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Microbial metabolism of partheniol by *Mucor circinelloides*

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Abstract

Six new partheniol metabolites were isolated from the biotransformation reaction with *Mucor circinelloides* ATCC 15242. These metabolites are: humula-1(10), 4, 7-trien-6α-ol **2**, maali-3-en-8α-ol **3**, aromadendrane-4α, 8α, 10α-triol **4**, maaliane-4α, 8α, 9α-triol **5**, maaliane-5α, 8α, 9α-triol **6**, 5(9), 6-tricyclohumulane-4α, 8α, 10α-triol **7**. The structural assignments of these metabolites were made possible by different spectroscopic means.

Keywords: Partheniol; Guayulins A and B; Humulane; Maaliane; Aromadendrane; *Mucor circinelloides*; Biotransformation

1. Introduction

Partheniol is a fungistatic sesquiterpene isolated from *Parthenium argentatum x P. tomentosa* (Maatooq and Hoffmann, 1996). Chemically, partheniol is 8α-hydroxy-6-bicyclogermacrene (6-bicyclohumula-1(10)-4-diene-8α-ol). Partheniol possesses a cyclopropyl ring system and two endocyclic double bonds. Transannular cyclization or the Cope rearrangement (March, 1985) can lead to different derivatives similar to those observed with cyclodeca-1,5-dienes (Sorm, 1971; Takeda, 1974. Microorganisms, particularly fungi, have recently been successfully used as in vitro models for the prediction of mammalian drug metabolites (Clark et al., 1985).

This article describes the metabolism of partheniol by *Mucor circinelloides* ATCC 15242. The fermentation methods, the isolation and the structural identification of the metabolites are reported.

2. Results and discussion

For the biotransformation of partheniol, several microorganisms were subjected to screening experiments. It was found that *Mucor circinelloides* ATCC 15242 demonstrated the best results and was able to convert partheniol, **1,** into several metabolites. Scale up of this reaction afforded the isolation of six metabolites, **2–7**.

Metabolite **2** (*m*/*z* 220) analyzed for $C_{15}H_{24}O$, which is similar to that of the substrate. The ^{1}H NMR spectrum indicated an opening of the cyclopropyl ring, since the characteristic upfield double doublets were absent (δ 1.42 and 0.73). The ^{13}C NMR spectrum showed six olefinic carbon signals at δ 26.5, 127.1, 128.4, 137.2, 138.7 and 139.3, indicating the formation of a new double bond. These new carbon signals were found to be at 127.1 and 126.5 ppm and these were assigned to C-7 and C-8, respectively. This was supported by the absence of the proton and carbon signals characteristic for the 8-position of the substrate. The carbon signal at δ 75.2 which was correlated with the proton doublet at δ 3.96 (J = 8 Hz) was assigned to the hydroxylated C-6. The relatively large coupling constant of this proton doublet (J = 8 Hz) indicated the likely presence of an equatorial proton and an axial hydroxyl group at this location. This indicated that **2** could be obtained after several enzymes-mediated steps started by dehydration followed by rearrangement to open the cyclopropane ring then hydration at 6-position. Therefore, the structure of metabolite **2** was assigned to be humula-1(10), 4, 7-trien-6α-ol.

Metabolite **3** was obtained as the acetate. The EI–MS showed *m*/*z* 262 corresponding to a molecular formula $C_{17}H_{26}O_2$, in addition to 219 for [M– acetyl]$^+$ and 202 for [M–HOAc]$^+$. This pointed to a metabolite with a molecular weight similar to that of the substrate. The three-methyl singlets at δ 0.98, 1.05 and 1.12 were assigned to 14, 12 and 13-methyl groups, respectively, while the downfield methyl singlet at δ 1.68 was assigned to 15-methyl group. The deshielding effect of C-1(10)

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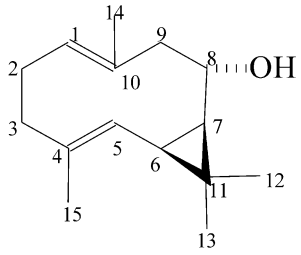

