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Absolute stereostructures of inoterpenes A–F from sclerotia of *Inonotus obliquus*

Seikou Nakamura [a], Junko Iwami [a], Hisashi Matsuda [a], Shuichi Mizuno [b], Masayuki Yoshikawa [a,*]

[a] *Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan*
[b] *Keitenkai Tohwa Hospital, 1-3-1 Moritsune honmachi, Minami-ku, Kokura, Kitakyusyu 802-0971, Japan*

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ABSTRACT

Six new lanostane-type triterpenes, inoterpenes A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**), were isolated from the sclerotia of *Inonotus obliquus* together with six known constituents. The chemical structures of new triterpenes **1**–**6** were characterized on the basis of chemical and physicochemical evidence including the application of the modified Mosher's method.

1. Introduction

Inonotus obliquus (Hymenochaetaceae) is a white-rot fungus and widely distributed in Europe, Russia, north regions of Japan, and so on. The sclerotia of this fungus (Kabanoanatake in Japanese, Charga in Russian) have been used traditionally for treating cancer, heart, liver, and stomach diseases, and tuberculosis. Recently, several chemical and biological studies on *I. obliquus* have been reported.[1–10] For example, the extracts from this fungus were reported to possess anti-tumor,[2–4] anti-tumor promoting,[5] antioxidant,[6,7] and anti-inflammatory properties.[8] In addition, several lanostane-type triterpenes were isolated as chemical constituents from the sclerotia of this fungus.[5,9,10] During the course of characterization studies on the bioactive constituents of traditional medicines[11–17] and medicinal foodstuffs,[18,19] we found that the methanolic (MeOH) extract of the sclerotia of *I. obliquus* showed an inhibitory effect on invasion of human fibrosarcoma HT1080 cells through matrigel-coated filter. From the MeOH extract, six new lanostane-type triterpenes, inoterpenes A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**), were isolated together with six known constituents. In this paper, we describe the isolation and absolute stereostructure elucidation of these six new lanostane-type triterpenes.

2. Results and discussion

The sclerotia of *I. obliquus* were extracted with MeOH to give a MeOH extract (21.7% from the sclerotia). The MeOH extract was partitioned into an EtOAc–H_2O mixture to furnish an EtOAc-soluble fraction (10.2%) and aqueous layer. The aqueous layer was further extracted with *n*-BuOH to give *n*-BuOH- and H_2O-soluble fractions (4.4% and 7.1%, respectively). The EtOAc-soluble fraction was subjected to normal- and reversed-phase column chromatographies, and finally HPLC to give inoterpenes A (**1**, 0.0004%), B (**2**, 0.0004%), C (**3**, 0.0024%), D (**4**, 0.0034%), E (**5**, 0.0005%), and F (**6**, 0.0042%), lanosterol (**7**, 0.38%),[20] 3β,21-dihydroxylanosta-8,24-diene (**8**, 0.013%),[21] 3β-hydroxylanosta-8,24-dien-21-al (**9**, 0.054%),[22] trametenolic acid (**10**, 0.24%),[22] inotodiol (**11**, 0.70%),[22] and 3β,25-dihydroxylanosta-8,23-diene (**12**, 0.0012%) (Chart 1).[23]

Inoterpenes A (**1**) and B (**2**) were obtained as a white powder with positive optical rotation (**1**: $[\alpha]_D^{23}$ +141.0; **2**: $[\alpha]_D^{22}$ +132.1 in $CHCl_3$). The IR spectra of **1** and **2** showed absorption band due to a hydroxyl function (3450 cm^{-1}). The common molecular formula $C_{30}H_{52}O_3$ of **1** and **2** was determined from EIMS (*m*/*z* 460 $[M]^+$) and by high-resolution (HR) EIMS measurement. The ^1H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra of **1** and **2**, which were assigned by various NMR experiments,[24] showed signals assignable to eight methyls [**1**: δ 0.69, 0.81, 0.88, 0.99, 1.01, 1.17, 1.22 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 0.92 (3H, d, *J*=6.1 Hz, H_3-21); **2**: δ 0.70, 0.81, 0.88, 0.98, 1.00, 1.17, 1.22 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 0.91 (3H, d, *J*=6.1 Hz, H_3-21)], two methines bearing an oxygen function [**1**: δ 3.24 (1H, dd, *J*=4.3, 11.6 Hz, H-3), 3.29 (1H, m, H-24); **2**: δ 3.24 (1H,

* Corresponding author. Tel.: +81 75 595 4633; fax: +81 75 595 4768.
E-mail address: myoshika@mb.kyoto-phu.ac.jp (M. Yoshikawa).

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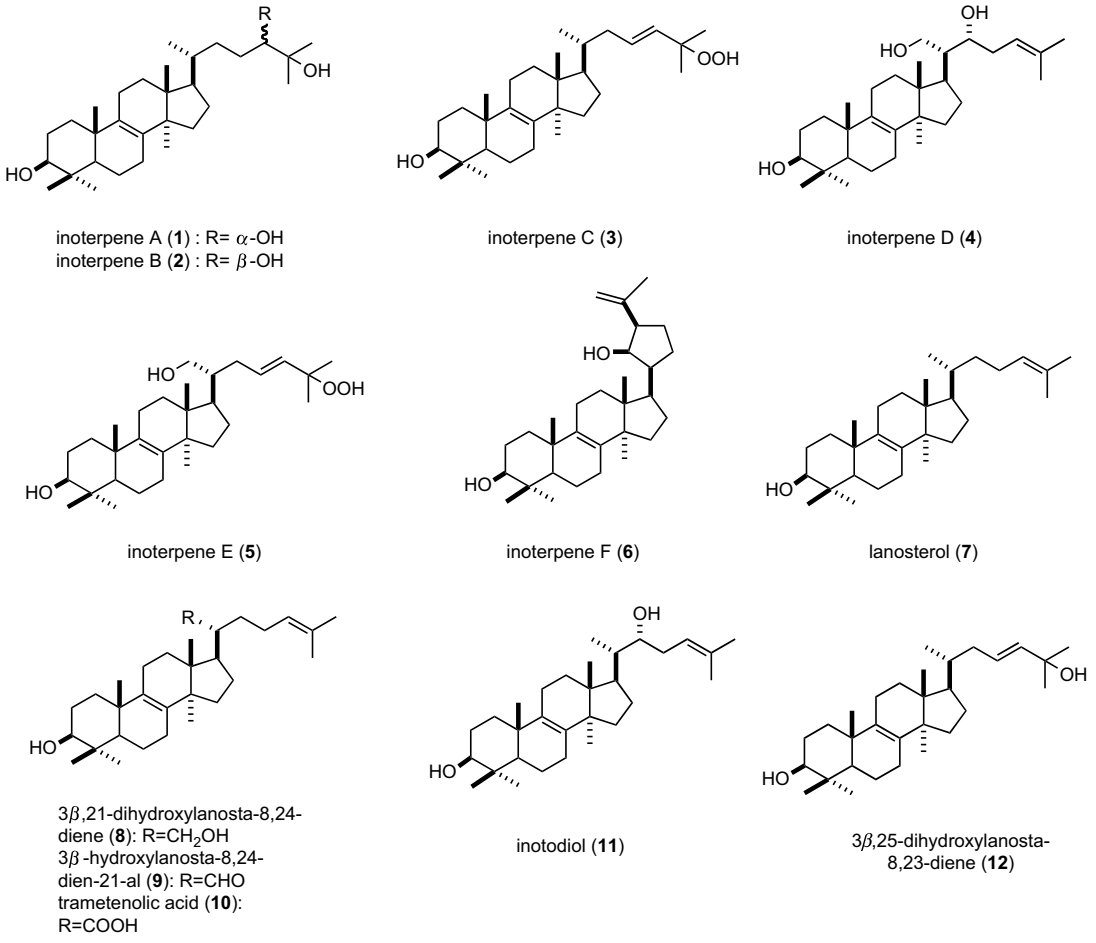

