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REARRANGED CARYOPHYLLENES BY BIOTRANSFORMATION WITH *CHAETOMIUM COCHLIODES*

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Key Word Index—*Chaetomium cochliodes*; Ascomycotina; microbial hydroxylation; sesquiterpene; caryophyllene; clovane; punctaporonin.

Abstract—Biotransformation of caryophyllene by *Chaetomium cochliodes* DSM 1909 (=ATCC 10195) leads to 4,5-epoxy-caryophyllene-7,12-diol as the main product. The hydroxylation of the geminal methyl groups is not very stereospecific, but as with *Diploida gossypina* the main product possesses the 11*R*-configuration. Side-reactions are ring contraction or formation of clovanes, probably via epoxide rearrangements. The use of mono- or diepoxy-caryophyllene as the substrate does not increase the yields significantly. Instead, diepoxy-caryophyllene gave a punctatin derivative. The same molecular framework, but with a different configuration, was observed among the fermentation products from *Diplodia gossypina*, which supports the assumption that a caryophyllane is the precursor of this class of antibiotics.

INTRODUCTION

The only biotransformations of caryophyllene published to date were carried out by Devi [1] with *Pseudomonas cruciviae* resulting in 1-hydroxy-14-norcaryophyllene and by Ishida *et al.* [2] and Asakawa *et al.* [3] in rabbits. In a preceding paper we described a biotransformation of caryophyllene with *Diplodia gossypina* [4]. As in the transformation of humulene with *Diplodia gossypina* ATCC 10936 and *Chaetomium cochliodes* Palliser ATCC 10195 (=DSM 1909) distinct differences concerning yield, enantioselectivity and optical purity were observed between both strains [5] we were interested to test whether these differences do also exist for caryophyllene as the substrate. Clark and Hufford [6] reported the activity of *Chaetomium cochliodes* in the biotransformation of the sesquiterpenoid costunolide. The second aim of this work was to produce compounds either known as natural products or previously undescribed substances for biological tests.

RESULTS

Diplodia gossypina ATCC 10936 and *Chaetomium cochliodes* ATCC 10195 were selected for biotransformation of caryophyllene on a preparative scale after a screen. We report here the results of the biotransformation with *C. cochliodes* and compare the products obtained with the metabolites formed by *D. gossypina* [4].

Fermentation of caryophyllene (**1**) with *C. cochliodes* ATCC 10195 led to nine different products (**2**, **5**, **7**, **13**, **20**, **21** and **26**). As in the biotransformation with *D. gossypina* ATCC 10936 the first step is the epoxidation of caryophyllene at the 4,5-double bond resulting in the epoxide **2**. The next step in the degradation of caryophyllene is the hydroxylation of one of the geminal methyl groups at C-11 leading preferably to the 11-*R*-configuration in alcohol **5**. Hydroxylation at C-7 resulted in **3** as the main product of the biotransformation. While the epimeric 11*S*-alcohol **4** is not observed with *D. gossypina*, it is formed with *C. cochliodes*. This incomplete selectivity is mirrored in a 5:1 ratio of **3** and **4**. The configuration at C-11 of **4** follows from NOE experiments with irradiation at the 11-Me signal and confirmed the ^{13}C NMR shift predictions using the γ-effect of a hydroxyl function and the known shifts of the geminal methyls in caryophyllene. Although **3** is five-fold more abundant, only **4** is rearranged to the isocaryophyllene derivative **13**. The stereochemistry of the 3,4-double bond follows from the NOE observed at 3-H when the resonance of 15-H is saturated. Again only **4** is rearranged to the derivative **20**. This rearrangement was also observed in the biotransformation of caryophyllene with *D. gossypina*. NOE experiments failed to establish the configuration of C-4 in **20**. Compound **3** is involved in the rearrangement to the clovane derivative **26** as depicted in path A of Scheme 1. ^{1}H-{^{1}H}-COSY and extensive selective decoupling experiments together with NOE difference experiments served to elucidate the structure **26** unambiguously.