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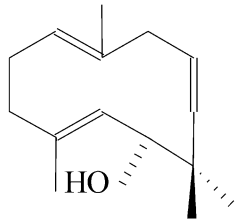

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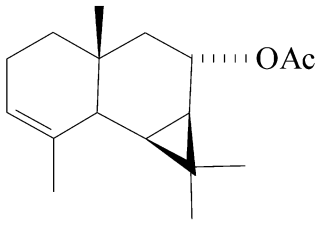

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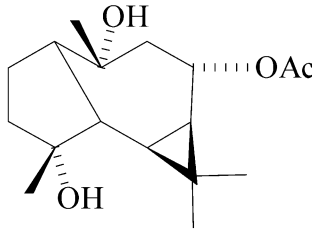

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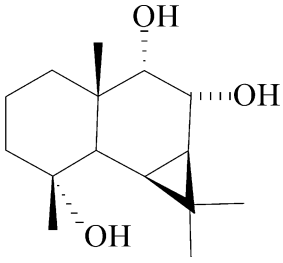

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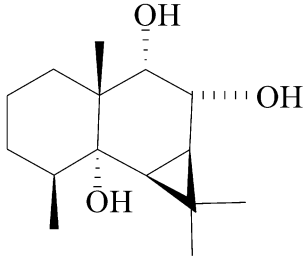

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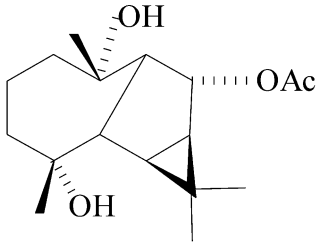

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double bond on 14-methyl, was absent and this pointed to a possible saturation of this double bond. Moreover, only two olefinic carbon signals at δ 118.3 and 134.4 were found. For these reasons, transannular cyclization was suggested between C-10 and C-5. This was supported by the appearance of H-8 signal as a multiplet (δ 4.87) indicating the presence of two protons at C-9. The appearance of H-6 signal as a double doublet indicated the presence of one proton at C-5. The shielding of 14-methyl group and its appearance as a singlet, in the ^{1}H NMR spectrum, proved that C-10 was quaternary and should be involved in the new ring formation. The other side of the new bond proved not to be C-4, since the 15-methyl was deshielded (δ 1.68). For these reasons, 5-position had to be involved in this cyclization and the double bond had to be moved to the 3-position. Furthermore, the olefinic methine carbon signal at δ 118.3 (DEPT) correlated to the broad proton triplet at δ 5.28 had to be assigned to C-3. The relative stereochemistry for 14-position was assigned to beβ based on comparison of its ^{13}C NMR chemical shifts with maaliane analogs (Atta-Ur-Rahman and Ahmad, 1992). The stereochemistry of the proton at the 5-position is not assigned. These results indicated that metabolite **3** was maali-3-en-8α-ol.

Metabolite **4** was isolated as the monoacetate, after acetylation during its purification. The acetate EI–MS gave m/z 236 which was assigned to $[M–HOAc]^+$. The ^1H NMR spectrum showed four methyl singlets at Δ 1.08, 1.09, 1.24 and 1.30, assigned to 12, 13,15 and 14-methyl groups, respectively. This referred to a saturation of both double bonds, since both 14 and 15-methyl groups were shielded and the olefinic carbon signals were absent. The two triplets at δ 0.62 and 0.87 were assigned to the cyclopropyl protons at C-6 and C-7, respectively. This indicated the presence of a methine group at C-5 and suggested the formation of a bridge at this position. The two hydroxylated quaternaries (DEPT) at δ 73.1 and 80.2, are in agreement with those for C-4 and C-10 of α-hydroxylated aromadendrane analogs (Atta-Ur-Rahman and Ahmad, 1992; Canigueral et al., 1994). This was supported by the observed downfield shift of the two-methyl proton signals at C-14 and C-15 (δ 1.30 and 1.24) due to the deshielding effect of the hydroxyl groups. The DEPT experiment proved the presence of four methine and three methylene groups, which required the formation of a new ring, most likely between C-1 and C-5. This was supported by the appearance of H-6 as triplet, while H-8 was a multiplet (set of three doublets), which indicated the presence of two protons at C-9. The stereochemistry of the protons at 1 and 5-positions are not assigned. Therefore the structure of metabolite **4** was assigned to be aromadendrane-4α, 8α, 10α-triol.

