# Phylogeny and Biotransformation. Part 5*: Biotransformation of Isopinocampheol

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*Dedicated to Professor Fritz Wagner on the occasion of his 65th birthday*

Biotransformation, Phylogeny, Isopinocampheol, Enantioselectivity, Microbial Hydroxylation

Biotransformation of isopinocampheol with 100 bacterial and fungal strains yielded 1-, 2-, 4-, 5-, 7-, 8- and 9-hydroxy-isopinocampheol besides three rearranged monoterpenes, one of them bearing the novel isocarane skeleton. A pronounced enantioselectivity between (+)- and (–)-isopinocampheol was observed. The phylogenetic position of the individual strains could be seen in their ability to form the products from (+)-isopinocampheol. The formation of 1,3-dihydroxypinane is a domain of bacteria, while 3,5- or 3,7-dihydroxypinane was mainly formed by fungi, especially those of the phylum Zygomycotina. The activity of Basidiomycotina towards oxidation of isopinocampheol was rather low. Such informations can be used in a more effective selection of strains for screening.

## Introduction

In the course of our investigation of the biotransformation capabilities of different groups of microorganisms we tested the monoterpene isopinocampheol. We were interested in the different products formed by microbial cooxidation and the activity of the different phyla towards this substrate. Isopinocampheol was chosen because of the importance of pinane monoterpenes as biologically active compounds or their synthons. Our experiences with other substrates told us that terpene hydrocarbons are rather poor substrates probably because of their low polarity (Abraham and Arfmann, 1992). Using oxygenated compounds usually leads to a faster reaction and to higher yields. Pinene gives almost no products under our screening conditions so we used isopinocampheol which is readily accessible in both enantiomeric forms. The aim of this study was to find the hydroxylation sites at this molecule, to compare the enantioselectivity of the strains and to get synthons for further synthetic studies.

## Results

Monoterpene alcohols like menthol, geraniol or nerol are only slowly attacked by microorganisms while their esters react much better (Madyastha and Renganathan, 1983). Led by this finding we used isopinocampheol-benzoate for screening of 100 strains (Abraham, 1992). Surprisingly the results were rather disappointing and we switched to

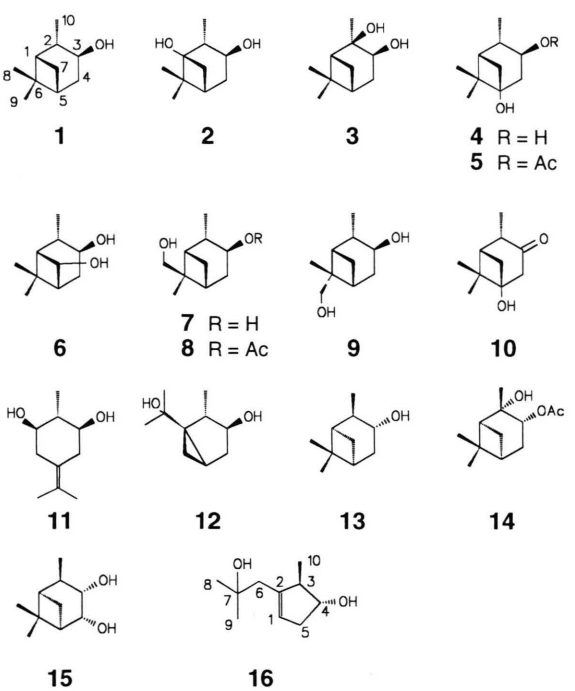


Fig. 1. Isopinocampheol and its biotransformation products.

* For part 4: see Abraham, 1994b.

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Table I. $^1$H NMR data of **1, 2, 4–9** ($CDCl_3$, 400 MHz).

