Three New Lanostane Triterpenoids from *Inonotus obliquus*

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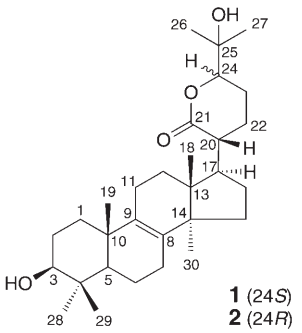
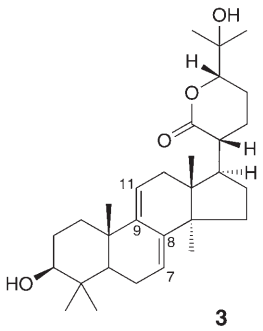
Three new lanostane-type triterpenoids, inonotsulides A, B, and C (**1**–**3**, resp.) were isolated from the sclerotia of *Inonotus obliquus* (PERS.: FR.) (Japanese name: *Kabanoanatake*; Russian name: *Chaga*). Their structures were determined to be (20*R*,24*S*)-3*β*,25-dihydroxylanost-8-en-20,24-olide (**1**), (20*R*,24*R*)-3*β*,25-dihydroxylanost-8-en-20,24-olide (**2**), and (20*R*,24*S*)-3*β*,25-dihydroxylanosta-7,9(11)-dien-20,24-olide (**3**) on the basis of chemical transformation, NMR spectroscopy including 1D and 2D (^{1}H,^{1}H-COSY, NOESY, HMQC, HMBC), EI-MS, and single-crystal X-ray analysis.

Introduction. – *Inonotus obliquus* (PERS.: FR.) Pil. (= *Fuscoporia obliqua* (PERS.: FR.) AOSHIMA), called *kabanoanatake* (in Japan), and *chaga* or *tchaga* (in Russia), is a white-rot fungus belonging to the family *Hymenochaetaceae Donk* [1], and the distribution of this mushroom is recognized in Europe, Asia, and North America [2]. The imperfect form of *I. obliquus* occurs parasitically on trunks, usually of *Betula* (birch), and more rarely also on *Ulmus*, *Alnus*, and *Fraxinus*. Only after the tree dies is the perfect form with pores and basidia produced under the bark. *I. obliquus* is widely distributed in Hokkaido forests of *Betula platyphylla* var. *japonica* (Japanese name: *shirakaba*) in Japan [3][4]. In Eastern Europe, especially in Russia, the sclerotia of this mushroom have been used as a folk medicine for cancer since the 16th or 17th century [5][6]. Also, the Khanty of West Siberia use this fungus to prevent and treat heart, liver, tuberculosis, and stomach disease [7]. Recently, we reported structure determination of two new lanostane-type triterpenoids isolated from the sclerotia, inonotsuoxides A and B ((22*R*,24*S*)-*22,25-epoxylanost-8-ene-3β,24-diol* and (22*S*)-22,25-epoxy epimer), and the results of *in vivo* mouse skin carcinogenesis test using DMBA/TPA model about inotodiol, the most abundant triterpene of this sclerotia [8]. We continued the study to search for new lanostane-type triterpenoids in order to clarify the whole process. Careful examination of the sclerotia of *I. obliquus* has led to the isolation of three new lanostane-type triterpenes named inonotsulides A, B, and C (**1**–**3**, resp.). The structures of the new compounds **1**–**3** were determined on the basis of chemical and spectroscopic methods, including X-ray analysis. This report deals with the structure determination of these compounds.

Results and Discussion. – Sclerotia of *I. obliquus* was extracted with $CHCl_3$, and the extract was separated by silica-gel column chromatography, medium-pressure liquid



1 (24*S*)
2 (24*R*)



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chromatography (MPLC), and high-pressure liquid chromatography (HPLC), and three new triterpenes, **1**–**3**, were obtained. The molecular formula of compound **1** was determined as $C_{30}H_{48}O_4$ (M^+; *m*/*z* 472.3558) by HR-EI-MS analysis. The IR spectrum indicated OH and C=O groups ($\tilde{\nu}_{max}$ 3403 and 1708 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra ($CDCl_3$) of **1** (*Table 1*) exhibited signals of seven tertiary Me, ten CH_2, six sp^3-CH groups, including two oxymethines (δ(H) 3.24 (*dd*, 1 H); δ(C) 78.9 (*d*)); and (δ(H) 4.16 (*dd*, 1 H); δ(C) 84.9 (*d*)), five sp^3-C-atoms including one C–O moiety (δ(C) 71.5 (*s*)), and a tetrasubstituted C=C bond (δ(C) 133.8 (*s*); 134.8 (*s*)) and a C=O group (δ(C) 174.9 (*s*)). The ^{1}H- and ^{13}C-NMR data suggested that the compound **1** was a lanost-8-ene derivative. One of the OH groups (β) was at C(3) as indicated by the chemical shifts and the coupling constants (δ(H) 3.24 (*dd*, $J(3,2\alpha) = 4.6$, $J(3,2\beta) = 11.7$ Hz); δ(C) 78.9 (*d*)), and the other was at C(25) (δ(H) 1.23, 1.29 (*s*, Me(26), Me(27)); δ(C) 23.9 (*q*, C(26)); 25.9 (*q*, C(27)); 71.5 (*s*, C(25))). Acetylation of **1** with Ac_2O/pyridine gave a monoacetate **1a** ($C_{32}H_{50}O_5$; *m*/*z* 514.3651), of which the acetoxymethine H-atom signal appeared at δ(H) 4.50 (*dd*); the other OH group (δ(H) 4.16 (*dd*, H–C(24))) resisted acetylation. The planar structure of **1** was determined by HMBC and ^{1}H,^{1}H-COSY spectra. The HMBC spectrum of **1** (*Table 1*) indicated a long-range correlation between Me(18) (δ(H) 0.77) and each of C(12), C(13), C(14), and C(17), between Me(19) (δ(H) 0.98) and C(1), C(5), C(9), and C(10), between Me(26) (δ(H) 1.23) and C(24), C(25), and C(27), between Me(27) (δ(H) 1.29) and C(24), C(25), and C(26), between Me(28) (δ(H) 1.00) and C(3), C(4), C(5), and C(29), between Me(29) (δ(H) 0.81) and C(3), C(4), C(5), and C(28), between Me(30) (δ(H) 0.93) and C(8), C(13), C(14), and C(15), between H–C(20) (δ(H) 2.52) and C(13), C(16), C(17), C(21) (δ(C) 174.9), C(22), and C(23), between H–C(24) (δ(H) 4.16) and C(21), C(22), C(25) (δ(C) 71.5), C(26), and C(27), respectively. In the ^{1}H,^{1}H-COSY spectrum (*Table 1*), H–C(24) correlated with CH_2(23) (δ(H) 1.78–1.85); and H–C(20) with H_α–C(17) and CH_2(22). Based on the molecular formula of $C_{30}H_{48}O_4$ and the spectral data, mentioned above we suggested that the structure of **1** was of a lanost-8-ene-3β,25-diol with a δ-lactone ring at C(20) and C(24). Alkaline hydrolysis furnished a trihydroxycarboxylic acid derivative **1b**, ($C_{30}H_{50}O_5$; *m*/*z* 490.3662), of which the signal of H–C(24) appeared at δ(H) 3.15 (*dd*, in CD_3OD), and the signal of the carboxylic acid C-atom C(21) at δ(C)