Metabolite **5** gave EI–MS m/z 254, which analyzed for $C_{15}H_{26}O_3$. The ^{13}C NMR displayed no olefinic signals. The ^1H NMR showed four methyl signals at δ 0.91 (C-14), 1.0 (C-12), 1.12 (C-13) and 1.29 (C-15), which confirmed the saturation of the two double bonds. The DEPT experiment discriminated the carbon signals into four methyl groups, three methylene groups, five methine groups and three quaternaries. The quaternary hydroxylated carbon signal at δ 72.0 was assigned to an α-hydroxylated C-4 (Atta-Ur-Rahman and Ahmad, 1992). This was supported by the relatively downfield shift of the 3H-15 signal (δ 1.29), which referred to a possible oxygenation in its vicinity. The methine carbon signal at δ 77.9 was correlated to the proton doublet at δ 3.99 ($J = 7$ Hz) and was assigned to position-9 with an α-hydroxylation. This is supported by the appearance of H-8 as a double doublet at δ 3.18 ($J = 10$, 7 Hz) which concluded the presence of a β-proton at C-9. The presence of the 3H-14 signal as a singlet and H-6 proton signal as a double doublet at δ 0.73 ($J = 10$, 8 Hz), suggested that the bridge formation had to be between the 5 and 10-positions. The carbon signal at δ 14.6 which was assigned to 14-methyl group is in agreement with a β orientation for this group in maaliane skeletons (Atta-Ur-Rahman and Ahmad, 1992). The stereochemistry of the proton at the 5-position is not assigned. Based on these arguments the structure of metabolite **5** was concluded to be maaliane-4α, 8α, 9α-triol.

Metabolite **6** gave spectral data similar to that of metabolite **4**. The 3H-15 signal appeared as a doublet at δ 1.15, which indicated the absence of the hydroxyl group at C-4. This hydroxyl group was found to be at C-5, based on the appearance of the cyclopropyl proton at C-6 as a broad doublet at δ 0.53 ($J = 10$ Hz), rather than a double doublet or a triplet. Therefore, the structure of metabolite **6** was concluded as maaliane-5α, 8α, 9α-triol.

Metabolite **7** was obtained as the acetate. The spectroscopic data of **7** acetate are very close to those of metabolite **4** acetate. The major significant difference is the appearance of H-8 signal as a triplet at δ 4.77, rather than a multiplet. This indicated that a methine group must be present at C-9 rather than a methylene group. The stereochemistry of the proton at 5 and 9-positions are not assigned. For this reasons, the structure of metabolite **7** was concluded to be 5(9), 6-tricyclohumulane-4α, 8α, 10α-triol.

In conclusion, six partheniol metabolites were isolated from the biotransformation reactions with *Mucor circinelloides* f. *circinelloides* ATCC 15242 (**2–7**). Several enzymatic systems seem to be involved in these reactions, where hydroxylation, hydration, dehydration, C–C bond cleavage and transannular cyclizations were evidenced. Dioxygenation was the predominant feature (**4–7**). A new double bond formation was evidenced (**2**), and a cyclopropane ring opening was featured (**2**). The fungus demonstrated its ability to induce transannular cyclization at positions 1–5 (aromadenderane skeleton, **4**), 5–9 (5(9), 6-tricyclohumulane skeleton, **7**) and 5–10 (maaliane skeleton, **3**, **5**, **6**). The 5–9 cyclization was unusual and unexpected, however, the 9-position proved to be hydroxylated (**5** and **6**). This indicated that 9-position is enzymatically accessible and this hydroxylation might be the first step for a subsequent cyclization. Compound **3** could be produced by acidic transannular cyclization of partheniol, **1**, which could be enzymatically hydroxylated to give compounds **5** and **6**. However, the pH of the broth was 6.4, just before the extraction, which looks very mild acidity to induce such cyclization and pointed to an enzyme-mediated cyclization.

