



BIOTRANSFORMATIONS OF ACYCLIC TERPENOID, (\pm)-CIS-NEROLIDOL AND NERYLACETONE, BY PLANT PATHOGENIC FUNGUS, *GLOMERELLA CINGULATA*

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Abstract—Microbial transformations of (\pm)-cis-nerolidol and nerylacetone were investigated using the plant pathogenic fungus, *Glomerella cingulata*. Both (\pm)-cis-nerolidol and nerylacetone were mainly oxidized at the remote double bond. (\pm)-cis-Nerolidol was transformed into (Z)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol while nerylacetone was transformed into (Z)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one as the major metabolite. In addition, the biotransformation of nerylacetone resulted in hydration at the remote double bond and reduction of the carbonyl group and produced (Z)-6,10-dimethyl-5,9-undecadien-2-ol, (Z)-10-hydroxy-6,10-dimethyl-5-undecen-2-one and (Z)-6,10-dimethyl-5-undecen-2,9,10-triol. The structures of the metabolic products were determined by spectroscopic data.

INTRODUCTION

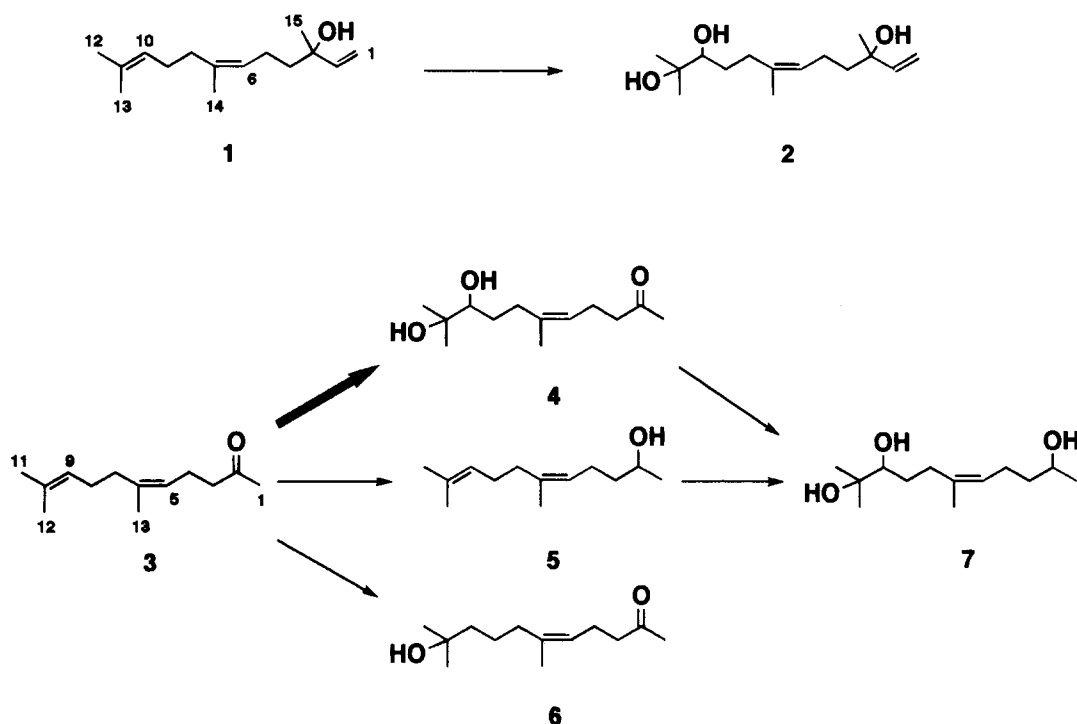
We have investigated the microbial transformation of terpenoids using a plant pathogenic fungus. In our previous papers, we reported the biotransformation of cyclic terpenoids, 1,8-cineole [1], ($-$)- α -bisabolol [2], (+)-cedrol [3], ($-$)-globulol [4] and ($-$)-nopol [5] into novel terpenoids by *Glomerella cingulata*. So far, there are no reports about the biotransformation of acyclic terpenoids using *G. cingulata*. Acyclic terpenoids are widely distributed in nature, and they are important biosynthetic precursors of cyclic terpenoids in organisms such as higher plants. If it is possible to produce new cyclic terpenoids from acyclic terpenoids by microbial transformation it may become a valuable technique for synthetic organic chemistry. Therefore, we have tested the biotransformation of two acyclic terpenoids, (\pm)-cis-nerolidol (1) and nerylacetone (3), using *G. cingulata*. There have been reports about the microbial transformation of 1 [6, 7] and 3 [6, 8–10]. Compound 1 was transformed into (Z)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol (2) and (6Z, 10E)-3,7,11-trimethyl-1,6,10-dodecatrien-3,12-diol by *Aspergillus niger* [6] and into the 12-carboxylic acid of 1 by *Rhodococcus rubropertinctus* [6]. *Streptomyces cinnamonensis* transformed 1 into small amounts of four metabolites [(Z)-10,11-epoxy-3,7,11-trimethyl-1,6-dodecadien-3,14-diol, 2; 3,7,11-trimethyl-1,10-dodecadien-3,6,7-triol and 3,6-epoxy-3,7,11-

