Constituents of the Leaves of Vitex cannabifolia

Rep. Fac. Sci. Kagoshima Univ. (Math., Phys. & Chem.),
No. 26, 57-61, 1993.

Constituents of the Leaves of *Vitex cannabifolia*

Tetsuo IWAGAWA*, Akemi NAKAHARA*, Ayako MIYAUCHI*, Hiroaki OKAMURA* and Munehiro NAKATANI*

(Received Sept. 6, 1993)

Abstract

From the leaves of *Vitex cannabifolia* methyl *p*-hydroxybenzoate and four glucosides, aucubin, agnuside, negundoside, and maltoglucoside have been isolated.

Key Words: Verbenacea, *Vitex cannabifolia*, leaves, methyl *p*-hydroxybenzoate, aucubin, agnuside, negundoside, maltoglucoside.

Introduction

Vitex cannabifolia Sieb. et Zucc. is a deciduous tree native to China. Its fruits have been used for treating colds under the name of Bokeishi. The methanolic extract of the leaves was dissolved in H_2O, and extracted with CH_2Cl_2 and *n*-BuOH, successively. The CH_2Cl_2 extract inhibited germination of cress and the *n*-BuOH extract showed antimicrobial activity against *Aeromonas salmonishida*, a fish pathogenic bacterium. From the CH_2Cl_2 extract compound (**1**) was isolated as a weak germination inhibitor by repeated open-column chromatography. On the other hands, the *n*-BuOH extract was fractionated by a combination of column chromatography on activated charcoal, ODS, and silicagel, and finally HPLC, giving six glucosides (**2**)～(**7**). In our previous paper [1], we reported that the structure of nishindaside **2** was corrected with regard to the configuration at C-3, and its new isomer, isonishindaside **3** was also characterized. In this paper, we describe the isolation and structural elucidation of compounds, **1**, **4**, **5**, **6**, and **7**.

Results and Discussion

The molecular formulas of **1**～**7** were determined by a combination of mass spectrometry and the ^1H, and ^{13}C NMR data (Table 1.). The presence of a β-glucopyranose unit in each of

* Department of Chemistry, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890, Japan.

the compounds **4**～**7** was readily established by ^{1}H and ^{13}C NMR spectra. Upon treatment with sulphuric acid compounds **3-5** turned black like other iridoids.

Compound **1** which acted as a weak germination inhibitor was isolated as needles, mp 128-129° and presented the molecular formula $C_8H_8O_3$. The IR spectrum showed the presence of a hydroxyl group at 3300 cm^{-1}, an α, β-unsaturated ester carbonyl at 1680 cm^{-1}, and a *p*-substitued phenyl group at 1600, 1580, and 850 cm^{-1}. In the ^{1}H NMR spectrum, an A_2B_2 system at δ 6.88 and 7.95 (J=8.8 Hz) due to *p*-substituted phenyl protons and a singlet at δ 3.89 to carbomethoxyl protons appeared.

Therefore compound **1** was determined to be methyl *p*-hydroxybenzoate on the basis of the above results, and the IR spectrum was in good agreement of that of an authentic sample of methyl *p*-hydoxybenzoate [2].

Compound **4** was isolated as an amorphous powder with a molecular formula $C_{15}H_{22}O_9$. The IR spectrum showed absorption bands for a hydroxyl group at 3300 cm^{-1} and a double bond at 1650 cm^{-1}, and the UV maxima at 206 nm was characteristic of the double bond of a non-conjugated iridoid enol-ether system. In the ^{1}H NMR spectrum, two double doublets at δ 6.30 (1H, J=1.8 and 5.9 Hz) and δ 5.09 (1H, J=3.9 and 6.1 Hz) due to olefinic protons at C-3 and C-4 respectively, were observed. The proton at C-4 was further coupled to a proton at δ 2.62-2.67 (1H, *m*, H-5) which in turn was coupled to a proton at δ 4.25-4.44 (1H, *m*, H-6). The chemical shift of latter proton indicated the presence of an hydroxyl group at C-6. The proton at C-6 was also coupled to an olefinic proton at δ 5.76 (1H, br *s*, H-7). The coupled protons at δ 4.12 and 4.36 (AB, J= 15.4 Hz) suggested the presence of hydroxymethylene which was positioned at C-10 due to the weak allylic coupling with H-7. In addition, the proton at C-5 was also coupled to a proton at δ 2.89 (1H, *t*-like, J=7.3 Hz, H-9), which in turn was coupled to an acetal proton at δ 4.95 (1H, *d*, J=7.0 Hz, H-1). From the findings described above, compound 4 was suggested to be aucubin. This was also supported by comparison of the ^{13}C NMR data [3].