3. Experimental

3.1. General instrumentation

Melting points are uncorrected. ^1H NMR and ^{13}C NMR were measured on a Bruker WM 250 NMR Spectrometer, at 250 and 62.5 MHz, respectively, with $CDCl_3$ or 1:1 $CDCl_3$– CD_3OD as the solvent and TMS as the internal standard. The chemical shifts are expressed in ppm. DEPT and HETCOR experiments were determined on a Bruker WM 300 NMR spectrometer. EI–MS (70 eV) and GC–EI–MS determined on Hewlett

Packard 5988A spectrometer, equipped with a Hewlett Packard RTE-6/VM data system. The GC column is 25 meter HP-5 capillary, 0.2 mm ID, film thickness 0.33 μm, cross-linked 5% phenyl-methyl silicone, helium with head pressure of 18 psi, 1 μl injection, split ratio 1:50; injector 200 °C, detector 300 °C, temperature program was 70 °C, hold for 1 min 20 °C/min. IR was conducted on Beckman Acculab I IR spectrometer. ORDs were measured on Autopole III automatic polarimeter (Rudolph Scientific).

3.2. Substrate material

Partheniol was obtained by alkaline hydrolysis of guayulin-B obtained from the resin of *Parthenium argentatum x P. tomentosa*. Six grams of guayulin-B were dissolved in 600 ml 10% NaOH/MeOH, and the mixture was refluxed for 2 h. The reaction was terminated by reducing the volume to 100 ml under vacuum then 500 ml of water was added and the mixture was extracted with EtOAc 3×600 ml. After drying over anhydrous Na_2SO_4 followed by solvent evaporation under vacuum, 3.1 g were recovered. Final purification was conducted by flash column chromatography, 200 g Si gel, 63–200 μm, 3.5×45 cm. Isocratic elution with 10% EtOAc/hexane, was adopted to give 2.8 g needles of partheniol. The identity of partheniol was confirmed cochromatographically (R_f = 0.23 in 10% EtOAc/hexane, Si gel GF_{254} TLC plates) and by ^1H and ^{13}C NMR and mass spectrometry (Maatooq and Hoffmann, 1996).

3.3. Fermentation methods

The following cultures were grown according to the standard two-stages fermentation protocol (Betts et al., 1974).

Aspergillus niger UA 172-1, *Aspergillus fumigatus* ATCC 9197, *Rhizopus arrhizus* ATCC 2433, *Rhizopus stolonifer* ATCC 6227, *Gibberella suabinetti* ATCC 20193, *Calonectria decora* ATCC 14767, *Septomyxa affinis* ATCC 6737, *Coniothyrium hellebori* ATCC 12527, *Rhodotorula glutinis* 15125, *Nocardia* species NRRL 5646, *Nocardia corallina* var. *taoka* ATCC 31338, *Mucor circinelloides* ATCC 15242, *Streptomyces antibioticus* ATCC 11891, *Streptomyces coeruleorubidus* ATCC 31276, *Streptomyces griseus* ATCC 1-0137, *Curvularia lunata* ATCC 2178, *Pseudomonas aeruginosa* ATCC 60690, *Pseudomonas* species ATCC 112996, *Penicillium notatum* ATCC 36740, *Corynebacterium mediolanum* ATCC 14004, *Cunninghamella echinulata* var. *elegans* ATCC 8688, *Mycobacterium* species NRRL B3683, *Staphylococcus aureus* ATCC 27664, *Bacillus cereus* UI 1477 and *Bacillus subtilis* ATCC 21394. Screening experiments were done in 125 ml DeLong culture flasks. The culture flasks held one fifth of their volume of the following medium: 2% glucose, 0.5% soybean meal, 0.5% yeast extract, 0.5% NaCl and 0.5% K_2HPO_4. The pH of the medium was adjusted to 7 using 6 N HCl before autoclaving for 20 min at 121°C and 15 psi. After inoculation with the microorganism, stage I culture were incubated at 27°C and 250 rpm for 72 h before being used to inoculate stage II culture flasks. Usually, 10% inoculum volumes are recommended. For screening scale experiments, 10 mg of partheniol in 0.1 ml of 1:1 dimethylformamide (DMF)–dichloromethane (DCM) mixture was added to 24-h-old stage II cultures, which were incubated again and sampled periodically for analysis.