trimethyl-1,10-dodecadien-7-ol] [7]. Compound 3 was transformed into (Z)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one (4), (Z)-6,10-dimethyl-5-undecen-2,9,10-triol (7) and (Z)-9,10-dihydroxy-6-methyl-5-undecen-2-one by *Diplodia gossypina* [8], into (Z)-9,10-epoxy-6,10-dimethyl-5-undecen-2-one, (Z)-11-hydroxy-6,10-dimethyl-5-undecen-2-one and five minor metabolites (including 4) by *Mucor circinelloides* [6], into 4 and three minor metabolites by *Fusarium solani* [9] and into 10-hydroxy-9,13-epoxynerylacetone and 9S-hydroxy-10,13-epoxynerylacetone by *Corynespora cassicola* [10]. So far, (Z)-6,10-dimethyl-5,9-undecadien-2-ol (5) and (Z)-10-hydroxy-6,10-dimethyl-5-undecen-2-one (6) had not been obtained from the biotransformation of 3. The present paper is the first report of selective production of 2 by the biotransformation of 1 and the production of 5 and 6 by the biotransformation of 3.

RESULTS AND DISCUSSION

From a time-course experiment of the microbial transformation of 1 by *G. cingulata*, a small amount of 1 was incubated with *G. cingulata* for 12 days. One major product (2) and minor products were detected by TLC and GC analysis. These products were not detected by TLC and GC analysis of the culture of *G. cingulata* to which no substrate (1) was fed, or from a mixture of 1 and the medium stirred for 12 days. From the above result, it is demonstrated that *G. cingulata* transformed 1 into 2 and some other minor products. The time-course of

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concentration changes of **1** and **2** was monitored by TLC and quantitatively measured by a GC method (Fig. 1). The starting substrate **1** was mainly transformed into **2**, and about 90% of **1** was consumed after eight days. The major metabolite **2** accounted for *ca* 85% of recovered material after eight days.

In order to isolate these metabolites, a large scale incubation of **1** using *G. cingulata* was carried out for 10 days. After the biotransformation, the culture was extracted as described in the Experimental, and metabolite **2** was isolated from the methylene dichloride extract. The structure of **2** was determined by spectral data.

The metabolite **2** had a molecular formula $C_{15}H_{28}O_3$ based on its mass spectrum. Its 1H and ^{13}C NMR signals indicated the presence of a secondary hydroxyl group (δ_H 3.30; δ_C 77.60/77.69) and a trisubstituted double bond [δ_H 5.19; δ_C 125.79/125.85(CH) and 134.87/134.97(C)] bearing a methyl group (δ_H 1.68; δ_C 27.45). From the spectral data, the metabolite **2** was elucidated to be (*Z*)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol (a mixture of diastereoisomers). Metabolite **2** had been obtained previously by microbial transformation of **1** [6, 7]. However, there had been no report on the selective production of **2** by biotransformation of **1**. In the present biotransformation of **1** by *G. cingulata*, a specific oxidation at the remote double bond proceeded and produced **2** as the major metabolite.

The biotransformation of **3** by *G. cingulata* during a time-course experiment produced four major products (**4**–**7**) which were detected by TLC and GC analysis. Compounds **4**–**7** were not detected in the culture of *G. cingulata* to which no substrate (**3**) was fed, nor were they found in a mixture of **3** and the medium stirred for 12 days. The time-course of the amounts of **3**–**7** observed by TLC and quantitatively measured by a GC method is shown in Fig. 2. The starting substrate (**3**) was transformed in various metabolites and *ca* 85% of **3** was consumed after 12 days. The major metabolite **4** was *ca* 55% after four days, but decreased after that. Compound **5** appeared at two days and accounted for about 15% at 12 days. Compounds **6** and **7** appeared at three days, and reached *ca* 8–10% at 12 days.

