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MICROBIAL OXIDATION OF TRICYCLIC SESQUITERPENOIDS CONTAINING A DIMETHYLCYCLOPROPANE RING

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Key Word Index—*Diplodia gossypina*; Deuteromycotina; *Bacillus megaterium*; Aeroendospora; *Mycobacterium smegmatis*; Actinobacteria; calarene; globulol; biotransformation; microbial hydroxylation.

Abstract—Calarene was oxidized by *Bacillus megaterium* and *Diplodia gossypina* to give allylic calarenols and calarendiols in which either of the geminal methyl groups of the cyclopropane ring was hydroxylated. Globulol was hydroxylated faster and in higher yields than calarene by both strains. In the case of this compound, vicinal diols were formed and, again, either of the geminal methyl groups was oxidized. Only bacterial strains caused 9-hydroxylation. *Mycobacterium smegmatis* produced globulol-14-exo-14-oic acid in good yields. Of the geminal methyl groups, the one in the exo-orientation was attacked preferentially by all of the strains tested. Some of the metabolites formed are stereoisomers of known natural products.

INTRODUCTION

Sesquiterpenes are widespread in nature and possess a multitude of biological activities. Some of the sesquiterpenoids, mostly hydrocarbons and alcohols, are available in larger quantities from essential oils at low to moderate prices. The idea to oxidize these compounds to biologically active natural products or their synthons, more precisely chirons, is attractive. Because of the low functionality of these starting materials chemical transformations are hampered. The use of microorganisms for this purpose is gaining more and more interest. A review on biotransformations of sesquiterpenoids, although incomplete, appeared recently [1].

In a continuation of our studies on microbial oxidation of sesquiterpenoids [2–6], we concentrated our efforts on the hydroxylation of the geminal methyl groups of the cyclopropane ring of tricyclic sesquiterpenes with an aromadendrane or aristolane skeleton. Such oxidation is chemically difficult because (i) it involves non-activated carbon atoms, (ii) the instability of the cyclopropane system compared to larger rings due to its strained bonds and (iii) the requirement of regioselectivity leading to exo- or endo-alcohols. These difficulties are typical of chemical transformations on such complex compounds, however microorganisms can oxidize unreactive carbons under mild conditions with high selectivity. Although these advantages are known, no biotransformations of tricyclic sesquiterpenes containing a dimethylcyclopropane ring have been reported so far. From a small screen, we selected two bacteria and one fungus for use in fermentations on a preparative scale.

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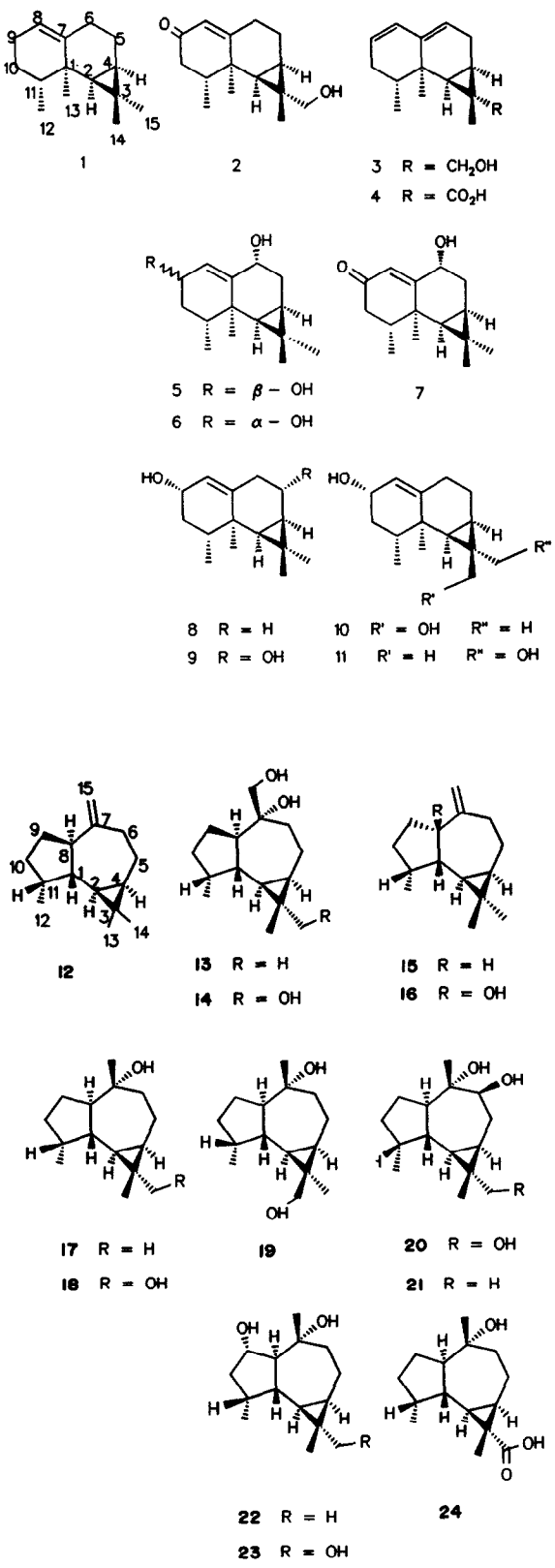
RESULTS AND DISCUSSION

Commercially available calarene (**1**), aromadendrene (**12**), allo-aromadendrene (**15**), and globulol (**17**) were used as substrates. We selected *Diplodia gossypina* ATCC 10936, *Bacillus megaterium* DSM 32, and *Mycobacterium smegmatis* DSM 43061 for use in the fermentation studies.