Chart 1.

dd, J=4.3, 11.6 Hz, H-3), 3.35 (1H, m, H-24)], and a tetrasubstituted olefin. The proton and carbon signals due to the tetracarbocyclic moieties (C-1–C-21, C-28–C30) in the ^{1}H and ^{13}C NMR spectra of **1** and **2** were superimposable on those of lanosterol (**7**).[20] As shown in Figure 1, the double quantum filter correlation spectroscopy (DQF COSY) experiment on **1** and **2** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple-bond connectivity (HMBC) experiment, long-range correlations were observed between the following protons and carbons: H-18 and C-13, 14, 17; H-19 and C-1, 5, 9, 10; H-21 and C-17, 20, 22; H-24 and C-22, 25, 26, 27; H-26, 27 and C-24, 25; H-28, 29 and C-3, 4, 5; H-30 and C-8, 14, 15. On the basis of these evidences, the planar structures of **1** and **2** were characterized, and these triterpenes were found to be stereoisomeric at the 24-position with each other. Next, the relative stereostructures of the tetracarbocyclic moieties in **1** and **2** were characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: H-3α and H-5, H_3-28; H-17 and H_3-30; H_3-18 and H_3-19; H_3-28 and H_3-30. The absolute configurations of the 24-positions in **1** and **2** were characterized by the application of the modified Mosher's method.[25] Namely, **1** gave the 3,24-di-(*S*)-MTPA ester (**1a**) by treatment with (−)-2-methoxy-2-trifluoromethylphenylacetyl cholride [(−)-MTPACl] in pyridine. In addition, the 3,24-di-(*R*)-MTPA ester (**1b**) was obtained from **1** using (+)-MTPACl in pyridine. As shown in Figure 2, the signals due to protons attached to the 1- and 19-positions in the 3,24-di-(*S*)-MTPA ester (**1a**) were observed at higher fields compared with those of the 3,24-di-(*R*)-MTPA ester (**1b**) [Δδ: negative], while the signals due to protons attached to the 28- and 29-positions in **1a** were observed at lower fields compared with those of **1b** [Δδ: positive]. Thus, the absolute configuration at the 3-position in **1** was determined to be *S*. On the other hand, the signals due to protons attached to the 26- and 27-positions in the 3,24-di-(*S*)-MTPA ester (**1a**) were observed at lower fields compared with those of the 3,24-di-(*R*)-MTPA ester (**1b**) [Δδ: positive], while the signal due to proton attached to the 21-position in **1a** was observed at higher field compared with that of **1b** [Δδ: negative]. Thus, the absolute configuration at the 24-position in **1** was determined to be *S*. By using the same method, the absolute configurations at the 3- and 24-positions in **2** were determined to be *S* and *R*, respectively. Finally, a mixture (1:1) of **1** and **2** was derived from **7** by oxidation with a catalytic amount of microencapsulated (MC) OsO_4 in the presence of *N*-methylmorpholine *N*-oxide (Fig. 3), so that the absolute configurations of the 20-positions in **1** and **2** were determined to be *R*. Thus, the absolute stereostructures of inoterpenes A (**1**) and B (**2**) were elucidated as shown.

Inoterpene C (**3**) was obtained as a white powder with positive optical rotation ($[\alpha]_D^{23}$ +36.1 in MeOH) and was shown to possess a hydroperoxide group by its positive response to the *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent.[26,27] The IR spectrum of **3** showed absorption band due to a hydroxyl function (3450 cm^{-1}). The molecular formula $C_{30}H_{50}O_3$ of **3** was determined from EIMS (*m*/*z* 458 $[M]^+$) and by HREIMS measurement. The ^{1}H NMR (pyridine-d_5) and ^{13}C NMR (Table 1) spectra[24] of **3** showed signals assignable to eight methyls [δ 0.78, 0.97, 1.09, 1.10, 1.26, 1.59, 1.59 (3H each, all s, H_3-18, 30, 19, 29, 28, 26, 27), 1.03 (3H, d,

Table 1
^{13}C NMR data[a] of inoterpenes A (**1**), B (**2**) C (**3**), D (**4**), E (**5**), and F (**6**)

Position	1	2	3	4	5	6
1	35.6	35.6	36.1	35.5	35.6	35.6
2	28.0	27.8	28.4	27.8	27.8	27.8
3	79.0	79.0	78.1	79.0	79.0	79.0
4	38.9	38.9	39.5	38.9	38.9	38.9
5	50.4	50.4	50.9	50.3	50.4	50.4
6	18.3	18.3	18.7	18.2	18.2	18.2
7	28.7	28.4	28.7	27.4	27.8	26.5
8	134.4	134.4	134.4	134.0	134.4	134.4
9	134.4	134.4	135.1	134.6	134.4	134.4
10	37.0	37.0	37.4	37.0	37.0	37.0
11	21.0	21.0	21.3	21.0	21.0	20.9
12	26.5	26.5	26.8	26.5	26.5	27.2
13	44.5	44.5	44.8	44.6	44.3	44.8
14	49.8	49.8	50.1	49.6	49.9	49.5
15	31.0	31.0	31.2	30.7	30.8	30.7
16	30.8	30.8	31.2	30.7	30.6	30.6
17	50.5	50.6	50.5	47.2	44.1	49.2
18	15.8	15.8	16.1	15.8	16.1	17.1
19	19.1	19.2	19.5	19.2	19.2	19.1
20	36.8	36.3	37.1	43.0	43.5	50.6
21	18.8	18.5	19.0	63.4	63.6	75.2
22	33.6	33.1	39.9	75.3	34.6	30.5
23	27.9	28.3	128.4	29.8	130.5	26.4
24	79.6	78.8	137.3	120.9	134.9	52.9
25	73.3	73.2	81.2	135.6	81.9	144.1
26	23.2[b]	23.2[b]	25.2[b]	18.1	24.5	112.5
27	26.5[b]	26.6[b]	25.4[b]	26.0	24.5	23.5
28	28.2	28.0	28.6	28.0	28.0	28.0
29	15.4	15.4	16.4	15.4	15.4	15.4
30	24.3	24.3	24.4	24.3	24.4	24.3

[a] Measured in $CDCl_3$ for **1**, **2**, **4**–**6** and pyridine-d_5 for **3** at 125 MHz.
[b] Interchangeable.

J=6.1 Hz, H_3-21)], a methine bearing an oxygen function [δ 3.47 (1H, m, H-3)], and two olefinic protons [δ 5.86 (1H, ddd like, J=5.5, 8.6, 15.9 Hz, H-23), 5.99 (1H, d, J=15.9 Hz, H-24)]. The proton and carbon signals due to the tetracarbocyclic moiety (C-1–C-21, C-28–C30) in the ^{1}H and ^{13}C NMR spectra of **3** were superimposable on those of **1**, **2**, and lanosterol (**7**),[20] while the signals due to the side chain part resembled those of known tetracarbocyclic triterpenes having the 25-hydroperoxide group such as floralginsenoside E[28] and floralquinquenoside A.[29] In addition, the planar structure of **3** was characterized by means of DQF COSY and HMBC experiments (Fig. 1). Finally, **3** was derived from **7** by photo-induced oxidation with O_2 (Fig. 3). This evidence led us to formulate the absolute stereostructure of inoterpene C (**3**) as shown.