The ^{1}H and ^{13}C NMR spectra of **5**. $C_{22}H_{26}0_{11}$, were similar to those of **4**, except for resonances ascibed to an additional *p*-hydroxybenzoyl group [δ 6.84 and 7.92 (AB, J=8.6 Hz); δ 167.9, 163.7, 133.0 × 2, 122.2, 116.3 × 2] which is frequently encountered in *Vitex* species. This acyl group was attached to C-10, since C-10 methylene protons resonances were characteristically shifted downfield by 0.77 ppm when compared to those of **4** . This was confirmed by the downfield shift (Δ2.2 ppm) of the C-10 signal in the ^{13}C NMR spectrum when compared to that of 4. Compound **5** is thus concluded to be agnuside [4].

Compound **6** was isolated as needles, mp 155-159° with a molecular formula $C_{23}H_{28}O_{12}$. The IR spectrum showed the presence of a hydroxyl group at 3400 cm^{-1}, an α, β-unsaturated ester carbonyl at 1700 cm^{-1}, a conjugated carboxyl group at 1690 cm^{-1}, and a *p*-substituted phenyl group at 1600, 1510, and 850 cm^{-1}. In the ^{1}H NMR spectrum, resonances at δ 7.08 (1H, *br s*) was assigned to the ptoton at C-3 β to the carboxyl function, and the resonance at δ 1.25 (3H, *s*) to a methyl group on carbon bearing a hydroxyl group. The resonances at δ 6.80 and 7.84 (AB, J=9.0 Hz) were assigned to a *p*-hydroxybenzoyl group, while a series of resonances in the δ 3.30-4.96 range were assigned to a glucose residue. The other protons were assigned by the ^{1}H-^{1}H COSY spectrum: δ 1.40 and 2.15 (1H each, *m*, H-6), 1.56-1.69 (2H, *m*, H-7), 2.20

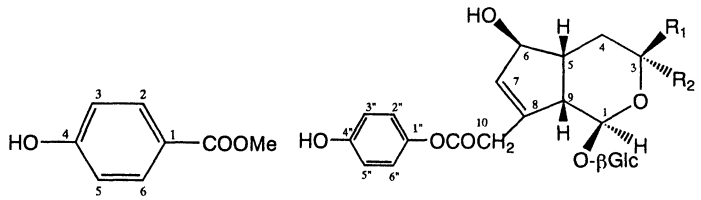
(1H, *dd*, J=2.6 and 9.5 Hz, H-9), 2.92 (1H, *m*, H-5), 5.47 (1H, *d*, J=2.9 Hz, H-1). Since the signal due to H-9 appeared as a double doublet, the C-8 was tertiary and hence the hydroxyl and methyl groups could be located at C-8. The configulation of the hydroxyl group was determined as β by the chemical shift of δ 52.5 for C-9 [5]. The placement of the *p*-hydroxybenzoyl group at C-2″ was confirmed by comparison of the ^{13}C NMR spectrum with that of **2**. Thus, a downfield shift of C-2″ (Δ1.7 or 2.2 ppm) and an upfield shift of C-1″ (Δ2.1 ppm) were observed. Therefore the structure of **6** was concluded to be negundoside [4].

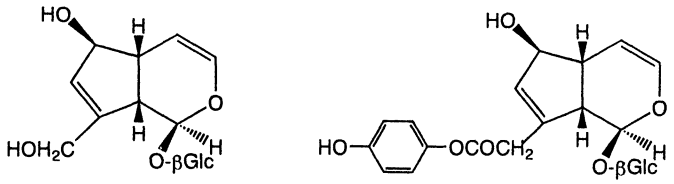
Compound **7** was obtained as a syrup with a molecular formula $C_{12}H_{10}O_8$. The IR spectrum contained absorption bands corresponding to a hydroxyl group at 3350 cm^{-1}, and a conjugated carbonyl at 1640 cm^{-1}. One carbonyl, two olefins, one glucose, and one methyl signals in the ^{13}C NMR spectrum, suggesting that **7** is monocyclic and have an ether oxygen. The ^1H NMR spectrum showed the presence of an olefinic methyl group at δ 2.47 (3H, *s*) and conjugated olefinic protons at δ 6.45 and 8.00 (AX, J=5.7 Hz), besides that of the glucose moiety. On the basis of the results, compound **7** could be assumed to have a 4-pyranone skeleton. To determine the location of the methyl group and the glucose, the NOEDS measurements were performed. Irradiation of the proton at δ 8.00 enhanced the signal of the methyl protons, suggesting **7** to be maltoglucoside [6].

