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Studies on the Constituents of *Vitex rotundifolia* L. fil.

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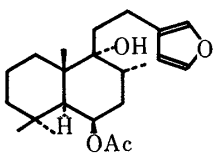
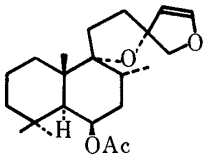
Two labdane-diterpenes, vitexilactone and previtexilactone, instead of the previously described rotundifuran and prerotundifuran, have been isolated from the fruits of *Vitex rotundifolia* L. fil. (Verbenaceae) together with *p*-hydroxybenzoic acid, vanillic acid and three flavones.

Keywords——*Vitex rotundifolia*; *V. trifolia* var. *ovata*; Verbenaceae; *p*-hydroxybenzoic acid; vanillic acid; vitexicarpin; luteolin; artemetin; vitexilactone; previtexilactone

Vitex rotundifolia L. fil. (= *V. trifolia* L. var. *ovata* MAKINO) is a small wild shrub of the family Verbenaceae, which is widely distributed in Asia. The fruits of this plant have been used in the treatment of headaches. The presence of the diterpenes, rotundifuran and prerotundifuran, and the flavone vitexicarpin (=casticin) in the fruits has been recorded.[1-4] We wish to report the isolation of phenolic acid, flavones, and two diterpenes, vitexilactone and previtexilactone, from the fruits of *V. rotundifolia*.



rotundifuran

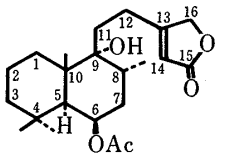


prerotundifuran

Hexane followed by methanol extraction of the dried fruits furnished a brown resinous material. Fractionation of the methanolic extract by silica gel column chromatography afforded five crystalline substances, **1**—**5**.

Substance **1** was identified as vitexicarpin (=casticin) by direct comparison with an authentic specimen.[2]

Substance **2** was isolated as colorless needles, mp 144—146 °C, $[\alpha]_D$ −12.4° ($CHCl_3$). Field desorption mass spectrometry (FD-MS) and elemental analysis of **2** established the molecular formula as $C_{22}H_{34}O_5$. The infrared (IR) spectrum of **2** exhibited the presence of hydroxyl (3520 cm^{-1}), acetoxyl (1725 cm^{-1}), and α,β-unsaturated γ-lactone (1748, 1780 cm^{-1}) groups. The four methyl signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum were similar to the corresponding resonances in the spectrum of rotundifuran and hence a labdane skeleton was assigned to **2**. The signal at δ 5.37 (q) was assigned to a proton on a carbon bearing an acetoxyl group (δ 2.05). The absence of any resonance due to a carbinyl proton indicated the tertiary nature of the hydroxyl group. Signals at 4.75 (2H) and δ 5.83 (1H) were assigned to methylene protons and an olefinic proton of the α, β-unsaturated γ-lactone system, respectively. The signal at δ 5.83 appeared as a broad quintet, but collapsed to a triplet when the doublet at δ 4.75 was irradiated. Additional decoupling experiments provided evidence that the olefinic proton was coupled to protons of another methylene group (δ 2.50) which was itself linked to a methylene group. From these results, it was concluded that



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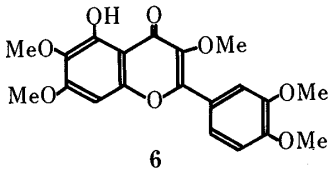

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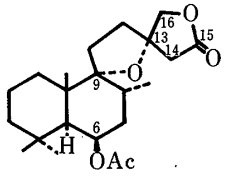


7

2 is vitexilactone. Vitexilactone has been previously isolated from *V. cannabifolia*.[5]

Substances **3** and **4** were confirmed to be *p*-hydroxybenzoic acid and vanillic acid, respectively, by direct comparison with authentic samples.

The flavone **5** was isolated as yellow needles, $C_{15}H_{10}O_6$, mp 328 °C (dec.), whose NMR spectrum exhibited six proton signals in the aromatic/alkene region; the absence of aliphatic proton(s) suggested a polyphenolic compound. Substance **5** was identified as luteolin by comparison of the ^{1}H-NMR and IR spectra with those of an authentic sample.

