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Lactones 1. Hydroxylation of Dihydro-β-campholenolactone by *Fusarium culmorum*

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Abstract: The stereospecific hydroxylation of racemic dihydro-β-campholenolactone (**1**) by several fungal strains has been evaluated. The 6-hydroxy derivative as a major product and 5-hydroxy as a minor one were isolated from transformation of **1** with *Fusarium culmorum*.

INTRODUCTION

Compounds with the lactone moiety are widely occurring natural products[1,2]. They usually exhibit specific biological activity, for example: allergenic, cytostatic or antimicrobial[3,4]. The lactone ring is also frequently present in natural compounds which exhibit feeding deterrent properties against insects[5-8]. Antifeedants with the lactone moiety isolated from natural sources usually possess one or more additional functional groups, mainly hydroxy or carbonyl.

Due to our interest in synthesis of terpene and sesquiterpene lactones as structural analogues of antifeedants occurring in plants we have been investigating methods for additional functionalisation of lactones. Chemical methods were found to be inefficient and not sufficiently stereospecific. On the other hand, we have noticed, in literature examples, biohydroxylations of ketones[9-12], hydrocarbons[13], alcohols[14,15], esters[15], lactones[14,16,17] and other groups[18] carried out by fungi as well as by plants.

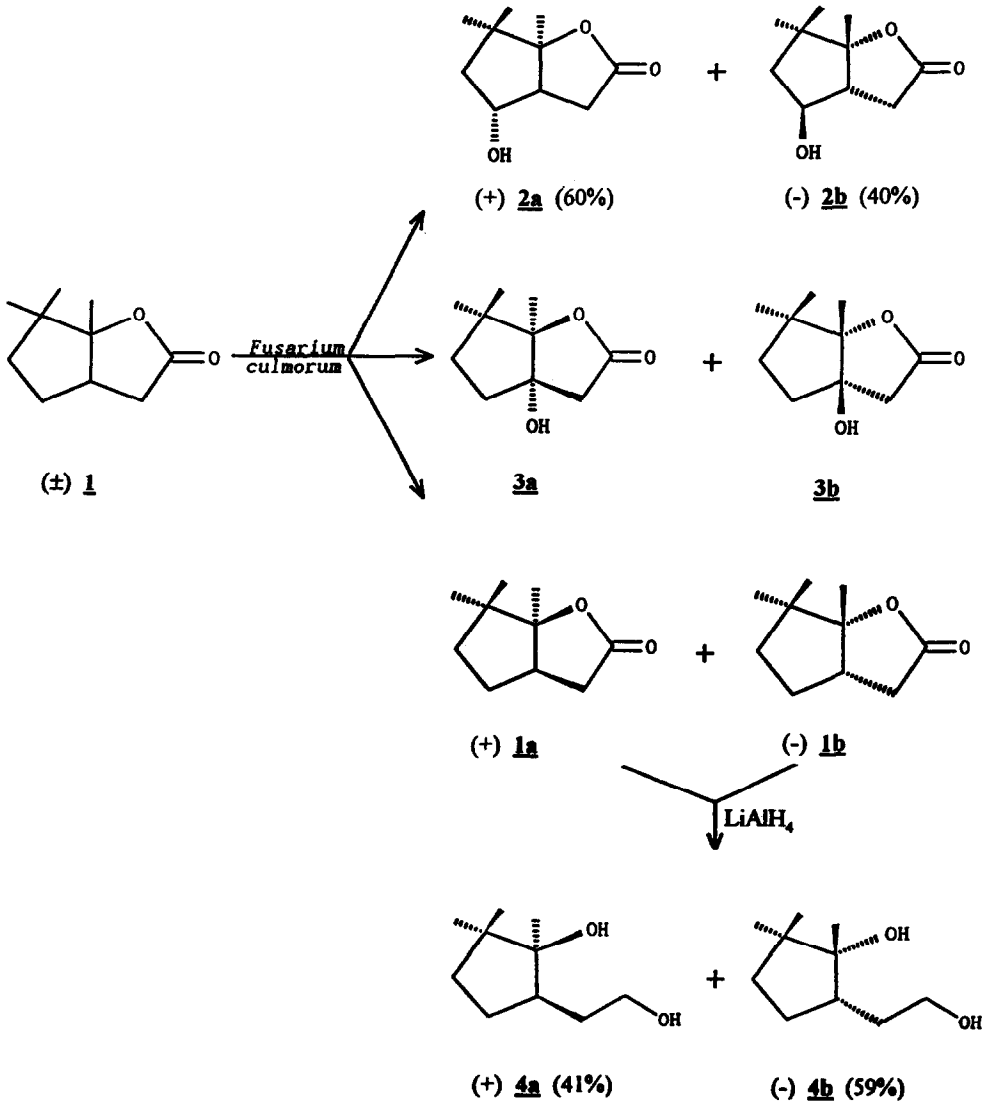
Having several fungi species at our disposal, we have tried to apply them to the functionalization of selected terpene lactones. Here we present the results of the biohydroxylation of dihydro-β-campholenolactone (**1**).

