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Microbial Hydroxylation of Activated Acyclic Monoterpene Hydrocarbons

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Abstract: While fermentation of myrcene and ocimene led to products in very low yields, good yields were obtained by protection and activation of the diene moiety by sulfur dioxide. Microbial hydroxylations of the sulfolenes **1** and **14** yielded 8-hydroxycompounds in up to 60% yield. Bacteria favour the 8*Z*- while fungi produce mainly the 8*E*-alcohol. The bacterium *Sebekia benihana NRRL 11111* oxidized myrcene sulfone **1** to 5*R*-hydroxy-myrcene sulfone **10**, a compound which can be converted to the pheromon ipsdienol simply by heating. Some unusual isomerizations were found all occurring in low yield. Addition of hydroquinone to the acyclic double bond of the substrate was observed in the incubations with some strains.

Introduction

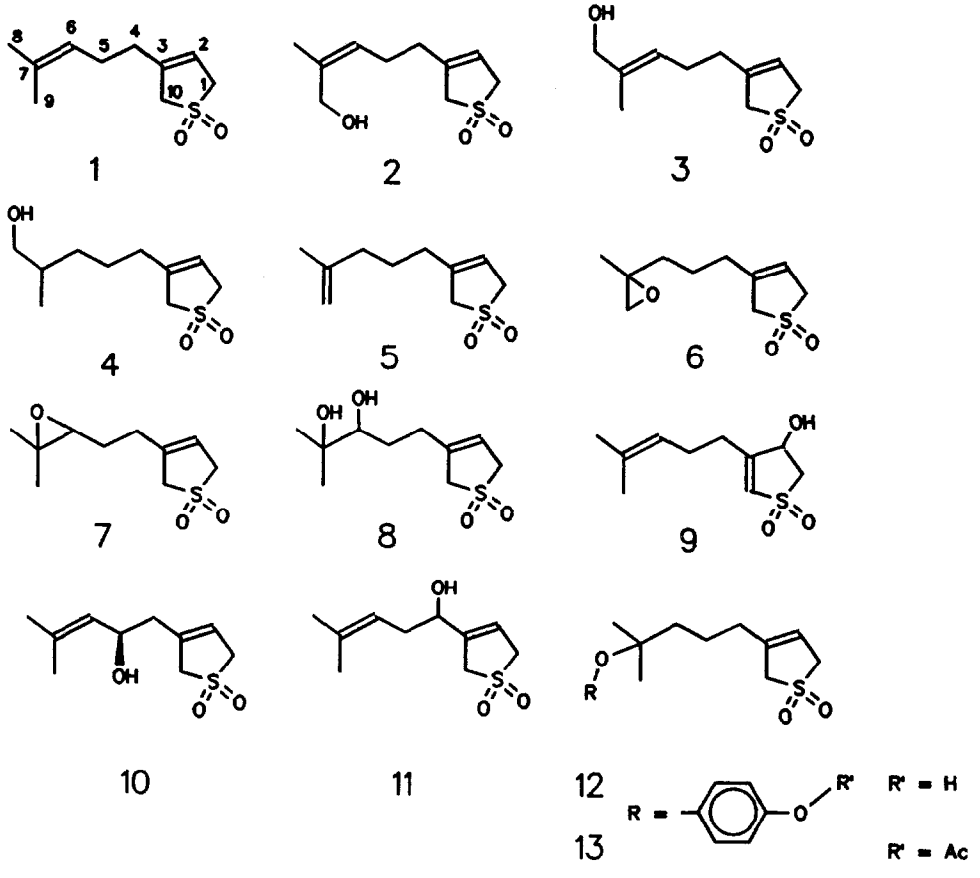
A wide range of biological active terpenoids contain terminal allylic alcohols, e. g. 8*Z*-hydroxy-myrcene **24**, a flavor compound from *Thymus sp.*[1], 8*E*-hydroxy-myrcene **25**, a pheromon of *Dendroctonus ponderosae*[2] and a synthon for zoapatanol[3,4] or its derivatives[5]. One of the drawbacks of its chemical synthesis from their hydrocarbons 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. Biotransformations on the contrary have the advantage of proceeding with mild conditions and high regioselectivity without producing toxic wastes. In continuation of our efforts to use microorganisms for omega-hydroxylations[6] we tested acyclic monoterpene hydrocarbons **23** and **26** as substrates.