Diplodia gossypina ATCC 10936 oxidized calarene at the allylic carbons C-6 and C-9 and at one of the geminal methyl groups on the cyclopropane ring. A dehydrogenation to the unusual calarane-6,8-diene nucleus was also observed. In three out of the four metabolites isolated one of the geminal methyl groups was oxidized. To determine the configuration of C-3 we performed NOE experiments, but irradiating the resonances due to H-2, H-4 or H-15 showed no suitable enhancements of signals. To circumvent this problem, we used the different chemical shifts of C-14 and C-15 in calarene to determine which one was hydroxylated to yield **3**. All proton signals of **1** were assigned with the aid of the COSY 2D NMR spectrum. Because of a ^4J coupling between the methyl singlets at δ_H 1.02 and 0.98 these resonances had to be attributed to H-14 and H-15. The 2D ^{13}C, ^1H-COSY spectrum allowed the assignment of all carbons (Table 1). Cross-peaks between δ_H 1.02 and δ_C 16.5 and between δ_H 0.98 and δ_C 30.0 were found. To decide which of these is due to the exo-methyl group, DNOE experiments were performed. Irradiation at δ_H 0.74 (H-4) and 0.57 (H-2) in the DNOE spectrum enhanced the resonance of the methyl singlet at δ_H 0.98 identifying this methyl group as being in the exo-position. With this assignment of the substrate in hand, we identified **3** as the exo-alcohol because the resonance of the adjacent methyl group was at δ_C 12.8. A high-field shift of 3.7 ppm is well in the range of the predicted γ-effect at C-14 of the hydroxy group. A shift change of 17.2 ppm (from δ 30.0 to 12.8) that would have occurred if **3** was the endo-alcohol, is very unreasonable.



Fermentation of calarene with *B. megaterium* DSM 32 led to seven metabolites. One of them was identified as debilone (**7**) known as a natural product from *Aristolochia debilis* [7] and *Nardostachys chinensis* [8]. The only mono-ol isolated from the culture broth was 9α-hydroxycalarene (**8**) which has been described earlier as a photooxidation product of calarene [9]. Metabolite **8** is further oxidized by the bacterium. To a minor extent, hydroxylation at C-5 occurred leading to 5α,9α-dihydroxycalarene (**9**). Two pairs of epimeric diols were formed. One consisted of the allylic diols 6,9-dihydroxycalarene (**5** and **6**). While **5** had been isolated previously from *Diplodia gossypina*, **6** was not formed by this fungus. The stereochemistry of the isomers can easily be derived from the coupling constants of H-9. Since the 9β-hydroxy group in **5** is the only OH-9β formed by this strain, its formation by reduction of debilone (**7**) is more likely than direct hydroxylation of the methylene group. The other pair of epimers are the 9,15-hydroxy-calarenes **10** and **11**. Unfortunately, no ^{13}C NMR spectrum of **10** could be obtained due to lack of material. Its stereochemistry at C-3 was elucidated instead by DNOE experiments. Irradiation at δ_H 3.75 and 3.69 enhanced the multiplet at δ_H 1.50–1.48 (H-5 and H-11), requiring the endo-orientation of the hydroxymethyl group (Table 2). Calarene was not metabolized by *M. smegmatis* DSM 43061, so no comparison of its product could be made.

The difficulties of biotransformation of sesquiterpene hydrocarbons was also mirrored in the microbial transformation of aromadendrene (**12**) and allo-aromadendrene (**15**). In both cases only one of the three selected strains was able to attack the substrate. *Bacillus megaterium* oxidized aromadendrene (**12**) at the double bond. The less polar metabolite proved to be the diol **13** and the more polar product was identified as the triol **14**. The stereochemistry at C-7 was deduced from the fact that a long-range coupling between H-6 and H-15′ was observed in the COSY 2D NMR spectrum requiring a dihedral angle close to 180° which is only possible with β-orientation of the hydroxymethyl group (Table 3). The configuration of the second hydroxymethyl group was determined from the shift of the adjacent methyl group in the ^{13}C NMR spectrum as explained above [10]. Epimeric compounds were isolated from *Wyethia arizonica* [11] differing in the configuration at C-7 and C-8. The same epimeric diol was found recently in *Pulicaria paludosa* [12].

The fermentation of allo-aromadendrene **15** by *M. smegmatis* resulted in only one product. This metabolite was identified as 8-hydroxy-allo-aromadendrene (**16**). It is again a natural compound which was isolated from the red alga *Laurencia subopposita* [13] and from *Cassinia subtropica* [14]. The optical rotation of **16** revealed that it is the enantiomer of the metabolite from *L. subopposita*, unfortunately no optical rotation of the product from *C. subtropica* was reported.

As it seems to be a general rule that biotransformations of sesquiterpene hydrocarbons proceed only in low yields [15] and as our results corroborate this finding, we used the alcohol globulol (**17**) as the substrate. The aim was to study the regioselectivity of the three strains.

