Tyromycic Acids B−E, New Lanostane Triterpenoids from the Mushroom *Tyromyces fissilis*[⊥]

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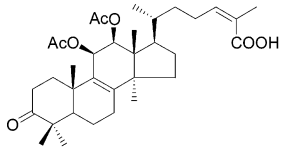
Received June 11, 2003

Four new triterpenoids, tyromycic acids B−E (**1**−**4**), were isolated from a methanol-soluble extract of the fruit bodies of the Japanese inedible mushroom *Tyromyces fissilis*. Tyromycic acids B−D possess a lanostane skeleton, while tyromycic acid E is based on a rare 14(13→12)*abeo*-lanostane skeleton. Their structures were determined by spectral data analysis and by single-crystal X-ray crystallography.

Tyromyces species belonging to the family Polyporaceae have been shown to be a good source of biologically active compounds. Previously, tyromycic acid, a new lanostane triterpenic acid, was isolated from *T. abbidus*.[1] Later, tyromycin A, 1,16-bis[4-methyl-2,5-dioxo-3-furyl]hexadecane, was isolated from *T. lacteus* as a inhibitor of leucine and cysteine aminopeptidases.[2] 4-But-3-enoxymethyl benzoate was obtained by fermentation of a *Tyromyces* species and showed the inhibition of phospholipase A2.[3] In the course of the investigation of the biologically active substances from Basidiomycetes,[4−6] we have studied the chemical constituents of the Japanese inedible mushroom *T. fissilis* and have isolated four new triterpenoids, tyromycic acids B−E (**1**−**4**). This paper describes their isolation and structural elucidation.



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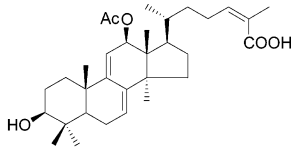

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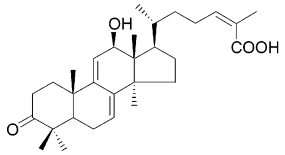

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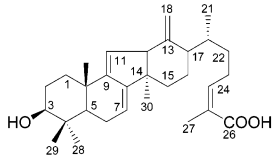

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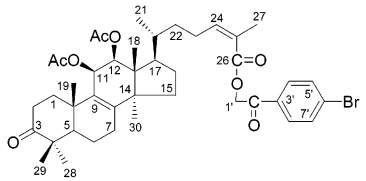

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Results and Discussion

The MeOH extract of the fruit bodies of *T. fissilis* was chromatographed on silica gel, DIOL, and reversed-phase (C_{18}) column chromatography, followed by preparative reversed-phase HPLC, to afford tyromycic acids B−E (**1**−**4**).

Tyromycic acid B (**1**) was obtained as an oil; its molecular formula was determined to be $C_{34}H_{50}O_7$ by HRFABMS. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of an olefinic proton, five tertiary methyls, one secondary methyl, one vinylic methyl, and two acetoxy groups. The ^{13}C NMR spectral data of **1** (Table 2) revealed resonances for 34 carbons including one ketone, four olefinic carbons, and one conjugated carboxylic group, which was confirmed by absorptions at 209 nm and at 2500−3400 and 1702 cm^{-1}, in its UV and IR spectra, respectively. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of (24*Z*)-3-oxo-12α-acetoxylanosta-8,24-dien-26-oic acid,[7] suggesting the presence of a (24*Z*)-3-oxo-lanosta-8,24-dien-26-oic acid unit with two acetoxy groups in **1**. The presence of two acetoxy groups at C-11 and C-12 was supported by the long-range correlation between C-11 and CH_3CO-11 and between C-12 and CH_3CO-12 in the HMBC spectrum and from the low-field chemical shifts of C-11 and C-12 in the ^{13}C NMR spectrum (Table 2). The relative configurations of H-11 and H-12 were both deduced to be equatorial by a NOESY experiment which showed NOE correlations between H-11/H-12 and H-17 and between H-12/H-11, H-17, and H-30 and from the coupling constants of H-11 and H-12 (d, J = 6.0 Hz).