The hexane extract of the fruits of *V. rotundifolia* furnished, after extensive chromatography, the flavone artemetin **6**, and a diterpene lactone **7**, designated as previtexilactone.

The flavone **6** was isolated as yellow needles, $C_{20}H_{20}O_8$, mp 158 °C. Compound **6** was identified as artemetin by comparison of the ^{1}H-NMR and IR spectral data with those of an authentic sample.

Previtexilactone **7** was isolated as colorless prisms, mp 214—215 °C. Its composition was determined as $C_{22}H_{34}O_5$ on the basis of elemental and FD-MS spectral analyses. The IR spectrum of **7** indicated the presence of acetyl (1720 cm^{-1}) and γ-lactone (1780 cm^{-1}) groups; no hydroxyl band was present.

As in the case of **2**, there were signals in the ^{1}H-NMR spectrum of **7** associated with four methyl groups, an acetoxyl group (δ2.07), methylene protons of the γ-lactone (δ 4.18 and 4.36), and a proton on a carbon bearing an acetoxyl group (δ 5.36) (see Experimental). In addition, a doublet of doublets appeared at δ 2.48 and 2.92, and these signals were assigned to methylene protons adjacent to a lactone-carbonyl group. The coupling patterns of both methylene groups in the γ-lactone moiety suggested that these were germinal protons in isolated methylenes. The absence of any olefinic proton signals argued against the presence of a double bond in **7**. These observations were in full accord with the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **7** (Table I).

Thus, **7** can be regarded as a spiro-ether derivative formed by intramolecular addition of the C_9 hydroxyl group of **2** to the double bond of an α, β-unsaturated γ-lactone. Previously, **7** has been derived by alkali treatment of **2**[5] in the course of the study on the structure of **2**.

TABLE I. ^{13}C-NMR Data for Vitexilactone (**2**) and Previtexilactone (**7**)[a]

	2	**7**		**2**	**7**
C-1	18.552	18.669	C-11	43.563	36.517
C-2	25.362	29.296	C-12	33.582	37.809
C-3	31.586	34.227	C-13	170.317	93.875 (85.773)
C-4	33.993	34.227	C-14	114.955	43.915
C-5	47.673	48.788	C-15	170.965	174.722
C-6	69.689	70.276	C-16	73.094	78.376
C-7	43.739	42.858	OCOMe	170.317	170.317
C-8	32.055	31.233		23.601	23.777
C-9	76.441	85.773 (93.875)	-Me	16.086, 18.963,	17.201, 19.785,
C-10	36.048	37.809		21.899, 33.582	21.899, 33.112

a) Run at 25.1 MHz in $CDCl_3$ solution; chemical shifts are given in ppm relative to tetramethylsilane.

