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BIOTRANSFORMATION OF SHIROMODIOL DIACETATE, MYLI-4(15)-EN-9-ONE AND MYLIOL BY *ASPERGILLUS NIGER*

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Key Word Index—Biotransformation; *Aspergillus niger*; shiromodiol diacetate; myli-4(15)-en-9-one; myliol.

Abstract—Microbiol biotransformation of shiromodiol diacetate from *Neolitsea serisea* koids, and of myli-4(15)-en-9-one and myliol from the liverwort *Mylia taylorii*, were carried out with *Aspergillus niger* IFO 4407. *A. niger* hydroxylated the allyl position (C-2) of shiromodiol diacetate, and one of the geminal dimethyl groups of myli-4(15)-en-9-one and myliol regioselectively. The structures of these transformants were elucidated by spectral analysis and confirmed by X-ray analysis. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

We are interested in the biotransformation of sesquiterpenoids as a means of obtaining new biologically active terpenoids. *Aspergillus niger* has been widely used for the biotransformation of various terpenoids because of its broad substrate specificity and high regioselectivity. The use of *A. niger* has made it possible to hydroxylate many terpenoids with a variety of structures in a single step. In previous studies, it was shown that sesquiterpenoids such as cerdrol [1], caryolan-1-ol [2], costunolide [3], *cis*-nerolidol [4] and α-bisabolol [5] are converted by *A. niger* to new metabolites with high yields. Three sesquiterpenoids, shiromodiol diacetate (**1**) [6] isolated from *Neolitsea serisea* koids, and myli-4(15)-en-9-one (**2**) [7] and myliol (**8**) [8] obtained from the liverwort *Mylia taylorii*, were selected as substrates for the present study because there are no reports on their biotransformants. In this paper, we describe the transformation of **1**, **2** and **3** by *A. niger* together with the stereochemistry of **1**.

RESULTS AND DISCUSSION

The stereochemistry of **1** has not been reported. The structure of **1** was confirmed by detailed NMR analyses including H-H and C-H COSY experiments and its configuration determined by X-ray analysis (Fig. 1). Incubation of **1** with *A. niger* afforded **1a** which has a hydroxyl group on the germacrane ring. The hydroxylation of **1** was supported by the IR (3600 cm^{-1}) and HREIMS (*m/z* 354.2046, calcd 354.2042 for $C_{19}H_{30}O_6$) data.

The ^1H and ^{13}C NMR spectra of **1a** showed the presence of an oxymethine signal at δ_H 4.63 (1H, *m*) and δ_C 66.9 (*d*) attributed to a secondary alcohol and the absence of an allyl methylene signal observed in **1** [δ_H 2.28 (2H, *m*, H-2), δ_C 24.4 (*t*, C-2)]. The oxymethine group was assigned to the 2-position on the basis of 2D NMR experiments. The stereochemistry of **1a** was confirmed by single-crystal X-ray analysis (Fig. 2). From these results, the structure of **1a** was determined to be 2β-hydroxyshiromodiol diacetate.

A. niger also hydroxylated compounds **2** and **3** at one of the geminal dimethyl groups on the cyclopropane ring to form **2a** and **3a**, respectively. The HREI mass spectrum of **2a** indicated a molecular formula $C_{15}H_{20}O_2$ (*m/z* 232.1452, calcd 232.1464) corresponding to a monohydroxylated form of **2**. This was supported by the IR data (3400 cm^{-1}). On comparison of the NMR spectra of **2** with those of **2a**, it was clear that one [δ_H 1.02 (3H, *s*, H-12), δ_C 30.2 (*q*, C-12)] of the geminal dimethyls in **2** had been replaced by a hydroxymethyl [δ_H 3.32 (2H, *d*) and δ_C 73.3 (*d*)] in **2a**. In the HMBC experiment, the hydroxymethyl carbon atom had long-range coupling with H-6 and H-13 (methyl), and correlated with C-11. The stereochemistry of the hydroxymethyl group (H-12) on the cyclopropane ring was elucidated with the NOESY spectrum which showed correlation contours between H-12 and H-6 [H-13 (methyl) also gave a cross peak with H-1]. The complete structure of **2a** was unambiguously confirmed to be 12β-hydroxymyli-4(15)-en-9-one on the basis of X-ray analysis (Fig. 3).