To confirm the stereostructure of compound **1**, the *p*-bromophenacyl derivative (**5**) of **1** was prepared. The ^{1}H and ^{13}C NMR spectral data of **5** resembled those of **1**, except for the signals at δ_H 7.80 (d, J = 8.8 Hz), 7.64 (d, J = 8.8 Hz), and 5.35 (d, J = 1.9 Hz), together with signals at δ_C 191.1, 132.2, and 129.3, corresponding to the *p*-bromophenacyl group of **5**. Furthermore, a suitable crystal of **5** was obtained and then X-ray crystallographic analysis was carried out. The absolute configuration of **1** was determined as shown in Figure 1. On the basis of the above data, the structure of tyromycic acid B (**1**) was established as (24*Z*)-3-oxo-11*R*,12*S*-diacetoxylanosta-8,24-dien-26-oic acid.

The molecular formula of tyromycic acid C (**2**) was determined to be $C_{32}H_{48}O_5$ by high-resolution FABMS. The IR and UV spectra indicated the presence of conjugated carboxylic acid (2500−3400, 1714 cm^{-1}) and conjugated diene (237 and 245 nm) absorptions. The ^{1}H NMR spectral data of **2** (Table 1) revealed the signals of three olefinic protons, five methyls, one secondary methyl, one vinylic methyl, and one acetoxy group. The ^{13}C NMR spectrum of **2** (Table 2) contained 32 carbon signals, including resonances for a carboxylic carbon (δ_C 171.0), six olefinic carbons, one acetoxy group, and two oxygenated carbons. The ^{1}H and ^{13}C NMR spectral data of **2** were typical for a lanosta-7,9(11),24-trien-26-oic acid unit.[8] The acetoxy group

[⊥] Dedicated to the late Dr. Monroe. E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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10.1021/np030264+ CCC: $27.50
Published on Web 01/24/2004

Table 1. ^{1}H NMR Data of Tyromycic Acids B−E (**1**−**4**)

position	**1**	**2**	**3**	**4**
1	1.86 m	1.78 m	2.30 ddd (3.3, 5.8, 13.2)	1.90 m
	1.64 m	1.41 ddd (1.9, 9.3, 12.4)	1.79 dt (4.4, 13.2)	1.53 m
2	2.61 ddd (6.3, 12.9, 15.9)	2.00 m	2.78 dt (5.8, 14.8)	1.66 m
	2.35 ddd (3.0, 5.5, 15.9)	1.65 m	2.37 ddd (3.3, 4.4, 14.8)	1.73 m
3		3.38 brs		3.27 dd (4.4, 11.3)
5	1.44 dd (2.8, 12.6)	1.59 m	1.55 dd (3.6, 12.1)	1.45 dd (4.4, 11.5)
6	1.73 m	2.07 dd (7.4, 12.4)	2.22 dd (12.1, 17.3)	2.19 td (4.7, 17.3)
	1.67 m		2.08 ddd (3.6, 6.9, 17.3)	2.12 dd (12.2, 17.3)
7	2.19 m	5.62 t (4.1)	5.57 d (6.9)	5.29 d (5.8)
11	5.66 d (6.0)	5.02 s	5.23 s	5.22 s
12	5.45 d (6.0)	5.46 s	4.26 s	2.99 s
15	1.73 m	1.82 m	1.74 m	1.40 ddd (2.5, 6.6, 12.1)
	1.30 m	1.62 m	1.36 ddd (2.2, 9.3, 12.1)	1.19 dd (2.5, 12.1)
16	1.97 m	2.04 m	2.01 dd (7.7, 13.5)	1.56 m
	1.53 m	1.53 m	1.48 m	1.10 m
17	1.86 m	1.91 t (7.7)	1.88 dd (8.2, 17.9)	1.88 m
18	0.97 s	0.69 s	0.56 s	4.83 d (2.8)
				4.65 d (2.8)
19	1.26 s	1.04 s	1.23 s	0.99 s
20	1.48 m	1.47 s	1.51 s	1.33 m
21	0.90 d (6.9)	0.92 d (6.6)	1.09 d (5.2)	0.84 d (6.6)
22	1.60 m	1.60 m	1.62 m	1.56 m
	1.07 m	1.14 m	1.18 ddd (4.9, 9.1, 18.4)	1.02 m
23	2.54 m	2.45 m	2.57 m	2.52 m
	2.47 m		2.49 m	2.43 m
24	6.08 dt (1.4, 7.6)	5.93 t (7.1)	6.10 dt (1.4, 7.7)	6.00 dt (1.4, 7.7)
27	1.92 s	1.87 d (1.1)	1.92 s	1.88 d (1.4)
28	1.09 s	0.94 s	1.13 s	1.01 s
29	1.08 s	0.95 s	1.09 s	0.90 s
30	1.07 s	1.03 s	0.93 s	1.06 s
CH_3CO-11	2.08 s			
CH_3CO-12	1.96 s	2.06 s		