Inoterpene D (**4**), obtained as a white powder with positive optical rotation ($[\alpha]_D^{28}$ +66.0 in $CHCl_3$), showed absorption band due to a hydroxyl function (3450 cm^{-1}) in the IR spectrum. The HREIMS analysis revealed the molecular formula of **4** to be $C_{30}H_{50}O_3$. The ^{1}H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra of **4** showed signals assignable to seven methyls [δ 0.78, 0.81, 0.85, 0.98, 1.00, 1.68, 1.75 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27)], a methylene and two methines bearing an oxygen function [δ 3.23 (1H, dd, J=4.4, 11.6 Hz, H-3), 3.72 (1H, dd, J=10.7, 10.7 Hz, H-21a), 4.02 (1H, dd, J=3.8, 10.7 Hz, H-21b), 3.83 (1H, m, H-22)], and an olefinic proton [δ 5.22 (1H, m, H-24)]. The proton and carbon signals due to the tetracarbocyclic moiety (C-1–C-21, C-28–C30) in the ^{1}H and ^{13}C NMR spectra of **4** were similar to those of 3β,21-dihydroxylanosta-8,24-diene (**8**).[21] As shown in Figure 1, long-range correlations in the HMBC experiment of the side chain on **4** were observed between the following proton and carbon: H-21 and C-20, 22; H-22 and C-21, 24; H-23 and C-22, 24, 25; H-24 and C-26, 27; H-26, 27 and C-24, 25, so that the planar structure of **4** was clarified (Fig. 1). Next, the relative stereostructure in **4** was clarified by the NOESY experiment on the 21,22-acetonide derivative (**4a**). Namely, treatment of **4** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid (*p*-TsOH) yielded **4a**. The relative stereostructure of the tetracarbocyclic moiety in **4a** was characterized by NOESY experiment (Fig. 1). On the other hand, the difference NOESY experiment on side chain moiety of **4a** showed NOE correlations between the following proton pairs: H-20 and H-22, H_3-2′; H-22 and H_3-2′ (Fig. 4), so that the geometry of the 20- and 22-protons was determined to be *syn* form. Finally, the absolute configuration of **4** was characterized by the application of the modified Mosher's method.[25] Namely, the pivaloyl ester (**4b**), which was derived from **4** upon reaction with pivaloyl chloride in pyridine, gave the 3,22-di-(*S*)-MTPA ester (**4c**) by treatment with (−)-MTPACl in pyridine. In addition, the 3,22-di-(*R*)-MTPA ester (**4d**) was obtained from **4b** using (+)-MTPACl in pyridine. As shown in Figure 4, the signal due to proton attached to the 19-position in the 3,22-di-(*S*)-MTPA ester (**4c**) was observed at higher field compared with that of the 3,22-di-(*R*)-MTPA ester (**4d**) [$\Delta\delta$: negative], while the signals due to protons attached to the 28- and 29-positions in **4c** were observed at lower fields compared with those of **4d** [$\Delta\delta$: positive]. Thus, the absolute configuration at the 3-position in **4** was determined to be *S*. On the other hand, the signals due to protons attached to the 24-, 26-, and 27-positions in the 3,22-di-(*S*)-MTPA ester (**4c**) were observed at lower fields compared with those of the 3,22-di-(*R*)-MTPA ester (**4d**) [$\Delta\delta$: positive], while the signal due to proton attached to the 21-position in **4c** was observed at higher field compared with that of **4d** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 22-position of **4** was determined to be *R* and the total stereostructure of inoterpene D (**4**) was elucidated as shown.

Inoterpene E (**5**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{26}$ +30.8 in $CHCl_3$) and was shown to possess a hydroperoxide group.[26,27] The HREIMS analysis revealed the

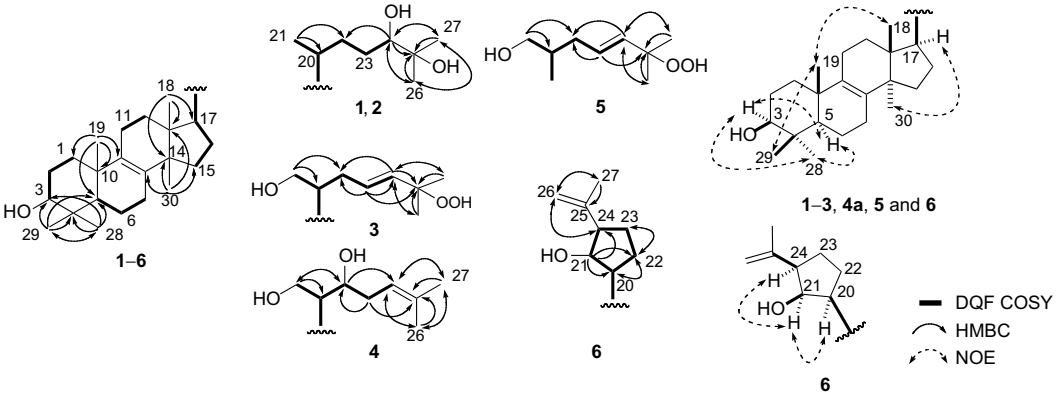

Figure 1. Selected DQF, HMBC, and NOE correlations.

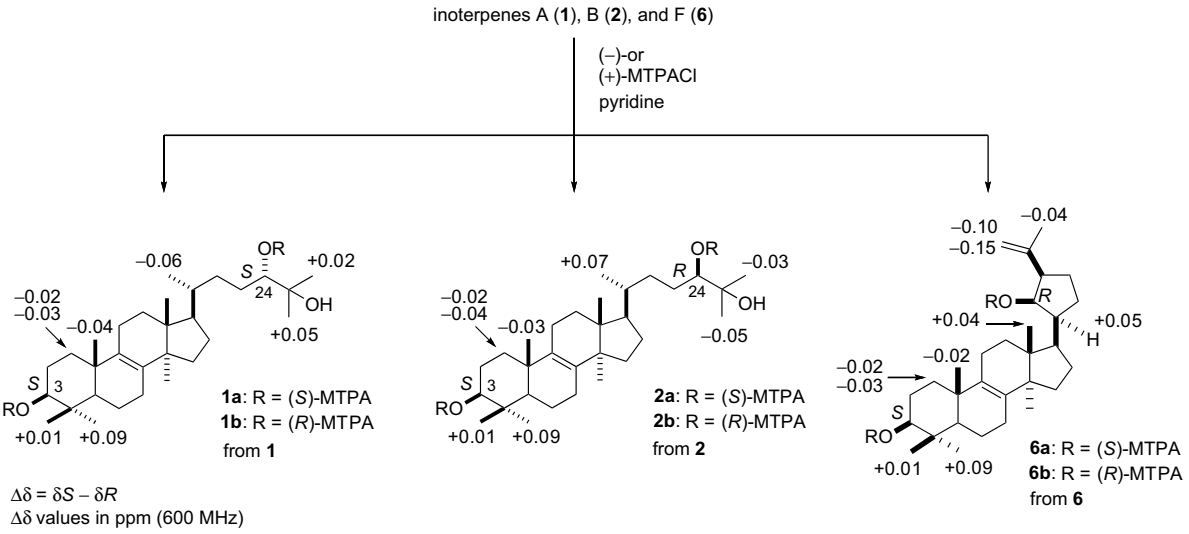

Figure 2.

