

Constituents of the Leaves of *Vitex cannabifolia*

著者	IWAGAWA Tetsuo, NAKAHARA Akemi, MIYAUCHI Ayako, OKAMURA Hiroaki, NAKATANI Munehiro
journal or publication title	鹿児島大学理学部紀要. 数学・物理学・化学
volume	26
page range	57-61
別言語のタイトル	ニンジンボク (<i>Vitex cannabifolia</i>) の葉の含有成分
URL	http://hdl.handle.net/10232/6512

Constituents of the Leaves of *Vitex cannabifolia*

著者	IWAGAWA Tetsuo, NAKAHARA Akemi, MIYAUCHI Ayako, OKAMURA Hiroaki, NAKATANI Munehiro
journal or publication title	鹿児島大学理学部紀要. 数学・物理学・化学
volume	26
page range	57-61
別言語のタイトル	ニンジンボク (<i>Vitex cannabifolia</i>) の葉の含有成分
URL	http://hdl.handle.net/10232/00010070

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 H₂O, and extracted with CH₂Cl₂ and *n*-BuOH, successively. The CH₂Cl₂ extract inhibited germination of cress and the *n*-BuOH extract showed antimicrobial activity against *Aeromonas salmonicida*, a fish pathogenic bacterium. From the CH₂Cl₂ 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 ¹³C 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 ^1H 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 $\text{C}_8\text{H}_8\text{O}_3$. 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*-substituted phenyl group at $1600, 1580, \text{ and } 850\text{ cm}^{-1}$. In the ^1H NMR spectrum, an A_2B_2 system at δ 6.88 and 7.95 ($J=8.8\text{ Hz}$) 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*-hydroxybenzoate [2].

Compound 4 was isolated as an amorphous powder with a molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_9$. 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 ^1H 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, $J=15.4\text{ Hz}$) 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, $J=7.3\text{ Hz}$, H-9), which in turn was coupled to an acetal proton at δ 4.95 (1H, *d*, $J=7.0\text{ Hz}$, 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 ^1H and ^{13}C NMR spectra of 5, $\text{C}_{22}\text{H}_{26}\text{O}_{11}$, were similar to those of 4, except for resonances ascribed to an additional *p*-hydroxybenzoyl group [δ 6.84 and 7.92 (AB, $J=8.6\text{ Hz}$); δ 167.9, 163.7, $133.0 \times 2, 122.2, 116.3 \times 2$] 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 ($\Delta 2.2\text{ ppm}$) 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 $\text{C}_{23}\text{H}_{28}\text{O}_{12}$. 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 $1600, 1510, \text{ and } 850\text{ cm}^{-1}$. In the ^1H NMR spectrum, resonances at δ 7.08 (1H, *br s*) was assigned to the proton 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, $J=9.0\text{ Hz}$) 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 ^1H - ^1H COSY spectrum: δ 1.40 and 2.15 (1H each, *m*, H-6), 1.56-1.69 (2H, *m*, H-7), 2.20

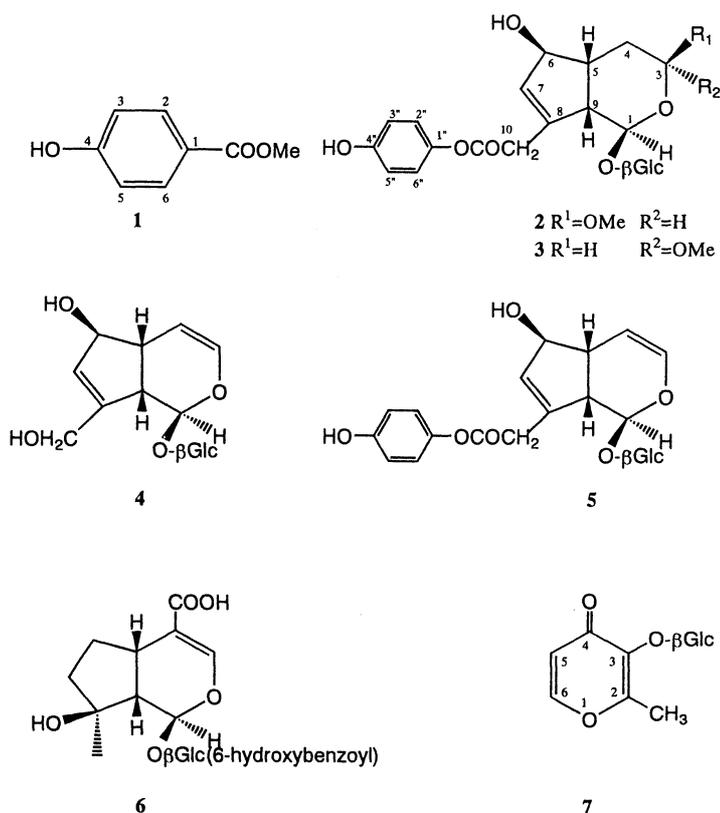
(1H, *dd*, $J=2.6$ and 9.5 Hz, H-9), 2.92 (1H, *m*, H-5), 5.47 (1H, *d*, $J=2.9$ Hz, H-1). Since the signal due to H-9 appeared as a double doublet, the C-8 was tertiary and hence the hydroxyl and methyl groups could be located at C-8. The configuration of the hydroxyl group was determined as β by the 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'' ($\Delta 1.7$ or 2.2 ppm) and an upfield shift of C-1'' ($\Delta 2.1$ 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 $\text{C}_{12}\text{H}_{10}\text{O}_8$. 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	
COO	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 chromatographed 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, $[\alpha]_D -107.7^\circ$ ($MeOH$; 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, $[\alpha]_D -75.6^\circ$ (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°, $[\alpha]_D -119.2^\circ$ (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 grateful 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.