Addition of glucose to the fermentation medium did not alter the pattern of metabolites dramatically. While in the glucose free medium the rearranged compound **21** was formed, it was not detected in the same medium with additionally 1% of glucose. Instead the monoepoxide **2**,

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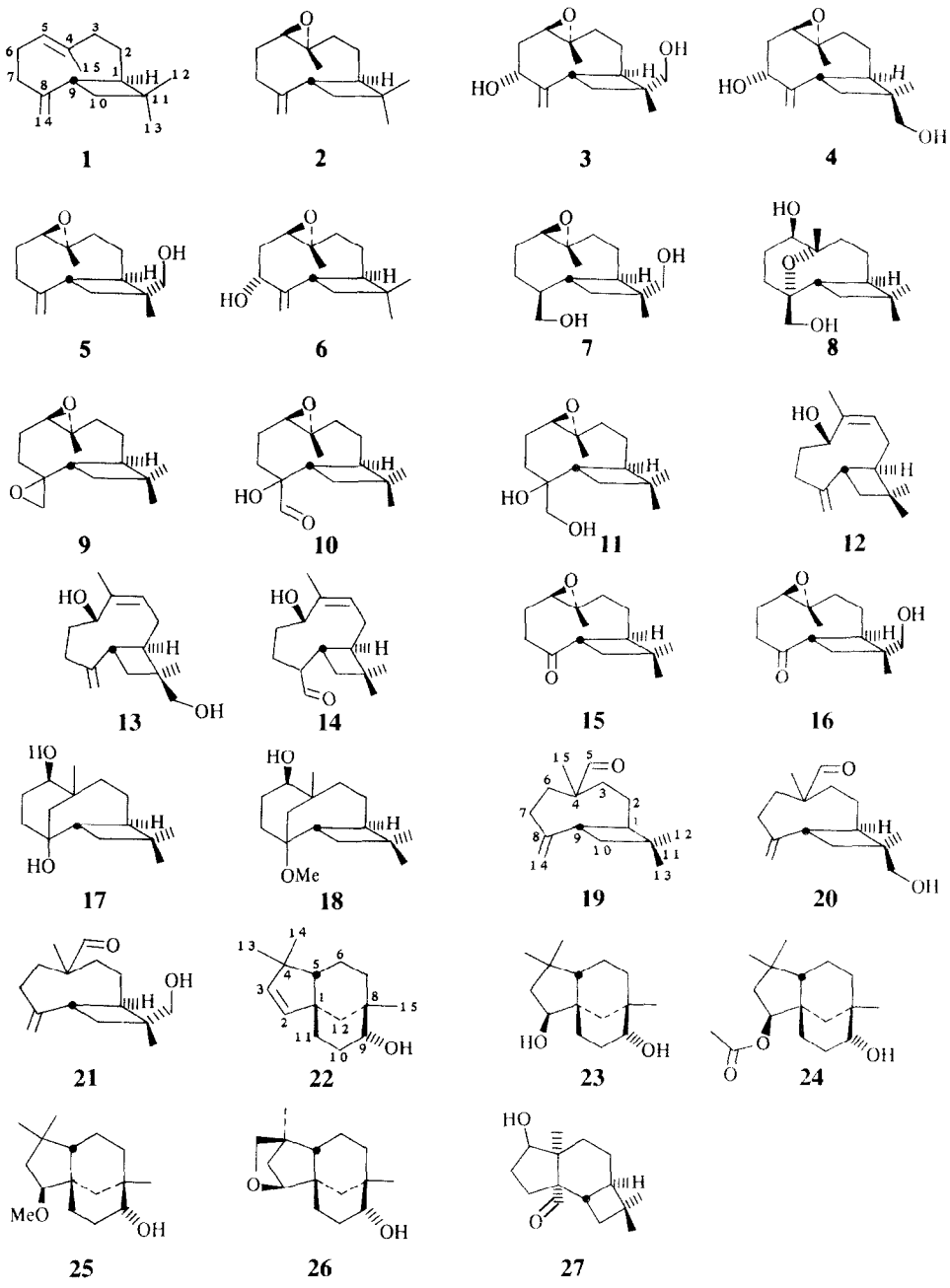
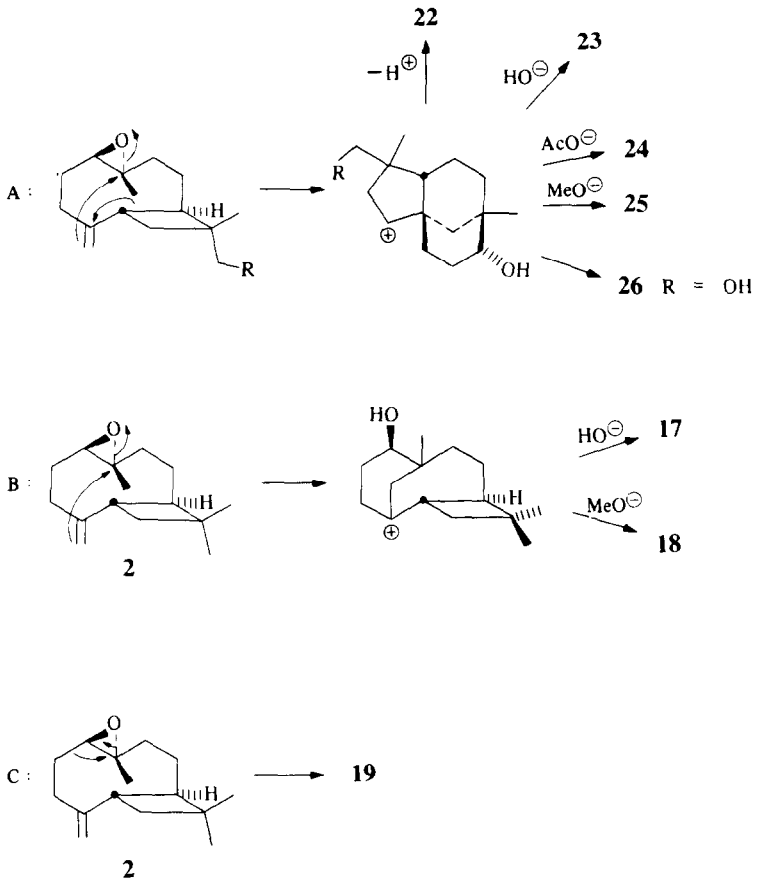


