Available online at www.sciencedirect.com
SCIENCE DIRECT
PHYTOCHEMISTRY
Phytochemistry 67 (2006) 1455–1459
www.elsevier.com/locate/phytochem

Microbiological transformation of two labdane diterpenes, the main constituents of *Madia* species, by two fungi

Mamdouh S.A. Haridy [a], Ahmed A. Ahmed [b,*], Matsumi Doe [c]

[a] *Department of Botany, Faculty of Science, El-Minia University, El-Minia 61519, Egypt*
[b] *Department of Chemistry, Faculty of Science, El-Minia University, El-Minia 61519, Egypt*
[c] *Analytical Division, Faculty of Science Osaka City University 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan*

Received 21 March 2006; received in revised form 21 May 2006
Available online 12 July 2006

Abstract

Microbial transformation of 13*R*,14*R*,15-trihydroxylabd-7-ene (**5**) and 13*R*,14*R*,15-trihydroxylabd-8(17)-ene (**6**) by the fungus *Debaryomyces hansenii* gave **1** (13*R*,14*R*,15-trihydroxy-6-oxolabd-8-ene) and **3** (7α,13*R*,14*R*,15-tetrahydroxy-labd-8(17)-ene), respectively. While, microbial transformation of **5** by *Aspergillus niger* afforded **2** (3β,13*R*,14*R*,15-tetrahydroxy-labd-7-ene), and 13*R*,14*R*,15-trihydroxylabd-8,17-ene (**6**) gave **3** and **4** (3*R*,14*R*,15-3-oxotetrahydroxy-labd-7-ene). The structures of the new compounds, **1** and **2**, were assigned by 1D and 2D high-field NMR spectroscopic methods. Antimicrobial activity of these compounds were tested and their MIC were determined.

Keywords: Labdane diterpenes; Oxidation; *Madia* species; Asteraceae; Microbial transformation; *Debaryomyces hansenii*; *Aspergillus niger*; NMR

1. Introduction

Madia is an Asteraceae genus (tribe Helenieae-Madiinae Benth) characterized by sophisticated features of their inflorescences (Karis and Ryding, 1994). Depending on the species, glandular trichomes and resinous excretions are obvious on stems, leaves and bracts, hence the trivial name, tarweed, is derived. Little is known so far about the external accumulation of flavonoid aglycones and terpenoids, a phenomenon that is widespread in the family (Wellenweber and Valant-Vetschera, 1996; Bohm et al., 1992). The genus comprises 18 species of annual or perennial herbs growing in the United States and Chile. Labdane derivatives are the main constituents of the genus *Madia* (Bohlmann et al., 1982). Previous work on the resinous leaf exudates of five *Madia* species afforded five labdane diterpenes, including an oxidative new one (Wellenweber et al., 2003).

Biotransformation has been extensively applied in the fermentation industry to produce L-aspartic and L-malic acids from fumaric acid (Vanek et al., 1999). Additionally, microorganisms are able to transform a huge variety of organic compounds, such as terpenes, steroids and alkaloids (Orden et al., 2005; Fraga et al., 1998; Lin and Rosazza, 1998). Our isolation of large amounts of the two compounds, **5** and **6** from five *Madia* species, *M. anomala* Greene, *M. capitata* Nutt., *M. citrigracilis* Keck, *M. dissitiflora* Torr. & Gray and *M. sativa* Mol (Wellenweber et al., 2003), permitted us to examine their biotransformation using two types of fungi, *Debaryomyces hansenii* and *Aspergillus niger*. These fungi were used for biotransformation of several compounds (El-Hassane et al., 2000; Galal et al., 1999). Compounds **5** and **6** were subjected to metabolic transformations leading to metabolites that are potentially more or less active against microbes compared to the starting material. Therefore, the objectives of the current study were microbial transformation of the compounds **5** and **6** by *Debaryomyces hansenii* and *Aspergillus*

* Corresponding author. Tel.: +208 634 5267; fax: +208 634 2601.
E-mail address: abdellaahmed@yahoo.com (A.A. Ahmed).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.05.036

niger and determination antibacterial activity of these compounds as well as their metabolic products.

