—Articles—

New Lanostane Triterpenoids, Inonotsutriols D, and E from *Inonotus Obliquus*

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Two new lanostane-type triterpenoids, inonotsutriols D (**1**) and E (**2**), were isolated from the sclerotia of *Inonotus obliquus* (Pers.: Fr.) Pil. (Japanese name: kabanoanatake; Russian name: chaga). Their structures were determined to be lanost-8-ene-3β,22*R*,24*R*-triol (**1**) and lanost-8-ene-3β,22*R*,24*S*-triol (**2**) on the basis of spectral data, including 2D NMR analysis. In addition, major compounds inotodiol (**3**), trametenolic acid (**4**), 3β-hydroxylanosta-8,24-dien-21-al (**5**), and inonotsuoxide A (**6**) were evaluated for their cancer cell growth inhibitory activity using the murine P388 leukemia cell line.

Key words——*Inonotus obliquus*; kabanoanatake; sclerotium; inonotsutriol D; inonotsutriol E; lanost-8-ene-3β,22*R*,24*R*-triol; lanost-8-ene-3β,22*R*,24*S*-triol; 2D NMR; murine P388 leukemia cell line

INTRODUCTION

Inonotus obliquus (PERS.: Fr.) Pil. (= *Fuscoporia obliqua* (PERS.: Fr.) Aoshima), commonly known as kabanoanatake in Japan and chaga or tchaga in Russia, is a white-rot fungus belonging to the family *Hymenochaetaceae Donk*[1] and is widely distributed in Europe, Asia, and North America.[2] The imperfect form of *I. obliquus* occurs parasitically on trunks, usually of *Betula* (birch), and more rarely 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 *Betula platyphylla* var. *japonica* (Japanese name: *shirakaba*) forests in Hokkaido, Japan.[3,4]

Lanost-8-ene-3β,22*R*-diol (inotodiol) was isolated by Ludwiczak and Wrecino.[5] Kahlos *et al*. isolated 3β-hydroxylanosta-8,24-dien-21-oic acid (trametenolic acid) and 3β-hydroxylanosta-8,24-dien-21-al,[6] as well as 3β,22,25-trihydroxylanosta-8,23-diene[7] and 3β,22-dihydroxylanosta-8,24-dien-7-one.[8] His group also reported that inotodiol exhibits significant anticancer activity against Walker 256 carcinosarcoma and MCF-7 human mammary adenocarcinoma in vitro and leukemia P388 in vivo,[9] and that 3β-hydroxylanosta-8,24-dien-21-al, 3β,21-dihydroxylanosta-8,24-diene, and trametenolic acid have antifungal activity.[10] Mizuno *et al*. reported the antitumor and hypoglycemic activities of polysaccharides from *I. obliquus*.[11] Babitskaya *et al*. reported melanin complex of *I. obliquus* has antioxidant and genoprotective effects[12] and Burczyk *et al*. reported that the aqueous extract of *I. obliquus* inhibited growth of human cervical and uterine cancer cells (HeLa S3).[13] Shin *et al*. isolated 3β-hydroxylanosta-8,24-diene-21,23-lactone, 21,24-cyclopentalanost-8-ene-3β,21,25-triol, and lanost-8-ene-3β,22,25-triol from the sclerotia of *I. obliquus*.[14,15]

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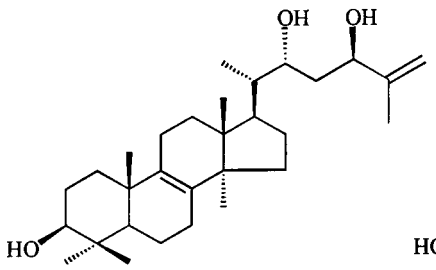
I. obliquus has been found to possess the following activities: anti-cancer,[16,17] anti-breast cancer,[18] anti-inflammatory and anti-nociceptive effects,[19,20] anti-oxidant,[21,22] anti-mutation,[23] anti-inflammatory,[24,25] inhibition of oxidative DNA damage in human lymphocytes,[25] platelet aggregation inhibitory activity,[26] and blocker of IκBα kinase activation.[27]

Recently, we reported the structures of new lanostane-type triterpenoids isolated from the sclerotia of *I. obliquus*: inonotsuoxides A and B (22*R*,25-epoxylanost-8-ene-3β,24*S*-diol and its 22*S*,25-epoxy epimer);[28] inonotsulides A, B, and C ((20*R*,24*S*)-3β,25-dihydroxylanost-8-en-20,24-olide, its (20*R*,24*R*)-olide epimer, and (20*R*,24*S*)-3β,25-dihydroxylanosta-7,9(11)-dien-20,24-olide);[29] lanosta-8,23*E*-diene-3β,22*R*,25-triol and lanosta-7:9(11),23*E*-triene-3β,22*R*,25-triol;[30] and inonotsutriols A, B, and C ((20*R*,24*S*)-cyclopentalanost-8-ene-3β,21*R*,25-triol, its 24*R*-hydroxyl epimer, and (20*R*,24*S*)-cyclopentalanosta-7:9(11)-diene-3β,21*R*,25-triol).[31] In addition, the anti-tumor promoting activity of inotodiol[28] and 3β-hydroxylanosta-8,24-dien-21-al,[30] which are abundant triterpene constituents in this sclerotium, has been reported.