molecular formula of **5** to be $C_{30}H_{50}O_4$. The ^1H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[24] of **5** showed signals assignable to seven methyls [δ 0.72, 0.80, 0.89, 0.97, 1.00, 1.32, 1.33 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27)], a methylene and a methine bearing an oxygen function [δ 3.24 (1H, dd, J=4.1, 11.7 Hz, H-3), 3.64 (1H, dd, J=6.2, 11.7 Hz, H-21a), 3.78 (1H, dd, J=2.8, 11.7 Hz, H-21b), and two olefinic protons [δ 5.59 (1H, d, J=15.1 Hz, H-24), 5.81 (1H, ddd like, J=7.9, 7.9, 15.1 Hz, H-23)]. The proton and carbon signals due to the tetracarbocyclic moiety in the ^1H and ^{13}C NMR spectra of **5** were similar to those of 3β,21-dihydroxylanosta-8,24-diene (**8**),[21] while the signals due to the side chain moiety resembled those of floralginsenoside E.[28] Finally, **5** was derived from **8**[21] by photoinduced oxidation with O_2 (Fig. 3). These evidence and the examination of the DQF COSY and HMBC data on **5** (Fig. 1) led us to elucidate the absolute stereostructure of inoterpene E (**5**) as shown.

Inoterpene F (**6**), a white powder with positive optical rotation ($[\alpha]_D^{27}$ +38.8 in $CHCl_3$), exhibited absorption bands assignable to hydroxyl (3450 cm^{-1}) and olefin (1655 cm^{-1}) functions in the IR spectrum. The HREIMS analysis revealed the molecular formula of **6** to be $C_{30}H_{48}O_2$. The ^1H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[24] of **6** showed signals assignable to six methyls [δ 0.78, 0.81, 0.89, 0.98, 1.00, 1.81 (3H each, all s, H_3-18, 29, 30, 19, 28, 27)], a methylene [δ 4.84, 5.00 (1H each, both s-like, H_2-26)], and two methines bearing an oxygen function [δ 3.23 (1H, dd, J=4.4, 11.6 Hz, H-3), 3.97 (1H, d like, J=ca. 5 Hz, H-21)]. The proton and carbon signals of **6** in the ^1H and ^{13}C NMR spectra were similar to those of inonotsutriol A,[10] except for the signals due to the E ring part (C-21–C-27). As shown in Figure 1, long-range correlations in the HMBC experiment of the E ring part (C-21–C-27) on **6** were observed between the following proton and carbon: H-20 and C-22; H-21 and C-17, 20, 22, 24; H-22 and C-17, 20, 23; H-24 and C-25, 26; H-26 and C-24, 27; H-27 and C-25, 26, so that the planar structure of **6** were clarified. Next, the relative stereostructure of the tetracarbocyclic moiety in **6** was characterized by NOESY experiment (Fig. 1). In addition, the relative stereostructure of the E ring part in **6** was characterized by difference NOESY experiment, which showed NOE correlations between the following proton pairs: H-20α and H-21α; H-21α and H-24α (Fig 1). Finally, the absolute configuration of **6** was

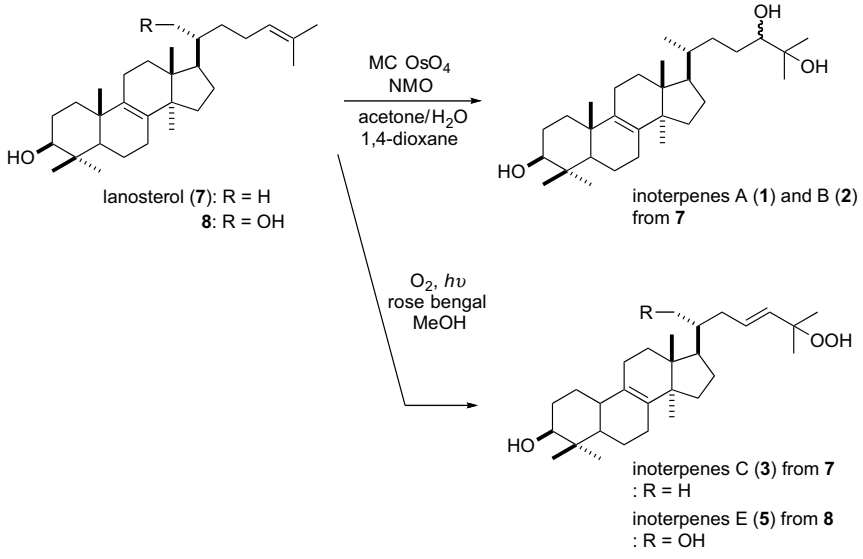

Figure 3.

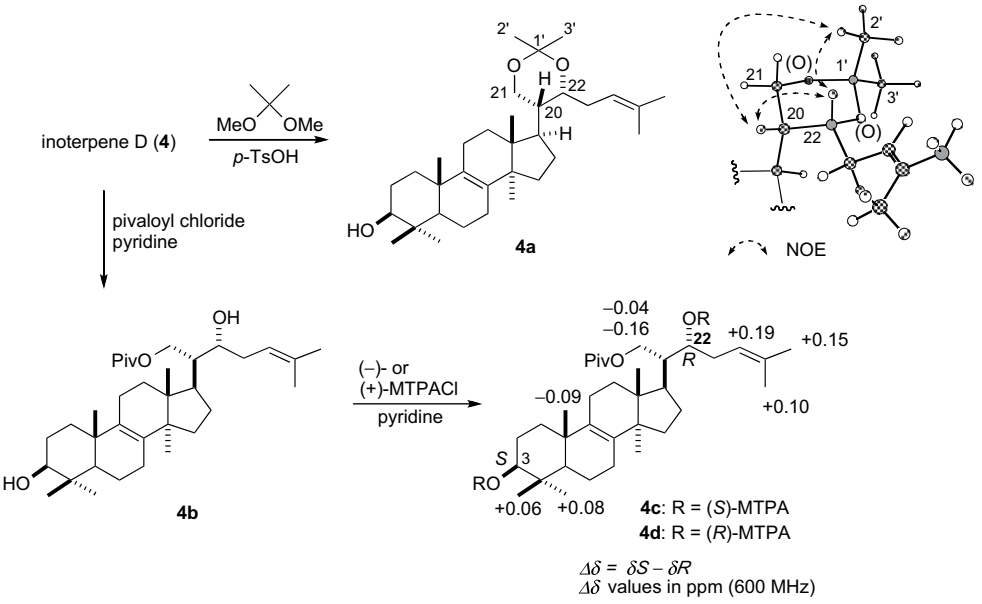

Figure 4.

characterized by the application of the modified Mosher's method.[25] Namely, **6** gave the 3,21-di-(*S*)-MTPA ester (**6a**) by treatment with (−)-MTPACl in pyridine. In addition, the 3,21-di-(*R*)-MTPA ester (**6b**) was obtained from **6** using (+)-MTPACl in pyridine. As shown in Figure 2, the signals due to protons attached to the 1- and 19-positions in the 3,21-di-(*S*)-MTPA ester (**6a**) were observed at higher fields compared with those of the 3,21-di-(*R*)-MTPA ester (**6b**) [$\Delta\delta$: negative], while the signals due to protons attached to the 28- and 29-positions in **6a** were observed at lower fields compared with those of **6b** [$\Delta\delta$: positive]. Thus, the absolute configuration at the 3-position in **6** was determined to be *S*. On the other hand, the signals due to protons attached to the 26- and 27-positions in the 3,21-di-(*S*)-MTPA ester (**6a**) were observed at higher fields compared with those of the 3,21-di-(*R*)-MTPA ester (**6b**) [$\Delta\delta$: negative], while the signals due to protons attached to the 18- and 20-positions in **6a** were observed at lower fields compared with those of **6b** [$\Delta\delta$: positive]. Thus, the absolute configuration at the 21-position in **6** was determined to be *R* and the total stereostructure of inoterpene E (**6**) was elucidated as shown.

