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Diterpenoids from the fruits of *Vitex trifolia*

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Abstract

An abietane-type diterpene, named vitetrifolin A, and two labdane-type diterpenes, named vitetrifolins B and C, were isolated from the acetone extract of the fruits of *Vitex trifolia* L. (Viticis Fructus; Verbenaceae) along with three known diterpenes, rotundifuran, dihydrosolidagenone and abietatriene 3β-ol. The structures of these compounds were elucidated on the basis of spectroscopic analysis, X-ray crystallographic analysis and chemical evidence.

Keywords: Viticis Fructus; *Vitex trifolia*; Verbenaceae; Labdane-type diterpene; Abietane-type diterpene; Vitetrifolin

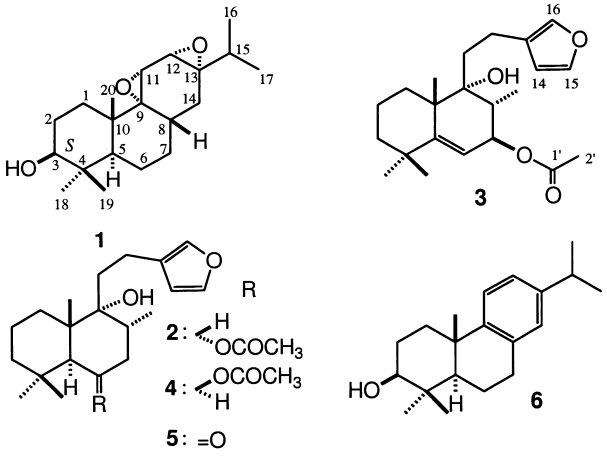
1. Introduction

Vitex trifolia L. (Verbenaceae) grows widely throughout Southeast Asia, Micronesia, Australia and East Africa. The fruits of this plant and of *V. rotundifolia* L. f. are called "Viticis Fructus" and are used as a folk medicine for headaches, colds, migraine and eyepain (Kimura and Kimura, 1981).

In preceding papers we have reported the isolation and structure elucidation of eight iridoids, eleven diterpenes and seven phenolic compounds from the MeOH extract of *V. rotundifolia* L. f., which exhibited stronger anti-oxidative activity than 3-*tert*-butylhydroxyanisole (Ono et al., 1997, 1998a,b, 1999). The present paper describes the isolation and structure elucidation of a new abietane-type diterpene, named vitetrifolin A, two new labdane-type diterpenes, named vitetrifolins B and C, and three known diterpenes, rotundifuran, dihydrosolidagenone and abietatriene 3β-ol, from the acetone extract of the fruits of *V. trifolia* L.

2. Results and discussion

The acetone extract of the fruits of *V. trifolia* L. was partitioned between hexane and H_2O. The hexane soluble fraction was successively subjected to silica gel column chromatography (CC) and HPLC on silica gel to afford an abietane-type diterpene (**1**) and two labdane-type diterpenes (**2** and **3**), along with three known diterpenes (**4–6**).



Compounds **4–6** were identified as rotundifuran (Asaka et al., 1973), dihydrosolidagenone (Anthonsen et

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al., 1969) and abietatriene 3β-ol (Chamy et al., 1991; Urones et al., 1998), respectively, based on their physical and spectral data, although the detailed NMR spectral data of **4** and **5** have not been reported in the literature.

Compound **1**, trivially named vitetrifolin A showed $[M]^+$ and $[M–H_2O]^+$ fragment ion peaks at *m/z* 320 and 302, respectively, in the EIMS. The ^1H NMR spectrum indicated signals due to three tertiary methyl groups (δ 1.08, 0.99, 0.79), two secondary methyl groups (δ 0.96, 0.90) and three oxygenated methine protons (δ 3.32, 3.21, 3.01). The ^{13}C NMR spectrum showed 20 carbon signals, including two oxygenated methine carbons (δ 77.9, 54.5) and two oxygenated quaternary carbons (δ 67.7, 59.0). These ^1H and ^{13}C NMR spectroscopic signals were assigned with the aid of ^1H–^1H COSY, HMQC and HMBC spectral analysis as shown in Tables 1 and 2, and the planar structure of **1**, an abietane-type diterpene, possessing two epoxy rings, was characterized. The relative stereochemistry of **1** was determined by analysis of difference NOE spectra, in which correlations were observed between H_3-18 and H-3, H_3-18 and H-5, H_3-18 and H_α-6, H_3-20 and H_3-19, H_3-20 and H-8, and H_3-20 and H-11. However, the configuration of the epoxy ring between C-13 and C-14 could not be confirmed. Finally, the complete relative stereochemistry of **1** was elucidated by X-ray crystallography. The ORTEP drawing of the structure of **1** is shown in Fig. 1. Furthermore, the absolute configuration of **1** was determined by application of Mosher's method (Dale and Mosher, 1973). The hydroxyl group at C-3 was esterified with (+)- and (−)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) to afford the (+)-MTPA ester (**1a**) and the (−)-MTPA ester (**1b**), respectively. The Δδ values [δ**1a**–δ**1b**] are shown in Fig. 2. The absolute configuration of **1** was thus determined, and the structure of vitetrifolin A was completely elucidated as **1**.