its diols **4** and **7** and the rearranged diol **13** were produced which were absent in the glucose free medium.

It was obvious that the epoxide **2** is the key intermediate for all metabolites of caryophyllene. To increase the yields, we synthesized 4,5-epoxy-caryophyllene (**2**) and used it as the substrate. The substrate was stable for 10 days in the medium without microorganism. Biotransformation of **2** with *C. cochliodes* led to **5**, already known from the fermentation of the hydrocarbon, and to the diol ether **8** which requires an additional epoxide at C-8, 14 for its formation, together with **12**, **15**, **16**, **18**, **19** and **22**–**26**. The isolation of kobusone (**15**) and of 13-hydroxy-kobusone (**16**) strongly suggest diol formation at C-8, 14 and subsequent diol cleavage which leads to these norsesquiterpenoids. Caryophyllenol-I (**12**) is a rearrangement product of **2** as is caryolandiol **17** (path B in Scheme 1). The corresponding monomethyl ether **18** is also isolated but in much lower yields. Another rearrangement of **2** consists in ring contraction to give **19** (path C). No less than five different clovane derivatives were formed. All of them can be derived from the 9-hydroxy-2-clovyl cation as shown in path A in Scheme 1.

Although the yields of the biotransformation of **2** were not extraordinarily good and the fermentation time was quite long, we also synthesized the diepoxide **9** and used it as the substrate. The synthesis led to two compounds



Scheme 1. Proposed reaction paths to the rearranged sesquiterpenoids.

epimeric at C-8 which were not separated for the purpose of the biotransformation. After 72 hr no starting material was detected and the fermentation was stopped. The main product was kobusone (**15**). Diol **11** was also isolated which is very probably not only the precursor of diol ether **8** but also of aldehyde **10** which were also found in the fermentation broth. Aldehyde **14** seems to be the result of a two-fold epoxide rearrangement. The most interesting product of this fermentation is the punctatin derivative **27**. Its constitution followed from the ^{13}C NMR which required a tricyclic sesquiterpene skeleton with an aldehyde and a secondary alcohol group. The ^{1}H NMR, especially ^{1}H–{^{1}H}-COSY, led to the punctatane skeleton [7–10]. Irradiation at 14-H gave NOEs at 1-H, 7-H, 10α-H, and 15-H. These results required *cis*-annellation of the cyclopentane and *trans*-annellation of the cyclobutane ring. Because no NOE was observed between 5-H and 15-H the hydroxy group is likely to have a *cis*-configuration relative to 15-H, so **27** is 5-deoxy-10,11-dihydro-12-oxypunctatin D [9]. The formation of a compound of the punctatin class, but with different stereochemistry at C-4 and C-8, was also observed in the biotransformation of caryophyllene with *D. gossypina* [4]. It indicates again that a caryophyllane could be the precursor of this unusual class of sesquiterpenoids.

Biotransformation of caryophyllene with *C. cochliodes* resulted in products all derived from 4,5-epoxycaryophyllene. The products are similar but no means identical to the ones produced by *D. gossypina*. One difference is the lower stereoselectivity of *C. cochliodes* in the hydroxylation of the geminal methyl groups, another is the rearrangement of the 4,5-epoxide to 6-hydroxy-isocaryophyllene derivatives or the formation of clovane compounds which were not observed with *D. gossypina*. Both strains possess the ability to form punctatin derivatives, but with different stereochemistry. While *D. gossypina* forms the 1*S*,2*S*,8*S*-punctatan, this skeleton has the natural 1*R*,2*S*,8*R*-geometry with *C. cochliodes*. This again demonstrates the usefulness of biotransformation in the preparation of rare compounds. The use of caryophyllene mono- or diepoxide as the substrate does not result in higher yields. This is in strong contrast to the biotransformation of humulene and humulene-diepoxide with both strains [5].

