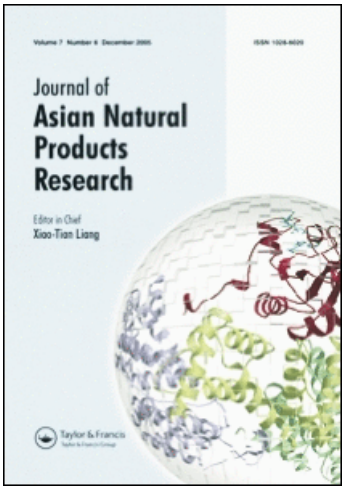

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Three New Triterpenoids from *Fuscoporia Obliqua*

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THREE NEW TRITERPENOIDS FROM *FUSCOPORIA OBLIQUA*

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Three new lanostane triterpenoids, fuscoporianol A (**1**), B (**2**), and C (**3**) were isolated from the petroleum ether extracts of *Fuscoporia obliqua* and their structures have been determined on the basis of chemical, spectroscopic methods and X-ray crystallographic analysis as 25-methoxy-21, 22-cyclolanosta-8-ene-3β, 21α-diol (**1**), 3β, 22α-dihydroxy-lanosta-8, 23E-diene-25-peroxide (**2**), 3β, 22α, 25-trihydroxy-lanosta-8, 23E-diene (**3**).

Keywords: *Fuscoporia obliqua*; Fungi; Polyporaceae; Triterpenoids; Fuscoporianol A (**1**), B (**2**), and C (**3**)

INTRODUCTION

Fuscoporia obliqua Fr Te.LáT, also named *Poria obliqua* and *Inonotus obliquus* [1,2], is the birch tree fungus growing in many countries. *Poria obliqua* has been used in Poland for treatment of some tumors [3] and Japanese scientists found the hot-water extracts of the mycelium of this fungus has inhibitory effect on AIDS virus multiplication [4]. In order to find new active compound and novel structure, we carried out systematic chemical studies on this fungus. Various triterpenoids and steroids have been isolated. This paper describes the isolation and structure elucidation of three new lanostane triterpenoids, fuscoporianol A (**1**), B (**2**), and C (**3**) from the petroleum ether extracts of this fungus.

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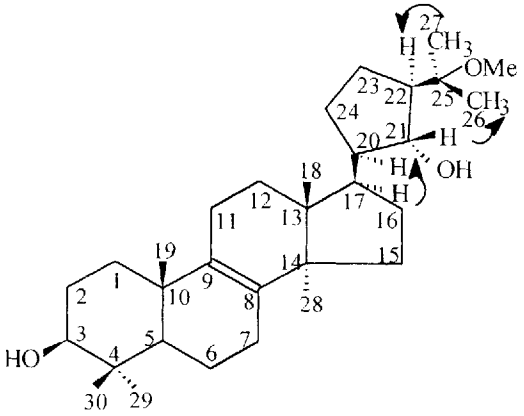
RESULTS AND DISCUSSION

Compound **1**, m.p. 178–179°C, $[\alpha]_D^{20}$ +42 ($CHCl_3$, C 0.05), was obtained as colorless needles and showed a violet spot on TLC with 1% vanillin H_2SO_4 after heating. The molecular formula $C_{31}H_{52}O_3$ was established by HREIMS measurement, which gave $[M^+]$ at 472.3882 (calcd. 472.3916). The IR absorption at 3420 cm^{-1} suggested the presence of hydroxyl group. In the EIMS spectrum, the intense peaks at *m/z* 314, 299, 281 and 274 together with the ^1H NMR spectrum, which clearly showed seven tertiary methyl signals (δ 0.74, 0.80, 0.90, 0.97, 0.99, 1.12 and 1.13 respectively), one methoxyl signal (δ 3.22, H-31) and two oxymethine (δ 3.66, t, J = 8 Hz, H-21), 3.22 (m, H-3) revealed the characteristics of lanostane skeleton. Comparison of the ^1H NMR and ^{13}C NMR spectral data of compound **1** with those of lanosterol, the signal of 21-methyl was unobserved, and one more signal of methylene appeared instead. The DEPT and HREIMS spectra displayed compound **1** had 31 carbon signals, that is, signals due to two oxymethine carbons at δ 79.0 (C-3) and δ 78.6 (C-21), two olefinic quaternary carbons at δ 134.7 (C-8) and δ 134.3 (C-9), an oxymethyl carbon at δ 49.4 (C-31) and a quaternary carbon bearing oxygen at δ 76.7 (C-25). Thus, the structure of compound **1** was established as **1**.