Biotransformation of globulol with *D. gossypina* ATCC 10936 gave three products. Two of them had similar polarity and were identified as the epimeric alcohols **18** and **19**. The ^{13}CNMR spectra of both compounds corroborated the results obtained from the assignments of the geminal methyl groups in calarene. The endomethyl group in **18** had δ_C 11.4 while the exo-methyl group in **19** resonated at δ_C 20.4. In accordance with this,

Table 1. ^{13}C NMR data of **1–3, 5–9, 11** (75 MHz, $CDCl_3$, **7** in C_6D_6)

C	1	2	3	5	6	7	8	9	11
1	36.9 0*	38.2 0	34.8 0	31.9 0	35.6 0	37.4 0	37.2 0	37.2 0	36.6 0
2	33.7 +	30.3 +	34.0 +	32.0 +	35.7 +	32.0 +	33.2 +	34.0 +	29.9 +
3	18.6 0	25.8 0	25.0 0	18.8 0	18.4 0	18.8 0	18.8 0	18.4 0	n.d.
4	19.8 +	15.3 +	15.0 +	15.1 +	15.3 +	17.3 +[c]	19.6 +	29.1 +	15.7 0
5	20.9 −	19.6 −	22.2 −	28.2 −	27.6 −	27.9 −	20.8 −	66.9 +	20.1 −
6	30.3 −	30.4 −	122.1 +[a]	73.7 +	72.7 +	72.3 +	29.8 −	40.3 −	29.4 −
7	144.3 0	173.3 0	141.2 0	149.9 0	147.3 0	169.0 0	147.7 0	144.3 0	146.7 0
8	120.4 +	125.3 +	125.5 +[a]	126.1 +	127.9 +	126.8 +	124.4 +	126.7 +	124.6 +
9	25.8 −	198.9 0	129.1 +[a]	64.4 −	67.2 +	198.1 0	68.0 +	67.7 +	67.5 +
10	27.4 −	42.5 −	32.4 −	36.4 −	36.5 −	43.0 −	37.4 −	37.0 −	36.8 −
11	36.9 +	36.4 +	28.7 +	31.1 +	31.7 +	37.2 +	35.4 +	35.1 +	35.2 +
12	16.1 +	16.0 +	16.8 +	16.2 +[b]	16.3 +	16.9 +	16.0 +	16.3 +	16.2 +
13	23.1 +	21.6 +	21.0 +	24.2 +	25.0 +	23.8 +	23.0 +	22.9 +	22.6 +
14	16.5 +	12.8 +	10.5 +	17.1 +[b]	16.5 +	14.8 +[c]	16.5 +	15.9 +	11.8 +
15	30.0 +	73.1 −	74.3 −	29.5 +	29.3 +	29.2 +	29.8 +	29.5 +	73.4 −

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

Table 2. ^1H NMR data of the biotransformation products **2–6, 9–11** (400 MHz, $CDCl_3$, **2** in C_6D_6) of calarene

H	2	3	4	5	6	9	10	11
2	0.47 *d*	0.81 *d*	n.d.*	0.68 *d*	0.64 *d*	0.72 *d*	0.74 *d*	0.68 *d*
4	0.64 *ddd*	1.0 *m*	n.d.	0.88 *m*	0.81 *ddd*	0.78 *dd*	1.04 *ddd*	0.94 *ddd*
5	1.19 *dddd*	2.60 *br dd*	2.27 *dd*	1.69 *ddd*	1.64 *ddd*	3.73 *ddd*	1.48 *dddd*	1.45 *dddd*
5′	1.69 *dddd*	2.24 *dd*	2.66 *dd*	2.22 *ddd*	2.18 *ddd*	—	2.14 *dddd*	2.07 *dddd*
6	1.98 *dddd*	5.28 *m*	5.27 *m*	4.12 *m*	4.01 *dd*	2.25 *m*	1.84 *ddd*	1.79 *m*
6′	1.55 *ddd*	—	—	—	—	2.25 *m*	2.27 *dddd*	2.29 *m*
8	5.81 *br s*	5.89 *dd*	5.89 *br d*	5.64 *dd*	5.42 *m*	5.33 *m*	5.29 *m*	5.28 *m*
9	—	5.57 *m*	5.60 *m*	4.12 *m*	4.16 *ddd*	4.16 *ddd*	4.21 *m*	4.20 *dddd*
10	2.1 *m*	1.91 *br dd*	1.93 *br dd*	1.76 *ddd*	1.50 *ddd*	1.42 *ddd*	1.39 *ddd*	1.41 *ddd*
10′	2.25 *m*	2.04 *dt*	2.05 *dt*	1.60 *dddd*	1.67 *m*	1.79 *m*	1.81 *dddd*	1.82 *ddd*
11	2.1 *m*	1.77 *dqd*	n.d.	1.97 *dqd*	1.69 *m*	1.78 *m*	1.50 *m*	1.80 *m*
12	0.78 *d*	1.01 *d*	0.97 *d*	1.00 *d*	0.98 *d*	1.00 *d*	0.98 *d*	1.00 *d*
13	0.86 *s*	0.98 *s*	1.03 *s*	1.23 *s*	1.31 *s*	1.15 *s*	1.14 *s*	1.15 *s*
14	0.85 *s*	1.15 *s*	1.30 *s*	1.06 *s*	1.03 *s*	1.04 *s*	3.75 *d*	1.10 *s*
14′							3.69 *d*	
15	2.91 *d*	3.36 *d*	—	0.96 *s*	0.91 *s*	0.99 *s*	1.10 *s*	3.29 *d*
15′	3.10 *d*	3.30 *d*						3.17 *d*

*Not determined.

