Two New Iridoids from Viticis trifoliae Fructus (Fruit of *Vitex rotundifolia* L.)

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Two new iridoids, called viteoids I and II, were isolated from Viticis trifoliae Fructus (fruit of *Vitex rotundifolia* L. fil.) along with six known iridoids, eucommiol, iridolactone, pedicularis-lactone, agnuside, VR-I and 1-oxo-eucommiol. The chemical structures of viteoids I and II were determined on the bases of spectroscopic data.

Key words *Vitex rotundifolia* L.; iridoid; viteoid; Verbenaceae; Viticis trifoliae Fructus

Vitex rotundifolia L. fil. (Verbenaceae) is widely distributed in Asia, and its fruit (Viticis trifoliae Fructus) is used in folk medicine for headaches.[1] The presence of diterpenes,[2–5] flavones,[5–8] lignan,[9] phenylpropanoides,[8,9] 3,4-dihydroxybenzoic acid[8] and iridoids[7,9] in this fruit have been reported.

In the course of our studies on natural antioxidants,[10] the MeOH extract of this fruit showed stronger antioxidative activity than *tert*-butylhydroxyanisol (BHA), which is a synthetic antioxidant, using the ferric thiocyanate method.[11] Therefore, we reexamined the components to determine the compounds responsible for the antioxidative effect.

The powdered fruit was extracted with MeOH at room temperature, and the MeOH extract was defatted with hexane. The residue, which showed a stronger antioxidative effect than the hexane-soluble fraction, was subjected successively to Diaion HP20, Sephadex LH20 and silica-gel column chromatography, and HPLC on octadecyl silica (ODS) to give two new iridoids (**7**, **8**) along with six known iridoids (**1**—**6**).

Compounds **1**, **2**, **3**, **4** and **5** were identified as eucommiol,[12] iridolactone,[13] pedicularis-lactone,[14] agnuside[7,15] and VR-I (10-*o*-vanilloyl aucubin),[9] respectively, using physical data and spectral data.

Compound **7**, called viteoid I, was obtained as a colorless oily substance, and it exhibited $[M+Na]^+$ and $[M+H]^+$ ion peaks at *m/z* 207 and 185, respectively, in the positive FAB-MS; the high-resolution (HR) positive FAB-MS indicated the molecular formula of **7** to be $C_9H_{12}O_4$. The infra-red (IR) spectrum of **7** showed strong absorptions at 3371 and 1740 cm^{-1} due to hydroxyl groups and an α,β-unsaturated lactone group, respectively. The ^{13}C-NMR spectrum showed signals for one carbonyl carbon (δ 172.7), two quaternary sp^2 carbons (δ 174.3, 137.2), two methine carbons (δ 84.1, 47.5) and four methylene carbons (δ 71.2, 61.4, 38.7, 35.7). The ^1H-NMR spectrum, which was similar to those of **1**—**3**, exhibited signals for four oxymethylene protons (δ 4.88 (2H), 3.73 (2H)), two methylene protons (δ 1.82, 1.75), one oxymethine proton (δ 4.55) and one methine proton (δ 2.76). These ^1H- and ^{13}C-NMR signals could be assigned with the aid of ^1H–^1H shift correlated 2D-NMR (COSY) and ^1H–^{13}C heteronuclear shift correlated 2D-NMR (HETCOR) spectra as