2. Results and discussion

Compound **1** was isolated as a colorless oil with $[\alpha]_D^{25}$ 12° ($c = 0.25$, $CHCl_3$). Its IR spectrum displayed absorption bands at 3350, 1634 cm^{-1}. A further band at 1665 cm^{-1} suggested the presence of an enone moiety. The positive FABMS exhibited an ion peak $[M+H]^+$ at *m/z* 339 ($C_{20}H_{35}O_4$), followed by a fragments at *m/z* 307 $[(M+H)-CH_3OH]^+$ and 289 $[307 - H_2O]^+$. HRFABMS spectrum showed an $[M+H]^+$ ion at *m/z* 339.2531. The ^1H NMR spectrum of **1** displayed five tertiary methyl signals, including an olefinic methyl appearing at δ 1.77 (3H, br *s*, H-17), which showed in the ^1H–^{13}C COSY spectrum correlation with a methyl carbon signal at δ 11.3; a methyl attached to an oxygen-bearing carbon δ 1.24 (3H, *s*, H-16); three methyls resonating at δ 0.88 (3H, *s*, H-18), 0.92 (3H, *s*, H-19) and 1.09 (3H, *s*, H-20). Additionally, ^1H–^1H COSY showed the presence of a $-CH_2(O)-CH(O)-$ fragment at δ 3.75 (2H, *m*, H-15) and 3.52 (1H, *t*, H-14), both correlated in ^1H–^{13}C COSY spectrum with two carbon signals at δ 63.3 (*t*, C-15) and 74.9 (*d*, C-14), respectively. A second $-CH_2-CH-$ moiety was detected at δ 2.49 (H-6a), 2.35 (H-6b) and 1.68 (H-5). The downfield chemical shift of the two former signals required the presence of a neighboring keto group. The ^{13}C NMR spectrum of **1**, in agreement with the molecular formula, revealed signals corresponding to 20 carbon atoms in the molecule. Analysis of the ^{13}C NMR and DEPT (90 and 135) spectral data with the aid of the ^1H–^{13}C COSY spectrum led to the deduction of the multiplicities of the carbon atoms and established the presence of five methyls (δ 22.0, 11.3, 21.3, 32.5, 18.2), two tetrasubstituted olefinic carbons (δ 130.2, 167.9), seven aliphatic methylenes (δ 18.6, 41.3, 35.9, 35.2, 23.5, 37.9, 63.3), two methines (δ 50.3, 74.9), one oxygenated nonprotonated carbon (δ 74.3), one carbonyl carbon (δ 200.3), and two quaternary carbons (δ 33.1, 74.9). The placement of the keto group at C-7 was established from HMBC experiments, Fig. 1. The carbonyl

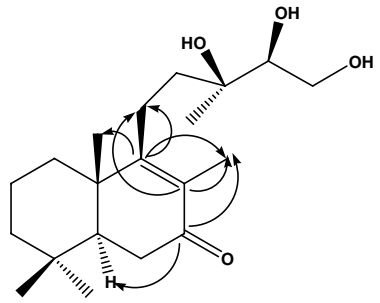


Fig. 1. Selected HMBC correlations of compound **1**.

