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

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Received July 22, 2003; accepted August 27, 2003

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, tyromycin acid (3) and trametenolic acid B (4). Their structures were identified by 2D NMR, IR, and UV spectroscopy.

Key words Tyromyces fissilis; tyromycin 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, tyromycin 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 tyromycin acid F (1) was determined to be C30H42O3 by high resolution (HR)-FAB-MS. The 1H-NMR spectral data of 1 (Table 1) showed the presence of three olefinic protons, one exo-methylene (δH 4.66 and 4.89, each d, J=2.2 Hz at H-18), four quaternary methyls, one secondary methyl (δH 0.85, d, J=6.6 Hz), and one olefinic methyl (δH 1.88, d, J=1.4 Hz). The 13C-NMR spectral data of 1 (Table 1) indicated the signals of one ketone and one carboxylic acid (δC 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 acid5) suggests that compound 1 is a triterpenoid with a 14(13 fi 12)abeo-lanostane skeleton.

Inspection of 1H–1H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra of 1 indicated that two olefinic protons (δH 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 (δH 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 C24—25 was determined to be Z partly based on a comparison of its spectral data with those of neokadsuranic acid5) 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 (24Z)-3-oxo-14(13 fi 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 source5) and 1 was the second example.

Tyromycin acid G (2) was obtained as an oil with the molecular formula C32H46O5 based on HR-FAB-MS. The 1H- and 13C-NMR spectral data (Table 1) of compound 2 were similar to those of tyromycin acid (3)1) except for the presence of two extra olefinic protons (δH 6.03 and 6.17, each d, J=12.8 Hz) and one extra methyl group (δH 0.82, s). Therefore compound 2 was determined to be (24Z)-3-oxo-14(13 fi 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 source5) and 1 was the second example.

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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 (24Z)-12b-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 CHCl3 as a solvent. The 1H- and 13C-NMR spectra were recorded on a Varian Unity 600 (600 MHz for 1H-NMR and 150 MHz for 13C-NMR) spectrometer, using CDCl3 as a solvent. Chemical shifts were evaluated using TMS (δ: 0.00) as an internal standard (1H-NMR), and δ: 77.03 (ppm) from CDCl3 as a standard (13C-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 SiO2 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 CH3CN:H2O as an eluent to give 3 (6 mg). Subfraction 3 (139.2 mg) was also separated on a reverse-phase column using CH3CN to yield 4 (3.0 mg). Fraction 2 (1572 mg) was fractionated on a reverse-phase column using CH3OH:H2O (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, [α]_D^20 = −103.1° (c=0.10, CHCl3). IR (KBr) cm⁻¹: 3400—2500, 2925, 2706, 1637, 1457, 1195, 842. UV λ_max (MeOH)
Tyromycin Acid G (2): Oil, \([\alpha]_D^{20} = -99.1^\circ (c = 0.08, \text{CHCl}_3)\). IR (KBr) cm\(^{-1}\): 3600—2500, 2927, 1708, 1642, 1457, 1376, 1243. UV \(\lambda_{\text{max}}\) (MeOH) nm: 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\(_5\)Na: 533.3243).

Acknowledgments The authors thank Miss Y. Okamoto (TBU, Japan) for measurement of the mass spectra and Mr. K. Takase (Kansai Fungus Association) for collection of mushrooms.

References