EXPERIMENTAL

The microorganisms were precultivated at 27° and 140 r.p.m. in 100 ml Erlenmeyer flasks containing 20 ml of the following medium: 1% of universal peptone (Merck), 2% of malt extract and 0.3% of yeast extract. After 48 hr 10 μl of terpene solved in 10 μl of DMF were added to the cultures. Every day, starting after 24 hr after the substrate addition, samples were taken and analysed as follows. To 1 ml of culture broth 0.2 ml of EtOAc were added and shaken for 2 min. prior to centrifugation. 10 μl of the extract were developed on HPTLC plates with CH_2Cl_2–Me_2CO (9:1) [11]. The spots were made visible by spraying with anisaldehyde–H_2SO_4 in HOAc and heating to 110° for 1 min. For biotransformations on a preparative scale the microorganisms were grown in 5 × 100 ml flasks, transferred after 48 hr into 1 l flasks containing 200 ml of the medium and incubated for

Table 1. ^{1}H NMR data of compounds **10**, **13**, **15**, **17**, **18**, **20**, and **25**–**27** ($CDCl_3$, **27** in C_6D_6)

H	**10**	**13**	**15**	**17**	**18**	**20**	**25**	**26**	**27**
1	2.04 *dd*	2.24 *ddd*	1.95 *ddd*	1.89 *ddd*	1.62 *m*	1.79 *ddd*	—	—	1.79 *m*
2	1.66 *m*	2.40 *m*	1.65 *m*	1.37 *m*	1.33 *dddd*	1.67 *m*	3.32 *dd*	3.67 *d*	1.23 *m*
2′	1.53 *m*	2.14 *ddd*	1.53 *m*	1.53 *m*	1.46 *m*	1.48 *m*	—	—	
3	2.11 *ddd*	5.41 *br t*	1.0 *m*	1.14 *m*	1.22 *ddd*	1.71 *m*	1.71 *dd*	1.66 *br d*	1.70 *m*
3′	1.02 *m*	—	2.16 *ddd*	1.41 *m*	1.60 *m*		1.46 *dd*	1.59 *dd*	1.23 *m*
5	3.27 *dd*	4.57 *dd*	2.70 *dd*	3.44 *dd*	3.37 *ddd*	9.42 *s*	1.41 *m*	1.50 *m*	4.06 *t*
6	2.29 *dddd*	1.77 *m*	2.41 *m*	2.03 *dddd*	2.14 *dddd*	1.83 *m*	1.44 *m*		1.83 *dddd*
6′	1.36 *m*		1.45 *m*	1.77 *dddd*	1.78 *dddd*	1.54 *m*	1.31 *m*	to	1.31 *dddd*
7	1.66 *m*	2.33 *m*	2.56 *m*	1.65 *ddd*	1.92 *dddd*	2.19 *m*	1.40 *m*		2.18 *ddd*
7′		1.77 *m*		1.49 *m*	1.68 *m*		1.10 *m*	1.08	1.17 *ddd*
9	2.20 *ddd*	2.43 *ddd*	3.06 *ddd*	2.22 *ddd*	2.00 *dddd*	2.74 *ddd*	3.31 *dd*	3.31 *ddd*	1.59 *ddd*
10	1.36 *m*	1.82 *dd*	2.08 *dd*	1.51 *m*	1.62 *m*	2.00 *ddd*	1.98 *dddd*	1.93 *dddd*	1.38 *dd*
10′	1.26 *dd*	1.58 *dd*	1.66 *dd*			1.65 *dd*	1.60 *dddd*	1.54 *dddd*	1.47 *dd*
11	—	—	—	—	—	—	1.69 *ddd*	1.45 *ddd*	—
11′	—	—	—	—	—	—	1.09 *ddd*	0.97 *dddd*	—
12	0.98 *s*	3.66 *d*	1.04 *s*	1.02 *s*	1.02 *s*	3.68 *d*	1.61 *d*	1.18 *d*	0.97 *s*
12′		3.62 *d*				3.65 *d*	0.99 *d*	1.5 *m*	
13	0.93 *s*	1.11 *s*	1.04 *s*	1.00 *s*	1.01 *s*	1.07 *s*	1.02 *s*	1.08 *s*	0.90 *s*
14	9.20 *s*	4.88 *s*	—	1.47 *m*	1.45 *br d*	4.72 *q*	0.85 *s*	3.76 *br d*	9.80 *s*
14′	—	4.85 *s*	—	1.40 *m*	1.73 *d*	4.52 *m*		3.23 *dd*	—
15	1.32 *s*	1.67 *s*	1.31 *s*	0.92 *s*	1.00 *s*	1.02 *s*	0.96 *s*	1.00 *s*	0.71 *s*
OMe	—	—	—	—	3.18 *s*	—	3.36 *s*		

