Tubiferic Acid, a New 9,10-Secocycloartane Triterpenoid Acid Isolated from the Myxomycete *Tubulifera arachnoidea*

Yukihito IPPONGI,^a Takashi OHTSUKI,^a Kazufumi TOUME,^a Midori A. ARAI,^a Yukinori YAMAMOTO,^b and Masami ISHIBASHI^{*,a}

^a Graduate School of Pharmaceutical Sciences, Chiba University; 1–33 Yayoi-cho, Inage-ku, Chiba 263–8522, Japan; and ^b Yamamoto Laboratory; 1010–53 Ohtsu-ko, Kochi 781–5102, Japan.

Received September 11, 2010; accepted November 8, 2010; published online November 9, 2010

Tubiferic acid (1), a new triterpenoid acid having a 2,6-dimethyl-4,5-dihydroxy-2-hexenoic acid moiety as a side chain, was isolated from field-collected fruit bodies of the myxomycete *Tubulifera arachnoidea*, and its structure was elucidated by spectral data. Tubiferic acid (1) had the same unique 9,10-secocycloartane carbon skeleton as tubiferal A (2).

Key words myxomycete; *Tubulifera arachnoidea*; triterpenoid; 9,10-secocycloartane

During our search for bioactive natural products from myxomycetes,¹⁾ we have recently isolated a number of new bioactive secondary metabolites such as a chlorinated polyene-pyrone²⁾ and a naphthoquinone pigment.³⁾ In 2004 we reported isolation of a cytotoxic triterpenoid aldehyde lactone, named tubiferal A (2), having a unique triterpenoid carbon skeleton with a consecutive 6-7-6-5 ring system, from field-collected fruit bodies of *Tubifera dimorphotheca*.⁴⁾ We recently investigated field-collected fruit bodies of *Tubulifera arachnoidea* (= *Tubifera ferruginosa*), and here we describe the isolation and structure elucidation of a new triterpenoid acid, named tubiferic acid (1). This compound (1), with a 2,6-dimethyl-4,5-dihydroxy-2-hexenoic acid moiety as a side chain, had the same unique triterpenoid carbon skeleton as tubiferal A (2).

Table 1. ¹H- and ¹³C-NMR Spectral Data of Tubiferic Acid (1) in CD₃OD

Position	δ_{H} (J in Hz)	δ_{C}
1	2.15 (m), 2.00 (m)	30.8
2	1.66 (m, α -H), 1.38 (m, β -H)	28.3
3	3.39 (dd, 10.9, 3.4)	76.9
4		40.9
5		138.3
6	2.36 (m, α -H), 2.26 (m, β -H)	28.1
7	1.47 (m), 1.57 (m)	26.5
8	2.21 (m)	47.5
9		137.8
10		129.6
11	5.23 (br d, 5.0)	121.7
12	1.93 (br d, 5.0, α -H), 2.14 (m, β -H)	38.8
13		45.9
14		48.6
15	1.38 (m) (2H)	34.4
16	1.67 (m), 1.96 (m)	27.6
17	2.07 (m)	48.0
18	0.77 (s) (3H)	15.3
19	2.64 (d, 14.0), 2.74 (d, 14.0)	42.9
20	1.91 (m)	37.6
21	0.93 (d, 6.6) (3H)	12.0
22	3.50 (dd, 9.0, 1.4)	76.5
23	4.30 (t, 9.0)	69.0
24	6.66 (dd, 9.0, 1.4)	143.3
25		132.1
26		172.2
27	1.89 d (1.4) (3H)	13.4
28	1.04 s (3H)	25.4
29	0.91 s (3H)	20.7
30	0.78 s (3H)	18.5

Results and Discussion

Fruit bodies of the myxomycete *Tubulifera arachnoidea* were extracted with MeOH, and the EtOAc-silane fraction of the MeOH extract was subjected to octadecyl silane (ODS) column chromatography to give a new compound, tubiferic acid (1), in 0.016% yield.

The molecular formula of 1 was suggested to be C₃₀H₄₆O₅ by high resolution-electrospray ionization (HR-ESI)-MS data [m/z 485.3294 (M–H)[–], Δ +2.2 mmu]. Compound 1 showed IR absorption bands at 3410 and 1700 cm^{–1}, implying the presence of hydroxyl and carboxyl groups. The ¹H-NMR spectrum of 1 in CD₃OD (Table 1) showed signals due to one secondary methyl (δ_{H} 0.93, d, J =6.6 Hz) and five tertiary methyl groups at (δ_{H} 0.77, 0.78, 0.91, 1.04, 1.89), three sp^3 oxymethines (δ_{H} 3.39, 3.50, 4.30), and two sp^2 methines (δ_{H} 5.23, 6.66). The ¹³C-NMR spectrum revealed signals of six olefinic carbons (δ_{C} 121.7, 129.6, 132.1, 137.8, 138.3, 143.3) and one carboxyl group (δ_{C} 172.2), thus accounting for four out of eight unsaturation degrees. The remaining four were therefore ascribable to four rings.