J (Hz): **2**: 2,4 = 9.5; 4,5α = 3.5; 4,5β = 9.5; 5,5′ = 14.5; 5α,6 = 13.7; 5α,6′ = 5.7; 5β,6 = 8; 5β,6′ = 1.5; 6,6′ = 13.7; 6,8 = 1.5; 11,12 = 6.5; 15,15′ = 10. **3**: 2,4 = 10; 4,5 = 6; 5,5′ = 20; 5′,6 = 5.5; 6,8 = 2; 8,9 = 9.5; 9,10′ = 5; 10,10′ = 18; 10,11 = 11.5; 10′,11 = 5; 11,12 = 6.5; 15,15′ = 10. **4**: 4,5 = 7; 5,5 = 20; 5′,6 = 6; 8,9 = 10; 9,10′ = 5; 10,10′ = 17; 10,11 = 11; 10′,11 = 5; 11,12 = 7. **5**: 2,4 = 9.5; 4,5 = 3.5; 4,5′ = 9.5; 5,5′ = 15.8; 5,6 = 2.2; 5′,6 = 4.5; 6,8 > 0; 8,9 = 4.5; 8,10 = 1.5; 9,10 = 4.7; 9,10′ = 1.5; 10,10′ = 14.5; 10,11 = 13; 10′,11 = 3.3; 11,12 = 7. **6**: 2,4 = 9; 4,5 = 4; 4,5′ = 10; 5,5′ = 15; 5,6 = 3; 5′,6 = 2; 8,9 = 2; 9,10 = 10; 9,10′ = 7; 10,10′ = 13; 10,11 = 13; 11,12 = 6.5. **9**: 2,4 = 10; 4,5 = 2; 5,6 = 8; 5,6′ = 7; 8,9 = 2; 9,10 = 10; 9,10′ = 10; 10,10′ = 13; 10,11 = 13; 11,12 = 7. **10** and **11**: 2,4 = 10; 4,5 = 10; 4,5′ = 4; 5,5′ = 14; 5,6 = 7; 5,6′ = 1; 5′,6 = 3; 5′,6′ = 1; 8,9 = 2; 9,10 = 10; 9,10′ = 7; 10,10′ = 13; 10,11 = 13; 10′,11 = 3; 11,12 = 7. **10**: 6,6′ = 13; 14,14′ = 11. **11**: 15,15′ = 11.

deshielding of the exo-methyl group was similar to the deshielding of the hydroxymethyl groups in **18** (δ_{exo} 73.5 in **18** and δ_{endo} 64.3 in **19**) (Table 4). Compound **18** was further hydroxylated to the triol **20**. It is interesting to note that an epimeric natural product of metabolite **19** exists. This diol had been isolated from *Flourensia cernua* and named flourensadiol [16]. It is again epimeric at C-7 and C-8. Fluorensadiol is believed to be one of the toxic components of *F. cernua*.

Fermentation of globulol with *B. megaterium* DSM 32 resulted in the same three metabolites as those produced by *D. gossypina*. In addition, however, three new products 6β-hydroxy-globulol (**21**, the diol of **20**) and the 9α-hydroxy derivatives of globulol and **21**, i.e. the diol **22** and

Table 3. ^{1}H NMR data of the biotransformation products **13**, **14**, **16** and **18–24** ($CDCl_3$)

H	**13**	**14**	**16**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	1.26 *m*	1.31 *m*	1.70 *dd*	1.28 *m*	1.36 *m*	1.30 *m*	1.24 *m*	1.53 *q*	1.57 *m*	1.30 *m*
2	0.52 *dd*	0.66 *dd*	0.19 *dd*	0.67 *dd*	0.73 *dd*	0.66 *dd*	0.50 *dd*	0.51 *dd*	0.66 *dd*	1.56 *dd*
4	0.62 *ddd*	0.79 *ddd*	0.52 *ddd*	0.76 *ddd*	0.79 *ddd*	0.83 *ddd*	0.64 *ddd*	0.59 *ddd*	0.74 *ddd*	1.62 *m*
5	0.92 *m*	0.98 *m*	1.47 *dddd*	0.98 *m*	1.03 *m*	1.24 *m*	1.14 *ddd*	0.98 *m*	1.03 *m*	1.00 *m*
5′	1.80 *m*	1.82 *m*	1.85 *dddd*	1.83 *m*	1.93 *m*	1.90 *m*	1.89 *ddd*	1.82 *dddd*	1.84 *dddd*	1.85 *m*
6	1.32 *ddd*	1.34 *ddd*	2.28 *dddd*	1.57 *dd*	1.54 *dd*	3.46 *dd*	3.44 *dd*	1.55 *m*	1.58 *m*	1.60 *m*
6′	2.09 *m*	2.11 *m*	2.63 *ddddd*	1.79 *m*	1.78 *m*			1.6 *m*	1.64 *ddd*	1.76 *m*
8	2.06 *m*	2.09 *m*	—	1.95 *ddd*	1.93 *m*	1.82 *m*	1.82 *m*	2.00 *dd*	2.04 *dd*	2.02 *ddd*
9	1.49 *m*	1.51 *m*	1.72 *ddd*	1.46 *m*	1.48 *m*	1.55 *m*	1.70 *m*	4.59 *q*	4.59 *dt*	1.48 *m*
9′	1.78 *m*	1.79 *m*	2.31 *ddd*	1.80 *m*	1.78 *m*	1.82 *m*	1.81 *m*			1.80 *m*
10	1.24 *m*	1.26 *m*	1.35 *dddd*	1.28 *m*	1.29 *m*	1.32 *m*	1.29 *m*	1.69 *t*	1.70 *t*	1.29 *m*
10′	1.63 *m*	1.63 *m*	2.00 *dddd*	1.69 *m*	1.68 *m*	1.70 *m*	1.50 *m*			1.69 *m*
11	1.99 *m*	2.01 *m*	2.54 *dddq*	2.05 *m*	2.05 *m*	2.05 *m*	2.03 *m*	2.23 *m*	2.25 *m*	2.05 *m*
12	0.91 *d*	0.91 *d*	0.96 *d*	0.92 *d*	0.94 *d*	0.92 *d*	0.90 *d*	0.91 *d*	0.92 *d*	0.91 *d*
13	1.01 *s*	1.08 *s*	1.00 *s*	1.10 *s*	3.65 *d*	1.10 *s*	1.01 *s*	1.00 *s*	1.11 *s*	1.23 *s*
13′					3.70 *d*					
14	0.96 *s*	3.19 *d*	0.97 *s*	3.28 *d*	1.13 *s*	3.20 *d*	0.97 *s*	1.01 *s*	3.28 *d*	—
14′		3.41 *d*		3.39 *d*		3.43 *d*			3.33 *d*	
15	3.62 *d*	3.63 *d*	4.85 *ddd*	1.12 *s*	1.12 *s*	1.05 *s*	1.04 *s*	1.40 *s*	1.40 *s*	1.12 *s*
15′	3.57 *dd*	3.59 *dd*	4.99 *dd*							