The racemic form of this lactone was obtained as a product of the reaction of (±)-α-campholenic acid with sulphuric acid[19]. The (+)-enantiomer of lactone **1** was synthesized by the reaction of pure (+)-α-campholenic acid with oxalic acid[20]. Hirata *et al.*[20] estabilished the structure of this isomer as (1S, 5R) *cis* isomer **1a**.

In our studies, (±)-lactone **1** obtained from (±)-α-campholenenitrile[21] was used. By carrying out biotransformations on the racemic form of **1** we were able to check not only the ability of the fungi applied to transform this substrate but also their enantiospecificity.

RESULTS AND DISCUSSION

A preliminary screening procedure showed that only four fungi species from among the fourteen tried transformed lactone **1** (see Table in the Experimental). Three of them: *Fusarium culmorum*, *Nigrospora oryzae* and *Claviceps purpurea* transformed the substrate to a very high extent (according to GC over 80%). In the case of *Fusarium culmorum* and *Nigrospora oryzae*, the formation of two products was observed: the major one (above 85% according to GC) with R_f = 0.32 (**2**) on TLC plate (silica gel, hexane : acetone : n-propanol, 50:18:0.2) and the second one (about 1,5% according to GC) with R_f = 0.38 (**3**). They were isolated from the product mixture as a result of the preparative biotransformation of lactone **1** with *Fusarium culmorum*.



The structures of the products were established based on IR, ^{1}H NMR and ^{13}C NMR data. The IR spectrum of the compound with R_f = 0.32 exhibited the presence of a secondary hydroxy group (absorption bands at 3524, 1244 and 1072 cm^{-1}) and the γ-lactone ring (1784 cm^{-1}) in the molecule. The presence of the hydroxy group in this product was also confirmed by an intensive signal of the carbon with the hydroxy group at 79.23 ppm in the ^{13}C-NMR spectrum and by a doublet (J=6.7 Hz) for the H-6 methine proton at 4.0 ppm in the ^{1}H NMR spectrum.

The ^{1}H NMR spectrum of this product was the most informative about its structure. The shape of the signal of the methine proton H-6 indicates that it is coupled with only one proton of the methylene group CH_2-7. The coupling constant with the other proton of this group as well as with methine proton H-5 is close to 0 Hz. The analysis of the dihedral angles between vicinal C-H bonds, carried out on the Dreiding model of **2**, indicated that such small coupling constants are possible when proton H-6 is located in the α-position (*trans* to the lactone ring), as is shown in figures **2a** and **2b**. The α-position of the hydroxy group is additionally confirmed by the chemical shifts of methyl groups at C-7 (δ=1.31 ppm) and C-1 (δ=1.10 ppm) which are situated *cis* to it. Singlets of these groups are shifted downfield by 0.08 and 0.26 ppm respectively in comparison with their location in the ^{1}H NMR spectrum of the starting lactone.

The second, minor product of the biotransformation of lactone **1** (R_f = 0.38, hexane : acetone : n-propanol, 50:18:0.2) was formed in very small amount (about 2%). The spectral data obtained from IR, ^{1}H NMR and ^{13}C NMR spectroscopy showed that it is also an hydroxy lactone with the hydroxy group at C-5. The absorption bands at: 3452, 1384 and 1132 cm^{-1} confirm the presence of a tertiary hydroxy group and the band at 1784 cm^{-1} confirms the γ-lactone moiety.

The position of the singlet for the methyl group at C-1 (δ=1.08) and its downfield shift by 0.24 ppm (in comparison with the location of the singlet for this group in the ^{1}H-NMR spectrum of lactone **1**) confirms the *cis* orientation of the hydroxy group in relation to this group.

To check the enantioselectivity of this biotransformation the optical rotations of products and untransformed substrate have been measured. These measurements showed that both hydroxy lactones **2** and **3** as well as unreacted lactone **1** were optically active. Hydroxy lactone **2** was found to be dextrorotatory ($[\alpha]_{546}^{20}$ = +7.8°, EtOH, c=4.6), whereas hydroxy lactone **3** ($[\alpha]_{546}^{20}$ = –4.0°, EtOH, c=0.5) and the untransformed lactone **1** ($[\alpha]_{546}^{20}$ = –9.3° , EtOH, c=4.3) were laevorotatory.

These data suggest that the biotransformation of lactone **1** by *Fusarium culmorum* proceeded with some enantiospecificity. Moreover, the fact that the mixture of enantiomers of the untransformed lactone **1** is laevorotatory indicates that the (+)-isomer **1a** (1S, 5R) of starting lactone was transformed to a greater extent than the (–)-enantiomer **1b** (1R, 5S).

