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Studies on the Constituents of *Vitex cannabifolia*

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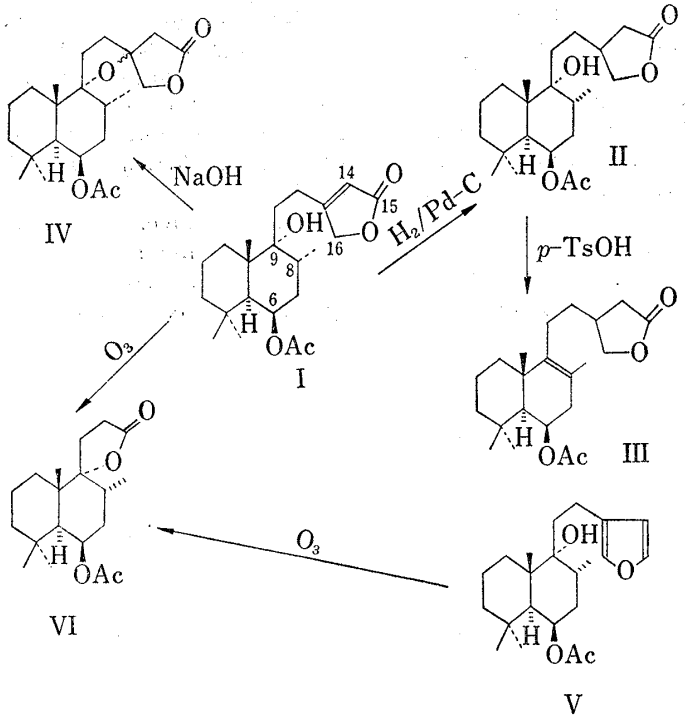
A new diterpenoid, named vitexilactone (I), was isolated together with a known iridoid glycoside, agnuside, a known flavonoid, artemetine, and *p*-hydroxybenzoic acid from the leaves of *Vitex cannabifolia* Sieb. et Zucc. (Verbenaceae). The structure of vitexilactone was established as I by the chemical and spectral examinations.

Vitex cannabifolia Sieb. et Zucc. (Verbenaceae) is a shrub growing in China and cultivated in Japan. The fruits are used as an antiinflammatory under the name of Bokeishi.

This paper deals with the structure of a new diterpenoid, named vitexilactone (I), isolated from the leaves as well as a known iridoid glycoside, agnuside,[2] a known flavonoid, artemetine,[3] and *p*-hydroxybenzoic acid.

Vitexilactone (I) $C_{22}H_{34}O_5$, mp 150—151°, $[\alpha]_D$ −12.6° ($CHCl_3$) has an absorption maximum at 210 nm (log ε 4.38) in the ultraviolet (UV) spectrum and the bands at 3430, 1780, 1745, 1705 and 1635 cm^{-1} in the infrared (IR) spectrum, showing the presence of a hydroxy group and α,β-unsaturated γ-lactone. I was not acetylated by acetic anhydride and pyridine, indicating that the hydroxy group has a tertiary nature. The nuclear magnetic resonance (NMR) spectrum (δ in $CDCl_3$) of I shows the presence of a secondary methyl group [0.98 (3H, d, J=6 Hz)], a secondary acetoxy group [2.05 (3H, s) and 5.38 (1H, q, J=2.5 Hz)] and three tertiary methyl groups [0.97, 1.05 and 1.23 (each 3H, s)]. The signals at δ 4.79 (2H, d, J=1 Hz) and 5.85 (1H, t, J=1 Hz) are due to the protons of the β-substituted $\Delta^{\alpha,\beta}$-butenolide.[4]



Catalytic hydrogenation of I with palladized charcoal in methanol afforded a dihydroderivative (II) $C_{22}H_{36}O_5$, mp 104—105.5°, IR (in KBr), 1773 and 1720 cm^{-1}.

Treatment of II with *p*-toluenesulfonic acid in methanol gave a olefinic compound (III) $C_{22}H_{32}O_4$.