J (Hz): **13** and **14**: 1,2 = 11; 2,4 = 10; 4,5 = 7; 4,5′ = 10; 5′,6′ = 6,6′ = 13; 6,13 > 0; 6′,15′ = 1; 11,12 = 7; 15,15′ = 11. **14**: 5,5′ = 13; 14,14′ = 11. **16**: 1,2 = 8.2; 1,11 = 7; 2,4 = 10.5; 4,5 = 9; 4,5′ = 6.8; 5,5′ = 14.5; 5,6 = 10.3; 5,6′ = 8.5; 5′,6 = 9; 5′,6′ = 3.8; 6,15 = 0.7; 6′,15 = 1.7; 6′,15′ = 1; 9,9′ = 13.8; 9,10 = 8.8; 9,10′ = 5; 9′,10 = 6.8; 9′,10′ = 10.5; 10,10′ = 13.3; 10,11 = 8.5; 10′,11 = 8.8; 11,12 = 7; 15,15′ = 1.7. **18**: 1,2 = 11; 1,8 = 12; 2,4 = 10; 4,5 = 10; 4,5′ = 7; 5,6 = 6,6′ = 13; 8,9 = 11; 8,9′ = 11; 11,12 = 7. **19**: 1,2 = 11; 2,4 = 10; 4,5 = 11; 4,5′ = 7; 5,6 = 6,6′ = 13; 5,6′ = 2; 11,12 = 7; 13,13′ = 11 **20**: 1,2 = 10; 2,4 = 9; 4,5 = 10; 4,5′ = 7; 5,6 = 11; 5′,6 = 2; 11,12 = 7; 14,14′ = 11. **21**: 1,2 = 11; 2,4 = 9; 2,13 > 0; 4,5 = 11; 4,5′ = 6; 4,13 > 0; 5,5′ = 15; 5,6 = 11; 5′,6 = 2; 11,12 = 7. **22**: 1,2 = 11; 1,8 = 1,11 = 9; 2,4 = 9; 4,5 = 11; 4,5′ = 7; 5,5′ = 15; 5′,6 = 7; 5′,6′ = 2; 8,9 = 8; 9,10 = 11,12 = 7. **23**: 1,2 = 10.6; 1,8 = 9.6; 2,4 = 9.7; 4,5 = 10.6; 4,5′ = 6.2; 5,5′ = 15; 5,6′ = 6; 5′,6 = 6.2; 5′,6′ = 2; 6,6′ = 13; 8,9 = 9,10 = 7.5; 11,12 = 7.3; 14,14′ = 10.9. **24**: 1,2 = 2,4 = 10.5; 1,8 = 11; 8,9 = 8,9′ = 7.8; 11,12 = 7.

Table 4. ^{13}C NMR data of **14**–**21**, **23** and **24** (75 MHz, $CDCl_3$)

C	14	15	16	17	18	19	20	21	23	24
1	37.3 +*	42.2 +	49.0 +	39.7 +	38.9 +	39.5 +	39.2 +	40.2 +	38.7 +	38.8 +
2	24.8 +	23.6 +	23.3 +[b]	28.3 +	25.2 +	28.9 +	24.5 +	27.9 +	25.6 +	30.7 +
3	26.3 0		n.d.	19.5 0	26.5 0	25.1 0	26.8 0	20.0 0	26.7 0	26.5 0
4	23.4 +	24.9 +	25.4 +[b]	26.7 +	23.7 +	27.1 +	20.2 +	23.8 +	23.2 +	28.8 +
5	19.2 –	22.2 –	21.2 –	20.2 –	19.8 –	20.0 –	25.2 –	25.4 –	19.8 –	19.2 –
6	37.1 –	35.8 –	32.0 –	44.6 –	44.3 –	44.3 –	79.0 +	79.5 +	44.1 –	43.0 –
7	76.0 0	152.5 0	152.9 0	75.3 0	75.4 0	75.6 0	77.9 0	78.2 0	76.1 0	74.9 0
8	54.9 +	50.8 +	88.5 0	57.0 0	56.7 +	56.9 +	53.0 +	53.5 +	57.8 +	55.6 +
9	25.6 –	28.3 –	36.5 –	26.1 –	26.0 –	25.9 –	27.7 –	28.2 –	74.4 +	25.7 –
10	34.6 –	31.3 –	30.6 –	34.6 –	34.6 –	34.5 –	34.7 –	34.8 –	45.4 –	34.3 –
11	36.2 +	37.8 +	34.3 +	36.3 +	36.4 +	36.7 +	36.4 +	36.4 +	33.4 +	35.9 +
12	15.5 +	16.4 +[a]	16.6 +	16.0 +	16.0 +	16.0 +	15.7 +	15.9 +	16.4 +	15.6 +
13	11.3 +	15.9 +[a]	15.9 +	15.8 +	11.4 +	64.3 –	11.4 +	15.8 +	11.6 +	9.5 +
14	72.8 –	28.7 +	28.6 +	28.7 +	73.5 –	24.0 +	72.6 –	28.6 +	73.3 –	179.2 0
15	61.1 –	109.7 –	111.7 –	20.2 +	20.2 +	20.2 +	13.1 +	13.4 +	23.0 +	19.6 +

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

the triol **23**, were produced. In contrast to these strains *M. smegmatis* DSM 43061 formed the acid **24** in 46% yield as the sole product.