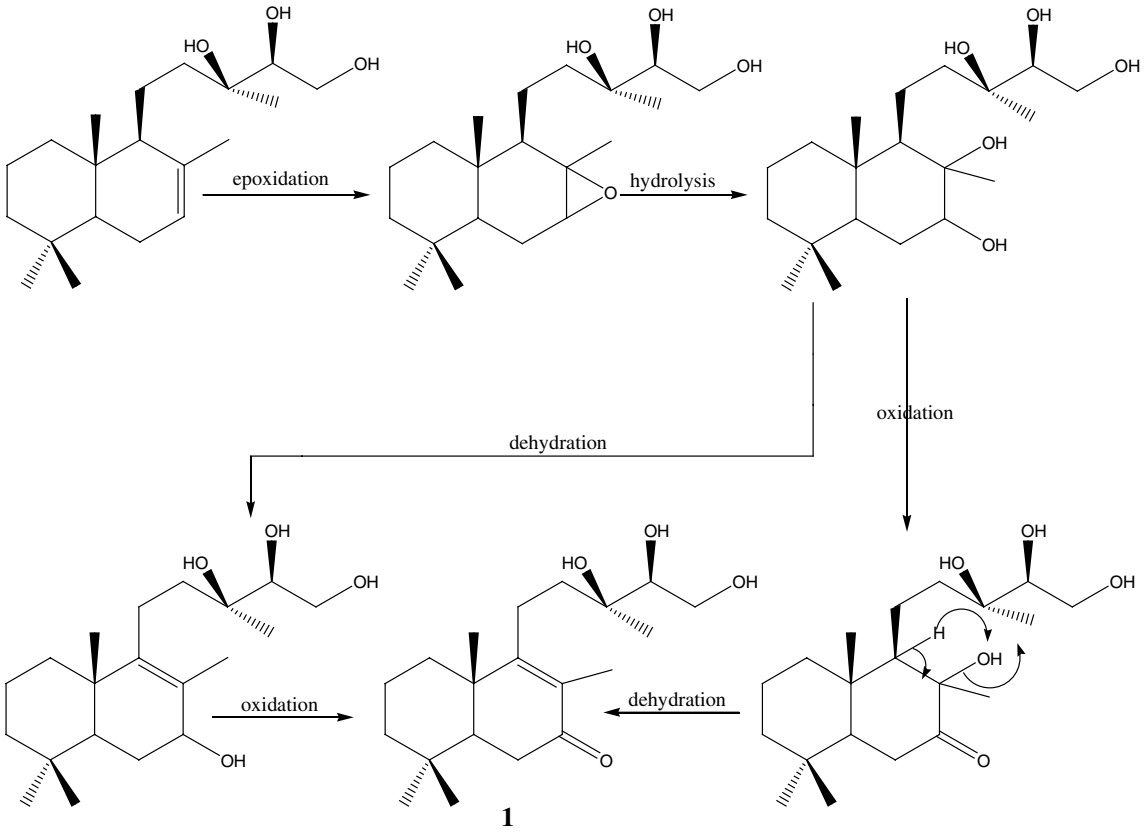
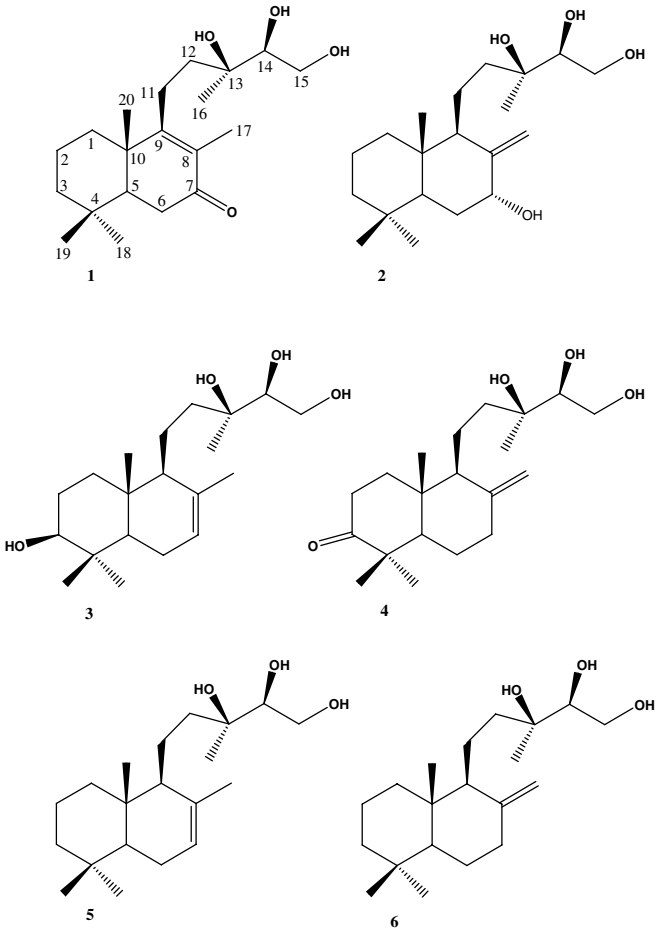


Fig. 2. Suggested biogenetic pathway of compound **1**.

signal at 200.3 showed correlations with the olefinic methyl at δ 1.77 (H-17), the proton signal at δ 1.68 (H-5) and the two protons signals at δ 2.49 (H-6a) and 2.35 (H-6b). Also, the presence of the double bond at C_8–C_9 was supported from the HMBC correlations of the carbon signal at δ 167.9 (C-9) with the proton signals at δ 2.33 (H-11) 2.28 (H-11), 1.09 (H-20), 1.77 (H-17), the carbon signal at δ 130.2 (C-8) showed correlations with the proton signals at δ 2.33 (H-11a), 2.28 (H-11b) and 1.77 (H-17). Furthermore, comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of **5** permitted the assignment of **1** as 13*R*,14*R*,15-trihydroxy-7-oxolabd-8-ene, a newly reported natural product. The suggested biogenetic pathway of compound **1** was given in Fig. 2.