Table 2. ^{13}C NMR Data of Tyromycic Acids B−E (**1**−**4**)

position	**1**	**2**	**3**	**4**
1	35.1 t	32.6 t	36.5 t	35.1 t
2	34.3 t	26.5 t	34.7 t	27.3 t
3	216.5 s	76.5 d	216.4 s	79.1 d
4	47.4 s	38.4 s	47.4 s	38.8 s
5	51.9 d	44.2 d	50.5 d	48.8 d
6	19.0 t	24.0 t	23.6 t	23.2 t
7	26.4 t	124.0 d	121.4 d	114.0 d
8	143.7 s	142.3 s	141.6 s	152.1 s
9	133.0 s	149.9 s	145.8 s	153.3 s
10	36.7 s	38.4 s	37.0 s	35.3 s
11	68.8 d	118.0 d	122.1 d	125.4 d
12	72.9 d	79.4 d	74.5 d	61.5 d
13	53.7 s	49.0 s	49.0 s	149.3 s
14	47.1 s	52.9 s	51.3 s	45.4 s
15	31.3 t	31.0 t	31.6 t	36.0 t
16	26.2 t	27.0 t	26.3 t	24.8 t
17	49.2 d	52.5 d	51.4 d	47.9 d
18	13.0 q	11.7 q	9.9 q	113.8 t
19	21.1 q	21.7 q	22.0 q	20.2 q
20	34.6 d	35.5 d	34.3 d	36.8 d
21	20.6 q	20.8 q	20.5 q	17.8 q
22	35.0 t	36.3 t	35.3 t	33.7 t
23	27.7 t	28.3 t	27.5 t	27.1 t
24	146.8 d	143.5 d	146.9 d	146.6 d
25	126.0 s	128.9 s	125.9 s	126.1 s
26	172.7 s	171.0 s	173.2 s	173.0s
27	20.6 q	21.1 q	20.5 q	20.6 q
28	21.8 q	23.3 q	22.4 q	28.0 q
29	25.6 q	28.9 q	25.3 q	15.7 q
30	26.0 q	25.9 q	25.2 q	27.0 q
CH_3CO-11	21.3 q, 170.5 s			
CH_3CO-12	21.1 q, 170.3 s	21.7 q, 172.9 s		

was attached at C-12 by comparing the NMR data of **2** with those of **1** and by the long-range correlation between the acetoxy group and C-12 in the HMBC spectrum. The other oxygenated carbon was determined to be C-3 from the chemical shift at 76.5 ppm in the ^{13}C NMR spectrum.[9,10] The stereochemistry of the hydroxyl group was established