J(Hz): **10**: 1,2 = 10; 1,9 = 10; 2,3 = 2′,3 = 4; 3,3′ = 12; 5,6 = 6; 5,6′ = 10; 6,6′ = 12; 9,10 = 10; 9,10′ = 11; 10,10′ = 12. **13**: 1,2 = 9.5; 1,2′ = 6.5; 1,9 = 9.5; 2,2′ = 16; 2,3 = 2,3′ = 8; 5,6 = 9; 5,6′ = 8; 9,10 = 9; 9,10′ = 10; 10,10′ = 11. **15**: 1,2 = 9; 1,2′ = 1.5; 1,9 = 10.5; 2,3 = 2,3′ = 3.5; 3,3′ = 13; 5,6 = 10; 5,6′ = 5; 9,10 = 10.5; 9,10′ = 7.5; 10,10′ = 10.5. **17**: 1,2 = 6.3; 1,2′ = 12.5; 1,9 = 9; 5,6 = 3; 5,6′ = 3; 6,6′ = 15; 6,7 = 5.5; 6,7′ = 12; 9,10′ = 8; 9,10 = 12.5. **20**: 1,2 ≈ 4; 1,2′ ≈ 12; 1,9 ≈ 10; 1,13 > 0; 3,15 > 0; 5,6 > 0; 5,15 > 0; 6,6′ = 15.6; 6,7 = 9.9; 6,7′ = 2.5; 6′,7 = 2.6; 6′,7′ = 7.8; 7,7′ = 15.5; 9,10 = 8.1; 9,10′ = 9.8; 10,10′ = 11.1; 10,13 > 0; 10′,13 > 0; 12,12′ = 10.9; 12,13 > 0; 14,14′ = 2.1. **25**: 2,3 = 5.6; 2,3′ = 10.5; 3,3′ = 12.0; 9,10 = 9,10′ ≈ 3; 10,10′ = 14.5; 10,11 = 13.5; 10,11′ ≈ 5; 10′,11 = 4.7; 10′,11′ ≈ 3; 11,11′ = 13.5; 12,12′ = 12.9. **26**: 2,3′ = 2.2; 3,3′ = 10.0; 9,10 = 2.8; 9,10′ = 2.8; 10,10′ = 14.5; 10,11 = 4.9; 10,11′ = 2.8; 10′,11 = 4.6; 10′,11′ = 2.4; 11,11′ = 13.1; 12,12′ = 13.2; 14,14′ = 7.4. **27**: 1,9 = 12.8; 5,6 = 5,6′ = 8.5; 6,6′ = 13.5; 6,7 = 5; 6,7′ = 10; 7,7′ = 14; 9,10 = 11; 9,10′ = 7; 10,10′ = 9.5.

Table 2. ^{13}C NMR data of compounds **6**, **10**–**14** (75 MHz, $CDCl_3$)

C	**6**	**10**	**11**	**12**	**13**	**14**
1	45.0 + *	45.9 + [a]	46.3 + [a]	50.5 + [a]	52.6 +	49.8 +
2	27.4 −	24.3 −	24.9 −	32.6 − [b]	26.5 −	29.8 − [a]
3	39.8 − [a]	34.0 − [b]	40.5 −	125.9 +	125.2 +	126.2 +
4	59.0 0	58.8 0	59.2 0	137.8 0	137.5 0	137.8 0
5	59.4 +	60.6 +	61.3 +	69.7 +	69.9 +	69.4 +
6	39.2 − [a]	28.2 −	28.3 −	34.5 − [b]	36.7 − [a]	28.1 − [a]
7	73.4 +	30.5 − [b]	32.2 −	28.7 − [b]	32.3 − [a]	22.2 − [a]
8	158.2 0	78.4 0	73.8 0	154.7 0	153.1 0	59.2 +
9	56.4 +	45.6 + [a]	49.3 + [a]	42.7 + [a]	42.6 +	36.0 +
10	37.6 − [a]	40.2 − [b]	35.4 −	39.8 −	32.8 − [a]	41.2 −
11	32.8 0	34.1 0	33.0 0	33.2 0	38.5 0	34.9 0
12	29.9 +	29.4 +	29.4 +	30.0 +	25.2 +	30.3 +
13	22.6 +	22.6 +	22.3 +	22.7 +	67.4 −	22.8 +
14	111.3 −	199.9 +	69.1 −	109.7 −	110.1 −	204.2 +
15	16.6 +	16.2 +	16.2 +	15.5 +	16.9 +	15.6 +

*Amplitude of signals in DEPT-135 spectrum (Me or CH = +; CH_2 = −; quat. C = 0).
[a, b]Assignments may be interchanged.

another period of 24 hr. The substrate (200 mg/flask dissolved in 0.2 ml of DMF) was then added aseptically. The epoxides **2** and **9** were prepared with *m*-chloroperbenzoic acid (90% peracid).