3.4. Sampling

Samples of 1 ml each were taken after 12, 24, 36 and 48 h and every other day for 2 weeks following substrate addition. Each sample was extracted by shaking with 0.5 ml EtOAc and spun at 3000×g for 1 min in a desk-top centrifuge. EtOAc extract from all samples were spotted on Si gel GF_{254} TLC plates, developed in a suitable EtOAc/hexane or acetone/DCM solvent systems, and visualized after spraying with 0.001% vanillin/H_2SO_4, followed by heating for 5–10 s with a heat gun.

3.5. Preparative scale conversion

Six 2-l stage II cultures received 1.44 g of partheniol in 9 ml of 1:1 DMF-DCM mixture (600 μg substrate per ml of culture medium). After incubation with *Mucor circinelloides* f. *circinelloides* ATCC 15242 for 28 days under the usual condition the cultures were combined and exhaustively extracted with 3×2 l of 10% MeOH/EtOAc. The extract was dried over anhydrous Na_2SO_4

Table 1
^{13}C NMR data of partheniol metabolites[a]

C#	**1**	**2**	**3**[b]	**4**[b]	**5**	**6**	**7**[b]
1	126.9	128.4	28.9	55.0	39.6	38.3	36.9
2	25.3	22.4	25.6	24.1	27.8	27.1	25.4
3	46.3	40.4	118.3	41.0	46.8	46.7	45.4
4	129.2	138.7	134.4	73.1	72.0	45.5	72.6
5	125.3	139.3	42.1	48.5	47.7	70.5	47.9
6	36.0	75.2	24.2	28.1	26.8	29.0	32.6
7	28.8	127.1	22.1	27.3	19.7	25.8	27.9
8	72.6	126.5	66.6	69.5	63.1	63.2	69.3
9	40.5	40.1	36.8	51.0	77.9	78.3	51.7
10	136.9	137.2	40.8	80.2	38.4	38.5	81.7
11	20.5	41.2	18.3	21.4	17.2	17.1	22.8
12	29.2	17.1	28.1	31.0	28.5	28.0	31.8
13	16.5	17.9	15.4	16.1	15.2	15.4	15.1
14	15.5	15.5	13.8	20.1	14.6	14.2	19.5
15	20.8	26.8	22.8	23.5	22.6	20.3	24.7
Others		–	171.0	170.1	–	–	170.9
		–	21.1	20.7	–	–	21.5

[a] At 62.5 MHz, using $CDCl_3$ as a solvent (except **4, 5** in 1:1 $CDCl_3$ CD_3OD), TMS is the internal standard and the chemical shifts (δ) are expressed in ppm.

[b] As acetate.

Table 2
^{1}H NMR data of partheniol metabolites[a]

