Biotransformation of Cycloartane-Type Triterpenes by the Fungus *Glomerella fusarioides*

Toshihiro Akihisa,*,† Kenji Watanabe,† Risa Yoneima,† Takashi Suzuki,§ and Yumiko Kimura§

College of Science and Technology, Nihon University, 1-8 Kanda Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan, and College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

Received November 4, 2005

Biotransformation of three cycloartane-type triterpenes, cycloartenol (**1**), 24-methylenecycloartanol (**2**), and cycloartenone (**3**), by the fungus *Glomerella fusarioides* was studied. Compound **1** was converted to **3**, cycloart-25-ene-3β,24-diol (**4**), and cycloartane-3β,24,25-triol (**5**). Compound **2** was metabolized to cycloeucalenol (**6**) and two new compounds, 24-methylcycloartane-3β,24,24^1-triol (**7**) and 24^1-methoxy-24-methylcycloartane-3β,24-diol (**8**). Compound **3** was converted into two new metabolites, 4α,4β,14α-trimethyl-9β,19-cyclopregnane-3,20-dione (**9**) and 25-hydroxy-24-methoxycycloartan-3-one (**14**), and four known compounds, viz., cycloartane-3,24-dione (**10**), 24-hydroxycycloart-25-en-3-one (**11**), (23*E*)-25-hydroxycycloart-23-en-3-one (**12**), and 24,25-dihydroxycycloartan-3-one (**13**). The structures of four new metabolites, **7**, **8**, **9**, and **14**, were established by spectroscopic methods.

In the course of our search for potential antitumor-promoters (cancer chemopreventive agents),[1,2] we have investigated fungal transformation products of two lupane-type triterpenes, betulin and betulonic acid,[3] and an *ent*-beyerane-type diterpene, isosteviol.[4] Several hydroxylated metabolites were obtained that might be more potent than the substrates.[1,2] Cycloartane-type triterpenes such as cycloartenol (**1**) and 24-methylenecycloartanol (**2**) occur abundantly in higher plants, especially as the feruloyl esters in rice bran.[5,6] They are considered to be good resources to develop potent antitumor-promoters.[1] In this paper we report the fungal transformation of compounds **1**−**3**. Cycloartenone (**3**) was semisynthesized from **1** by chemical oxidation. Since *Glomerella fusarioides* is known to transform eburicoic acid [3β-hydroxy-24-methyllanosta-8,24(24^1)-dien-21-oic acid], a lanostane-type tetracyclic triterpene, to its 3,4-*seco*-derivatives efficiently,[7] it was selected for the transformation of **1**, **2**, and **3**.

Results and Discussion

To evaluate the ability of *G. fusarioides* to transform compounds **1**, **2**, and **3**, preliminary experiments were conducted in 500 mL flasks containing 5-day-old cultures of the fungus. After addition of the substrates to the mycelia of the fungus suspended in water, the fermentation was continued for 10 more days, after which metabolites in the ethyl acetate (EtOAc) extract of the broth were detected by TLC. The metabolites were not present in the control experiments undertaken without either the mycelium or the substrate.

^{1}H and ^{13}C NMR spectra along with DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments were used to elucidate the structures of the four new metabolites, **7**, **8**, **9**, and **14**. Seven known metabolites, **4**−**6** and **10**−**13**, were identified by comparison of spectroscopic data with that published in the literature.

Incubation of **1** with *G. fusarioides* on a preparative scale resulted in the formation of **3** (2.2% yield based on weight relative to starting material), cycloart-25-ene-3β,24-diol (**4**; 0.8% yield),[8] and cycloartane-3β,24,25-triol (**5**; 1.0% yield)[9] (unmetabolized **1**: 60.8%).