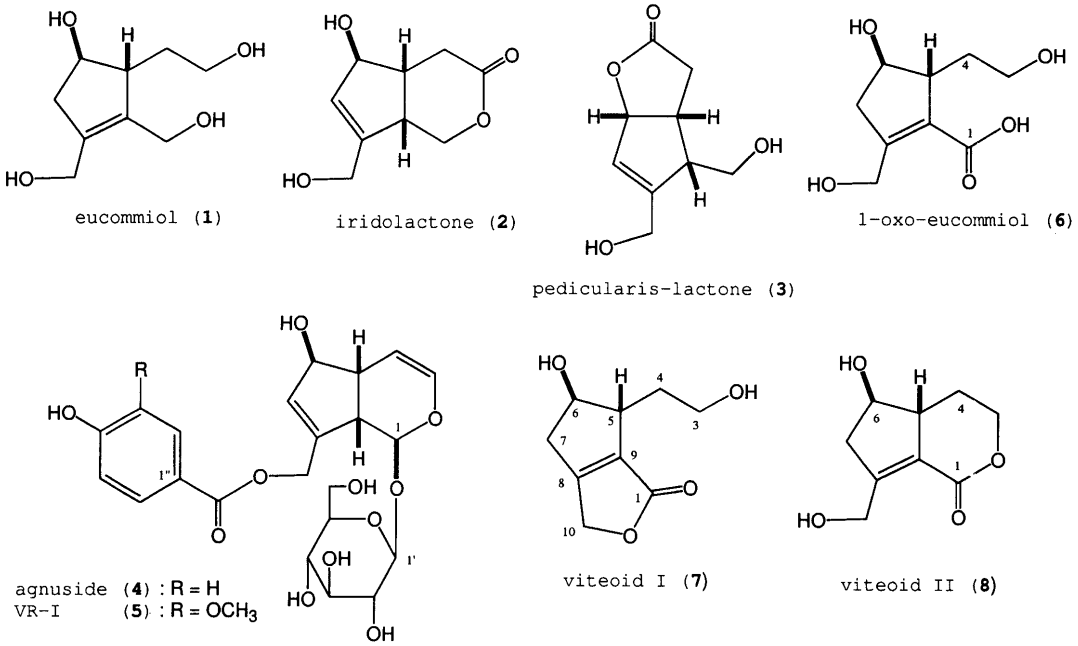

Fig. 1. Structures of **1**—**8**

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Table 1. ^{13}C-NMR Data for **6**—**8** (in CD_3OD)

	6[a]	**7**[b]	**8**[a]
C-1	167.4	172.7	165.9
C-3	60.6[c]	61.4	70.9
C-4	35.3	35.7	29.1
C-5	54.5	47.5	51.1
C-6	75.0	84.1	79.2
C-7	44.0	38.7	42.2
C-8	158.4	174.3	161.7
C-9	130.0	137.2	123.6
C-10	61.5[c]	71.2	60.4

δ in ppm from TMS. *a*) 125 MHz. *b*) 100 MHz. *c*) Assignments may be interchanged.

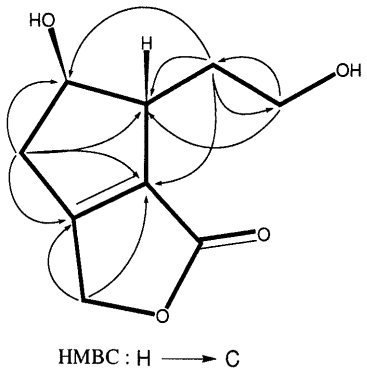

Fig. 2. HMBC Correlations of **7**

shown in Table 1. From these data, **7** was suggested to be an iridoid derivative, having an α,β-unsaturated lactone structure.

The connectivities of the quaternary carbons were determined from the ^{1}H-detected heteronuclear multiple-bond multiple-quantum coherence (HMBC) spectrum. In this spectrum, the correlations were as shown in Fig. 2.

The stereostructure of **7** was characterized on the basis of the difference nuclear Overhauser effect (NOE) experiment and the coupling constant values of the H-6 signal in the ^{1}H-NMR spectrum. In the NOE spectrum of **7**, irradiation of the signal of H-6 gave enhancements of the signals of H_2-4. Moreover, the stable conformation of **7** with minimum steric energy was simulated using CAChe,[16] and is illustrated in Fig. 3. The coupling constant values (J=4.7, 4.7, 7.7 Hz), calculated from the dihedral angles for H-6 of this conformation using a Karplus equation,[17] were approximately coincident with those (J=3.7, 3.7, 6.7 Hz) in the ^{1}H-NMR spectrum. Consequently, the relative stereostructure of **7** was concluded to be as shown in Fig. 1.

