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Sesterterpenoid from *Gentianella alborosea*

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Abstract

The structure of a new type of sesterterpenoid, designated as alborosin, isolated from *Gentianella alborosea*, has been deduced from a spectroscopic investigation.

Keywords: *Gentianella alborosea*; Gentianaceae; Hercampuri; Alborosin; Sesterterpenoid

1. Introduction

Gentianella alborosea and *G. nitida* (Gentianaceae), commonly known as 'Hercampuri' or 'Hircampure', are biennial medicinal plants growing in the Andes region. The aqueous extracts of the whole plants have been used in traditional Peruvian folk medicine as a remedy for hepatitis, as a cholagogue and in treatment of obesity (Senatore, Feo & Zhou, 1991). Recently, we described a novel sesterterpenoid, nitidasin (**1**), from the extract of *G. nitida* (Kawahara et al., 1997). During our ongoing research on the plants mentioned above, we have isolated a novel sesterterpenoid, named alborosin **2**, from the $CHCl_3$ extract of *G. alborosea*. The paper describes the structure elucidation of **2**, occurring in the plant together with xanthones and phenolic compounds.

2. Results and discussion

Alborosin (**2**), colorless amorphous powder, had the molecular formula $C_{25}H_{38}O_3$ as shown by the high resolution electron-impact ionization (EI) mass spectrometry, which had a molecular ion at m/z 386.2825 (calc. 386.2823) $(M)^+$. The IR and UV spectra indicated the presence of an α, β-unsaturated carbonyl group. The ^{1}H-NMR spectrum of **2** exhibited thirty-eight non-exchangeable protons, including two tertiary (δ 1.19 and 2.29) and four secondary (δ 0.79, 0.96, 1.02 and 1.05) methyl groups, and an olefinic proton (δ 6.72). The ^{13}C-NMR spectrum of **2** displayed signals corresponding to six methyls, six methylenes, seven methines, one tertiary and one quaternary C atoms, (olefinic moiety) and three carbonyl groups (δ 218.6, 210.7 and 196.7). The olefinic moiety and the three carbonyl groups accounted for four of the seven unsaturations, thus implying that **2** consisted of a three ring system with a structure related to nitidasin (**1**).

Interpretation of the ^{1}H–^{1}H COSY and HOHAHA spectra of **2** suggested the presence of a quaternary carbon (δ 38.6), two methyl groups (δ 19.9 and 26.7) and the above three carbonyl carbons. The structure was deduced from the PFGHMBC spectrum (see Fig. 1). A methyl group at δ 0.79 (H_3-20) was correlated to the methylene carbon at δ 32.2 (C-4). The methine proton at δ 2.84 (H-6) showed correlations to the carbonyl group at δ 196.7 (C-11), which was further correlated to the singlet methyl group at δ 2.29 (H_3-22), and the quaternary olefinic carbon at δ 156.0

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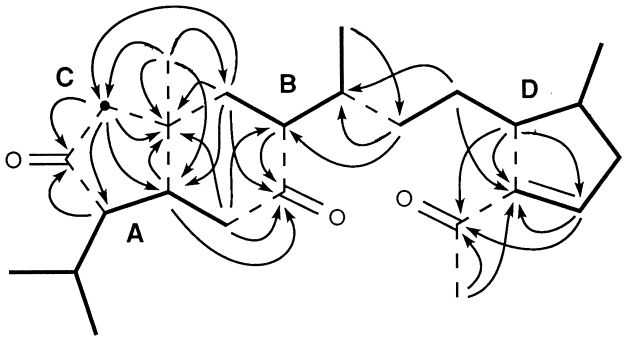


Fig. 1. Major long-range H–C correlations and spectral structure of alborosin (**2**).

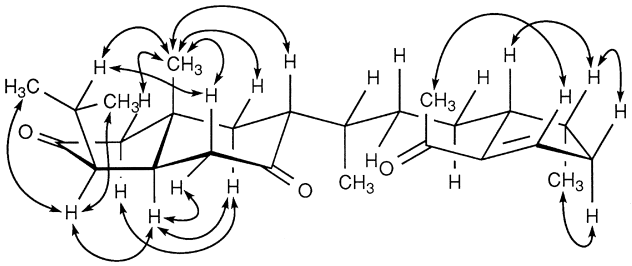


Fig. 2. NOESY correlations of alborosin (**2**).

