10*E*-9-hydroxy-β-farnesene sulfone) **16:** R_f 0.44. MS *(m/z):* 266.1349 ($[M-H_2O]^+$, 266.1341 calc. f. $C_{15}H_{22}O_2S$) (39%), 202 (12), 135 (57), 91 (100).

$[\alpha]^{27}$ = 589 nm −8.3° | 578 nm −8.5° | 546 nm −9.5° | 436 nm −17.5° (*c* = 1.00).

3-(7′*E*-4′,8′-Dimethyl-14′-hydroxy-7′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen (= 14-hydroxy-6,7-dihydro-β-farnesene sulfone) **17:** R_f 0.38. MS *(m/z):* 286.1601 ($[M]^+$, 286.1603 calc. f. $C_{15}H_{26}O_3S$) (8%), 152 (12), 135 (37), 109 (64), 82 (94), 69 (94), 41 (100).

$[\alpha]^{27}$ = 589 nm 0° | 578 nm 0° | 546 nm −0.1° | 436 nm −0.7° (*c* = 1.00).

3-(3′*E*,7′*E*-4′,8′-Dimethyl-1′-hydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*,10*E*-4-hydroxy-β-farnesene sulfone) **18:** R_f 0.48. MS *(m/z):* 284.1448 ($[M]^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (1%), 266 (2), 220 (5), 137 (71), 95 (61), 83 (81), 69 (100).

$[\alpha]^{27}$ =

589 nm +20.7° | 578 nm +21.4° | 546 nm +24.7° | 436 nm +44.1° | 365 nm +60.7° (*c* = 1.00).

3-(3′*E*,7′*Z*-4′,8′-Dimethyl-1′,9′-dihydroxy-nona-3′,7′-dienyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*,10*Z*-4,12-dihydroxy-β-farnesene sulfone) **19:** R_f 0.15. MS *(m/z):* 300.1402 ($[M]^+$, 300.1395 calc. f. $C_{15}H_{24}O_4S$) (0.2%), 283 (1), 218 (1), 135 (9), 107 (13), 95 (17), 93 (34), 91 (100).

$[\alpha]^{27}$ = 589 nm +15.0° | 578 nm +15.6° | 546 nm +17.7° | 436 nm +30.6° (*c* = 0.33).

3-(3′*Z*-4′-Methyl-7′-hydroxy-3′-heptenyl)-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*-10-hydroxy-11,12,13-trinor-β-farnesene sulfone) **20:** R_f 0.29. MS *(m/z):* 244.1144 ($[M]^+$, 244.1433 calc. f. $C_{12}H_{20}O_3S$) (1%), 226 (9), 149 (11), 135 (10), 95 (100), 67 (78).

1,1-Dimethyl-2-(2′-(3″-(2″,5″-dihydro-1″,1″-dioxo-thiophenyl))-ethyl)-3-methyliden-4-cyclohexene (7(14),8-tetradehydro-cyclo-β-farnesene sulfone) **21:** R_f 0.72. MS *(m/z):* 266.1350 ($[M]^+$, 266.1341 calc. f. $C_{15}H_{22}O_2S$) (2%), 251 (30), 107 (26), 91 (37), 79 (100).

$[\alpha]^{27}$ = 589 nm −14.8° | 578 nm −14.8° | 546 nm −16.8° | 436 nm −31.8° (*c* = 0.5).

2-(2′-(3″-(2″,5″-dihydro-1″,1″-dioxo-thiophenyl))-ethyl)-1,3,3-trimethyl-7-oxabicyclo[2.2.0]heptane (7,10-epoxy-cyclo-β-farnesene sulfone) **22:** R_f

0.52. MS *(m/z):* 284.1446 ([M]$^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (5%), 220 (14), 177 (23), 161 (19), 153 (96), 135 (95), 71 (100).

$[\alpha]^{27}$ =

589 nm	578 nm	546 nm	436 nm	365 nm
−14.5°	−14.7°	−16.6°	−14.4°	−17.3°

(c = 1.00).

2-(2′*E*,6′*Z*-3′,7′-Dimethyl-8′-hydroxy-octa-2′,6′-dienyl)-3-methyl-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*,10*Z*-12-hydroxy-α-farnesene sulfone) **24:** R_f 0.46. MS *(m/z):* 284.1436 ([M]$^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (1%), 266 (2), 220 (2), 134 (25), 91 (77), 81 (100).

2-(2′*E*-3′,7′-Dimethyl-6′,7′-epoxy-2′-octenyl)-3-methyl-2,5-dihydro-1,1-dioxo-thiophen (= 6*E*-10,11-epoxy-10,11-dihydro-α-farnesene sulfone) **25:** R_f 0.56. MS *(m/z):* 284.1440 ([M]$^+$, 284.1446 calc. f. $C_{15}H_{24}O_3S$) (8%), 220 (4), 203 (20), 81 (100).

$[\alpha]^{27}$ =

589 nm	578 nm	546 nm	436 nm	365 nm
−15.8°	−16.4°	−19.0°	−33.0°	−50.4°

(c = 0.5).

2-(2′*Z*-3′,7′-Dimethyl-6′,7′-dihydroxy-2′-octenyl)-3-methyl-2,5-dihydro-1,1-dioxo-thiophen (= 6*Z*-10,11-dihydroxy-10,11-dihydro-α-farnesene sulfone) **26:** Only isolated as 2,2-dimethyl-1,3-dioxolane (acetonide) **27. 27:** R_f 0.65. MS *(m/z):* 342.1867 ([M]$^+$, 342.1865 calc. f. $C_{18}H_{30}O_4S$) (0.5%), 327 (100), 285 (15), 263 (6), 135 (15), 119 (22), 105 (29), 85 (46), 81 (61).

$[\alpha]^{27}$ =

589 nm	578 nm	546 nm
0°	+0.2°	+0.3°

(c = 1.00).

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