The metabolite **2** was isolated as reddish gum with $[\alpha]_D^{25}$ -21° ($c = 0.13$, $CHCl_3$). Its IR spectrum displayed absorption bands at 3550, 2920, 1445 cm^{-1}. The molecular formula was determined to be $C_{20}H_{36}O_4$ by negative FABMS, which showed an ion peak $[M-H]^+$ at *m*/*z* 339, followed by a fragment at *m*/*z* 305 $[(M-H)-2OH]^+$. The high resolution mass spectrum exhibited a molecular ion peak $[M-H]^+$ at *m*/*z* 339.2532 (calc. 339.2535). Comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with those of **5**, permitted the assignment of **2** as 3β,13*R*,14*R*,15-tetrahydroxy-labd-7-ene. The presence of an additional hydroxyl group in **2** was suggested from the downfield signal at δ 3.24 and showed a correlation with a carbon signal at δ 79.2. in the HMQC spectrum. The hydroxyl group was placed at C-3 on the basis of the HMBC spectrum, and important correlations were observed between C-3 with H-1 (δ 1.91), H-5 (δ 1.15), H-18 (δ 0.98) and H-19 (δ 0.86). Moreover, the ^{1}H and ^{13}C NMR showed H-14 at δ 3.52 (δ 74.8, *d*) and H-15 at δ 3.76 (δ 63.8, *t*), an olefinic proton at δ 5.40 (br *s*, H-7) and an olefinic methyl at δ 1.69 (br *s*, H-17). The other proton and carbon signals were determined from ^{1}H–^{1}H COSY, HMQC and HMBC. The hydroxyl group at C-3 was placed in the equatorial position due to the large coupling constant between H-3*ax* and H-2*ax* (11.0 Hz) and the NOE effect between H-3 and H-18. Therefore, compound **2** was identified as 3β,13*R*,14*R*,15-tetrahydroxy-labd-7-ene, a newly reported natural product. The structure of compound **3** (Jolad et al., 1990) and **4** (Konishi et al., 1999) was determined from comparison of their NMR data with that in the literature.

Antibacterial activity of the compounds **1**–**6** was determined against Gram-positive bacteria *B. cereus* UK 89 and *S. aureus* ATCC 25929 as well as Gram-negative bacterium *E. coli* UK 61. The minimum inhibitory concentrations (MIC) of the compounds were estimated (Table 3). Results showed that Gram-negative bacterium *E.coli* UK61 was more sensitive to the tested compounds than Gram-positive bacteria *B. cereus* and *S. aureus* which may, in part, be related to the differences in the chemical structures of their cell envelopes. It was also clear that compound **3** was the most active compound against the tested bacteria. Its MIC was less than 2 mg/mL. In contrast, compound **4** exhibited lower activity against the tested bacteria, its MIC was more than 2.5 mg/mL, compared with the original compound **6**. Compounds **1** and **2** had a lower activity against the tested bacteria compared with the original compound **5**. Their MIC were more than 2.5 mg/mL.



Results of evaluation of antifungal activity of compounds **1**–**6** against *Penicillium italicum* and *Rhizopus stolonifer* revealed that none of these compounds exhibited any activity at the concentration (5 mg/mL) against fungi tested.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in $CHCl_3$ with a Perkin–Elmer 2435 polarimeter. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer. NMR spectra were recorded with a Bruker AVANCE 600 spectrometer (600 MHz for 1H, 150 MHz for 13C). NMR chemical shifts were referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. FABMS were recorded on a JEOL JMS-700T spectrometer. HPLC was performed in the reverse phase on Knauer instrument, pump type 64, detector: different refractometer (Knauer system), column: RP-8, 250 × 25 mm (Knauer system), flow rate = 17 ml/min, elution with MeOH–H_2O.

