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RING-A CLEAVAGE OF 3-OXO-OLEAN-12-EN-28-OIC ACID BY THE FUNGUS *CHAETOMIUM LONGIROSTRE*

NOBORU SHIRANE,* YUTAKA HASHIMOTO, KAZUO UEDA, HIDEYUKI TAKENAKA and KENJI KATOH

Aburahi Laboratories, Shionogi & Co. Ltd, Koka-cho, Koka-gun, Shiga 520-34, Japan

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Key Word Index—*Chaetomium longirostre*; Chaetomiaceae; fungus; biotransformation; enzymic Baeyer–Villiger oxidation; triterpenes; 3-oxo-olean-12-en-28-oic acid; 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid; 3,4-*seco*-olean-12-en-4,21β-diol-3,28-dioic acid; A-homo-3*a*-oxa-olean-12-en-3-one-28-oic acid.

Abstract—3-Oxo-olean-12-en-28-oic acid was transformed by the filamentous fungus *Chaetomium longirostre* into 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid and the 21β-hydroxylated compound. A cell-free preparation derived from the fungus converted 3-oxo-olean-12-en-28-oic acid into 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid. The ring-A cleavage activity was detected in the soluble fraction of the cell-free preparation and showed a requirement for NADPH. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Microorganisms metabolize various xenobiotics, and fungal biotransformations of steroids are among the earliest examples of their practical use as biocatalysts. Advances in genetic engineering have raised the possibility of utilizing the biocatalysts as reagents in organic synthesis [1]. To obtain a site specific mono-oxygenase which is stable, self sufficient and stereo-selective, we screened fungi which transform triterpene into more polar compounds. We report here that *Chaetomium longirostre* cleaves ring-A of 3-oxo-olean-12-en-28-oic acid efficiently, and the enzyme system responsible for the cleavage is active in the cell-free preparation of the fungus.

RESULTS AND DISCUSSION

Mycelium of *C. longirostre* converted 3-oxo-olean-12-en-28-oic acid (**1**) into two more polar compounds (**2** and **3**). The time-course experiment of the conversion revealed that 1 g fresh wt of mycelia metabolized *ca* 2 μmol of **1** within 24 hr (Fig. 1). The metabolites **2** and **3** were detected after 3 and 6 hr incubations, respectively.

The metabolites were isolated and the structures were deduced from their spectral data (Experimental and Table 1). The signals in the ^{13}C NMR spectrum of **1** were assigned on the basis of the 2D NMR spectra and by comparison with the signals reported by Seo *et al.* [2] (Table 1). The molecular formula of **2** was determined as $C_{30}H_{48}O_5$ from its HR mass spectrum (Experimental) indicating that two protons and two oxygens were introduced into **1**. In the ^{1}H NMR spectrum of **2**, signals of seven singlet methyl groups were observed showing that no methyl group in the original compound **1** was oxygenated. The presence of two carboxyl groups in **2** was inferred by the ^{13}C NMR spectrum (δ 178.9 and 181.8) (Table 1). The 2D NMR spectral data, including COLOC and HMBC, gave the gross structure of **2**. In the COLOC spectrum, all the methyl proton signals proved ^2J and ^3J cross-correlations with those of the related carbons (Fig. 2). These findings suggested **2** to be 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid derived from **1** via oxidative cleavage of the ring-A. The ROESY spectrum of **2** supported the deduced structure. Treatment of **2** with diazomethane afforded the corresponding dimethyl ester (**4**); δ_H 3.61 (3H, *s*, CO_2Me), δ_H 3.62 (3H, *s*, CO_2Me), δ_C 52.2 (CO_2Me) and δ_C 52.4 (CO_2Me) (Experimental; Table 1). Thus, the structure of **2** was established to be 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid, which has not been reported previously.