| | **1** | **2** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|
| 1-H | 1.78 ddd | – | 1.59 dd | 1.71 dd | 2.01 ddd | 1.92 ddd | 1.95 ddd | 1.92 ddd |
| 2-H | 1.92 m | 2.05 qd | 1.68 m | 1.97 ddq | 2.14 m | 1.98 ddq | 2.16 m | 1.98 ddq |
| 3-H | 4.04 ddd | 4.12 ddd | 3.99 ddd | 5.08 ddd | 3.78 ddd | 4.07 ddd | 5.07 ddd | 4.02 ddd |
| 4α-H | 2.49 dddd | 2.39 dddd | 2.32 ddd | 2.51 ddd | 2.49 dddd | 2.49 dddd | 2.58 dddd | 2.51 dddd |
| 4β-H | 1.69 ddd | 1.58 ddd | 1.68 m | 1.75 dd | 1.79 ddd | 1.77 ddd | 1.72 ddd | 1.76 ddd |
| 5-H | 1.92 m | 1.79 ddd | – | – | 2.14 m | 2.08 ddd | 2.08 m | 2.09 ddd |
| 7-H | 1.02 d | 1.50 d | 1.42 d | 1.55 d | – | 1.10 d | 1.13 d | 1.11 d |
| 7′-H | 2.34 dddd | 2.24 ddd | 2.22 ddd | 2.29 ddd | 4.40 dd | 2.32 dddd | 2.33 ddd | 2.38 dddd |
| 8-H | 1.20 s | 1.12 s | 1.06 s | 1.16 s | 0.94 s | 3.69 ABq | 3.73 ABq | 1.32 s |
| 9-H | 0.90 s | 0.92 s | 0.86 s | 1.00 s | 1.14 s | 1.00 s | 1.05 s | 3.52 d |
| 9′-H | | | | | | | | 3.46 d |
| 10-H | 1.11 d | 1.16 d | 1.04 d | 1.11 d | 1.18 d | 1.14 d | 1.11 d | 1.11 d |

*J* (Hz): **1:** 1,2 = 2.0; 1,5 = 5.8; 1,7-exo = 5.8; 2,3 = 4.4; 2,10 = 7.3; 3,4β = 4.5; 3,4α = 8.8; 4α,4β = 14.0; 4β,5 = 2.4; 4α,5 = 3.4; 4α,7-exo = 2.3; 5,7-exo = 6; 7,7′ = 9.7. **2:** 2,3 = 5; 2,10 = 7.5; 3,4β = 4.5; 3,4α = 9.2; 4,4′ = 13; 4α,5 = 3; 4α,7′ = 2; 4β,5 = 3; 4β,8 > 0; 5,7′ = 7.5; 7,7′ = 9.5. **4:** 1,2 = 2; 2,3 = 5.5; 2,10 = 7; 3,4α = 9.5; 3,4β = 5.5; 4α,4β = 13; 4α,7′ = 3; 7,7′ = 9.5. **5:** 1,2 = 2; 1,7′ = 6; 2,3 = 4.5; 2,10 = 7; 3,4α = 9; 3,4β = 5; 4α,4β = 14; 4α,7′ = 2; 7,7′ = 10. **6:** 1,2 = 2.0; 1,5 = 6; 1,7′ = 6; 2,3 = 3.5; 2,10 = 7; 3,4β = 3.5; 3,4α = 9; 4,4 = 15; 4β,5 = 3; 4α,5 = 2; 4α,7′ > 0; 5,7′ = 6. **7, 9:** 1,2 = 2; 1,5 = 6; 1,7′ = 6; 2,3 = 5; 2,10 = 7; 3,4β = 5; 3,4α = 10; 4α,4β = 14; 4β,5 = 3; 4α,5 = 3; 4α,7′ = 2; 5,7′ = 6; 7,7′ = 10. **8:** 1,2 = 2; 1,5 = 6; 1,7′ = 6; 2,3 = 4; 2,10 = 7; 3,4β = 5; 3,4α = 9; 4α,4β = 14; 4β,5 = 2; 4α,5 = 3; 4α,7′ = 2; 5,7′ = 6; 7,7′ = 10. **9:** 9,9′ = 10.

Table I (continued): $^1$H NMR data of **10–12, 15** and **16** ($CDCl_3$, 400 MHz).

| | **10** | **11** | **12** | **15** | **16** |
|---|---|---|---|---|---|
| 1-H | 1.96 dd | 1.42 dq | – | 1.83 m | 5.39 s br |
| 2-H | 2.30 qd | 3.11 ddd | 1.97 q br | 1.83 m | – |
| 3-H | – | 1.84 dd | 3.94 dd | 3.95 m | 2.58 m |
| 3′-H | – | 2.67 dd | – | – | – |
| 4α-H | 2.65 m | – | 2.29 ddd | 3.95 m | – |
| 4β-H | 2.65 m | – | 1.63 d br | – | 4.00 ddd |
| 5-H | – | 2.67 dd | 0.77 ddd | 2.06 ddd | 2.68 dd br |
| 5′-H | | 1.84 dd | | | 2.22 d br |
| 6-H | – | 3.11 ddd | – | – | 2.23 s |
| 7-H | 1.63 d | 1.04 d | 1.06 dd | 0.85 d | – |
| 7′-H | 2.53 dddd | | 0.86 dd | 2.37 ddd | – |
| 8-H | 0.87 s | – | 1.25 s | 1.08 s | 1.24 s |
| 9-H | 1.26 s | 1.62 s | 1.13 s | 1.27 s | 1.24 s |
| 10-H | 1.20 d | 1.62 s | 0.93 d | 1.18 d | 1.01 d |