Careful examination of the sclerotia of *I. obliquus* has led to the isolation of two new lanostane-type triterpenes named inonotsutriols D (**1**) and E (**2**). The



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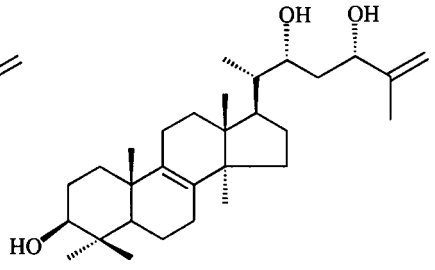

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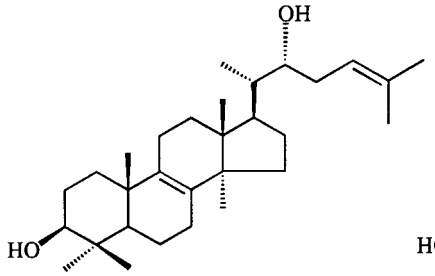

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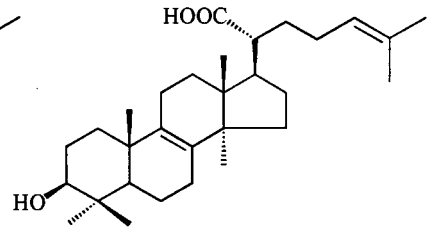

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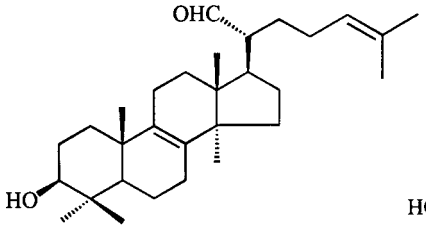

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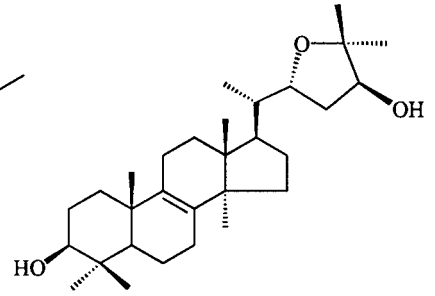

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structures of compounds **1** and **2** were determined on the basis of spectroscopic data, including 2D NMR spectra. This report deals with the structure determination of inonotsutriols D (**1**) and E (**2**), and the P388 cancer cell growth inhibitory activity of abundant triterpenoids inotodiol (**3**), trametenolic acid (**4**), 3β-hydroxylanosta-8,24-dien-21-al (**5**), and inonotsuoxide A (**6**).

Results and discussion

The sclerotia of *I. obliquus* were extracted with $CHCl_3$ and the extract was separated by silica gel column chromatography, medium-pressure liquid chromatography (MPLC), and high-pressure liquid chromatography (HPLC) to obtain two new triterpenes (**1** and **2**). The molecular formula of inonotsutriol D (**1**) was determined to be $C_{30}H_{50}O_3$ (M^+; *m/z* 458.3766, calcd 458.3760) by HREIMS. The IR spectrum showed the presence of hydroxyl groups (ν_{max} 3399 cm^{-1}). The ^1H and ^{13}C NMR spectra ($CDCl_3$) of **1** (Table 1) exhibited signals assignable to six tertiary methyl groups; one secondary methyl group [δ_H 0.94 (3H, d, J = 6.7 Hz)]; nine sp^3 methylenes; one terminal methylene [δ_H 4.84 (1H, dq, J = 3.8, 1.8 Hz), 5.01 (1H, quint, J = 1.2 Hz)]; six sp^3 methines including three oxymethines [δ_H 3.24 (1H, dd, J = 11.7, 4.5 Hz); δ_C 79.0 (d)], [δ_H 3.96 (1H, dt, J = 9.2, 3.0 Hz); δ_C 74.6 (d)], [δ_H 4.26 (1H, dd, J = 9.2, 3.5 Hz); δ_C 76.5 (d)]; four sp^3 quaternary carbons; and one tetrasubstituted double bond [δ_C 134.1 (s), 134.6 (s)]. Compound **1** showed the same carbon composition ($C_{30}H_{50}O_3$) and fragment ion peaks similar to those of inonotsuoxides A and B,[28] and inonotsutriols A and B.[31] In addition, ^1H and ^{13}C NMR chemical shifts of rings A-D (C-1 – C-19) were in good agreement with those of inonotsuoxides A and B, inonotsutriols A and B, and inonotsulides A and B[29] which have a common lanost-8-ene skeleton. The planar structure of **1** was determined from HMBC and ^1H-^1H COSY spectra. The HMBC spectrum of **1** (Table 1, Fig. 1) indicated long-range correlations between Me-18 (δ_H 0.72) and 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-21 (δ_H 0.94) and C-17, C-20, and C-22; between Me-27 (δ_H 1.76) and C-24, C-25, and C-26; between H_2-26 (δ_H 4.84, 5.01) and C-24, C-25, and C-27; 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; and between Me-30 (δ_H 0.87) and C-8, C-13, C-14, and C-15. The chemical shifts in the ^1H and ^{13}C NMR spectra of **1** resembled those of inonotsuoxide A (**6**) except those of H-22, H-23α, H-23β, H-24, H_2-26, and Me-27 signals and C-22, C-24, and C-27 signals. Partial structural units are shown by bold lines in the ^1H-^1H COSY spectrum (Fig. 1). Acetylation of **1** with Ac_2O/pyridine gave triacetate (**1a**) ($C_{36}H_{56}O_6$, *m/z* 584.4069, calcd. 584.4077) in which the acetoxymethine proton signal appeared at δ_H 4.49 (dd), δ_H 4.88 (dt), and δ_H 5.22 (dd). The results indicate that compound **1** is a side-chain-cleaved inonotsuoxide A, and its chemical name is suggested to be lanost-8-ene-3β,22,24-triol. The relative configuration at C-17 and C-20 was established as *R* and *S*, because of the observed NOEs from Me-18 to H-20β and from H-17α to Me-21 and Me-30, and the coupling constants of H-17 [δ_H 1.46 (ddd, $J_{17,16\alpha}$ = 9.6 Hz, $J_{17,16\beta}$ = 7.3 Hz, $J_{17,20}$ = 12.7 Hz)] and H-20 [δ_H 1.70 (1H, dqd, $J_{20,17}$ = 12.7 Hz, $J_{20,21}$ = 6.7 Hz, $J_{20,22}$ = 3.0 Hz)]. The absolute configuration at C-22 was determined to be *R* (Figs. 2 and 3) because of the significant NOEs for H-20β/H-22, Me-21/H-23α, H-22/H-23β and H-22/H-24, and the coupling constants of H-22 [δ_H 3.96 (1H, dt, $J_{22,20}$, $J_{22,23\beta}$ = 3.0 Hz, $J_{22,23\alpha}$ = 9.2 Hz)]. The configuration of H-24 was *R* due to the significant NOEs for H-24/

