Donnaienin, a New Acetogenin Bearing a Hydroxylated Tetrahydrofuran Ring

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A novel Annonaceous acetogenin, donnaienin (**1**), was isolated from the roots of *Goniothalamus donnaiensis*. Its structure and stereochemistry were elucidated on the basis of spectral data and chemical evidence. This compound represents an unusual type of Annonaceous acetogenin, bearing a hydroxylated tetrahydrofuran ring.

The Annonaceous acetogenins are a class of potent bioactive compounds found in various plant species of the family Annonaceae. Since 1982, more than 250 acetogenins have been discovered. Most of the previously known acetogenins belong to several classical types usually containing an unsubstituted tetrahydrofuran (THF) ring.[1−4] A previous investigation of the EtOH extract of the roots of *Goniothalamus donnaiensis* Finet et Gagnep has resulted in the isolation of several compounds.[5] In this paper, we report on the identification of a novel acetogenin, donnaienin (**1**), which is only the second acetogenin to be found with a hydroxylated THF ring.[6]

Donnaienin (**1**) was isolated as a white amorphous powder. The molecular formula of **1** was determined as $C_{35}H_{64}O_8$ by FABMS and elemental analysis. The existence of five OH groups was indicated by an IR hydroxyl absorption at 3429 cm^{-1} and give successive losses of H_2O from the MH^+ in the FABMS spectrum. This was confirmed by the acetylation of **1** to produce **1a**. In the ^{1}H-NMR spectrum of **1a**, five signals appeared downfield from δ 3.47, 3.62, 3.70, 3.85, and 4.38 to δ 4.82, 4.93 (2H), 5.01, and 5.10. As with other acetogenins, the presence of the methyl substituted α,β-unsaturated γ-lactone with a C-4−OH group in **1** was suggested by the IR carbonyl absorption (1746 cm^{-1}) and the corresponding resonances in the ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1). However, the NMR data (δ 3.75, 4.14, and 4.38 in ^{1}H-NMR spectrum, and δ 89.0 in ^{13}C-NMR spectrum) indicated that this compound exhibited an unusual structural feature. The ^{1}H−^{1}H COSY spectrum of **1** showed that the signal at δ 3.75 had cross peaks with the signals at δ 3.62 and 4.38, while the signal at δ 4.14 had three cross peaks with the signals at δ 2.11, 1.90, and 3.47. In the ^{1}H−^{13}C COSY spectrum of **1**, the proton at δ 3.75 correlated with the carbon at δ 89.0, and the proton at δ 4.14 correlated with the carbon at δ 81.2. As a result of these observations, a hydroxylated THF ring in **1** was established. By comparison of the NMR data of **1** (Table 1) with those of known acetogenins,[1−4] it was deduced that **1** had two flanking hydroxyl groups adjacent to the hydroxylated THF ring and another isolated hydroxyl group with the addition of C-4−OH on the hydrocarbon chain.

Table 1. ^{1}H-NMR (500 MHz, *J* in Hz) and ^{13}C-NMR (125 Hz) Data of Compound **1**

position	δ_H (in $CDCl_3$)	δ_H (in CD_3COCD_3)	δ_C (in $CDCl_3$)
1			174.8
2			131.0
3a	2.41, m	2.23, ddd (14.8, 7.7, 1.3)	33.3
3b	2.52, m	2.38, ddd (14.8, 4.5, 1.6)	
4	3.85, m	3.77, m	69.8
5	1.47, m	1.39, m	37.1
6−9	1.2−1.6, m	1.2−1.5, m	25−32
10	3.70, m	3.52, m	72.0
11	1.71, m	1.65, m	29.4[a]
12	1.71, m	1.65, m	29.7[a]
13	3.62, m	3.44, dt (8.2, 3.9)	71.5
14	3.75, m	3.64, dd (3.9, 2.9)	89.0
15	4.38, m	4.26, dt (5.9, 2.9)	73.6
16a	2.11, m	2.00, ddd (12.8, 9.0, 5.9)	37.6
16b	1.90, m	1.79, ddd (12.8, 6.4, 2.7)	
17	4.14, m	4.04, ddd (9.0, 6.3, 3.9)	81.2
18	3.47, m	3.38, dt (8.2, 3.9)	73.8
19	1.50, m	1.41, m	34.2
20−30	1.2−1.6, m	1.2−1.6, m	25−29
31	1.26, m	1.26, m	22.7
32	0.88, t (6.8)	0.85, t (6.8)	14.1
33	7.20, m	7.37, q (1.4)	152.1
34	5.07, m	5.04, dq (1.5, 6.8)	78.1
35	1.43, d, (6.8)	1.34, d (6.8)	19.0

[a] Assignments may be interchangeable.

