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ROSEOLIDE A, A NOVEL DIMERIC DRIMANE SESQUITERPENOID FROM THE BASIDIOMYCETE *ROSEOFORMES SUBFLEXIBILIS*

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Abstract : Roseolide A (**1**), a novel dimeric sesquiterpenoid containing drimane skeletons, has been isolated from the wood-rotting fungus *Roseoformes subflexibilis* (Berk. et Curt.) Aoshi. The chemical structure was elucidated on the basis of 2D NMR spectroscopy, chemical transformation to a known compound, and X-ray crystallographic analysis.

Basidiomycetes are potentially rich sources of structurally and pharmacologically intriguing substances.[1] In the cource of our program designated to discover cytotoxic compounds from Basidiomycetes, a novel dimeric drimane sesquiterpenoid, named roseolide A (**1**), has been isolated from the wood-rotting fungus *Roseoformes subflexibilis* (Berk. et Curt.) Aoshi. We wish to report herein the structure elucidation of **1** on the basis of 2D NMR spectroscopy, transformation to a known compound, and X-ray crystallographic analysis.

The chloroform extract (4.86 g) of the fruiting bodies (300 g) of *R. subflexibilis* was subjected to silica gel [deactivated with 10%(w/w) water] column chromatography. The fractions eluted with a mixture of chloroform-methanol (99:1) contained 5,6-dihydroergosterol (fungisterol), ergosterol peroxide, and roseolide A (**1**). The lattest was further purified by column chromatography on silica gel (hexane-ethyl acetate, 10:1→1:1), followed by recrystallization from ethyl acetate-chloroform to afford **1** (120 mg) as colorless prisms: m.p. 255-258°C, $[\alpha]_D^{19}$ -4.0° (c 2.3, $CHCl_3$).

Roseolide A (**1**) showed a molecular ion peak at *m/z* 724 (M^+) in the FD-MS. The HREI-MS exhibited a fragment ion peak at *m/z* 363.2174 corresponding to the empirical formula $C_{21}H_{31}O_5$, although it did not give a molecular ion. These results together with the ^{13}C and ^1H NMR spectral data indicated the molecular