Table 1 NMR spectral data of inonotsutriol D (**1**) in $CDCl_3$

Position		**1**							
		δ_H[a]		*J*/Hz	^{1}H-^{1}H COSY	NOE	δ_C		HMBC (C)[b]
1	α	1.23	td	12.6 (1β, 2β), 3.8 (2α)	1β, 2α, 2β	1β, 2α, 3, 5	35.6	(t)	2, 10, 19
	β	1.74	dt	12.6 (1α), 3.8 (2α, 2β)	1α, 2α, 2β	2α, 2β, 19			2, 3, 10
2	α	1.67	m		1α, 1β, 2β, 3	1α, 1β, 2β, 3	27.8	(t)	10
	β	1.58	qd	11.7 (1α, 2α, 3), 3.8 (1β)	1α, 1β, 2α, 3	1β, 2α, 19, 29			1, 3
3		3.24	dd	11.7 (2β), 4.5 (2α)	2α, 2β	1α, 2α, 5, 28	79.0	(d)	2, 4, 29
4							38.9	(s)	
5		1.05	dd	13.0 (6β), 2.3 (6α)	6α, 6β	1α, 3, 6α, 7, 28	50.4	(d)	6, 7, 10, 19, 29
6	α	1.68	m		5, 6β, 7	5, 6β, 7, 28	18.2	(t)	7, 10
	β	1.51	m		5, 6α, 7	6α, 7, 19, 29			5
7		2.04	m		6α, 6β	5, 6α, 6β, 30	26.5	(t)	9
8							134.1	(s)	
9							134.6	(s)	
10							37.0	(s)	
11		2.02	m		12α, 12β	12α, 12β, 19	21.0	(t)	8, 9, 12
12	α	1.71	m		11, 12β	11, 12β, 30	30.9	(t)	13, 18
	β	1.69	m		11, 12α	11, 12α, 18, 21			13, 18
13							44.8	(s)	
14							49.4	(s)	
15	α	1.21	ddd	14.2 (15β), 9.3 (16α), 2.5 (16β)	16α, 16β	15β, 16α, 30	30.9	(t)	
	β	1.63	m		16α, 16β	15α			8, 14, 30
16	α	1.78	m		15α, 15β, 16β, 17	15α, 16β, 17, 22, 30	27.2	(t)	
	β	1.45	m		15α, 15β, 16α, 17	16α, 18, 20			17
17		1.46	ddd	12.7 (20), 9.6 (16α), 7.3 (16β)	16α, 16β, 20	16α, 21, 22, 30	47.3	(d)	15, 18, 20
18		0.72	s			11, 12β, 16β, 19, 20, 21	15.8	(q)	12, 13, 14, 17
19		0.98	s			2β, 6β, 11, 18, 29	19.1	(q)	1, 5, 9, 10
20		1.70	dqd	12.7 (17), 6.7 (21), 3.0 (22)	17, 21, 22	16β, 18, 21, 22	42.6	(q)	
21		0.94	d	6.7 (20)	20	12β, 17, 18, 20, 23α	12.7	(q)	17, 20, 22
22		3.96	dt	9.2 (23α), 3.0 (20, 23β)	20, 23α, 23β	16α, 16β, 17, 20, 23β, 24	74.6	(d)	21
23	α	1.54	m		22, 23β, 24	21	34.6	(t)	22, 24, 25
	β	1.52	m		22, 23α, 24	22, 24			22, 24, 25
24		4.26	dd	9.2 (23α), 3.5 (23β)	23α, 23β	22, 23β, 26B, 27	76.5	(d)	22, 23, 25, 26, 27
25							147.6	(s)	
26	A	4.84	brs			26B, 27	110.8	(t)	24, 25, 27
	B	5.01	brs			24, 26A			24, 25, 27
27		1.76	brs			24, 26A	17.9	(q)	24, 25, 26
28		1.00	s			3, 5, 6α, 29	28.0	(q)	3, 4, 5, 29
29		0.81	s			2β, 6β, 19, 28	15.4	(q)	3, 4, 5, 28
30		0.87	s			7, 12α, 15α, 16α, 17	24.4	(q)	8, 13, 14, 15