The molecular formula of **3** was determined as $C_{30}H_{48}O_6$ from its HR mass spectrum (Experimental), indicating that an oxygen was introduced into **2**. Treatment of **3** with diazomethane afforded the corresponding dimethyl ester (**5**); δ_H 3.62 (3H, *s*, CO_2Me), δ_H 3.63 (3H, *s*, CO_2Me) and δ_C 52.3 (CO_2Me) (Table 1; Experimental). The main feature differentiating the ^{1}H NMR spectrum of **5** from that of **4** was the appearance of a signal at δ_H 3.48 (1H, *dd*) in the spectrum of **5**. This signal suggested that **5** was a hydroxylated compound. The position of the hydroxyl group was suggested as 21β by the HMBC spectrum

*Author to whom correspondence should be addressed.

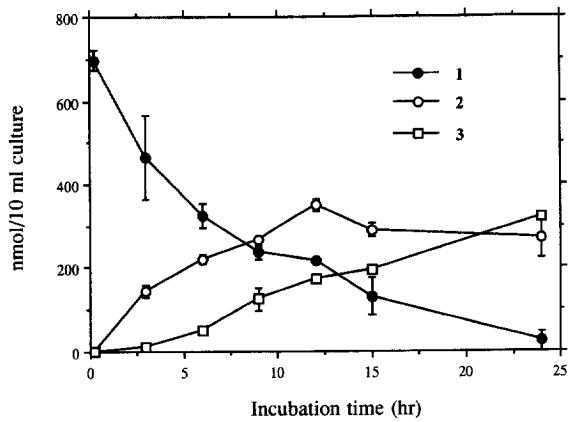


Fig. 1. Time course of 3-oxo-olean-12-en-28-oic acid transformation by *Chaetomium longirostre*: (●) 3-oxo-olean-12-en-28-oic acid (**1**); (○) 3,4-*seco*-olean-12-en-4-ol-3,28-dioic acid (**2**); (□) 3,4-*seco*-olean-12-en-4,21β-diol-3,28-dioic acid (**3**).

and the coupling constants ($J = 11.6$ and 5.0 Hz) of the signal at δ_H 3.48. The stereochemistry of the C-21 position was proved by the ROESY spectrum. All the ^1H and ^{13}C NMR signals of **5** were assigned on the basis of the 2D NMR spectra (Table 1; Experimental). Thus, the structure of **3** was established to be a 21β-hydroxylated derivative of **2**, which has not been reported previously.

Recently, an A-*seco* triterpene acid, 3,4-*seco*-olean-4(23),18-dien-3-oic acid, was obtained from a small

Table 1. ^{13}C NMR (100 MHz, CD_3OD) spectral data for compounds **1**, **2**, **4**, **5** and **6**