1

Key important NOEs

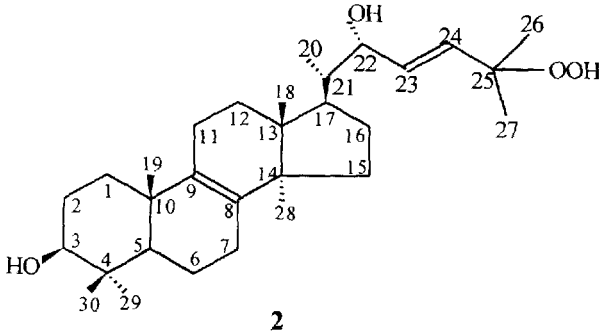
The broad triple peak of H-21 (δ 3.66, J = 8 Hz) and the correlation with δ 1.92 (m, H-20) and 1.85 (m, H-22) in ^1H–^1H COSY spectrum were also consistent with the structure of **1**. Furthermore, the coupling constant of

H-21 (brs, t, $J=8$ Hz) suggested that H-21 with H-20 and H-21 with H-22 were all *trans* quasi-axial orientation. In the NOESY spectrum of compound **1**, H-21 with H-26 and H-20 with 17-H showed cross peaks, considering the known configuration of lanostane skeleton (18, 19-methyl were β configuration, 28-methyl was α configuration and H-17 was α configuration), the stereostructure of compound **1** is established as shown.

The ^{13}C and ^{1}H NMR chemical shifts were assigned by ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, DEPT spectra and related literatures [5, 6].

Compound **2**, m.p. 196–198°C, $[\alpha]_D^{20}$ +56.4 ($CHCl_3$, C 0.055), was obtained as colorless flat crystals and showed a violet spot on TLC with 1% vanillin H_2SO_4 after heating. The molecular formula $C_{30}H_{50}O_4$, was established by HREIMS measurement, which gave [M^+] at 474.3723 (calcd. 474.3709). The IR absorption at 3400 cm^{-1} indicated the presence of hydroxyl group. The intense peaks at *m/z* 343, 329, 311, 299, 281 and 109 in the EIMS, together with the ^{1}H NMR spectrum, which clearly showed eight methyl signals (δ 0.81, 0.98, 1.06, 1.07, 1.23, 1.31, 1.55 and 1.55 respectively) and two oxymethine signals (δ 4.60, dd, $J=3.7$, 6.5 Hz, H-21), 3.43 (dd, $J=7$, 10 Hz, H-3), also revealed the characteristics of lanostane skeleton. When compared with the ^{1}H NMR spectral data of lanosterol, the side chain clearly contained the trans double bond (δ 6.35, d, $J=15$ Hz and δ 6.15, dd, $J=15$, 6.5 Hz). The ^{13}C NMR, DEPT and HREIMS spectra also displayed 30 signals of carbons, including two oxymethine carbons at δ 78.1 (C-3) and δ 74.1 (C-21), four olefinic carbons at δ 134.4 (C-8), 134.4 (C-9) and δ 136.7 (C-23), 129.9 (C-24), one quaternary carbon bearing oxygen at δ 81.2 (C-25). Furthermore, the intense peak at *m/z* 441 ($M^+ - 33$) in EIMS together with *m/z* 373 ($M^+ - 101$) revealed the existence of –OOH in 25-position. Thus, the structure of compound **2** may be established as **2**.

The ^{13}C and ^{1}H NMR data were assigned by reference to related literatures [5,6].



2

The relative configuration of compound **2** was determined by X-ray crystallographic analysis, together with the known configuration of lanostane skeleton, the structure of this compound was shown as **2**.

Single crystal X-ray diffraction data were collected by using a MAC Science DIP 2030k Image Plate with graphite monochromate, Mokα radiation. The crystal belongs to monoclinic, space group $P2_1$. Accurate cell parameters are as follow; $a = 8.505$ (1), $b = 10.446$ (1), $c = 15.763$ (1) Å, $\beta = 90.358$ (4)°, $V = 1400.41$ (23) Å^3, $Z = 2$. There were 1546 reflections of which 1500 were observed, the positions of 31 nonhydrogen atoms were obtained directly from E-map. The structure was solved with NOMCSDP software package. Positions of the other nonhydrogen atoms were obtained and the kind of atoms were determined by using the least square calculation and the difference Fourier method in turn. Positions of all hydrogen atoms were obtained by geometric calculation and difference Fourier method.

Analysis result indicated that compound **2** belongs to lanostane triterpenoid and had the molecular formula of $C_{30}H_{50}O_4$ (the final reliable factors were $Rf = 0.045$, $Rw = 0.051$, $S = 6.729$). In this compound, ring A is chair, ring B and C are semi-chair and ring D is envelope configuration. Furthermore, ring A with B and ring C with D were all *trans*-fused. The result also showed that 3, 22-position were substituted by hydroxyl and 25-position by peroxide group. Its X-ray structure is displayed in Figure 1.