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2) *a*) E. Winde and Hänsel, *Arch. Pharm.* **293**, 556 (1960); *b*) R. Hänsal, C.H. Leuckert, H. Rimpler, and K.D. Schaaf, *Phytochemistry* **4**, 19 (1965).
3) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer Verlag, Berlin. Heidelberg. New-York, 1970, p. 308.
4) C.A. Henrick and P.R. Jefferies, *Tetrahedron*, **21**, 3219 (1965).

The NMR spectrum of III shows an olefinic methyl group at δ 1.60 (3H, s) instead of the secondary methyl group, indicating the vicinal nature of the hydroxy group and the secondary methyl group. Furthermore, the treatment of I with 1% sodium hydroxide in aqueous methanol gave a crystalline substance (IV) $C_{22}H_{34}O_5$, mp 216—218°. The signals at δ 2.69 (center, 2H, q, J=17 Hz) and 4.23 (center, 2H, q, J=9 Hz) in the NMR spectrum of IV indicate the formation of a spiro ether.[5]

Finally, the structure of I was established by the chemical correlation with rotundifuran (V), isolated from *Vitex rotundifolia* L..[6] Ozonolysis of I afforded a γ-lactone (VI) $C_{19}H_{30}O_4$, mp 128—130°, $[\alpha]_D$ −24.4° (EtOH), IR (in KBr), 1765 and 1725 cm^{-1}, which was identified with VI obtained from rotundifuran (V) by the same procedure. Consequently, the structure including the absolute configuration of vitexilactone was formulated as I.

Experimental

All melting points were determined on a Yanagimoto Micro Melting Point Apparatus and uncorrected. IR spectra were measured with a Hitachi Model EPI-G2. NMR spectra were measured with a Varian Model T-60 NMR Spectrometer. Specific rotations were measured with a JASCO Model DIP-SL.

Isolation of Vitexilactone (I)——550 g of the dried leaves of *Vitex cannabifolia* collected from Tsumura botanical garden were extracted with $CHCl_3$ under reflux for three times. The combined extracts were concentrated and chromatographed on silica gel (150 g) using benzene (2 liters) and benzene–ether (9: 1). The fractions (fr.) No. 16—32 (each fr. 250 ml) eluted with benzene–ether (9: 1) were combined and concentrated under reduced pressure. The residue was rechromatographed on silica gel (50 g) using benzene–ether (9: 1) and 250 ml fractions were collected. Fr. 4—18 were combined, concentrated under reduced pressure and the residue was crystallized from MeOH to give I as colorless needles (yield, 500 mg), mp 150—151°, $[\alpha]_D^{25}$ −12.6° (c=1.23, in $CHCl_3$), UV λ_{max}^{EtOH}: 210 nm (log ε 4.38), IR ν_{max}^{KBr} cm^{-1}: 3430, 1780, 1745, 1705, 1635. NMR (δ in $CDCl_3$): 0.89 (3H, d, J=6 Hz, >CH-C$\underline{H}_3$), 0.97, 1.05, 1.23 (each 3H, s, 3×-$\overset{|}{\underset{|}{C}}$-C$\underline{H}_3$), 2.05 (3H, s, -OCOC$\underline{H}_3$), 4.79 (2H, d, J=1 Hz, C_{16}-H), 5.38 (1H, q, J=2 Hz, C_6-H), 5.85 (1H, t, J=1 Hz, C_{14}-H). *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 70.02; H, 8.98. Mass Spectrum m/e: 318 (M^+–AcOH).

Catalytic Hydrogenation of Vitexilactone (I)——140 mg of I in MeOH (10 ml) was shaken with hydrogen in the presence of 5% Pd- charcoal (100 mg) as a catalyst. After uptake of hydrogen corresponding to one mole per mole of I, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (5 g) using a mixture of benzene and ether. The fractions eluted with benzene–ether (95: 5) gave colorless needles (II), mp 104—105.5° $[\alpha]_D^{29}$ −28.8° (c=0.42, in EtOH), UV λ_{max}^{EtOH}: 205 nm (log ε 3.46), IR ν_{max}^{KBr} cm^{-1}: 3500, 1773, 1720, NMR (δ in $CDCl_3$): 0.85 (3H, d, J=6 Hz, >CH-C$\underline{H}_3$), 0.95, 1.00, 1.22 (each 3H, s, 3×-$\overset{|}{\underset{|}{C}}$-C$\underline{H}_3$), 2.02 (3H, s, -OCOC$\underline{H}_3$), 3.7—4.6 (2H, m, C_{16}-H), 5.39 (1H, q, J=2 Hz, C_6-H). *Anal.* Calcd. for $C_{22}H_{36}O_5$: C, 69.44; H, 9.54. Found: C, 69.87; H, 9.59. Mass Spectrum m/e: 320 (M^+–AcOH).