Results and Discussion

Attempts to oxidize myrcene **23** with microorganisms led always to a slow conversion of the substrate while metabolites could be detected in very low yield only. Myrcene reacts only slowly with microorganisms, so *Nocardia rubropertincta DSM 43197* formed 8*E*-hydroxy-myrcene in only 0.3% yield while the corresponding *Z*-alcohol was not detected. Yamazaki and coworkers reported the formation of diols with *Aspergillus niger JTS 191* but also in low yield[7]. The same experience was made with the biotransformations of the sesquiterpene hydrocarbons humulene[8], cedrene[9] or isolongifolene[10]. The presumable cause for these results is the lack of a polar center for the attachment of the substrate to the active sites of the enzymes and the sensitivity of the conjugated diene to oxygen and acids.

Our results with sesquiterpenes told us that a polar function in the substrate makes the

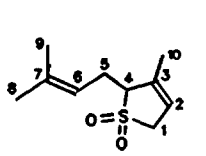
biotransformation to proceed faster and in higher yields[11]. We tried to protect the dienyl moiety of myrcene by a dienophile resulting in a polar substrate which can be cleaved easily after the biotransformation. For this purpose we choose sulfur dioxide as dienophile which reacts under pressure with the diene to a sulfolene[12]. These sulfolenes were completely stable under fermentation conditions but could be cleaved easily simply by heating. This protection of myrcene is used for the production of myrcene-hydrate in industrial scale.



We were interested to test whether this method of protection and activation of dienes can be applied also to the biotransformation of acyclic monoterpenes with conjugated double bonds. The fermentations of the sulfone of myrcene **1** resulted in far better yields than that of the hydrocarbon. *Streptomyces albus DSM 40763* formed 8*Z*-hydroxy-myrcenesulfone **2** (24% yield) and 8*E*-hydroxy-myrcene sulfone **3** (20% yield). *Nocardia sp. DSM 43130* produced even more of the *Z*-alcohol (**2** (37%) and **3** (5%)). Fungi on the contrary made more *E*-alcohol, so *Mucor indicus CBS 226.29* gave 7% of **2** and 21% of **3** and *Absidia coerulea IFO 4011* yielded only 8*E*-hydroxy-myrcene sulfone **3** in 55% yield[13]. In lower yields some other hydroxylation products were detected. *Mucor indicus CBS 226.29* and *Rhizopus arrhizus ATCC 10260* formed 4-hydroxy-myrcene sulfone **11** and the later strain

also oxidized the substrate in 5-position to 5-hydroxy-myrcene sulfone **10**. This compound is the protected form of the pheromon ipsdienol which can be generated by heating. Its absolute configuration and optical purity was determined by the modified Mosher procedure[14]. The main enantiomer was always *R*-configurated. Unfortunately, the yields were as low as the optical purity of 4% e.e. was. Higher yield (20%) and higher enantiomeric excess (82% e.e.) could be reached by using the bacterium *Sebekia benihana NRRL 11111*. Beside hydroxylation products epoxidations were observed. The simplest is the formation of 6,7-epoxy-myrcene sulfone **7** which is opened by some strains to the diol **8**. In rather low yields an isomerization occurred to **5** which was epoxidized to **6**. The formation of **9** by *Nocardia sp. DSM 40350* could also be explained by epoxidation with a subsequent isomerization. Hydrogenation of **2** and/or **3** to **4** and formation of their acetates are further side reactions. The addition of hydroquinone to myrcene sulfone to give **12** is a rather unusual microbial reaction. This was only observed with *Streptomyces albus DSM 40763* and *Absidia coerulea IFO 4011*.

Biotransformations of another acyclic monoterpene hydrocarbon, ocimene **26**, yielded no products.



