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ORIGINAL ARTICLE

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Chemical constituents of *Inonotus obliquus* II: a new triterpene, 21,24-cyclopentalanosta-3β,21,25-triol-8-ene from sclerotium

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Abstract A new lanostane-type triterpene with a cyclopentanol partial structure in the side chain was isolated from the sclerotium of the wood rotting fungus *Inonotus obliquus* along with four known compounds: lanosterol, inotodiol, trametenolic acid, and 3β-hydroxy-8,24-dien-lanosta-21,23-lactone. The new compound was determined to be 21,24-cyclopentalanosta-3β,21,25-triol-8-ene by spectroscopic analyses.

Key words *Inonotus obliquus* · Sclerotium · Antitumor · Lanostane type · Triterpene

Introduction

Inonotus obliquus (kabanoanatake in Japanese, charga in Russian, *Fuscoporia obliqua* in alternate taxon) is a white-rot fungus belonging to the Hymenochaetaceae. In Japan, the fungus is found only in Hokkaido and in a part of an alpine belt in mainland Japan.[1]

In Eastern Europe, the sclerotium of this fungus has long been used since the sixteenth or seventeenth century as a folk medicine for cancer.[2] Also, the Khanty of West Siberia use this fungus to prevent and treat heart disease, liver disease, stomach disease, and tuberculosis.[3]

Extractives from the sclerotium of *Inonotus obliquus* have been known to have a positive effect on controlling cancer, human immunodeficiency virus 1 (HIV-1), and stomach ulcers.[4] Antitumor experiments with *n*-hexane extractives of *Inonotus obliquus* have been conducted; and it has been reported that triterpenoids, especially inotodiol (**2**), have a significant anticancer effect on walker 256 carcinosarcoma and MCF-7 human mammary adenocarcinoma.[5]

In a previous report[6] four triterpenes, including a new compound, have been reported. They were lanosterol (**1**), inotodiol (**2**), trametenolic acid (**3**), and 3β-hydroxy-8,24-dien-lanosta-21,23-lactone (**4**). In this paper, we describe the isolation of another new triterpene (**5**) from the sclerotium of *Inonotus obliquus*. Biogenesis of compound **5** is discussed in relation to the compounds isolated so far.

Results and discussion

The four isolated compounds – lanosterol (**1**), inotodiol (**2**), trametenolic acid (**3**), 3β-hydroxy-8,24-dien-lanosta-21,23-lactone (**4**) – were identified in a comparison using field desorption-mass spectrometry (FD-MS), election ionization-mass spectrometry (EI-MS), and ^{1}H-nuclear magnetic resonance (^{1}H-NMR), respectively, and from ^{13}C-NMR spectral data in the literature.[5–7] Compound **5** was suggested also to have a lanostane-type triterpene by comparing its NMR spectral data with those of lanosterol (**1**) (Table 1). Compound **5** exhibited a molecular ion peak at m/z 458.3780 in an FD-MS spectrum and it was formulated as $C_{30}H_{50}O_3$ by EI-high resolution (HR)-MS. In the EI-MS spectrum of compound **5**, the ions of m/z 425(100), 407(90), and 389(13) were $[M^+-(CH_3 + H_2O)]$, $[M^+-(CH_3 + 2H_2O)]$, and $[M^+-(CH_3 + 3H_2O)]$, respectively. The fragmentation indicated that compound **5** has three hydroxyl groups, and it was confirmed by MS of its acetate. The difference in the molecular ions of compound **5** (458) and its acetate (584) was 126, which corresponds to three acetyl units (42 × 3), indicating that compound **5** has three hydroxyl groups in its structure.

In the ^{1}H-NMR spectrum of compound **5**, two singlets representing the protons of two methyl groups of the isopropyl group, appeared in a higher field (δ1.18, 1.21) than those of the isopropenyl group of lanosterol (**1**) (δ1.60, 1.68). The olefinic proton (δ5.10, t), which was exhibited in

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Table 1. Comparison of NMR spectral data of lanosterol (**1**) and compound **5** (in $CDCl_3$)

^{1}H-NMR		^{13}C-NMR		
Lanosterol	**5**	Lanosterol	**5**	**5** (in C_5D_5N)
		C-1 36. 0	35.7	36.7
		C-2 28. 1	27.7	27.5
H-3: 3.24,3.22, *dd*,	3.24,3.22, *dd*,	C-3 79. 4	78.9	78.6
J = 4.43	J = 4.43	C-4 39. 3	38.9	40.0
H-5: 1.05,m	1.04–1.07,m	C-5 50. 8	50.5	51.5
		C-6 18. 6	18.3	19.2
		C-7 27. 0	29.0	28.9
		C-8 134.8	134.4	134.4
		C-9 134.8	134.6	134.7
		C-10 37.4	37.1	37.9
		C-11 21.4	21.0	21.8
		C-12 31.4	27.2	25.2
		C-13 44.9	44.6	45.6
		C-14 50.2	49.4	50.2
		C-15 31.2	30.9	31.7
		C-16 28.6	26.2	27.3
H-17: 1.48,m	1.70–1.80,m	C-17 50.8	47.5	50.0
		C-18 15.8	24.4	25.1
		C-19 19.5	15.5	16.8