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Long range ^{1}H-^{13}C correlations from H to C observed in the HMBC experiments.

H-26B, Me-27, and the coupling constants of H-24 [δ_H 4.26 (1H, dd, $J_{24,23\alpha}$ = 9.2 Hz, $J_{24,23\beta}$ = 3.5 Hz)]. One of the hydroxyl groups was C-3β as shown by the chemical shift and the coupling constants [δ_H 3.24 (1H, dd, $J_{3,2\alpha}$ = 4.5 Hz and $J_{3,2\beta}$ = 11.7 Hz.); δ_C 79.0 (d)]. Other NOEs were observed from Me-19 to H-2β, H-6β, Me-18, and Me-29; from H-5α to H-1α, H-3α, H-7α, nd Me-28; from H-6β to Me-19 and Me-29; from H-7α to H-5α and Me-30; from H-12α to Me-30; from H-11β to Me-19; and from Me-18 to H-20β and Me-21. Therefore, rings A, B, and C in **1** adopted chair/twist, chair/twist, and chair conformations (Fig. 2). These

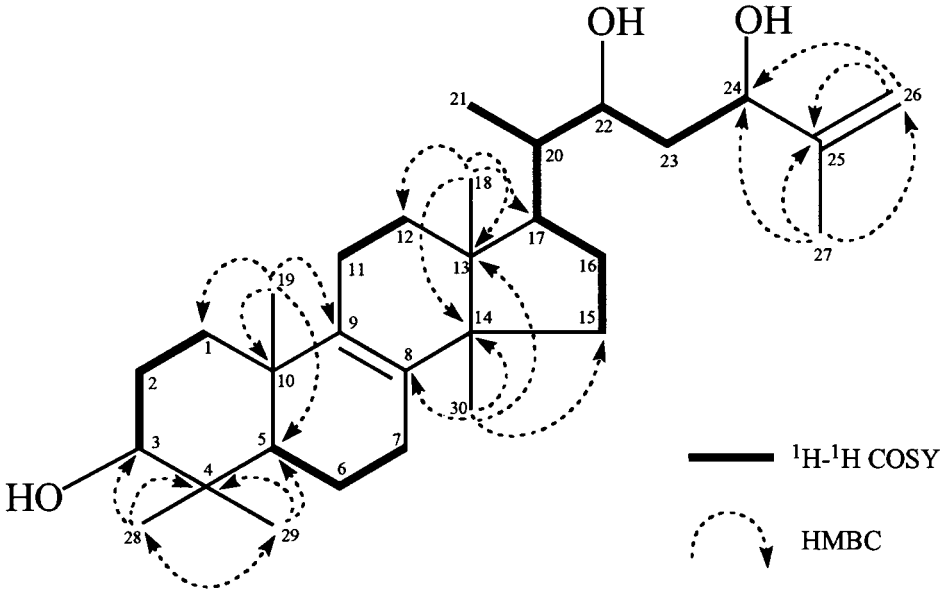

Fig. 1 Selected ^{1}H-^{1}H COSY and HMBC correlations for innotsutriol D (**1**)