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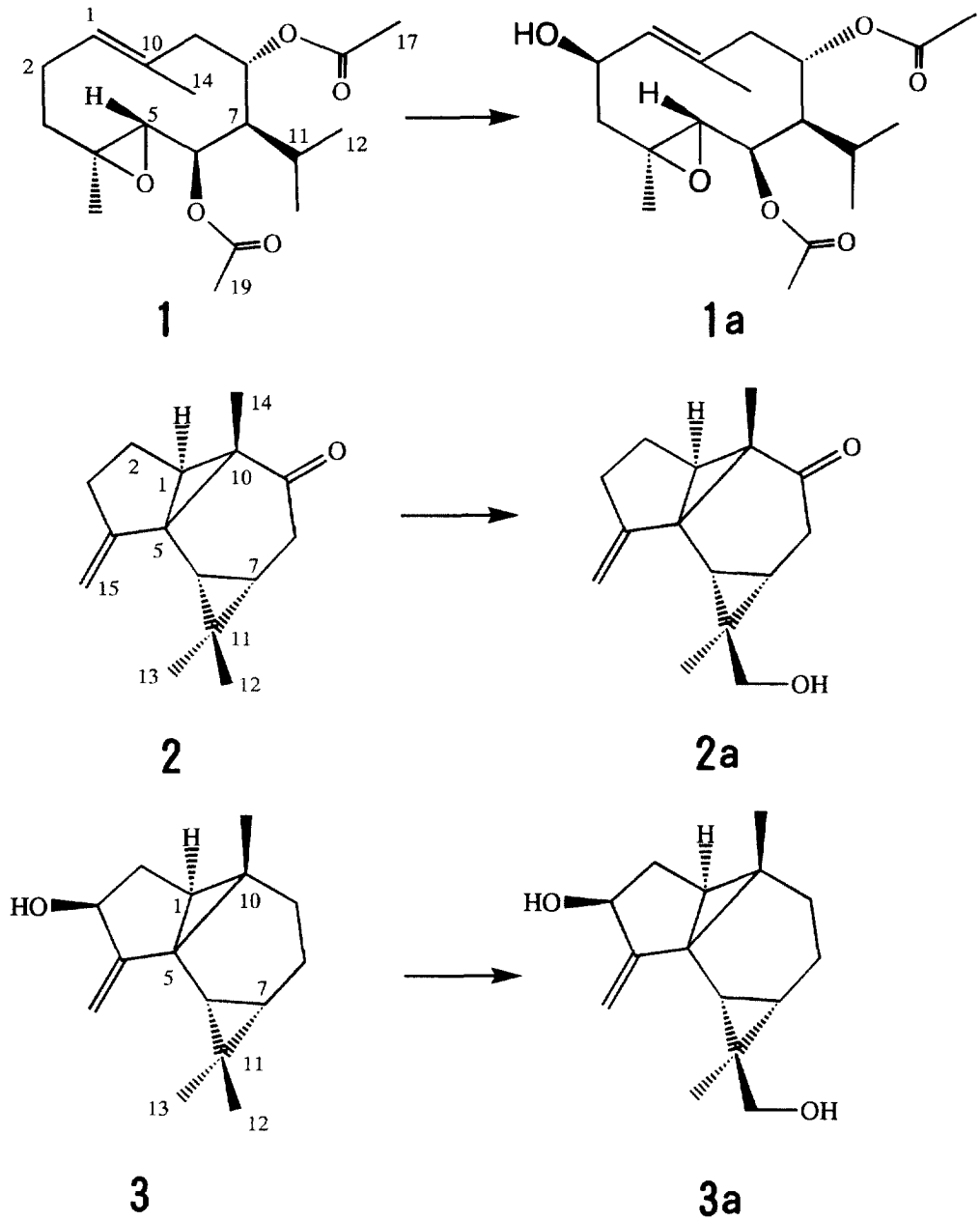

Compound **3a** was assigned the molecular formula $C_{15}H_{22}O_2$ (*m/z* 234.1636, calcd 234.1620). Its ^1H and ^{13}C NMR spectra also showed the presence of a hydroxymethyl group [δ_H 3.24 (2H, *m*) and δ_C 74.3 (*d*)] in addition to the loss of a signal attributed to one of the geminal dimethyl groups in **3**. The position of hydroxymethyl group was elucidated by an HMBC experiment and a comparison of the NMR data of **3a** with those of **3**. The stereostructure of **3a** was confirmed by the NOESY spectrum which showed the same correlations as observed for **2a**. The structure of **3a** was thus determined to be 12β-hydroxymyliol.