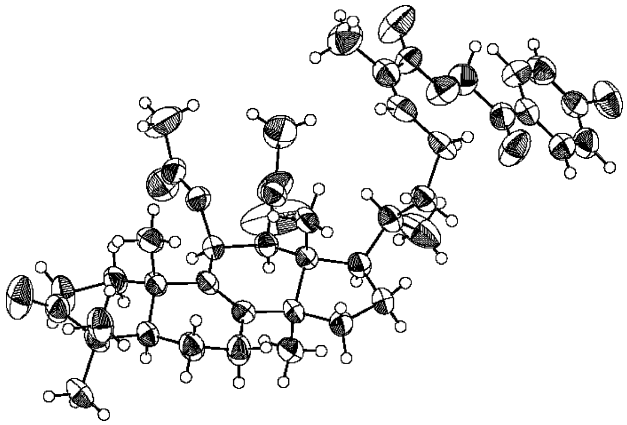
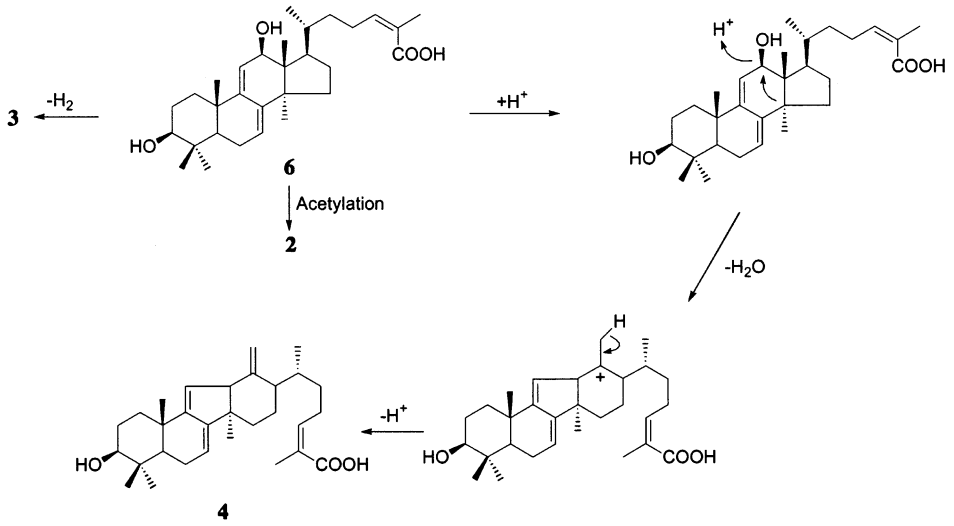


Figure 1. ORTEP drawing of tyromycic acid B *p*-bromophenacyl ester (**5**).

to be β-equatorial, and the acetoxy groups were determined to be β-axial by the NOE correlations between H-3 and H-5, H-28, and between H-12 and H-17, H-30 in the NOESY spectrum, respectively. In addition, the C-24, C-25 double bond was determined to be *Z* from a NOE correlation between H-24 and H-27. Accordingly, compound **2** was assigned as 3β-hydroxy-12β-acetoxylanosta-7,9(11),24(*Z*)-trien-26-oic acid.

In the high-resolution FABMS, tyromycic D (**3**) gave a molecular ion at *m*/*z* 468.3277, indicating an element formula of $C_{30}H_{44}O_4$. The ^{1}H and ^{13}C NMR spectral data of **3** were similar to those of **2** and tyromycic acid[2] except for the presence of a hydroxy group and a ketone group at C-3 (δ_C 216.4) in place of an acetoxy group at C-12 and a hydroxy group in **2**, respectively. The relative configuration of the hydroxy group at C-12 was determined to be axial by the NOE correlations between H-12 and H-17, H-21, and H-30 in the NOESY spectrum. Comparison of the ^{13}C NMR data of **3** with those of daldinialanone[11] suggested

Scheme 1. Biogenetic Pathway Proposed for Compound **4**



that the ketone group is located at C-3. Thus, the structure of **3** was established as 3-oxo-12β-hydroxylanosta-7,9(11),-24(*Z*)-trien-26-oic acid.