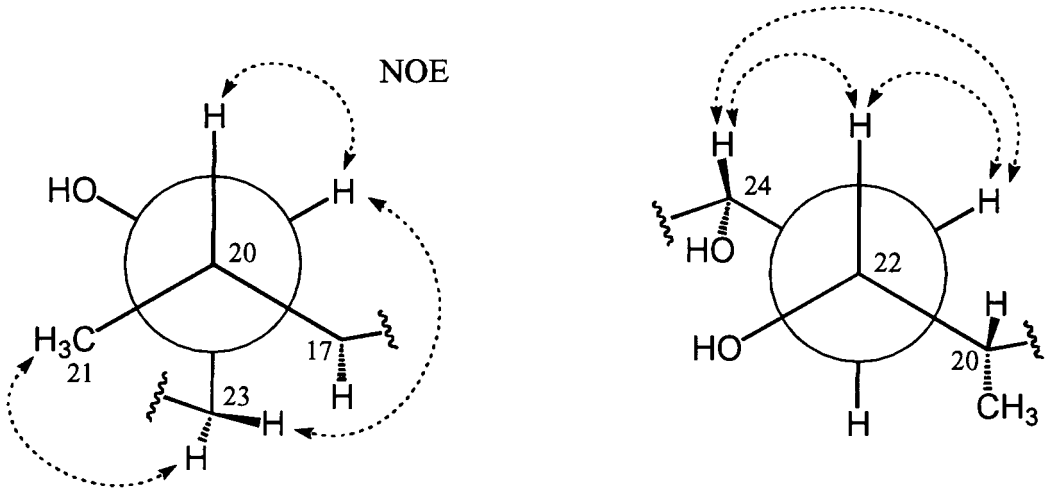

Fig. 2 Partial conformation for innotsutriol D (**1**)

Table 2 NMR spectral data of inonotsutriol E (**2**) in $CDCl_3$

Position	**2**							
	δ_H[a]		J/Hz	^{1}H-^{1}H COSY	NOE	δ_C		HMBC (C)[b]
1 α	1.23	td	12.6 (1β, 2β), 3.8 (2α)	1β, 2α, 2β	1β, 2α, 3, 5	35.6	(t)	
β	1.74	dt	12.6 (1α), 3.8 (2α, 2β)	1α, 2α, 2β	2α, 2β, 19			
2 α	1.68	m		1α, 1β, 2β, 3	1α, 1β, 2β, 3	27.8	(t)	
β	1.58	m		1α, 1β, 2α, 3	1β, 2α, 19, 29			
3	3.23	dd	11.8 (2β), 4.5 (2α)	2α, 2β	1α, 2α, 5, 28	79.0	(d)	2, 29
4						38.9	(s)	
5	1.05	dd	12.5 (6β), 2.5 (6α)	6α, 6β	1α, 3, 6α, 7, 28	50.4	(d)	6, 10, 19, 29
6 α	1.68	m		5, 6β, 7	5, 6β, 7, 28	18.2	(t)	
β	1.50	m		5, 6α, 7	6α, 7, 19, 24			
7	2.04	m		6α, 6β	5, 6α, 6β, 30	26.5	(t)	
8						134.1	(s)	
9						134.6	(s)	
10						37.0	(s)	
11	2.02	m		12α, 12β	12α, 12β, 19	21.0	(t)	
12 α	1.73	m		11, 12β	11, 12β, 30	30.9	(t)	
β	1.68	m		11, 12α	11, 12α, 18, 21			18
13						44.8	(s)	
14						49.4	(s)	
15 α	1.18	m		16α, 16β	15β, 16α, 30	30.9	(t)	
β	1.63	m		16α, 16β	15α			
16 α	1.78	m		15α, 15β, 16β, 17	15α, 16β, 17, 22, 30	27.3	(t)	13
β	1.41	m		15α, 15β, 16α, 17	16α, 18, 20			
17	1.44	ddd	12.7 (20), 9.6 (16α), 7.3 (16β)	16α, 16β, 20	16α, 21, 22, 30	47.2	(d)	
18	0.71	s			11, 12β, 16β, 19, 20, 21	15.7	(q)	12, 13, 14, 17
19	0.98	s			2β, 6β, 11, 18, 29	19.1	(q)	1, 5, 9, 10
20	1.67	dqd	12.7 (17), 6.7 (21), 3.2 (22)	17, 21, 22	16β, 18, 21, 22	42.5	(d)	
21	0.93	d	6.7 (20)	20	12β, 17, 18, 20, 23α	12.6	(q)	17, 20, 22
22	3.98	ddd	10.2 (23α), 3.2 (20), 2.8 (23β)	20, 23α, 23β	16α, 16β, 17, 20, 23β	70.3	(d)	
23 α	1.67	m		22, 23β, 24	21, 24	33.1	(t)	
β	1.58	m		22, 23α, 24				24
24	4.36	t	4.8 (23α, 23β)	23α, 23β	23α, 23β, 26B	73.5	(d)	22, 23, 25, 26, 27
25						147.3	(s)	
26 A	4.94	brs			26B, 27	110.2	(t)	24, 27
B	5.11	brs			24, 26A			24, 27
27	1.73	brs			26A	19.3	(q)	24, 25, 26
28	1.00	s			3, 5, 6α, 29	28.0	(q)	3, 4, 5, 29
29	0.81	s			2β, 6β, 19, 28	15.4	(q)	3, 4, 5, 28
30	0.87	s			7, 12α, 15α, 16α, 17	25.0	(q)	8, 13, 14, 15

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Long range ^{1}H-^{13}C correlations from H to C observed in the HMBC experiments.

data suggested that inonotsutriol D (**1**) is lanost-8-ene-3β,24*R*,24*R*-triol.