Incubation of **2** afforded three metabolites, cycloeucalenol (**6**; 1.9% yield),[10] 24-methylcycloartane-3β,24,24^1-triol (**7**; 0.7% yield), and 24^1-methoxy-24-methylcycloartane-3β,24-diol (**8**; 2.6% yield). The molecular formula of **7** was determined to be $C_{31}H_{54}O_3$ by HREIMS ([M]$^+$, *m*/*z* 474.4064). The diagnostic MS fragment ion at *m*/*z* 315 [M − $C_9H_{19}O_2$ (side-chain)]$^+$ suggested that **7** possesses a C_9-saturated side-chain with two hydroxyl groups. The ^{1}H signals of C-21 (δ_H 0.90, d, J = 7.3 Hz), C-26 (δ_H 0.93, d, J = 6.9 Hz), and C-27 (δ_H 0.94, d, J = 6.9 Hz) methyl groups were observed as doublets, suggesting that the hydroxyl groups were located not at C-20 and C-25 but at other carbons most probably C-24 (δ_C 76.1, s) and C-24^1 (δ_C 65.8/65.9, t) probably as a 1,2-glycol functionality. This was supported by an HMBC experiment, which provided cross-correlations for H-24^1 (with C-23, C-24, and C-25) and H-25 (with C-24, C-24^1, C-26, and C-27). The ^{13}C and ^{1}H NMR signals for the ring system of **7** (Table 1) were very similar to those of **2**. This determined the structure of compound **7** as 24-methylcycloartane-3β,24,24^1-triol. Some of the ^{13}C and ^{1}H signals relevant to the side-chain moiety of compound **7** (Table 1) were double-peaks, which indicated that **7** is a mixture of C-24 stereoisomers.

Compound **8**, [M]$^+$, *m*/*z* 488.4228 ($C_{32}H_{56}O_3$) by HREIMS, appeared to be an *O*-methyl derivative of compound **7** since the ^{13}C and ^{1}H NMR signals were very similar to those of **7**, whereas **8** displayed signals indicative of a methoxyl group (δ_C 49.3/49.4, q; δ_H 3.22/3.23, 3H, s) (Table 1). The methoxyl group was shown to be present as a CH_2OMe by the fragment ion at *m*/*z* 428 [M − CH_2OMe − Me]$^+$ in the EIMS. The HMBC spectrum of **8** exhibited cross correlations for H-24^1 (with C-23, C-25, and C-O*Me*) and H-O*Me* (with C-24), suggesting that the methoxyl group was located at C-24^1. On the basis of these observations, metabolite **8** was identified as 24^1-methoxy-24-methylcycloartane-3β,24-diol.

Incubation of **3** afforded a mixture of metabolites. Isolated components were 4α,4β,14α-trimethyl-9β,19-cyclopregnane-3,20-dione (**9**; 0.7% yield), cycloartane-3,24-dione (**10**; 0.6% yield),[11] 24-hydroxycycloart-25-en-3-one (**11**; 1.8% yield),[8] (23*E*)-25-hydroxycycloart-23-en-3-one (**12**; 0.8% yield),[12,13] 24,25-dihydroxycycloartan-3-one (**13**; 2.0% yield),[9,14] and 25-hydroxy-24-methoxycycloartan-3-one (**14**; 0.8% yield). Compounds **9** and **14** are new; **10**−**13** are known compounds.

Metabolite **9** had the elemental composition $C_{24}H_{36}O_2$ (HREIMS: [M]$^+$, *m*/*z* 356.2714). Comparison of the ^{13}C and ^{1}H NMR data of **9** (Table 1) with those of **3** and progesterone[15] suggested that **9** possesses a 3-oxo-cycloartane ring system and a pregnane side-chain. The presence of the diagnostic fragment ion at *m*/*z* 313 [M − C_2H_3O (side-chain)]$^+$ in the EIMS and cross-correlations for H-21 (with C-17 and C-20) and H-17 (with C-13, C-17, C-18, and C-20) in the HMBC spectrum of **3** supported the proposed structure. The combined evidence confirmed that metabolite **9** was 4α,4β,14α-trimethyl-9β,19-cyclopregnane-3,20-dione.