In order to isolate these metabolites (**4**–**7**), a large scale incubation of **3** using *G. cingulata* was carried out for 10 days. After the biotransformation, the culture was extracted as described in the Experimental. Metabolites **4**–**7**

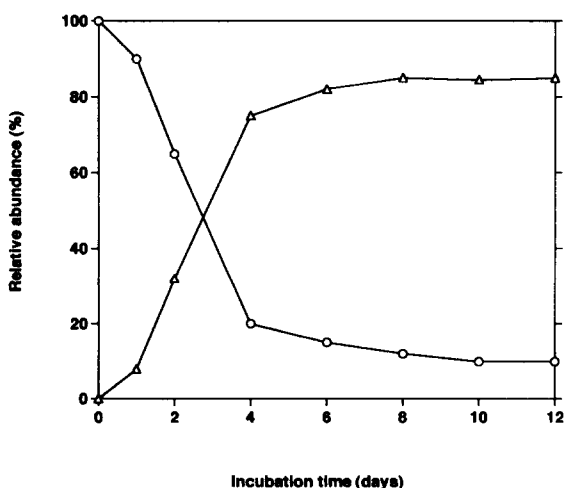


Fig. 1. Time-course for the biotransformation of **1** by *G. cingulata*. \circ = (\pm)-*cis*-Nerolidol (**1**); \triangle = (*Z*)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol (**2**).

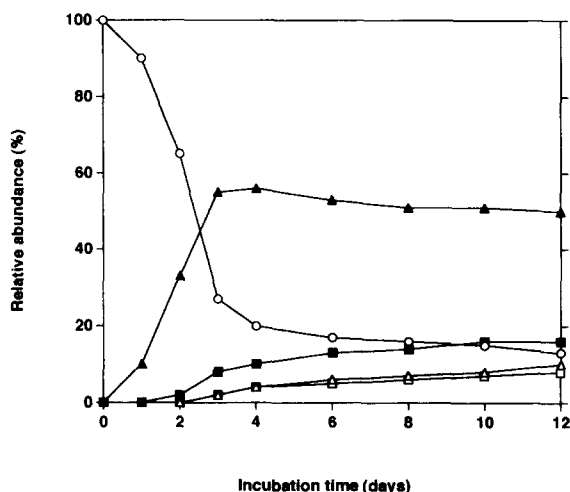


Fig. 2. Time-course for the biotransformation of 3 by *G. cingulata*. ○ = Nerylacetone (3); ▲ = (Z)-9,10-dihydroxy-6,10-dimethyl-5-undecen-diol-2-one (4); ■ = (Z)-6,10-dimethyl-5,9-undecadien-2-ol (5); △ = (Z)-10-hydroxy-6,10-dimethyl-5-undecen-2-one (6); □ = (Z)-6,10-dimethyl-5-undecen-2,9,10-triol (7).

were isolated and their structures were determined by spectral data.

The major metabolite 4 ($[\alpha]_D^{20} + 5.50^\circ$) had a molecular formula $C_{13}H_{24}O_3$ based on its mass spectrum. Its spectral data indicated the presence of a secondary hydroxyl group ($\delta_H 3.31$; $\delta_C 77.69$), a tertiary hydroxyl group ($\delta_C 72.88$; $\nu_{max} 3424, 1163\text{ cm}^{-1}$), a trisubstituted double bond [$\delta_H 5.08$; $\delta_C 124.16$ (CH) and 136.04 (C)] bearing methyl group ($\delta_H 1.68$; $\delta_C 23.25$) and carbonyl group ($\delta_C 209.34$; $\nu_{max} 1709\text{ cm}^{-1}$). The comparison of spectral data between 4 and metabolites of 3 by other microorganisms [6, 8–10] indicated that 4 was (+)-(Z)-9,10-dihydroxy-6-10-dimethyl-5-undecen-2-one. The enantiomeric excess of 4 was 33%ee, which was shown by ^1H NMR analysis of the (R)-MTPA ester of 4.

The product 5 ($[\alpha]_D^{20} + 4.45^\circ$) had a molecular formula $C_{13}H_{24}O$ based on its mass spectrum. Its spectral data indicated the presence of a secondary hydroxyl group ($\delta_H 3.80$; $\delta_C 67.85$), two trisubstituted double bonds [$\delta_H 5.14$; $\delta_C 124.74$ (CH) and 135.69 (C), and $\delta_H 5.12$; $\delta_C 124.22$ (CH) and 131.57 (C)] and no carbonyl group. From the spectral data, metabolite 5 was elucidated as (+)-(Z)-6,10-dimethyl-5,9-undecadien-2-ol. The enantiomeric excess of 5 was 71%ee, which was determined by ^1H NMR analysis of the (R)-MTPA ester of 5.