3. Experimental

3.1. General

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz) and JEOL ECA-600K (600 MHz) spectrometers with tetramethylsilane as an internal standard; ^{13}C NMR spectra, JNM-LA500 (125 MHz) and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector; and HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 250×4.6 mm i.d. and 250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd, 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd, 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm) and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

3.2. Plant material

The sclerotia of *I. obliquus* were collected in Hokkaido, Japan, in March 2007. The plant was identified by one of the authors (M.Y.). A voucher of the plant is on file in our laboratory [2007, Japan-IO-1].

3.3. Extraction and isolation

The sclerotia of *I. obliquus* (1.4 kg) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (304 g, 21.7% from the sclerotia). The aliquot (267.5 g) from the extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (125.4 g, 10.2%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (54.0 g, 4.4%) and an H_2O-soluble fraction (88.1 g, 7.1%). The EtOAc fraction (125.4 g) was subjected to ordinary-phase silica gel column chromatography (CC) [3.0 kg, *n*-hexane–EtOAc (2:1→1:1, v/v)→EtOAc→$CHCl_3$–MeOH (50:1→20:1→10:1→5:1, v/v)→MeOH] to give 14 fractions {Fr. 1 (1.9 g), Fr. 2 (0.11 g), Fr. 3 (6.4 g), Fr. 4 [1.1 g=lanosterol (**7**)], Fr. 5 [0.66 g=3β-hydroxylanosta-8,24-dien-21-al (**9**)], Fr. 6 (14.0 g), Fr. 7 [0.52 g=inotodiol (**11**)], Fr. 8 (3.3 g), Fr. 9 (3.4 g), Fr. 10 [0.16 g=trametenolic acid (**10**)], Fr. 11 (5.2 g), Fr. 12 (18.9 g), Fr. 13 (43.1 g), and Fr. 14 (5.2 g)}. Fraction 3 (6.4 g) was further subjected to ordinary-phase silica gel CC [300 g, *n*-hexane–EtOAc (50:1→10:1→5:1, v/v)] to afford 14 fractions {Fr. 3-1, Fr. 3-2 [3.596 g=lanosterol (**7**)], Fr. 3-3, Fr. 3-4, Fr. 3-5, Fr. 3-6, Fr. 3-7, Fr. 3-8 (187 mg), Fr. 3-9, Fr. 3-10 (149 mg), Fr. 3-11, Fr. 3-12 (69 mg), Fr. 3-13, and Fr. 3-14}. Fraction 3-8 (187 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish inoterpene C (**3**, 30 mg). Fraction 3-10 (149 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish 3β,25-dihydroxylanosta-8,23-diene (**12**, 14 mg). Fraction

3-12 (69 mg) was purified by HPLC [MeOH–H_2O (85:15, v/v)] to afford inoterpene A (**1**, 4.8 mg) and inoterpene B (**2**, 5.0 mg). Fraction 6 (14.0 g) was subjected to reversed-phase silica gel CC [700 g, MeOH–H_2O (70:30→80:20→90:10, v/v)→MeOH] to afford nine fractions {Fr. 6-1, Fr. 6-2, Fr. 6-3, Fr. 6-4, Fr. 6-5, Fr. 6-6 (1.440 g), Fr. 6-7 [7.232 g=inotodiol (**11**)], Fr. 6-8, and Fr. 6-9}. Fraction 6-6 (580 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish inoterpene F (**6**, 20 mg) and inotodiol (**11**, 116 mg). Fraction 8 (3.3 g) was subjected to reversed-phase silica gel CC [165 g, MeOH–H_2O (60:40→70:30→80:20→90:10, v/v)→MeOH] to afford seven fractions [Fr. 8-1, Fr. 8-2, Fr. 8-3, Fr. 8-4 (1.278 g), Fr. 8-5 (2.190 g), Fr. 8-6, and Fr. 8-7]. Fraction 8-4 (510 mg) was separated by HPLC [MeOH–H_2O (90:10, v/v)] to afford six fractions {Fr. 8-4-1, Fr. 8-4-2, Fr. 8-4-3 (21 mg), Fr. 8-4-4, Fr. 8-4-5, and Fr. 8-4-6 [42 mg=trametenolic acid (**10**)]}. Fraction 8-4-3 (21 mg) was purified by HPLC [MeOH–H_2O (80:20, v/v)] to furnish inoterpene E (**5**, 2.6 mg). Fraction 8-5 (200 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish 3β,21-dihydroxylanosta-8,24-diene (**8**, 15 mg), trametenolic acid (**10**, 35 mg), and inotodiol (**11**, 48 mg). Fraction 9 (3.4 g) was subjected to reversed-phase silica gel CC [170 g, MeOH–H_2O (70:30→80:20→90:10, v/v)→MeOH] to afford nine fractions {Fr. 9-1, Fr. 9-2, Fr. 9-3, Fr. 9-4, Fr. 9-5, Fr. 9-6 (107 mg), Fr. 9-7 [2.225 g=trametenolic acid (**10**)], Fr. 9-8, and Fr. 9-9}. Fraction 9-6 (107 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish trametenolic acid (**10**, 9.9 mg). Fraction 11 (5.2 g) was subjected to reversed-phase silica gel CC [260 g, MeOH–H_2O (10:90→20:80→40:60→60:40→80:20, v/v)→MeOH] to afford 19 fractions {Fr. 11-1, Fr. 11-2, Fr. 11-3, Fr. 11-4, Fr. 11-5, Fr. 11-6, Fr. 11-7, Fr. 11-8, Fr. 11-9, Fr. 11-10, Fr. 11-11, Fr. 11-12, Fr. 11-13, Fr. 11-14, Fr. 11-15, Fr. 11-16 (1.960 g), Fr. 11-17 (415 mg), Fr. 11-18, and Fr. 11-19}. Fraction 11-16 (240 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish inoterpene D (**4**, 5.1 mg) and trametenolic acid (**10**, 21 mg). Fraction 11-17 (415 mg) was purified by HPLC [MeOH–H_2O (90:10, v/v)] to furnish inotodiol (**11**, 5.1 mg). Fraction 12 (18.9 g) was subjected to reversed-phase silica gel CC [950 g, MeOH–H_2O (20:80→40:60→60:40→80:20, v/v)→MeOH] to afford 16 fractions {Fr. 12-1, Fr. 12-2, Fr. 12-3, Fr. 12-4, Fr. 12-5, Fr. 12-6, Fr. 12-7, Fr. 12-8, Fr. 12-9, Fr. 12-10, Fr. 12-11, Fr. 12-12 (493 mg), Fr. 12-13 (164 mg), Fr. 12-14, Fr. 12-15, and Fr. 12-16}. Fraction 12-12 (493 mg) was purified by HPLC [MeOH–H_2O (85:15, v/v)] to furnish inoterpene D (**4**, 19 mg). Fraction 12-13 (164 mg) was purified by HPLC [MeOH–H_2O (85:15, v/v)] to furnish inoterpene A (**1**, 3.2 mg) and inoterpene B (**2**, 3.7 mg). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, MS) with reported values.