* To whom correspondence should be addressed. Tel: +81-3-3259-0806. Fax: +81-3-3293-7572. E-mail: akihisa@chem.cst.nihon-u.ac.jp.
† College of Science and Technology, Nihon University.
§ College of Pharmacy, Nihon University.

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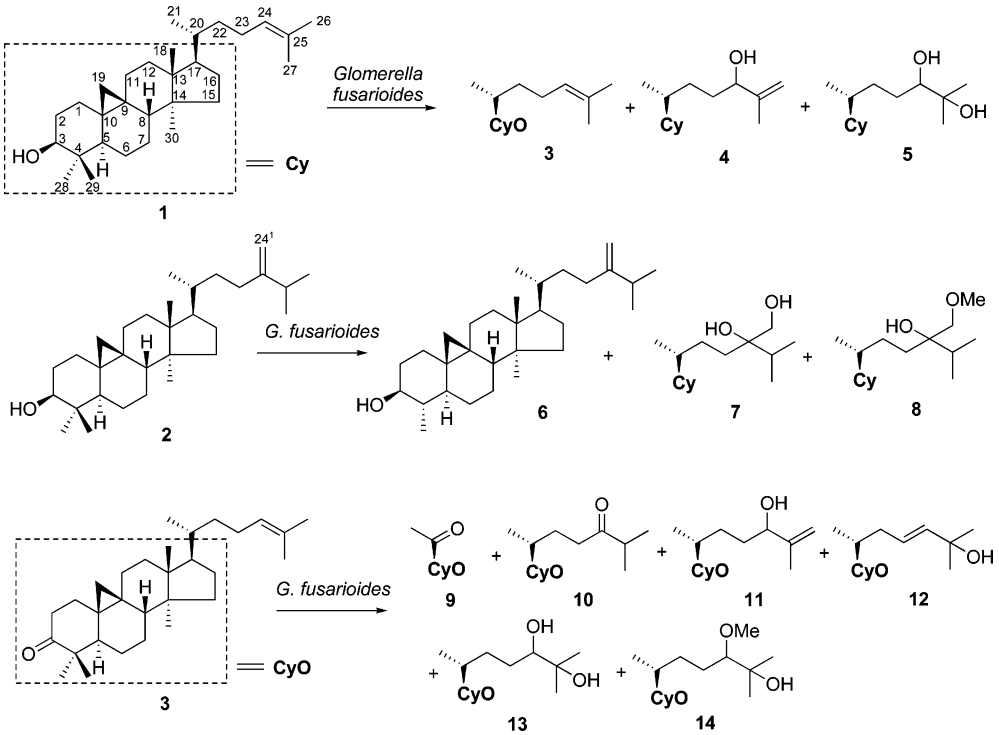

Figure 1. Structures of cycloartenol (**1**), 24-methylenecycloartanol (**2**), cycloartenone (**3**), and metabolites **4**−**14**.

The molecular formula of metabolite **14** was $C_{31}H_{52}O_3$ (HREIMS: $[M]^+$, *m/z* 472.3908). The diagnostic fragment ions at *m/z* 354 $[M - C_6H_{13}O_2$ (cleavage of C-22−C-23 bond) $- H]^+$ and 313 $[M - C_9H_{19}O_2$ (side-chain)$]^+$ in the EIMS and *O*-methyl signals (δ_C 49.0/49.1, q; δ_H 3.23/3.24, 3H, s) in the NMR spectra (Table 1) of **14** suggested the presence of a methoxyl group at C-24 or C-25 and a hydroxyl group at C-25 or C-24 in the C_8 side-chain.[16] Analysis of the $^1H-^1H$ COSY spectrum and observation of cross-correlations for H-24 (with C-25 and 24-O*Me*) and 24-O*Me* (with C-24) in the HMBC spectrum of **14** suggested that it possesses a 24-methoxy-25-hydroxy side-chain. The ^{13}C and ^1H NMR signals for the ring system of **14** were almost superimposable with those of **3**. Thus, the structure of metabolite **14** had to be 25-hydroxy-24-methoxycycloartan-3-one.