Extraction and purification. Culture medium and mycelia were separated by filtration and both extracted × 3 with EtOAc. The solvent was evapd 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 purified further by prep. TLC.

General. NMR: the ^{1}H NMR spectra were obtained at

Table 2a. ^{13}C NMR data of the rearranged sesquiterpenoids **17–22** and **24–27** (75 MHz, $CDCl_3$)

C	**17***	**18***	**19***	**20***	**21***	**22**	**24**	**25**	**26**	**27***
1	44.2 + †	48.5 +[a]	52.6 +	52.4 +	47.0 +	50.10[a]	44.70	44.50	47.60[a]	47.6 +
2	20.75 −	23.6 −[b]	22.7 −	22.4 −	23.2 −	136.6 +	82.3 +	90.3 +	84.4 +	23.1 −
3	35.8 −	37.3 −	31.4 −	31.5 −	31.1 −	139.0 +	44.6 −	44.3 −	42.5 −	37.3 −
4	39.40	37.80	49.10	49.10	49.10	48.00[a]	34.80	37.20	44.40[a]	58.60
5	72.2 +	75.0 +	206.0 +	206.0 +	206.1 +	49.9 +	50.5 +	50.8 +	46.5 +	75.0 +
6	28.3 −	28.8 −	30.1 −	30.1 −	30.0 −	33.6 −[b]	26.7 −	20.7 −	17.7 −	24.9 −
7	33.7 −	25.8 −[b]	29.9 −	29.8 −	29.7 −	33.9 −	33.3 −	33.4 −	33.1 −	29.4 −
8	70.70	77.30	153.10	152.60	152.40	n.d.	38.30	34.90	34.40	47.60
9	38.7 +	48.8 +[a]	40.2 +	40.2 +	39.7 +	74.5 +	75.0 +	75.3 +	75.0 +	40.0 +
10	34.3 −	39.1 −	37.7 −	33.0 −	32.5 −	27.5 −[b]	27.5 −	26.3 −	25.0 −[b]	33.2 −
11	35.00	37.00	34.30	39.00	39.10	21.3 −	20.9 −	26.8 −	29.4 −[b]	38.60
12	30.5 +	31.0 +	30.1 +	67.6 −	71.6 −	35.7 −[b]	35.7 −	36.7 −	30.3 −[b]	30.2 +
13	20.81 +	20.1 +	22.2 +	25.0 +	17.7 +	24.9 +	25.4 +	31.4 +	71.2 −	20.1 +
14	42.8 −	42.9 −	107.2 −	107.5 −	107.6 −	32.8 +	31.5 +	25.5 +	15.7 +	206.2 +
15	26.7 +	30.2 +	22.4 +	22.4 +	22.3 +	28.4 +	28.3 +	28.4 +	28.4 +	17.3 +

*Numbering of carbons according to the numbering of caryophyllene.

†Amplitude of signals in DEPT-135 spectrum (Me or CH = +; CH_2 = −; quat. C = 0).

[a, b]Assignments may be interchanged; acetate in **24**: 168.0 (0), 21.2 (+); methoxy in **18**: 49.4 (+), in **25**: 58.2 (+).

400 MHz and the ^{13}C NMR spectra at 75.5 MHz, $CDCl_3$ was the solvent unless otherwise stated and TMS the int. standard [12]. Mass spectra were recorded with 70 eV. IR spectra and optical rotations were measured in $CHCl_3$ if not stated otherwise. TLC solvents: A = *n*-hexane–EtOAc (1:2) or B = hexane–EtOAc (1:4).

Biotransformation of 1.8 g of caryophyllene (**1**) with *C. cochliodes* ATCC 10195 in the medium with additional 1% glucose gave, after 168 hr, recovered **1** (23 mg), **2** (2 mg), **3** (250 mg), **4** (50 mg), **5** (10 mg), **7** (12 mg), **13** (15 mg), **20** (12 mg), and **26** (4 mg).