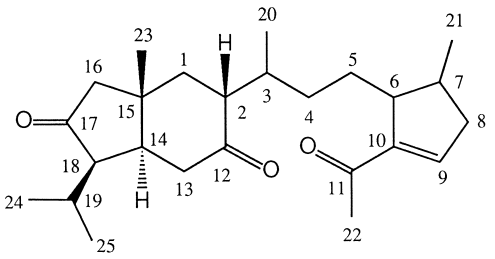
(C-10). The remaining singlet methyl group (δ 1.19) showed correlations to the quaternary carbon at δ 38.6 (C-15), the methylene carbon at δ 38.2 (C-1), the methylene carbon at δ 54.9 (C-16) and the methine carbon at δ 47.6 (C-14). The methylene protons at δ 2.61 and 2.65 and the methine proton at δ 2.49 were all correlated to the carbonyl group at δ 210.7 (C-12). The methine proton at δ 1.98 and the methylene protons at δ 2.02 and 2.28 showed correlations to the remaining carbonyl group at δ 218.6 (C-17). These data were in agreement with the structure of alborosin as shown in **2**. The assignments of the ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

From the analysis of the correlation peaks in the NOESY spectrum (see Fig. 2), the relative stereochemistry at H-2, H-14, C-15 and H-18 was deduced. The H_3-23 showed cross peak to H-2 and H-19, and H-14

Table 1
^{1}H- and ^{13}C-NMR chemical shifts and heteronuclear multiple bond (HMBC) correlations of alborosin (**2**) in $CDCl_3$

Position		^{1}H[a]	J (Hz)	^{13}C[b]	HMBC (^{1}H)[c]
1	(α)	1.46 *t*	12.8	38.2 *t*	2, 16, 23
	(β)	1.85 *dd*	6.2, 12.8		
2		2.49 *ddd*	3.5, 6.2, 12.8	49.6 *d*	1, 3, 4, 20
3		2.26 *m*		30.8 *d*	2, 4, 5, 20
4	(2H)	1.15 *m*		32.2 *t*	3, 5, 6, 20
5	(2H)	1.42 *m*		25.8 *t*	4, 6, 7
6		2.84 *m*		44.9 *d*	4, 5, 7, 8, 9, 21
7		2.45 *m*		37.1 *d*	5, 6, 8, 9, 21
8		2.17 *ddt*	2.2, 8.8, 17.6	40.2 *t*	6, 7, 9, 21
		2.51 *ddd*	2.2, 8.1, 17.6		
9		6.72 *br, t*	2.2	144.3 *d*	6, 8
10				156.0 *s*	5, 6, 8, 9, 22
11				196.7 *s*	6, 9, 22
12				210.7 *s*	1, 2, 13, 14
13	(α)	2.65 *dd*	5.5, 15.3	40.2 *t*	14
	(β)	2.61 *t*	15.3		
14		2.32 *m*		47.6 *d*	1, 13, 16, 18, 19, 23
15				38.6 *s*	1, 13, 14, 16, 18, 23
16	(α)	2.02 *d*	17.2	54.9 *t*	1, 23
	(β)	2.28 *d*	17.2		
17				218.6 *s*	16, 18
18		1.98 *m*		55.9 *d*	13, 14, 16, 19, 24, 25
19		1.95 *m*		27.8 *d*	14, 18, 24, 25
20	(3H)	0.79 *d*	6.6	16.1 *q*	2, 3, 4
21	(3H)	1.05 *d*	7.0	15.2 *q*	7, 8
22	(3H)	2.29 *s*		26.7 *q*	
23	(3H)	1.19 *s*		19.9 *q*	1, 14, 16
24	(3H)	1.02 *d*	6.6	20.9 *q*	18, 19, 25
25	(3H)	0.96 *d*	6.2	25.2 *q*	18, 19, 24

[a] 600 MHz.
[b] 150 MHz.
[c] Optimized for $^nJ_{CH} = 6$ Hz.