As discussed above, some of the ^{13}C and ^1H NMR signals of **7**, and of **8** and **14** (Table 1), and of **4**, **5**, **11**, and **13**, were observed as double-peaks, which suggested mixtures of C-24 stereoisomers. Isolation of individual stereoisomers[16] was not undertaken due to an insufficient amount of metabolites obtained.

Thus our results show that biotransformation of three cycloartane-type triterpenes, **1**−**3**, by the filamentous fungus *G. fusarioides* yielded metabolites with C-3 hydroxyl group-oxidized (**3**), side-chain-oxygenated (**4**, **5**, **7**, **8**, **10**−**14**), C-4 demethylated (**6**), and side-chain-degraded (**9**) structures, although in low transformation rates. All of the C-24-hydroxylated (**4**, **5**, **7**, **8**, **11**, and **13**) and -methoxylated (**14**) metabolites obtained were mixtures of C-24 stereoisomers with almost equal proportions of the isomers. Such nonstereospecific hydroxylation has been observed also in the biotransformation of acyclic sesquiterpenes by *G. cingulata*.[17]

Demethylation of compound **2** at C-4 to give cycloeucalenol (**6**) is one of the possible biosynthetic sequences leading to sterols in algae and higher plants.[18] This study has shown that *G. cingulata* can also use **2** as a substrate for C-4 demethylation to give **6**, which can be further metabolized to give obtusifoliol [4α,14α,24-trimethylcholesta-8,24(24^1)-dien-3β-ol] and other sterols.

Transformation of **2** by *G. fusarioides* afforded two new metabolites, **7** and **8**, possessing a 1,2-glycol group at C-24 in the side-chain. A triterpene possessing a 1,2-glycol group at C-24 has previously been synthesized from a 24-methylenated lanostane-type compound by OsO_4 oxidation.[7] Compound **3** was converted by *G. fusarioides* into a new side-chain-degraded metabolite, **9**, which possessed a pregnane-type C_2 side-chain, along with five side-chain-oxygenated metabolites, **10**−**14**, of which **14** was a new compound. This seems to be the first example of bioconversion of a cycloartane-type triterpene into one with a pregnane-type side-chain. Some sterols have previously been reported to be metabolized into progesterone and some other pregnanes by fungi such as *Mycobacterium aurum*.[19] Two methoxylated metabolites, **8** and **14**, appear to be formed from their 1,2-glycol homologues, **7** and **13**, respectively, by methylation of one of the hydroxyl groups of the glycol functionality. This kind of biomethylation has recently been observed for three flavonoids possessing catechol functional groups, quercetin, fisetin, and catechin.[20] We are now in the process of evaluating the antitumor-promoting activities of the metabolites (**4**−**14**) of cycloartanes **1**−**3** (vide supra).

Experimental Section

General Experimental Procedures. Crystallizations were performed from MeOH, and melting points (uncorrected) were determined using a Yanagimoto micromelting point apparatus. Optical rotations were measured on a JASCO P-1030 polarimeter in $CHCl_3$ at 25 °C. IR spectra were obtained on a JASCO FTIR-300E spectrometer in KBr disks. NMR spectra were recorded with a JEOL ECX-500 spectrometer at 500 MHz (^1H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$. Chemical shifts are in δ (ppm) relative to tetramethylsilane (TMS). EIMS and HREIMS were recorded on a JEOL JMS-BU20 spectrometer (70 eV, direct inlet system). Silica gel (Kieselgel 60, 230−400 mesh, Merck) was used for open column chromatography. Column chromatography fractions were monitored by TLC (silica gel 60 F_{254}, Merck). Reversed-phase preparative HPLC (with refractive index detector) was carried out on a 25 cm × 10 mm i.d. C_{18} column (Pegasil ODS II column, 5 μm; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C [eluent: MeOH−AcOH (100:1) (HPLC I) or MeOH−H_2O−AcOH (95:5:1) (HPLC II) at a flow rate of 3.0 mL/min].