(1R,4R,5R,7R9S,11S)-4,5-*Epoxy*-8(14)-*caryophyllen*-7,12-*diol* (**4**). R_f 0.20 (B). ^1H NMR: 1.08 (3H, *s*, 12-H), 1.32 (3H, *s*, 15-H), 2.49 (1H, *ddd*, $J = 9, 9, 9$ Hz, 9-H), 2.65 (1H, *dd*, $J = 10, 6$ Hz, 5-H), 3.56 (1H, *d*, $J = 11$ Hz, 13-H), 3.66 (1H, *d*, $J = 11$ Hz, 13'-H), 4.05 (1H, *dd*, $J = 9, 7$ Hz, 7-H), 5.33 (1H, *br s*, 14-H), 5.49 (1H, *br s*, 14'-H). MS *m/z* (rel. int.): $[M]^+$ 252.1730 (3) (252.1725 calc. for $C_{15}H_{24}O_3$), 221 (30), 43 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{-74.4^\circ \quad -77.7^\circ \quad -88.1^\circ \quad -150.7^\circ \quad -242.9^\circ} (c\ 1.00)$$

(1R,5R,9S,11S)-3Z,8(14)-*Caryophylladiene*-5,12-*diol* (**13**). For ^1H and ^{13}C NMR see Tables 1 and 2.

(1R,9S,11S)-4,11-*Dimethyl*-4-*formyl*-11-*hydroxymethyl*-8-*methylene*-*bicylo*[6,2,0]*decane* (**20**). R_f 0.56 (**B**). For ^1H and ^{13}C NMR see Tables 1 and 2. MS *m/z* (rel. int.): $[M]^+$ 236.1775 (9) (236.1776 calc. for $C_{15}H_{24}O_2$), $[M-Me]^+$ 221 (13), $[M-CH_2OH]^+$ 205 (16), 41 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{-6.8^\circ \quad -7.1^\circ \quad -8.2^\circ \quad -15.2^\circ \quad -25.6^\circ} (c\ 1.00)$$

(1S,2S,4R,5S,8R,9R)-2,13-*Epoxy*-9-*clovanol* (**26**). R_f 0.46 (B). For ^1H and ^{13}C NMR see Tables 1 and 2. MS *m/z* (rel. int.): $[M]^+$ 236.1778 (3) (236.1776 calc. for $C_{15}H_{24}O_2$), 149 (100). Biotransformation of 400 mg of caryophyllene with *C. cochliodes* ATCC 10195 gave, after 216 hr, recovered **1** (20 mg), **3** (100 mg), **5** (8 mg), **20** (5 mg), **21** (4 mg), and **26** (2 mg).

(1R,9S,11R)-4,11-*Dimethyl*-4-*formyl*-11-*hydroxymethyl*-8-*methylene*-*bicyclo*[6,2,0]*decane* (**21**). ^1H NMR: 1.02 (3H, *s*, 15-H), 1.08 (3H, *s*, 13-H), 2.21 (2H, *m*, 7-H), 2.72 (1H, *ddd*, $J = 9, 9, 9$ Hz, 9-H), 3.50 (2H, *s*, 12-H), 4.52 (1H, *br s*, 14-H), 4.72 (1H, *br s*, 14'-H). For ^{13}C NMR see Table 2.

Biotransformation of 2.8 g of 4,5-epoxy-caryophyllene with *C. cochliodes* ATCC 10195 gave, after 240 hr, recovered **2** (435 mg), **5** (35 mg), **6** (25 mg), **8** (5 mg) [17], caryophyllenol-I **12** (70 mg) [15], **15** (8 mg), **16** (5 mg), **17** (80 mg) [13, 14], **18** (12 mg), **19** (30 mg), **22** (20 mg), **23** (13 mg) [16], **24** (10 mg), **25** (14 mg), and **26** (5 mg).

(1R,4R,5R,7R,9S)-4,5-*Epoxy*-8(14)-*caryophyllen*-7-*ol* (**6**). R_f 0.51 (B). ^1H NMR: 1.02 (6H, *s*, 12- and 13-H), 1.32 (3H, *s*, 15-H), 2.43 (1H, *ddd*, $J = 10, 10, 9$ Hz, 9-H), 2.63 (1H, *dd*, $J = 10, 6$ Hz, 5-H), 4.08 (1H, *dd*, $J = 10, 7$ Hz, 7-H), 5.30 (1H, *br s*, 14-H), 5.48 (1H, *br s*, 14'-H). For ^{13}C NMR see Table 2. MS *m/z* (rel. int.): $[M]^+$ 236.1779 (6) (236.1776) calc. for $C_{15}H_{24}O_2$), 43 (100).