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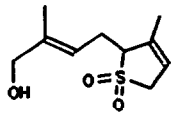

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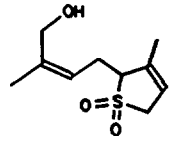

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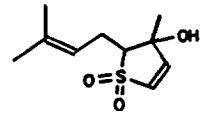

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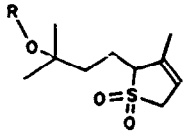
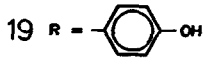
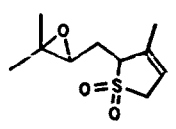


18 R = H

19 R = 



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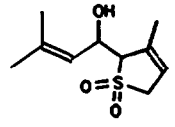

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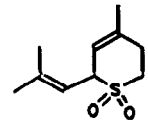

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Protection and activation of ocimene with sulfur dioxide to ocimene sulfone **14** and subsequent biotransformation led to some good yields. *Streptomyces anandii DSM 40535* produced 22% of 8*E*-hydroxy-ocimene sulfone **15** and 11% of 8*Z*-hydroxy-ocimene sulfone **16**. *Corynespora cassiicola DSM 62475* yielded 28% of **15** and 5% of **16**. The sulfolene **14** is a chiral compound and it is worth mentioning that some strains produced optical active **15** and **16** displaying the opposite sign of rotation than the recovered substrate. Again hydroxylation in 5-position to 5-hydroxy-ocimene sulfone **21** was found. Epoxidation at the 7,8-double bond to **20** and at the 2,3-double bond with subsequent isomerization to **17** occurred. While *Streptomyces albus DSM 40763* did not perform hydrations at this substrate *Penicillium diversum CBS 320.48* was leading to **18** and **19**, the analogue of **12**. The rearrangement of the substrate to the thia-3-cyclohexene-1,1-dioxide **22** was surprising.

Table 1: ^{1}H NMR Data of Monoterpene Sulfone Derivatives **2** - **13** and **15** - **22** ($CDCl_3$, 400 MHz)

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1-H	3.81 m	3.81 m	3.80 m	3.80 m	3.80 m	3.82 m	3.80 s br	3.63 dd	3.80 m	3.84 m
1'-H								3.24 dd		
2-H	5.72 m	5.72 m	5.71 m	5.69 m	5.70 m	5.78 m	5.74 m	4.90 dd	5.79 m	5.96 m
4-H	2.26 m	2.26 m	2.2 m	2.18 t	2.20 m	2.35 m	2.29 m	2.50 m	2.44 dd	4.26 t
4'-H							2.49 m	2.31 m	2.34 dd	-
5-H	2.26 m	2.26 m	1.1	1.61 tt	1.58 m	1.79 dddd	1.65 dddd	2.26 m	4.51 ddd	2.32 t
5'-H						1.59 dddd	1.50 dddd			
6-H	5.23 m	5.36 m	to	2.03 t	1.58 m	2.70 dd	3.35 dd	5.09 t	5.20 d br	5.10 t
7-H	-	-	1.7 m	-	-	-	-	-	-	-
8-H	1.79 s	3.98 s	3.44 m	4.74 m	2.60 m	1.30 s	1.23 s	1.70 s	1.74 s	1.76 s
8'-H				4.67 m						
9-H	4.09 s	1.66 s	0.92 d	1.71 s	1.31 s	1.28 s	1.18 s	1.62 s	1.70 s	1.66 s
9'-H										
10-H	3.71 s	3.71 s	3.69 s	3.69 s	3.68 s	3.73 s	3.71 s	6.35 s	3.80 s	3.83 d
10'-H										3.74 d

	12	**13**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1-H	3.70 s	3.70 s	3.75 m	3.74 d br	6.65 d	3.75 d br	3.75 d br	3.75 m	3.78 d	3.09 dd
1'-H			3.66 m	3.62 d br	-	3.68 d br	3.67 d br		3.70 d	
2-H	5.70 s	5.71 s	5.70 m	5.72 m	6.59 d	5.68 m	5.68 m	5.71 m	5.80 m	2.72 ddd br
2'-H	-	-	-	-	-	-	-	-	-	2.58 ddd br
4-H	2.20 t	2.22 m	3.58 t	3.59 m	3.26 dd	3.56 d br	3.55 d br	3.72 m	3.56 d	5.21 dq
5-H	1.64 m	1.66 m	2.62 t	2.68 m	2.65 ddd	1.98 m	2.14 m	2.27 ddd	4.73 m	4.38 d br
5'-H					2.52 ddd		2.03 m	1.79 ddd	-	-
6-H	1.64 m	1.66 m	5.51 t	5.25 t	5.27 t br	1.79 ddd	1.94 m	3.02 dd	5.54 d br	5.07 dqq
6'-H			-		-	1.64 ddd	1.79 ddd	-	-	-
8-H	1.22 s	1.26 s	4.07 d	1.84 s	1.77 s	1.24 s	1.25 s	1.35 s	1.78 s	1.84 s
8'-H			3.99 d							
9-H	1.22 s	1.26 s	1.72 s	4.17 d	1.72 s	1.24 s	1.25 s	1.32 s	1.71 s	1.79 s
9'-H				4.07 d						
10-H	3.81 s	3.81 s	1.87 s	1.88 s	1.42 s	1.83 s	1.86 s	1.89 s	1.91 s	1.78 s
10'-H										
12-H	6.82 m	6.93 m	-	-	-	-	6.83 m	-	-	-
13-H	6.72 m	7.00 m	-	-	-	-	6.71 m	-	-	-
OAc	-	2.28 s	-	-	-	-	-	-	-	-