*J* (Hz): **10:** 1,2 = 2; 1,7′ = 6; 2,10 = 7; 4,7′ = 3; 7,7′ = 10. **11:** 1,2 = 2,3′ = 10; 1,7 = 7; 2,3 = 4; 3,3′ = 13. **12:** 2,10 = 7.5; 3,4β < 1; 3,4α = 7; 4,4 = 13.5; 4α,5 = 2; 5,7 = 8.5; 5,7′ = 4.5; 7,7 = 4.5. **15:** 1,5 = 6; 2,10 = 7; 4,5 = 2; 5,7′ = 6; 7,7′ = 10. **16:** 2,10 = 7; 2,3 = 3; 3,4 = 6; 3,4′ = 3; 4,4′ = 17.

the alcohol **1** (Fig. 1). Here far better yields were obtained and 14 compounds could be characterized. Main compound of most biotransformations was the 5-hydroxy-isopinocampheol **4.** Here yields up to 50% could be found without any attempt of optimization. The structure elucidation of this compound and the others pinanes could be done mainly by using $^1$H and $^{13}$C NMR after assignment of the signals of **1.** From the 2D $^1$H-detected long-range $^{13}$C–$^1$H shift correlated NMR spectrum (HMBC) (Summers *et al.*, 1986) all carbons of **1** could be assigned (Table I). Because a long-range coupling between 4β-H and 8-H ($\delta_H$ 1.20) could clearly be seen in the COSY-45 $^1$H{$^1$H} NMR requiring dihedral angles close to 180°, the resonance at $\delta_C$ 27.7 could be assigned to C-8 from the HETCOR NMR, agreeing completely with the assignment of isopinocampheol (Coxon *et al.*, 1984). 1-Hydroxy-isopinocampheol **2** was also one of the common products. Again $^{13}$C

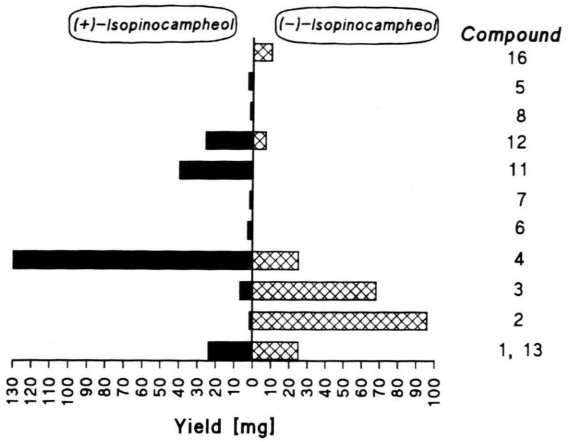


Fig. 2. Biotransformation of (+)- and (−)-isopinocampheol (400 mg) with *Mortierella isabellina* DSM 63355.

NMR was useful in the location of the hydroxylation site (Table II). Further 2-hydroxy-isopinocampheol **3** was isolated, which is a known compound from the oxidation of α-pinene. The *cis*-orientation of the diol was proved by the formation of the acetonide. Less often and in lower amount the 7-hydroxy-isopinocampheol **6** was formed. Its configuration at C-7 was deduced from the couplings of 7-H to 1-H and 5-H and the $^4J$-coupling to 4α-H requiring a dihedral angle close to 180°. This compound may have been found in the reduction of chrysanthenone with diborane already, but its stereochemistry was not given (Chretien-Bessiere, 1964).

Hydroxylation at the geminal methyl groups was also observed leading to compounds **7** and **9.** The $^{13}$C NMR of **7** proved to be very useful in the decision which of these two compounds is hydroxylated at C-9. Two methyl carbons could be seen, one of them at $\delta_C$ 20.4 belonging to the unaffected C-10 and the other one at $\delta_C$ 18.4. In caryophyllene derivatives it was found that the remaining methyl group at a geminal dimethyl moiety in a cyclobutane ring is shielded in the $^{13}$C NMR by about 5 ppm if the adjacent methyl group is hydroxylated (Abraham *et al.*, 1990). Using this empirical rule it could be deduced that C-8 is hydroxylated, so C-9 is shifted from $\delta_C$ 23.7 to 18.4, which is pretty good in the expected range. To confirm the assignment a DNOE was performed in the $^1$H NMR with **9** resulting in an enhancement of 7-H after irradiation at H-8 ($\delta_H$ 1.32). The assignments were further corroborated by the shifts of H-8 or H-9 methyl groups in the $^1$H NMR. In caryophyllene derivatives the remaining methyl group was deshielded by about $\Delta\delta_H$ 0.08 after hydroxylation of the adjacent one. H-9 is shifted from $\delta_H$ 0.90 (**1**) to $\delta_H$ 1.00 and H-8 is shifted from $\delta_H$ 1.20 (**1**) to $\delta_H$ 1.32 (**9**), in perfect agreement with the prediction.