Treatment of II with *p*-Toluenesulfonic Acid——II (96 mg) and p-toluenesulfonic acid (44 mg) were dissolved in 10 ml of MeOH and refluxed for 5 hr. The reaction mixture was concentrated under reduced pressure and the residue was purified by the preparative thin-layer chromatography (PTLC) to give III as a colorless oil. IR ν_{max}^{Direct} cm^{-1}: 1780, 1725, NMR (δ in $CDCl_3$): 1.00 (6H, s, 2×-$\overset{|}{\underset{|}{C}}$-C$\underline{H}_3$), 1.30 (3H, s, -$\overset{|}{\underset{|}{C}}$-C$\underline{H}_3$), 1.60 (3H, br s, =$\overset{|}{C}$-C$\underline{H}_3$), 2.01 (3H, s, -OCOC$\underline{H}_3$), 5.50 (1H, m, C_6-H). $C_{22}H_{34}O_4$ [Mass Spectrum m/e: 302 (M^+−AcOH)].

Treatment of Vitexilactone (I) with Sodium Hydroxide——To the solution of I (50 mg) in MeOH (4 ml) was added 2 ml of 2% NaOH and the mixture was allowed to stand at room temperature for 18 hr, acidified with 2% HCl, and then extracted with ether. The ethereal extract was concentrated and the residue was crystallized from a mixture of ether and isopropyl ether to give IV as colorless prisms, mp 216—218°, $[\alpha]_D^{25}$ −18.5° (c=0.27, in EtOH), IR ν_{max}^{KBr} cm^{-1}: 1785, 1722, NMR (δ in $CDCl_3$): 0.80 (3H, d, J=6 Hz, >CHC$\underline{H}_3$), 0.96, 1.00, 1.22 (each 3H, s, 3×-$\overset{|}{\underset{|}{C}}$-C$\underline{H}_3$), 2.03 (3H, s, -OCOC$\underline{H}_3$), 2.40—3.20 (center 2.60, 2H, q, J=17 Hz, C_{14}-H), 4.23 (center, 2H, q, J=9 Hz, C_{16}-H), 5.36 (1H, q, J=2 Hz, C_6-H). *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81, H, 9.05; Found: 69.51, H. 9.08. Mass Spectrum m/e: 318 (M^+–AcOH).

Ozonolysis of Vitexilactone (I)——Ozonized O_2 was passed through a solution of I (250 mg) in $CHCl_3$ (40 ml) at 0° for 2 hr and then 3 ml of water was added to the reaction mixture. The mixture was refluxed for 2 hr and then concentrated under reduced pressure. The residue was chromatographed on silica gel (13 g) using benzene–ether (100: 15) to give γ-lactone (VI) as colorless needles (from ether–n-hexane), mp

5) J.W.B. Fulke, M.S. Henderson, and R.Mc Crindle, *J. Chem. Soc.*(*C*), **1968**, 807.
6) Y. Asaka, T. Kamikawa, and T. Kubota, *Chemistry Letters* **1973**, 937.

128—130°, $[\alpha]_D^{25}$ −24.4° (c=0.62, in EtOH). IR ν_{max}^{KBr} cm^{-1}: 1765, 1725. NMR (δ in $CDCl_3$): 0.90 (3H, d, J=6 Hz, >CHC$\underline{H}_3$), 0.97, 1.00, 1.26 (each 3H, s, 3×-C(-)-C$\underline{H}_3$), 2.07 (3H, s, -OCOC$\underline{H}_3$), 5.45 (1H, m, $W_{1/2}$= 6 Hz, C_6–H). *Anal.* Calcd. for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.54; H, 9.31. This was identified with an authentic sample of VI derived from rotundifuran (V) by the mixed melting point and the comparison of IR and NMR spectra.