The preferred attack of the geminal methyl groups is at the exo-site. This is in agreement with the results reported by Hebda and colleagues on the biotransformation of the unnatural 1,4,4-trimethyltricyclo[5.4.0.$0^{3,5}$]undec-7-en-9-one [17]. They found, as did we, that no strain exclusively formed the endo-alcohol.

The formation of 6β-hydroxy-globulol (**21**) is thought-provoking. Biotransformations seldom lead to hydroxylations near existing alcohol groups. One theory is that the alcohol of the substrate serves as an anchor to the active site of the enzyme. The fixing site, however, is most probably not identical with the active site, so a minimal distance is necessary between the polar group of the substrate and its reaction site. The formation of **20** is in accord with this model because the 7-hydroxy group can serve as the attachment site for the hydroxylation at C-14 while the 14-hydroxy group in **18** may be the new fixing site for the hydroxylation of C-6. This theory, however, fails in the case of the formation of **21**. Here the fixing site and the reacting site are so close together that it is difficult to believe that they are located in different parts of the enzyme. In this connection, it is interesting that we observed the formation of **21** only with bacteria, while **20** is formed by fungal and bacterial strains.

The biotransformations described here resulted in some metabolites which are stereoisomers of natural products. Since some of these compounds are biologically active it would be interesting to test whether their isomers display different activity or are active at all. The formation of the globulols oxidized at C-13 or C-14 proceeded in good yield so these compounds may be attractive for use as chiral auxiliaries in chemical syntheses.

EXPERIMENTAL

^1H and ^{13}C NMR: 400 and 75.5 MHz, respectively, $CDCl_3$ unless otherwise stated, TMS the int. standard; MS: 70 eV; IR and optical rotations: $CHCl_3$; Mps: uncorr; TLC: *n*-hexane–EtOAc (1:2).

The microorganisms were precultivated at 27° and 140 r.p.m. in 100 ml conical flasks containing 20 ml of the following medium: 1% universal peptone (Merck), 2% malt extract and 0.3% yeast extract. After 48 hr, 10 μl of terpene dissolved in 10 μl EtOH were added to the cultures. Every day, starting 24 hr after the substrate addition, samples were taken and analysed as follows: to 1 ml culture broth 0.2 ml EtOAc was added and the mixture was shaken for 2 min prior to centrifugation. The extract (10 μl) was chromatographed on HPTLC plates with CH_2Cl_2–Me_2CO (9:1). The terpenoids 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 100 ml flasks, transferred after 48 hr into 2 l flasks containing 400 ml of the medium and incubated for another period of 24 hr. The substrate (200 mg per flask dissolved in 0.2 ml of DMF) was then added aseptically.

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.

Biotransformations of calarene (**1**). (a) Fermentation of **1** (1800 mg) with *Diplodia gossypina* ATCC 10936 resulted, after

40 hr, in unutilized **1** (42 mg), **2** (15 mg), **3** (20 mg), **4** (3 mg) and **5** (7 mg).

(1R,2R,3S,4R,11R)-3-*Hydroxymethyl*-1,3,11-*trimethyltricyclo*-[5.4.0.0^{2,4}]*undec*-7-*en*-9-*one* (**2**). [13-*Hydroxy*-1(10)-*aristolen*-2-*one*]. R_f 0.27. ^{1}H NMR (C_6D_6): NOE: irradiation at δ2.91 or 3.10 caused no useful signal enhancement.

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 549\text{ nm}}{+57.3^\circ\quad +60.5^\circ\quad +71.3^\circ}\ (c\ 1.00).$$

[(1R,2R,3S,4R,11R)-1,3,11-*Trimethyltricyclo*[5.4.0.0^{2,4}]-*undeca*-6,8-*dien*-3-*yl*]*methanol* (**3**). R_f 0.57. UV λ_{max}^{MeOH} nm: 236, 230; MS *m/z*: 218.1670 (218.1671 calc. $C_{15}H_{22}O$, $[M]^+$ 14%), 200 (4), 187 (6), 185 (15), 157 (46), 145 (56), 105 (78), 91 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{-70.6^\circ\quad -73.8^\circ\quad -84.8^\circ\quad -154.0^\circ}\ (c\ 1.00).$$

(1R,2R,3S,4R,11R)-1,3,11-*Trimethyltricyclo*[5.4.0.0^{2,4}]*undeca*-6,8-*diene*-3-*carboxylic acid* (**4**). R_f 0.52. MS *m/z*: 232.1463 (232.1463 calc. $C_{15}H_{20}O_2$, $[M]^+$ 31%), 217 (6), 204 (7), 159 (52), 105 (67), 91 (79), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{-33.8^\circ\quad -35.4^\circ\quad -40.4^\circ\quad -79.4^\circ}\ (c\ 1.00).$$

(1R,2S,4R,6R,9R,11R)-1,3,3,11-*Tetramethyltricyclo*[5.4.0.0^{2,4}]-*undec*-7-*ene*-6,9-*diol* (**5**). R_f 0.20. MS *m/z*: 236.1778 (236.1776 calc. $C_{15}H_{24}O_2$, $[M]^+$ 5%), 221 (4), 218 (19), 200 (9), 105 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{+72.8^\circ\quad +76.8^\circ\quad +87.4^\circ\quad +162.0^\circ}\ (c\ 1.00).$$

(b) Biotransformation of calarene (**1**) (800 mg) with *Bacillus megaterium* DSM 32 yielded, after 168 hr, unutilized **1** (152 mg), **5** (10 mg), **6** (10 mg), debilone **7** (6 mg), **8** (16 mg), **9** (9 mg), **10** (4 mg) and **11** (7 mg).