The gas chromatographic analysis with the chiral column (CP-Cyclodextrin-β-2,3,6, M-19), confirmed these suggestions and allowed determination of the enantiomeric composition of the hydroxy lactone **2** as well as of the untransformed lactone **1**. The analysis of hydroxy lactone **2** showed that this product consists of two enantiomers in the ratio of 60 to 40%. The larger contribution (60%) should be ascribed to the isomer **2a** (1S, 5S, 6R) which is formed from the (+)-isomer of the starting lactone **1a** and the minor one (40%) to isomer **2b** (1R, 5R, 6S) formed from the (–)-enantiomer of the starting lactone **1b**. The (+)-rotation sign of the mixture of **2a** and **2b** isolated from the biotransformation indicates that the enantiomer **2a** is dextrorotary whereas **2b** is laevorotatory.

In order to confirm these assignments, it was necessary to know the enantiomeric composition of the lactone which remained after biotransformation. Attempts to separate the enantiomers of **1** with the use of the CP-Cyclodextrin-β-2,3,6 column failed. Fortunately, the enantiomeric diols obtained by reduction of lactone **1** could be separated on this column. As we expected, the analysis showed that the mixture of diols consists of two enantiomers in the ratio of 59 to 41%.

The optical rotation of this mixture, ($[\alpha]_{546}^{20} = -13.20°$, EtOH, c=1.06) also showed the predominance of (–)-(1R, 5S) enantiomer (**4b**) over (+)-(1S, 5R) enantiomer (**4a**) obtained earlier in the pure form[20]. Taking into account both these facts, the larger contribution (59%) should be ascribed to the diol **4b** formed from the (–)-lactone **1b**, and the smaller one (41%) to diol **4a** formed from the (+)-lactone **1a**.

CONCLUSIONS

To sum up the results presented above, it can be pointed out that:

1. the enantiospecificity of *Fusarium culmorum* towards dihydro-β-campholenolactone (**1**) is relatively low,
2. the stereospecificity of the biohydroxylation of this substrate by this species is very high (100% according to the GC) and
3. the regiospecificity of this hydroxylation is also very high. The formation of small amounts of 5-hydroxy-lactone (**3**) may be a result of rearrangement of the intermediate radical formed in the course of the biohydroxylation[22,23].

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as internal standard on a Brüker AMX 300 spectrometer. IR spectra were recorded in CCl_4 on a Specord M-80 Carl Zeiss Jena spectrometer. Optical rotations were measured in 0,5 dm cells on a Polamat-A Carl Zeiss Jena spectropolarimeter. Analytical thin-layer chromatography (TLC) was carried out using aluminium foil coated with silica gel 60 F_{254} Merck, various developing systems were applied. The spots were made visible by spraying with a 10% solution of phosphomolybdic acid and cerium sulfate in 10% sulfuric acid and then by heating. Preparative column chromatography was carried out on silica gel 60 (230-400 mesh, Merck) with a mixture of hexane : acetone : n-propanol (50:16:0.2) as an eluent. Analytical gas chromatography was performed on a Hewlett-Packard HP 5890 A II apparatus using a HP-1 capilary column (25mm, 0.32 mm) and a Chrompack CP-cyclodextrin B-2,3,6 M-19 chiral column (25 m, 0.25 mm). Melting points (uncorrected) were determined on Boetius apparatus. (±) Dihydro-β-campholenolactone **1** (bp. 95-96 °C/1 mm Hg, lit.[19] bp. 75.5-78.5 °C/0.5 mm Hg, was obtained from (±)-α-campholenenitrile as described earlier[21].

Screening procedure:

The microorganisms were cultivated at 28° C in 250 ml Erlenmayer flasks containing 100 ml of the following medium: 3% solution of malto and 1% solution aminobak in deionized water. After 5 days of growth, 10 mg of substrate in 1 ml of acetone was added to the shaken cultures. The transformations were continued for 14 days. The products were extracted with chloroform and analyzed by TLC (eluent: hexane :

acetone : n-propanol, 50:18:0.2) and GC (capillary column). The results of GC analysis are presented in the Table.

Table. The compositions (in % according to GC) of the product mixtures obtained by incubation of lactone **1** with various fungal strains .