The minor compound inonotsutriol E (**2**) had the same molecular formula $C_{30}H_{50}O_3$ (M^+; *m/z* 458) as **1** by EIMS. The IR, ^1H, and ^{13}C NMR spectra (Table 2) resembled those of **1** except H-23α (δ_H 1.67), H-24 (δ_H 4.36), H-26A (δ_H 4.94), and H-26B (δ_H 5.11) in the ^1H NMR spectrum and C-22 (δ_C 70.3), C-24 (δ_C 73.5), and C-27 (δ_C 19.3) in the ^{13}C NMR spectrum. HMBC and ^1H-^1H COSY spectra of **2** closely resembled those of **1**, therefore, it was revealed that compound **2** was the same lanost-8-ene-3β,22,24-triol of **1**. The *R* configuration at C-22 in **2** was deduced from the chemical shift and the coupling constants [δ_H 3.98 (1H, ddd, $J_{22,20\beta}$ = 3.2 Hz, $J_{22,23\alpha}$ = 10.2 Hz, $J_{22,23\beta}$ = 2.8 Hz)], and from NOEs from H-22 to H-16α, H-16β, H-17α, H-20β, and H-23β, which is the same as that of **1**. On the other hand, the absolute configuration at C-24 was *S* because of the NOEs of H-24/H-23α, H-24/H-23β, and H-24/H-26B (Figs. 4 and 5), and the chemical shift and the coupling constants [δ_H 4.36 (1H, t, $J_{24,23\alpha}$, $J_{24,23\beta}$= 4.8 Hz)]. These data deduced the structure of inonotsutriol E (**2**) as lanost-8-ene-3β,24*R*,24*S*-triol, which is the C-24 epimer of **1**.

Cancer cell growth inhibitory activities of main triterpene constituents: inotodiol (**3**), trametenolic acid (**4**), 3β-hydroxylanos-8,24-dien-21-al (**5**), and inonotsuoxide A (**6**) were examined using the murine P388 leukemia cell line (Table 3). Compounds **4**-**6** exhibited moderate cytotoxic activity, while inotodiol (**3**) was inactive.

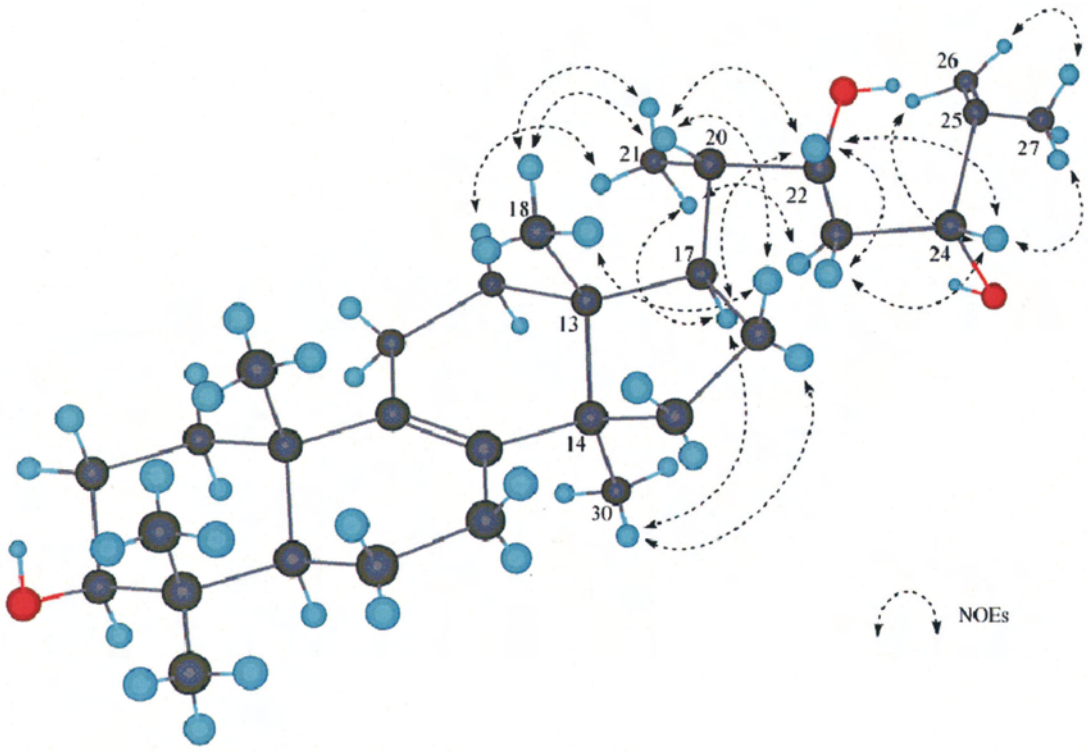

Fig. 3 Observed NOEs correlations of innotsutriol D (**1**) (graphical representation using Chem 3D)

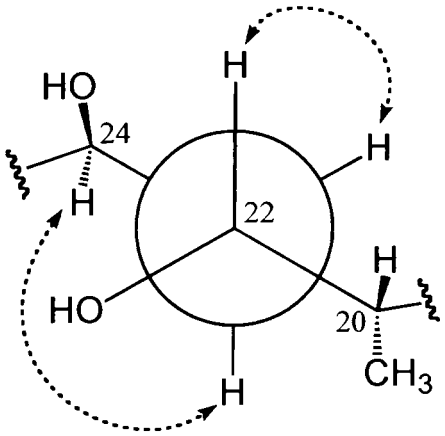

Fig. 4 Partial conformation for innotsutriol E (**2**)