Isolation of Rotundifuran (V) from *Vitex rotundifolia*——The dried leaves of *V. rotundifolia* (800 g) were extracted with petroleum ether under reflux for 3 times. The combined extracts were concentrated under reduced pressure and the residue (11.5 g) was chromatographed on silica gel (300 g) using a mixture of benzene and ether.

The benzene–ether (99: 1) eluate was purified by PTLC with silica gel HF_{254} (Merck) plate to give rotundifuran (V) as a colorless oil, NMR (δ in $CDCl_3$); 0.93 (3H, d, J=6 Hz, >CH-C$\underline{H}_3$), 0.95, 1.00, 1.26 (each 3H, s, 3×-C(-)-C$\underline{H}_3$), 2.03 (3H, s, -OCOC$\underline{H}_3$), 5.42 (1H, m, $W_{1/2}$=5 Hz, C_6–H), 6.28 (1H, d/d, J=2/1 Hz), 7.23 (1H, m) 7.40 (1H, d/d, J=2/2 Hz) (furan ring). *Anal.* Calcd. for $C_{22}H_{34}O_4$ [Mass Spectrum m/e: 302 (M^+—AcOH); 81 (100%)]. yield. 1 g. All of physical data were identical with those of rotundifuran in the literature.[6]

Ozonolysis of Rotundifuran (V)——Rotundifuran (V) was ozonized as in the case of I, giving γ-lactone (VI), mp 126—128°, $[\alpha]_D^{30}$ −27.7° (c=0.42, in EtOH). *Anal.* Calcd. for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.63; H, 9.38. (The melting point of VI was not given in the literature[6]).

Isolation of Agnuside, Artemetine and *p*-Hydroxybenzoic Acid——The fresh leaves of *V. cannabifolia* were extracted with MeOH for three times. The combined extracts were concentrated to dryness and water (1 liter) was added to the residue. The insoluble part was filtrated off and the filtrate was extracted with AcOEt. The aqueous layer was concentrated under reduced pressure to give a dark brown mass (74 g). A part of this mass (20 g) was chromatographed on charcoal (125 g) using water (6 liters) and MeOH (6 liters). The methanolic eluate was concentrated and the residue (2.86 g) was rechromatographed on silica gel (100 g). The $CHCl_3$–MeOH (85: 15) eluate gave an amorphous powder, which was acetylated with acetic anhydride and pyridine to give agnuside hexaacetate, mp 133—135°. *Anal.* Calcd. for $C_{32}H_{36}O_{16}$: C, 56.82; H, 5.32. Found: C, 56.47; H, 5.26. This was identified with an authentic sample isolated from *V. rotundifolia* by the mixed melting point and the comparison of IR spectra.

The AcOEt extract (5 g) was chromatographed on silica gel (300 g) using a mixture of $CHCl_3$ and MeOH. The $CHCl_3$–MeOH (97:3) eluate gave colorless needles, mp 216—218°, which was identified with *p*-hydroxybenzoic acid. The $CHCl_3$–MeOH (95: 5) eluate gave pale yellow needles, mp 167—168°. UV λ_{max}^{EtOH} nm: 256, 273, 347, NMR (δ in d_6-DMSO): 3.78 (3H, s), 3.88 (3H, s), 3.92 (6H, s), 3.98 (3H, s) (5×OCH_3), 7.00 (1H, s, C_8–H), 7.26 (1H, d, J=9 Hz, C_5'–H), 7.70 (1H, d, J=1 Hz, C_2'–H), 7.82 (1H, d/d, J=9/1 Hz, C_6'–H), 12.59 (1H, s, C_5–O$\underline{H}$). *Anal.* Calcd. for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 61.97; H, 4.83. These spectral data were identical with those of artemetine.[3]

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