Only with bacteria the metabolite **10** is formed although it always appeared only in low amount. With (+)-isopinocampheol **1** often two rearrangement products were observed. One proved to be the achiral menthene-diol **11.** Its configuration followed from the fact that in the $^1$H NMR only six signals were observed while the $^{13}$C NMR displayed only 7 resonances requiring a $C_s$-symmetry of this molecule. It is probably formed from **2** by formation of the cation at C-1 followed by the cleavage of the C(1)–C(6) bond. The other rearrangement product is more complicated. Its composition determined by HR-MS and the $^{13}$C

Table II. $^{13}$C NMR data of **1–4, 6, 7, 11, 12, 15** and **16** ($CDCl_3$, 75.5 MHz).

| | **1** | **2** | **3** | **4** | **6** | **7** | **11** | **12** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 47.9 +[a] | n.d. | 54.1 + | 45.6 + | 52.6 + | 43.5 + | 46.5 + | 38.0 0 | 48.3 + | 40.3 − |
| C-2 | 47.8 + | 52.3 + | 73.7 0 | 42.0 + | 40.9 + | 47.1 + | 73.4 + | 44.8 + | 44.4 + | 52.0 + |
| C-3 | 71.7 + | 73.2 + | 69.3 + | 72.4 + | 69.8 + | 71.4 + | 37.5 − | 79.7 + | 80.3 + | 79.5 + |
| C-4 | 39.1 − | 37.8 − | 38.2 − | 44.8 − | 33.6 − | 38.3 − | 126.0 0 | 36.9 − | 82.0 + | 42.3 − |
| C-5 | 41.8 + | 35.8 + | 40.6 + | 75.4 0 | 47.0 + | 37.3 + | 37.5 − | 27.8 + | 48.9 + | 124.5 + |
| C-6 | 38.2 0 | 43.0 0 | 39.6 0 | 42.9 0 | 32.3 0 | 43.7 0 | 73.4 + | 70.4 0 | 38.1 0 | 70.9 0 |
| C-7 | 34.4 − | 41.8 − | 28.0 − | 42.0 − | 67.6 + | 34.2 − | 14.2 + | 14.5 − | 32.0 − | 143.9 0 |
| C-8 | 27.7 + | 22.6 + | 27.8 + | 22.6 + | 27.3 + | 69.5 − | 123.5 0 | 27.3 + | 28.5 + | 29.5 + |
| C-9 | 23.7 + | 21.6 + | 24.1 + | 20.8 + | 23.3 + | 18.4 + | 20.0 + | 27.4 + | 24.4 + | 30.0 + |
| C-10 | 20.8 + | 15.0 + | 29.5 + | 20.3 + | 20.1 + | 20.4 + | 20.0 + | 20.0 + | 19.7 + | 16.3 + |

[a] Amplitude of signals in DEPT-135 spectrum ($CH_3$ or CH = +; $CH_2$ = −; quat. C = 0).

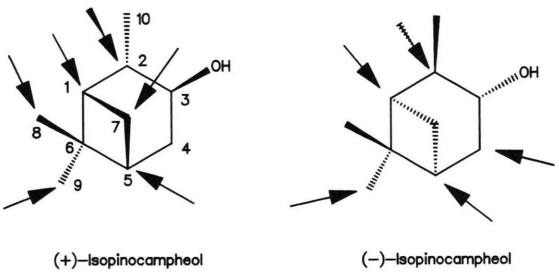


Fig. 3. Hydroxylation sites at (+)- and (–)-isopinocampheol.