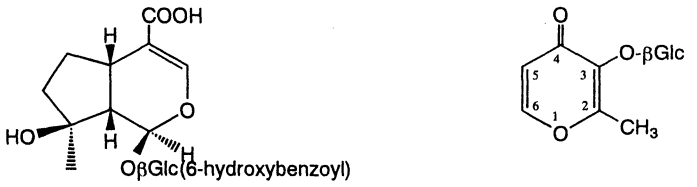
Table 1 ^{13}C NMR spectral data of compounds **4-6** (CD_3OD, TMS as int. standard)

C	1	4	5	6	7
1	122.3	97.8	98.0	97.9	
2	132.0				143.7
3	115.3	141.6	141.8	151.1	164.6
4	160.4	105.8	105.6	114.0	177.2
5	115.3	46.3	46.4	31.3	117.4
6	132.0	82.9	82.9	30.3	157.2
7		130.3	132.5	41.3	
8		148.1	142.9	79.9	
9		49.7	48.4	52.5	
10		61.5	63.7	24.4	
11				170.2	
1′		100.0	100.3	95.1	105.5
2′		74.9	74.9	76.2	75.5
3′		77.9[§]	78.0[§]	75.0	78.1[§]
4′		71.6	71.5	71.9	71.2
5′		78.3[§]	78.3[§]	78.6	78.6[§]
6′		62.7	62.8	62.8	62.6
1″			122.2	122.3	
2″, 6″			133.0	132.9	
3″, 5″			116.3	116.2	
4″			163.7	163.4	
*C*OO	167.5	167.9	167.9	167.4	
Me	52.1				15.8

§ These values may be interchangeable in any column.







Experimental

Extraction and isolation. The extraction procedure was described in our previous work [1]. The CH_2Cl_2 extract (19.2 g) was chromatographyed on a column of SiO_2 with MeOH-CH_2Cl_2 (1 : 99) to give **1** (46 mg). A portion (37 g) of the *n*-BuOH extract was subjected to chromatography on a column of activated charcoal (100 g), frs (100 ml) being collected as follows: 1 (H_2O), 2-3 (MeOH-H_2O, 1 : 1), 4-5 (MeOH), 6-7 (CH_2Cl_2-MeOH, 1 : 1), and 8-11 (CH_2Cl_2). The combined frs 2-3 (1.7 g) was subjected to ODS CC. The frs eluted with MeOH-H_2O (1 : 4) was applied to HPLC with MeOH-H_2O (1 : 19 to 1 : 9) to afford **7** (2.5 mg) and **4** (9.7 mg). Further elution of the frs 2-3 with MeOH-H_2O (2 : 3) gave crude **6**, which was purified by use of HPLC (ODS, MeOH-H_2O, 1 : 1) to yield pure **6** (5.2 mg). From the part (1.15 g) of frs 5-8, **1** (5.4 mg) and **2** (2.8 mg) were isolated as described earlier. Compound **5** (97.4 mg) was isolated from the frs. 5-8 by the same procedure as in the case of **1** and **2**.

Compound (**1**). Needles, mp 128-129° (lit. 126-128°), UV λ_{max}^{MeOH} nm (ε): 213 (15600), 256 (27100); IR ν_{max}^{Nujol} cm^{-1}: 3300, 1680, 1600, 1580, 850, ^1H NMR ($CDCl_3$): δ 3.89 (3H, *s*, COOMe), 6.88 and 7.95 (AB, J=8.8 Hz, aromatic H); EI-MS *m/z*: 152 $[M]^+$.

Compound (**4**). Amorphous powder, $[\alpha]_D$ -107.7° (MeOH; *c* 0.117), UV λ_{max}^{MeOH} nm (ε): 206 (4200); IR ν_{max}^{KBr} cm^{-1}: 3300, 1650; ^1H NMR (CD_3OD): δ 2.62-2.67 (1H, *m*, H-5), 2.89 (1H, *t*-like, J= 7.3 Hz, H-9), 3.64 (1H, *dd*, J=5.3 and 11.9 Hz, H-6′), 3.85 (1H, *dd*, J=1.8 and 11.9, H-1′), 4.12 and 4.34 (AB, J=15.4 Hz, H-10), 4.25-4.44 (1H, *m*, H-6), 4.67 (1H, *d*, J=8.1 Hz, H-1′), 4.95 (1H, *d*,

J=7.0 Hz, H-1), 5.09 (1H, *dd*, J=3.9 and 6.1 Hz, H-4), 5.76 (1H, *br s*, H-7), 6.30 (1H, *dd*, J=1.8 and 5.9 Hz, H-3); FAB-MS *m/z*: 369 $[M+Na]^+$.

