Four New Halimane-Type Diterpenes, Vitetrifolins D—G, from the Fruit of *Vitex trifolia*

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Four new halimane-type (rearranged labdane-type) diterpenes, vitetrifolins D—G were isolated from the fruit of *Vitex trifolia* L. (Verbenaceae). Their chemical structures were determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Vitex trifolia*; Verbenaceae; *Viticis fructus*; rearranged labdane; halimane; diterpene

In a previous paper,[1)] we reported the isolation and structure elucidation of three new diterpenes, vitetrifolins A—C, along with three known diterpenes from the acetone extract of the fruit of *Vitex trifolia* L. (Verbenaceae), which are called "Viticis Fructus" and are used as folk medicine for headaches, colds, migraine, eye pain, *etc.*[2)]

The present paper describes the isolation and structure characterization of four new halimane-type (rearranged labdane-type) diterpenes (**1**—**4**)[3)] from the acetone extract of the fruit of *V. trifolia*. Repeated chromatography of the acetone extract led to the isolation of **1**—**4**.

Compound **1**, called vitetrifolin D, was obtained as a colorless syrup. In the electron impact (EI)-MS, **1** did not show a molecular ion peak but showed fragment ion peaks at *m/z* 346 $[M-CH_3COOH]^+$ and 286 $[M-CH_3COOH\times2]^+$. The molecular formula of **1** was determined to be $C_{24}H_{38}O_5$ by high-resolution (HR) positive FAB-MS. The ^{1}H-NMR spectrum of **1** indicated signals due to four tertiary methyl groups (δ 1.25, 1.07, 1.07, 0.91), one secondary methyl group (δ 0.90, d, J=6.5 Hz), two acetyl groups (δ 2.03, 1.99), one vinylic group (δ 5.84, dd, J=11.0, 17.0 Hz; 5.18, dd, J=1.5, 17.0 Hz; 5.06, dd, J=1.5, 11.0 Hz), and two oxygenated methine protons (δ 5.61, d, J=3.5 Hz; 4.86, dd, J =3.5, 13.0 Hz). The ^{13}C-NMR spectrum of **1** gave 24 carbon signals, including two carbonyl groups (δ 170.8, 170.7), one tetra-substituted olefinic bond (δ 141.5, 132.7), one vinylic group (δ 144.5, 112.1), one oxygenated quaternary carbon (δ 73.1), and two oxygenated methine carbons (δ 72.8, 66.2), suggesting **1** to be a diterpene with a 3-hydroxy-3-methyl-1-propenyl group and two acetyl groups. These ^{1}H- and ^{13}C-NMR spectroscopic signals were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) techniques as shown in Tables 1 and 2, and the planar structure of **1** was determined as illustrated in Fig. 1. The relative stereostructure was defined on the basis of difference nuclear Overhauser effect (NOE) experiments and the coupling constant values of the signals due to H-6, H-7, and H-8 in the ^{1}H-NMR spectrum. In the difference NOE spectra of **1**, which were mainly carried out in C_5D_5N, because the ^{1}H-NMR spectral signals were rather congested in $CDCl_3$, NOEs were observed between the respective protons, as illustrated in Fig. 2. Moreover, when the configuration at C-13 was postulated to be *S**, the stable conformation of **1** with minimum steric energy was simulated using CAChe

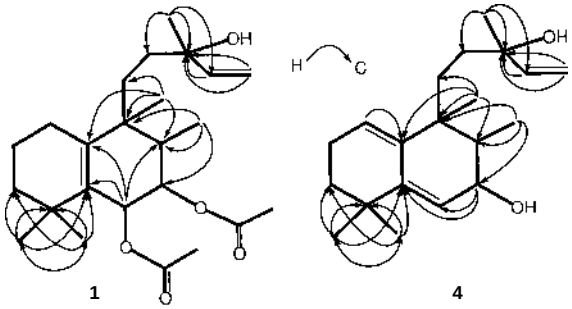

Fig. 1. ^{1}H–^{13}C Long-Range Correlations Observed for **1** and **4** in the HMBC Spectra (in $CDCl_3$, 500 MHz)