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**, *Together with ^{1}H,^{1}H-COSY and HMBC* (H → C) *Correlations.* At 500 (^{1}H) and 125 (^{13}C) MHz in $CDCl_3$; δ in ppm, J in Hz.

Position		δ(H)	^{1}H,^{1}H-COSY	NOE	δ(C)	HMBC (C)
1	α	1.19–1.26 (*m*)	1β, 2α, 2β	1α, 1β, 3, 5, 11	35.5 (*t*)	2, 10
	β	1.71–1.77 (*m*)	1α, 2α, 2β	1α, 11, 19		
2	α	1.64–1.72 (*m*)	1α, 1β, 2β, 3	1α, 2β, 3	27.8 (*t*)	1, 3
	β	1.55–1.63 (*m*)	1α, 1β, 2α, 3	2α, 19, 29		
3		3.24 (*dd*, *J* = 11.7, 4.6)	2α, 2β	1α, 2α, 5, 28	78.9 (*d*)	1, 4, 28, 29
4		–			38.8 (*s*)	
5		1.06 (*dd*, *J* = 12.8, 2.9)	6α, 6β	1α, 3, 6α, 7, 30	50.3 (*d*)	1, 3, 4, 6, 7, 9, 10, 19, 28, 29
6	α	1.66–1.72 (*m*)	5, 6β, 7	5, 28	18.2 (*t*)	5, 7, 10
	β	1.49–1.54 (*m*)	5, 6α, 7	19, 29		
7		2.02–2.07 (*m*, 2 H)	6α, 6β	5, 30	26.4 (*t*)	5, 8, 9
8		–			133.8 (*s*)	
9		–			134.8 (*s*)	
10		–			37.0 (*s*)	
11		1.94–2.05 (*m*, 2 H)	12α, 12β	1α, 1β, 12β, 18	20.8 (*t*)	8, 9, 10, 12, 13
12	α	1.67–1.73 (*m*)	11	12β, 30	29.0 (*t*)	11, 13, 14
	β	1.57–1.63 (*m*)	11	11, 12α, 18, 19, 20		
13		–			22.5 (*s*)	
14		–			49.6 (*s*)	
15	α	1.23–1.29 (*m*)	15β, 16α, 16β	16α, 30	30.3 (*t*)	13, 14, 16, 17, 30
	β	1.59–1.65 (*m*)	15α, 16α, 16β	16β, 18		
16	α	1.85–1.92 (*m*)	15α, 15β, 16β, 17, 20	15α, 17, 30	25.7 (*t*)	13, 14, 15, 17, 20
	β	1.39–1.45 (*m*)	15α, 15β, 16α, 17, 20	15β, 18, 20, 22α		
17		2.32 (*q*, *J* = 9.4)	16α, 16β, 20	16α, 22α, 23, 30	43.5 (*d*)	12, 13, 16, 18, 20, 21, 22
18		0.77 (*s*)		11, 12β, 15β, 16β, 19, 20, 22β	26.5 (*q*)	12, 13, 14, 17
19		0.98 (*s*)		1β, 2β, 6β, 12β, 18, 29	19.1 (*q*)	1, 5, 9, 10
20		2.52 (*ddd*, *J* = 9.4, 8.0, 6.4)	16α, 16β, 17, 22α, 22β	12β, 16β, 18, 22β, 24	39.6 (*d*)	13, 16, 17, 21, 22, 23
21		–			174.9 (*s*)	17, 20, 22
22	α	1.68–1.73 (*m*)	20, 22β, 23	16β, 17, 26, 27	23.2 (*t*)	17, 20, 21
	β	2.00–2.06 (*m*)	20, 22α, 23	18, 20, 24, 26, 27		
23		1.78–1.85 (*m*, 2 H)	22α, 22β, 24	17, 24, 26, 27	20.5 (*t*)	20, 22, 24, 25
24		4.16 (*dd*, *J* = 10.1, 5.9)	23	20, 22β, 23, 26, 27	84.9 (*d*)	21, 22, 25, 26, 27
25		–			71.5 (*s*)	23, 24, 26, 27
26		1.23 (*s*)		22α, 22β, 23, 24	23.9 (*q*)	24, 25, 27
27		1.29 (*s*)		22α, 22β, 23, 24	25.9 (*q*)	24, 25, 26
28		1.00 (*s*)		3, 6α	27.9 (*q*)	3, 4, 5, 29
29		0.81 (*s*)		2β, 6β, 18, 19	15.4 (*q*)	3, 4, 5, 28
30		0.93 (*s*)		5, 7, 12α, 15α, 16α, 17	24.5 (*q*)	8, 13, 14, 15

180.7. The absolute configuration of **1** at C(20) was established as (*R*) on the basis of significant NOEs for H_β–C(20)/H_β–C(16), Me(18) and H_β–C(22). The absolute configuration at C(24) was determined to be (*S*) based on significant NOEs for H–C(24)/H_β–C(20), H_β–C(22), Me(26), and Me(27). Other NOEs were observed from Me(19) to H_β–C(2) and Me(29), from H_α–C(5) to H_α–C(7), from H_β–C(6) to Me(19) and Me(29), from H_α–C(7) to Me(30), from H_α–C(12) to Me(30), from H_β–C(11) to Me(18), and from Me(18) to H_β–C(20). Therefore, A, B, and C rings in **1** have chair, half-chair, and half-chair conformation, respectively (*Fig. 1*). These data established the structure of inonotsulide A (**1**) as (20*R*,24*S*)-3β,25-dihydroxylanost-8-en-20,24-olide. To confirm the structure indicated by spectroscopic methods, single-crystal X-ray analysis of **1a** was performed. *Fig. 2* shows the ORTEP view of **1a**. Two molecules, A and B, which spin at C(24) were obtained crystallographically. Relative orientations of the side chain are different in *Fig. 1* (NOESY) and *Fig. 2* (ORTEP). Therefore, it is suggested that the side chain in the solid state (*Fig. 2*) turns about 90° degrees when it is in $CDCl_3$ (*Fig. 1*).