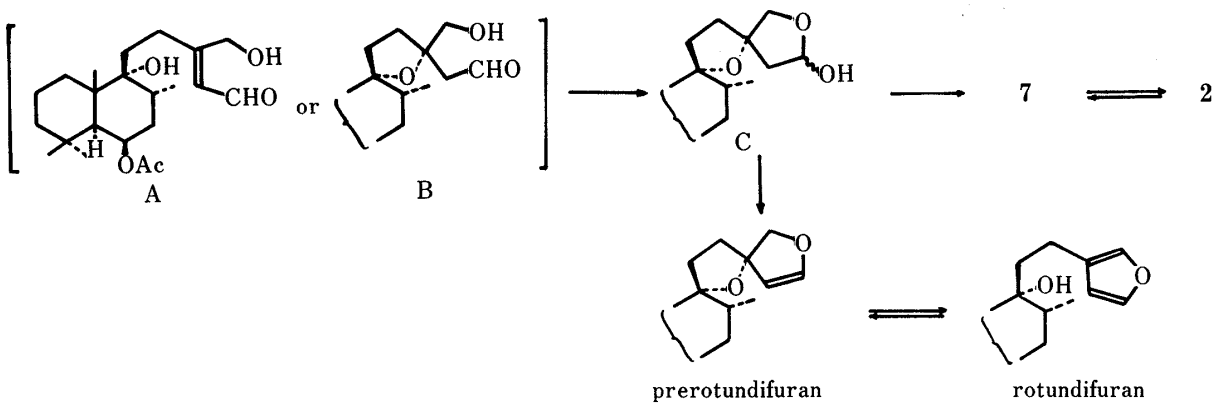


Chart 1

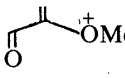
It has been demonstrated that prefuranoid diterpenes are readily converted into the respective furanoids. A postulated hemiacetal (C) should undergo elimination to the prefuranoid diterpenes. Oxidation of the hemiacetal (C) would give the γ-lactone moiety present in **7** (Chart 1).

Experimental

Melting points were taken on a Yamato MP-21 melting point apparatus and are uncorrected. NMR spectra were run on a JEOL JNM-FX-100 using tetramethylsilane as an internal standard. Chemical shifts are given in δ units relative to tetramethylsilane. IR spectra were recorded on a JASCO A-100S grating infrared spectrophotometer.

Isolation of Substances 1—5——Dried and powdered fruits of *V. rotundifolia* L. fil. were exhaustively extracted with *n*-hexane and methanol. The methanol extract was evaporated under reduced pressure. The residual crude syrup was chromatographed over a column of silica gel, which was eluted in the following order: **1**, $CHCl_3$; **2**, $CHCl_3$–MeOH (98 : 2); **3**, $CHCl_3$–MeOH (95 : 5); **4**, $CHCl_3$–MeOH (9 : 1); **5**, $CHCl_3$–MeOH (7 : 3). Fractions of about 50 ml were collected and monitored by thin-layer chromatography (TLC).

The fraction eluted with a 98 : 2 mixture of $CHCl_3$–MeOH afforded a mixture of flavonoids, which was rechromatographed on silica gel using the same solvent system to give **1**.

Vitexicarpin (=Casticin) (**1**) : **1** afforded yellow needles from MeOH, mp 188—189 °C (lit,[2] mp 189 °C). *Anal.* Calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.85. Found; C, 60.88; H, 4.79. MS *m/z* (rel intensity): 374 (100%, M^+), 359 (36%, M^+-CH_3), 84 (40%, ). The identity of this compound with an authentic sample of vitexicarpin was confirmed by comparison of the NMR and IR spectra.

Vitexilactone (**2**): The $CHCl_3$–MeOH (95 : 5) eluate was collected, and further purified on a silica gel column (solvent system: *n*-hexane–EtOAc (2 : 1)). The eluate was combined and concentrated to give vitexilactone, mp 144—146 °C, after recrystallization from *n*-hexane, $[\alpha]_D^{25}$ −12.4° (c = 1.11 in $CHCl_3$). *Anal.* Calcd for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.37; H, 9.07. EI-MS *m/z* (rel intensity): 318 (80%, $M^+-CH_3CO_2H$), 303 (36%, $M^+-CH_3CO_2H-CH_3$). FD-MS *m/z*: 378 (M^+), 318 ($M^+-CH_3CO_2H$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (–OH), 1725 (–OAc), 1748, 1780 (α,β-unsaturated γ-lactone). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (2H, d, J = 7 Hz, C_7-$\underline{H}_2$), 0.97 (3H, s, C_4-Me), 1.01 (3H, s, C_4-Me), 1.26 (3H, s, C_{10}-Me), 1.78 (2H, t, J = 9 Hz, C_{11}-$\underline{H}_2$), 2.05 (3H, s, –OCOMe), 2.15 (1H, m, C_8-$\underline{H}$), 2.50 (2H, br t, J = 8 Hz, C_{12}-$\underline{H}_2$), 4.75 (2H, d, J = 2 Hz, C_{16}-$\underline{H}_2$), 5.37 (1H, q, J = 2 Hz, C_6-$\underline{H}$), 5.83 (1H, quintet, J = 2 Hz, C_{14}-$\underline{H}$). ^{13}C-NMR: Table I.

p-Hydroxybenzoic Acid (**3**): The subsequent $CHCl_3$–MeOH (9 : 1) eluate containing *p*-hydroxybenzoic acid was combined and recrystallized from H_2O, mp 213—214 °C; the mixed melting point with an authentic sample was undepressed.