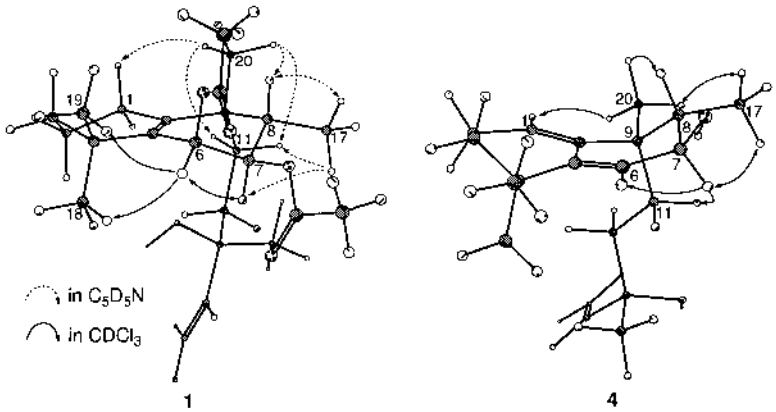

Fig. 2. CAChe Drawings and Selected NOE Correlations Observed in Difference NOE Spectra of **1** and **4**

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CONFLEX,[4] and is illustrated in Fig. 2. The coupling constant values for H-6 (d, J=3.5 Hz), H-7 (dd, J=3.5, 13.0 Hz), and H-8 (dq, J=13.0, 6.5 Hz) supported this conformation. Consequently, vitetrifolin D was elucidated to be (*rel* 6*S*,7*R*, 8*S*,9*R*)-6,7-diacetoxy-5(10),14-halimadien-13-ol (Fig. 3).

Compound **2**, called vitetrifolin E, was obtained as colorless needles, and **3**, called vitetrifolin F, was obtained as colorless syrup. Both compounds showed an $[M+Na]^+$ ion peak at *m/z* 387, which was 42 mass units $[CH_3CO-H]$ smaller than that of **1** in the positive FAB-MS. The ^{1}H-NMR spectra of **2** and **3** were similar to that of **1**, except that the signal due to an acetyl group disappeared and signals assignable to H-7 in **2** and H-6 in **3** were shifted upfield by 1.13 ppm and 1.40 ppm, respectively, in comparison with those of **1**. Therefore **2** and **3** were recognized as 7-deacetyl and 6-deacetyl derivatives of **1**, respectively. This was confirmed by the acetylation of **2** and **3** with acetic anhydride in pyridine to give **1**. Accordingly, the structures of vitetrifolins E and F were concluded to be (*rel* 6*S*,7*R*,8*S*,9*R*)-6-acetoxy-5(10),14-halimadien-7,13-diol and (*rel* 6*S*,7*R*,8*S*,9*R*)-7-acetoxy-5(10),14-halimadien-6,13-diol (Fig. 3), respectively.

Compound **4**, called vitetrifolin G, was obtained as colorless needles, and the UV (λ_{max} 240 nm) spectrum showed the presence of a heteroannular diene group. The ^{1}H-NMR spectrum was analogous to that of **1**, apart from the appearance of signals due to two olefinic protons (*ca*. 5.52, *ca*. 5.53) and the loss of signals due to two acetyl groups and an oxygenated methine proton. Acetylation of **4** in the same manner as that used for **2** and **3** afforded a monoacetate (**5**), whose ^{1}H-NMR spectrum showed a downfield shift of 1.29 ppm for

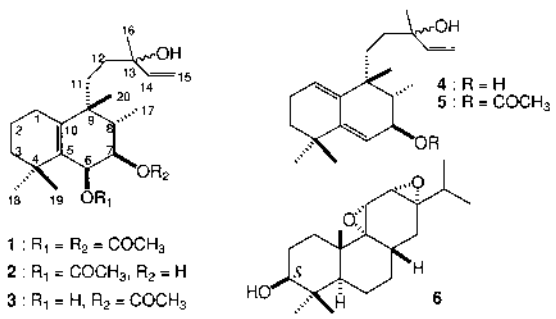

Fig. 3. Structures of **1**—**6**

Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**4** (125 MHz)