NMR spectrum pointed to a bicyclic diol. The $^1H$ NMR spectrum showed three protons resonating at high field which is characteristic for a cyclopropane moiety. Extensive $^1H$ NMR studies, especially two-dimensional techniques led to structure **12.** The relative stereochemistry was deduced in the following way: From the COSY-45 the resonance at $\delta_H$ 0.77 was assigned to 5-H because of its couplings to the methylene of the cyclopropane and to one of the protons at C-4 ($\delta_H$ 2.29). 3-H coupled with the same proton with $^3J = 2$ Hz which must be the α-proton in syn-configuration while 4β-H showed a coupling constant smaller than 1 Hz. The 4α-H coupled with 5-H with 2 Hz, while 4β-H displayed a coupling to 5-H which is smaller than 1 Hz. Inspection of the Dreiding model revealed that such a coupling pattern requires a β-position of the cyclopropane, *i.e.* the α-position of 5-H. Here the dihedral angle between 4β-H and 5-H is almost 90° resulting in the observed small coupling between these protons. Compound **12** has the novel isocarane skeleton and is probably also formed from **2** *via* the carbocation at C-2 and rearrangement of the C(5)–C(6)-bond to C(5)–C(1). This reaction path is in agreement with the observed configuration of **12.**

The question whether these rearranged products were formed enzymatically or chemically in the medium could not be solved completely. Different ratios of **11** and **12** in different biotransformations (Fig. 10) and different amounts of **11** or **12** in the fermentation of (+)-isopinocampheol **1** and (–)-isopinocampheol **13** (Fig. 2) are not in agreement with a chemical formation, so the enzymatical pathway is more likely.

To test the enantioselectivity of the strains used some fermentations were also performed with (–)-isopinocampheol **13.** Interestingly almost the same products were isolated although their individual amounts were different from the fermentations with **1** (Table III). However with **13** as the substrate some new products could be detected which were not observed with **1.** One of them is the acetate **14** only formed from *ent*-**3.** *Botryosphaeria rhodina* (= *Diplodia gossypina*) ATCC 10936 formed another hydroxylation product not observed with **1.** The mass spectrum revealed a composition of $C_{10}H_{18}O_2$ pointing to a diol and the $^{13}C$ NMR displayed only one triplet requiring

Table III. Yields in the biotransformation of isopinocampheol with different strains.

| Strain (fermentation time) | Biotransformation of (+)-isopinocampheol **1** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| *Rhizopus arrhizus* ATCC 11145 (45 h) | 8 | | | 157 | | | | | | | 57 | 52 |
| *Bacillus sphaericus* ATCC 13805 (72 h) | 37 | | | 181 | | | | | 3 | 2 | | 53 |
| *Bacillus megaterium* DSM 32 (210 h) | 219 | | | 35 | | | | | | | 8 | |
| *Botryosphaeria rhodina* ATCC 10936 (72 h) | 128 | 36 | | 104 | | 10 | | | 5 | | 5 | 27 |
| *Mortierella isabellina* DSM 63355 (45 h) | 24 | 2 | 7 | 130 | 3 | 5 | 2 | 2 | | | 40 | 26 |
| *Nocardia* sp. DSM 43130 (138 h) | 60 | 2 | 70 | 72 | | 20 | 7 | | 3 | | | 10 |

| | Biotransformation of (–)-isopinocampheol **13** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *ent*-**2** | *ent*-**3** | *ent*-**4** | *ent*-**5** | *ent*-**6** | *ent*-**7** | *ent*-**8** | *ent*-**9** | *ent*-**10** | *ent*-**11** | *ent*-**12** | **13** | **14** | **15** | **16** |
| *Botryosphaeria rhodina* ATCC 10936 (163 h) | 20 | 15 | 80 | | | | | 9 | | | 17 | 71 | 3 | 16 | |
| *Mortierella isabellina* DSM 63355 (68 h) | 96 | 68 | 25 | | | | | | | | 7 | 25 | | | 10 |
| *Nocardia* sp. DSM 43130 (165 h) | | | 50 | | | | | | | | 7 | 160 | | | |

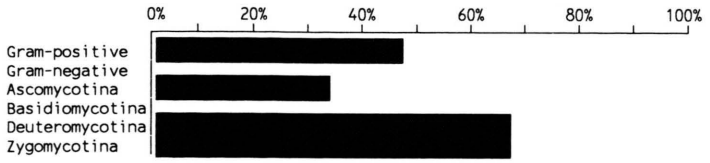


Fig. 4. Frequency of saponification of (1*S*)-isopinocampheol-benzoate.