H#	1	2	3[b]	4[b]	5	6	7[b]
1	4.91 *dd*, 6,9	4.96 *br dd*	–	–	–	–	–
3		–	5.28 br *t*	–	–	–	–
5	4.36 *d*, 11	5.16 *d*, 10	–	–	–	–	–
6	1.42 *dd*, 11,9	3.96 *d*, 8	0.73 *dd*, 10,8	0.87 *t*, 9,8	0.73 *dd*, 10,8	0.53 *d*, 10	0.21 *t*, 10,9
7	0.73 *dd*, 11,9	4.98 *d*,7	0.65 *dd*, 10,8	0.62 *t*,9,8	0.68 *dd*, 10,8	0.71 *dd*, 10,8	0.91 *t*,10,9
8	3.6 *ddd* 10,10,5	5.72 *m*	4.87 *m*	4.49 *m*	3.18 *dd*,10,7	3.11 *dd*,10,8	4.77 *t*,9,5
9	—	–	–	–	3.93 *d*, 7	3.86 *d*, 8	–
12	1.12 *s*	1.03 *s*	1.05 *s*	1.08 *s*	1.05 *s*	1.09 *s*	1.08 *s*
13	1.19 *s*	1.13 *s*	1.12 *s*	1.09 *s*	1.12 *s*	1.09 *s*	1.09 *s*
14	1.49 *s*	1.56 *s*	0.98 *s*	1.30 *s*	0.91 *s*	0.82 *s*	1.33 *s*
15	1.63 *s*	1.64 *s*	1.68 *s*	1.24 *s*	1.29 *s*	1.15 *d*, 8	1.21 s
Ac	–	–	2.04	2.04	–	–	2.02

[a] At 250 MHz, using $CDCl_3$ as a solvent (except **4, 5** in 1:1 $CDCl_3$–CD_3OD), TMS is the internal standard, the chemical shifts (δ) are expressed in ppm and the coupling constant J in Hz. *d* = doublet, *dd* = double doublets, *m* = multiplet, *t* = triplet and *s* = singlet, *br* = broad.
[b] As acetate.

and evaporated under reduced pressure to yield a crude dark oily residue of 1.7 g.

3.6. Isolation and purification of the metabolites

The extract (1.7 g) was column chromatographed by the flash method, 200 g Si gel 63–200 μm, 2.2×45 cm. The elution was achieved by EtOAc/hexane using 1-l volume each of 10, 15, 20, 25, 35, 50 and 100%. Twenty-four fractions (200–300 ml each) were collected.

Fraction 3 afforded 70 mg of metabolite **2** as needles by crystallization from the chromatographic solvent. Further purification was done on 1 mm-thick Si gel GF_{254} plates, developed in 5% acetone/DCM, to give 60 mg pure compound. Metabolite **2** possesses a higher R_f value than the substrate (R_f = 0.6 for **2** and 0.5 for the substrate in 25% EtOAc/hexane). Frs. 4–13, 340 mg, contained the recovered substrate mainly.

Frs. 14–16 (128 mg) were acetylated using acetic anhydride–pyridine 1:1 mixture. After the usual work-up, the crude acetate was subjected to prep. TLC on 1 mm-thick Si gel GF_{254} plates, using 10% EtOAc/hexane as a solvent. This afforded 29 mg of metabolite **3** acetate as a solid gum.

Frs 17–19 (92 mg) were treated the same way as frs. 14–16 except that the chromatographic solvent was 10% acetone/DCM. This gave 38 mg of **4** acetate as needles.

Frs 20–22 (210 mg) were subjected to multiple developments (8 times) on prep. TLC using 25% acetone/DCM. This afforded 58 mg of **5** and 55 mg of **6**, as needles and possess R_f = 0.3, 0.28 in 50% acetone/DCM, respectively.

Fr. 23 (310 mg) was purified by MPLC chromatography, 50 g Si gel, 15–25 μm, 15×460 mm, by isocratic elution with 50% acetone/DCM. This gave 34 mg material which was acetylated followed by multiple development (3 times) on prep. TLC using 50% acetone/DCM (R_f = 0.16). This afforded 11 mg of **7** acetate as a solid gum.

3.7. (+) Humula-1(10),4, 7-trien-6α-ol, 2

Needles, mp 62–63 °C, $[\alpha]_D^{25}$, +18.24° ($CHCl_3$; *c*. 1.50), IR $\upsilon_{max}^{cm^{-1}}$: 3420, 2940, 2870, 1645, 1450, 1390, 1320, 1020, 990, 970, 930, 880, 850 and 800. EI–MS, 70 eV, *m*/*z* (relative intensity): 220 $[M]^+$ (3), 205 $[M–CH_3]^+$ (2), 202 $[M–H_2O]^+$ (1), 121 (28), 93 (100), 80 (42), 43 (30) and 41 (52). ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