C	**1**[a]	**1**[b]	**2**[a]	**3**[a]	**4**[a]
1	26.0	26.2	25.9	25.8	122.4
2	19.5	19.7	19.5	19.6	23.2
3	39.4	39.6	39.5	39.5	36.4
4	34.7	34.8	34.5	34.6	33.4
5	132.7	132.7	132.2	136.4	143.3
6	66.2	66.4	70.0	65.0	120.8
7	72.8	73.2	71.3	75.2	72.1
8	36.5	37.0	38.8	35.9	48.0
9	42.9	43.4	42.8	43.0	41.2
10	141.5	142.4	141.8	139.1	138.6
11	29.4	30.1	29.5	29.4	24.8
12	38.7	39.9	38.9	38.7	36.6
13	73.1	72.3	73.1	73.1	73.3
14	144.5	146.7	144.6	144.6	145.1
15	112.1	111.6	112.1	112.0	111.7
16	28.2	28.4	28.1[c]	28.1[c]	27.9
17	11.1	11.3	11.1	11.1	11.7
18	29.3	29.2	29.3	29.7[d]	26.6
19	27.9	28.0	27.8	29.2[d]	28.4
20	28.2	28.4	28.3[c]	28.3[c]	21.5
CO	170.8	171.0	173.2	170.7	
CO	170.7	170.8			
$COCH_3$	21.5	21.3	21.6	21.2	
$COCH_3$	21.0	20.9			

δ in ppm from TMS. *a*) In $CDCl_3$. *b*) In C_5D_5N. *c*, *d*) Assignments in each column may be interchangeable.

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**4** (500 MHz)

H	**1**[a]	**1**[b]	**2**[a]	**3**[a]	**4**[a]
1a	*ca*. 2.03	2.23 ddd (4.5, 4.5, 17.0)	*ca*. 2.02	*ca*. 2.00	*ca*. 5.52
1b	*ca*. 2.03	2.03 ddd (6.0, 10.0, 17.0)	*ca*. 2.02	*ca*. 2.00	
2a	*ca*. 1.63	*ca*. 1.61	*ca*. 1.64	*ca*. 1.63	*ca*. 2.23
2b	*ca*. 1.63	*ca*. 1.56	*ca*. 1.59	*ca*. 1.63	*ca*. 2.16
3a	*ca*. 1.48	1.48 ddd (3.5, 13.0, 13.0)	*ca*. 1.51	*ca*. 1.50	1.50 ddd (6.0, 10.5, 13.5)
3b	*ca*. 1.48	1.39 m	*ca*. 1.47	*ca*. 1.50	*ca*. 1.42
6	5.61 d (3.5)	6.03 d (3.0)	5.51 d (3.5)	4.21 d (3.0)	*ca*. 5.53
7	4.86 dd (3.5, 13.0)	5.29 dd (3.0, 13.0)	3.73 dd (3.5, 12.0)	4.91 dd (3.0, 12.5)	3.91 d (9.5)
8	2.06 dq (13.0, 6.5)	2.34 dq (13.0, 6.5)	1.83 dq (12.0, 6.5)	2.19 dq (12.5, 6.5)	*ca*. 1.42
11a	*ca*. 1.49	1.81 ddd (2.0, 13.0, 13.0)	*ca*. 1.44	*ca*. 1.55	*ca*. 1.25
11b	*ca*. 1.43	1.73 ddd (5.0, 13.0, 13.0)	1.36 m	*ca*. 1.41	*ca*. 1.19
12a	*ca*. 1.48	1.88 ddd (5.0, 13.0, 13.0)	*ca*. 1.42	*ca*. 1.41	*ca*. 1.36
12b	1.19 m	*ca*. 1.56	1.11 m	1.19 m	*ca*. 1.25
14	5.84 dd (11.0, 17.0)	6.15 dd (10.5, 17.5)	5.84 dd (11.0, 17.5)	5.85 dd (11.0, 17.0)	5.79 dd (10.0, 17.5)
15a	5.18 dd (1.5, 17.0)	5.54 dd (2.0, 17.5)	5.18 dd (1.5, 17.5)	5.18 dd (1.5, 17.0)	5.14 d (17.5)
15b	5.06 dd (1.5, 11.0)	5.16 dd (2.0, 10.5)	5.06 dd (1.5, 11.0)	5.06 dd (1.5, 11.0)	5.01 d (10.0)
16	1.25 s	1.44 s	1.24 s	1.26 s	1.20 s
17a	0.90 d (6.5)	1.08 d (6.5)	1.02 d (6.5)	0.92 d (6.5)	1.06 d (6.5)
18	1.07 s	1.02 s	1.05 s	1.16 s[c]	0.96 s
19	0.91 s	1.03 s	0.94 s	1.09 s[c]	1.11 s
20	1.07 s	1.13 s	1.05 s	1.05 s	1.01 s
$COCH_3$	2.03 s	2.10 s	2.07 s	2.17 s	
$COCH_3$	1.99 s	2.09 s			