The time courses for the biotransformation of **1**, **2** and **3** by *A. niger* are shown in Figs 4 and 5. The conversion rates (measured by GC) reached maxima at 4 days after incubation. The increasing amounts of all transformants seemed to be proportional to the decrease in the amount of the corresponding substrates. Increasing the incubation time did not improve the production of transformants and gave no further oxidized products.

EXPERIMENTAL

General experimental details

^1H and ^{13}C NMR: Bruker ARX 400 in $CDCl_3$; EIMS and HREIMS: JEOL MStation at 70 eV; CC:

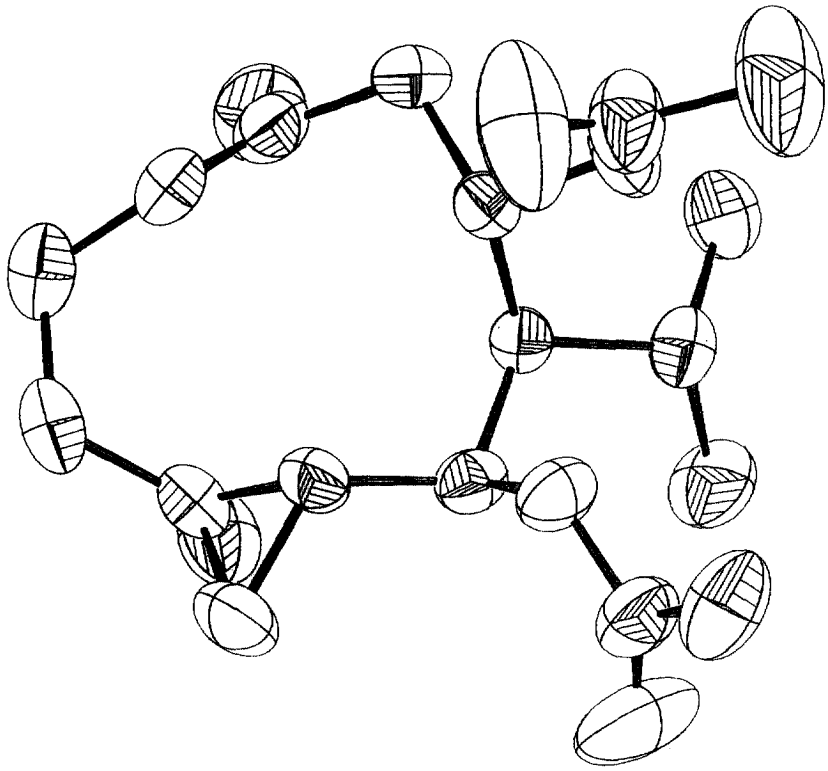

Fig. 1. ORTEP drawing of **1**.

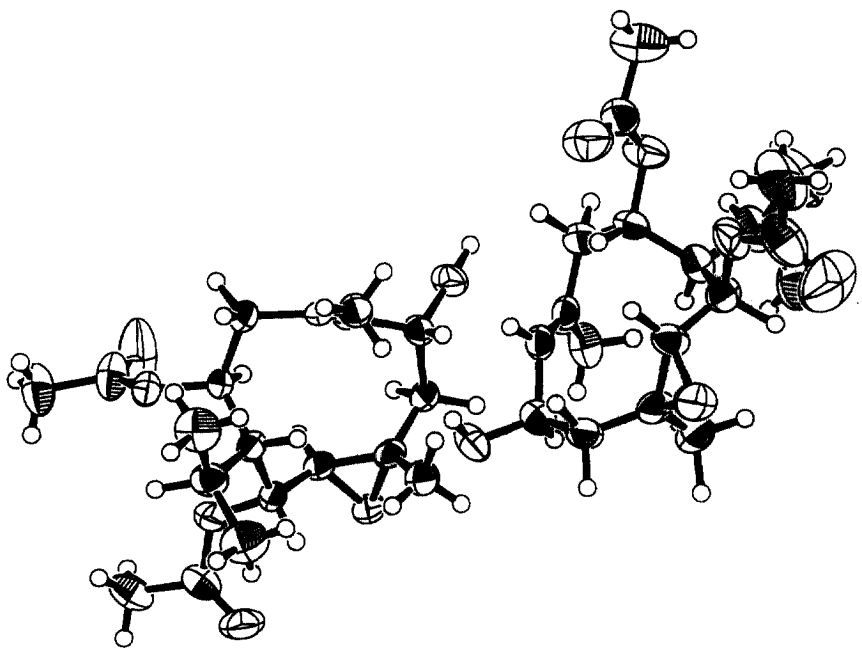

Fig. 2. ORTEP drawing of **1a**.