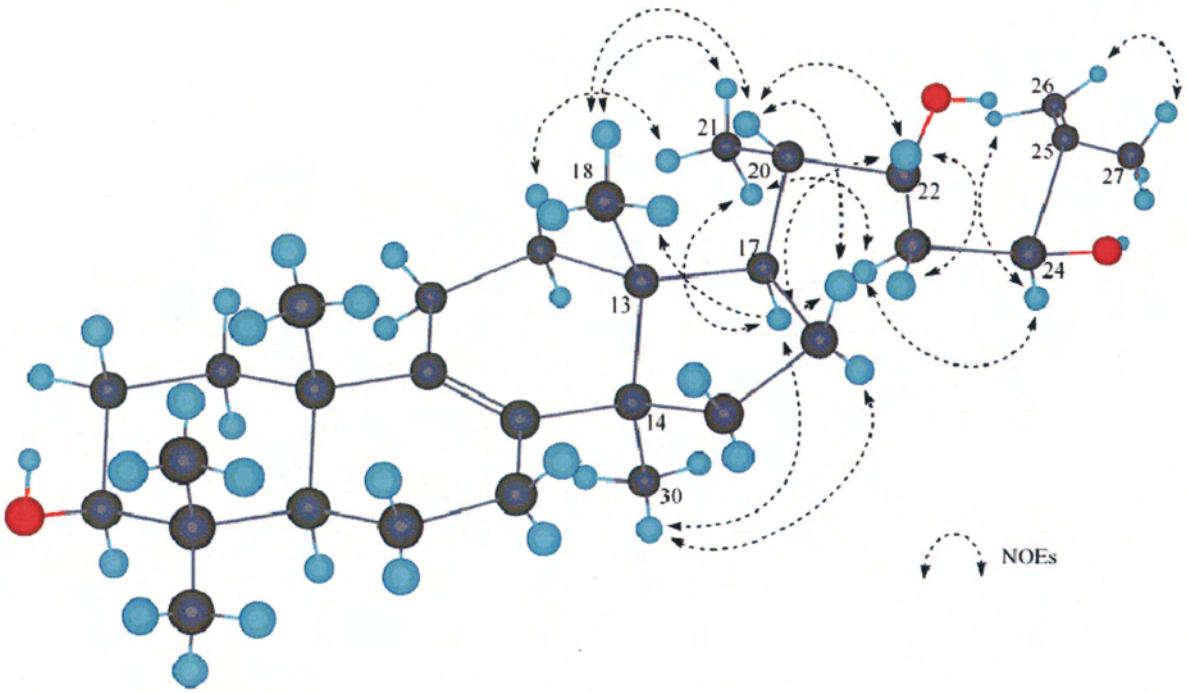

Fig. 5 Observed NOEs correlations of innotsutriol E (**2**)
(graphical representation using Chem 3D)

Table 3 Cytotoxity of the metabolites from the scelerotia of *Inonotus obliquus* against P388 cells

Compounds		Cell line P388 IC_{50} (mM)[a]
inotodiol	(**3**)	>100
trametenolic acid	(**4**)	27
3β-hydroxylanosta-8,24-dien-21 al	(**5**)	22
inonotsuoxide A	(**6**)	30
5-FU[b]		0.33

[a] DMSO was used for vehicle

[b] Positive control

EXPERIMENTAL

General

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. IR spectra were recorded using a Perkin-Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and TMS, as the internal standard. EIMS was recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over silica gel (70-230 mesh, Merck) and medium-pressure liquid chromatography (MPLC) was carried out with silica gel (230-400 mesh, Merck). HPLC was run on a JASCO PU-1586 instrument equipped with a differential refractometer (RI 1531). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}, Merck). Preparative TLC was carried out on Merck silica gel F_{254} plates (20 x 20 cm, 0.5 mm thick).

Material

Inonotus obliquus is succeeded in culture in Salada Melon Co. Ltd., Nayoro City, Hokkaido, Japan. Sclerotium (4 kg) of *I. obliquus* was obtained from the above company in April 2005. A voucher specimen (CG-03) was deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Assay for cytotoxicity using P388 cell line

Cytotoxicity of inotodiol (**3**), trametenolic acid (**4**), 3β-hydroxylanos-8,24-dien-21-al (**5**), and inonotsuoxide A (**6**) was examined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. P388 and HL-60 cells were cultured in Eagle's Minimum Essential Medium (10% fetal calf serum) suspension at 37 ºC in 5% CO_2. The test material was dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 10 mM and the solution was diluted with the Essential Medium to concentrations of 200, 20, and 2 μmol. Each solution was combined with the cell suspension (1×10^5 cells/ml) in the medium. After incubation at 37 ºC for 72 h in 5% CO_2, the grown cells were labeled

with 5 mg/ml MTT in phosphate buffered saline (PBS) and the absorbance of formazan dissolved in 20% sodium dodecyl sulfate (SDS) in 0.1 N HCl was measured at 540 nm using a microplate reader (Model 450, BIO-RAD). Each absorbance value was expressed as percentage relative to the control cell suspension that was prepared without the test substance using the same procedure as that described above. All assays were performed three times. Semilogarithmic plots were constructed from the averaged data and the dose required to inhibit cell growth by 50% (IC_{50}) was determined.