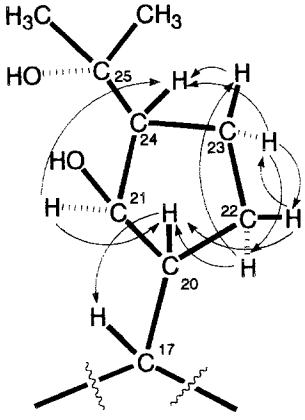

Fig. 1. Correlation of the partial structure of compound **5** in ^{1}H-^{1}H COSY

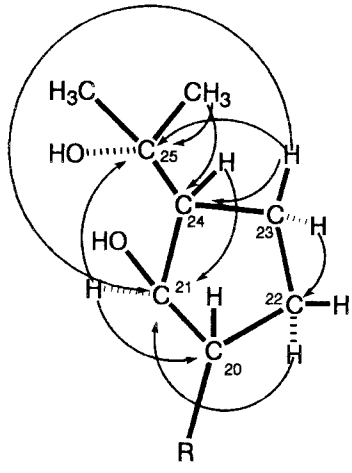

Fig. 2. Correlation of the partial structures of compound **5** in HMBC

lanosterol (**1**), did not appear in the spectrum of compound **5**. Instead, a peak corresponding to one methine proton appeared in a higher field, δ1.88 (m), suggesting that the double bond of the isopropenyl group of the side chain in lanosterol (**1**) was substituted in compound **5**. The signal of a methyl group that appeared at δ0.91 (*dd*) in lanosterol (**1**) did not appear in compound **5**. On the other hand, a peak corresponding to one methine proton bearing one hydroxyl group appear at δ3.68–3.71 (m). These data suggested that compound **5** have a cyclopentanol partial structure in the side chain (Fig. 1).

With the two-dimensional homonuclear chemical shift correlated spectroscopy (^{1}H-^{1}H COSY) of compound **5**, the methine proton bearing a hydroxyl group in the side chain (C-21, δ3.68–3.71, m) proved to have a correlation with two methine protons at δ1.80–2.0 (C-20, 1H, m) and at δ1.88 (C-24, 1H, m). There was also a correlation between the two methine protons at δ1.80–2.0 (C-20, 1H, m) and δ1.70–1.80 (C-17, m). The protons of two methylene groups were observed at δ2.05 (C-23, m) and δ1.6–1.75 (C-22, m), which correlated with methine protons at δ1.88 (C-24, m) and δ1.80–2.0 (C-20, m), respectively. These data supported the partial structure of the cyclopentanol in the side chain as shown in Fig. 1. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of compound **5** showed signals of carbons of two secondary alcohols at δ79.0 (C-3) and δ78.6 (C-21), one tertiary alcohol at δ72.6 (C-25), two methine carbons at δ57.4 (C-24) and δ48.7 (C-20), and two methylene carbons at δ26.5 (C-23) and δ24.5 (C-22). The heteronuclear multiple bond coherence (HMBC) and heteronuclear quantum multiple coherence (HQMC) spectra of compound **5** satisfied the correlation of the proposed partial structures, as shown in Fig. 2. On the basis of these data, it was concluded that

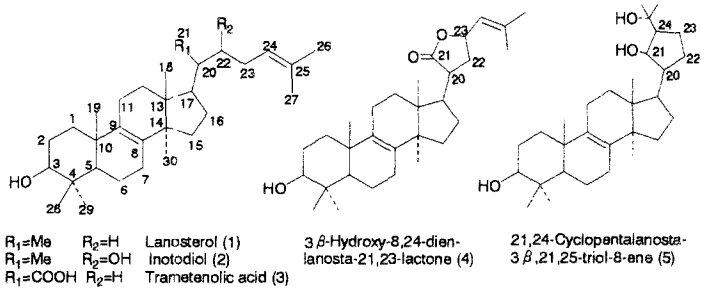

Fig. 3. Structures of isolated compounds from the sclerotium of *Inonotus obliquus*