(1R,2S,4R,6R,9S,11R)-1,3,3,11-*Tetramethyltricyclo*[5.4.0.0^{2,4}]-*undec*-7-*ene*-6,9-*diol* (**6**). R_f 0.13. MS *m/z*: 236.1774 ($[M]^+$ 236.1776 calc. for $C_{15}H_{24}O_2$) (2%), 218 (8), 203 (7), 133 (38), 43 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}\quad 365\text{ nm}}{-35.2^\circ\quad -36.6^\circ\quad -40.8^\circ\quad -68.8^\circ\quad -104.0^\circ}\ (c\ 0.50).$$

(1R,2S,4R,9S,11R)-1,3,3,11-*Tetramethyltricyclo*[5.4.0.0^{2,4}]-*undec*-7-*en*-9-*ol* (**8**). R_f 0.57. MS *m/z* 220.1828 (220.1827 calc. $C_{15}H_{24}O$, $[M]^+$ 44%), 205 (20), 202 (88), 131 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{+27.6^\circ\quad +29.1^\circ\quad +33.2^\circ\quad +55.7^\circ}\ (c\ 1.00).$$

(1R,2S,4R,5S,9S,11R)-1,3,3,11-*Tetramethyltricyclo*[5.4.0.0^{2,4}]*undec*-7-*ene*-5,9-*diol* (**9**). R_f 0.22. MS *m/z*: 236.1777 (236.1776 calc. $C_{15}H_{24}O_2$, $[M]^+$ 5%), 218 (13), 203 (7), 91 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}}{+9.4^\circ\quad +10.6^\circ\quad +12.2^\circ}\ (c\ 1.00).$$

(1R,2R,3R,4R,9S,11R)-3-*Hydroxymethyl*-1,3,11-*trimethyltricyclo*[5.4.0.0^{2,4}]*undec*-7-*en*-9-*ol* (**10**). R_f 0.20. ^{1}H NMR: NOE: irradiation at δ3.75 led to enhancements at δ1.81 and 1.84. MS *m/z*: 236.1777 (236.1776 calc. $C_{15}H_{24}O_2$, $[M]^+$, 3%), 218 (45), 203 (17), 91 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{-3.2^\circ\quad -3.2^\circ\quad -3.6^\circ\quad -1.0^\circ}\ (c\ 0.50).$$

(1R,2R,3S,4R,9S,11R)-3-*Hydroxymethyl*-1,3,11-*trimethyltricyclo*[5.4.0.0^{2,4}]*undec*-7-*en*-9-*ol* (**11**). R_f 0.32. MS *m/z*: 236.1777 (236.1776 calc. $C_{15}H_{24}O_2$, $[M]^+$ 6%), 218 (13), 203 (8), 107 (77), 58 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}}{+11.0^\circ\quad +12.0^\circ\quad +13.4^\circ}\ (c\ 1.00).$$

Biotransformation of aromadendrene (**12**). Fermentation of **12** (800 mg) with *Bacillus megaterium* DSM 32 yielded, after 168 hr, unused **12** (134 mg), **13** (4 mg) and **14** (7 mg).

(1S,2S,4R,7R,8R,11R)-3,3,11-*Trimethyl*-7-*hydroxymethyl*-*tricyclo*[6.3.0.0^{2,4}]*undecan*-7-*ol* (**13**). R_f 0.33. MS *m/z*: 238.1932 ($[M]^+$, 238.1933 calc. for $C_{15}H_{26}O_2$) (8%), 220 (13), 207 (36), 189 (47), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{-19.6^\circ\quad -19.6^\circ\quad -22.4^\circ\quad -37.2^\circ}\ (c\ 0.50).$$

(1S,2S,3S,4R,7R,8R,11R)-3,7-*Bis*(*hydroxymethyl*)-3,11-*dimethyltricyclo*[6.3.0.0^{2,4}]*undecan*-7-*ol* (**14**). R_f 0.08. MS *m/z*: 254.1883 ($[M]^+$, 254.1882 calc. for $C_{15}H_{26}O_3$) (2%), 236 (4), 223 (33), 205 (30), 187 (24), 177 (13), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}}{-25.9^\circ\quad -26.9^\circ\quad -30.5^\circ}\ (c\ 1.00).$$

Biotransformation of allo-aromadendrene (**15**). Incubation of **15** (1200 mg) [18] with *Mycobacterium smegmatis* DSM 43061 yielded, after 144 hr, unused **15** (762 mg) and **16** (10 mg).

1-*Hydroxy*-*alloaromadendrene* (**16**). R_f 0.68.

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}}{-85.5^\circ\quad -89.0^\circ\quad -94.1^\circ}\ (c\ 1.00).$$

Biotransformation of globulol (**17**). (a) Incubation of **17** (1 g) with *Diplodia gossypina* ATCC 10936 yielded, after 48 hr, unused **17** (95 mg), **18** (182 mg), **19** (20 mg) and **20** (20 mg).