Compound **3**, m.p. 241°C, $[\alpha]_D^{20}$ +308.5 ($CHCl_3$, C 0.07), was obtained as colorless slice crystals and showed violet spot turning to yellow green on TLC with 1% vanillin H_2SO_4 after heating. The molecular formula $C_{30}H_{50}O_3$ was established by HREIMS measurement, which gave [M$^+$] at

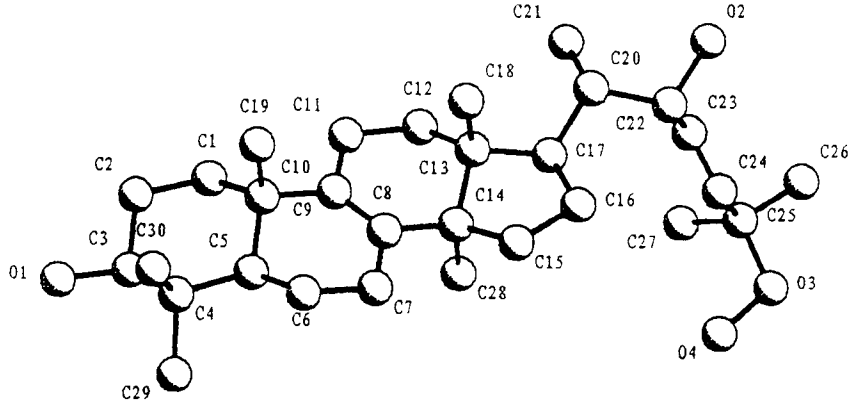
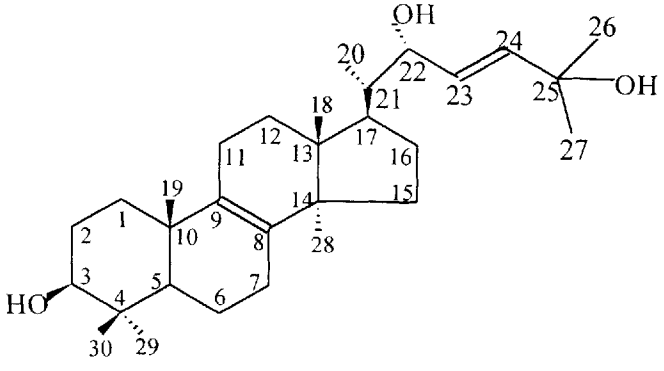


FIGURE 1 The X-ray crystallographic structure of compound **2**.

m/z 458.3748 (calcd. 458.3759). In the EIMS spectrum, the base peak at *m/z* 343 together with the intense peaks at *m/z* 440 (M^+-H_2O), 425 ($M^+-H_2O-CH_3$), 325, 311, 299, 281 and 109 revealed the characteristics of lanostane skeleton. The IR, ^1H NMR, ^{13}C NMR, DEPT spectra were very similar to those of compound **2** except 16 amu less for the molecular weight, thus we deduced the structure of this compound as **3**.

A natural product with identical structure to compound **3** has been reported by Finn scientists [7], but the configuration of C-22 has not confirmed. In order to determine the configuration of this compound, we conducted the reduction of compound **2** by using $NaBH_4$ in methanol. After 15 min, only one product was obtained. Comparison by TLC, mixed melting points as well as IR spectrum indicated that this product was identical to compound **3**. Thus the structure of compound **3** was established as **3**. The assignment of ^{13}C and ^1H NMR data was by reference to related literatures [5,6].



3

EXPERIMENTAL SECTION

General Experimental Procedures Mps were determined on an X_4 micro-melting point apparatus and are uncorrected. IR spectra were run on Perkin-Elmer-683 infrared spectrophotometer in KBr pellets. ^1H NMR (500 Hz), ^{13}C NMR (125 Hz) were determined on Bruker AM 500 spectrometer using TMS as internal standard. EIMS on a VG ZAB-2F instrument. TLC was performed on silica gel F_{254}. Separation and purification were performed by column chromatography on silica gel (200–300 mesh).

Plant Material *Fuscoporia Obliqua Fr Te.LáT* was collected in Jiling province, China and was taxonomically identified by Professor Bai Wang of the Scientific Institute of Chang Bai Mountain Protection, Jiling province, China. A voucher specimen is deposited in the herbarium of Department of Botany, Institute of Materia Medica, Chinese Academy of Medical Science, Beijing.

Extraction and Isolation Air-dried fungi 1.35 kg were thoroughly extracted with petroleum ether in Soxhlet apparatus under reflux. The extract was concentrated under reduced pressure and was subjected to column chromatography and gradiently eluted with petroleum and acetone to yield compound **1** (25 mg), **2** (28 mg) and **3** (20 mg). TLC condition: Compound **1**: petroleum ether : ethyl acetate = 3 : 1, $Rf1 = 0.7$. Compound **2** and **3**: petroleum ether : ethyl acetate = 1 : 1, $Rf2 = 0.72$, $Rf3 = 0.42$.

