Tyromycic Acids F and G: Two New Triterpenoids from the Mushroom *Tyromyces fissilis*

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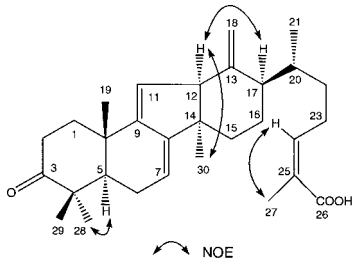
Phytochemical examination of the methanol extract of the fruit bodies of the Japanese fungus *Tyromyces fissilis* led to the isolation of two new lanostane derivatives called tyromycic acids F (1) and G (2), together with two known compounds, tyromycic acid (3) and trametenolic acid B (4). Their structures were identified by 2D NMR, IR, and UV spectroscopy.

Key words *Tyromyces fissilis*; tyromycic acid; triterpene

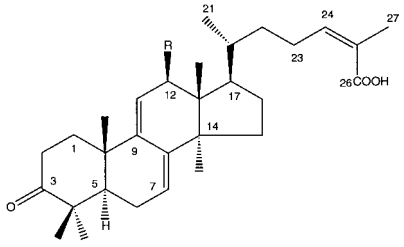
Tyromyces sp. have been chemically investigated since 1967 by Gaudermer, who reported the isolation and structural elucidation of tyromycic acid (**3**) from *Tyromyces albidus*.[1] Later, 4-but-3-enoxymethyl benzoate was obtained by fermentation of a *Tyromyces* species that inhibited phospholipase A_2.[2] Tyromycin A, 1,16-bis-[4-methyl-2,5-dioxo-3-furyl]hexadecane, was isolated from *Tyromyces lacteus* as an inhibitor of leucine and cysteine aminopeptidases.[3] In the course of screening of biologically active constituents from Japanese inedible mushrooms, we previously reported the isolation of tyromycic acids B—E from *Tyromyces fissilis*.[4] Further fractionation of its methanol extract resulted in the isolation of two new triterpenoids called tyromycic acids F (**1**) and G (**2**), together with two known lanostane triterpenoids, tyromycic acid (**3**) and trametenolic acid B (**4**). This paper describes their isolation and structural elucidation.

The methanol extract of *T. fissilis* was fractionated on a silica gel, DIOL column and finally reverse-phase HPLC to afford four compounds (**1**—**4**). The molecular formula of tyromycic acid F (**1**) was determined to be $C_{30}H_{42}O_3$ by high resolution (HR)-FAB-MS. The ^{1}H-NMR spectral data of **1** (Table 1) showed the presence of three olefinic protons, one *exo*-methylene (δ_H 4.66 and 4.89, each d, J=2.2 Hz at H-18), four quaternary methyls, one secondary methyl (δ_H 0.85, d, J=6.6 Hz), and one olefinic methyl (δ_H 1.88, d, J=1.4 Hz). The ^{13}C-NMR spectral data of **1** (Table 1) indicated the signals of one ketone and one carboxylic acid (δ_C 172.5), which was confirmed by the IR spectrum with absorption bands at 2500—3600 and 1706 cm^{-1}. Comparison of its spectral data with those of neokadsuranic acid B[5] suggests that compound **1** is a triterpenoid with a 14(13→12)*abeo*-lanostane skeleton. Inspection of ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra of **1** indicated that two olefinic protons (δ_H 5.35 and 5.29) were assigned at C-7 and C-11, respectively. In addition, the other olefinic proton in the low-field position (δ_H 6.03) was determined to be C-24 due to conjugation with the carboxylic acid. The stereochemistry of **1** was deduced by a nuclear Overhauser effect spectroscopy (NOESY) experiment, which showed an nuclear Overhauser effect (NOE) correlation between 1) H-5 and H-28, and 2) H-12, H-17, and H-30, indicating that H-5, H-12, and H-17 were in an α-face. Further, the geometry of $C_{24—25}$ was determined to be *Z* partly based on a comparison of its spectral data with those of neokadsuranic acid[5] and partly based on the NOE correlation between H-24 and H-27 in the NOESY spectrum. Therefore tyromycic acid F (**1**) was determined to be (24*Z*)-3-oxo-14(13→12)*abeo*-lanosta-7,9(11),13(18),24-tetraen-26-oic acid, as shown in Chart 1. In addition, 14(13→12)*abeo*-lanostane structure is a rare skeleton from natural source[5] and **1** was the second example.