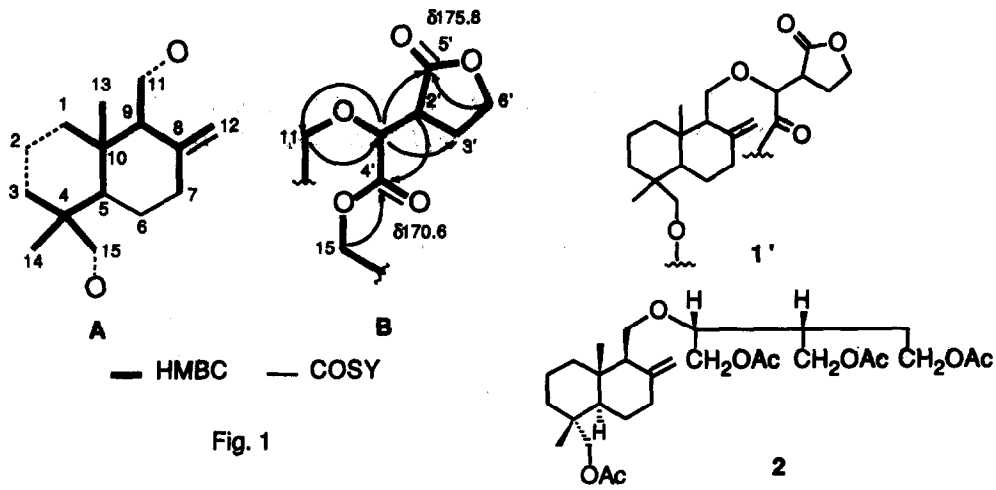


Fig. 1

Table 1. $^{13}C^{a}$ and $^{1}H^{b}$ NMR Spectral Data for 1

position	$\delta_C{}^c$	$\delta_H{}^c$
1	39.8 (t)	~1.32 (m[d], Hα), 1.79 (br d, *J*=11.8, Hβ)
2	18.5 (t)	1.50 (m[d]), ~1.56 (m[d])
3	35.5 (t)	1.24 (m), ~1.56 (m[d])
4	37.3 (s)	
5	46.7 (d)	1.78 (br d, *J*= 11.8)
6	23.5 (t)	~1.32 (m[d], Hα), ~1.56 (m[d], Hβ)
7	37.4 (t)	1.96 (ddd, *J*= 12.5, 12.5, 5.0, Hα), 2.26 (m, Hβ)
8	147.9 (s)	
9	55.4 (d)	~2.39 (dd[d], *J*= 9.0, 3.9)
10	39.6 (s)	
11	70.8 (t)	3.71 (dd, *J*= 9.0, 3.9, Hpro-*R*), 3.86 (dd, *J*= 9.0, 9.0, Hpro-*S*)
12	107.4 (t)	4.30 (br s, H*cis*), 4.76 (br s, H*trans*)
13	15.1 (q)	0.82 (s)
14	17.9 (q)	0.79 (s)
15	71.2 (t)	3.73 (d, *J*= 10.4), 3.89 (d, *J*= 10.4)
1'	78.4 (d)	4.20 (d, *J*= 4.2)
2'	42.0 (d)	3.14 (ddd, *J*= 10.0, 9.0, 4.2)
3'	24.6 (t)	2.21 (dddd, *J*= 16.7, 9.0, 9.0, 8.0, Hβ), ~2.37 (m[d], Hα)
4'	170.6 (s)	
5'	175.8 (s)	
6'	66.7 (t)	4.25 (ddd, *J*= 9.0, 8.0, 8.0, Hα), 4.34 (ddd, *J*= 9.0, 9.0, 4.2, Hβ)

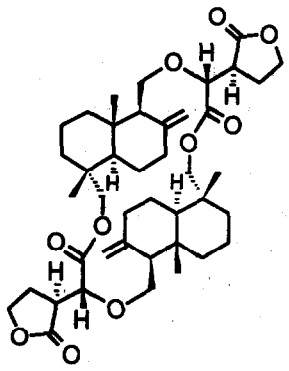
a) Recorded at 125 MHz in $CDCl_3$ relative to the solvent peak at δ 77.1. Multiplicities were determined by DEPT experiments. b) Recorded at 500 MHz in $CDCl_3$. Coupling constants in hertz (Hz) are given in parentheses. c) Assignments are based on COSY, HMBC, and NOE experiments. d) Overlapping signals.

formula of $C_{42}H_{60}O_{10}$ for **1**, and also suggested that **1** might be a symmetrical dimer possessing identical half-molecules.

The ^{13}C and ^{1}H NMR spectral data of **1** are given in Table 1. ^{13}C NMR spectrum revealed only half of carbon signals and the multiplicities were determined by DEPT experiments to be as follows: 2 x CH_3, 7 x CH_2, 3 x CH, 3 x CH_2O, 1 x CHO-, 2 x O-$\dot{C}$=O, and 3 x C. Several of these groups were connected by a combined analysis of ^{1}H-^{1}H COSY and HMQC spectra as following partial structures : -CH_2CH_2-, -CH_2CH_2O-, -CH_2CHCHO-, and -$CHCH_2O$-. As shown in Fig. 1, the HMBC experiments coupled with the fact that **1** possesses esters (1725 cm^{-1}, δ_C170.6) and lactone rings (1765 cm^{-1}, δ_C175.8) clarified the connectivities of above partial structures, and revealed the presence of a sesquiterpene structure **A**, presumably with a drimane skeleton, and a partial structure **B**.[2] The ring size of the lactone moiety is probably, though not certainly, γ rather than δ as judged from the IR data. The HMBC spectrum was also indicative of the connectivity of partial structures **A** and **B**, exhibiting cross peaks for H-1'(δ4.20)/C-11(δ70.8) and H-11(δ3.71, 3.86)/C-1'(δ78.4). The half-molecule of **1** was thus concluded to be **1'**. The absolute configuration of the half-molecule **1'** was established by chemical transformation to a known compound **2**, which was obtained by reduction of **1** with $LiAlH_4$, followed by acetylation with Ac_2O/pyridine. The physico-chemical and spectral data of **2** were identical to those of the tetraacetate derived from cryptoporic acid B.[3] The results led to conclusion that the absolute configurations of the sesquiterpene and γ-lactone parts were the same as those of cryptoporic acid B. Finally, the further interpretation of HMBC spectrum of **1** clarified the position of dimerization to be placed between C-15 and C-4' in another half-molecule through an ester linkage, in which the long range couplings were observed between H-15(δ3.73, 3.89)/C-4'(δ170.6). Consequently, the absolute structure of roseolide A was represented as **1**. The proposed structure **1** for roseolide A was further confirmed by X-ray crystallographic analysis.[4] The ORTEP drawing of **1** is shown in Fig. 2.