C	1	2	4	5	6
1	40.24 (*t*)	35.49 (*t*)	35.70 (*t*)	35.48 (*t*)	39.26 (*t*)
2	35.08 (*t*)	30.01 (*t*)	30.12 (*t*)	29.90 (*t*)	32.74 (*t*)
3	220.46 (*t*)	178.87 (*s*)	177.34 (*s*)	177.15 (*s*)	178.27 (*s*)
4	48.51 (*s*)	76.17 (*s*)	76.27 (*s*)	76.05 (*s*)	88.38 (*s*)
5	56.55 (*d*)	53.06 (*d*)	53.39 (*d*)	53.15 (*d*)	55.90 (*d*)
6	20.74 (*t*)	23.39 (*t*)	23.53 (*t*)	23.29 (*t*)	24.28 (*t*)
7	33.48 (*t*)	33.35 (*t*)	33.49 (*t*)	33.29 (*t*)	33.35 (*t*)
8	40.53 (*s*)	40.41	40.67 (*s*)	40.42 (*s*)	40.60 (*s*)*
9	48.19 (*d*)	40.32 (*d*)	40.58 (*d*)	40.34 (*d*)	48.79 (*d*)
10	37.93 (*s*)	42.19 (*s*)	42.40 (*s*)	42.18 (*s*)	41.13 (*s*)*
11	24.60 (*t*)	24.20 (*t*)	24.41 (*t*)	24.20 (*t*)	24.78 (*t*)
12	123.49 (*d*)	123.87 (*d*)	124.27 (*d*)	124.49 (*d*)	123.07 (*d*)
13	145.25 (*s*)	144.85 (*s*)	144.93 (*s*)	143.67 (*s*)	145.63 (*s*)
14	43.04 (*s*)	43.47 (*s*)	43.61 (*s*)	43.39 (*s*)	43.08 (*s*)
15	28.86 (*t*)	28.88 (*t*)	29.07 (*t*)	28.90 (*t*)	28.95 (*t*)
16	24.08 (*t*)	24.09 (*t*)	24.30 (*t*)	25.38 (*t*)	24.10 (*t*)
17	47.67 (*s*)	47.65 (*s*)	48.37 (*s*)	49.85 (*s*)	47.55 (*s*)
18	42.82 (*d*)	42.69 (*d*)	42.98 (*d*)	42.29 (*d*)	43.21 (*d*)
19	47.21 (*t*)	47.13 (*t*)	47.21 (*t*)	47.72 (*t*)	47.55 (*t*)
20	31.62 (*s*)	31.60 (*s*)	31.79 (*s*)	37.10 (*s*)	31.69 (*s*)
21	34.91 (*t*)	34.93 (*t*)	35.03 (*t*)	73.62 (*d*)	35.18 (*t*)
22	33.80 (*t*)	33.80 (*t*)	33.80 (*t*)	40.98 (*t*)	34.03 (*t*)
23	27.02 (*q*)	32.84 (*q*)*	33.14 (*q*)*	32.90 (*q*)*	32.60 (*q*)
24	21.90 (*q*)	28.34 (*q*)*	28.51 (*q*)*	28.29 (*q*)*	26.12 (*q*)
25	15.52 (*q*)	20.44 (*q*)	20.62 (*q*)	20.39 (*q*)	17.28 (*q*)
26	17.60 (*q*)	17.81 (*q*)	17.88 (*q*)	17.66 (*q*)	17.68 (*q*)
27	26.29 (*q*)	26.09 (*q*)	26.27 (*q*)	25.87 (*q*)	26.10 (*q*)
28	181.77 (*s*)	181.77 (*s*)	180.23 (*s*)	178.71 (*s*)	183.63 (*s*)
29	33.56 (*q*)	33.58 (*q*)	33.71 (*q*)	29.50 (*q*)	33.69 (*q*)
30	23.96 (*q*)	23.99 (*q*)	24.15 (*q*)	17.50 (*q*)	24.10 (*q*)
CO_2Me	—	—	52.24 (*q*)	52.29 (*q*)	—
CO_2Me	—	—	52.39 (*q*)	52.29 (*q*)	—

*Assignments may be reversed in each vertical column.

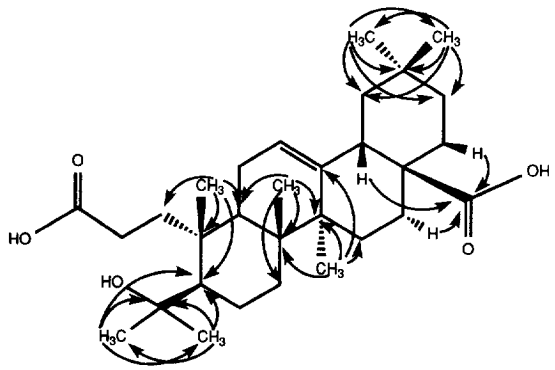


Fig. 2. The COLOC correlations for compound **2**.

perennial shrub *Vahilia capensis*, which is a medicinal plant that has been used widely in Botswana to cure sore eyes [3]. Two A-*seco* triterpene acids named secobryononic acid and secoisobryononic acid were obtained from the stem bark of *Sandricum koetjape* [4]. Local people in Indonesia use this plant against colic and leucorrhoea [5]. Although the biological activities of these 3,4-*seco*-type triterpenes are unknown, antibacterial activities have been shown on 3,4-*seco* acids derived from eburicoic acid [6] and lupane [7]. Laskin *et al.* [6] reported that the fungus *Glomerella fusarioides* cleaved the ring-A of eburicoic acid. We used eburica-8,24(24′)-dien-3-one as a substrate of *C. longirostre*, but the fungus did not cleave the ring-A of the steroid (data not shown). This result suggests that the enzyme of *G. fusarioides* which is responsible for the ring-A cleavage of eburicoic acid is different from the enzyme of *C. longirostre*.