Tyromycic acid G (**2**) was obtained as an oil with the molecular formula $C_{32}H_{46}O_5$ based on HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) of compound **2** were similar to those of tyromycic acid (**3**)[1] except for the pres-



1



2: R= OAc
3: R= H

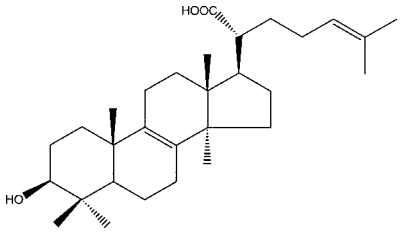


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Chart 1. Structures of Compounds **1**—**4**

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Table 1. ^{1}H- and ^{13}C-NMR ($CDCl_3$) Spectral Data for Compounds **1**—**3**

Position	Compound **1**		Compound **2**		Compound **3**
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_C
1	2.19 ddd (2.8, 5.5, 13.5)	35.9 t	2.24 m	36.3 t	36.6 t
	1.82 dt (4.4, 13.5)		1.87 m		
2	2.78 dt (5.8, 14.8)	34.7 t	2.75 dt (5.8, 14.6)	34.6 t	34.8 t
	2.34 ddd (3.0, 4.4, 14.8)		2.36 ddd (3.0, 4.7, 14.6)		
3		216.2 s		216.2 s	216.9 s
4		47.8 s		47.4 s	47.5 s
5	1.55 dd (4.1, 11.8)	50.2 d	1.56 dd (3.9, 12.1)	50.3 d	50.7 d
6	2. 25 m	23.9 t	2.22 m	23.6 t	23.7 t
	2.13 m		2.08 m		
7	5.35 d (5.8)	113.7 d	5.60 d (6.6)	122.0 d	119.9 d
8		151.8 s		141.2 s	144.5 s
9		152.4 s		146.4 s	142.9 s
10		35.1 s		37.1 s	37.2 s
11	5.29 s	126.4 d	5.06 s	118.4 d	117.3 d
12	3.00 s	61.5 d	5.44 s	77.6 d	37.8 t
13		148.9 s		47.7 s	43.8 s
14		45.4 s		51.8 s	50.3 s
15	1.42 ddd (2.5, 6.6, 13.2)	36.0 t	1.74 m	31.5 t	31.5 t
	1.19 m		1.38 m		
16	1.58 m	24.9 t	2.20 m	26.6 t	27.9 t
	1.10 m		1.47 m		
17	1.91 m	47.8 d	1.85 m	51.1 d	50.8 d
18	4.89 d (2.2)	114.2 t	0.67 s	11.1 q	15.7 q
	4.66 d (2.2)				
19	1.20 s	19.5 q	1.23 s	21.8 q	22.0 q
20	1.34 m	37.0 d	1.43 m	34.7 d	36.2 d
21	0.85 d (6.6)	17.8 q	0.91 d (6.6)	19.9 q	18.3 q
22	1.56 m	33.7 t	1.58 m	35.2 t	35.7 t
	1.20 m		1.14 m		
23	2.52 m	27.3 t	2.54 m	27.5 t	26.9 t
	2.44 m		2.46 m		
24	6.03 dt (1.4, 8.0)	147.0 d	6.08 dt (1.4, 7.8)	146.7 d	147.2 d
25		125.8 s		125.8 s	125.7 s
26		172.5 s		171.8 s	172.3 s
27	1.88 d (1.4)	20.6 q	1.92 d (1.4)	20.6 q	20.6 q
28	1.13 s	22.3 q	1.13 s	22.5 q	22.5 q
29	1.09 s	25.2 q	1.09 s	25.3 q	25.4 q
30	1.06 s	27.0 q	1.00 s	25.3 q	25.3 q
12-OAc			2.27 s	21.8, 171.0	