Cryptoporic acids (CAs) A-G are drimane sesquiterpenoid ethers of isocitric acid recently isolated from the wood-rotting fungus *Cryptoporus volvatus* by Asakawa *et al*, of which CA-D is a symmetrical dimer of CA-B.[3] It is noteworthy that the position of dimerization in **1** was placed between C-15 and C-4', instead of C-15 and C-5' in CA-D.

Roseolide A (**1**) exhibited little activity against P388 leukemia cell growth, however, cryptoporic acids have been reported to inhibit germination of rice seeds, release of superoxide anion, and tumor promotion of okadaic acid[3]. Therefore, further biological studies seem worthwhile to evaluate the pharmacological activity of **1**.



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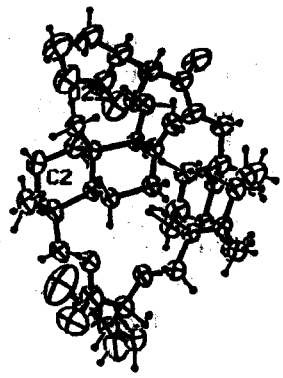

Fig. 2. ORTEP drawing of **1**

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References and Notes

1) W. A. Ayer, and L. M. Browne, *Tetrahedron*, **37**, 2199(1981); W. Steglich, *Pure Appl. Chem.*, **53**, 1233(1981).

2) Following cross peaks were observed: 13-CH_3(δ0.82)/C-1(δ39.8), C-5(δ46.7), C-9(δ55.4), C-10(δ39.6); 14-CH_3(δ0.79)/C-3(δ35.5), C-4(δ37.3), C-5, C-15(δ71.2); H_2-11(δ3.71, 3.86)/C-8(δ147.9), C-9, C-10; H_2-12(δ4.30, 4.76)/C-7(δ37.4), C-9; H-1'(δ4.20)/C-2'(δ42.0), C-3'(δ24.6), C-4'(δ170.6), C-5'(δ175.8); H-2'(δ3.14)/C-1'(δ78.4), C-2', C-4'; H-6'(δ4.25, 4.34)/C-5'.

3) T. Hashimoto, M. Tori, Y. Mizuno, and Y. Asakawa, *Tetrahedron Letters*, **28**, 6303(1987); T. Hashimoto, M. Tori, and Y. Asakawa, *Trans. mycol. Soc. Japan*, **29**, 281(1988); T. Hashimoto, M. Tori, Y. Mizuno, Y. Asakawa, and Y. Fukazawa, *J. Chem. Soc., Chem. Commun.*, 258(1989); Y. Asakawa, T. Hashimoto, Y. Mizuno, M. Tori, and Y. Fukazawa, *Phytochemistry*, **31**, 579(1992).

4) Colorless prisms of **1** for X-ray crystallographic analysis were grown in a $CHCl_3$-CH_3OH solution. Crystal data of **1** are as follows: M.F.=$C_{42}H_{60}O_{10}$, M.W.=724.934, orthorhombic space group $P2_12_12_1$, a=18.703(3)Å, b=18.947(3), c=11.840(2), Z=4, V=4195(1)(Å)^3, D_{calc}=1.148(g/ml^3) and refined by full-matrix least squares to R=7.74, Rw=8.97. These calculations were carried out by Micro VAX II system at Osaka University of Pharmaceutical Sciences.

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