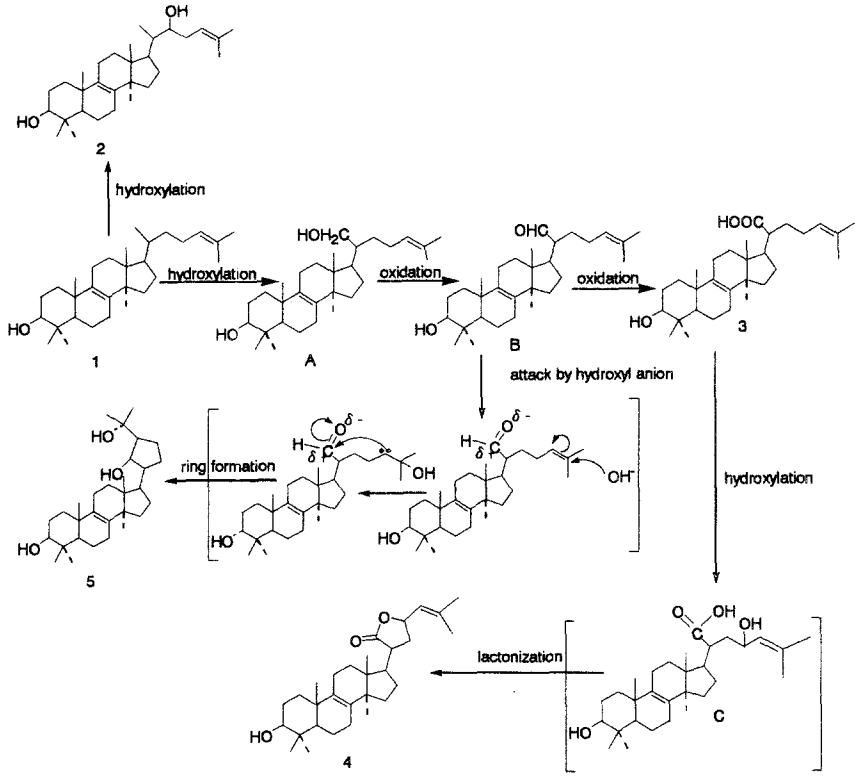

Fig. 4. Proposed biogenetic pathway of compound **5** in relation to the compounds isolated so far. 1, lanosterol; 2, inotodiol; 3, trametenolic acid; 4, 3β-hydroxy-8, 24-dien-lanosta-21,23-lactone; 5, 21,24-cyclopentalanosta-3β,21, 25-triol-8-ene; A, 3β,21-dihydroxylanosta-8,24-diene; B, 3β-hydroxylanosta-8,24-dien-21-al; C, not isolated

compound **5** is 21,24-cyclopentalanosta-3β,21,25-triol-8-ene (Fig. 3).

The proposed biogenesis of compound **5**, in relation to the isolated compounds so far, is as follows. Lanosterol (**1**) was hydroxylated at C-21 to yield 3β,21-dihydroxylanosta-8,24-diene (A).[8] 3β,21-Dihydroxylanosta-8,24-diene (A) was oxidized to yield 3β-hydroxylanosta-8,24-dien-21-al (B).[7] Attack by hydroxyl anions at C-25 of 3β-hydroxylanosta-8,24-diene-21-al (B) and successive attacks of the newly formed anion of C-24 on the cationic carbonyl carbon of C-21 formed the cyclopentanol partial structure of compound **5**, 21,24-cyclopentalanosta-3β,21,25-triol-8-ene, as shown in Fig. 4.

Experiment

The NMR spectra were measured on a Brucker AMX-500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) using deuterated chloroform ($CDCl_3$), $CDCl_3/CD_3OD$ (20:1, v/v), and pentadeuterated pyridine (C_5D_5N) as solvents and tetramethylsilane (TMS) as an internal standard. Two-dimensional (2D) NMR was performed with ^{1}H-^{1}H, ^{1}H-^{13}C COSY, HQMC, and HMBC. FD-MS data were obtained using a JEOL JMS-SX102A mass spectrometer; and EI-MS and EI-HR-MS spectra were obtained using a JEOL JMS-AX500 mass spectrometer. Thin-layer chromatography (TLC) was performed on a Wakogel B-10 and a Silicagel 70 plate-wako, using as developing solvents *n*-hexane/ethyl acetate (HEA, 10:1 and 4:1, v/v), chloroform/methanol (CM, 10:1, v/v) and toluene/formic acid/ethylformate (SGIII, 5:1:4, v/v).