3.8. (−) Maali-3-en-8α-ol acetate, **3**

Solid gum, $[\alpha]_D^{25}$, −11.6° ($CHCl_3$; *c*. 0.50), IR $\upsilon_{max}^{cm^{-1}}$: 3430, 2950, 2880, 1635, 1440, 1370, 1240, 1030, 890 and 800. GC–EI–MS, 70 eV, *m*/*z* (relative intensity): 262 $[M]^+$ (2), 219 $[M–Ac]^+$ (3), 202 $[M–HOAc]^+$ (2), 187 $[M–HOAc–CH_3]^+$ (2), 185 (10), 157(12), 143 (9), 119 (10), 43 (100) and 41 (15). ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

3.9. (−) Aromadendrane-4α, 8α, 10α-triol 8-acetate, **4**

Needles, mp 168–170 °C, $[\alpha]_D^{25}$, − 2.4° ($CHCl_3$; *c*. 1.50), IR $\upsilon_{max}^{cm^{-1}}$: 3490, 3400, 2990, 2940, 1705, 1420, 1370, 1270, 1240, 1130, 1105, 1020, 960, 940 and 915. EI–MS, 70 eV, *m*/*z* (relative intensity): 236 $[M–HOAc]^+$ (2), 218 $[M–HOAc–H_2O]^+$ (2), 200 $[M–HOAc–2H_2O]^+$ (1), 185 $[M–HOAc–2H_2O–CH_3]^+$ (2), 178 (4), 160(3), 145 (3), 121 (3), 93 (4), 79 (4), 72 (3), 55 (4), 43 (100) and 41 (11). ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

3.10. (+) Maaliane-4α, 8α, 9α-triol, **5**

Needles, mp 155–156°C, $[\alpha]_D^{25}$, +24.5° ($CHCl_3$; *c*. 1.00), IR $\upsilon_{max}^{cm^{-1}}$: 3380, 2920, 2860, 1450, 1370, 1250, 1150, 1040, 1020 and 900. EI–MS, 70 eV, *m*/*z* (relative intensity): 254 $[M]^+$(1), 239 $[M–CH_3]^+$ (8), 236 $[M–H_2O]^+$ (3), 221$[M–H_2O–CH_3]^+$ (2), 218 $[M–2H_2O]^+$ (8), 193 (10), 175 (12), 125 (13), 107 (12), 83 (13), 59 (16), 55 (23), 43 (100) and 41 (33). ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

3.11. (+) Maalian-5α, 8α, 9α-triol, **6**

Needles, mp 167–169 °C, $[\alpha]_D^{25}$, +4.8° ($CHCl_3$; *c*. 0.75), IR $\upsilon_{max}^{cm^{-1}}$: 3390, 2920, 2860, 1450, 1365, 1070, 1020 and 900. EI–MS, 70 eV, *m*/*z* (relative intensity): 239 $[M–H_3]^+$ (2), 236 $[M–{}_2O]^+$ (2), 218 $[M–H_2O]^+$ (8), 203 $[M–H_2O–H_3]^+$ (8), 125 (32), 83 (20), 43 (100) and 41 (28). ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

3.12. (+) 5(9), 6-Tricyclohumulan-4α, 8α, 10α-triol 8-acetate, **7**

Solid gum, $[\alpha]_D^{25}$, +5.4° ($CHCl_3$; *c*. 0.50), IR $\upsilon_{max}^{cm^{-1}}$: 3420, 2950, 2880, 1460, 1370, 1250, 1170, 1130, 1050, 1030, 970, 830 and 740. EI–MS, 70 eV, *m*/*z* (relative intensity): 236 $[M–HOAc]^+$ (1), 221 $[M–HOAc–CH_3]^+$ (1), 218 $[M–HOAc–H_2O]^+$ (5), 200 $[M–HOAc–2H_2O]^+$ (4), 175 (5), 160(7), 145 (10), 107 (8), 105 (10), 72 (5), 43 (100) and 41 (12). ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively.

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