Vanillic Acid (**4**): The $CHCl_3$–MeOH (7 : 3) eluate containing vanillic acid was combined and concentrated, and the residue was recrystallized from H_2O, mp 205—206 °C. The identity of this compound with an authentic sample of vanillic acid was confirmed by comparison of the IR and NMR spectra.

Luteolin (**5**): The subsequent 7 : 3 $CHCl_3$–MeOH eluate containing luteolin was combined and concentrated. The residue was recrystallized from MeOH to give yellow needles, mp 328 °C (dec.). *Anal.* Calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.87; H, 3.49. FD-MS *m/z*: 286 (M^+). ^{1}H-NMR (CD_3OD) δ; 6.15 (1H, d, J = 2 Hz, C_6-$\underline{H}$), 6.39 (1H, d, J = 2 Hz, C_8-$\underline{H}$), 6.48 (1H, s, C_3-$\underline{H}$), 6.85 (1H, d, J = 9 Hz, $C_{5'}$-$\underline{H}$), 7.32 (1H, d, J = 2 Hz, $C_{2'}$-H), 7.33 (1H, dd, J = 9, 2 Hz, $C_{6'}$-$\underline{H}$).

Isolation of Substances 6 and 7——The *n*-hexane extract of the fruits of *V. rotundifolia* after removal of the precipitate was evaporated, and the residue was chromatographed over a silica gel column, which was eluted in the following order: 1, *n*-hexane; 2, *n*-hexane–AcOEt (9 : 1); 3, *n*-hexane–AcOEt (1 : 1); 4, AcOEt; 5, AcOEt–MeOH (9 : 1).

Previtexilactone (**7**): The *n*-hexane–AcOEt (9 : 1) eluate was evaporated, and the residue was further purified over a silica gel column with 6 : 1 *n*-hexane–AcOEt. Upon removal of the solvent, these fractions gave an oily residue, which crystallized upon addition of hexane. Recrystallization from CH_2Cl_2–*n*-hexane yielded colorless prisms, mp 214—215 °C. *Anal.* Calcd for $C_{22}H_{34}O_5 \cdot 1/4\ H_2O$: C, 68.99; H, 9.08. Found: C, 69.21; H, 9.08. EI-MS *m/z* (rel intensity): 318 (100%, $M^+ - CH_3CO_2H$), 303 (4%, $M^+ - CH_3CO_2H - CH_3$). FD-MS *m/z*: 378 (M^+), 318 ($M^+ - CH_3CO_2H$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (–OAc), 1780 (γ-lactone). ^{1}H-NMR ($CDCl_3$): 0.81 (3H, d, $J=7$ Hz, C_8-Me), 0.96 (3H, s, C_4-Me), 0.99 (3H, s, C_4-Me), 1.24 (3H, s, C_{10}-Me), 1.78 (2H, t, $J=9$ Hz, C_{11}-H_2), 2.07 (3H, s, OCOMe), 2.48 (1H, d, $J=17$ Hz, C_{14}-HH), 2.92 (1H, d, $J=17$ Hz, C_{14}-HH), 4.18 (1H, d, $J=9$ Hz, C_{16}-HH), 4.36 (1H, d, $J=9$ Hz, C_{16}-HH), 5.36 (1H, q, $J=1.5$ Hz, C_6-H). ^{13}C-NMR: Table I.

Artemetin (**6**): The subsequent 9 : 1 AcOEt–MeOH eluate, which gave a positive test for flavonoids, was collected and evaporated. The crude material was recrystallized from MeOH to give yellow needles, mp 158 °C (lit.[6] mp 158—159 °C). *Anal.* Calcd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 62.13; H, 5.08. EI-MS *m/z* (rel intensity): 388 (100%, M^+), 373 (67%, $M^+ - CH_3$). The identity of this compound with an authentic sample of artemetin was confirmed by comparison of the ^{1}H-NMR and IR spectra, and TLC behavior.

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