Several preliminary experiments were conducted to examine the properties of the ring-A cleavage enzyme system. A cell-free preparation was obtained from mycelium of *C. longirostre* as described in the Experimental section. The cell-free preparation conducted the ring-A cleavage when supplemented with NADPH, but not with other pyridine nucleotides (Table 2). The enzyme activity was completely recovered in the supernatant of the ultracentrifuged (100 000 *g*, 30 min) cell-free preparation (Table 2). These findings indicated that the enzymes concerned with the ring-A cleavage are soluble and require NADPH as a cofactor. In an attempt to enhance the enzymes in the cell of *C. longirostre*, compound **1** was added to the growth culture at 8 hr before harvest. However, the ring-A cleavage activity was not increased in the treated mycelium, which suggests that the ring-A cleavage enzymes are not inducible.

Because the oxidation of ketones to lactones or esters was conducted by enzymic Baeyer–Villiger oxidation [8-11], the mechanism involving the ring-A cleavage of **1** by *C. longirostre* might be an oxygenation of the compound into the intermediate lactone as a first step, followed by hydrolysis. We found another metabolite **6** in a reaction mixture which contained **1**, NADPH and partially purified enzyme solution obtained from *C. longirostre*. The metabolite was isolated by preparative TLC and the structure was elucidated from the spectral data. The molecular formula of **6** was determined as $C_{30}H_{46}O_4$ from its HR mass spectrum (Experimental), indicating that an oxygen was introduced into **1**. The signals in the ^1H and ^{13}C NMR spectra of **6** have been assigned on the basis of the HSQC and by comparison with signals of **1**. The signals of H-2α and H-2β in the ^1H NMR spectrum of **6** appeared downfield (δ_H 2.57–2.91) compared to the signals (δ_H 2.34–2.60) of **1**. The conspicuous differences between the ^{13}C NMR spectra of **6** and **1** were the signals of C-3 (δ 178.3 and 220.5) and C-4 (δ 88.4 and 48.5). These results indicated that the metabolite **6** was a compound which has an oxygen between C-3 and C-4 of the substrate **1**. Thus, the structure of **6** was established to be A-homo-3*a*-oxa-olean-12-en-3-one-28-oic acid, which has not been reported previously. This finding supports a possible ring-A cleavage mechanism described above. According to these results, a possible transformation sequence of **1** by *C. longirostre* is shown in Scheme 1. In view of the result of the time-course experiment showing that a 3-hr time lag was observed between the accumulation

Table 2. Coenzyme requirement of 3-oxo-olean-12-en-28-oic acid converting enzymes*

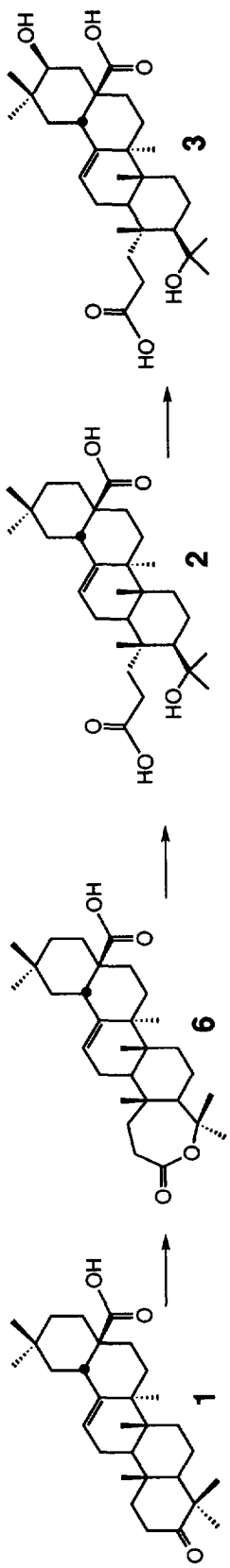
Crude enzyme preparation	Coenzyme (1 μmol)	Compound **2** (nmol)	Compound **3** (nmol)
Cell-free	None	1.8	ND
Cell-free	NADPH	8.2	ND
Cell-free	NADH	1.6	ND
Cell-free	FAD	0.8	ND
Cell-free	FMN	0.9	ND
Soluble fraction†	NADPH	8.0	ND
Microsome fraction‡	NADPH	ND§	ND

*The assay system contained 1 ml of cell-free preparation and 100 nmol of compound **1** with or without 1 μmol of reduced pyridine nucleotides. The reaction mixture was incubated for 2 hr at 30°.