Analysis of the 2D-NMR data of 1 (Fig. 1) showed the presence of three partial structures, A, B, and C. The ¹H–¹H correlation spectroscopy (COSY) spectrum showed proton connectivities for H₂–1/H₂–2/H–3, H₂–6/H₂–7/H–8, and H–11/H₂–12 for the partial structure A, and a proton network for H₂–15/H₂–16/H–17/H–20(H₃–21)/H–22/H–23/H–24 in partial structure C was also suggested from the ¹H–¹H COSY spec-

* To whom correspondence should be addressed. e-mail: mish@faculty.chiba-u.jp

Fig. 1. Three Partial Structures (A—C) and Key 2D-NMR Data for Compound **1**

Fig. 2. Connection of Three Partial Structures (A—C) by HMBC Correlations

trum. The heteronuclear multiple bond connectivity (HMBC) spectrum of **1** revealed the key correlations for H₂-1/C-10, H₂-1/C-5, H₃-28/C-3, H₃-28/C-4, H₃-28/C-5, H₃-29/C-3, H₃-29/C-4, H₂-6/C-4, H₂-6/C-8, H₂-6/C-10, H₂-19/C-9, H₂-19/C-10, H₂-19/C-11, H-11/C-8, and H-11/C-19, leading to the partial structure **A**. The HMBC correlations for H₃-18/C-13, H₃-18/C-14, H₃-30/C-13, and H₃-30/C-14 suggested the partial structure **B**, while the HMBC correlations for H₃-21/C-17, H₃-21/C-20, H₃-21/C-22, H-22/C-21, H-22/C-24, H-23/C-24, H-23/C-25, H-24/C-26, H-24/C-27, H₃-27/C-24, H₃-27/C-25, and H₃-27/C-26 corroborated the partial structure **C**.

These three partial structures were then connected by further analysis of the HMBC spectral data, as shown in Fig. 2. Partial structures **A** and **B** were connected between C-12 and C-13 positions and between C-8 and C-14 positions on the basis of the HMBC correlations observed for H₃-18/C-12, H₃-30/C-8, and H-11/C-13, whereas partial structures **B** and **C** were connected at C-14 and C-15 positions and at C-13 and C-17 positions by HMBC cross peaks for H₃-18/C-17, H₃-30/C-15, and H-17/C-13. HMBC correlation observed from H-8 to C-15 also supported the connection of these partial structures, suggesting that C-8 in partial structure **A** was correlated with C-15 in partial structure **C** through C-14 in partial structure **B**.

From the results described above, the whole planar structure of tubiferic acid was constructed as **1**, which possesses the same carbon skeleton with a 6-7-6-5 ring system contained in tubiferal A (**2**); this carbon skeleton may correspond to a 9,10-secocycloartane.^{5,6)}

The hydroxyl group on the C-3 position of **1** was suggested to be equatorially oriented, since the H-3 signal was observed as a doublet of doublet ($J=10.9, 3.4$ Hz), implying that H-3 was α -axial and coupled with the 2β -axial hydrogen of C-2 with a large coupling constant (10.9 Hz). This assignment was also supported by nuclear Overhauser effect (NOE) observations from H-3 to H-2 α (δ_{H} 1.66) and H₃-28 (α -equatorial methyl group attached on C-4) as shown in Fig. 3a. The

Fig. 3. Assignment of NOE Correlations Observed for Compound **1**
Stereochemical relationship between A/B and C/D rings is arbitrary.

geometry of the $\Delta^{24,25}$ -double bond was assigned as *Z* on the basis of the high-field resonance of the C-27 methyl carbon (δ_{C} 13.4)⁷⁾ as well as the NOE correlation observed from H₃-27 (δ_{H} 1.89) to H-23 (δ_{H} 4.30). NOE correlations observed for other positions were assigned as shown in Fig. 3, on which bases we propose that the stereochemistry of C/D rings of **1** was parallel to that of tubiferal A (**2**), although the stereochemistry of a diol moiety in the side chain of **1** remains undefined. Since we could not obtain the NOE correlations between A/B and C/D rings, their stereochemical relationship is arbitrary.