ence of signals of an acetoxyl group (δ_H 2.27; δ_C 21.8, 171.0). The location of the acetoxyl group was determined to be at C-12 by the correlation between the acetoxyl group and C-12 in the HMBC spectrum and the low-field position of C-12 (δ_C 77.6). The stereochemistry of H-12 was found to be in an α-face based on the NOE correlation between H-12 and H-17, and H-30. Thus tyromycic acid G (**2**) was determined to be (24*Z*)-12β-acetoxylanosta-7,9(11),24-trien-26-oic acid, as shown in Chart 1.

Compounds **3** and **4** were identified as tyromycic acid[1)] and trametenolic acid B,[6)] respectively, based on a comparison of their spectral data with those reported in the literature.[1,6)]

It is noteworthy that *Tyromyces* species are rich sources of lanostane and rearranged lanostane carboxylic acids.

Experimental

UV spectra were obtained on a Shimadzu UV-1650PC spectrophotometer in MeOH. IR spectra were recorded on a JASCO FT/IR-5300 spectrophotometer. Specific optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as a solvent. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR) spectrometer, using $CDCl_3$ as a solvent. Chemical shifts were evaluated using TMS (δ: 0.00) as an internal standard (^{1}H-NMR), and δ: 77.03 (ppm) from $CDCl_3$ as a standard (^{13}C-NMR). Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. Preparative HPLC was performed on a Shimadzu liquid chromatograph model LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II column. Column chromatography was carried out on silica gel 60 (0.2—0.5 mm, 0.04—0.063 mm, Merck).

Fungal Material Fruit bodies of *T. fissilis* were collected in October 2002 in Aichi prefecture, Higashikamo-gun, Japan, and identified by Kazuyuki Takase (Kansai Fungus Association). A voucher specimen (Taka-02-1) has been deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

Extraction and Isolation Dried fruit bodies of *T. fissilis* (159 g) were extracted with MeOH. The filtrate was concentrated under reduced pressure to give a residue (7.5 g), which was subjected to SiO_2 column chromatography using hexane : EtOAc (1 : 1) to give six fractions. Fraction 1 (1680 mg) was rechromatographed on silica gel with the same solvent system to afford three subfractions. Subfraction 2 (248 mg) was purified on a reverse-phase column using $CH_3CN : H_2O$ as an eluent to give **3** (6 mg). Subfraction 3 (139.2 mg) was also separated on a reverse-phase column using CH_3CN to yield **4** (3.0 mg). Fraction 2 (1572 mg) was fractionated on a reverse-phase column using $CH_3OH : H_2O$ (9 : 1) and then a DIOL column using hexane : EtOAc (2 : 1) to afford tyromycic acids F (**1**) (17.4 mg) and G (**2**) (5.7 mg).

Tyromycic Acid F (**1**): Oil, $[\alpha]_D^{20}$ −103.1° (c=0.10, $CHCl_3$). IR (KBr) cm^{-1}: 3400—2500, 2925, 1706, 1637, 1457, 1195, 842. UV λ_{max} (MeOH)

(log ε): 214 (4.2). ^{1}H- and ^{13}C-NMR ($CDCl_3$) data: see Table 1. HR-FAB-MS: *m*/*z* 450.3095 $[M]^+$ (Calcd for $C_{30}H_{42}O_3$: 450.3134).

Tyromycic Acid G (**2**): Oil, $[\alpha]_D^{20}$ −99.1° (*c*=0.08, $CHCl_3$). IR (KBr) cm^{-1}: 3600—2500, 2927, 1708, 1642, 1457, 1376, 1243. UV λ_{max} (MeOH) nm (log ε): 221 (3.8), 235 (3.8), 244 (3.8). ^{1}H- and ^{13}C-NMR ($CDCl_3$) data: see Table 1. HR-FAB-MS: *m*/*z* 533.3207 $[M+Na]^+$ (Calcd for $C_{32}H_{46}O_5Na$: 533.3243).

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