Compound **2**, named vitetrifolin B, gave the same intense EIMS fragment ion peak at *m/z* 302 $[M–CH_3COOH]^+$ as did **4**. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **4**, although the splitting patterns and chemical shifts of the signals due to H-5 (δ 1.94, *d*, *J* = 11.5 Hz), H-6 (δ 5.09, *dt*, *J* = 5.0,11.5 Hz) and H_α-7 (δ 1.42, *q*, *J* = 11.5 Hz) were different from those (H-5: δ 1.66, *d*, *J* = 2.5 Hz; H-6: δ 5.39, *dt*, *J* = 2.5, 3.0 Hz; H_α-7: δ 1.66, *dt*, *J* = 3.0, 13.5 Hz) of 4. In the difference NOE spectra, NOEs were observed between H_3-19 and H-6, and between H_3-20 and H-6, instead of the NOE correlation between H_3-18 and H-6 seen in **4**. Other NOE correlations between H_3-18 and H-5, H_3-20 and H_3-19, H_3-20 and H-8 and H_3-20 and H-11, were the same as those of **4**. Thus, **2** was concluded to be the C-6 epimer of **4**.

Compound **3**, named vitetrifolin C, gave signals analogous to those of **1** and **2**, with additional signals due to one tri-substituted double bond group, and the loss

Table 1
^1H NMR spectral data for **1**–**5** (in $CDCl_3$, 500 MHz)[a]

H	1	2	3	4	5
1a	*ca.* 1.65	*ca.* 1.49	*ca.* 1.80	*ca.* 1.49	*ca.* 1.61
1b	*ca.* 1.57	*ca.* 1.49	*ca.* 1.59	*ca.* 1.49	*ca.* 1.51
2a	*ca.* 1.65	*ca.* 1.56	*ca.* 1.80	*ca.* 1.66	*ca.* 1.61
2b	*ca.* 1.60	*ca.* 1.49	*ca.* 1.59	*ca.* 1.49	*ca.* 1.51
3a	3.21 *dd* (4.5,11.5)	*ca.* 1.33	1.46 *ddd* (3.5,5.5,13.5)	1.32 *dt* (13.5,2.5)	*ca.* 1.27
3b		1.22 *dt* (3.5,12.5)	*ca.* 1.29	1.17 *dt* (3.0,13.5)	*ca.* 1.06
5	1.20 *dd* (6.5,12.0)	1.94 *d* (11.5)		1.66 *d* (2.5)	2.81 *s*
6a	*ca.* 1.71	5.09 *dt* (5.0,11.5)	5.60 *d* (3.5)	5.39 *dt* (2.5,3.0)	
6b	*ca.* 1.49				
7a	1.78 *ddt* (5.0,12.0,3.5)	1.78 *dt* (11.5,5.0)	5.14 *dd* (3.5,9.5)	1.66 *dt* (3.0,13.5)	2.40 *t* (13.0)
7b	*ca.* 1.14	1.42 *q* (11.5)		1.54 *dt* (13.5,3.0)	2.06 *dd* (5.0,13.0)
8	1.94 *tt* (5.0,12.0)	1.99 *m*	2.19 *dq* (9.5,6.5)	2.12 *m*	2.18 *ddq* (5.0,13.0,6.5)
11a	3.32 *d* (3.0)	1.86 *ddd* (6.0,11.5,14.5)	1.93 *ddd* (5.5,12.0,14.5)	1.92 *ddd* (6.0,11.0,14.5)	1.96 *ddd* (6.0,11.0,14.5)
11b		1.70 *ddd* (6.0,11.5,14.5)	1.73 *ddd* (5.5,12.0,14.5)	1.77 *ddd* (6.0,11.0,14.5)	1.78 *ddd* (6.0,11.0,14.5)
12a	3.01 *d* (3.0)	2.49 *ddd* (6.0,11.5,14.5)	2.59 *ddd* (5.5,12.0,14.5)	2.54 *ddd* (6.0,11.0,14.5)	2.55 *ddd* (6.0,11.0,14.5)
12b		2.44 *ddd* (6.0,11.5,14.5)	2.52 *ddd* (5.5,12.0,14.5)	2.49 *ddd* (6.0,11.0,14.5)	2.51 *ddd* (6.0,11.0,14.5)
14a	*ca.* 1.67	6.27 *d* (1.5)	6.28 *s*	6.28 *d* (1.5)	6.29 *d* (1.5)
14b	1.21 *t* (12.0)				
15	*ca.* 1.47	7.35 *t* (1.5)	7.35 *t* (1.5)	7.35 *t* (1.5)	7.37 *t* (1.5)
16	0.96 *d* (6.5)	7.22 *t* (1.5)	7.22 *br s*	7.23 *dt* (1.5,1.0)	7.25 *d* (1.5)
17	0.90 *d* (6.5)	0.92 *d* (6.5)	1.07 *d* (6.5)	0.94 *d* (7.5)	1.01 *d* (6.5)
18	0.99 *s*	1.04 *s*	1.11 *s*	0.96 *s*	0.98 *s*
19	0.79 *s*	0.90 *s*	1.16 *s*	1.00 *s*	1.24 *s*
20	1.08 *s*	1.02 *s*	1.27 *s*	1.26 *s*	0.92 *s*
2′		2.03 *s*	2.10 *s*	2.05 *s*	