Compound **1** Recrystallized from methanol, colorless needles, m.p. 178–179°C, $[\alpha]_D^{20}$ +42 ($CHCl_3$, C 0.05), IR (KBr) υ max: 3420 cm^{-1}; for ^1H NMR data see Table II; for ^{13}C NMR data see Table I; CIMS *m/z* 473($M^+ + 1$); EIMS *m/z* 472 (30), 457 (10), 440 (40), 425 (100), 407 (91), 314 (13), 299 (52), 281 (39), 274 (21), 109 (46), 73 (61). HREIMS *m/z* 472.3882 $C_{31}H_{52}O_3$ (calcd. 472.3916).

Compound **2** Recrystallized from methanol, colorless slice crystals, m.p. 196–198°C, $[\alpha]_D^{20}$ +56.4 ($CHCl_3$, C 0.055), IR (KBr) υ max: 3400 cm^{-1}; for ^1H NMR data see Table II; for ^{13}C NMR data see Table I; HREIMS *m/z* 474.3723, $C_{30}H_{50}O_4$ (calcd. 474.3709). EIMS *m/z* 474 (12), 441 (49), 425 (30), 407 (38), 311 (51), 109 (100).

TABLE I ^{13}C NMR spectral data for compounds **1**, **2** and **3**

No.	**1**	**2**	**3**	*No.*	**1**	**2**	**3**
1	35.8	36.1	36.1	17	48.8	43.7	43.7
2	27.9	27.8	27.8	18	16.7	16.2	16.2
3	79.0	78.1	78.1	19	18.7	19.4	19.4
4	39.0	39.5	39.5	20	56.5	47.5	47.9
5	50.5	50.9	50.9	21	78.6	13.4	13.4
6	18.3	18.7	18.7	22	47.5	74.1	74.0
7	28.7	26.9	26.9	23	24.3	136.7	141.0
8	134.7	134.4	134.4	24	26.5	129.9	126.3
9	134.3	134.4	134.4	25	76.7	81.2	69.8
10	37.1	37.4	37.4	26	24.4	25.6	30.9
11	21.0	21.3	21.3	27	19.1	25.2	30.9
12	26.8	27.6	27.6	28	28.0	28.6	28.6
13	44.6	45.1	45.1	29	24.3	24.3	24.3
14	49.4	49.7	49.7	30	15.4	16.3	16.3
15	30.8	31.4	31.4	31	49.4		
16	27.5	31.4	31.4				

TABLE II ^{1}H NMR spectral data for compounds **1**, **2** and **3***

No.	**1**	**2**	**3**	*No.*	**1**	**2**	**3**
3	3.22, m	3.43, dd (J = 7,10)	3.43, dd (J = 7,10)	23		6.16, dd (J = 6.5,15)	6.31, dd (J = 6.5,15)
17	1.78, m			24		6.35, d	6.25, d
18	0.74, s	0.81, s	0.81, s			(J = 15)	(J = 15)
19	0.97, s	0.98, s	0.93, s	26	1.22, s	1.55, s	1.55, s
20	1.92, m			27	1.13, s	1.55, s	1.54, s
21	3.66, t	1.31, d	1.29, d	28	0.90, s	1.07, s	1.07, s
	(J = 8.0)	(J = 6.7)	(J = 6.7)	29	0.99, s	1.23, s	1.23, s
22	1.85, m	4.60, dd	4.61, dd	30	0.80, s	1.06, s	1.05, s
		(J = 3.7, 6.5)	(J = 3.7, 6.5)	31	3.21s		

*For **1** was tested in $CDCl_3$, **2** and **3** in pyridine-d_6, coupling constants were showing in parentheses.

Compound **3** Recrystallized from methanol, colorless slice crystals, m.p. 241°C, $[\alpha]_D^{20}$ +308.5 ($CHCl_3$, C 0.07), IR(KBr) υ max: 3400 cm^{-1}; for ^{1}H NMR data see Table II; for ^{13}C NMR data see Table I; HREIMS m/z 458.3748 $C_{30}H_{50}O_3$ (calcd. 458.3760). EIMS m/z 458 (27), 440 (34), 425 (68), 407 (73), 343 (100), 325 (89),311 (39), 109 (11).

Reduction of **2** To a solution of **2** (5 mg) dissolved in methanol (3 ml), was added $NaBH_4$ (10 mg) at room temperature, and stirred for 15 min. After evaporated methanol, the residue was washed by water and recrystallized with methanol to obtain **3** (4.0 mg).

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