Compound **6** was obtained as a colorless oily substance, and **8**, named viteoid II, was also obtained as a colorless oily substance. Both compounds could be interconverted in aqueous methanol at room temperature, indicating the occurrence of an equilibrium state. Compound **8** exhibited an $[M+H]^+$ ion peak at m/z 185 in the positive FAB-MS; the HR positive FAB-MS indicated the molecular formula of **8** to be $C_9H_{12}O_4$. The respective ^{13}C-NMR spectra of **6** and **8** showed signals for one carbonyl carbon, two quaternary sp^2 carbons, two methine carbons and four methylene carbons as shown in Table

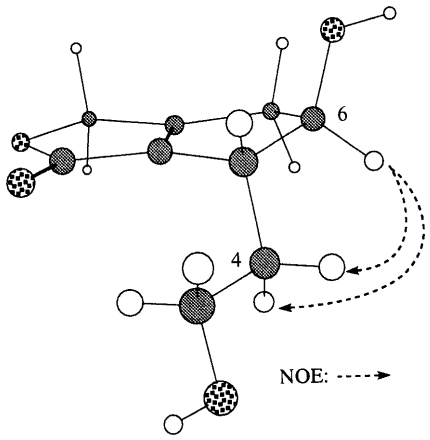

Fig. 3. CAChe Drawings of **7** and NOE Correlations

1. From these data, **6** and **8** were also assumed to be iridoid derivatives. The ^{1}H-NMR spectra of **6** and **8** were assigned on the basis of COSY spectra. When comparing the chemical shifts between **6** and **8**, marked downfield shifts were observed by *ca.* 0.81 and 0.69 ppm at H_2-3 signals of **8**. This suggested that **8** formed a δ-lactone ring between the hydroxyl group at C-3 and the carboxylic group at C-1, while **6** took an open ring form. The difference NOE spectra of **6** and **8** showed NOEs between the following sets of protons in **6** [H_b-4→H-6; H-6→H_b-4] and in **8** [H_α-4→H-6; H_β-4→H-5; H-6→H_α-4]. Accordingly, the relative stereostructure of **6** and viteolides II (**8**) was concluded to be as shown in Fig. 1, and **6** was identical with the compound which was very recently isolated from *Crescentia cujete* by Kaneko *et al.*[18]

As far as we know, viteolides I (**7**) and II (**8**) are novel iridoids, but **7** and **8** might be artifacts produced from **6** during the extraction and/or isolation procedures, and **1**, **2**, **3** and **6** are the first examples of isolation from the fruit of *Vitex rotundifolia* L. fil. Compound **5** showed stronger antioxidative activity than BHA using the ferric thiocyanate method.[11]

Further investigations of the constituents of this fruit are in progress.

Experimental

All instruments and materials used were the same as cited in the preceding report[19] unless otherwise specified.

Isolation of 1—8 Powdered fruit of *Vitex rotundifolia* L. fil. (2914 g) purchased from Uchida Wakanyaku Co., Ltd. was extracted with MeOH (4 l × 2) at room temperature and the solvent was removed under reduced pressure to afford a brown syrup (185.2 g). This syrup was partitioned between hexane (1110 ml) and MeOH (800 ml). Concentration of the hexane layer furnished fraction (fr.) 1 (17.7 g). The MeOH layer was filtered through absorbent cotton and the filtrate was evaporated to give fr. 2 (158 g). Fraction 2 was chromatographed over Diaion HP 20 (40% MeOH→70% MeOH→90% MeOH→MeOH→acetone) to afford fr. 3 (48.6 g), fr. 4 (12.4 g), fr. 5 (20.3 g), fr. 6 (15.8 g) and fr. 7 (17.3 g). Chromatography of fr. 3 over silica-gel [Merck Art. 7734 ($CHCl_3$–MeOH–H_2O, 10:2:0.1→8:2:0.2→7:3:0.5→6:4:1→4:6:1→MeOH)] furnished fr. 8 (273 mg), fr. 9 (177 mg), fr. 10 (142 mg), fr. 11 (671 mg), fr. 12 (1877 mg), fr. 13 (4347 mg), fr. 14 (1917 mg) and fr. 15 (45.52 g). Fraction 11 was chromatographed over silica-gel [Merck, Art. 9385 ($CHCl_3$–MeOH–H_2O, 14:2:0.1→10:2:0.1→8:2:0.2→7:3:0.5)] to give fr. 16 (19 mg), fr. 17 (67 mg), fr. 18 (117 mg), fr. 19 (58 mg), fr. 20 (82 mg), fr. 21 (80 mg), fr. 22 (94 mg) and fr. 23 (142 mg). HPLC (YMC-pack S-5 120A ODS, 20 mm i.d. × 250 mm, 10% MeOH) of fr. 17 gave **7** (12 mg) and **8** (3 mg). Fraction 18 and fr. 20 were each subjected