†Supernatant of ultracentrifuged cell-free preparation (100 000 *g*, 30 min).

‡Precipitate of ultracentrifuged cell-free preparation (100 000 *g*, 30 min).

§Not detected.



Scheme 1. Possible transformation pathway of 3-oxo-olean-12-en-28-oic acid by *Chaetomium longirostre*.

of **2** and **3** (Fig. 1), the hydroxylation at C-21β may occur after the cleavage of ring-A.

EXPERIMENTAL

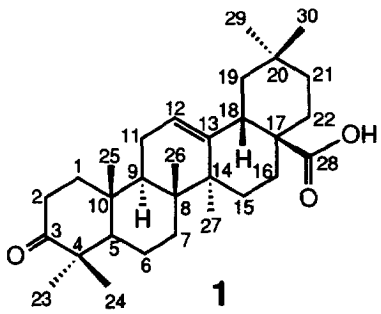
Mps were determined on a Yanagimoto micro melting-point apparatus. IR spectra were measured with a JASCO FT IR-300 spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL JNM A-400 spectrometer. Chemical shifts are given in ppm with TMS as int. standard, and coupling constants (J) are given in Hz. LSIMS (*m*-NBA, Cs^+ ion) were recorded on a HITACHI M-90 spectrometer. CC: silica gel 60 (70–230 mesh), Diaion HP-20. Prep. TLC: silica gel 60 F_{254} (0.5 mm). HPLC: Develosil ODS-HG-5 column (150 × 4.6 mm i.d.). Prep. HPLC: Develosil ODS-HG-5 column (250 × 20 mm i.d.) equipped with a guard column (50 × 20 mm i.d.).

Chemicals. NADPH and NADH were purchased from Kojin. EDTA, dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma. FAD, FMN and oleanolic acid were purchased from Nacalai Tesque. All other chemicals were of analyt. grade.

Organisms and growth conditions. Chaetomium longirostre RF-1095 was provided by Dr Y. Kawamura, Shionogi Research Laboratories, Shionogi & Co., Ltd, Japan, and was maintained on potato–dextrose agar medium (PDA, Nissui) as a slant. Seed culture was grown in a 500-ml flask containing 10 ml potato–dextrose broth medium (PDB; 39.5 g of Nissui PDA powder was suspended in 1 l H_2O and the containing agar was removed by filtration). After incubation at 24° on a reciprocal shaker (120 rpm, 7 cm stroke) for 3 days, the culture was transferred in 4-l culture flasks (Hario) containing 3 l PDB and incubated for 38 hr under aeration by bubbling and stirring. The cells were harvested and washed with H_2O by filtration giving 75 g fr. wt mycelia.

Time course experiment of 3-*oxo-olean*-12-*en*-28-*oic acid* (**1**) *transformation by* C. longirostre. Mycelia (50 g fr. wt.) was suspended in 1 l H_2O containing 50 mg **1** (110 μM) and was evenly dispensed into 5 flasks followed by incubation at 24° on a reciprocal shaker (120 rpm, 7 cm stroke). To the portions (10 ml) of culture, 200 μg oleanolic acid was added as int. standard and the culture was extracted with 10 ml EtOAc. The organic layer was dried by passage through Na_2SO_4, evapd, and then subjected to HPLC. The amount of each compound was estimated using a standard curve for the purified compounds.

