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IRIDOID AND PHENOLIC GLUCOSIDE FROM *VITEX ROTUNDIFOLIA*

ISAO KOUNO, MASAAKI INOUE, YOSHIKO ONIZUKA, TAMAYO FUJISAKI and NOBUSUKE KAWANO*

Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi 1–14, Nagasaki 852, Japan

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Key Word Index—*Vitex rotundifolia*; Verbenaceae; iridoid glucoside; agnuside; eurostoside; phenylbutanone glucoside.

Abstract—The phenylbutanone glucoside, as well as the known compounds agnuside and eurostoside, were isolated from the leaves of *Vitex rotundifolia*, although eurostoside was obtained as a *trans*- and *cis*-mixture. The structures were established on the basis of spectroscopic data.

INTRODUCTION

Vitex rotundifolia L.f. is widely distributed on the sea coast in Asia. The presence of the iridoid glucoside, agnuside [1], and the diterpene, rotundifuran [2] have been recorded. This report deals with a new constituent of *V. rotundifolia*.

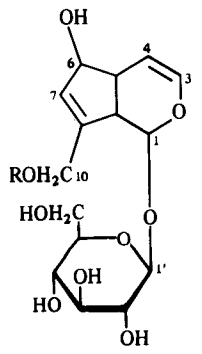
RESULTS AND DISCUSSION

From the methanol extract of the leaves of *V. rotundifolia*, two iridoid glucosides **1** and **2** and a phenol glucoside **3** were isolated.

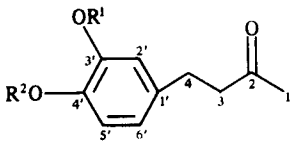
The ^{1}H and ^{13}C NMR spectra of compound **1** were identical with those of agnuside [3, 4], which was isolated as a major component from this plant [1].

Compound **2** analysed for $C_{24}H_{28}O_{11}$ [FABMS, *m/z* 515 $[M+H]^+$]. The ^{1}H NMR spectrum of compound **2** is very similar to that of agnuside except for the signals of the aromatic and olefinic protons. Although these low field signals are too complex to clarify, two sets of para-substituted cinnamoyl moieties were elucidated by the proton–proton decoupling experimental, i.e. the trans-olefin system ($J=16.1$ Hz) at δ6.36 and 7.65 (each doublet), and the *cis*-olefin system ($J=12.6$ Hz) at 5.81 and 6.90 (each doublet) were found, along with the *para*-substituted benzene ring protons (7.46, 6.80, each *d*, $J=8.7$ Hz, 1H; 7.63, 6.75, each *d*, $J=8.9$ Hz, 1H). As the compound **2** gave a hexa-acetate (**2a**) (EIMS, *m/z* 744 $[M]^+$), these findings indicated **2** has a *p*-hydroxycinnamoyl moiety instead of the *p*-hydroxybenzoyl found in agnuside (**1**). The position of the cinnamoyl moiety was established as C-10, as the signals of the glucose moiety in the ^{13}C NMR spectrum and the signals of H-6 (δ4.45) and H-10 (δ4.70) in the ^{1}H NMR spectrum of **2** were similar to those of agnuside (**1**) [3, 4]. It was concluded that **2** is a 1:1 mixture of 10-*O*-*trans*- and *cis*-*p*-hydroxycinnamoyl aucubin. Compound **2** was separated into *cis*- and *trans*-compounds by HPLC, but it was still a mixture after leaving it at room temperature. The *trans*-compound **2** is known from *Euphrasia rostkoviana* [5], *Penstemon whippleanus* [6], and *Vitex agnuscustus* [7], and named eurostoside.

Compound **3** analysed for $C_{16}H_{22}O_8$ (FABMS, *m/z* 365 $[M+Na]^+$). The keto group was suggested by an IR absorption at 1700 cm^{-1}, and a methyl ketone signal at δ2.11 in the ^{1}H NMR spectrum. Moreover, the ^{1}H NMR spectrum of **3** showed four equivalent protons at δ2.74 (*br s*, 4H) and the trisubstituted benzene ring protons at 6.60 (*dd*, $J=10.6$ and 2.2 Hz, 1H), 6.69 (*d*, $J=2.2$ Hz, 1H) and 7.07 (*d*, $J=10.6$ Hz, 1H). The ^{13}C NMR of **3** indicated the



1 R = *p*-hydroxybenzoyl
2 R = *trans* and *cis* *p*-hydroxycinnamoyl



3 R^1 = H, R^2 = Glu
3a R^1 = Ac, R^2 = Glu(OAc)$_4$
4 R^1 = Glu, R^2 = H

*Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR data of myzodendrone* and compound **3** (22.5 MHz)

Position *n*-chain	Myzodendrone	**3**
C-1	30.2 *q*	30.0 *q*
C-2	165.6 *s*†	211.4 *s*
C-3	45.9 *t*	45.6 *t*
C-4	30.2 *t*	30.0 *t*
Benzene ring		
C-1′	124.5 *s*	138.3 *s*
C-2′	117.0 *d*	117.0 *d*
C-3′	134.2 *s*	144.8 *s*
C-4′	146.5 *s*	148.2 *s*
C-5′	118.9 *d*	119.3 *d*
C-6′	119.0 *d*	120.7 *d*
Glucose		
C-1″	104.3 *d*	104.5 *d*
C-2″	74.9 *d*	74.7 *d*
C-3″	77.6 *d*	77.5 *d*
C-4″	71.4 *d*	71.1 *d*
C-5″	78.3 *d*	78.0 *d*
C-6″	62.5 *t*	62.4 *t*

All values are in (ppm) relative to TMS (CD_3OD).
*Data obtained from ref. [8].
†This assignment is thought to be incorrect.

presence of a benzene ring, two methylene carbons and a sugar group which was confirmed as glucose by chromatographic comparison of the hydrolysis product of **3** with an authentic sample. Compound **3** gave a penta-acetate (**3a**) upon usual acetylation. All these findings indicated **3** is 3′,4′-dihydroxyphenylbutanone glucoside in comparison with myzodendrone (**4**) [4-(3′,4-dihydroxyphenyl)-butan-2-one-3′-*O*-glucoside] which was isolated from *Myzodendron punctulatum* [8].