3.3.1. *Inoterpene A (**1**)*

A white powder; $[\alpha]_D^{23}$ +141.0 (*c* 0.21, $CHCl_3$); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.69, 0.81, 0.88, 0.99, 1.01, 1.17, 1.22 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 0.92 (3H, d, *J*=6.1 Hz, H_3-21), 3.24 (1H, dd, *J*=4.3, 11.6 Hz, H-3), 3.29 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; EIMS *m*/*z* 460 $[M]^+$; HREIMS *m*/*z* 460.3909 (calcd for $C_{30}H_{52}O_3$ $[M]^+$, 460.3916).

3.3.2. *Inoterpene B (**2**)*

A white powder; $[\alpha]_D^{22}$ +132.1 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.70, 0.81, 0.88, 0.98, 1.00, 1.17, 1.22 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 0.91 (3H, d, *J*=6.1 Hz, H_3-21), 3.24 (1H, dd, *J*=4.3, 11.6 Hz, H-3), 3.35 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; EIMS *m*/*z* 460 $[M]^+$; HREIMS *m*/*z* 460.3912 (calcd for $C_{30}H_{52}O_3$ $[M]^+$, 460.3916).

3.3.3. *Inoterpene C (**3**)*

A white powder; $[\alpha]_D^{23}$ +36.1 (*c* 2.10, MeOH); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.78, 0.97, 1.09, 1.10, 1.26, 1.59, 1.59 (3H each, all s, H_3-18, 30, 19, 29, 28, 26, 27), 1.03 (3H, d, *J*=6.1 Hz, H_3-21), 3.47 (1H, m, H-3), 5.86 (1H, ddd like, *J*=5.5, 8.6, 15.9 Hz, H-23), 5.99 (1H, d, *J*=15.9 Hz, H-24); ^{13}C NMR (pyridine-d_5, 125 MHz) see Table 1; EIMS *m*/*z* 458 $[M]^+$; HREIMS *m*/*z* 458.3764 (calcd for $C_{30}H_{50}O_3$ $[M]^+$, 458.3760).

3.3.4. *Inoterpene D (**4**)*

A white powder; $[\alpha]_D^{28}$ +66.0 (*c* 1.50, $CHCl_3$); IR (KBr) ν_{max} 3450, 2940 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.78, 0.81, 0.85, 0.98, 1.00, 1.68, 1.75 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 3.23 (1H, dd, *J*=4.4, 11.6 Hz, H-3), 3.72 (1H, dd, *J*=10.7, 10.7 Hz, H-21a), 3.83 (1H, m, H-22), 4.02 (1H, dd, *J*=3.8, 10.7 Hz, H-21b), 5.22 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; EIMS *m*/*z* 458 $[M]^+$; HREIMS *m*/*z* 458.3769 (calcd for $C_{30}H_{50}O_3$ $[M]^+$, 458.3760).

3.3.5. *Inoterpene E (**5**)*

A white powder; $[\alpha]_D^{26}$ +30.8 (*c* 0.17, $CHCl_3$); IR (KBr) ν_{max} 3450, 2940 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72, 0.80, 0.89, 0.97, 1.00, 1.32, 1.33 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 3.24 (1H, dd, *J*=4.1, 11.7 Hz, H-3), 3.64 (1H, dd, *J*=6.2, 11.7 Hz, H-21a), 3.78 (1H, dd, *J*=2.8, 11.7 Hz, H-21b), 5.59 (1H, d, *J*=15.1 Hz, H-24), 5.81 (1H, ddd like, *J*=7.9, 7.9, 15.1 Hz, H-23); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; EIMS *m*/*z* 474 $[M]^+$; HREIMS *m*/*z* 474.3711 (calcd for $C_{30}H_{50}O_4$ $[M]^+$, 474.3709).

3.3.6. *Inoterpene F (**6**)*

A white powder; $[\alpha]_D^{27}$ +38.8 (*c* 0.85, $CHCl_3$); IR (KBr) ν_{max} 3450, 2950, 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.78, 0.81, 0.89, 0.98, 1.00, 1.81 (3H each, all s, H_3-18, 29, 30, 19, 28, 27), 3.23 (1H, dd, *J*=4.4, 11.6 Hz, H-3), 3.97 (1H, d like, *J*=ca. 5 Hz, H-21), 4.84, 5.00 (1H each, both s-like, H_2-26); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; EIMS *m*/*z* 440 $[M]^+$; HREIMS *m*/*z* 440.3663 (calcd for $C_{30}H_{48}O_2$ $[M]^+$, 440.3654).

3.3.7. *Preparation of the (S)- and (R)-MTPA esters (**1a** and **1b**) from **1***

A solution of **1** (1.9 mg, 0.004 mmol) in pyridine (1 mL) was treated with (−)-MTPACl (0.015 mL, 0.08 mmol), and the mixture was stirred at rt for 6 h. The reaction mixture was poured into water (1 mL) and the whole was extracted with EtOAc (6 mL). The EtOAc extract was washed with brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by reversed-phase silica gel CC [MeOH–H_2O (60:40, v/v)→MeOH] to give (*S*)-MTPA ester derivative (**1a**, 2.8 mg, 76%). Through a similar procedure, the (*R*)-MTPA ester derivative (**1b**, 2.0 mg, 75%) was obtained from **1** (1.4 mg, 0.003 mmol) using (+)-MTPACl.

*3.3.7.1. (S)-MTPA ester derivative (**1a**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.64, 0.84, 0.86, 0.93, 0.98 (3H each, all s, H_3-18, 29, 30, 28, 19), 0.84 (3H, d, *J*=6.0 Hz, H_3-21), 1.17, 1.23 [3H each, all s, H_3-26, 27 (interchangeable)], 1.33, 1.76 (1H each, both m, H_2-1), 4.72 (1H, dd, *J*=4.8, 11.7 Hz, H-3), 4.95 (1H, m, H-24); positive-ion FABMS *m*/*z* 915 $[M+Na]^+$; HRFABMS *m*/*z* 915.4616 (calcd for $C_{50}H_{66}O_7F_6Na$ $[M+Na]^+$, 915.4610).

*3.3.7.2. (R)-MTPA ester derivative (**1b**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.67, 0.83, 0.84, 0.87, 1.02 (3H each, all s, H_3-18, 29, 28, 30, 19), 0.90 (3H, d, *J*=6.0 Hz, H_3-21), 1.15, 1.18 [3H each, all s, H_3-26, 27 (interchangeable)], 1.35, 1.79 (1H each, both m, H_2-1), 4.74 (1H, dd, *J*=2.8, 12.0 Hz, H-3), 4.95 (1H, m, H-24); positive-ion FABMS *m*/*z* 915 $[M+Na]^+$; HRFABMS *m*/*z* 915.4617 (calcd for $C_{50}H_{66}O_7F_6Na$ $[M+Na]^+$, 915.4610).

*3.3.8. Preparation of the (S)- and (R)-MTPA esters (**2a** and **2b**) from **2***

(*S*)- and (*R*)-MTPA esters, **2a** (3.7 mg, 91%) and **2b** (4.3 mg, 98%), were each obtained from **2** (2.1 mg, 0.005 mmol for **2a**, 2.3 mg, 0.005 mmol for **2b**) by using a similar procedure as that used to obtain **1a** and **1b**.