3.2. Microorganisms

The fungal cultures of *Penicillium italicum*, *Rhizopus stolonifer* and *Aspergillus niger* were obtained from the culture collection of the Botany Department, Faculty of Science, El-Minia University and the yeast *Debaryomyces hansenii* (strain CBS 767) was obtained from Centraalbureau voor Schimmelcultures, Holland. These fungi were maintained on YMPGA (yeast extract–malt extract–peptone–glucose–agar) slants (Lodder, 1970) at 4 °C. The bacterial cultures *B. cereus* UK 89 and *E. coli* UK 61 were obtained from Prof. J. Klein, der Universität Kaiserslautern, Germany. *S. aureus* ATCC 25929 was obtained from the American Type Culture Collection, Rockville, MD, USA. Bacterial cultures were maintained on nutrient agar slants at 4 °C.

3.3. Media

YMPG (yeast extract–malt extract–peptone–glucose) liquid medium (Lodder, 1970) was used for screening experiments.

3.4. Fermentation procedures

Screening experiments were performed in 250 ml Erlenmyer flasks containing 50 ml YMPG medium. The medium was autoclaved at 121 °C for 15 min. Sterile culture media were inoculated with a cell suspension of yeast *Debaryomyces hansenii* isolate (48-h-old culture) or spore suspension of *Aspergillus niger* (2-week-old culture) and kept on rotary shaker (150 rpm/min) at room temperature (28 °C) for 48 h. After incubation, compounds **5** (30 mg) and **6** (30 mg) were added as a solution in methanol (5 mg/50 ml medium). Six flasks were prepared for each compound. Both substrate and organism controls were made. After two weeks incubation, cultures were filtered

Table 2
^{13}C NMR spectral data of compounds **1**–**4** (600 MHz, $CDCl_3$, δ-values)

Carbons	**1**	**2**	**3**	**4**[a]
C-1	18.6 *t*	37.2 *t*	38.7 *t*	37.6 *t*
C-2	41.3 *t*	27.3 *t*	19.2 *t*	34.9 *t*
C-3	35.9 *t*	79.2 *d*	42.0	215.6 *s*
C-4	33.1 *s*	38.8	33.2	47.7
C-5	50.3 *d*	49.6	47.6	55.0
C-6	35.2 *t*	20.9	30.8	25.2
C-7	200.3 *s*	122.1	74.0 *d*	38.0 *t*
C-8	130.2 *s*	134.8	149.7	147.9
C-9	167.9 *s*	55.1	51.3 *d*	56.7
C-10	41.1 *s*	36.8	39.3	39.6
C-11	23.5 *t*	23.4	16.6	18.3
C-12	37.9 *t*	42.0	37.7	38.1
C-13	74.3 *s*	74.9	74.7	74.3
C-14	74.9 *d*	74.8	75.3	78.3
C-15	63.3 *t*	63.8	63.3	64.0
C-16	22.0 *q*	22.4	22.0	23.7
C-17	11.3 *q*	22.0 *q*	109.9 *t*	108.1 *t*
C-18	21.3 *q*	15.1	33.0	26.1
C-19	32.5 *q*	27.9	21.3	21.6
C-20	18.2 *q*	13.6	13.3	14.0

[a] In pyridine-d_5.

Table 1
^{1}H NMR spectral data of compounds **1**–**4** (600 MHz, $CHCl_3$, δ-values)

	1	**2**	**3**	**4**[a]
H-1	1.68 *m*	1.91 *m*	1.63 br *dd* (12.5, 12.0)	1.96 *m*
	1.58 *m*	1.11 *m*	1.11 *ddd* (13, 13, 3)	1.42 *ddd* (13, 13, 5)
H-2	1.47 *m*	1.60 *m*	1.58 *m*	2.53 *m*
	1.20 *m*		1.52 *m*	2.34 *m*
H-3	2.00 *m*	3.24 *dd* (11, 3.0)	1.40 *m*	
	1.86 *m*		1.20 *ddd* (14, 14, 8)	
H-5	1.68 *dd* (14.5, 3)	1.15 *m*	1.59 *m*	1.50 *m*
H-6	2.49 *dd* (17.5, 3)	1.50 *m*	1.86 br *d* (12.5)	1.50 *m*
	2.35 *dd* (17.5, 4.5)	1.20 *m*	1.56 *m*	1.34 *dddd* (13, 13, 13, 4)
H-7		5.40 br *s*	4.36 br *s*	2.38 *m*
				1.91 *m*
H-9		1.54 *m*	2.09 br *d* (7.5)	1.64 *t* (5)
H-11	2.33 *ddd* (11, 11, 6.5)	1.98 *m*	1.57 *m*	1.83 *m*
	2.28 *m*		1.33 *m*	
H-12	1.68 *m*	1.74 *m*	1.64 *m*	2.11 *m*
		1.51 *m*	1.34 *m*	1.75 *m*
H-14	3.52 *t* (4.5)	3.52 *t* (3.5)	3.51 br *t* (4)	4.13 *dd* (8.3)
H-15	3.75 *m*	3.76 *d* (3.5)	3.74 br *d* (4)	4.30 *dd* (10.5, 3)
				4.17 *dd* (10.5, 8)
H-16	1.24 *s*	1.18 *s*	1.16 *s*	1.54 *s*
H-17	1.77 br *s*	1.69 br *s*	5.05 br *s*	4.95 br *s*
			4.67 br *s*	4.97 br *s*
H-18	0.88 *s*	0.98 *s*	0.80 *s*	0.94 *s*
H-19	0.92 *s*	0.86 *s*	0.87 *s*	1.11 *s*
H-20	1.09 *s*	0.77 *s*	0.66 *s*	0.76 *s*