Chemicals and Materials. Cycloartenol (**1**) and 24-methylene-cycloartanol (**2**) were prepared from γ-oryzanol (a mixture of the ferulates of triterpene alcohols and phytosterols derived from rice bran; supplied by Wako Pure Chemicals Co., Osaka, Japan) by the method described in the literature.[5] Cycloartenone (**3**)[21] was derived from **1** by

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectroscopic Data ($CDCl_3$) for Four Cycloartane-Type Triterpenes (**7**, **8**, **9**, **14**)

	7			**8**			**9**			**14**		
C no.	δ_C		δ_H[b]	δ_C	δ_H[b]		δ_C	δ_H[b]		δ_C	δ_H[b]	
1	32.0	t	1.57 (α), 1.25 (*β*)	32.0	t	1.57 (α), 1.24 (*β*)	33.4	t	1.88 (α, dt, 4.3, 14.0), 1.55 (*β*)	33.3	t	1.85 (α, dt, 4.3, 13.4), 1.56 (*β*)
2	30.4	t	1.76 (α) 1.59 (*β*)	30.4	t	1.75 (α) 1.56 (*β*)	37.4	t	2.31 (α, ddd, 3.0, 4.3, 14.0) 2.72 (*β*, dt, 6.4, 14.0)	37.5	t	2.30 (α, ddd, 2.4, 4.3, 13.7) 2.70 (*β*, dt, 6.7, 13.7)
3	78.8	d	3.28 (dd, 4.5, 11.3)	78.8	d	3.29 (dd, 4.1, 11.0)	216.4	s		213.0	s	
4	40.5	s		40.5	s		50.2	s		50.2	s	
5	47.1	d	1.30 (dd, 4.5, 8.6)	47.1	d	1.30 (dd, 3.8, 8.9)	48.2	d	1.72 (dd, 4.6, 12.5)	48.4	d	1.72 (dd, 4.3, 12.2)
6	21.1	t	1.60 (α), 0.78 (b, dq, 2.7, 12.7)	21.1	t	1.61 (α), 0.79 (*β*, dq, 2.7, 12.7)	21.3	t	1.57 (α), 0.98 (*β*, dq, 2.8, 12.5)	21.5	t	1.55 (α), 0.95 (*β*)
7	26.0	t	1.07 (α), 1.32 (*β*)	26.0	t	1.06 (α), 1.33 (*β*)	25.9	t	1.16 (α, dq, 2.8, 12.5), 1.43 (*β*)	25.9	t	1.13 (α), 1.38 (*β*)
8	48.0	d	1.50 (dd, 4.8, 12.8)	48.0	d	1.51 (dd, 4.8, 12.0)	47.1	d	1.62 (dd, 4.9, 12.5)	47.9	d	1.58 (dd, 3.7, 11.8)
9	20.0	s		20.0	s		20.7	s		21.1	s	
10	26.1	s		26.1	s		26.2	s		26.0	s	
11	26.4	t	1.98 (α, ddd, 7.6, 7.6, 8.7), 1.10 (*β*)	26.5	t	1.98 (α), 1.11 (*β*)	26.5	t	2.14 (α), 1.30 (*β*, ddd, 3.7, 10.4, 14.3)	26.8	t	2.05 (α), 1.17 (*β*)
12	32.9	t	1.62 (2H)	32.9	t	1.61 (2H)	31.9	t	1.96 (α), 1.77 (*β*, ddd, 5.5, 10.4, 13.3)	32.8	t	1.67 (2H)
13	45.3	s		45.3	s		47.0	s		45.3	s	