The product 6 has a molecular formula $C_{13}H_{24}O_2$ based on its mass spectrum. Its spectral data indicated the presence of a tertiary hydroxyl group ($\delta_C 70.80$; $\nu_{max} 3423, 1159\text{ cm}^{-1}$), a trisubstituted double bond [$\delta_H 5.08$; $\delta_C 123.37$ (CH) and 136.45 (C)] bearing a methyl group ($\delta_H 1.68$; $\delta_C 23.25$) and a carbonyl group ($\delta_C 208.77$; $\nu_{max} 1713\text{ cm}^{-1}$). From the spectral data, the metabolite 6 was elucidated to be (Z)-10-hydroxy-6,10-dimethyl-5-undecen-2-one.

The metabolite 7 had a molecular formula $C_{13}H_{26}O_3$ based on its mass spectrum. Its spectral data indicated

the presence of two secondary hydroxyl groups ($\delta_H 3.28$; $\delta_C 67.35/67.08$, and $\delta_H 3.28$; $\delta_C 77.82/77.15$), a tertiary hydroxyl group ($\delta_C 72.99/72.94$; $\nu_{max} 3387, 1128\text{ cm}^{-1}$), a trisubstituted double bond [$\delta_H 5.16$; $\delta_C 125.44/125.98$ (CH) and $135.33/134.93$ (C)] and no carbonyl group. The comparison of the spectral data between 7 and 3–6 indicated that 7 was (Z)-6,10-dimethyl-5-undecen-2,9,10-triol (a mixture of diastereoisomers).

Metabolites 4 and 7 were previously obtained by the biotransformation of 3 by other microorganisms [6, 8, 9]. However, 5 and 6 had not been obtained during the biotransformation of 3 by other microorganisms.

As in the case of the biotransformation of 1 by *G. cingulata*, compound 3 was oxidized at the remote double bond to produce 4 as a major metabolite. Both major metabolites 2 and 4 were possibly formed *via* epoxydation and subsequent hydrolysis, although the intermediate epoxide could not be isolated. In addition, 3 was transformed into small amounts of 5–7 by side reactions. These minor metabolite (5–7) were formed by hydration of the remote double bond and reduction of the carbonyl group. During the biotransformations by *G. cingulata*, the remote double bond of 1 was oxidized, while by contrast that of 3 was either oxidized or hydrated. This difference in product formation by *G. cingulata* with 1 and 3 may be explained by the influence of the substituent.

EXPERIMENTAL

Preculture of *Glomerella cingulata*. Spores of *Glomerella cingulata*, which had been preserved at low temp., were inoculated into sterilized culture medium (1.5% saccharose, 1.5% glucose, 0.5% polypeptone, 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05% KCl, 0.1% K_2HPO_4 and 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water) in a shaking flask, and the flask was shaken at 27° for 3 days.

Time-course experiment. Precultured *G. cingulata* were transferred into a 100 ml Erlenmeyer flask containing 50 ml of medium, and stirred for 3 days. After the growth of *G. cingulata*, 1 (100 mg) and 3 (100 mg) were added to the medium, respectively, and cultured for 12 more days. Every other day 5 ml culture medium was removed and acidified to pH 2 with 1 M HCl and satd with NaCl. The culture medium was then extracted with Et_2O and the extract was analysed by GC and TLC. The ratios between the substrate and metabolic products were determined on the basis of the GC peak areas and are shown in Figs 1 and 2.

Biotransformation of cis-nerolidol (1) for 10 days. Precultured *G. cingulata* were transferred into a 3 l stirred fermentor containing 1.5 l of medium. Cultivation was carried out at 27° and stirring for 3 days under aeration. After the growth of *G. cingulata*, compound 1 (3.32 g) was added to the medium and the culture continued for 10 more days.