The positions of the hydroxylated THF ring and hydroxyl groups on the hydrocarbon chain were determined by careful analysis of the EIMS fragments of **1** and its TMSi derivative **1b** (Figure 1). The position of oxygenation in the THF ring of **1**, however, could not be assigned yet, because the hydroxyl group could be replaced either at C-15 or at C-16. In order to solve this assignment, the formaldehyde acetal derivative **1c** of **1** was prepared. In the ^{1}H-NMR spectrum of **1c**, the H-10 signal shifted downfield from δ 3.70 to 3.86, suggesting that the formal was formed at C-10 to C-13. This was confirmed by the acetylation of **1c** (**1d**). In the ^{1}H-NMR spectrum of **1d**, the signals at δ 3.47 and 4.40 resonated downfield at δ 5.00 and 5.13, respectively, while the signal at δ 3.62 was unchanged. Thus, the ^{1}H-NMR chemical shift at δ 3.62 could be assigned to H-13. That the formal formed at C-10 to C-13 instead of C-13 to C-15 may be due to the steric hindrance of the THF ring. With regard to the correlations in the COSY spectra and the coupling constant (because of the bad resolution of the ^{1}H-NMR spectrum of **1** in $CDCl_3$, the ^{1}H-NMR spectrum of **1** in CD_3COCD_3 was mea-

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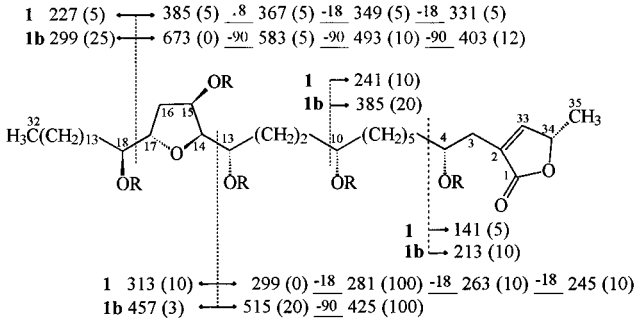


Figure 1. Diagnostic EIMS (*m*/*z*) fragmentations of **1** (R═H) and **1b** (R═TMSi) (numbers in parentheses are percent intensities).

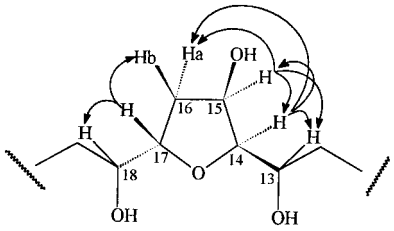
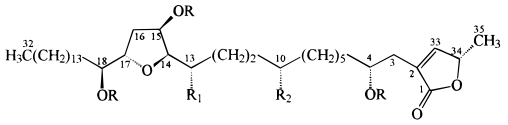


Figure 2. NOE correlations of the THF system of **1**.

sured, Table 1), the position of the hydroxyl group was then established at C-15.