Isolation of metabolites. Mycelia (50 g fr. wt.) was suspended in 1 l H_2O containing 50 mg **1** (110 μM) and were evenly dispensed into 5 flasks followed by incubation at 24° on a reciprocal shaker (120 rpm, 7 cm stroke). After incubation for 15 hr, the culture (1 l) was filtered through filter paper (No. 1, Toyo Roshi). The filtrate was used to isolate the transformed products since most of the metabolites were detected in the filtrate. The transformed products were adsorbed in a



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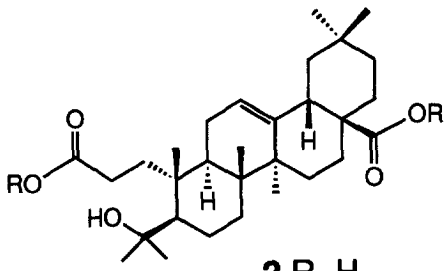
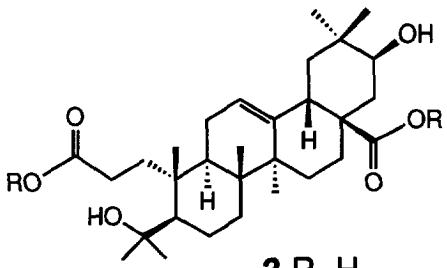
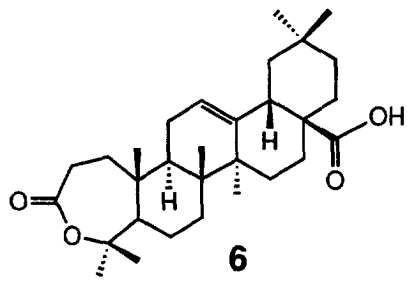


2 R=H
4 R=Me



3 R=H
5 R=Me



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diaion HP-20 column (100 ml bed vol.) by passage through the filtrate, and eluted with MeOH. The eluate was evapd to dryness *in vacuo* and residual materials were subjected to prep. TLC. They were then purified by prep. HPLC. Elution with CH_3CN–H_2O (3:1) afforded **2** (15 mg) and **3** (14 mg).

Conversion of 3-oxo-olean-12-en-28-oic acid by cell-free preparation of C. longirostre. Mycelia (150 g fr. wt.) was suspended in 120 ml 20 mM Tris–HCl buffer (pH 7.5), containing 0.1 mM EDTA, 1 mM DTT and 1 mM PMSF. The cells were ruptured with the aid of a glass-bead homogenizer (Biospec Products) containing 300 g glass beads (diam. 0.5 mm). The cells were disrupted ×7 for 60 sec each at 180-sec intervals while the outer jacket around the vessel was filled with ice–water. Glass beads were removed by filtration and cell debris was removed by centrifugation at 3000 *g* for 20 min. The resulting supernatant (cell-free prep.) was used for enzymic conversion of **1**. The cell-free prep. (1 ml) was added with 1 μmol reduced pyridine nucleotide and 0.1 μmol **1**, and then incubated for 2 hr at 30°. The products were extracted with 10 ml EtOAc and then subjected to HPLC.

3-Oxo-olean-12-en-28-oic acid (**1**). A soln of 5 g oleanolic acid in 328 ml Me_2CO–$CHCl_3$ (16:5) was added with 7 ml Jones reagent (13.7 g CrO_3, 11.6 ml H_2SO_4 in 30 ml H_2O) and was allowed to stand in ice for 30 mn. The reaction was stopped by addition of 2 ml 2-PrOH and 25 ml H_2O followed by extraction of the products with EtOAc. The product was purified by silica gel chromatography (70–230 mesh silica gel, 100 g; mobile phase, $CHCl_3$) giving 4.4 g **1**: mp 170–173°; $[\alpha]_D^{25}$ +96.6° (MeOH, *c* 0.89). IR ν_{max}^{KBr} cm^{-1}: 3335, 3200, 2944, 2863, 2647, 1724, 1708, 1460, 1386, 1366. ^1H NMR (CD_3OD): δ 0.87 (3H, *s*, H-26), 0.90 (3H, *s*, H-29), 0.94 (3H, *s*, H-30), 1.04 (3H, *s*, H-24), 1.06 (3H, *s*, H-25), 1.07 (3H, *s*, H-23), 1.17 (3H, *s*, H-27), 2.37 (1H, *ddd*, J = 3.7, 7.0, 16.1 Hz, H-2α), 2.56 (1H, *ddd*, J = 7.4, 10.8, 16.1 Hz, H-2β), 2.86 (1H, *dd*, J = 3.9, 13.9 Hz, H-18), 5.26 (1H, *t*, J = 3.6 Hz, H-12). ^{13}C NMR (CD_3OD): Table 1. LSIMS *m/z*: 909 $[2M+H]^+$, 455 $[M+H]^+$, 437 $[MH-H_2O]^+$, 409 $[M-CO_2H]^+$. HR-LSIMS: $[M+H]^+$ at *m/z* 455.3523 ($C_{30}H_{47}O_3$ requires 455.3523).