[a] Coupling constants (*J*) in Hz are given in parentheses. All assignments are based on ^1H–^1H COSY.

Table 2
^{13}C NMR spectral data for **1**–**5** (in $CDCl_3$)[a]

C	1[b]	2[c]	3[b]	4[b]	5[b]
1	28.9	32.8	30.6	33.6	31.6
2	26.5	18.4	17.9	18.6	18.2
3	77.9	43.0	39.7	43.6	42.3
4	39.0	33.1	35.8	33.9	32.1
5	49.8	48.2	153.9	47.4	57.9
6	21.2	72.3	118.6	70.3	211.9
7	32.6	37.1	75.1	36.1	48.0
8	31.7	35.2	38.3	31.8	38.8
9	67.7	76.4	78.3	76.9	76.6
10	37.3	44.9	45.2	43.6	48.2
11	49.6	34.9	33.8	34.8	34.3
12	54.5	21.5	21.0	21.4	21.5
13	59.0	125.3	125.6	125.4	125.0
14	29.0	110.8	110.9	110.8	110.7
15	34.4	142.9	142.8	142.9	143.1
16	18.0	138.5	138.5	138.4	138.6
17	17.1	15.7	13.0	16.0	15.9
18	27.6	36.5	32.5	33.6	33.0
19	15.3	22.8	31.0	23.7	21.9
20	19.5	17.7	25.2	19.0	18.1
1′		170.5	171.4	170.5	
2′		22.0	21.4	21.9	

[a] δ in ppm from TMS.
[b] At 125 MHz.
[c] At 100 MHz.

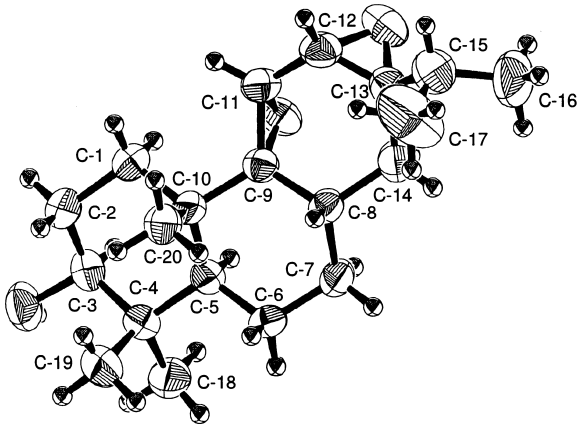

Fig. 1. ORTEP drawing of **1**.