Isolation of metabolite 2. After the fermentation, culture medium and mycelia were sepd by filtration. The medium was acidified to pH 2 with 1 M HCl, satd with

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (**1** was recorded at 67.80 MHz, **2** was recorded at 125.65 MHz, residual CHCl_3 used as int. ref. $\delta = 77.00$)

C	1	2
1	111.66 (CH_2)	111.73/111.59 (CH_2)
2	145.00 (CH)	145.19/144.77 (CH)
3	73.36 (C)	73.42/73.36 (C)
4	42.34 (CH_2)	42.25/42.31 (CH_2)
5	22.50 (CH_2)	22.37/22.33 (CH_2)
6	124.96 (CH)	125.79/125.85 (CH)
7	135.57 (C)	134.87/134.97 (C)
8	31.90 (CH_2)	29.41/29.48 (CH_2)
9	26.51 (CH_2)	28.43/28.47 (CH_2)
10	124.24 (CH)	77.60/77.69 (CH)
11	131.54 (C)	73.00 (C)
12	25.68 (CH_3)	26.35 (CH_3)
13	17.59 (CH_3)	23.61/23.34 (CH_3)
14	23.35 (CH_3)	27.45 (CH_3)
15	27.77 (CH_3)	28.15 (CH_3)

Chemical shifts in ppm; multiplicities were determined by the DEPT pulse sequence.

NaCl and extracted with CH_2Cl_2 . The mycelia was also extracted with CH_2Cl_2 . The CH_2Cl_2 extracts were mixed, the solvent was evapd and crude extract (3.00 g) was obtained. The extract was sep'd into the neutral part (2.77 g) and acidic part (0.08 g) in the usual manner. The neutral part was chromatographed on Si-60 columns with a hexane-EtOAc gradient (19:1–1:1) and substrate **1** (210 mg) and metabolite **2** (1.20 g) were isolated.

Compound 2. Oil; $[\alpha]_{\text{D}}^{20} + 3.80^\circ$ (CHCl_3 ; c 1.2). FABMS(POS) m/z : 257 $[\text{MH}]^+$. IR ν_{max} cm^{-1} : 3392, 2971, 1459, 1377, 1160, 1078, 996, 923, 758. ^1H NMR (500.00 MHz, CDCl_3 , TMS as int. standard): δ 1.15, 1.19 (each 3H, s, H-12, 13), 1.28 (3H, 2s, H-15), 1.68 (3H, s, H-14), 3.30 (1H, dt, $J = 1.5$, 11 Hz, H-10), 5.05 (1H, m, H-1), δ 5.19 (1H, m, H-6), 5.22 (1H, 2dd, $J = 1.5$, 17 Hz, H-1'), 5.91 (1H, 2dd, $J = 11$, 17 Hz, H-2); ^{13}C NMR: see Table 1.

Biotransformation of nerylacetone 3 for 10 days. Pre-cultured *G. cingulata* were transferred into a 3 l stirred fermentor containing 1.5 l of medium. Cultivation was carried out at 27° with stirring for 3 days under aeration. After the growth of *G. cingulata*, compound **1** (3.03 g) was

Table 2. ^1H NMR spectral data of compounds **3–7** (500.00 MHz, CDCl_3 , TMS as int. standard)

H	3	4	5	6	7
1	2.14 s	2.14 s	1.18 d (6.5)	2.14 s	1.19 m
2	—	—	3.80 sextet (6.5)	—	3.79 m
3	2.46 t (7)	2.49 t (7)	1.48 m	2.45 t (7)	1.29–1.66 m
4	2.27 q (7)	2.20–2.40 m	2.08 m	2.26 q (7)	2.24–2.42 m
5	5.09 dt (1, 7)	5.07 t (7)	5.14 dt (1.5, 7)	5.08 t (7)	5.17/5.15 t (7)
7	2.60 m	2.20–2.40 m	2.05 m	2.03 m	2.24–2.42 m
8	2.60 m	1.59 m	2.05 m	1.45 m	1.29–1.66 m
		1.38 m			
9	5.12 m	3.30 dd (2, 11)	5.12 m	1.45 m	3.30/3.27 dd (1.5, 11)
11	1.69 brs	1.15 s	1.69 brs	1.21 s	1.14 s
12	1.62 brs	1.20 s	1.61 brs	1.21 s	1.19 s
13	1.69 brs	1.67 brs	1.68 brs	1.67 m	1.69/1.68 brs

Chemical shifts in ppm; coupling constants in Hz.

