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

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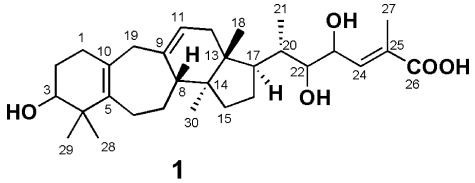
Tubiferic acid (1), a new triterpenoid acid having a 2,6-dimethyl-4,5-dihydroxy-2-hexenoic acid moiety as a side chain, was isolated from field-collected fruit bodies of the myxomycete *Tubulifera arachnoidea*, and its structure was elucidated by spectral data. Tubiferic acid (1) had the same unique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



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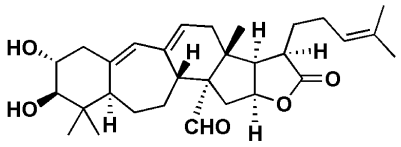

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Results and Discussion

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 $C_{30}H_{46}O_5$ 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 ^{1}H-NMR spectrum of **1** in CD_3OD (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 ^{13}C-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 ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum showed proton connectivities for H_2-1/H_2-2/H-3, H_2-6/H_2-7/H-8, and H-11/H_2-12 for the partial structure **A**, and a proton network for H_2-15/H_2-16/H-17/H-20(H_3-21)/H-22/H-23/H-24 in partial structure **C** was also suggested from the ^{1}H–^{1}H COSY spec-

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Tubiferic Acid (**1**) in CD_3OD

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

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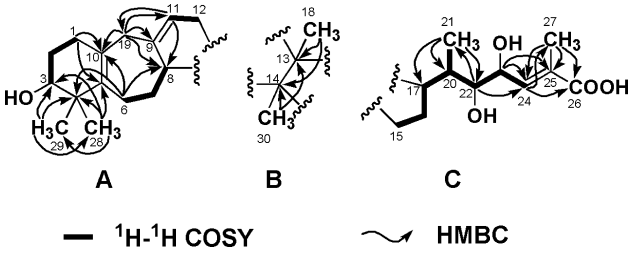

Fig. 1. Three Partial Structures (**A**—**C**) and Key 2D-NMR Data for Compound **1**

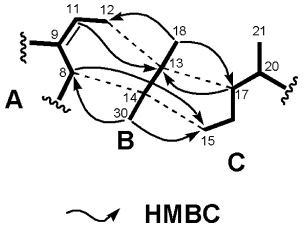

Fig. 2. Connection of Three Partial Structures (**A**—**C**) by HMBC Correlations

trum. The heteronuclear multiple bond connectivity (HMBC) spectrum of **1** revealed the key correlations for H_2-1/C-10, H_2-1/C-5, H_3-28/C-3, H_3-28/C-4, H_3-28/C-5, H_3-29/C-3, H_3-29/C-4, H_2-6/C-4, H_2-6/C-8, H_2-6/C-10, H_2-19/C-9, H_2-19/C-10, H_2-19/C-11, H-11/C-8, and H-11/C-19, leading to the partial structure **A**. The HMBC correlations for H_3-18/C-13, H_3-18/C-14, H_3-30/C-13, and H_3-30/C-14 suggested the partial structure **B**, while the HMBC correlations for H_3-21/C-17, H_3-21/C-20, H_3-21/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, H_3-27/C-24, H_3-27/C-25, and H_3-27/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 H_3-18/C-12, H_3-30/C-8, and H-11/C-13, where as 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 H_3-18/C-17, H_3-30/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-2α (δ_H 1.66) and H_3-28 (α-equatorial methyl group attached on C-4) as shown in Fig. 3a. The

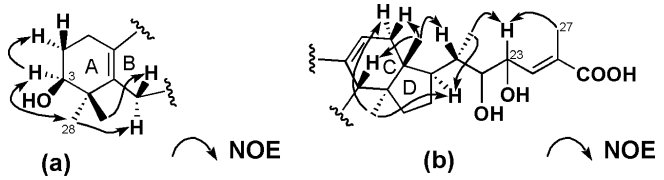

Fig. 3. Assignment of NOE Correlations Observed for Compound **1**
Stereochemical relationship between A/B and C/D rings is arbitrary.

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 H_3-27 (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×3), and the combined MeOH extracts (1.24 g) suspended in 10% aqueous MeOH (100 ml) were partitioned with hexane (1300 ml×3), EtOAc (100 ml×3) and *n*-BuOH (100 ml×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 (2.0×27.5 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 ^{1}H-NMR spectral analysis, α-spinasterol[8] (3.7 mg) was obtained from less polar fraction of the same ODS column eluted with MeOH/$CHCl_3$ (80 : 20). From the *n*-BuOH soluble fraction, D-trehalose[9] (9.0 mg) was isolated by Sephadex LH-20 column chromatography (14×620 mm, 100% MeOH).

Tubiferic Acid (**1**): Colorless solid; $[\alpha]_D^{19}$ +19.5 (c=0.5, MeOH); IR ν_{max} (ATR) 3410, 2940, 1700, 1650, 1570, 1460, and 1380 cm^{-1}; ^{1}H- and ^{13}C-NMR data in Table 1; HR-ESI-MS *m/z* 485.3294 [M−H]$^-$, Calcd for $C_{30}H_{45}O_5$, 485.3272.

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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 acerinol[5] 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).