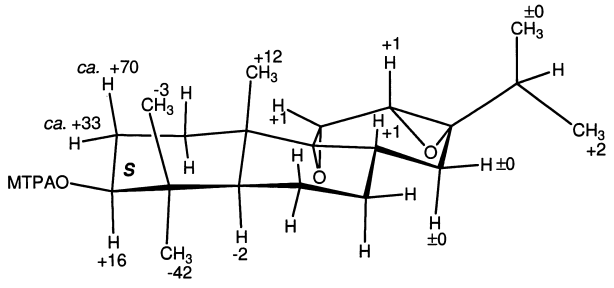

Fig. 2. ^{1}H NMR chemical shift difference values (Hz) for the MTPA esters of **1** [$\Delta\delta$: $\delta(+)$-MTPA (**1a**)− $\delta(-)$-MTPA (**1b**)].

of signals due to one methine group and one methylene group in the ^{1}H and ^{13}C NMR spectra. ^{1}H and ^{13}C NMR spectral assignments were made with the aid of ^{1}H–^{1}H COSY and HMQC spectra as shown in Tables 1 and 2, and the planar structure of **3** was defined. The stereostructure of **3** was characterized on the basis of difference NOE spectra, in which correlations were observed as shown in Fig. 3, and by analysis of the coupling constants of the signals due to H-6 (*d*, $J=3.5$ Hz), H-7 (*dd*, $J=3.5$, 9.5 Hz) and H-8 (*dq*, $J=9.5$, 6.5 Hz) in the ^{1}H NMR spectrum. The structure of vitetrifolin C was thus determined to be **3**.

Although the absolute configurations for **2** and **3** have not been confirmed, it is presumably the same as that of **1**, based on biogenetic considerations.

To the best of our knowledge, **1–3** are novel diterpenoids. Furthermore, the isolation of **5** as a natural product and isolation of **4** and **6**, previously isolated from the fruits of *V. rotundifolia*. L. f. (Asaka et al., 1973; Ono et al., 1999), are reported here for the first time from *V. trifolia* L.

3. Experimental

3.1. General

^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz and 100 MHz; NOE: 400 MHz and 500 MHz; HMQC: 500 MHz; HMBC: 500 MHz (TMS as int. standard). CC: silica gel 60 (230–400 mesh, Merck). HPLC: YMC pack SIL-06 (250 mm × 20 mm i.d., YMC Co., Ltd.).

3.2. Plant material

Fruits of *V. trifolia* L. were collected in July 1998 at the Medical Plant Garden of Kumamoto University, Kumamoto prefecture, Japan. A voucher specimen is

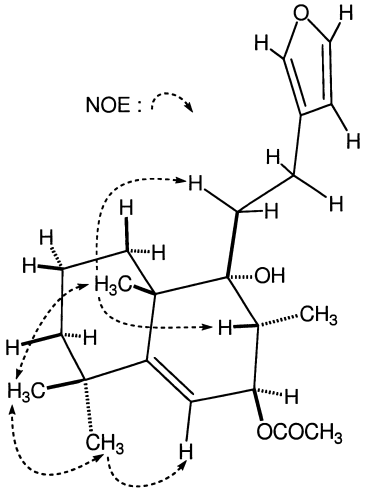

Fig. 3. NOEs observed for **3** in the difference NOE spectra.

deposited in the Laboratory of Chemistry, Research Institute of General Education, Kyushu Tokai University.

3.3. Extraction and isolation

Fruits of *V. trifolia* L. (1996 g) were 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 hexane–soluble fr. was subjected to silica gel chromatography, elution with [hexane–aceone (1:0, 20:1, 10:1, 5:1, 3:1, 2:1, 1:1, 0:1), MeOH] to give 21 frs. Fr. 11 (692 mg) and fr. 12 (99 mg) were each subjected to HPLC (hexane–acetone, 7:1) to yield **2** (31 mg), **3** (9 mg), **4** (281 mg) and **5** (9 mg) from fr. 11, and **6** (8 mg) from fr. 12. Similar HPLC (hexane–acetone, 4:1) of fr. 17 (339 mg) as used for fr. 11 gave **1** (8 mg).

Vitetrifolin A (**1**). Colorless needles (hexane–AcOEt), mp 174–175°C, $[\alpha]_D^{19}$ −11.7° (acetone; *c* 0.9); HR FABMS (positive) *m/z* 343.2249 $[M+Na]^+$(calcd for $C_{20}H_{32}O_3Na$: 343.2249); EIMS *m/z* 320 $[M]^+$, 302 $[M-H_2O]^+$; ^1H NMR spectral data: Table 1; ^{13}C NMR spectral data: Table 2.

Vitetrifolin B (**2**). Colorless syrup, $[\alpha]_D^{29}$ −56.3° (acetone; *c* 1.4); HR FABMS (positive) *m/z* 385.2350 $[M+Na]^+$(calcd for $C_{22}H_{34}O_4Na$: 385.2355); EIMS *m/z* 302 $[M-CH_3COOH]^+$; ^1H NMR spectral data: Table 1; ^{13}C NMR spectral data: Table 2.