Applying Born's rule,[7] the relationship at C-17/18 as threo was indicated by the chemical shifts of H-18 (δ 3.47) and C-18 (δ 73.8). Also, taking account of the deshielding effect and γ-effect of the C-15−OH, the relationship at C-13/14 was deduced as threo from the chemical shifts of H-13 (δ 3.62 (3.40 + 0.22) and C-13 [δ 71.5 (74.0−2.5)]. If this were not the case, the signal of H-13 should have appeared at ca. δ 4.00 (3.80 ± 0.20),[7,8] and the signal of C-13 should have resonated at ca. δ 69.0 (71.5−2.5).[7,9] The configurational assignment of the THF ring as trans and the relationship at C-14/15 as threo were determined by NOE difference experiments. As illustrated in Figure 2, when the proton at δ 3.75 (H-14) was irradiated, the δ 3.62 (H-13), 4.38 (H-15), and 2.11 (H-16a) signals showed enhancements, but NOE enhancements between H-14 and H-17 were not observed. When the proton at δ 4.14 (H-17) was irradiated, the protons at δ 3.47 (H-18) and 1.90 (H-16b) showed enhancements, but the correlations between H-15 and H-17 were not observed. The large difference in the shifts of the two formal protons in **1c** of δ 4.58 and 5.10 showed that they resided in very different chemical environments, indicating a cis relationship between the alcohol centers at C-10 and C-13 of **1** (i.e., the configurations of C-10/13 must be either *S*/*S* or *R*/*R*).[10]

The absolute stereochemistry of **1** was determined by advanced Mosher ester methodology.[11] Because the yield of formaldehyde acetal derivative was low, the acetonide derivative **1e** of **1** was prepared. In addition, the (*R*)- and (*S*)-tri-MTPA esters of **1e** (**1es** and **1er**) were prepared and their ^{1}H-NMR signals assigned by the COSY spectra, and the corresponding $\Delta\delta_{1es-1er}$ values were calculated (Table 2). The data indicated that the respective absolute configurations at C-18, C-15, and C-4 were *S*, *R*, and *R*. The configurational assignment of the C-34 as *S* was made by comparing the $\Delta\delta_{1es-1er}$ data with those of synthetic model compounds.[12] Combining with the relative stereochemistry previously established, the absolute configurations of **1** at the remaining stereocenters were assigned as 10*S*, 13*S*, 14*R*, and 17*S*. Compound **1** gave cytotoxicity IC_{50}



	1	1a	1b	1c	1d	1e	1es	1er
R =	H	Ac	TMSi	H	Ac	H	(*S*)-OMTPA	(*R*)-OMTPA
R_1 =	H	Ac	OTMSi	O–	O–	O–	O–	O–
R_2 =	H	Ac	OTMSi	OCH_2–	OCH_2–	$OC(CH_3)_2$–	$OC(CH_3)_2$–	$OC(CH_3)_2$–

values against KB, HCT-8, and Bel human tumor cell lines of >10, >10, and 6.7 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Materials and methods were described previously.[5]

Plant Material. The plant material was previously described.[5]

Extract and Isolation. The procedures employed for isolation and derivatization were described previously.[5] A large Si gel column was used to separate the aqueous MeOH-soluble fraction F005 (91 g) into 210 fractions. The amorphous powder, compound **1**, was afforded from fraction nos. 140−150 by repeated column chromatography and preparative TLC (35 mg).

Donnaienin (1): white amorphous powder; mp 90−92 °C, $[\alpha]^{20}_D$ 0° (*c* 0.25, MeOH); IR ν_{max} 3429 (OH), 2920 and 2850 (CH), 1746 (lactone C═O), 1469 (CH) cm^{-1}, ^{1}H-NMR (500 MHz, $CDCl_3$ or CD_3COCD_3) data, see Table 1; ^{13}C-NMR (125 MHz, $CDCl_3$) data, see Table 1; FABMS *m*/*z* $[MH]^+$ 613 (100), with 5 × H_2O loses at 595 (20), 577 (7), 559 (4), 541 (10), and 523 (5); EIMS, see Figure 1; *anal.* C 68.40%, H 10.55%; calcd for $C_{35}H_{64}O_8$, C 68.63%, H 10.46%.