[a] In pyridine-*d*.

Table 3
Antimicrobial activity of compounds **1**–**6**

Microorganism	MIC – minimum inhibitory concentration (mg/mL)					
	1	**2**	**5**	**3**	**4**	**6**
B. cereus UK 89	2.8	3.1	2.2	1.6	2.8	2.1
S. aureus ATCC 25929	2.7	3.2	2.3	1.8	2.9	2.4
E. coli UK 61	2.6	2.6	2.0	1.2	2.6	2.2

These data are average of four replicates. *B.*, *Bacillus*; *S.*, *Staphylococcus*; *E.*, *Escherichia*.

and filtrates were extracted with methylene chloride to afford **1** (7.5 mg), **2** (9.0 mg), **3** (6.5 mg) and **4** (10.5 mg). After evaporation of the solvent, the residues were chromatographed on Sephadex LH-20 column eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5).

3.5. Antimicrobial assay

Antimicrobial activity of the compounds, **1**–**6**, was determined against Gram-positive bacteria *B. cereus* UK 89 and *S. aureus* ATCC 25929, Gram-negative bacterium *E. coli* UK 61 and fungi *Penicillium italicum* and *Rhizopus stolonifer* using the agar well diffusion method (Vanden-Berghie and Vlietinck, 1991). Fresh cultures of bacteria (24-h-old cultures) and fungi (7-days-old cultures) were suspended in saline solution in a concentration of approximately 10^5 cfu/mL. 0.5 ml of the suspension was spread over the surface of nutrient agar (for bacterial) and potato dextrose agar (for fungi). The tested samples were dissolved in methylene chloride and serial dilutions (0.1–5 mg/mL) were prepared. 0.2 ml portions were dropped in the wells and after incubation at 35 °C for 24 h in case of bacteria or at 28 °C for 48 h in case of fungi zones of inhibition around the wells were observed and then measured. Minimum inhibitory concentration were determined as the lowest concentration of compound preventing any visible growth.

13*R*,14*R*,15-trihydroxy-7-oxolabd-8-ene (**1**) colorless oil; $[\alpha]_D^{25}$ 12° ($c = 0.25$, $CHCl_3$); IR (ν_{max}^{KBr}): 3445, 2938, 1634 cm^{-1}; positive FABMS $[M+H]^+$ *m/z* 339, $[(M+H)-CH_3OH]^+$ *m/z* 307, $[307 - H_2O]^+$ *m/z* 289; HRFABMS *m/z* 339.2531 (calc. for $C_{20}H_{35}O_4$, 339.2536); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

3β,13*R*,14*R*,15-tetrahydroxylabd-7-ene (**2**) white powder; $[\alpha]_D^{25}$ −21° ($c = 0.13$, $CHCl_3$); IR (ν_{max}^{KBr}) 3550, 2920, 1445; negative FABMS $[M-H]^+$ *m/z* 339, $[M-H-2HO]^+$ *m/z* 305; HRFABMS: *m/z* 339.2532 (calc. for $C_{20}H_{35}O_4$, 339.2535); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