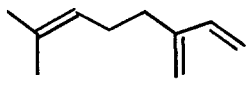
J(Hz): **4**:8,9=7; **5**:4,5=5,6=7; **7**:4,5=8.7;4',5=6.5; 4,5'=6.5;4',5'=7.5;5,5'=13.5;5,6=4.5;5',6=7.7; **8**:4,5=9.3;4,5'=6.7; 4',5=5.3;4',5'=9.5;5,5'=13.7;5,6=10.5;5',6=2.3; **9**:1,1'=14;1,2=7;1',2=4;5,6=7; **10**:4,4'=15;4,5=5;4',5=5,6=8; **11**:4,5= 5,6=7;10,10'=15; **12**:4,5=7; **15**:4,5=6.5;8,8'=11; **16**:1,1'=12;5,6=7;9,9'=16; **17**:1,2=7;4,5=4,5'=7;5,5'=14;5,6=5',6=7; **18**:1,1'=12;4,5=9;5,6=5',6'=4;5,6'=5',6= 12;6,6'=14; **19**:1,1'=12;4,5=9;5,6=4;5',6'=12;6,6'=13; **20**:4,5=9; 4,5'=5; 5,5'=12;5,6=3;5',6=7; **21**:1,1'=12;4,5=9;5,6=10; **22**:1,2=7;1,2'=6;2,2'=17;4,5=4,10=1;5,6=10;6,8=6,9=1.

Table 2: ^{13}C NMR of Monoterpene Sulfone Derivatives ($CDCl_3$, 75.5 MHz)

	2	3	4	6	7	10	11	12	13	15	16	18	19
C-1	56.8 -[a]	57.0 -	56.9 -	57.0 -	57.1 -	56.8 -	56.8 -	57.1 -	57.1 -	55.8 -	56.1 -	56.0 -	56.0 -
C-2	117.4 +	117.4 +	117.0 +	117.3 +	117.8 +	119.4 +	117.8 +[b]	117.2 +	117.3 +	117.4 +	117.6 +	116.9 +	117.1 +
C-3	138.0 0	138.2 0	138.6 0	138.2 0	137.7 0	136.1 0	140.7 0	138.5 0	138.4 0	138.7 0[c]	137.8 0	138.4 0	138.6 0
C-4	32.9 -	32.6 -	32.5 -	32.8 -	30.2 -	41.0 -	71.2 +	41.4 -	41.6 -	67.1 +	67.5 +	67.4 +	67.6 +
C-5	24.8 -	24.9 -	24.9 -	22.3 -	26.4 -	66.7 +	34.4 -	21.5 -	21.4 -	25.9 -	25.6 -	22.2 -	22.3 -
C-6	125.5 +	123.4 +	24.1 -	35.8 -	63.2 +	126.7 +	117.9 +[b]	33.3 -	33.3 -	119.2 +	120.9 +	39.6 -	38.6 -
C-7	136.1 0	136.5 0	35.3 +	56.4 0	58.5 0	135.8 0	137.2 0	79.7 0	80.2 0	138.4 0[c]	139.3 0	70.5 0	79.5 0
C-8	21.0 +	68.4 -	67.2 -	53.6 -	24.8 +	25.7 +	25.9 +	26.5 +	26.5 +	68.2 -	21.9 +	29.6 +	26.8 +
C-9	60.6 -	13.7 +	16.4 +	20.8 +	18.8 +	18.1 +	18.1 +	26.5 +	26.5 +	14.0 +	61.5 -	28.9 +	25.9 +
C-10	57.5 -	57.7 -	57.6 -	57.7 -	57.8 -	58.3 -	55.4 -	57.8 -	57.8 -	18.2 +	18.0 +	18.0 +	18.1 +
C-11	-	-	-	-	-	-	-	152.0 0	152.7 0	-	-	-	152.0 0
C-12	-	-	-	-	-	-	-	125.0 +	121.9 +	-	-	-	115.5 +
C-13	-	-	-	-	-	-	-	115.6 +	124.4 +	-	-	-	125.1 +
C-14	-	-	-	-	-	-	-	148.1 0	146.4 0	-	-	-	148.2 0