Kiesel gel (230–400 mesh, Merck). *A. niger* IFO 4407 was obtained from the Institute for Fermentation (Osaka, Japan) and grown on the Czapek agar at 28°. Compound **1** was isolated from *Neolitsea serisea* koids (Lauvaceae), and **2** and **3** were obtained from *Mylia taylorii* (liverwort) by methods reported previously.

Biotransformation of **1**, **2** *and* **3**. A slant culture of *A. niger* was inoculated into a test tube containing 5

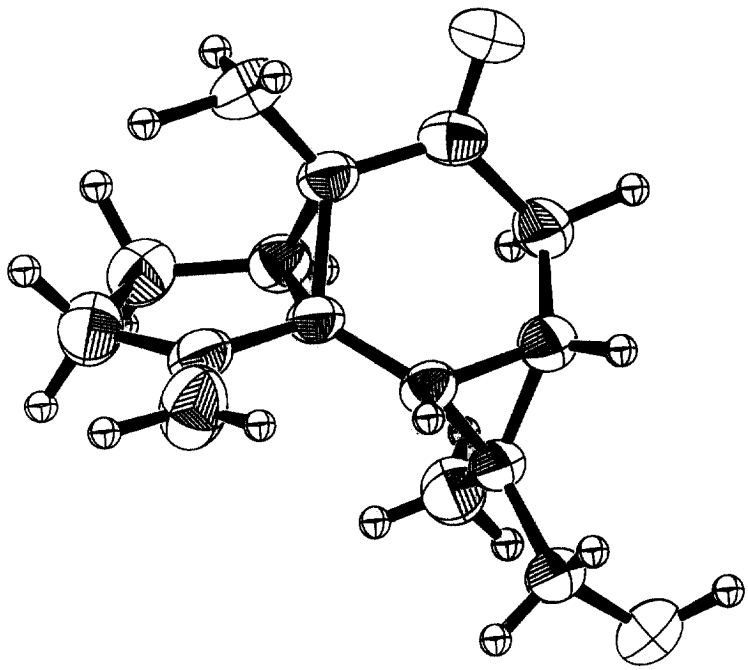

Fig. 3. ORTEP drawing of **2a**.

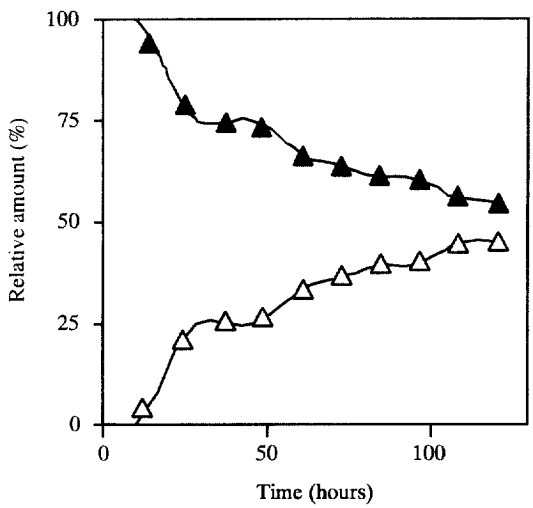

Fig. 4. Time course for biotransformation of shiromodiol diacetate (**1**) by *A. niger*, ▲, **1**; △, 2-hydroxyshiromodiol diacetate (**1a**).