to HPLC under the same conditions as for fr. 17 to give **3** (5 mg) and **6** (17 mg) from fr. 18, and **2** (19 mg) from fr. 20. Fraction 12 was chromatographed over silica-gel [Merck, Art. 9385 ($CHCl_3$–MeOH–H_2O, 10:2:0.1→8:2:0.2→7:3:0.5)] to give fr. 24 (128 mg), fr. 25 (43 mg), fr. 26 (92 mg), fr. 27 (140 mg), fr. 28 (562 mg) and fr. 29 (192 mg). HPLC (YMC-pack S-5 120A ODS, 20 mm i.d. × 250 mm, 10% MeOH) of fr. 27 gave **1** (9 mg). Fraction 4 was chromatographed over Sephadex LH 20 (60% MeOH) to give fr. 30 (0.2 g), fr. 31 (9.2 g), fr. 32 (0.4 g), fr. 33 (0.4 g), fr. 34 (0.2 g) and fr. 35 (1.2 g). Fraction 31 was chromatographed over silica-gel [Merck, Art. 9385 ($CHCl_3$–MeOH, 40:1→30:1→20:1→10:1)] furnished fr. 36 (538 mg), fr. 37 (242 mg), fr. 38 (17 mg), fr. 39 (1366 mg), fr. 40 (199 mg), fr. 41 (375 mg), fr. 42 (1033 mg), fr. 43 (860 mg) and fr. 44 (1195 mg). Similar HPLC (60% MeOH) of fr. 42 as for fr. 17 gave fr. 45 (301 mg), fr. 46 (111 mg), fr. 47 (66 mg), fr. 48 (150 mg), fr. 49 (83 mg) and fr. 50 (7 mg). Fraction 45 was subjected to HPLC [Kusano C.I.G. prepacked column Si-10, 22 mm i.d. × 100 mm ($CHCl_3$–MeOH–H_2O, 14:2:0.1)] to give **4** (63 mg) and **5** (24 mg).

1: A colorless oil, $[\alpha]_D^{28}$ −37.7° (c= 0.9, MeOH). Positive FAB-MS m/z: 189 $[M+H]^+$.

2: A colorless oil, $[\alpha]_D^{28}$ −15.4° (c= 2.0, MeOH). Positive FAB-MS m/z (%): 207 (15) $[M+Na]^+$, 185 (100) $[M+H]^+$.

3: A white powder, $[\alpha]_D^{28}$ +7.5° (c= 0.6, MeOH). Positive FAB-MS m/z (%): 207 (100) $[M+Na]^+$, 185 (41) $[M+H]^+$.

4: A white powder, $[\alpha]_D^{25}$ −94.4° (c=1.1, MeOH). Positive FAB-MS m/z (%): 449 (57) $[M+H-18(H_2O)]^+$, 287 (100) $[449-162\ (\text{hexose})]^+$.

5: A white powder, $[\alpha]_D^{25}$ −66.1° (c=4.3, MeOH). Positive FAB-MS m/z: 479 $[M+H-18(H_2O)]^+$. ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 7.60 (1H, dd, J=1.8, 8.5 Hz, H-6″), 7.58 (1H, d, J=1.8 Hz, H-2″), 6.86 (1H, d, J=8.5 Hz, H-5″), 6.34 (1H, dd, J=1.8, 6.1 Hz, H-3), 5.84 (1H, s, H-7), 5.11 (1H, dd, J=4.3, 6.1 Hz, H-4), 5.09 (1H, d, J=15.3 Hz, H_a-10), 5.03 (1H, d, J=7.3 Hz, H-1), 4.93 (1H, d, J=15.3 Hz, H_b-10), 4.70 (1H, d, J=7.9 Hz, H-1′), 4.47 (1H, br d like, J=5.5 Hz, H-6), 3.90 (3H, s, OCH_3), 3.84 (1H, dd, J=1.8, 11.6 Hz, H_a-6′), 3.66 (1H, dd, J=6.1, 11.6 Hz, H_b-6′), 3.39 (1H, dd, J=8.7, 8.7 Hz, H-3′), *ca.* 3.31 (2H, H-4′ and H-5′), 3.24 (1H, dd, J=7.9, 8.5 Hz, H-2′), 3.01(1H, t like, J=7.3 Hz, H-9), 2.71 (1H, m, H-5). ^{13}C-NMR (in CD_3OD, 100 MHz) δ: 167.8 (C=O), 153.0 (C-3″), 148.8 (C-4″), 142.9 (C-8), 141.7 (C-3), 132.5 (C-7), 125.2 (C-6″), 122.4 (C-1″), 116.1 (C-5″), 113.6 (C-2″), 105.5 (C-4), 100.2 (C-1′), 97.8 (C-1), 82.8 (C-6), 78.2, 77.9 (C-3′, C-5′), 74.9 (C-2′), 71.5 (C-4′), 63.8 (C-10), 62.7 (C-6′), 56.4 (OCH_3), 48.6 (C-9), 46.1 (C-5).