14	48.8	s		48.9	s		49.3	s		48.7	s	
15	35.5	t	1.29 (2H)	35.6	t	1.29 (2H)	35.5	t	1.42 (2H)	35.6	t	1.32 (2H)
16	28.2	t	1.92 (α), 1.29 (*β*)	28.3	t	1.94 (α), 1.30 (*β*)	21.9	t	1.68 (α), 2.32 (*β*)	28.1	t	1.95 (α), 1.32 (*β*)
17	52.1/52.2	d	1.61	52.3	d	1.60	61.1	d	2.98 (t, 9.2)	52.4/52.5	d	1.60
18	18.0	q	0.97 (s)	18.0	q	0.97 (s)	19.9	q	0.93 (s)	18.1	q	1.00 (s)
19	29.9	t	0.33 (1H, d, 4.4, *exo*) 0.55 (1H, d, 4.4, *endo*)	29.9	t	0.33 (1H, d, 4.1, *exo*) 0.55 (1H, d, 4.1, *endo*)	29.4	t	0.57 (1H, d, 4.4, *exo*) 0.82 (1H, d, 4.4, *endo*)	29.6	t	0.57 (1H, d, 4.2, *exo*) 0.79 (1H, d, 4.2, *endo*)
20	36.6/36.7	d	1.38	37.0/37.2	d	1.33	210.3	s		36.0	d	1.30
21	19.3	q	0.90 (d, 7.3)	18.3/18.4	q	0.90 (d, 6.5)	31.2	q	2.12 (s)	18.2/18.4	q	0.89/0.90 (d, 6.4)
22	30.5/30.7	t	1.36, 1.62	28.9	t	1.36, 1.57				33.7	t	1.25, 1.52
23	29.2/29.3	t	1.05, 1.40	29.4/29.5	t	1.05, 1.52				28.2	t	1.16, 1.41
24	76.1	s		79.8/79.9	s					77.6	d	3.37/3.42 (br d, 10.0)
25	32.5/32.6	d	1.87 (sept. 6.9)	32.1	d	1.95 (sept. 6.9)				76.9	s	
26	17.0/17.1	q	0.93 (d, 6.9)	17.5	q	0.92 (d, 6.9)				18.8	q	1.10 (s)
27	16.8/16.9	q	0.94 (d, 6.9)	17.6	q	0.95/0.96 (d, 6.9)				20.8	q	1.13 (s)
28	25.4	q	0.97 (s)	25.5	q	0.97 (s)	22.2	q	1.05 (s)	22.2	q	1.05 (s)
29	14.0	q	0.81 (s)	14.0	q	0.81 (s)	20.7	q	1.10 (s)	20.8	q	1.10 (s)
30	18.3	q	0.89 (s)	19.3	q	0.90 (s)	19.3	q	0.98 (s)	19.3	q	0.91 (s)
24[1]	65.8/65.9	t	3.47/3.48 (1H, d, 11.3) 3.60/3.62 (1H, d, 11.3)	63.9/64.2	t	3.55/3.56 (1H, d, 11.3) 3.60 (1H, d, 11.3)						
OMe				49.3/49.4	q	3.22/3.23 (s)				49.0/49.1	q	3.23/3.24 (s)

[a] Figures in parentheses denote *J* values (hertz). *J* values not included were not determined. [b] Assignment interchangeable.

chemical oxidation with CrO_3 in pyridine.[22] Potato-dextrose agar (PDA), corn steep liquor, and yeast extract were from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan), and glucose was from Nacalai Tesque, Inc. (Kyoto, Japan).