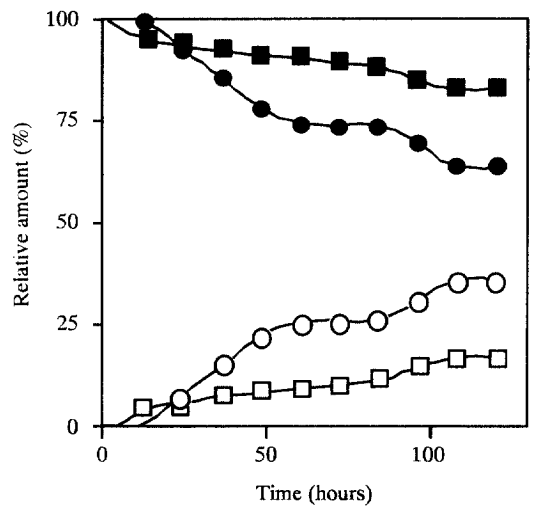

Fig. 5. Time course for biotransformation of myli-4(15)-en-9-one (**2**) and myliol (**3**) by *A. niger*. ■, **2**; ●, **3**; □, 12-hydroxymyli-4(15)-en-9-one (**2a**); ○, 12-hydroxymyliol (**3a**).

ml of the medium (2% Corn steep Liquor, 1% Glucose, pH 5.5). This tube was cultured for 24 h at 28° on a reciprocal shaker (150 rpm). The pre-culture was transferred into a 500-ml Sakaguchi flask containing 100 ml of the medium described above. This flask was shaken at 28° for 24 h on a reciprocal shaker (100 rpm). 100 mg of substrate was dissolved in 2 ml of MeOH, and the soln added to the flask. Cultivation was carried out for a further 4 days at 28°.

Time courses for the biotransformation of **1**, **2** *and* **3**. The culture broth (3 ml) was sampled every 12 h during the fermentation. The whole broth was extracted with CH_2Cl_2 and the extract was analyzed by capillary GC (TC-1 25 m × 0.25 mm, 200°–310°, 4° min^{-1}).

Isolation of **1a**, **2a** *and* **3a**. The culture broth after 5 days incubation with the substrate was filtered. The filtrate was neutralized (pH 7.0) with 6 N NaOH and extracted twice with CH_2Cl_2. The organic layer was dried over Na_2SO_4. The mycelia was soaked in MeOH and filtered to remove the mycelia. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2. The CH_2Cl_2-soluble material was combined with the organic layer from the filtrate. An oily residue (**1a**:

Table 1. ^{1}H NMR chemical shifts of compounds **1**, **1a**, **2**, **2a**, **3** and **3a** (400 MHz, $CDCl_3$)

H	**1**	**1a**	**2**	**2a**	**3**	**3a**
1	5.33 *dd* (9.4, 1.4)	5.43 *dd* (9.3, 1.2)	2.34 *d* (5.9)	2.33 *d* (5.9)	1.29 *m*	1.37 *m*
2	2.28 *m*	4.63 *m*	1.67 *m*	1.96 *m*	1.36 *m*	1.33 *m*
	2.28 *m*		2.09 *m*	2.11 *m*	2.41 *m*	2.43 *m*
3	1.18 *m*	1.23 *d* (1.6)	2.21 *m*	2.23 *m*	4.73 *brs*	4.73 *brs*
	2.19 *m*	2.53 *dd* (12.5, 5.4)	2.58 *m*	2.57 *m*		
5	2.95 *d* (7.0)	2.97 *d* (6.9)				
6	4.92 *dd* (7.0, 0.4)	4.88 *dd* (6.8, 1.2)	1.34 *d* (9.9)	1.47 *d* (10.1)	1.07 *d* (9.5)	1.19 *d* (8.0)
7	1.48 *m*	1.33 *d* (9.4)	1.13 *dd* (19.1, 7.1)	1.20 *m*	0.54 *dd* (18.0, 9.5)	0.76 *dd* (17.6, 8.0)
8	5.42 *m*	5.47 *m*	2.09 *m*	2.10 *dd* (16.0, 7.3)	0.72 *m*	0.81 *m*
			2.42 *dd* (15.8, 9.2)	2.45 *dd* (16.0, 9.2)	1.72 *m*	1.71 *m*
9	1.92 *m*	1.95 *m*			1.60 *m*	1.56 *m*
	2.62 *m*	2.77 *dd* (13.0, 5.8)			1.60 *m*	1.67 *m*
10						
11	1.90 *m*	1.90 *m*				
12	0.94 *d* (6.6)	0.93 *d* (6.6)	1.02 *s*	3.32 *d* (3.7)	1.00 *s*	3.24 *q* (10.8)
13	1.11 *d* (6.6)	1.11 *d* (6.6)	1.23 *s*	1.32 *s*	0.93 *s*	0.96 *s*
14	1.77 *s*	1.87 *s*	1.02 *s*	1.07 *s*	1.00 *s*	1.12 *s*
15	1.20 *s*	1.19 *s*	5.11 *q* (2.1)	5.12 *dt* (10.8, 2.2)	5.09 *d* (2.6)	5.09 *d* (2.8)
					5.14 *d* (2.4)	5.16 *d* (2.4)
17	2.10 *s*	2.01 *s*				
19	2.07 *s*	2.08 *s*				