(1R,4R,5R,8S,9S)-8-*Methoxy*-5-*caryollanol* (**18**). R_f 0.47 (B). For ^1H and ^{13}C NMR see Tables 1 and 2. MS *m/z* (rel. int.): $[M]^+$ 252.2091 (2) (252.2089 calc. for $C_{16}H_{28}O_2$), $[M-MeOH]^+$ 220 (24), 141 (94), 73 (100).

(1R,9S)-4-*Formyl*-8-*methylene*-4,11,11-*trimethylbicylco* [6,2,0] *decane* (**19**). ^1H NMR: 0.98 (3H, *s*, 13-H), 1.00 (3H, *s*, 12-H), 1.02 (3H, *s*, 15-H), 2.18 (2H, *m*, 7-H), 2.65 (1H, *ddd*, $J = 9, 9, 9$ Hz, 9-H), 4.50 (1H, *m*, 14-H), 4.70 (1H, *m*, 14'-H), 9.44 (1H, *s*, 5-H). For ^{13}C NMR see Table 2. $[\alpha]_D - 0.1^\circ$ (*c* 1.00).

(1S,2S,5S,8R,9R)-2-*Acetoxy*-9-*clovanol* (**24**). R_f 0.54 (B). ^1H NMR: 0.92 (3H, *s*, 14-H), 0.95 (3H, *s*, 15-H), 1.05 (3H, *s*, 13-H), 1.78 (1H, *dd*, $J = 12, 6$ Hz, 3-H), 1.98 (1H, *m*, 10-H), 3.32 (1H, *m*, 9-H), 4.84 (1H, *dd*, $J = 8, 6$ Hz, 2-H). For ^{13}C NMR see Table 2. MS *m/z* (rel. int.): $[M]^+$ 280.2046 (3) (280.2038 calc. for $C_{17}H_{28}O_3$), $[M-H_2O]^+$ 262 (5), $[M-HOAc]^+$ 220 (76), 43 (100).

(1S,2S,5S,8R,9R)-2-*Methoxy*-9-*clovanol* (**25**): R_f 0.53 (B). For ^1H and ^{13}C NMR see Tables 1 and 2. MS *m/z* (rel. int.): $[M]^+$ 252.2091 (68) (252.2089 calc. for $C_{16}H_{28}O_2$), $[M-MeOH]^+$ 220 (18), 99 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436\ nm}{+4.3^\circ \quad +4.5^\circ \quad +5.3^\circ \quad +10.1^\circ} (c\ 1.00)$$

Biotransformation of 3 g of 4,5; 8,14-diepoxy-caryophyllene with *C. cochliodes* ATCC 10195 gave, after 72 hr, **8** (100 mg) [17], **10** (60 mg), **11** (19 mg) [13], **14** (15 mg), **15** (475 mg), and **27** (25 mg).

(1R,4R,5R,9S)-4,5-*Epoxy*-8-*hydroxy*-14-*caryophyllanal* (**10**). For ^1H NMR and ^{13}C NMR see Table 2. R_f 0.53 (A). MS *m/z* (rel. int.): $[M]^+$ 252.1724 (1) (252.1725 calc. for $C_{15}H_{24}O_3$), $[M-CHO]^+$ 223 (66), 55 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{-146.8^\circ \quad -153.2^\circ \quad -174.8^\circ \quad -309.3^\circ \quad -525.0^\circ} (c\ 1.00)$$

(1R,4R,9S)-5-*Hydroxy*-*caryophyll*-3Z-*en*-14-*al* (**14**). R_f 0.52 (A). ^1H NMR: 1.00 (3H, *s*, 12-H), 1.03 (3H, *s*, 13-H), 1.62 (3H, *s*, 15-H), 4.71 (1H, *dd*, $J = 12, 8$ Hz, 5-H), 5.56 (1H, *t*, $J = 7$ Hz), 9.67 (1H, *s*, 14-H). For ^{13}C NMR see Table 2. MS *m/z* (rel. int.): $[M]^+$ 236.1778 (20) (236.1776 calc. for $C_{15}H_{24}O_2$), 96 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+36.7^\circ \quad +38.1^\circ \quad +43.5^\circ \quad +69.1^\circ \quad +88.2^\circ} (c\ 1.00)$$

(1R,4R,5S,8R,9S)-8-*Formyl*-4,11,11-*trimethyltricyclo* [6.3.0.0^{1,9}] *undecan*-5-*ol* (**27**). R_f 0.53 (A). For ^1H and ^{13}C NMR see Tables 1 and 2. MS *m/z* (rel. int.): $[M]^+$ 236.1778 (6) (236.1776 calc for $C_{15}H_{24}O_2$), 41 (100).

$$[\alpha] = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+27.0^\circ \quad +28.4^\circ \quad +31.4^\circ \quad +41.2^\circ \quad +7.7^\circ} (c\ 1.00)$$

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