Fungus and Culture Conditions. Stock culture of the fungus *Glomerella fusarioides* IFO 8831 obtained from the Institute of Fermentation (IFO) (Osaka, Japan) was stored on PDA medium at 24 °C. A seed culture was grown in a 500 mL flask containing 300 mL of potato-dextrose broth medium (PDB; 39.5 g of PDA powder was suspended in 1 L of H_2O, and the agar was removed by filtration). After incubation at room temperature and stirring with a magnetic stirrer for 5 days, the whole culture was transferred into a 5 L culture flask containing 2.3 L of PDB and incubated for 3 days under aeration by bubbling and stirring. The cells were harvested by filtration and washed with H_2O. Yield of mycelia: 70 g (wet weight).

Biotransformation. Substrate (200 mg/5 mL DMSO) was introduced into the mycelium (70 g wet weight), then suspended in 3 L of H_2O in a 5 L culture flask, and incubated for 10 days at room temperature under aeration by bubbling and stirring. After incubation, the mycelium was filtered off and washed with EtOAc. The broth, after adjusting the acidity to pH 3−4 using dilute HCl, was extracted three times with EtOAc, and the organic layers were combined. Evaporation of the solvent in vacuo yielded the crude extract.

Biotransformation of Cycloartenol (1) and Isolation of Metabolites. Biotransformation of **1** by the procedure described above afforded a crude extract (214 mg), which was subjected to column chromatography on silica gel (12 g). The column was eluted with *n*-hexanes−EtOAc (19:1, 300 mL; 9:1, 360 mL; 1:1, 400 mL), which yielded fractions A (4.3 mg), B (16.6 mg), C (121.5 mg), D (7.4 mg), E (15.9 mg), and F (8.6 mg) in increasing order of polarity. Compounds from fractions B and C were identified as **3** and unmetabolized **1**, respectively, by MS and ^{1}H NMR analysis. HPLC II of fractions D and E yielded metabolites **5** [2.0 mg, retention time (t_R) 22.4 min] and **4** (1.6 mg, t_R 23.2 min), respectively.

Biotransformation of 24-Methylenecycloartanol (2) and Isolation of Metabolites. Biotransforamtion of **2** by *G. fusarioides* yielded a crude extract (248 mg), which was chromatographed on silica gel (15 g). Elution of the column with *n*-hexanes−EtOAc (19:1, 300 mL; 9:1, 100 mL; 4:1, 500 mL) yielded fractions A′ (14.2 mg), B′ (179.0 mg), C′ (13.0 mg), D′ (2.5 mg), and E′ (17.6 mg). Fraction B′ was subjected to HPLC I to yield **6** (3.8 mg, t_R 27.2 min), **7** (1.4 mg, t_R 8.4 min), and **8** (5.2 mg, t_R 18.4 min) in addition to unmetabolized **2** (125.6 mg).

(24*R*,*S*)-24-Methylcycloartane-3*β*,24,24^1-triol (7): fine needles, mp 175−177 °C; $[\alpha]^{25}_D$ +25.5 (*c* 0.10, $CHCl_3$); IR ν_{max} 3407 (OH), 3040 (cyclopropyl) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 474 $[M]^+$ (8), 456 $[M - H_2O]^+$ (30), 441 $[M - Me - H_2O]^+$ (23), 438 $[M - 2H_2O]^+$ (19), 423 (*m*/*z* 438 − Me) (30), 413 (13), 395 (8), 369 [M − $C_5H_{11}O_2$ (species formed by the cleavage of C-23−C-24 bond) − 2H]$^+$ (10), 334 [M − $C_9H_{16}O$ (ring A)]$^+$ (23), 316 (20), 315 [M − $C_9H_{19}O_2$ (side-chain)]$^+$ (19), 297 (*m*/*z* 315 − H_2O) (19), 273 [*m*/*z* 315 − C_3H_6 (ring D)] (4), 255 (*m*/*z* 273 − H_2O) (7), 95 (100); HREIMS *m*/*z* 474.4064 (calcd for $C_{31}H_{54}O_3$, 474.4073).