	Lactones		
Strains*	1	2	3
1. *Fusarium culmorum*	7.6	90.6	1.8
2. *Fusarium tricinctum*	100	-	-
3. *Fusarium equiseti*	100	-	-
4. *Fusarium oxysporum*	100	-	-
5. *Fusarium avenaceum*	95.6	4.4	-
6. *Beauveria bassiana*	100	-	-
7. *Syncephalastrum racemosum*	100	-	-
8. *Acremonium roseum*	100	-	-
9. *Nigrospora oryzae*	13.4	85.2	1.4
10. *Spricaria vidacea*	100	-	-
11. *Claviceps purpurea*	12.8	87.2	-
12. *Aphanocladium album*	100	-	-
13. *Mucor racemosus*	100	-	-
14. *Rhodotorula mucilaginosa*	100	-	-

*All strains are from local origin: Department of Phytopathology, Agricultural University, Wroclaw and Department of Biology and Botany, Medical Academy, Wroclaw.

Preparative biotransformation:

Lactone **1** (450 mg) in 10 ml of acetone was added to the culture of *Fusarium culmorum* (5 flasks with 500 ml of the medium in each) prepared as described for the screening procedure. After 14 days of shaking at 28° C the products were extracted with chloroform. The chloroform solution was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude product mixture (392 mg) was separated by column chromatography (silica gel, hexane:acetone:n-propyl alcohol, 50:16:0.2). The pure hydoxy lactones **2** (289 mg) and **3** (9 mg) and 94 mg of untransformed lactone **1** were isolated. Their physical and spectral data are given below:

6-Hydroxy-1,8,8-trimethyl-2-oxabicyclo[3.3.0]octan-3-one (mixture of enantiomers: 60% of **2a** and 40% of **2b**): $[\alpha]_{546}^{20}$ = +7.8° (EtOH, c=4.6); mp. 69.5-70.5 °C; ^{1}H NMR ($CDCl_3$, TMS), δ: 1.01 (s, 3H, CH_3-11), 1.10 (s, 3H, CH_3-9), 1.31 (s, 3H, CH_3-10), 1.56 (d, J=14.1 Hz, 1H, H_α-7), 1.96 (dd, J=14.1 Hz, J=6.7 Hz, 1H, H_β-7), 2.25 (s, 1H, OH), 2.45 (d, J=18.4 Hz, 1H, H_α-4), 2.54 (d, J=9.5 Hz, 1H, H-5), 2.88 (dd, J=18.4 Hz, J=9.5 Hz, 1H, H_β-4), 4.00 (d, J=6.7 Hz, 1H, H-6).^{13}C NMR ($CDCl_3$), δ: 19.81, 23.33, 26.44, 36.02, 45.50, 49.02, 54.80, 79.23, 100.02, 177.22. IR(cm^{-1}) : 3524 (s,b), 1784 (s), 1384 (s), 1376 (s), 1244 (s), 1072 (s).

5-Hydroxy-1,8,8-trimethyl-2-oxabicyclo[3.3.0]octan-3-one (mixture of enantiomers: **3a** and **3b**): $[\alpha]_{546}^{20}$ = –4.0° (EtOH, c=0.5), mp. 105-106 °C; ^{1}H NMR ($CDCl_3$, TMS) δ: 1.01 (s, 3H, CH_3-11), 1.08 (s, 3H, CH_3-9), 1.31 (s, 3H, CH_3-10), 1.50-1.70 (m, 2H, CH_2-7), 1.86 (s, 1H, OH), 2.00-2.20 (m, 2H, CH_2-6), 2.77 (d, J=17.7 Hz, 1H, CH_2-4), 2.86 (dd, J=17.7 Hz, J=1.5 Hz, 1H, CH_2-4).^{13}C NMR ($CDCl_3$) δ: 15.52, 23.40, 24.32, 37.46, 40.88, 45.30, 45.58, 79.63, 84.50, 190.02. IR (cm^{-1}) : 3452 (s, b), 1784 (s), 1424 (s), 1396 (s), 1384 (s), 1132 (s).

1,8,8-Trimethyl-2-oxabicyclo[3.3.0]octan-3-one (mixture of enantiomers: **1a**-41% and **1b**-59%): $[\alpha]_{546}^{20}$ = –9.3° (EtOH, c=4.3). Pure (+)-enantiomer[20] has $[\alpha]_D^{20}$ = +49.9° (EtOH, c=10). The composition of the mixture of enantiomers of lactone **1** remained after biotransformation was estabilished by the GC analysis

on a chiral column of the enantiomeric diols **4a** and **4b** obtained by the reduction of this lactone with $LiAlH_4$. The reduction was carried out in the standard manner. Starting from 30 mg of unreacted lactone **1**, the mixture of **4a** and **4b** (23,3 mg) was obtained as a solid: mp. 143-146 °C, $[\alpha]_{546}^{20} = -13.2°$ (EtOH, c=1.06). The GC analysis showed that this mixture consists of 59% of **4b** and 41% of **4a**. Pure (+)-enantiomer **4a** is characterized[20] by mp. 147-148 °C and $[\alpha]_D^{25} = +38,9°$ (EtOH, c=1.49).

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