[a]amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = -; quat. C = 0); [b, c]assignments may be interchanged

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 myrcene-6,7-diol from myrcene in 20% yield[15] but with myrcene sulfone **1** no reaction was observed.



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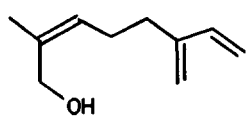

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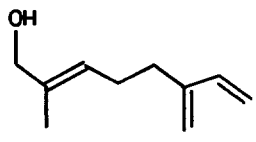

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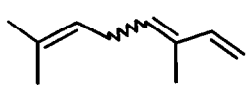

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Biotransformations of myrcene and ocimene were unsuccessful and led to products in very low yield. Protection and activation of the dienyl moiety of these monoterpenes by the addition of sulfurdioxide resulting in its sulfones produced stable and reactive substrates. After an extended

screen some microorganisms were found to oxidize these substrates at the omega-position in good yields.

It seems that the sulfone moiety of the molecule is sufficient polar to fix the substrates in the enzymes and it also seems to fit well into the active sites. Substrate concentrations up to 2g/l did not result in any toxic effects to the microorganisms. It is an interesting task to look whether this method can also be applied to higher terpenes, e. g. carotenoids.

Experimental

The microorganisms were cultivated at 27°C and 140 r.p.m. in 100 ml Erlenmeyer 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. Twenty-four 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 biotransformations on a preparative scale, the microorganisms were grown in five 100 ml flasks, transferred after 48 h into 1 liter 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: Culture medium and mycelia were separated by filtration 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 $CHCl_3$.

Biotransformations of myrcene sulfone **1**:

a) Incubation of **1** (5g) with *Streptomyces albus DSM 40763* yielded after 49 h **1** (1604mg), **2** (1930mg), acetate of **2** (7mg), **3** (2084mg), acetate of **3** (19mg), **6** (90mg), **7** (88mg),**10** (5mg), and **12** (50mg).

3-(3'Z-5'-Hydroxy-4'-methyl-3'-pentenyl)-thia-3-cyclopentene-1,1-dioxide **2**: Oil, R_f 0.25. MS (m/z): 216.0822 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(2%), 198(3), 151(15), 84(68), 43(100).

3-(3'E-5'-Hydroxy-4'-methyl-3'-pentenyl)-thia-3-cyclopentene-1,1-dioxide **3**: Oil, R_f 0.23. MS (m/z): 216.0820 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(0.7%), 152(2), 151(8), 84(61), 67(82), 43(100).

3-(4',5'-Epoxy-4'-methyl-pentyl)-thia-3-cyclopentene-1,1-dioxide **6**: Oil, R_f 0.36. MS (m/z): 216 (M^+)(15%), 198(20), 133(97), 85(100).

3-(3',4'-Epoxy-4'-methyl-pentyl)-thia-3-cyclopentene-1,1-dioxide **7**: Oil, R_f 0.32. MS (m/z): 216 (M^+)(13%), 198(6), 152(33), 85(100).

$$[\alpha]^{27} = \frac{589nm \quad 578nm \quad 546nm \quad 436nm}{+2.5° \quad +2.9° \quad +3.6° \quad +6.8°} \quad (c=1.00)$$