Acetate derivative of 1 (1a): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, 5, *J* = 6.8 Hz, H-32), 1.40 (3H, d, *J* = 6.8 Hz, H-35), 2.03 (3H, s, OCC*H*$_3$), 2.04 (3H, s, OCC*H*$_3$), 2.05 (3H, s, OCC*H*$_3$), 2.07 (3H, s, OCC*H*$_3$), 2.09 (3H, s, OCC*H*$_3$), 2.54 (2H, m, H-3), 3.95 (1H, m, H-14), 4.14 (1H,

Table 2. Partial ^{1}H-NMR (500 MHz, $CDCl_3$) Data of Compounds. **1es** and **1er**

position	1es	1er	$\Delta\delta_{1es-1er}$	position	1es	1er	$\Delta\delta_{1es-1er}$
3a	2.58	2.60	−0.02	14	3.89	3.91	−0.02
3b	2.60	2.68	−0.08	15	5.46	5.37	*R*[a]
4	5.32	5.37	*R*[a]	16a	2.05	1.99	+0.06
5	1.65	1.62	+0.03	16b	1.99	1.85	+0.14
33	6.73	6.97	−0.24	17	4.09	4.04	+0.05
34	4.86	4.90	−0.04	18	5.15	5.12	*S*[a]
35	1.28	1.31	−0.03	19	1.48	1.58	−0.10

[a] Absolute configuration of carbinol center.

m, H-17), 4.82 (1H, m, H-10), 4.93 (2H, m, H-13 and H-18), 5.01 (2H, m, H-15 and H-34), 5.10 (1H, m, H-4), and 7.08 (1H, br s, H-33).

TMSi derivative of 1 (1b). EIMS, see Figure 1.

Formal Derivative of 1 (1c). To **1** (5 mg in 5 mL of CH_2Cl_2) was added excessive polyformaldehyde and a few of crystals of *p*-toluenesulfonic acid, and the reaction mixture was stirred at room temperature for 24 h. The product (**1c**) was purified by preparative TLC: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, J = 6.9 Hz, H-32), 1.43 (3H, d, J = 6.9 Hz, H-35), 2.40 (1H, dd, J = 15.2, 8.2 Hz, H-3a), 2.53 (1H, dd, J = 15.2, 4.1 Hz, H-3b), 3.40 (1H, m, H-18), 3.63 (1H, m, H-13), 3.80 (1H, t, J = 3.4 Hz, H-14), 3.85 (1H, m, H-4), 3.86 (1H, m, H-10), 4.12 (1H, m, H-17), 4.40 (1H, m, H-15), 4.58 (1H, d, J = 7.5 Hz, formal proton), 5.06 (1H, dq, J = 1.3, 6.8 Hz, H-34), 5.10 (1H, d, J = 7.5 Hz, formal proton), and 7.18 (1H, d, J = 1.3 Hz, H-33).

Acetate derivative of 1c (1d): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, J = 6.8 Hz, H-32), 1.40 (3H, d, J = 6.8 Hz, H-35), 2.02 (3H, s, OCCH_3), 2.04 (3H, s, OCCH_3), 2.08 (3H, s, OCCH_3), 2.54 (2H, m, H-3), 3.63 (1H, m, H-13), 3.85 (1H, m, H-10), 3.92 (1H, m, H-14), 4.15 (1H, m, H-17), 4.55 (1H, d, J = 7.5 Hz, formal proton), 4.97 (1H, m, H-18), 5.00 (1H, m, H-34), 5.08 (1H, d, J = 7.5 Hz, formal proton), 5.10 (1H, m, H-4), 5.13 (1H, m, H-15), and 7.08 (1H, br s, H-33).

Acetonide derivative of 1 (1e): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, J = 6.8 Hz, H-32), 1.43 (3H, d, J = 6.8 Hz, H-35), 2.41 (1H, dd, J = 14.8, 8.3 Hz, H-3a), 2.52 (1H, dd, J = 14.8, 4.2 Hz, H-3b), 3.38 (1H, m, H-18), 3.75 (1H, m, H-13), 3.86 (1H, m, H-4), 3.95 (1H, m, H-14), 4.04 (1H, m, H-10), 4.14 (1H, m, H-17), 4.47 (1H, m, H-15), 5.05 (1H, q, J = 6.8 Hz, H-34), and 7.18 (1H, br s, H-33).

MTPA derivative of 1e (1es, 1er): ^{1}H-NMR (500 MHz, J in Hz, $CDCl_3$) data, for characteristic resonances, see Table 2.

Biological Evaluation. Cytotoxicity against human solid tumor cells was measured in five-day MTT tests at the Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, for the KB nasopharyngeal carcinoma, HCT-8 colon adenocarcinoma, and Bel hepatoma cell lines.

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