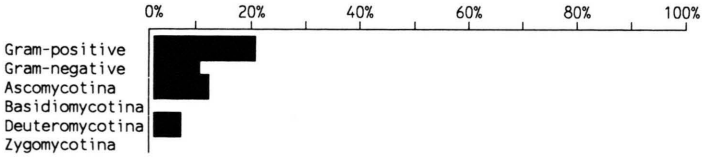


Fig. 5. Frequency of formation of 1-hydroxy-isopinocampheol **2.**

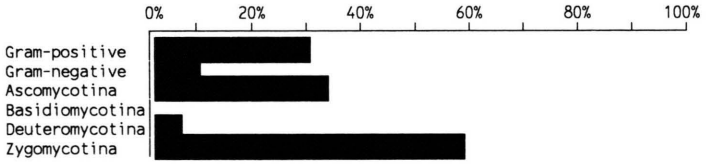


Fig. 6 Frequency of formation of 2-hydroxy-isopinocampheol **3.**

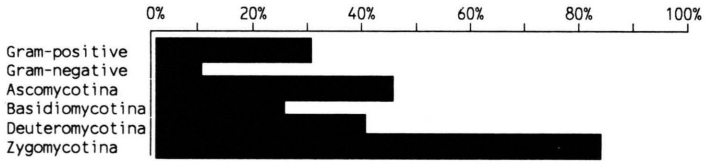


Fig. 7. Frequency of formation of 5-hydroxy-isopinocampheol **4.**

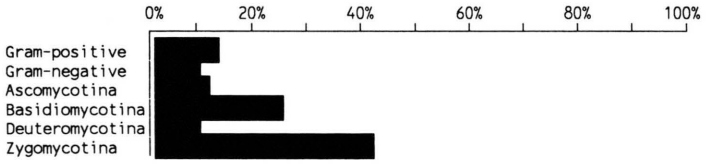


Fig. 8. Frequency of formation of 7-hydroxy-isopinocampheol **6.**

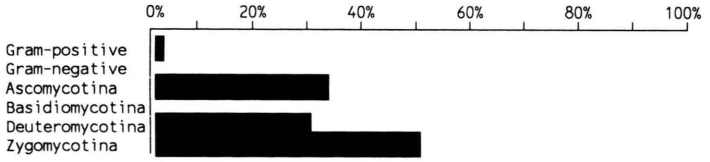


Fig. 9. Frequency of the rearrangement to 4(8)-menthene-2,6-diol **11.**

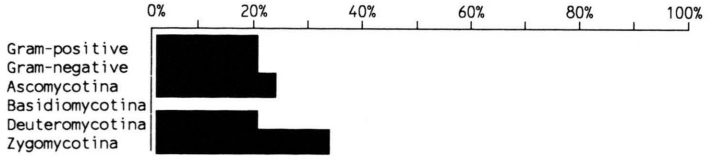


Fig. 10. Frequency of the rearrangement to **12.**

the hydroxylation at C-4 or C-7. C-2 and C-7 is shielded compared to **13,** while C-5 and C-3 are deshielded. This is only possible in 4-hydroxy-isopinocampheol **15.** The relative configuration at C-4 is determined from the fact that no long-range coupling between 4-H and 7-H is observed. This means that 4α-H is missing, demanding the structure of a *cis*-diol. Until now eight different 3,4-dihydroxy-pinanes are known, but (1*R*,2*R*,3*S*,4*R*,5*R*)-pinane-3,4-diol (**15**) is new (Uzarewicz and Segiet-Kujawa, 1978).

In the fermentation broth of **13** with *Mortierella isabellina* DSM 63355 another rearrangement product was isolated. Its composition is $C_{10}H_{18}O_2$ as determined by HR-MS. It contains a trisubstituted double bond as judged from the $^{13}C$ NMR so it is monocyclic. $^1H$ NMR spectra, especially COSY-45, was used to identify the compound as a (2′-hydroxy-2′-methyl-propyl)-3-methyl-cyclopentene-4-ol. From the data it could not be decided whether the hydroxy-isopropyl moiety is attached at C-1 or at C-2. Irradiation at $\delta_H$ 5.39 led to a NOE at 1-H and 4-H indicating that the large side chain is attached at C-2 so the structure is **16.** The formation of **16** from isopinocampheol obviously needs a number of complex rearrangements.

The pattern of activity of the different groups of microorganisms towards the oxidation of isopinocampheol is worthwhile for a closer look. In the saponification of the benzoate we find a rather strong activity of the Gram-positive bacteria. This activity is comparable to that one of the Deuteromycotina and Zygomycotina while the Basidiomycotina tested did not show this reaction at all (Fig. 4). The hydroxylation of **1** at C-1 is mostly done by bacteria (Fig. 5), but bacteria are not as active as fungi in the hydroxylation at C-2, C-5 and C-7 (Fig. 6–8). The rearrangement of **1** to **11** is also a domain of fungi (Fig. 9), while the rearrangement to **12** is done by strains belonging to almost all groups. The only exception here and in almost all other hydroxylations of isopinocampheol are the Basidiomycotina. This is an observation however which is only true for the substrate discussed here, while other substrates like the sesquiterpenes globulol or cedrol are preferably transformed by Basidiomycotina (Abraham, 1994 a).