Compound **2**. Mp 179–182°; $[\alpha]_D^{25}$ +70.2° (MeOH, *c*1.01). IR ν_{max}^{KBr} cm^{-1}: 3479, 3153, 2948, 2865, 2642, 1702, 1461, 1386. ^1H NMR (CD_3OD): δ 0.84 (3H, *s*, H-26), 0.91 (3H, *s*, H-29), 0.94 (3H, *s*, H-30), 1.08 (3H, *s*, H-25), 1.17 (3H, *s*, H-27), 1.25 (3H, *s*, H-23 or H-24), 1.27 (3H, *s*, H-23 or H-24), 2.01 (1H, *dt*, J = 13.4, 3.8 Hz, H-16α), 2.15 (1H, *ddd*, J = 15.4, 11.2, 4.5 Hz, H-2), 2.25 (1H, *ddd*, J = 14.4, 11.2, 4.5 Hz, H-1), 2.51 (1H, *ddd*, J = 15.4, 11.2, 4.5 Hz, H-2), 2.87 (1H, *dd*, J = 13.8, 4.0 Hz, H-18), 5.28 (1H, *t*, J = 3.5 Hz, H-12). ^{13}C NMR (CD_3OD): Table 1. LSIMS *m/z*: 1043 $[2M+3Na-2H]^+$, 1021 $[2M+2Na-H]^+$, 999 $[2M+Na]^+$, 533 $[M+2Na-H]^+$, 511 $[M+Na]^+$, 493 $[M+Na-H_2O]^+$, 471 $[MH-H_2O]^+$, 453 $[MH-2H_2O]^+$. HR-LSIMS: $[M+Na]^+$ at *m/z* 511.3394 ($C_{30}H_{48}O_5Na$ requires 511.3396).

Compound **3**. Mp 250–255°. IR ν_{max}^{KBr} cm^{-1}: 3312, 2948, 2874, 2586, 1701, 1543, 1458, 1388. LSIMS *m/z*: 1053 $[2M+2Na-H]^+$, 1031 $[2M+Na]^+$, 549 $[M+2Na-H]^+$, 527 $[M+Na]^+$, 509 $[M+Na-$

$H_2O]^+$, 487 $[MH - H_2O]^+$. HR-LSIMS: $[M + Na]^+$ at m/z 527.3356 ($C_{30}H_{48}O_6Na$ requires 527.3346).

Compound **4**. Compound **2** (10 mg) was dissolved in 1 ml MeOH followed by addition of a Et_2O soln of CH_2N_2 (2.5%, 1.2 ml), and the mixt. was kept at ambient temp. for 15 min. The product was purified by prep. HPLC to give a solid (8.7 mg): mp 108–111°; $[\alpha]_D^{25}$ +54.9° (MeOH, *c* 0.39). IR ν_{max}^{KBr} cm^{-1}: 3542, 2948, 2856, 1724, 1457, 1438, 1384, 1363. ^1H NMR (CD_3OD): δ 0.77 (3H, *s*, H-26), 0.91 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 1.07 (3H, *s*, H-25), 1.15 (3H, *s*, H-27), 1.23 (3H, *s*, H-23 or H-24), 1.25 (3H, *s*, H-23 or H-24), 2.01 (1H, *dt*, J = 14.8, 4.0 Hz, H-16α), 2.17 (1H, *ddd*, J = 15.1, 11.3, 4.5 Hz, H-2), 2.28 (1H, *ddd*, J = 14.2, 11.3, 4.5 Hz, H-1), 2.53 (1H, *ddd*, J = 15.1, 11.3, 4.5 Hz, H-2), 2.88 (1H, *dd*, J = 14.2, 4.2 Hz, H-18), 3.61 (3H, *s*, OMe-3 or -28), 3.62 (3H, *s*, OMe-3 or -28), 5.27 (1H, *t*, J = 3.6 Hz, H-12). ^{13}C NMR (CD_3OD): Table 1. LSIMS m/z: 1055 $[2M + Na]^+$, 1033 $[2M + H]^+$, 539 $[M + Na]^+$, 499 $[MH - H_2O]^+$. HR-LSIMS: $[M + Na]^+$ at m/z 539.3708 ($C_{32}H_{52}O_5Na$ requires 539.3709).