*3.3.8.1. (S)-MTPA ester derivative (**2a**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.66, 0.84, 0.87, 0.93, 0.98 (3H each, all s, H_3-18, 29, 30, 28, 19), 0.85 (3H, d, J=5.5 Hz, H_3-21), 1.14, 1.18 [3H each, all s, H_3-26, 27 (interchangeable)], 1.34, 1.75 (1H each, both m, H_2-1), 4.72 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.97 (1H, m, H-24); positive-ion FABMS m/z 915 $[M+Na]^+$; HRFABMS m/z 915.4615 (calcd for $C_{50}H_{66}O_7F_6Na$ $[M+Na]^+$, 915.4610).

*3.3.8.2. (R)-MTPA ester derivative (**2b**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.67, 0.83, 0.84, 0.87, 1.01 (3H each, all s, H_3-18, 29, 28, 30, 19), 0.78 (3H, d, J=5.5 Hz, H_3-21), 1.17, 1.23 [3H each, all s, H_3-26, 27 (interchangeable)], 1.36, 1.79 (1H each, both m, H_2-1), 4.72 (1H, dd, J=4.3, 12.2 Hz, H-3), 4.98 (1H, m, H-24); positive-ion FABMS m/z 915 $[M+Na]^+$; HRFABMS m/z 915.4615 (calcd for $C_{50}H_{66}O_7F_6Na$ $[M+Na]^+$, 915.4610).

*3.3.9. Synthesis of the mixture of **1** and **2** by OsO_4 oxidation*

A solution of **7** (40 mg, 0.094 mmol), MC OsO_4 (12 mg, containing about 10% by weight as OsO_4, 0.0047 mmol), and *N*-methylmorpholine *N*-oxide (33 mg, 0.28 mmol) in acetone–1,4-dioxane–H_2O (6:2:1, 4.5 mL) was stirred for 48 h at rt. The MC OsO_4 was filtered off, and the filtrate was concentrated to a quarter under reduced pressure and extracted with EtOAc. The EtOAc extract was washed with H_2O, saturated sodium thiosulfate, and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (2:1, v/v)] to give the mixture (1:1 by ^{1}H NMR) of **1** and **2** (35 mg). The mixture of **1** and **2** were identified by comparison of their ^{1}H NMR, ^{13}C NMR, and MS data with those of isolated compounds.

*3.3.10. Synthesis of **3** by photoinduced oxidation*

A solution of **7** (96 mg, 0.22 mmol) and rose bengal (10 mg) in MeOH (5 mL) was kept in a Pyrex photochemical reactor vessel. The solution bubbled through with O_2 was irradiated with a 400-W high-pressure mercury lamp in a water-cooled quartz immersion well for 2 h. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (5:1, v/v)] and HPLC [MeOH–H_2O (90:10, v/v)] to give inoterpene C (**3**, 5.0 mg, 5%). The obtained inoterpene C (**3**) was identified by comparison of their physical data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, MS) with those of isolated compound.

*3.3.11. Preparation of acetonide derivative **4a***

A solution of **4** (3.0 mg, 0.0066 mmol) and *p*-toluenesulfonic acid (ca. 2 mg) in dry DMF (1 mL) was treated with 2,2-dimethoxypropane (0.1 mL), and the mixture was stirred for 30 min at rt. The reaction mixture was poured into water (4 mL) and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc (8 mL). The EtOAc extract was washed with brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (2:1, v/v)] to give acetonide derivative (**4a**, 3.1 mg, quant.).

*3.3.11.1. Acetonide derivative **4a**.* Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.75, 0.81, 0.86, 0.93, 0.95, 1.40, 1.41, 1.61, 1.69 (3H each, all s, H_3-18, 29, 30, 19, 28, 3′[(–OC(O)(CHa_3)(*CH*b$_3$)), 2′[(–OC(O)(*CH*a$_3$)(CHb_3)), 26, 27), 1.66 (1H, m, H-20), 3.23 (1H, dd, J=4.0, 11.6, H-3), 3.87 (1H, m, H-22), 3.93, 3.98 (1H each, m, H_2-21), 5.16 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 79.0 (C-3), 16.0 (C-18), 19.2 (C-19), 42.0 (C-20), 63.5 (C-21), 75.3 (C-22), 121.6 (C-24), 132.6 (C-25), 18.2 (C-26), 25.8 (C-27), 28.0 (C-28), 15.4 (C-29), 24.2 (C-30), 99.9 [C-1′, –O*C*(O)(CH_3)$_2$], 22.7 [C-2′, –OC(O)(*C*Ha_3)(CHb_3)], 28.8 [C-3′, –OC(O)(CHa_3)(*C*Hb_3)]; EIMS m/z 498 $[M]^+$; HREIMS m/z 498.4065 (calcd for $C_{33}H_{54}O_3$ $[M]^+$, 498.4073).

*3.3.12. Preparation of pivaloyl ester (**4b**)*

A solution of **4** (6.9 mg, 0.015 mmol) in pyridine (1.0 mL) was treated with pivaloyl chloride (2 μL, 0.015 mmol) and the mixture was stirred for 2 h at 0 °C. The reaction mixture was poured into water (1.0 mL) and the whole was extracted with EtOAc (4 mL). The EtOAc extract was washed with brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (10:1→5:1→2:1, v/v)] to give **4b** (2.0 mg, 25%).

*3.3.12.1. Pivaloyl ester (**4b**).* Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.76, 0.81, 0.89, 0.97, 1.00, 1.63, 1.74 (3H each, all s, H_3-18, 29, 30, 19, 28, 26, 27), 1.22 (9H, s, (CH_3)$_3$CCOO), 3.22 (1H, dd, J=4.0, 11.6, H-3), 3.73 (1H, m, H-22), 4.13 (1H, dd, J=5.5, 11.6, H-21a), 4.38 (1H, dd, J=1.9, 11.6, H-21b), 5.17 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 79.0 (C-3), 15.9 (C-18), 19.2 (C-19), 42.2 (C-20), 63.0 (C-21), 72.9 (C-22), 121.5 (C-24), 18.0 (C-26), 26.0 (C-27), 28.0 (C-28), 15.4 (C-29), 24.4 (C-30), 27.4 [(CH_3)$_3$CCOO], 38.9 [(CH_3)$_3$*C*COO]; EIMS m/z 542 $[M]^+$; HREIMS m/z 542.4333 (calcd for $C_{35}H_{58}O_4$ $[M]^+$, 542.4335).

*3.3.13. Preparation of the (S)- and (R)-MTPA esters (**4c** and **4d**) from **4***

(*S*)- and (*R*)-MTPA esters, **4c** (1.0 mg, 51%) and **4d** (1.1 mg, 56%), were each obtained from **4** (each 1.1 mg, 0.002 mmol for **4c** and **4d**) by using a similar procedure as that used to obtain **1a** and **1b**.

*3.3.13.1. (S)-MTPA ester derivative (**4c**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.70, 0.89, 0.84, 0.98, 0.92, 1.56, 1.72 (3H each, all s, H_3-18, 29, 30, 28, 19, 26, 27), 1.18 (9H, s, (CH_3)$_3$CCOO), 3.86 (1H, dd, J=1.8, 12.2, H-21a), 3.98 (1H, dd, J=3.7, 12.2, H-21b), 4.70 (1H, dd, J=4.3, 11.6, H-3), 5.07 (1H, m, H-24), 5.25 (1H, m, H-22); positive-ion FABMS m/z 997 $[M+Na]^+$; HRFABMS m/z 997.5026 (calcd for $C_{55}H_{72}O_8F_6Na$ $[M+Na]^+$, 997.5029).