## Discussion

Isopinocampheol can be oxidized at almost all carbons by microorganisms. The hydroxylation sites at the two enantiomers are rather similar (Fig. 3), but significant differences in the yields between the two enantiomers were found indicating a pronounced enantioselectivity of the enzymes. A dependence of the biotransformation capability of a strain from its systematic position was observed. These preferences of certain phyla towards the formation of a metabolite can be used in a more effective screen, because for its production, one can focus to this particular phylum ignoring the others. The compounds discussed in this article form valuable intermediates for a broad diversity of compounds like enantiomeric ligands for boranes for chiral hydrogenations, synthons for flavours or bergamotanes.

## Experimental

One hundred of the most active strains (40 bacteria and 60 fungi) were selected from our strain collection. They were tested in a medium containing glucose (5 g/l), malt extract (5 g/l), peptone (2 g/l) and yeast extract (5 g/l) with isopinocampheol.

24 h after the substrate addition (0.5 g/l), samples were taken each day and analyzed as follows. To 1 ml of culture broth 0.2 ml of ethyl acetate was added, shaken for 2 min, centrifuged and 10 µl of the extract was developed on HPTLC with *n*-hexane–ethyl acetate 1:2. The spots were made visible by spraying with anisaldehyde/sulphuric acid in acetic acid and heating to 110 °C for 1 min. A video camera connected with a frame store board (SYNAPSE card) in a personal computer (1 MB RAM, 80286 processor with 80287 coprocessor) and SW 2000 software (Ultraviolet Products Gel Analysis System, Cambridge, U.K.), was used to determine the $R_f$ values of the biotransformation products and their intensity. Data management was done in a dBase file and a program was written for arrangement of the data for evaluation with the MULVA-4 program (Wildi and Orloci, 1990). Basic statistics were performed with some other statistic programs. The individual spots were identified using reference substances, unknown products were isolated and their structures elucidated.

*Extraction and purification*

Culture medium and mycelia were separated by filtration and both were extracted three times with ethyl acetate. The solvent was evaporated and the crude extract separated on Si-60 columns with a *n*-hexane/ethyl acetate gradient (changing from 9:1, v/v to 0:1, v/v). When necessary the collected fractions were further purified by preparative TLC.

*Analysis*

$^1$H NMR spectra were obtained at 400 MHz and the $^{13}$C NMR spectra at 75.5 MHz, deuterochloroform was the solvent and TMS the internal standard. Mass spectra were recorded with 70 eV. IR spectra were measured in chloroform. Melting points are uncorrected.

The yield of the individual strains with the substrate **1** or **13** is listed in Table III.

*(1 R,2 S,3 S,5 R)-Pinane-1,3-diol* (**2**): $R_f$ 0.40. MS *(m/z):* 170 (1%), 152.1202 (152.1201 calcd for $C_{10}H_{16}O$) ($M^+-H_2O$) (31), 137 (12), 114 (26), 100 (67), 70 (97), 43 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|---|
| | +19.9° | +20.6° | +22.9° | +33.9° | +46.3° | |

*(1 R,2 R,3 S,5 R)-Pinane-2,3-diol* (**3**): Acetonide of **3:** $R_f$ 0.75 (*n*-hexane/ethyl acetate 8:2). $^1$H NMR: 0.83 (s, 8-H), 1.28 (s, 9-H), 1.40 (s, 11-H), 1.47 (s, 10-H), 1.48 (s, 12-H), 1.63 (d, $J$ = 10 Hz, 7-H), 1.92 (m, 4β-, 5-H), 2.13 (m, 1-, 4α-, 7′-H), 4.18 (d, $J$ = 8 Hz, 3-H).