Experimental

General Optical rotations were measured with a JASCO P-1020 polarimeter. IR spectra were measured on ATR in a JASCO FT-IR 230 spectrophotometer. UV spectra were measured in a Shimadzu UV mini-1240 spectrometer. NMR spectra were recorded on JEOL JNM-A400 and JEOL JNM-ECP600 spectrometers with a deuterated solvent, the chemical shift of which was used as an internal standard. HR-ESI-MS were obtained on an Exactive (Thermo Scientific, Japan).

Organisms Fruit bodies of *Tubulifera arachnoidea* were collected and identified by Y.Y. in Konan-shi, Kochi Prefecture, Japan, in July 2008. A voucher specimen (#31366) is maintained by Y.Y. (Yamamoto Lab., Kochi, Japan).

Extraction and Isolation Air-dried fruiting bodies of *Tubulifera arachnoidea* (30.1 g) were extracted with MeOH (100 ml $\times$ 3), and the combined MeOH extracts (1.24 g) suspended in 10% aqueous MeOH (100 ml) were partitioned with hexane (1300 ml $\times$ 3), EtOAc (100 ml $\times$ 3) and *n*-BuOH (100 ml $\times$ 3) to give four fractions, a hexane layer (102 mg), EtOAc layer (211 mg), *n*-BuOH layer (346 mg), and water layer (612 mg). The EtOAc-soluble fraction (193 mg), which was positive for anisaldehyde reagent by TLC examination, was subjected to ODS column chromatography (2.0 $\times$ 27.5 cm, Chromatorex ODS C18; Fuji Silysia Chemical Ltd., Japan) eluted with 70–100% MeOH in water to give compound **1** (4.8 mg) in the fraction eluted with 83% MeOH in water. By TLC examination using anisaldehyde reagent as well as crude ¹H-NMR spectral analysis, α -spinasterol⁸⁾ (3.7 mg) was obtained from less polar fraction of the same ODS column eluted with MeOH/CHCl₃ (80:20). From the *n*-BuOH soluble fraction, D-trehalose⁹⁾ (9.0 mg) was isolated by Sephadex LH-20 column chromatography (14 $\times$ 620 mm, 100% MeOH).

Tubiferic Acid (**1**): Colorless solid; $[\alpha]_{\text{D}}^{19} +19.5$ ($c=0.5$, MeOH); IR ν_{max} (ATR) 3410, 2940, 1700, 1650, 1570, 1460, and 1380 cm⁻¹; ¹H- and ¹³C-NMR data in Table 1; HR-ESI-MS m/z 485.3294 [$\text{M}-\text{H}$]⁻, Calcd for C₃₀H₄₅O₅, 485.3272.

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS).

References and Notes

- 1) Ishibashi M., *Yakugaku Zasshi*, **127**, 1369–1381 (2007).
- 2) Shintani A., Ohtsuki T., Yamamoto Y., Hakamatsuka T., Kawahara N., Goda Y., Ishibashi M., *Tetrahedron Lett.*, **50**, 3189–3190 (2009).
- 3) Shintani A., Yamazaki H., Yamamoto Y., Ahmed F., Ishibashi M., *Chem. Pharm. Bull.*, **57**, 894–895 (2009).
- 4) Kamata K., Onuki H., Hirota H., Yamamoto Y., Hayashi M., Komiyama K., Sato M., Ishibashi M., *Tetrahedron*, **60**, 9835–9839 (2004).
- 5) The carbon skeletons of **1** and **2** were the same as that of acerinol,

which was an artificially generated compound from a cycloartane-triterpene by treatment with mineral acid: Kusano G., Uchida H., Murakami Y., Sakurai N., Takemoto T., *Yakugaku Zasshi*, **96**, 321—325 (1976).

- 6) The fission of the cyclopropane ring for acerinol⁵⁾ was likely to be assisted by 3,10-epoxy ring formation, although it was previously reported that an acerinol-related compound was isolated from *Cimicifuga heracleifolia* without acid treatment: Li J. X., Kadota S., Hattori M., Yoshimachi S., Shiro M., Oogami N., Mizuno H., Namba T., *Chem. Pharm. Bull.*, **41**, 832—841 (1993).
- 7) Carey L., Clough J. M., Pattenden G., *J. Chem. Soc. Perkin Trans. 1*, **1983**, 3005—3009 (1983).
- 8) Furuya T., Orihara Y., Tsuda Y., *Phytochemistry*, **29**, 2539—2543 (1990).
- 9) Matsuura H., Asakawa C., Kurimoto M., Mizutani J., *Biosci. Biotechnol. Biochem.*, **66**, 1576—1578 (2002).