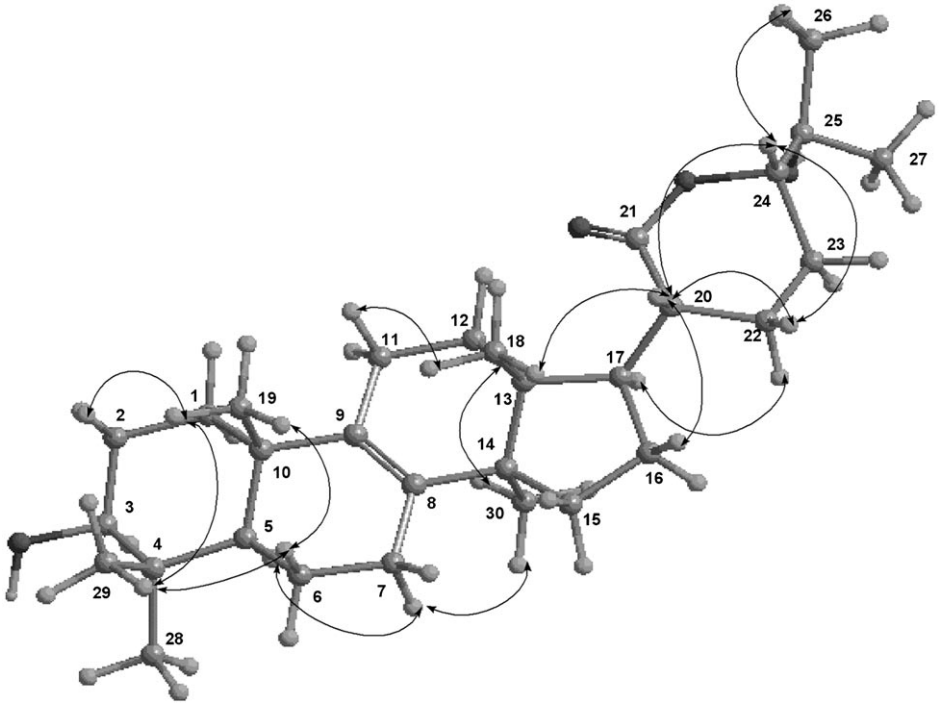


Fig. 1. *Key NOE correlations for* **1**

Compound **2** had the same molecular formula $C_{30}H_{48}O_4$ (M^+; *m*/*z* 472.3554) as **1** according to the HR-EI-MS analysis. The IR, and ^{1}H- and ^{13}C-NMR spectra (*Table 2*) resembled those of **1** except for H–C(24) (δ(H) 4.23, *dd*), H–C(20) (δ(H) 2.59, *ddd*), Me(18) (δ(H) 0.84), and Me(30) (δ(H) 0.91) in the ^{1}H-NMR spectrum, and for C(24) (δ(C) 84.4), C(20) (δ(C) 42.0), C(21) (δ(C) 174.7), and C(23) (δ(C) 21.7) in the

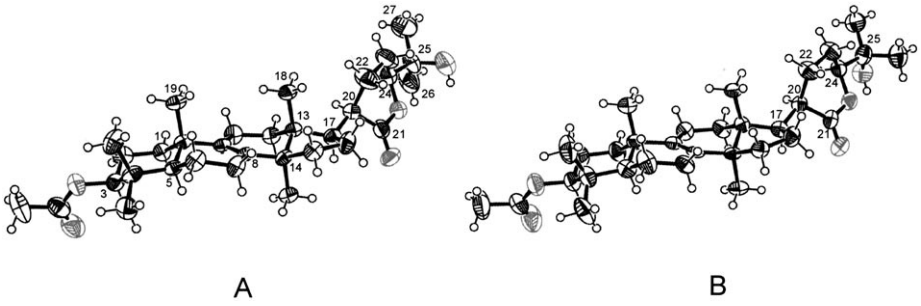


Fig. 2. *ORTEP Drawing of compound* **1a**

^{13}C-NMR spectrum. The HMBC and ^{1}H,^{1}H-COSY spectra closely resembled those of **1**. Alkaline hydrolysis furnished trihydroxy carboxylic acid derivative **2a** ($C_{30}H_{50}O_5$; *m/z* 490.3662), of which the signal of H−C(24) appeared at δ(H) 3.35 (1 H, br. *s*), and that of the carboxylic acid C-atom C(21) appeared at δ(C) 180.7. The (*R*)-configuration at both C(20) and C(24) in **2** were deduced from the chemical shifts and coupling constants (δ(H) 2.59 (*ddd*, J = 9.4, 6.6, 5.3 Hz, H−C(20)), and 4.23 (*dd*, J = 10.8, 4.5 Hz, H−C(24))), and NOEs were observed from H_β−C(20) to H_β−C(16), Me(18), and H_β−C(22). The observed NOEs from H−C(24) to H_α−C(17), H_α−C(22), and Me(26) indicated the (*R*)-configuration at C(24) (*Fig. 3*). These data established the structure of inonotsulide B (**2**) as (20*R*,24*R*)-3β,25-dihydroxylanost-8-en-20,24-olide, which is the C(24)-epimer of **1**.