6: A colorless oil, $[\alpha]_D^{28}$ −23.4° (c= 0.7, MeOH). ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 4.60 (1H, ddd, J=1.5, 3.0, 15.3 Hz, Ha-10), 4.56 (1H, ddd, J=1.5, 3.0, 15.3 Hz, H_b-10), 4.12 (1H, ddd, J=1.5, 1.5, 5.5 Hz, H-6), *ca.* 3.62 (2H, H_2-3), 2.99 (1H, m, H_a-7), 2.91 (1H, m, H-5), 2.58 (1H, d, J=18.9 Hz, H_b-7), 1.83 (1H, dddd, J=3.7, 6.7, 7.3, 13.7 Hz, H_a-4), 1.49 (1H, dddd, J=6.1, 7.3, 9.2, 13.7 Hz, H_b-4). ^{13}C-NMR δ: see Table 1.

7: A colorless oil, $[\alpha]_D^{27}$ −30.4° (c= 1.3, MeOH). HR positive FAB-MS m/z: 185.0816 $[M+H]^+$ (Calcd for $C_9H_{13}O_4$: 185.0814). Positive FAB-MS m/z (%): 207 (40) $[M+Na]^+$, 185 (100) $[M+H]^+$. IR (KBr) cm^{-1}: 3371 (OH), 1740 (C=O). ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 4.88 (2H, d like, J=1.2 Hz, H_2-10), 4.55 (1H, ddd, J=3.7, 3.7, 6.7 Hz, H-6), 3.73 (2H, m, H_2-3), 3.05 (1H, m, H_a-7), 2.76 (1H, br s, H-5), 2.52 (1H, m, H_b-7), 1.82 (1H, dddd, J=6.7, 6.7, 6.7, 14.0 Hz, H_a-4), 1.75 (1H, dddd, J=6.7, 6.7, 6.7, 14.0 Hz, H_b-4). ^{13}C-NMR δ: see Table 1.

8: A colorless oil, $[\alpha]_D^{28}$ −68.1° (c=0.4, MeOH). HR positive FAB-MS m/z: 185.0808 $[M+H]^+$ (Calcd for $C_9H_{13}O_4$: 185.0814). Positive FAB-MS m/z (%): 207 (32) $[M+Na]^+$, 185 (100) $[M+H]^+$. ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 4.64 (1H, ddd, J=1.2, 2.4, 16.5 Hz, H_a-10), 4.54 (1H, ddd, J=1.2, 2.4, 16.5 Hz, H_b-10), 4.43 (1H, ddd, J=2.4, 4.9, 11.6 Hz, H_α-3), 4.31 (1H, ddd, J=3.1, 11.6, 11.6 Hz, H_β-3), 4.09 (1H, ddd, J=7.3, 7.3, 7.6 Hz, H-6), 2.95 (1H, m, H_a-7), 2.86 (1H, m, H-5), 2.49 (1H, m, H_b-7), 2.23 (1H, dddd, J=2.4, 3.1, 4.8, 13.4 Hz, H_β-4), 1.65 (1H, dddd, J=4.9, 11.6, 11.6, 13.4 Hz, H_α-4). ^{13}C-NMR δ: see Table 1.

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