Extraction and isolation

The sclerotia of white-rot fungus *I. obliquus* (Pers.: Fr.) Pil. (4 kg) were extracted with chloroform (10 L) in 2005. Preliminary silica gel column chromatography (3 kg) of the chloroform extract (153.9 g) of *I. obliquus* sclerotia was reported to yield five fractions (**A** – **E**)[28]. Residue **C** (30.2 g) was recrystallized from MeOH to give inotodiol, and the filtrate (17.1 g) was rechromatographed on MPLC (230-400 mesh silica gel, 500 g) using *n*-hexane:EtOAc (5:1). This was followed by HPLC [ODS, MeOH:H_2O (85:15)], affording inonotsuoxides A (**6**) and B.[28] Subsequent elution on the same column with chloroform:EtOAc (5:1) gave a crystalline mass (272.8 mg) (Fr. Nos. 54-58), which was subjected MPLC (230-400 mesh silica gel, 30 g) using *n*-hexane:EtOAc (4:1) to afford a mixture of compounds **1** and **2** (52.6 mg) (Fr. Nos. 29-34). This mixture was separated by HPLC [ODS, MeOH:H_2O (90:10)] to give compounds **1** (19.1 mg) and **2** (10.1 mg).

Inonotsutriol D (1). Colorless prisms; mp 292-294 ºC (from MeOH-$CHCl_3$); HREIMS *m/z*: 458.3766 $[M]^+$ ($C_{30}H_{50}O_3$, calcd for 458.3760); IR (KBr) ν_{max} cm^{-1}: 3399 (OH), 2943, 2876, 1457, 1373, 1072, 1030; ^1H and ^{13}C NMR, see Table 1. EIMS *m/z* (rel. int.): 458 (9) $[M]^+$, 425 (3), 407 (3), 357 (75), 339 (18), 311 (13), 299 (8), 281 (7), 215 (6), 187 (8).

Inonotsutriol D triacetate (1a). A mixture of compound **1** (8.6 mg) and Ac_2O (2.2 mL) in pyridine (2 mL) was kept at room temperature overnight. Usual work-up gave a residue (10.8 mg) that was recrystallized from MeOH-$CHCl_3$ to yield corresponding inonotsutriol D triacetate (**1a**) (7.3 mg). Colorless prisms; HREIMS *m/z*: 584.4049 $[M]^+$ ($C_{36}H_{56}O_6$, calcd for 584.4077); ^1H NMR δ: 0.68 (3H, s, Me-18), 0.86 (3H, s, Me-30), 0.88 (6H, s, Me-28 and Me-29), 0.90 (3H, d, *J* = 6.7 Hz, Me-21), 1.00 (3H, s, Me-19), 1.73 (3H, s, Me-27), 2.03 (3H, s, C-22 OCO$\underline{CH_3}$), 2.06 (6H, s, C-3 and 22 OCO$\underline{CH_3}$), 4.49 (1H, dd, *J* = 11.7, 4.5 Hz, H-3α), 4.88 (1H, dt, *J* = 9.2, 3.0 Hz, H-22), 4.95 (2H, m), 5.22 (1H, dd, *J* = 9.2, 3.5 Hz, H-24). ^{13}C NMR δ: 12.9 (C-21), 15.7 (C-18), 16.5 (C-29), 17.5 (C-27), 18.1 (C-6), 19.2 (C-19), 21.0 (C-11), 21.2 (C-22 OCO$\underline{CH_3}$), 21.3 (C-3 and C-24 OCO$\underline{CH_3}$), 24.2 (C-2), 24.3 (C-30), 26.4 (C-7), 26.8 (C-16), 27.9 (C-28), 30.1 (C-23), 30.8 (C-15), 30.9 (C-12), 35.3 (C-1), 37.8 (C-4), 39.6 (C-20), 44.8 (C-13), 47.2 (C-17), 49.3 (C-14), 49.5 (C-14), 50.5 (C-5), 73.2 (C-22), 75.6 (C-27), 80.9 (C-3), 114.4 (C-26), 134.2 (C-8), 134.4 (C-9), 169.9 (C-24 O$\underline{C}OCH_3$), 170.4 (C-22 O$\underline{C}OCH_3$), 171.0 (C-3 O$\underline{C}OCH_3$).

Inonotsutriol E (2). Colorless prisms; mp 292-294 ºC (from MeOH-$CHCl_3$); HREIMS *m/z*: 458.3762 $[M]^+$ ($C_{30}H_{50}O_3$, calcd for 458.3760); IR (KBr) ν_{max} cm^{-1}: 3421 (OH), 2965, 2877, 1457, 1375, 1051, 1031; ^1H and ^{13}C NMR, see Table 2. EIMS *m/z* (rel. int.): 458 (9) $[M]^+$, 407 (3), 357 (75), 339 (18), 311 (13), 299 (8), 281 (7), 215 (6), 187 (8).

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