Tyromycic acid E (**4**) was obtained as an oil. High-resolution FABMS of **4** established the molecular formula as $C_{30}H_{44}O_3$. The ^{1}H NMR spectral data (Table 1) of **4** showed the presence of three olefinic protons, one exo-methylene, four tertiary methyls, one secondary methyl, and one vinylic methyl group. The ^{13}C NMR spectral data of **4** (Table 2) exhibited 30 carbon signals including one conjugated carboxylic group, eight olefinic carbons, and one oxygenated carbon. The ^{1}H and ^{13}C NMR data of **4** resembled those of neokadsuranic acid A isolated from the higher plant *Kadsura heteroclita*,[12] suggesting the presence of a 14(13→12)*abeo*-lanostane partial structure in **4**, for which there has been only one previous example as a natural product. The exomethylene group was located at C-13 due to the long-range correlations between H-18 and C-13, C-12, and C-17 in the HMBC spectrum. Furthermore, the hydroxyl group was placed at C-3 from the chemical shift of C-3 (δ_C 79.1) in the ^{13}C NMR spectrum and the long-range correlation between H-3 and C-2, C-4, and C-5 in the HMBC spectrum. By comparing the NMR data of **4** with those of **3**, three olefinic protons were located at C-7, C-11, and C-24, and the C-24, C-25 double bond was assigned as the *Z* form. To establish the stereochemistry of **4**, a NOESY experiment was carried out, in which the NOE correlations were observed between H-3/H-5, H-28; H-19 and H-29; H-5 and H-30; and H-17 and H-21, suggesting that H-3, H-5, H-17, H-21, H-28, and H-30 are all α-oriented. Thus, the structure of tyromycic acid E (**4**) was determined as (24*Z*)-3β-hydroxy-14(13→12)*abeo*-lanosta-7,9(11),13(18),24-tetraen-26-oic acid.

A biogenetic pathway for compound **4** is proposed as shown in Scheme 1. Starting from dihydrotyromycic acid D (**6**) as precursor, which has not been isolated from *T. fissilis*, tyromycic acid E (**4**) may be formed by elimination of one water molecule, followed by a hydrogen shift.

Previously, lanostane-type triterpene carboxylic acids have shown various bioactivities, such as cholesterol biosynthesis inhibitory activity and antinociceptive effects.[13,14] Tyromycic acids B−E (**1**−**4**) were tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and for their anti-HIV effects. However, these new triterpenoids showed neither antioxidant nor anti-HIV activities.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. UV spectra were obtained on a Shimadzu UV-1650PC instrument in EtOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using $CDCl_3$ as solvent. Chemical shifts are given relative to TMS (δ 0.00) as internal standard (^{1}H) and δ 77.03 (ppm) from $CDCl_3$ as standard (^{13}C). Mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer. X-ray reflection data were measured on a DIP Image diffractometer using Mo Kα radiation (λ = 0.71073 Å). Preparative medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd, Japan) and a Lobar column (Merck). Preparative HPLC was performed on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II column or 5 SL-II column. Column chromatography was carried out on silica gel 60 (0.2−0.5 mm, 0.04−0.063 mm, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, $CHCl_3$−MeOH, 1:1).

Fungal Material. Fruit bodies of *Tyromyces fissilis* were collected in October 2002 in Aichi Prefecture, Hagashikano-gun, Japan, and identified by Kazuyuki Takase. A voucher specimen (Taka-02-1) has been deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