59 mg, **2a**: 51 mg, **3a**: 28 mg) was obtained after evaporation. For the isolation of the transformant, each residue was purified by repeated silica gel CC (eluent system; benzene–Me_2CO and $CHCl_3$–MeOH). Fraction was monitored by TLC and GC. **1a** (28.9 mg), **2a** (48.0 mg) and **3a** (7.4 mg) were obtained as needles and recrystallized from $CHCl_3$. Unutilized **1** (49.9 mg), **2** (50.8 mg) and **3** (71.8 mg) were recovered, respectively, from culture broths.

Shiromodiol diacetate (**1**). Needles, mp 112°, $[\alpha]_D^{25}$ −61.9° ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 1735, 1240; EIMS 70 eV, *m/z* (rel. int): 338 $[M]^+$ (7), 278 (37), 236 (47), 218 (45), 201 (62), 175 (100), 160 (73), 139 (66), 109 (48), 93 (44); ^{1}H NMR: Table 1. ^{13}C NMR: Table 2.

Myli-4(15)-*en*-9-*one* (**2**). Needles, mp 145°, $[\alpha]_D^{25}$ +1.0° ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 2933, 1710, 1610, 1518, 1463, 1379, 1231; EIMS 70 eV, *m/z* (rel. int): 216 $[M]^+$ (94), 201 (26), 173 (85), 159 (53), 145 (100), 131 (80); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Myliol (**3**). Needles, mp 111°, $[\alpha]_D^{25}$ −20.0° ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 3610, 3420, 1654, 898, 890; EIMS 70 eV, *m/z* (rel. int): 218 (28), 200 (49), 175 (59), 157 (87), 143 (100), 131 (81), 91 (80); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

2β-*Hydroxyshiromodiol diacetate* (**1a**). Prism, mp 146°, $[\alpha]_D^{25}$ −94.5° ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 3600, 3025, 2933, 1735, 1240; HREIMS: 354.2046 (calcd 354.2042 for $C_{19}H_{30}O_6$); EIMS 70 eV, *m/z* (rel. int): 354 $[M]^+$ (2), 294 (5), 252 (6), 234 (17), 211 (74), 191 (39), 152 (64), 109 (62), 95 (63), 84 (100); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

12β-*Hydroxymyli*-4(15)-*en*-9-*one* (**2a**). Needles, mp 128°, $[\alpha]_D^{25}$ +60.0° ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 3400, 2933, 1709, 1608, 1524, 1464, 1427, 1374, 1217; HREIMS: 232.1452 (calcd 232.1464 for $C_{15}H_{20}O_2$); EIMS 70 eV, *m/z* (rel. int): 232 $[M]^+$ (11), 167 (35), 149 (100), 131 (27), 105 (21), 91 (24), 71 (28); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Table 2. ^{13}C NMR chemical shifts of compounds **1**, **1a**, **2**, **2a**, **3** and **3a** (100 MHz, $CDCl_3$)