Extraction and isolation

Sclerotium (ca. 1 kg) of *Inonotus obliquus* was obtained from the Hidaka local forestry office in 1996. Powdered sclerotium (900 g) was extracted five times with 95% etha-

nol (EtOH, 2l) at room temperature for 24h. The EtOH extracts were combined and concentrated under reduced pressures. The concentrated extracts (30.2g) were successively separated on silica gel column chromatography (CC, Wakogel C-200) with developing solvents of *n*-hexane, *n*-hexane/EtOAc (HEA, 25:1, v/v), EtOAc, EtOAc saturated with H_2O, and EtOH, successively. By monitoring with TLC using the developing solvent (SGIII), the extractives were separated into seven fractions. Crude lanosterol (**1**) was obtained from fractions 3 and 4 using a silica gel column (Wakogel C-200) with HEA (10:1, v/v) solvent. Finally, the purified lanosterol (**1**) (76mg) was isolated. Inotodiol (**2**), trametenolic acid (**3**), and compound **5** were isolated from fraction 5 and purified by silica gel column chromatography using HEA (8:1, 7:1, 7:3, v/v, respectively) solvent. The yields of the isolated compounds – inotodiol (**2**), trametenolic acid (**3**), and compound **5** – were 56, 48, and 6mg, respectively. Compound **4** was isolated from fraction 6 by silica gel column chromatography using HEA (1:1,v/v) solvent. The yield of compound 4 was 6mg.

The isolated compounds were acetylated by acetic anhydride and pyridine at 55°C for 24h.

Compounds **1–4** were described in a previous paper.[6]

Compound **5** was isolated as white powder: Rf value on TLC (SGIII): 0.56; FD-MS m/z 458; EI-HR-MS m/z 458.3780, $C_{30}H_{50}O_3$; EI-MS m/z [rel.int (%)]: 458(45) [M^+], 443(32) [M^+-CH_3], 425(100) [M^+-(CH_3 + H_2O)], 407(90) [M^+-(CH_3 + 2H_2O)], 389(13) [M^+-(CH_3 + 3H_2O)], 299(45), 281(28), 273(8), 255(7), 109(26), 95(24), 69(22), 55(14), 41(10); ^{1}H-NMR (in $CDCl_3$/CD_3OD, 20:1, v/v): 0.72 (s,3H,Me), 0.81 (s,3H,Me), 0.91 (s,3H,Me), 0.98 (s,3H,Me), 1.00 (s,3H,Me), 1.04,1.06 (*dd*, *J* = 1.97, 1H, 5-H), 1.18 (s,3H,Me), 1.22 (s,3H,Me), 2.05 (m,1H), 3.24,3.21 (*dd*, *J* = 4.43, 1H, 3-CHOH), 3.69 (m,1H); (in C_5D_5N): 1.03 (s,3H), 1.04 (s,3H), 1.07 (s,3H), 1.09 (s,3H), 1.10–1.15 (m,1H), 1.27 (s,3H), 1.30 (m,1H), 1.46 (s,3H), 1.50 (s,3H), 1.77 (m,1H), 2.18 (t,2H), 3.47 (t,1H), 4.13 (t,1H); ^{13}C-NMR (in $CDCl_3$/CD_3OD, 20:1, v/v): 15.5(C-19), 17.1(C-30), 18.3(C-6), 19.2(C-29), 21.0(C-11), 23.8(C-27), 24.4(C-18), 24.5(C-22), 26.2(C-16), 26.5(C-23), 27.2(C-12), 27.7(C-2), 28.0(C-28), 29.0(C-7), 30.3(C-26), 30.9(C-15), 35.7(C-1), 37.1(C-10), 38.9(C-4), 44.6(C-13), 47.5(C-17), 48.7(C-20), 49.4(C-14), 50.5(C-5), 57.3(C-24), 73.3(C-25), 78.9(C-3), 79.0(C-21), 134.4(C-8), 134.6(C-9); (in C_5D_5N): 16.8(C-19), 17.8(C-30), 19.2(C-6), 19.9(C-28), 21.8(C-11), 25.1(C-18), 25.2(C-12), 26.7(C-26), 27.3(C-16), 27.5(C-2), 28.9(C-7), 29.1(C-28), 29.2(C-21), 29.5(C-22), 30.7(C-27), 31.7(C-15), 36.7(C-1), 37.9(C-10), 40.0(C-4), 45.6(C-13), 49.5(C-20), 50.0(C-17), 50.2(C-14), 51.5(C-5), 59.1(C-23), 72.6(C-25), 78.6(C-24), 79.7(C-3).

Acetate of compound **5**: m/z 584; EI-HR-MS 584.3278, $C_{36}H_{56}O_6$; EI-MS m/z [rel.int (%)]: 584(28) [M^+], 542(100) [M^+-CH_3CO]; ^{1}H-NMR ($CDCl_3$:CD_3OD, 20:1, v/v and C_5D_5N): 0.68 (s,3H), 0.87 (s,3H), 0.88 (s,6H), 1.00 (s,3H), 1.17 (s,3H), 1.19 (s,3H), 1.26 (s,3H), 2.05–2.06 (s,6H), 4.49,4.51 (*dd*, *J* = 4.43, 1H), 5.00 (t,1H).

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