7α,13*R*,14*R*,15-tetrahydroxylabd-8(17)-ene (**3**) reddish gum; $[\alpha]_D^{25}$ −14° ($c =$ 0.31, $CHCl_3$); IR (ν_{max}^{KBr}): 3420, 3000, 1660, 1400 cm^{-1}; negative FABMS $[M-H]^+$ *m/z* 339, $[(M-H)-2OH]^+$ *m/z* 305; HRFABMS *m/z* 339.2539 (calc. for $C_{20}H_{35}O_4$, 339.2542); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

3*R*,14*R*,15-trihydroxy-3-oxolabd-8(17)-ene (**4**) white powder; $[\alpha]_D^{25}$ 11° ($c = 0.3$, $CHCl_3$) (lit., −14, $c = 0.5$, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3425, 2930, 1665 cm^{-1}; positive FABMS $[M+H]^+$ *m/z* 339; HRFABMS: *m/z* 339.2528 (calc. for $C_{20}H_{35}O_4$, 339.2535); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

References

Bohlmann, F., Jakupovic, J., King, R.M., Robinson, H., 1982. New labdane derivatives from *Madia sativa*. Phytochemistry 21, 1103–1107.

Bohm, B.A., Fong, C., Hiebert, M., Jamal, A., Crins, W.J., 1992. Non-polar flavonoids of *Calycadenia*, *Lagophylla* and *Madia*. Phytochemistry 31, 1261–1623.

El-Hassane, L., Yoshiaki, N., Toshihiro, H., Yoshinoli, A., 2000. Microbial transformation of dehydropinguisenol by *Aspergillus niger*. Phytochemistry 54, 455–460.

Fraga, B.M., González, P., Guillermo, R., Hernández, M.G., 1998. Microbiological transformation of manoyl oxide derivatives by *Mucor plumbeus*. J. Nat. Prod. 61, 1237–1241.

Galal, A.M., Ibrahim, A.S., Mossa, J.S., El-Feraly, F.S., 1999. Microbial transformation of parthenolide. Phytochemistry 51, 761–765.

Jolad, S.D., Hoffmann, J.J., Joseph, J., Timmermann, B.N., Bates, R.B., Camou, F.A., McLaughlin, S.P., 1990. Diterpenoids and acetogenins of *Blepharizonia plumosa*. Phytochemistry 29, 905–910.

Karis, P.O., Ryding, O., 1994. Tribe Helenieae. In: Bremer, K. (Ed.), Asteraceae: Cladistics and Classification. Timber Press, Portland, Oregon, pp. 521–537.

Konishi, T., Konoshima, T., Fujiwara, Y., Kiyosawa, S., Miyahara, K., Nishi, M., 1999. Stereostructures of new labdane-type diterpenes, excoecarins F, G1, and G2 from the wood of *Excoecaria agallocha*. Chem. Pharm. Bull. 47, 456–458.

Lin, A.H., Rosazza, J.P., 1998. Microbial transformation of isocupressic acid. J. Nat. Prod. 61, 922–926.

Lodder, J. (Ed.), 1970. Criteria and Methods used in Classification. The Yeast, A Taxonomic Study. North-Holland Publish Co., Amsterdam, p. 38.

Orden, A.A., Cifuente, D.A., Borkowski, E.J., Tonn, C.E., Sanz, M.K., 2005. Stereo- and regioselective hydroxylation of grindelic acid derivatives by *Aspergillus niger*. Nat. Prod. Res. 19, 625–631.

Vanden-Berghie, D.A., Vlietinck, A.J., 1991. In: Hostettman, K. (Ed.), Methods in Plant Biochemistry. Academic, London, pp. 47–69 (Chapter 3).

Vanek, T., Vslterovia, I., Vasiar, T., 1999. Biotransformation of (*S*)-(−) and (*R*)-(+) limonene using *Solanum aviculare* and *Diocorea deltoidea* plant cells. Phytochemistry 50, 1347–1351.

Wellenweber, E., Valant-Vetschera, K.M., 1996. In: Hind, D.J.N., Beentje, H., Smith, S.A.L. (Eds.), Proceedings of the International Compositae Conference, Kew, 1994, Systematic, vol. 1. Royal Botanic Gardens, Kew, pp. 169–185.

Wellenweber, E., Dörr, M., Dörsam, M., Hassan, A., Ahmed, A.A., Hegazy, M.F., Zeller, K.P., 2003. Flavonoids and terpenoids from the Resinous exudates of *Madia* species. Zeitchrift für Naturforschung, C: J. Biosci. 58, 153–160.