The minor compound **3** had the molecular formula of $C_{30}H_{46}O_3$ (M^+; *m/z* 470.3393) according to the HR-EI-MS analysis. The IR and UV spectra indicated OH and C=O groups ($\tilde{\nu}_{max}$ 3420 and 1706 cm^{-1}), and a heteroannular diene (λ_{max} 232, 237, 245 (ε 12,000, 14,500, and 9,000). The ^{1}H- and ^{13}C-NMR spectra (*Table 3*) resembled those of **1** except for H−C(7) (δ(H) 5.48 (*d*)), H−C(11) (δ(H) 5.32 (*d*)) in the ^{1}H-NMR spectrum, and C(7) (δ(C) 120.3 (*d*)), C(11) (δ(C) 116.9 (*d*)), C(8) (δ(C) 142.5 (*s*)), and C(9) (δ(C) 145.5 (*s*)) in the ^{13}C-NMR spectrum. The HMBC spectrum showed the long-range correlation between Me(19) (δ(H) 0.97) and C(9), between Me(30) (δ(H) 0.92) and C(8), between H_α−C(5) (δ(H) 1.10), and C(6) and C(7), and between CH_2(12) (δ(H) 2.06 and 2.23) and C(11). The configuration at C(20) and C(24) in **3** were deduced from the chemical shifts and coupling constants (δ(H) 2.52 (*ddd*, J = 9.4, 8.0, 6.4 Hz, H−C(20)); 4.16 (*dd*, J = 10.3, 5.8 Hz, H−C(24))), and NOEs observed from H−C(20) to H_β−C(16), Me(18), and H_β−C(22), and from H−C(24) to H−C(20), H_β−C(22), Me(26), and Me(27) indicated the (20*R*,24*S*)-configuration as in **1** (*Fig. 4*). These data established the structure of inonotsulide C (**3**) as (20*R*,24*S*)-3β,25-dihydroxylanosta-7,9(11)-dien-20,24-olide.

It is suggested that compounds **1**–**3** were biosynthesized from 3β-hydroxylanosta-8,24-dien-21-oic acid (trametenolic acid), which is the most abundant triterpene constituent in this sclerotia, by epoxidization *via* an attack at C(24) of the OH of the COOH group at C(21), and by subsequent opening and closure of the epoxy ring at C(21) and C(24) (*Scheme*).

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**, *Together with ^{1}H,^{1}H-COSY and HMBC* (H → C) *Correlations.* At 500 (^{1}H) and 125 (^{13}C) MHz in $CDCl_3$; δ in ppm, J in Hz.

Position		δ(H)	^{1}H,^{1}H-COSY	NOE	δ(C)	HMBC (C)
1	α	1.19–1.25 (*m*)	1β, 2	1β, 3, 5, 11	35.6 (*t*)	2, 3, 5, 9, 10, 19
	β	1.71–1.76 (*m*)	1α, 2	1α, 11, 19		
2	α	1.64–1.71 (*m*)	1α, 1β, 3	3, 6β, 19	27.8 (*t*)	1, 2, 4, 10
	β	1.55–1.63 (*m*)				
3		3.23 (*dd*, *J* = 11.6, 4.4)	2	1α, 2, 5, 28	78.9 (*d*)	1, 4, 28, 29
4		–			38.9 (*s*)	
5		1.05 (*dd*, *J* = 12.8, 2.3)	6α, 6β	1α, 3, 6α, 7, 12	50.4 (*d*)	1, 3, 4, 6, 7, 10, 19, 28, 29
6	α	1.67–1.71 (*m*)	5, 7	5, 6β, 7, 28	18.2 (*t*)	4, 5, 7, 8, 10
	β	1.50–1.54 (*m*)	5, 7	2, 6α, 7, 19, 29		
7		2.03–2.07 (*m*, 2 H)	6α, 6β	5, 6α, 6β, 12, 15α, 30	26.4 (*t*)	5, 6, 8, 9, 14
8		–			133.7 (*s*)	
9		–			134.9 (*s*)	
10		–			37.1 (*s*)	
11		1.99–2.03 (*m*, 2 H)	12	1α, 1β, 12, 18, 19	20.7 (*t*)	8, 9, 12, 13
12	α	1.55–1.63 (*m*)	11	5, 7, 12, 17, 20, 30	28.1 (*t*)	9, 11, 13, 14, 17, 18
	β	1.64–1.71 (*m*)				
13		–			44.7 (*s*)	
14		–			49.6 (*s*)	13, 14, 16, 17, 30
15	α	1.25–1.29 (*m*)	15β, 16α, 16β	7, 16α, 30	30.3 (*t*)	13, 14, 16, 17, 30
	β	1.71–1.76 (*m*)	15α, 16α, 16β	18		
16	α	1.95–2.00 (*m*)	15α, 15β, 16β, 17	15α, 16β, 17, 22α, 24	26.6 (*t*)	15, 17, 20
	β	1.44–1.49 (*m*)	15α, 15β, 16α, 17	16α, 18, 20, 22β		
17		2.26 (*q*, *J* = 9.4)	16α, 16β, 20	16α, 22α, 24, 30	45.5 (*d*)	12, 13, 16, 18, 20, 21, 22
18		0.84 (*s*)		11, 12, 15β, 16β, 19, 20	16.1 (*q*)	12, 13, 14
19		0.98 (*s*)		1β, 2, 6β, 11, 18, 29	19.1 (*q*)	1, 5, 9, 10
20		2.59 (*ddd*, *J* = 9.4, 6.6, 5.3)	17, 22α, 22β	12, 16β, 18, 22β, 23β	42.0 (*d*)	16, 17, 21, 22, 23
21		–			174.7 (*s*)	
22	α	1.77–1.83 (*m*)	20, 22β, 23α, 23β	16α, 1, 22β, 24	23.1 (*t*)	17, 20, 21, 23, 24
	β	1.94–1.99 (*m*)	20, 22α, 23α, 23β	16β, 20, 22α, 23β		
23	α	1.89–1.94 (*m*)	22α, 22β, 23β, 24	23β, 24, 26, 27	21.7 (*t*)	20, 22, 24, 25
	β	1.69–1.73 (*m*)	22α, 22β, 23α, 24	20, 22β, 23α, 26, 27		
24		4.23 (*dd*, *J* = 10.8, 4.5)	23α, 23β	16α, 17, 22α, 23α, 26, 27	84.4 (*d*)	22, 23, 25, 26, 27
25		–			71.8 (*d*)	
26		1.22 (*s*)		23α, 23β, 24	23.8 (*q*)	24, 25, 27
27		1.27 (*s*)		22β, 23α, 23β, 24	25.6 (*q*)	24, 25, 26
28		1.00 (*s*)		3, 6α	28.0 (*q*)	3, 4, 5, 29
29		0.81 (*s*)		6β, 19	15.4 (*q*)	3, 4, 5, 28
30		0.91 (*s*)		7, 12, 15α, 17	24.4 (*q*)	8, 13, 14, 15