3-(4'-(4''-Hydroxy-phenoxy)-4'-methyl-pentyl-)-thia-3-cyclopentene-1,1-dioxide **12**: Oil, R_f 0.46. Acetylation of **12** with Ac_2O and catalytic amounts of N,N-dimethyl-4-amino-pyridine yielded quantitatively **13**: MS (m/z): 352.1344 (M^+, 352.1345 calc. for $C_{18}H_{24}O_5S$)(9%), 337(2), 310(1), 193(9), 152(61), 110(100).

b) Fermentation of **1** (5g) with *Nocardia sp. DSM 43130* resulted after 97 h in **1** (200mg), **2** (1972mg), and **3** (246mg).

c) Biotransformation of **1** (200mg) with *Nocardia sp. DSM 40350* led after 175 h to **1** (73mg), **2** (3mg), **3** (20mg), acetate of **3** (3mg), **4** (31mg), acetate of **4** (6mg), **7** (2mg), and **9** (12mg).

3-(5'-Hydroxy-4'-methyl-pentyl)-thia-3-cyclopentene-1,1-dioxide **4**: Oil, R_f 0.23. MS (m/z): 218.0953 (M^+, 218.0977 calc. for $C_{10}H_{18}O_3S$)(0.5%).

$$[\alpha]^{27} = \frac{\begin{array}{cccc}589nm & 578nm & 546nm & 436nm\\ -2.2° & -2.3° & -2.7° & -3.3°\end{array}}{} \quad (c=1.00)$$

Not completely separated from **3**. Acetate of **4**: MS (m/z): 260.1085 (M^+, 260.1082 calc. for $C_{12}H_{20}O_4S$)(4%), 218(2), 200(3), 136(39), 43(100).

4-Hydroxy-3-(4'-methyl-3'-pentenyl-)-thia-2-cyclopentene-1,1-dioxide **9**: Oil, R_f 0.41. MS (m/z): 198 ([M-H_2O]$^+$)(1%), 148(54), 69(100).

$$[\alpha]^{27} = \frac{\begin{array}{ccccc}589nm & 578nm & 546nm & 436nm & 365nm\\ -3.7° & -3.8° & -4.1° & -7.3° & -11.4°\end{array}}{} \quad (c=1.00)$$

d) Incubation of **1** (200mg) with *Mucor indicus CBS 226.29* yielded after 49.5 h **1** (77mg), **2** (15mg), **3** (45mg), **7** (3mg), and **11** (7mg).

3-(1'-Hydroxy-4'-methyl-3'-pentenyl)-thia-3-cyclopentene-1,1-dioxide **11**: Oil, R_f 0.35. MS (m/z): 216 (M^+)(0.2%), 198(1), 152.1202 ([M-SO_2]$^+$, 152.1201 calc. for $C_{10}H_{16}O$)(32), 83(88), 69(97), 41(100).

$$[\alpha]^{27} = \frac{\begin{array}{cccc}589nm & 578nm & 546nm & 436nm\\ +6.8° & +6.8° & +7.8° & +13.7°\end{array}}{} \quad (c=1.00)$$

e) Fermentation of **1** (200mg) with *Absidia coerulea IFO 4011* resulted after 48 h in **1** (43mg) and **3** (89mg).

f) Fermentation of **1** (5g) with the same strain in a 10 l fermentor resulted after 47 h in **1** (600mg), **3** (875mg), acetate of **3** (75mg), **5** (35mg), **10** (81mg, α_D -6.4°, c=1.00), **11** (20mg), and **12** (20mg).

3-(4'-Methyl-4'-pentenyl)-thia-3-cyclopentene-1,1-dioxide **5**: Oil, R_f 0.60. MS (m/z): 200 (M^+)(27%), 135(87), 69(100).

g) Biotransformation of **1** (200mg) with *Rhizopus arrhizus ATCC 10260* led after 69.5 h to **1** (12mg), **2** (15mg), **3** (31mg), **8** (18mg), **10** (16mg, α_D -0.8°(c=0.50), and **11** (6mg).

3-(3',4'-Dihydroxy-4'-methyl-pentyl)-thia-3-cyclopentene-1,1-dioxide **8**: Oil, R_f 0.09. $[\alpha]_D$ +0.9° (c=1.00). MS (m/z): 219.0693 ([M-CH_3]$^+$, 219.0691 calc. for $C_9H_{15}O_4S$)(1%), 176(14), 67(78), 59(100).

h) Incubation of **1** (190mg) with *Sebekia benihana NRRL 11111* in TYG medium[16] yielded after 49 h **1** (14mg), **2** (11mg), **3** (22mg), **7** (22mg), and **10** (41mg).