*(1 S,2 S,3 S,5 S)-Pinane-3,5-diol* (**4**): $R_f$ 0.26. MS *(m/z):* 170.1090 (170.1311 calcd for $C_{10}H_{18}O_2$) (10%), 152 (18), 137 (14), 84 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|---|
| | +9.0° | +9.5° | +10.5° | +15.7° | +21.7° | |

*(1 S,2 S,3 S,5 S)-3-Acetoxy-pinane-5-ol* (**5**): $R_f$ 0.80. MS *(m/z):* 152 (22) ($M^+$–AcOH), 137 (14), 109 (24), 96 (26), 70 (41), 43 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 0.33). |
|---|---|---|---|---|---|---|
| | +20.7° | +23.4° | +26.7° | +46.5° | +48.9° | |

*(1 S,2 S,3 S,5 R,7 R)-Pinane-3,7-diol* (**6**): $R_f$ 0.57. MS *(m/z):* 170.1307 (170.1307 calcd for $C_{10}H_{18}O_2$) (0.5%), 155 (5), 152 (3), 84 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|---|
| | +11.1° | +11.7° | +13.0° | +19.2° | +25.1° | |

*(1 R,2 S,3 S,5 R,6 S)-Pinane-3,8-diol* (**7**): $R_f$ 0.59. MS *(m/z):* 170 (0.2%), 152.1201 (152.1201 calcd for $C_{10}H_{16}O$) (3), 139 (2), 137 (5), 95 (31), 82 (56), 68 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | ($c$ = 0.33). |
|---|---|---|---|---|---|
| | +12.6° | +14.4° | +18.6° | +35.4° | |

*(1 R,2 S,3 S,5 R,6 R)-8-Acetoxy-pinane-3-ol* (**8**): $R_f$ 0.78. MS *(m/z):* 170 (0.4%) ($M^+-C_2H_2O$), 152 (2), 121 (12), 82 (18), 67 (16), 43 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 0.33). |
|---|---|---|---|---|---|---|
| | +5.1° | +5.4° | +6.6° | +9.0° | +13.2° | |

*(1 R,2 S,3 S,5 R,6 R)-Pinane-3,9-diol* (**9**): $R_f$ 0.55. MS *(m/z):* 152.1201 (152.1201 calcd for $C_{10}H_{16}O$) ($M^+-H_2O$) (4%), 121 (10), 109 (15), 95 (33), 82 (61), 68 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|---|
| | +36.1° | +37.8° | +42.5° | +69.5° | +105.8° | |

Optical rotation in chloroform–methanol 9:1.

*(1 R,2 S,5 S)-5-Hydroxy-pinane-3-one* (**10**): $R_f$ 0.75 (*n*-hexane/ethyl acetate 1:3). IR $\nu_{max}$ cm$^{-1}$: 4215, 1713, 1603, 1304. MS *(m/z):* 168.1143 (168.1150 calcd for $C_{10}H_{16}O_2$) (3%), 153 (2), 126 (15), 125 (16), 83 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | ($c$ = 0.25). |
|---|---|---|---|---|
| | +2.4° | +2.8° | +3.2° | |

*Trans,trans-4(8)-Menthene-2,6-diol* (**11**): $R_f$ 0.44. M.p. 157 °C. MS *(m/z):* 170 (3%), 152 (24), 137 (15), 109 (23), 99 (100).

*(1 S,2 S,3 S)-1-(1′-Hydroxy-1′-methyl-propyl)-2-methylbicyclo[3.1.0]hexane-3-ol* (**12**): $R_f$ 0.53. M.p. 89–90 °C. MS *(m/z):* 170.1315 (170.1307 calcd for $C_{10}H_{18}O_2$) (0.3%), 155 (7), 152 (10), 137 (37), 109 (45), 94 (93), 59 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|---|
| | +2.6° | +2.7° | +3.0° | +3.7° | +5.3° | |

*(1 R,2 R,3 S,4 R,5 R)-Pinane-3,4-diol* (**15**): $R_f$ 0.45 (ethyl acetate). MS *(m/z):* 170.1312 (170.1307 calcd for $C_{10}H_{18}O_2$) (3%), 155 (3), 152 (13), 137 (4), 121 (21), 109 (36), 95 (59), 85 (100).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | 365 nm | ($c$ = 1.00, $CH_3OH$). |
|---|---|---|---|---|---|---|
| | +3.2° | +3.3° | +3.3° | +3.8° | +5.3° | |

*(3 R,4 R)-2-(2′-Hydroxy-2′-methyl-propyl)-3-methyl-cyclopentene-4-ol* (**16**): $R_f$ 0.33 (dichloromethane/methanol 9:1). MS *(m/z):* 152 (1%) ($M^+-H_2O$), 137 (4), 94 (68), 79 (100), 59 (73).

| $[\alpha]^{27}$ = | 589 nm | 578 nm | 546 nm | 436 nm | ($c$ = 1.00). |
|---|---|---|---|---|---|
| | −40.3° | −41.9° | −48.8° | −84.5° | |

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