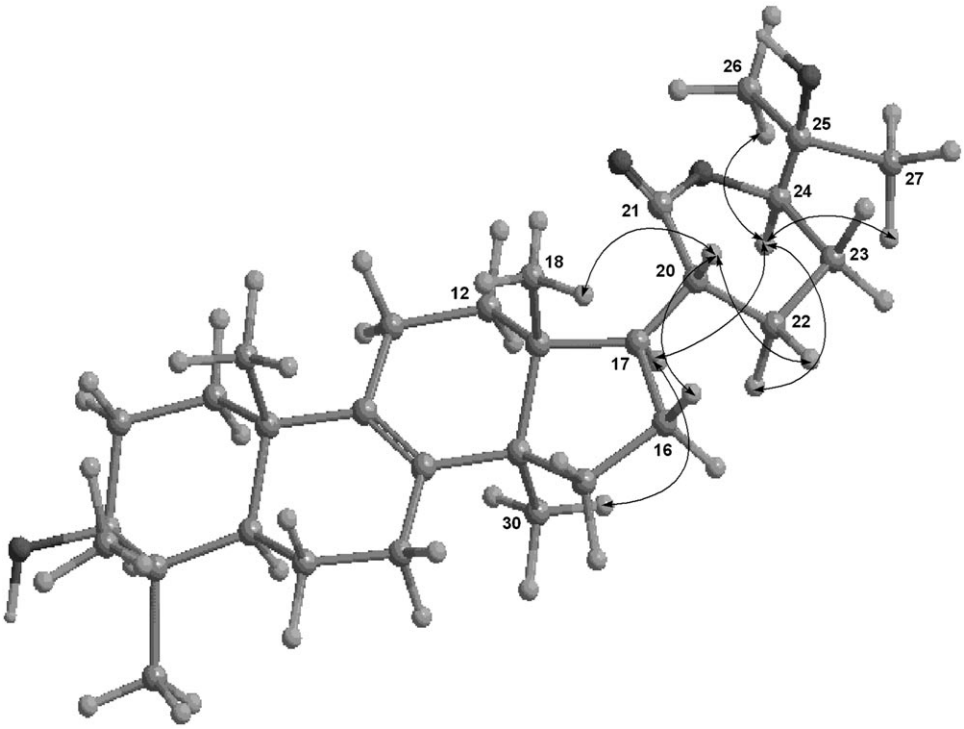


Fig. 3. *Key NOE correlations for* **2**

Experimental Part

General. Column chromatography (CC): silica gel (70–230 mesh, *Merck*). Medium-pressure liquid chromatography (MPLC): silica gel (230–400 mesh, *Merck*). HPLC: *JASCO PU-1586* instrument equipped with a differential refractometer (*RI 1531*). Fractions obtained from CC were monitored by TLC (silica gel *60* F_{254}, *Merck*). Prep. TLC: *Merck* silica-gel F_{254} plates (20 × 20 cm, 0.5-mm thick). M.p.: *Yanagimoto* micro-melting point apparatus; uncorrected. Optical rotations: *JASCO DIP-1000* digital polarimeter. UV Spectra: *Hitachi 150-20* spectrophotometer; λ_{max} in nm (ε). IR Spectra: *Perkin-Elmer 1720X* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian INOVA 500* spectrometer with standard pulse sequences, at 500 and 125 MHz, resp., $CDCl_3$ as the solvent and TMS as the internal standard. EI-MS: *Hitachi 4000 H* double-focusing mass spectrometer (70 eV).

Material. Inonotus obliquus is succeeded in culture in *Salada Melon Co. Ltd.*, Nayoro City, Hokkaido, Japan. Sclerotium (4 kg) of *Inonotus obliquus* was obtained in April, 2004, from the above office.

Extraction and Isolation. The sclerotia of white-rot fungus, *Inonotus obliquus* (PERS. Fr.) PIL. (4 kg) was extracted with $CHCl_3$ (10 l) employing an automatic percolator for 20 d at 60°. The $CHCl_3$ soln. was evaporated under reduced pressure, and the resulting dark brown residue (153.9 g) was subjected to CC (silica gel (3 kg); $CHCl_3$/AcOEt 20:1) to afford residues *A* (*Fr. 1–17*; 4.9 g), *B* (*Fr. 18–32*; 23.6 g), and *C* (*Fr. 33–51*; 10.9 g). Elution was continued with $CHCl_3$/AcOEt 5:1 to give residue *D* (*Fr. 52–87*; 34.7 g), and subsequent CC with $CHCl_3$/AcOEt 2:1 provided residue *E* (*Fr. 88–120*; 24.5 g). Residue *C* was submitted to CC (silica gel (70–230 mesh, 400 g); $CHCl_3$/AcOEt 10:1) to give crude inonotsuoxide A (*Fr. 76–89*; 1.87 g), and subsequent CC with $CHCl_3$/AcOEt 5:1 afforded crude inonotsuoxide B (*Fr. 101–123*; 2.55 g). Recrystallization from MeOH/$CHCl_3$ gave pure inonotsuoxides A (1.32 g) and B

Table 3. *^1H - and ^{13}C-NMR Data of* **3***, Together with $^1H,^1H$-COSY and HMBC* (H → C) *Correlations.* At 500 (^1H) and 125 (^{13}C) MHz in $CDCl_3$; δ in ppm, J in Hz.