δ in ppm from tetramethylsilane (TMS) (coupling constants [J] in Hz are given in parentheses). *a*) In $CDCl_3$. *b*) In C_5D_5N. c) Assignments in each column may be interchangeable. Assignments are based on ^{1}H–^{1}H COSY experiments.

the signal due to an oxyganated methine proton as compared with that of **4**. The ^{13}C-NMR spectrum of **4** showed 20 carbon signals, including one each of the vinylic group (δ 145.1, 111.7), conjugated diene group (δ 143.3, 138.6, 122.4, 120.8), oxygenated quaternary carbon (δ 73.3), and oxygenated methine carbon (δ 72.1). The ^{1}H- and ^{13}C-NMR signal assignments of **4** were made with the aid of similar NMR techniques as used for **1**, and the planar structure of **4** was elucidated as shown in Fig. 1. The stereostructure of **4** was characterized on the basis of difference NOE spectra, in which NOEs were observed between the respective protons, as illustrated in Fig. 2, and by analysis of the coupling constant of the signal due to H-7 (d, J=9.5 Hz) in the ^{1}H-NMR spectrum. The structure of vitetrifolin G was thus defined as (*rel* 7*S*,8*S*,9*R*)-1(10),5,14-halimatrien-7,13-diol.

Although the configurations of the hydroxyl group at C-13 and absolute configurations of **1**—**4** have not been confirmed, their absolute configurations are probably the same as that of vitetrifolin A (**6**)[1] from a biogenetic point of view.

Experimental

All instruments and materials used were the same as cited in previous reports[1,5,6] unless otherwise specified.

Extraction and Isolation Powdered fruit of *Vitex trifolia* L. (1996 g) was extracted with acetone (3.5 l) at room temperature for 2 weeks. The acetone extract (89.8 g) was partitioned between hexane (300 ml×3) and H_2O (700 ml). The aqueous layer was extracted with AcOEt (100 ml×2). The AcOEt-soluble fraction (15.88 g) was subjected to silica gel (Merck, Art. 1.09385) with a gradient of $CHCl_3$–MeOH (1 : 0→0 : 1) to afford fractions 1—10. Chromatography of fraction 3 (199 mg) on a Sephadex LH-20 column with MeOH furnished fractions 11—13. Fraction 11 (17 mg) was subjected to HPLC (column, COSMOSIL 5C18 AR-II, 250 mm×20 mm i.d., Nacalai Tesque Inc.; solv., 80% MeOH) to give **1** (2 mg). Fraction 5 (1373 mg) was subjected to high porous polymer (MCI gel CHP 20P) with a gradient of H_2O–MeOH (3 : 7→0 : 1) and acetone to afford fractions 14—18. Fractions 14 (24 mg) and 15 (56 mg) were each subjected to HPLC under the same conditions as for fraction 11 to give **3** (10 mg) from fraction 14, and **2** (14 mg) and **4** (4 mg) from fraction 15.