Extraction and Isolation. Dried fruit bodies of *T. fissilis* (159.2 g) were extracted with MeOH, and the methanolic extract was evaporated to give a residue (7.5 g), which was subjected to silica gel column chromatography using hexane−EtOAc (1:1) as solvent system, to afford six fractions. Fraction 2 (1.5 g) was chromatographed over a reversed-phase (C_{18}) column, using the solvent system MeOH−H_2O (9:1), to give six subfractions. Fraction 2-4 (232.6 mg) was purified by MPLC on a reversed-phase column using CH_3CN as eluent to yield tyromycic acid B (**1**) (131.6 mg) and tyromycic acid D (**3**) (47.2 mg). Fraction 2-5 (329 mg) was purified by MPLC with a DIOL column, using hexane−EtOAc (2:1) as solvent system, and afforded tyromycic acid C (**2**) (11.4 mg) and tyromycic acid E (**4**) (97.6 mg). Fraction 3 (286.8 mg) was treated in the same manner described above to give three subfractions. Fraction 3-2 (50.3 mg) was isolated by reversed-phase preparative HPLC, using MeOH as solvent, to give tyromycic acid E (**4**) (25.4 mg). Fraction 3-3 (34.6 mg) was purified by reversed-phase preparative HPLC, using CH_3CN as mobile phase, to give a further quantity of tyromycic acid D (**3**) (10.1 mg).

Tyromycic Acid B (1): oil; $[\alpha]_D^{20}$ −9.6° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209 (4.4) nm; IR (KBr) ν_{max} 2500−3400, 1735, 1702, 1637, 1245 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 593 $[M]^+$; HRFABMS *m*/*z* 593.3423 (calcd for $C_{34}H_{50}O_7Na$, 593.3454).

Tyromycic Acid C (2): oil; $[\alpha]^{20}_D$ −53.1° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 237 (4.1), 245 (4.2) nm; IR (KBr) ν_{max} 2500−3400, 1736, 1714, 1644, 1243 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 512 $[M]^+$; HRFABMS *m*/*z* 512.3474 (calcd for $C_{32}H_{48}O_5$, 512.3502).

Tyromycic Acid D (3): oil; $[\alpha]^{20}_D$ −52.2° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 222 (4.1), 229 (4.1), 237 (4.1), 246 (4.1), 289 (2.6) nm; IR (KBr) ν_{max} 2500−3400, 1695, 1639 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 468 $[M]^+$; HRFABMS *m*/*z* 468.3277 (calcd for $C_{30}H_{44}O_4$, 468.3240).

Tyromycic Acid E (4): oil; $[\alpha]^{20}_D$ −94.3° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 243 (3.8), 251 (3.8) nm; IR (KBr) ν_{max} 2500−3400, 1689, 1636 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 452 $[M]^+$; HRFABMS *m*/*z* 452.3281 (calcd for $C_{30}H_{44}O_3$, 452.3290).