Compound (**5**). Amorphous powder, $[\alpha]_D$ -75.6° (MeOH; *c* 0.41), UV λ_{max}^{MeOH} nm (ε): 209 (17000), 258 (20000); IR ν_{max}^{film} cm^{-1}: 3300, 1700, 1655, 1605, 860; ^1H NMR ($CDCl_3$): δ 2.70 (1H, *m*, H-5), 2.99 (1H, *t*-like, J=7.5 Hz, H-9), 3.65 (1H, *dd*, J=5.3 and 11.9 Hz, H-6′), 3.85 (1H, *dd*, J=1.8 and 12.1 Hz, H-6′), 4.70 (1H, *d*, J=7.7 Hz, H-1′), 4.91 and 5.09 (AB, J=14.7 Hz, H-12), 4.99 (1H, *d*, J=7.7, H-1), 5.12 (1H, *dd*, J=3.7 and 5.9 Hz, H-4), 5.83 (1H, *br s*, H-7), 6.34 (1H, *dd*, J=2.0 and 6.0 Hz, H-3), 6.84 and 7.92 (AB, J=8.6 Hz, aromatic H); FAB-MS *m/z*: 489 $[M+Na]^+$.

Compound (**6**). Needles, mp 155-159°, $[\alpha]_D$ -119.2° (MeOH; *c* 0.13), UV λ_{max}^{MeOH} nm (ε): 210 (21900), 254 (23400); IR ν_{max}^{film} cm^{-1}: 3400, 1700, 1690, 1640, 1600, 1510, 850; ^1H NMR ($CDCl_3$): δ 1.25 (3H, *s*, H-10), 1.40 (1H, *m*, H-6), 1.56-1.69 (2H, *m*, H-7), 2.15 (1H, *m*, H-6), 2.20 (1H, *dd*, J= 2.6 and 9.5 Hz, H-9), 2.89-2.95 (1H, *m*, H-5), 3.71 (1H, *m*, H-6′), 3.94 (1H, *dd*, J=1.5 and 13.1, H-6′), 4.96 (1H, *d*, J=8.1 Hz, H-1′), 5.47 (1H, *d*, J=2.9 Hz, H-1), 6.80 and 7.84 (AB, J=9.0 Hz, aromatic H), 7.08 (1H, *br s*, H-3); FAB-MS *m/z*: 519 $[M+Na]^+$.

Compound (**7**). Syrop, UV λ_{max}^{MeOH} nm (ε): 210 (5800), 259 (7600); IR ν_{max}^{KBr} cm^{-1}: 3350, 1640, 1620; ^1H NMR ($CDCl_3$): δ 2.47 (3H, *s*, Me), 3.25 (1H, *ddd*, J=2.2, 5.5, and 7.7 Hz, H-5′), 3.68 (1H, *dd*, J=5.5 and 11.9 Hz, H-6′) 3.82 (1H, *dd*, J=2.4, 11.9 Hz, H-6′) 4.81 (1H, *d*, J=7.3 Hz, H-1′), 6.45 and 8.00 (AB, J=5.7 Hz, H-5 and H-6); FAB-MS *m/z*: 311 $[M+Na]^+$.

Acknowledgements — We are garateful to Miss M. Saeki (Taiho Yakugyou, Co., Ltd) for FAB-MS measurements.

References

1. Iwagawa, T., Nakahara, A. and Nakatani, M. (1993), *Phytochemistry* **32**, 453.
2. Pouchert, C. J. (1981), "*The Aldrich Library of Infrared Spectra*" ed. III, pp. 1029
3. Bianco, A., Passacantilli, P., Nicoletti, M. and De Lima, R. A. (1982), *Tetrahedron* **38**, 359.
4. Dutta, P. K., Chowdhury, U. S., Chakravarty, A. K., Achari, B. and Pakrashi, S. C. (1983), *Tetrahedron* **39**, 3067.
5. Jensen, S. R. and Nielsen, B. J. (1982), *Phytochemistry* **21**, 1623.
6. Yasue, M., Kawamura, N. and Ishibashi, K. (1968), *Yakugaku Zasshi*, 88, 390.