Compound **5**. Compound **3** (4 mg) was dissolved in 0.5 ml MeOH followed by addition of a Et_2O soln of CH_2N_2 (2.5%, 0.6 ml), and the mixt. was kept at ambient temp. for 15 min. The products were purified by prep. HPLC to give a solid (3.1 mg): mp 148–152°; $[\alpha]_D^{25}$ +68.5° (MeOH, *c* 0.27); IR ν_{max}^{KBr} cm^{-1}: 3545, 3339, 2949, 2874, 1726, 1561, 1458, 1435, 1384. ^1H NMR (CD_3OD): δ 0.77 (3H, *s*, H-26), 0.89 (3H, *s*, H-30), 0.96 (3H, *s*, H-29), 1.08 (3H, *s*, H-25), 1.15 (3H, *s*, H-27), 1.24 (3H, *s*, H-23 or H-24), 1.25 (3H, *s*, H-23 or H-24), 2.02 (1H, *dt*, J = 13.6, 4.1 Hz, H-16α), 2.18 (1H, *ddd*, J = 15.3, 11.5, 4.6 Hz, H-2), 2.29 (1H, *ddd*, J = 15.9, 11.5, 4.6 Hz, H-1), 2.54 (1H, *ddd*, J = 15.3, 11.5, 4.6 Hz, H-2), 2.92 (1H, *dd*, J = 13.5, 3.5 Hz, H-18), 3.48 (1H, *dd*, J = 11.6, 5.0 Hz, H-21α), 3.62 (3H, *s*, OMe-3 or -28), 3.63 (3H, *s*, OMe-3 or -28), 5.30 (1H, *t*, J = 3.5 Hz, H-12). ^{13}C NMR (CD_3OD): Table 1. LSIMS m/z: 555 $[M + Na]^+$, 515 $[MH - H_2O]^+$. HR-LSIMS: $[M + Na]^+$ at m/z 555.3659 ($C_{32}H_{52}O_6Na$ requires 555.3659).

Compound **6**. Partially purified enzyme soln (3 ml; 14 mg protein) isolated from the cell-free prep. was added with 7 μmol NADPH and 7 μmol **1** and then incubated for 4 hr at 30°. The metabolite was extracted with 10 ml EtOAc and then isolated by prep. TLC giving 1.1 mg **6**: IR ν_{max}^{KBr} cm^{-1}: 2930, 2865, 2554, 1919, 1725, 1557, 1461, 1390. ^1H NMR (CD_3OD): δ 0.89 (3H, *s*, H-26), 0.90 (3H, *s*, H-29), 0.94 (3H, *s*, H-30), 1.15 (3H, *s*, H-25), 1.16 (3H, *s*, H-27), 1.43 (3H, *s*, H-24), 1.47 (3H, *s*, H-23), 2.57 – 2.91 (2H, *m*, H-2α, H-2β), 2.89 (1*H*, *dd*, J = 14.2, 4.4 Hz, H-18), 5.26 (1H, *t*, J = 3.5 Hz, H-12). ^{13}C NMR (CD_3OD): Table 1. LSIMS m/z: 1007 $[2M + 3Na - 2H]^+$, 985 $[2M + 2Na - H]^+$, 963 $[2M + Na]^+$, 515 $[M + 2Na - H]^+$, 493 $[M + Na]^+$. HR-LSIMS: $[M + Na]^+$ at m/z 493.3287 ($C_{30}H_{46}O_4Na$ requires 493.3291).

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