Preparation of the *p*-Bromophenacyl Ester (5) of Tyromycic Acid B (1). A mixture of **1** (16.4 mg), *p*-bromophenacyl bromide (15 mg), and K_2CO_3 (6 mg), in dry acetone (2 mL), was stirred at room temperature for 3 h. After removal of inorganics by filtration, the filtrate was concentrated under reduced pressure to a syrup, which was subjected to silica gel chromatography, followed by preparative HPLC, using hexane−EtOAc (1:4), to give compound **5** (9.4 mg) as white needles (MeOH); mp 183−185 °C; $[\alpha]^{20}_D$ −39.8° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 212 (4.3), 255 (4.2) nm; IR (KBr) ν_{max} 2956, 1736, 1704, 1646, 1586, 1247 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.80 (2H, d, J = 8.8 Hz, H-4′, H-8′), 7.64 (2H, d, J = 8.8 Hz, H-5′, H-7′), 6.04 (1H, dt, J = 1.4, 7.7 Hz, H-24), 5.75 (1H, d, J = 5.8 Hz, H-11), 5.44 (1H, d, J = 5.8 Hz, H-12), 5.35 (2H, d, J = 1.9 Hz, H-1′), 2.60 (1H, dd, J = 6.3, 10.2 Hz, H-2), 2.56 (1H, m, H-23), 2.45 (1H, m, H-23), 2.35 (1H, ddd, J = 3.0, 5.5, 10.2 Hz, H-2), 2.18 (2H, m, H-7), 2.07 (3H, s, *CH*$_3$CO-11), 1.96 (1H, m, H-16), 1.94 (3H, s, *CH*$_3$CO-12), 1.86 (1H, m, H-1), 1.84 (1H, m, H-17), 1.73 (1H, m, H-6), 1.71 (1H, m, H-15), 1.66 (1H, m, H-6), 1.64 (1H, m, H-1), 1.61 (1H, m, H-22), 1.54 (1H, m, H-16), 1.47 (1H, m, H-20), 1.44 (1H, dd, J = 2.8, 12.6 Hz, H-5), 1.28 (1H, m, H-15), 1.26 (3H, s, H-19), 1.19 (3H, d, J = 1.4 Hz, H-26), 1.12 (1H, m, H-22), 1.09 (3H, s, H-28), 1.08 (3H, s, H-29), 1.06 (3H, s, H-30), 0.96 (3H, s, H-18), 0.89 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 216.4 (s, C-3), 191.1 (s, C-2′), 170.4 (s, CH$_3$*CO*-11), 170.3 (s, CH$_3$*CO*-12), 167.0 (s, C-27), 145.7 (d, C-24), 143.7 (s, C-8), 133.0 (s, C-9), 132.2 (d, C-5′, C-7′), 129.3 (d, C-4′, C-8′), 129.1 (s, C-6′), 125.9 (s, C-25), 72.9 (d, C-12), 68.8 (d, C-11), 65.6 (t, C-1′), 53.7 (s, C-13), 51.9 (d, C-5), 49.2 (d, C-17), 47.4 (s, C-4), 47.1 (s, C-14), 36.7 (s, C-10), 35.2 (t, C-1), 35.1 (t, C-22), 34.7 (d, C-21), 34.3 (t, C-2), 31.3 (t, C-15), 27.6 (t, C-23), 26.5 (t, C-7), 26.3 (t, C-16), 26.0 (q, C-29), 25.6 (q, C-30), 21.9 (q, C-28), 21.6 (q, C-26), 21.4 (q, *CH*$_3$CO-11), 21.2 (q, C-19), 21.1 (q, *CH*$_3$CO-12), 20.6 (q, C-21), 19.1 (t, C-6), 13.0 (q, C-18); FABMS *m*/*z* 789 $[M + Na]^+$; HRFABMS *m*/*z* 789.2985 (calcd for $C_{42}H_{55}O_8BrNa$, 789.2978).

Crystal Data for 5. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL). Data reduction: maXus.[15] Program used to solve structure: SHELXL-97.[16] Refinement: on F^2 full matrix least-squares. Diffractometer: DIP image plate. $C_{42}H_{55}O_8Br$, MW 768, orthorhombic, $P2_12_12_1$, a = 6.5480(2) Å, b = 19.9720(8) Å, c = 29.568(2) Å, α = 90.00°, β = 109.75(2)°, γ = 90.00°, V = 3866.8(3) Å^3, Z = 4, Mo Kα radiation, λ = 0.71073 Å, μ = 1.115 mm^{-1}, 6981 reflections, 460 parameters; only coordinates of H atoms refined, R = 0.0936, R_w = 0.2433, S = 1.057.

Bioassays. An antioxidant activity test was carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.[18] Biological testing against human immunodeficiency virus type 1 (HIV-1) was carried out with MT-4 cells in culture as described previously.[19]

Acknowledgment. We thank Mr. K. Takase (Aichi Prefecture, Nishio-city, Japan) for the collection and identification of fungus and Miss Y. Okamoto (Tokushima Bunri University) for recording the mass spectra. Thanks are due to Prof. M. Baba at the Faculty of Medicine, Kagoshima University, Kagoshima, Japan, for anti-HIV testing.

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(17) Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (deposition number CCDC 226254). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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NP030264+