Position		δ(H)	$^1H,^1H$-COSY	NOE	δ(C)	HMBC (C)
1	α	1.40–1.46 (*m*)	1β, 2α, 2β	2α, 3, 5	35.7 (*t*)	2, 3, 5, 9, 10 19
	β	1.96–2.01 (*m*)	1α, 2α, 2β	11, 19		
2	α	1.68–1.75 (*m*)	1α, 1β, 2β, 3	1α	27.3 (*t*)	1, 3, 4, 10
	β	1.63–1.68 (*m*)	1α, 1β, 2α, 3	19, 29		
3		3.25 (*dd*, *J* = 11.5, 4.4)	2α, 2β	1α, 5, 28	78.9 (*d*)	1, 2, 4, 5, 28, 29
4		–			38.7 (*s*)	
5		1.10 (*dd*, *J* = 11.9, 4.5)	6	1α, 3, 6, 28, 30	49.1 (*d*)	3, 4, 6, 7, 10, 19, 28, 29
6		2.06–2.12 (*m*, 2 H)	5, 7	5, 7, 11, 18, 19, 28, 29	23.0 (*t*)	4, 5, 7, 8, 10
7		5.48 (br. *d*, *J* = 6.2)	6	6, 15α, 15β, 30	120.3 (*d*)	5, 6, 9, 14
8		–			142.5 (*s*)	
9		–			145.5 (*s*)	
10		–			37.3 (*s*)	
11		5.32 (br. *d*, *J* = 6.4)	12α, 12β	1β, 6, 12β, 16β, 19	116.9 (*d*)	8, 9, 10, 12, 13
12	α	2.20–2.26 (*m*)	11, 12β	17, 30	36.0 (*t*)	11, 13, 14
	β	2.04–2.09 (*m*)	11,12α	11, 18, 19, 20, 22		
13		–			43.8 (*s*)	
14		–			50.2 (*s*)	
15	α	1.44–1.49 (*m*)	15β, 16α, 16β	7, 30	31.0 (*t*)	8, 13, 14, 16, 17, 30
	β	1.63–1.67 (*m*)	15α, 16α, 16β	7, 18		
16	α	1.92–1.98 (*m*)	15α, 15β, 16β, 17	17, 30	25.9 (*t*)	13, 14, 17, 20
	β	1.38–1.45 (*m*)	15α, 15β ,16α, 17	11, 18, 20		
17		2.35 (*q*, *J* = 9.4)	16α, 16β, 20	12α, 16α, 22, 23, 30	44.1 (*d*)	13, 14, 18
18		0.63 (*s*)		6, 12β, 15β, 16β, 19, 20	16.4 (*q*)	12, 14, 17
19		0.97 (*s*)		1β, 2β, 6, 11, 12β, 18, 29	22.7 (*q*)	1, 5, 9, 10
20		2.52 (*ddd*, *J* = 9.4, 8.0, 6.4)	16β, 17, 18, 22β	12β, 16β, 18, 22β, 24	39.8 (*d*)	17, 21, 22, 23
21		–	20, 23		174.7 (*s*)	
22	α	1.67–1.72 (*m*)	22, 24	12β, 17, 20, 26	23.3 (*t*)	17, 20, 21, 23, 24
	β	2.02–2.08 (*m*)				
23		1.80–1.87 (*m*, 2 H)	23	17, 24, 27	20.6 (*t*)	20, 22, 24
24		4.16 (*dd*, *J* = 10.3, 5.8)		20, 22β, 23, 26, 27	84.9 (*d*)	22, 23, 25, 26, 27
25		–			71.5 (*s*)	
26		1.23 (*s*)		22, 24, 27	24.0 (*q*)	24, 25, 27
27		1.29 (*s*)		23, 24, 26	26.0 (*q*)	24, 25, 26
28		1.01 (*s*)		3, 5, 6	28.1 (*q*)	3, 4, 5, 29
29		0.88 (*s*)		2β, 6, 19	15.8 (*q*)	3, 4, 5, 28
30		0.92 (*s*)		5, 7, 12α, 15α, 16α, 17	25.7 (*q*)	8, 13, 14, 15

(1.98 g), resp. Residue *D* was recrystallized from MeOH/$CHCl_3$ to give trametenolic acid (see *Scheme*; 10.9 g), and the filtrate (23.8 g) was purified by CC (silica gel (70–230 mesh, 1.5 kg); $CHCl_3$/AcOEt 5 :1) to give a yellow residue *D1* (*Fr. 76–89*; 1.85 g). Residue *D1* was submitted to MPLC (230–400-mesh silica gel (100 g); hexane/AcOEt 2 :1) to give a crystalline solid (residue *D2*) (*Fr. 132–139*; 358.3 mg), which was separated with HPLC (*ODS*, 90% MeOH) to yield a crystalline mass (residue *D3*; *Fr. 5–6*;