Vitetrifolin C (**3**). Colorless syrup, $[\alpha]_D^{19}$ +93.4° (acetone; *c* 1.0); EIMS *m/z* $[M]^+$ absent, 252, 173; ^1H NMR spectral data: Table 1; ^{13}C NMR spectral data: Table 2.

3.4. Preparation of (+)-MTPA ester (*1a*) and (−)-MTPA ester (*1b*) of *1*

(+)-MTPA chloride (10 mg) or (−)-MTPA chloride (10 mg) was added to a solution of **1** (1 mg) in pyridine (0.2 ml), and the mixture was left to stand at room temperature for 1 h. The solvent was removed under an N_2 stream to give a residue, which was purified by chromatography over silica gel (hexane–AcOEt, 1:0, 10:1, 8:1, 4:1, 3:1) to furnish the corresponding ester.

1a. ^1H NMR spectral data (in $CDCl_3$, 500 MHz) δ:4.69 (*dd*, *J* = 4.5, 12.0 Hz, H-3), 3.56 (*s*, OCH_3), 3.32 (*d*, *J* = 3.0 Hz, H-11), 3.01 (*d*, *J* = 3.0 Hz, H-12), 1.94 (*tt*, *J* = 5.0, 12.0 Hz, H-8), 1.87 (*dq*, *J* = 12.0, 4.5 Hz, H_α-2), 1.79 (*m*, H-7), *ca*. 1.73 (H_β-2), 1.66 (*dd*, *J* = 5.0, 14.5 Hz, H_β-14), 1.31 (*dd*, *J* = 2.5, 12.5 Hz, H-5), 1.22 (*dd*, *J* = 12.0, 14.5 Hz, H_α-14), 1.11 (*s*, H_3-20), 0.96 (*d*, *J* = 6.5 Hz, H_3-17), 0.90 (*d*, *J* = 6.5 Hz, H_3-16), 0.82 (*s*, H_3-19), 0.82 (*s*, H_3-18).

1b. ^1H NMR spectral data (in $CDCl_3$, 500 MHz) δ: 4.66 (*dd*, *J* = 5.0, 11.0 Hz, H-3), 3.51 (*d* like, *J* = 1.0 Hz, OCH_3), 3.32 (*d*, *J* = 3.0 Hz, H-11), 3.01 (*d*, *J* = 3.0 Hz, H-12), 1.94 (*tt*, *J* = 5.0, 12.0 Hz, H-8), *ca*. 1.81 (H_α-2), *ca*. 1.79 (H-7), 1.66 (*dd*, *J* = 5.0, 14.5 Hz, H_β-14), *ca*. 1.60 (H-6), *ca*. 1.59 (H_β-2), *ca*. 1.48 (H-15), *ca*. 1.48 (H-6), 1.32 (*dd*, *J* = 2.5, 12.0 Hz, H-5), 1.22 (*dd*, *J* = 12.0, 14.5 Hz, H_α-14), 1.09 (*s*, H_3-20), 0.96 (*d*, *J* = 6.5 Hz, H_3-17), 0.91 (*s*, H_3-18), 0.90 (*d*, *J* = 6.5 Hz, H_3-16), 0.82 (*s*, H_3-19).

Table 3
X-ray diffraction data of **1**

Crystal data	
Dimensions (mm)	0.30×0.10×0.30
System	Monoclinic
Space group	$P2_1$(#4)
Z value	2
a (Å)	13.457 (2)
b (Å)	10.889 (1)
c (Å)	12.956 (1)
V (Å^3)	1880.8 (3)
μ(Cu K_α) (cm^{-1})	6.15
*D*calc(gcm^{-3})	1.163
F(000)	724.00
Refinement	
Total reflections	3112
Observed reflections	2743[I > 3.0σ(I)]
R	0.053
R_w	0.079

3.5. X-ray structure analysis of *1*

The reflection data were collected on a Rigaku AEC7R diffractometer using graphite-monochromated CuK_α radiation (λ = 1.54178 Å) with the $\omega - 2\theta$ scan technique to a maximum 2θ value of 120.1°C at room temperature (23±1°C). The structure was solved by the direct method using MITHRIL90 (Gilmore, 1990) and expanded using Fourier techniques (Beurskens et al., 1994). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. Experimental data are shown in Table 3.

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