(1S,2S,3S,4R,7R,8R,11R)-3-*Hydroxymethyl*-3,7,11-*trimethyl*-*tricyclo*[6.3.0.0^{2,4}]*undecan*-7-*ol* (14-*exohydroxyglobulol*) (**18**). R_f 0.23. mp: 113°. MS *m/z*: 238.1934 (238.1933 calc. $C_{15}H_{26}O_2$, $[M]^+$ 5%), 220 (16), 202 (12), 177 (20), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}\quad 365\text{ nm}}{-33.0^\circ\quad -34.1^\circ\quad -38.9^\circ\quad -64.9^\circ\quad -93.5^\circ}\ (c\ 1.00).$$

(1S,2S,3R,4R,7R,8R,11R)-3-*Hydroxymethyl*-3,7,11-*trimethyl*-*tricyclo*[6.3.0.0^{2,4}]*undecan*-7-*ol* (14-*endohydroxyglobulol*) (**19**). R_f 0.27. MS *m/z*: 238.1936 (238.1933 calc. $C_{15}H_{26}O_2$, $[M]^+$ 1%), 220 (11), 205 (8), 202 (5), 138 (25), 107 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}\quad 365\text{ nm}}{-34.2^\circ\quad -35.6^\circ\quad -40.0^\circ\quad -66.2^\circ\quad -100.8^\circ}\ (c\ 0.50).$$

(1S,2S,3S,4R,6S,7S,8R,11R)-3-*Hydroxymethyl*-3,7,11-*trimethyltricyclo*[6.3.0.0^{2,4}]*undecane*-6,7-*diol* (6,14-*exo*-*dihydroxyglobulol*) (**20**). R_f 0.05. Mp 164°. MS *m/z*: 239 ($[M-Me]^+$ 1%), 236.1777 (236.1776 calc. $C_{15}H_{24}O_2$, $[M-H_2O]^+$ 2), 218 (4), 205 (3), 175 (9), 154 (74), 70 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}\quad 365\text{ nm}}{-37.5^\circ\quad -38.7^\circ\quad -44.3^\circ\quad -73.2^\circ\quad -111.5^\circ}\ (c\ 0.75).$$

(b) Biotransformation of globulol (**17**) (400 mg) with *Bacillus megaterium* DSM 32 resulted, after 28 hr, in **18** (60 mg), **19** (2 mg), **20** (31 mg), **21** (8 mg), **22** (4 mg) and **23** (6 mg).

(1S,2S,4R,6S,7S,8R,11R)-3,3,7,11-*Tetramethyltricyclo*-[6.3.0.0^{2,4}]*undecane*-6,7-*diol* (6-*hydroxyglobulol*) (**21**). R_f 0.34. IR ν_{max} cm^{-1}: 3480. MS *m/z*: 238.1934 ($[M]^+$ 238.1933 calc. for $C_{15}H_{26}O_2$) (7%), 220 (11), 177 (15), 86 (81), 84 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\quad 578\text{ nm}\quad 546\text{ nm}\quad 436\text{ nm}}{-36.1^\circ\quad -37.7^\circ\quad -42.7^\circ\quad -71.2^\circ}\ (c\ 1.00).$$

(1S,2S,4R,7R,8S,9S,11R)-3,3,7,11-*Tetramethyltricyclo*-[6.3.0.0^{2,4}]*undecane*-7,9-*diol* (9-*hydroxyglobulol*) (**22**). R_f 0.28. MS *m/z*: 238.1934 (238.1933 calc. $C_{15}H_{26}O_2$, $[M]^+$ 6%), 220 (12), 202 (11), 177 (26), 81 (80), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\ \ 578\text{ nm}\ \ 546\text{ nm}\ \ 436\text{ nm}\ \ 365\text{ nm}}{-31.0^\circ\ \ -32.3^\circ\ \ -36.4^\circ\ \ -59.8^\circ\ \ -88.4^\circ}\ (c\,0.50).$$

(1S,2S,3S,4R,7R,8S,9S,11R)-3-*Hydroxymethyl*-3,7,11-*trimethyltricyclo*[6.3.0.0^{2,4}]*undecane*-7,9-*diol* (9,14-*exo*-*dihydroxyglobulol*) (**23**). R_f 0.11. MS *m/z*: 254.1879 ($[M]^+$ 254.1882 calc. for $C_{15}H_{26}O_3$) (3%), 239 (4), 236 (5), 218 (15), 161 (16), 55 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\ \ 578\text{ nm}\ \ 546\text{ nm}\ \ 436\text{ nm}\ \ 365\text{ nm}}{-38.6^\circ\ \ -40.4^\circ\ \ -45.8^\circ\ \ -76.0^\circ\ \ -115.0^\circ}\ (c\,0.50).$$

(c) Biotransformation of globulol (**17**) (400 mg) with *Mycobacterium smegmatis* DSM 43061 yielded, after 96 hr, unused **17** (31 mg) and **24** (210 mg).

(1S,2S,3S,4R,7R,8R,11R)-7-*Hydroxy*-3,7,11-*trimethyltricyclo*[6.3.0.0^{2,4}]*undecane*-3-*carboxylic acid* (**24**). Crystals, mp 205°, R_f 0.26. MS *m/z*: 252.1725 ($[M]^+$ 252.1725 calc. for $C_{15}H_{24}O_3$) (8%), 234 (61), 219 (49), 207 (40), 206 (75), 81 (100).

$$[\alpha]^{27}\ \frac{589\text{ nm}\ \ 578\text{ nm}\ \ 546\text{ nm}}{-22.1^\circ\ \ -22.7^\circ\ \ -25.8^\circ}\ (c\,1.00).$$

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