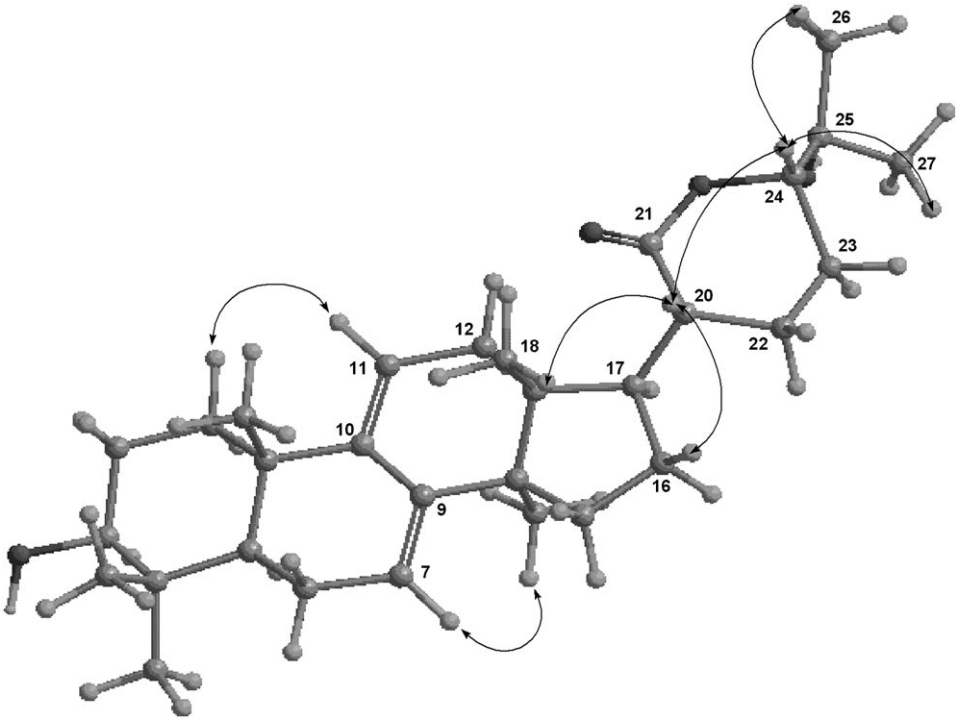


Fig. 4. *Key NOE correlations for* **3**

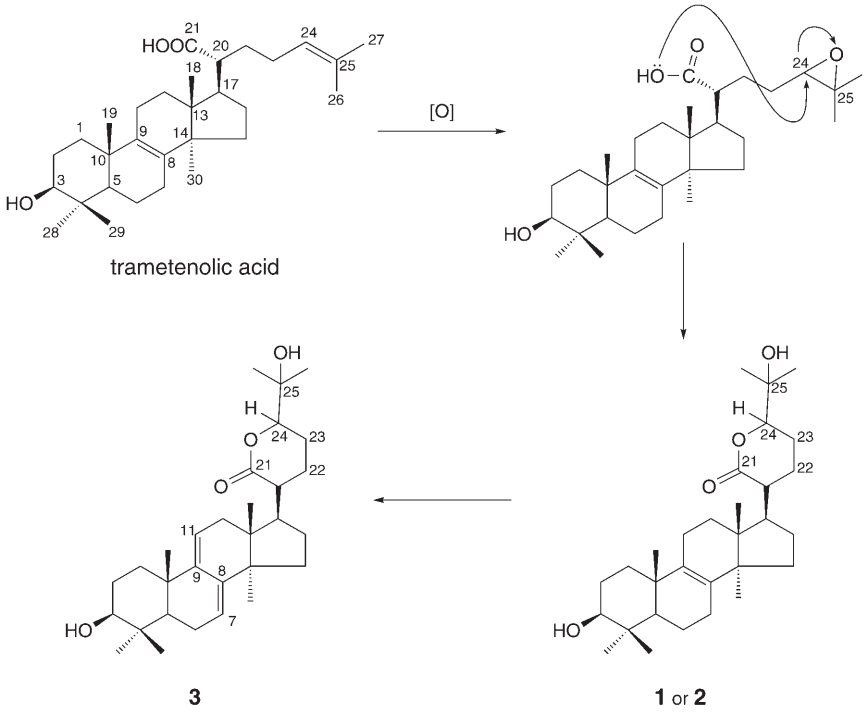
237.9 mg). Residue *D3* was separated by HPLC (*ODS*, 70% MeCN) to give compounds **1** (182.1 mg), **2** (14.1 mg), and **3** (6.3 mg). Inonotsuoxides A and B were identified by comparison of their spectral data with published data [8].

Inonotsulide A (=*(20*R,*24*S*)-3β,25-Dihydroxylanost-8-en-20,24-olide*; **1**). Colorless prisms. M.p. 241–243° (MeOH/$CHCl_3$). $[\alpha]_D^{20} = +62.5$ ($c = 0.16$, $CHCl_3$). IR (KBr): 3403 (OH), 2965, 1708 (δ-lactone), 1457, 1375, 1255, 1063, 747. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 472 (62, M^+), 457 (100, [M – Me]$^+$), 439 (46, [M – Me – H_2O]$^+$), 411 (13), 393 (11), 314 (19), 299 (19). HR-EI-MS: 472.3558 (M^+, $C_{30}H_{48}O_4^+$; calc. 472.3553).

Inonotsulide A Acetate (**1a**). A mixture of compound **1** (10.3 mg) and Ac_2O (1 ml) in pyridine (1 ml) was kept at r.t. overnight. Usual workup gave a residue (10.1 mg), which was recrystallized from MeOH/$CHCl_3$ to **1a** (9.8 mg). ^{1}H-NMR: 0.76 (*s*, Me(29)); 0.88 (*s*, Me(18)); 0.92 (*s*, Me(30)); 1.22 (*s*, Me(26)); 1.28 (*s*, Me(27)); 2.05 (OCOMe); 2.52 (*ddd*, $J = 9.4, 8.0, 6.4$, H–C(20)); 2.31 (*q*, $J = 9.4$, H_α–C(17)); 4.16 (*dd*, $J = 10.1$, 5.9, H–C(24)); 4.50 (*dd*, $J = 11.7$, 4.6, H_α–C(3)); 4.93 (*dd*, $J = 6.4$, 4.1, H–C(24)). ^{13}C-NMR: 16.5 (C(18), C(29)); 18.1 (C(6)); 19.2 (C(19)); 20.5 (C(23)); 20.8 (C(11)); 21.3 (OCO*Me*); 23.2 (C(22)); 23.9 (C(26)); 24.1 (C(2)); 24.5 (C(30)); 25.7 (C(16)); 25.9 (C(27)); 26.3 (C(7)); 27.9 (C(28)); 28.9 (C(12)); 30.3 (C(15)); 35.2 (C(1)); 36.9 (C(10)); 37.8 (C(4)); 39.7 (C(20)); 43.4 (C(17)); 44.5 (C(13)); 49.5 (C(14)); 50.4 (C(5)); 71.4 (C(25)); 80.9 (C(3)); 85.1 (C(24)); 133.9 (C(8)); 134.7 (C(9)); 171.1 (O*C*OMe); 175.0 (C(21)). EI-MS: 514 (61, M^+), 499 (78), 454 (17), 439 (100), 421 (16), 393 (15), 346 (9). HR-EI-MS: 514.3651 (M^+, $C_{32}H_{50}O_5^+$; calc. 514.3658).

Crystal Data of **1a**. $C_{32}H_{50}O_5$, M_r 514.72, monoclinic, space group: $P2_1$, $a = 7.050(5)$ Å, $b = 22.558(5)$ Å, $c = 21.145(5)$ Å, $\beta = 94.912(5)°$, $V = 3350(3)$ Å^3, $D_x = 1.020$ g/cm^3, $Z = 4$. $F(000) = 1128$, $\mu(MoK_\alpha) = 0.067$ mm^{-1}, measured independent reflections 12758, reflections 8491 ($I > 2\sigma(I)$), parameters used for refinement 666, $R_1 = 0.1121$ (for $I > 2\sigma(I)$), $wR_2 = 0.2579$ (for all data). The X-ray

Scheme. *Plausible Biogenetical Pathway to Compounds* **1**–**3**



diffraction data were collected with a *Bruker AXS SMART APEX CCD* camera using graphite-monochromated MoK_α radiation ($\lambda = 0.7103$ Å) at 293 K for **1a**. The crystal structures were solved by a direct method using the SHELXS-97 program [9]. Atomic scattering factors were taken from International Tables for X-Ray Crystallography [10]. Positional parameters of non-H-atoms were refined by a full-matrix least-squares method with anisotropic thermal parameters using the SHELXL-97 program [11]. The structural data were deposited with the following designation: **1a**: CCDC-648899. These can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). The H-atoms were calculated assuming idealized geometries but were not refined.