*3.3.13.2. (R)-MTPA ester derivative (**4d**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.71, 0.83, 0.84, 0.90, 1.01, 1.46, 1.57 (3H each, all s, H_3-18, 29, 30, 28, 19, 26, 27), 1.20 (9H, s, (CH_3)$_3$CCOO), 3.90 (1H, dd, J=3.1, 11.0 Hz, H-21a), 4.14 (1H, m, H-21b), 4.73 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.89 (1H, m, H-24), 5.25 (1H, m, H-22); positive-ion FABMS m/z 997 $[M+Na]^+$; HRFABMS m/z 997.5020 (calcd for $C_{55}H_{72}O_8F_6Na$ $[M+Na]^+$, 997.5029).

*3.3.14. Synthesis of **5** by photoinduced oxidation*

Inoterpene E (**5**, 6.1 mg, 6%) was obtained from 3β,21-dihydroxylanosta-8,24-diene (**8**, 95 mg, 0.21 mmol) by using a similar procedure as that used to obtain **3** from **7**.

*3.3.15. Preparation of the (S)- and (R)-MTPA esters (**6a** and **6b**) from **6***

(*S*)- and (*R*)-MTPA esters, **6a** (2.7 mg, 66%) and **6b** (1.8 mg, 47%), were each obtained from **6** (2.1 mg, 0.0048 mmol for **6a**, 2.0 mg,

0.0045 mmol for **6b**) by using a similar procedure as that used to obtain **1a** and **1b**.

*3.3.15.1. (S)-MTPA ester derivative (**6a**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.70, 0.84, 0.90, 0.93, 0.99, 1.73 (3H each, all s, H_3-18, 29, 30, 28, 19, 27), 1.33, 1.76 (1H each, both m, H_2-1), 4.63, 4.68 (1H each, both s-like, H_2-26) 4.72 (1H, dd, J=4.1, 12.4 Hz, H-3), 5.63 (1H, d like, J=ca. 4 Hz, H-21); positive-ion FABMS m/z 872 $[M+Na]^+$; HRFABMS m/z 872.4454 (calcd for $C_{50}H_{62}O_6F_6Na$ $[M+Na]^+$, 872.4451).

*3.3.15.2. (R)-MTPA ester derivative (**6b**).* A white powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.66, 0.82, 0.83, 0.88, 1.01, 1.77 (3H each, all s, H_3-18, 29, 28, 30, 19, 27), 1.35, 1.79 (1H each, both m, H_2-1), 4.73, 4.83 (1H each, both s-like, H_2-26) 4.74 (1H, dd, J=4.8, 11.6 Hz, H-3), 5.63 (1H, d like, J=ca. 4 Hz, H-21); positive-ion FABMS m/z 872 $[M+Na]^+$; HRFABMS m/z 872.4447 (calcd for $C_{50}H_{62}O_6F_6Na$ $[M+Na]^+$, 872.4451).

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References and notes

1. Saar, M. *J. Ethnopharmacol.* **1991**, *31*, 175–179.
2. Kim, Y. O.; Park, H. W.; Kim, J. H.; Lee, J. Y.; Moon, S. H.; Shin, C. S. *Life Sci.* **2006**, *79*, 72–80.
3. Park, J.-R.; Park, J.-S.; Jo, E.-H.; Hwang, J.-W.; Kim, S.-J.; Ra, J.-C.; Okezie, O. I.; Lee, Y.-S.; Kang, K.-S. *Biofactors* **2006**, *27*, 147–155.
4. Song, Y.; Hui, J.; Kou, W.; Xin, R.; Jia, F.; Wang, N.; Hu, F.; Zhang, H.; Liu, H. *Curr. Microbiol.* **2008**, *57*, 454–462.
5. Nakata, T.; Yamada, T.; Taji, S.; Ohishi, H.; Wada, S.; Tokuda, H.; Sakuma, K.; Tanaka, R. *Bioorg. Med. Chem.* **2007**, *15*, 257–264.
6. Nakajima, Y.; Sato, Y.; Konishi, T. *Chem. Pharm. Bull.* **2007**, *55*, 1222–1226.
7. Lee, I.-K.; Kim, Y.-S.; Jang, Y.-W.; Jung, J.-Y. J.; Yun, B.-S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6678–6681.
8. Kim, H.-G.; Yoon, D.-H.; Kim, C.-H,; Shrestha, B.; Chang, W.-C.; Lim, S.-Y.; Lee, W.-H.; Han, S.-G.; Lee, J.-O.; Lim, M.-H.; Kim, G.-Y.; Choi, S.; Song, W.-O.; Sung, J.-M.; Hwang, K.-C.; Kim, T.-W. *J. Med. Food* **2007**, *10*, 80–89.
9. Taji, S.; Yamada, T.; In, Y.; Wada, S.; Usami, Y.; Sakuma, K.; Tanaka, R. *Helv. Chim. Acta* **2007**, *90*, 2047–2057.
10. Taji, S.; Yamada, T.; Tanaka, R. *Helv. Chim. Acta* **2008**, *91*, 1513–1524.
11. Yoshikawa, M.; Xu, F.; Nakamura, S.; Wang, T.; Matsuda, H.; Tanabe, G.; Muraoka, O. *Heterocycles* **2008**, *75*, 1397–1405.
12. Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yasuda, D.; Yamaguchi, I.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2038–2042.
13. Zhang, H.; Matsuda, H.; Nakamura, S.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3272–3277.
14. Zhang, Y.; Nakamura, S.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Tetrahedron* **2008**, *64*, 7347–7352.
15. Morikawa, T.; Li, X.; Nishida, E.; Ito, Y.; Matsuda, H.; Nakamura, S.; Muraoka, O.; Yoshikawa, M. *J. Nat. Prod.* **2008**, *71*, 828–835.
16. Tanabe, G.; Sakano, M.; Minematsu, T.; Matusda, H.; Yoshikawa, M.; Muraoka, O. *Tetrahedron* **2008**, *64*, 10080–10086.
17. Muraoka, O.; Xie, W.; Tanabe, G.; Amer, M. F. A.; Minematsu, T.; Yoshikawa, M. *Tetrahedron Lett.* **2008**, *49*, 7315–7317.
18. Yoshikawa, M.; Morikawa, T.; Funakoshi, K.; Ochi, M.; Pongpiriyadacha, Y.; Matsuda, H. *Heterocycles* **2008**, *75*, 1639–1650.
19. Morikawa, T.; Funakoshi, K.; Ninomiya, K.; Yasuda, D.; Miyagawa, K.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2008**, *56*, 956–962.
20. Shin, Y.; Tamai, Y.; Terazawa, M. *J. Wood. Sci.* **2006**, *47*, 313–316.
21. Kahlos, K.; Hiltunen, R. *Acta Pharm. Fenn.* **1986**, *95*, 71–76.
22. Kahlos, K.; Hiltunen, R.; Schantz, M. V. *Planta Med.* **1984**, *50*, 197–198.
23. Leong, Y.; Harrison, L. J. *Phytochemistry* **1999**, *50*, 849–857.
24. The ^{1}H and ^{13}C NMR spectra of **1**–**6** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
25. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
26. Knappe, E.; Peteri, D. Z. *Anal. Chem.* **1962**, *190*, 380–386.
27. Yoshikawa, M.; Sugimoto, S.; Nakamura, S.; Sakumae, H.; Matsuda, H. *Chem. Pharm. Bull.* **2007**, *55*, 1034–1038.
28. Yoshikawa, M.; Sugimoto, S.; Nakamura, S.; Matsuda, H. *Chem. Pharm. Bull.* **2007**, *55*, 571–576.
29. Nakamura, S.; Sugimoto, S.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2007**, *55*, 1342–1348.