(24*R*,*S*)-24^1-Methoxy-24-methylcycloartane-3*β*,24-diol (8): fine needles, mp 168−171 °C; $[\alpha]^{25}_D$ +4.3 (*c* 0.10, $CHCl_3$); IR ν_{max} 3409 (OH), 3040 (cyclopropyl) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 488 $[M]^+$ (13), 470 $[M - H_2O]^+$ (45), 457 $[M - OMe]^+$ (100), 438 (*m*/*z* 470 − MeOH) (37), 428 $[M - CH_2OMe - Me]^+$ (26), 423 (*m*/*z* 438 − Me) (52), 395 (17), 369 [M − $C_6H_{13}O_2$ (species formed by the cleavage of C-23−C-24 bond) − 2H]$^+$ (17), 348 [M − $C_9H_{16}O$ (ring A)]$^+$ (14), 317 [M − $C_{10}H_{21}O_2$ (side-chain) + 2H]$^+$ (19), 297 [M − side-chain − H_2O]$^+$ (21); HREIMS *m*/*z* 488.4229 (calcd for $C_{32}H_{56}O_3$, 488.4228).

Biotransformation of Cycloartenone (3) and Isolation of Metabolites. Column chromatography on silica gel (15 g) of the crude extract (192 mg) eluted with *n*-hexanes−EtOAc [19:1, 500 mL; 4:1, 200 mL; 1:1, 350 mL] gave fractions A″ (109 mg), B″ (11 mg), and C″ (46 mg) in ascending order of polarity. Fraction A″ was subjected to further chromatography on silica gel, which yielded fractions A″-1 (86 mg) and A″-2 (14 mg), of which the former was identified as unmetabolized **3**. HPLC II of fraction A″-2 afforded **10** (1.2 mg, t_R 16.2 min), **11** (3.6 mg, t_R 13.8 min), and **14** (1.6 mg, t_R 12.9 min). Fraction B″ (11 mg), upon HPLC II, gave **9** (1.4 mg, t_R 6.9 min). HPLC II of fraction C″ (46 mg) afforded **12** (1.6 mg, t_R 16.5 min) and **13** (4.0 mg, t_R 8.7 min).

4α,4*β*,14α-Trimethyl-9*β*,19-cyclopregnane-3,20-dione (9): fine needles, mp 198−199 °C; $[\alpha]^{25}_D$ +8.3 (*c* 0.16, $CHCl_3$); IR ν_{max} 3055 (cyclopropyl), 1710 (>C═O) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 356 $[M]^+$ (100), 341 $[M - Me]^+$ (52), 313 $[M - C_2H_3O]^+$ (52), 271 (*m*/*z* 313 − C_3H_6 (ring D), 218 $[M - C_9H_{14}O$ (ring A)]$^+$ (100), 175 [*m*/*z* 218 − C_2H_3O (side-chain)] (70); HREIMS *m*/*z* 356.2714 (calcd for $C_{24}H_{36}O_2$, 356.2715).

(24*R*,*S*)-25-Hydroxy-24-methoxycycloartan-3-one (14): fine needles, mp 165−168 °C; $[\alpha]^{25}_D$ +16.4 (*c* 0.18, $CHCl_3$); IR ν_{max} 3445 (OH), 3060 (cyclopropyl), 1710 (>C═O) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 472 $[M]^+$ (15), 440 $[M - MeOH]^+$ (29), 425 (*m*/*z* 440 − Me) (15), 422 (*m*/*z* 440 − H_2O) (17), 407 (*m*/*z* 422 − Me) (5), 399 (11), 354 [M − $C_6H_{13}O_2$ (species formed by the cleavage of C-22−C-23 bond) − H]$^+$ (15), 334 [M − $C_9H_{14}O$ (ring A)]$^+$ (8), 313 [M − $C_9H_{19}O_2$ (side-chain)]$^+$ (50), 271 [*m*/*z* 313 − C_3H_6 (ring D)] (4), 73 (100); HREIMS *m*/*z* 472.3908 (calcd for $C_{31}H_{52}O_3$, 472.3916).

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