Alkaline Hydrolysis of **1**. Compound **1** (11.5 mg) was refluxed with a soln. of 0.01M KOH in MeOH (15 ml) on a steam bath for 2 h. Evaporation of the solvent under reduced pressure afforded a residue, which was diluted with H_2O (20 ml), and the resulting precipitate was extracted with Et_2O. Acidification of the aq. layer yielded **1b** (7.8 mg). ^{1}H-NMR (CD_3OD): 0.78 (*s*, Me(18)); 0.80 (*s*, Me(29)); 0.92 (*s*, Me(30)); 0.98 (*s*, Me(28)); 1.00 (*s*, Me(19)); 1.10 (*s*, Me(26) or Me(27)); 1.14 (*s*, Me(26) or Me(27)); 3.15 (*dd*, $J = 9.6$, 6.6, H_α–C(3)); 3.18 (*dd*, $J = 10.5$, 1.8, H–C(24)). ^{13}C-NMR (CD_3OD): 16.5 (C(29)); 16.8 (C(18)); 19.4 (C(6)); 19.7 (C(19)); 21.9 (C(11)); 24.8 (C(30)); 24.9 (C(26) or C(27)); 25.7 (C(26) or C(27)); 27.6 (C(7)); 28.0 (C(16)); 28.5 (C(2)); 28.6 (C(28)); 30.1 (C(12)); 30.3 (C(23)); 31.2 (C(22)); 31.5 (C(15)); 37.0 (C(1)); 38.3 (C(10)); 40.0 (C(4)); 45.5 (C(13)); 48.6 (C(17)); 50.1 (C(20)); 50.7 (C(14)); 52.0 (C(5)); 73.7 (C(25)); 79.6 (C(3)); 80.0 (C(24)); 135.5 (C(8)); 136.0 (C(9)); 180.7 (C(21)). EI-MS: 490 (47, M^+), 457 (87), 454 (17), 439 (100), 411 (42), 393 (26). HR-EI-MS: 490.3662 (M^+, $C_{30}H_{50}O_5^+$; calc. 490.3658).

Inonotsulide B (=*(20*R,*24*R*)-3β,25-Dihydroxylanost-8-en-20,24-olide*; **2**). Colorless prisms. M.p. 238–241° (from MeOH/$CHCl_3$). $[\alpha]_D^{20} = +33.6$ ($c = 0.20$, $CHCl_3$). IR (KBr): 3455 (OH), 2962, 1706 (δ-lactone), 1456, 1374, 1264, 1057, 1030. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 472 (68, M^+), 457 (100, $[M-Me]^+$), 439 (53, $[M-Me-H_2O]^+$), 411 (15), 393 (12), 314 (26), 299 (21). HR-EI-MS: 472.3554 (M^+, $C_{30}H_{48}O_4^+$; calc. 472.3553).

Alkaline Hydrolysis of **2**. Compound **2** (9.2 mg) was refluxed with a soln. of 0.01M KOH in MeOH (12 ml) on a steam bath for 2 h. Evaporation of the solvent under reduced pressure afforded a residue, which was diluted with H_2O (20 ml), and the resulting precipitate was extracted with Et_2O. Acidification of the aq. layer afforded **2b** (6.4 mg). ^{1}H-NMR (CD_3OD): 0.79 (*s*, Me(18)); 0.80 (*s*, Me(29)); 0.92 (*s*, Me(30)); 0.98 (*s*, Me(28)); 1.00 (*s*, Me(19)); 1.12 (*s*, Me(26) or Me(27)); 1.13 (*s*, Me(26) or Me(27)); 3.15 (*dd*, $J = 10.4, 5.8$, H_α–C(3)); 3.35 (br. *s*, H–C(24)). ^{13}C-NMR (CD_3OD): 16.1 (C(18)); 16.5 (C(29)); 19.4 (C(6)); 19.7 (C(19)); 22.0 (C(11)); 24.8 (C(30)); 25.3 (C(26) or C(27)); 25.4 (C(26) or C(27)); 27.6 (C(7)); 28.0 (C(16)); 28.5 (C(2)); 28.6 (C(28)); 29.7 (C(12) or C(22) or C(23)); 30.2 (C(12) or C(22) or C(23)); 30.3 (C(12) or C(22) or C(23)); 31.5 (C(15)); 37.0 (C(1)); 38.3 (C(10)); 40.0 (C(4)); 45.5 (C(13)); 48.7 (C(17)); 48.9 (C(20)); 50.7 (C(14)); 52.0 (C(5)); 73.8 (C(25)); 78.6 (C(24)); 79.7 (C(3)); 135.5 (C(8)); 136.0 (C(9)); 180.7 (C(21)). EI-MS: 490 (8, M^+), 457 (100), 454 (17), 439 (99), 411 (19), 393 (14), 314 (29). HR-EI-MS: 490.3658 (M^+, $C_{30}H_{50}O_5^+$; calc. 490.3658).

Inonotsulide C (=*(20*R,*24*S*)-3β,25-Dihydroxylanosta-7,9(11)-dien-20,24-olide*; **3**). Colorless prisms. M.p. 218–220° (from MeOH/$CHCl_3$). $[\alpha]_D^{20} = -26.1$ ($c = 0.26$, $CHCl_3$). UV (EtOH): 232 (12,000), 237 (14,500), 245 (9,000). IR (KBr): 3420 (OH), 2963, 1706 (δ-lactone), 1457, 1375, 1258, 1060, 1037, 755. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 470 (100, M^+), 455 (17 $[M-Me]^+$), 437 (9, $[M-Me-H_2O]^+$), 383 (4), 312 (64), 297 (22). HR-EI-MS: 470.3393 (M^+, $C_{30}H_{46}O_4^+$; calc. 470.3396).

This study was supported by a Grant-in-aid for High Technology from the *Ministry of Education, Science, Sports and Culture*, Japan.

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Received June 29, 2007