3-(2'-Hydroxy-4'-methyl-3'-pentenyl)-thia-3-cyclopentene-1,1-dioxide **10**: Oil, R_f 0.27. MS (m/z): 216 (M^+)(0.2%), 85(100).

$$[\alpha]^{27} = \frac{\begin{array}{ccccc}589nm & 578nm & 546nm & 436nm & 365nm\\ -18.7° & -19.7° & -22.8° & -39.2° & -64.2°\end{array}}{} \quad (c=1.00)$$

i) Fermentation of **1** (200mg) with *Aspergillus niger AC 3* resulted after 23 h in **1** (17mg), **3** (21mg), acetate of **3** (3mg), **5** (2mg), and **8** (16mg).

Biotransformations of ocimene sulfone **14**:

a) Fermentation of **14** (200mg) with *Streptomyces anandii DSM 40535* resulted after 166.5 h in **14** (45mg), **15** (48mg)(α_D -3.0°, c=1.00), **16** (24mg)(α_D -8.2°, c=1.00), **20** (5mg), and **21** (2mg).

2-(4'-Hydroxy-3'-methyl-2'E-butenyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **15**: Oil, R_f 0.20. MS (m/z): 216.0814 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(24%), 151(21), 134(44), 93(100).

2-(4'-Hydroxy-3'-methyl-2'Z-butenyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **16**: Oil, R_f 0.25. MS (m/z): 216.0811 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(98%), 198(21), 151(71), 134(99), 93(100).

2-(2',3'-Epoxy-3'-methyl-butyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **20**: Oil, R_f 0.42. MS (m/z): 216.0815 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(100%), 152(33), 108(36), 71(37).

2-(1'-Hydroxy-3'-methyl-2'-butenyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **21**: Oil, R_f 0.43. MS (m/z): 216.0816 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(12%), 85(100).

b) Biotransformation of **14** (200mg) with *Corynespora cassiicola DSM 62475* led after 72.5 h to **14** (9mg)(α_D +10.9°, c=1.00), **15** (61mg)(α_D -1.6°, c=1.00), **16** (11mg)(α_D -7.4°, c=1.00), **20** (4mg), and **21** (1mg).

c) Incubation of **14** (2.5g) with *Penicillium diversum CBS 320.48* yielded after 67 h **14** (192mg), **15** (610mg), **17** (4mg), **18** (270mg), **19** (17mg), **22** (3mg), and gliotoxin (15mg).

2-(3'-Methyl-2'-butenyl)-3-hydroxy-3-methyl-thia-4-cyclopentene-1,1-dioxide **17**: Oil, R_f 0.36. MS (m/z): 216.0824 (M^+, 216.0820 calc. for $C_{10}H_{16}O_3S$)(34%), 198(87), 183(72), 149(85), 119(100).

$$[\alpha]^{27} = \frac{\begin{matrix}589nm & 578nm & 546nm & 436nm & 365nm\end{matrix}}{\begin{matrix}+9.4° & +9.8° & +11.4° & +13.8° & +10.2°\end{matrix}} \quad (c=0.50)$$

2-(3'-Hydroxy-3'-methyl-butyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **18**: Oil, R_f 0.20. MS (m/z): 216 (3%), 203.0739 ($[M-CH_3]^+$, 203.0742 calc. for $C_9H_{15}O_3S$)(100), 152(45), 132(54).
2-(3'-(4''-Hydroxy-phenoxy)-3'-methyl-butyl)-3-methyl-thia-3-cyclopentene-1,1-dioxide **19**: Oil, R_f 0.44. MS (m/z): 310.1235 (M^+, 310.1239 calc. for $C_{16}H_{22}O_4S$)(2%), 295(2), 246(2), 201(55), 151(19), 136(44), 110(97), 81(100).
2-(2'-Methyl-propenyl)-4-methyl-thia-3-cyclohexene-1,1-dioxide **22**: Oil, R_f 0.51. $[\alpha]_D$ -1.6° (c=0.50). MS (m/z): 216 (M^+)(2%), 136(74), 121(100), 93(78).

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