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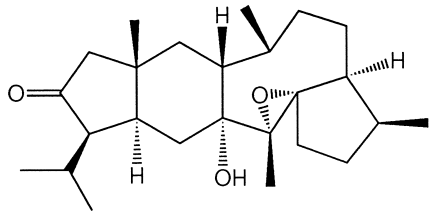

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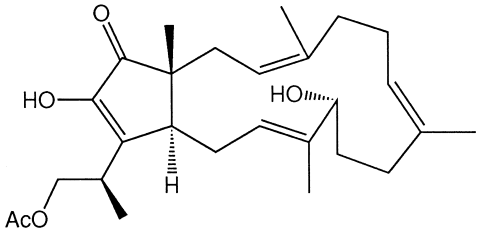

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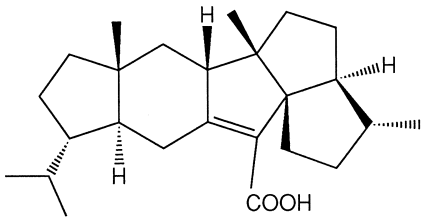

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showed cross peak to H_2-1α and H-18, thus supporting a *trans* ring junction between the five- and six-membered rings. Irradiation of H-6 gave a clear NOE with H-7 but not with H_3-21, suggesting a *cis* relationship between H-6 and H-7. However, the relative stereochemistries at H-3, H-6 and H-7 could not be assigned.

Recently a toxic sesterterpenoid, fusaproliferin (**3**) was isolated from the fungus, *Fusarium proliferatum* (Randazzo et al., 1993; Santini et al., 1996). A novel sesterterpenoid, retigeranic acid (**4**), was also isolated from lichens of *Lobaria retigera* group (Kaneda, Takahashi, Iitaka & Shibata, 1972). The carbon skeleton of alborosin (**2**) is similar to that of the above compounds except for the absence of carbon–carbon connectivity between C11 and C12. Thus, this unique tricyclic sesterterpenoid is considered to be derived from the cyclization of geranyl farnesyl pyrophosphate through a key intermediate, nitidasin (**1**), and final oxidative cleavage of the C11/C12 bond.

Alborosin (**2**) is the first example of a seco-type sesterterpenoid with the new ring skeleton shown, and is one of only two sesterterpenoids isolated from Gentianaceae (Kawahara et al., 1997).

3. Experimental

3.1. General

IR and UV spectra were recorded on JASCO FT/IR-5300 and Hitachi U-2000 spectrophotometers, respectively. Optical rotation data was measured on a JASCO DIP-370 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on JEOL α-600 and α-500 spectrometers, respectively with *J* values given in Hz. EI mass (EIMS) and high resolution EI mass (HR-EIMS) spectra were recorded on a JEOL JMS-D-300 spectrometer. Low pressure LC (LP-LC) was performed on a Nihon Seimitsu NP-FX-20 by a glass column (10 × 300 mm) packed with silica gel CQ-3 (30–50 μ; Wako).

3.2. Plant material

The aerial parts of *Gentianella alborosea* were collected in 1995, in Houaroz, Peru. A voucher specimen (No. PR003) has been deposited at the National Institute of Health Sciences, Japan.

3.3. Extraction and isolation

The aerial portions of *G. alborosea* (462 g) were crushed and extracted with MeOH (3 l × 3) to give an extract (96.5 g), which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction (8.6 g) was subjected to a silica gel column chromatography using a $CHCl_3$–MeOH (50:1) solvent system, followed by LP-LC with a *n*-hexane-EtOAc (8:1) to afford alborosin **2** (28 mg).

3.4. Alborosin (2)

Colorless amorphous powder; $[\alpha]_D^{25}$ — 69.6° ($CHCl_3$; *c* 0.24); IR ν_{max}^{KBr} cm^{-1}: 1740 (CO); 1700 (CO) and 1660 (CO), UV λ_{max}^{MeOH} (log ε): 235 sh (4.11). EIMS (70 eV) *m*/*z* (rel. int.): 386 (15), 179 (100). HR-EIMS *m*/*z* :

386.2825 $[M]^+$ ($C_{25}H_{38}O_3$ requires 386.2823); ^1H- and ^{13}H-NMR spectral data: Table 1.

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