Vitetrifolin D (**1**): A colorless syrup, $[\alpha]_D^{28}$ +107.8° (c=0.9, acetone). HR-FAB-MS *m/z*: 429.2567 $[M+Na]^+$ (Calcd for $C_{24}H_{38}O_5Na$: 429.2617). Positive FAB-MS *m/z*: 429 $[M+Na]^+$, 369 $[M+Na-CH_3COOH]^+$, 309 $[M+Na-CH_3COOH\times2]^+$. EI-MS *m/z* (rel. int.): 346 (61) $[M-CH_3COOH]^+$, 286 (100) $[M-CH_3COOH\times2]^+$. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2.

Vitetrifolin E (**2**): Colorless needles (hexane–acetone), mp 143—144 °C, $[\alpha]_D^{25}$ +126.6° (c=1.4, acetone). HR-FAB-MS *m/z*: 387.2464 $[M+Na]^+$ (Calcd for $C_{22}H_{36}O_4Na$: 387.2512). Positive FAB-MS *m/z*: 387 $[M+Na]^+$, 327 $[M+Na-CH_3COOH]^+$. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2.

Vitetrifolin F (**3**): A colorless syrup, $[\alpha]_D^{25}$ +94.6° (c=1.1, acetone). HR-FAB-MS *m/z*: 387.2495 $[M+Na]^+$ (Calcd for $C_{22}H_{36}O_4Na$: 387.2512). Positive FAB-MS *m/z*: 387 $[M+Na]^+$, 327 $[M+Na-CH_3COOH]^+$. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2.

Vitetrifolin G (**4**): Colorless needles (hexane–acetone), mp 146—147 °C, $[\alpha]_D^{25}$ −44.0° (c=0.4, acetone). UV λ_{max} (MeOH) nm (log ε): 240 (4.4). HR-FAB-MS *m/z*: 327.2238 $[M+Na]^+$ (Calcd for $C_{20}H_{32}O_2Na$: 327.2300). Positive FAB-MS *m/z*: 327 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2.

Computational Methods Caluculations were performed using CAChe (Version 4.1.1) with extended MM2 parameters[7] (Fujitsu Co., Japan), which was run on a Macintosh Powerbook G-3/400 personal computer. Energies were minimized with the conjugate gradient by varying the dihedral angles of (C-20)–(C-9)–(C-11)–(H-11) and between 0° and 360° in 15° increments. Convergence was obtained when the difference in the energies between two successive interactions was less than 0.00000001 kcal/mol. Drawing was perfomed using the Chem3D program (Cambridge Scientific Computing Inc., Cambridge, Massachusetts, U.S.A.)

Acetylation of 2, 3, and 4 Compounds **2** (2 mg), **3** (2 mg), and **4** (1 mg) in Ac_2O–pyridine (1 : 1, 0.2 ml) were each left to stand at room temperature overnight. After removal of the reagent under a stream of N_2, the residue was partitioned between ether (1 ml×3) and H_2O (1 ml). The ether layer was concentrated to afford an acetate (acetate of **2** [2 mg], acetate of **3** [2 mg], acetate (**5**) of **4** [1 mg]). The ^{1}H-NMR spectra of acetates of **2** and **3** were identical with that of **1**. **5**: ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 5.80 (1H, dd, J=11.0, 17.5 Hz), 5.57 (1H, dd, J=2.0, 3.0 Hz), 5.41 (1H, br s, $W_{h/2}$=6.0 Hz), 5.20 (1H, dd, J=2.5, 9.0 Hz), 5.16 (1H, dd, J=1.0, 17.5 Hz), 5.02 (1H, dd, J=1.0, 11.0 Hz), 2.08 (3H, s), 1.21 (3H, s), 1.09 (3H, s), 1.03 (3H, s), 0.96 (3H, s), 0.91 (3H, d, J=6.5 Hz).

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