Table 3. ^{13}C NMR spectral data for compounds **3–7** (125.65 MHz, CDCl_3 , residual CHCl_3 used as int. ref., $\delta = 77.00$)

C	3	4	5	6	7
1	29.80 (CH_3)	30.08 (CH_3)	23.41 (CH_3)	29.92 (CH_3)	23.76/23.73 (CH_3)
2	208.60 (C)	209.34 (C)	67.85 (CH)	208.77 (C)	67.35/67.08 (CH)
3	43.94 (CH_2)	43.64 (CH_2)	39.43 (CH_2)	43.53 (CH_2)	39.24/38.99 (CH_2)
4	22.23 (CH_2)	22.04 (CH_2)	24.22 (CH_2)	22.20 (CH_2)	24.16/23.66 (CH_2)
5	124.10 (CH)	124.16 (CH)	124.74 (CH)	123.37 (CH)	125.44/125.98 (CH)
6	136.39 (C)	136.04 (C)	135.69 (C)	136.45 (C)	135.33/134.93 (C)
7	31.80 (CH_2)	28.48 (CH_2)	31.89 (CH_2)	31.90 (CH_2)	28.45/28.10 (CH_2)
8	26.44 (CH_2)	29.38 (CH_2)	26.51 (CH_2)	22.52 (CH_2)	29.67/29.04 (CH_2)
9	123.28 (CH)	77.69 (CH)	124.22 (CH)	43.86 (CH)	77.82/77.15 (CH)
10	131.55 (C)	72.88 (C)	131.57 (C)	70.80 (C)	72.99/72.94 (C)
11	25.62 (CH_3)	26.31 (CH_3)	25.66 (CH_3)	29.19 (CH_3)	23.32/26.26 (CH_3)
12	17.55 (CH_3)	23.05 (CH_3)	17.58 (CH_3)	29.19 (CH_3)	23.16/22.94 (CH_3)
13	23.27 (CH_3)	23.25 (CH_3)	23.32 (CH_3)	23.21 (CH_3)	23.28 (CH_3)

Chemical shifts in ppm; multiplicities were determined by the DEPT pulse sequence.

added to the medium and the culture maintained for 10 more days.

Isolation of metabolites 4–7. After the fermentation, culture medium and mycelia were sep'd by filtration. The medium was acidified to pH 2 with 1 M HCl, sat'd with NaCl and extracted with CH_2Cl_2 . The mycelia was also extracted with CH_2Cl_2 . The CH_2Cl_2 extracts were mixed, and the solvent was evpd and crude extract (2.81 g) was obtained. The extract was sep'd into the neutral part (2.32 g) and acidic part (0.18 g) in the usual manner. The neutral part was chromatographed on Si-60 columns with a hexane–EtOAc gradient (19:1–1:1), and substrate **3** (224 mg) and metabolites **4** (598 mg), **5** (130 mg), **6** (120 mg) and **7** (93 mg) were isolated.

Compound 4. Oil; $[\alpha]_D^{20} + 5.50^\circ$ (CHCl_3 ; c 1.35). HRFABMS (POS.) m/z : 229.1811 $[\text{MH}]^+$, Calc. for $\text{C}_{13}\text{H}_{25}\text{O}_3$: 229.1804. IR ν_{max} cm^{-1} : 3424, 2970, 1709, 1376, 1163, 1078, 760. ^1H NMR: see Table 2; ^{13}C NMR: see Table 3.

Compound 5. Oil; $[\alpha]_D^{20} + 4.45^\circ$ (CHCl_3 ; c 0.85); HREIMS m/z : 196.1823 $[\text{M}]^+$, Calc. for $\text{C}_{13}\text{H}_{24}\text{O}$: 229.1828. IR ν_{max} cm^{-1} : 3351, 2966, 1451, 1377, 1126, 1083, 830. ^1H NMR: see Table 2; ^{13}C NMR: see Table 3.

Compound 6. Oil; HRFABMS (POS.) m/z : 213.1866 $[\text{MH}]^+$, Calc. for $\text{C}_{13}\text{H}_{25}\text{O}_2$: 213.1855. IR ν_{max} cm^{-1} : 3423, 2968, 1713, 1376, 1159, 759. ^1H NMR see Table 2; ^{13}C NMR: see Table 3.

Compound 7. Oil; $[\alpha]_D^{20} + 17.98^\circ$ (CHCl_3 ; c 1.05); HRFABMS (POS.) m/z 231.1953 $[\text{MH}]^+$, Calcd. for

$\text{C}_{13}\text{H}_{27}\text{O}_3$: 231.1961; IR ν_{max} cm^{-1} : 3387, 2970, 1459, 1377, 1218, 1128, 1077, 758; ^1H NMR: see Table 2; ^{13}C NMR: see Table 3.

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