C	**1**	**1a**	**2**	**2a**	**3**	**3a**
1	128.9 *d*	132.4 *d*	41.9 *d*	42.0 *d*	28.5 *d*	28.7 *d*
2	24.4 *t*	66.9 *d*	23.0 *t*	23.0 *t*	32.5 *t*	32.5 *t*
3	38.0 *t*	47.6 *t*	32.7 *t*	32.7 *t*	78.3 *d*	78.2 *d*
4	59.5 *s*	57.8 *s*	153.7 *s*	153.3 *s*	159.9 *s*	158.2 *s*
5	66.3 *d*	66.9 *d*	47.9 *s*	46.9 *s*	35.0 *s*	34.3 *s*
6	73.6 *d*	73.6 *d*	21.9 *d*	18.9 *d*	24.0 *d*	21.1 *d*
7	46.4 *d*	47.8 *d*	24.5 *d*	21.1 *d*	19.7 *d*	16.4 *d*
8	72.3 *d*	72.7 *d*	32.2 *t*	31.5 *t*	16.0 *t*	15.5 *t*
9	39.2 *t*	40.5 *t*	210.4 *s*	209.8 *s*	32.6 *t*	31.3 *t*
10	129.9 *s*	133.4 *s*	36.0 *s*	35.9 *s*	24.7 *s*	25.4 *s*
11	25.5 *d*	26.2 *d*	23.4 *s*	29.7 *s*	18.5 *s*	25.0 *s*
12	21.2 *q*	21.5 *q*	30.2 *q*	73.3 *t*	30.5 *q*	74.3 *t*
13	21.4 *q*	22.1 *q*	14.8 *q*	10.8 *q*	16.3 *q*	16.2 *q*
14	23.1 *q*	23.4 *q*	7.3 *q*	7.4 *q*	14.9 *q*	10.7 *q*
15	16.4 *q*	18.2 *q*	107.3 *t*	108.1 *t*	105.6 *t*	106.1 *t*
16	170.2 *s*	169.8 *s*				
17	21.0 *q*	21.0 *q*				
18	170.3 *s*	169.8 *s*				
19	21.2 *q*	21.3 *q*				

12β-*Hydroxymyliol* (**3a**). Needles, mp 161°, $[\alpha]_D^{25}$ −18.8° ($CHCl_3$; *c* 0.06). IR ν_{max} cm^{-1}: 3423, 2928, 1712, 1479, 1466, 1382, 1364, 1017; HREIMS: 234.1636 (calcd 234.1620 for $C_{15}H_{22}O_2$); EIMS 70 eV,

m/z (rel. int): 234 $[M]^+$ (39), 216 (41), 203 (49), 185 (70), 175 (68), 157 (100), 143 (92), 131 (73), 105 (69), 91 (75); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Crystallographic structure determination of **1**, **1a** *and* **2a**. **1**: $C_{19}H_{30}O_5$, M_r 338.4, orthorhombic, space group $P2_12_12_1$, $a = 13.772(6)$, $b = 14.180(8)$, $c = 9.988(4)$ Å $U = 1951(2)$ Å^3, $D_c = 1.15$ g/cm^3, $Z = 4$; **1a**: $C_{19}H_{30}O_6$, M_r 354.4, monoclinic, space group $P2_1$, $a = 8.095(1)$, $b = 12.443(2)$, $c = 19.738(1)$ Å, $\beta = 93.053(9)^\circ$, $U = 1985.4(4)$ Å^3, $D_c = 1.186$ g/cm^3, $Z = 4$; **2a**: $C_{15}H_{20}O_2$, M_r 232.3, monoclinic, space group $P2_1$, $a = 6.175(2)$, $b = 12.478(3)$, $c = 8.285(2)$ Å, $\beta = 93.14(3)^\circ$, $U = 637.4(3)$ Å^3, $D_c = 1.210$ g/cm^3, $Z = 2$. All unique diffraction intensities of **1** with $2\theta < 120^\circ$ ($2\theta < 60^\circ$ for **1a** and **2a**) were collected in the ω-2θ scan mode on a Rigaku AFC-7R four-circle automatic diffractometer (Mac Science MXC 18 diffractometer for **1a** and **2a**) using graphite monochromatized Mo–K_α radiation ($\lambda = 0.7107$ Å) (Cu–K_α radiation $\lambda = 1.54184$ Å, for **1a** and **2a**). Of the 2640 reflections collected for **1** (**1a**: 3355, **2a**: 1106), 2538 (**1a**: 2777, **2a**: 854) were judged to be observed after correction for Lorentz, polarization and background effects. The structure was solved by direct methods using CRYSTAN program system [9, 10] (Rigaku TEXAN program system [11] for **1a** and **2a**). Full-matrix least-squares refinements with anisotropic temperature factors for the non-hydrogen atoms and isotropic factors for the hydrogen atoms converged to a final *R*-factor of 0.055 (**1a**: 0.046, **2a**: 0.113) for the 2538 reflections (**1a**: 2777, **2a**: 854). The crystallographic data of **1**, **1a** and **2a** have been deposited with the Cambridge Crystallographic Data Centre.

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