The position of glucose was established as 4′ by the NOE enhancement (10%) of the signal at δ7.07 (H-5′) of the aromatic proton upon irradiation at δ4.68 (anomeric proton, H-1″) frequency. The β-configuration of C-1″ (anomeric carbon) was deduced by the signals of glucose in the ^{13}C NMR spectrum of **3** and the *J* value (J = 7.5 Hz) at δ4.68 of the anomeric proton signal in the ^{1}H NMR spectrum of **3**. Thus, it was concluded that compound **3** is 4-(3′,4′-dihydroxyphenyl)-butan-2-one-4′-*O*-β-D-glucoside.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded in CD_3OD solns unless otherwise specified and 90.0/22.5 MHz (^{1}H/^{13}C) using TMS as int. standard.

Extraction and isolation. The dried leaves of *V. rotundifolia* were collected on the sea coast in Nagasaki, Japan, and extracted with MeOH. The MeOH extracts (362 g) were dissolved in H_2O, and partitioned between *n*-hexane and H_2O, then *n*-BuOH and H_2O, successively. The *n*-BuOH soluble portion was extracted with EtOAc–MeOH (7:3) to give the soluble part (42.3 g). This was chromatographed on silica gel using the solvent system of $CHCl_3$–MeOH–H_2O (8:2:0.1), then purified by reversed phase chromatography [solvent system MeOH–H_2O (6:4)] to give the compounds **1** (23 g), **2** (345 mg) and **3** (290 mg), respectively. The ^{1}H and ^{13}C NMR spectra of **1** were identical with those of agnuside.

Compound **2**. Amorphous powder, $[\alpha]_D^{16}$ −49.6° (EtOH; *c* 0.9); FABMS *m/z* 515 $[M+Na]^+$; IR ν_{max}^{KBr} cm^{-1}: 3550, 1700 and 1610; ^{1}H NMR: δ5.00 (*d*, *J* = 7.4 Hz, 1H, H-1), 6.39 (*dd*, *J* = 2.0, 6.3 Hz, 1H, H-3), 5.11 (*dd*, *J* = 3.8, 6.3 Hz, 1H, H-4), 2.70 (*m*, 1H, H-5), 4.45 (*m*, 1H, H-6), 5.80 (*br s*, 1H, H-7), 2.96 (*br t*, *J* = 7.4 Hz, 1H, H-9), 4.70 (*d*, *J* = 7.1 Hz, 2H, H-10), 4.69 (*d*, *J* = 7.1 Hz, 1H, H-1′), *p*-hydroxy cinnamoyl moiety; H-*trans* 6.36 (*d*, *J* = 16.1 Hz, 1 H), 7.65 (*d*, *J* = 16.1 Hz, 1H), 7.46 (*d*, *J* = 8.7 Hz, 2H), 6.80 (*d*, *J* = 8.7 Hz, 2H); H-*cis* 5.81 (*d*, *J* = 12.6 Hz, 1H), 6.90 (*d*, *J* = 12.6 Hz, 1H), 7.63 (*d*, *J* = 8.9 Hz, 2H), 6.75 (*d*, *J* = 8.9 Hz, 2H); ^{13}C NMR: glucose moiety δ100.3 (*d*, C-1′), 74.9 (*d*, C-2′), 78.2 (*d*, C-3′), 71.5 (*d*, C-4′), 78.0 (*d*, C-5′) and 62.8 (*t*, C-6′).

Compound **3**. Amorphous powder. (Found: C, 52.41; H, 6.54. $C_{16}H_{22}O_8 \cdot 3/2H_2O$ requires: C, 52.03; H, 6.83%). $[\alpha]_D^{16}$ −39.6° (MeOH; *c* 0.4); FABMS *m/z* 365 $[M+Na]^+$; IR ν_{max}^{KBr} cm^{-1}: 3500–3100, 1702, 1600 and 1510; ^{1}H NMR: δ2.11 (*s*, 3H, H-1), 2.74 (*br s*, 4H, H-3 and 4), 3.35–3.90 (*m*, 6H, sugar protons), 4.68 (*d*, *J* = 7.5 Hz, 1H, anomeric proton H-1″), 6.60 (*dd*, *J* = 10.6 and 2.2 Hz, 1H, H-6′), 6.99 (*d*, *J* = 2.2 Hz, 1H, H-2′), 7.07 (*d*, *J* = 10.6 Hz, 1H, H-5′).

Acetate of compound **3**. Prepared with Ac_2O–pyridine; **3a** colourless oil, $[\alpha]_D^{16}$ −11.2° ($CHCl_3$; *c* 0.3); EIMS *m/z* 552 $[M]^+$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1713, 1600 and 1509; ^{1}H NMR ($CDCl_3$): δ2.03, 2.04, 2.07 and 2.08 (each *s*, 3H, acetyl signals), 2.12 (*s*, 3H, H-1), 2.25 (*s*, 3H, 3′-OAc), 2.79 (*m*, 4H, H-3 and 4), 3.96 (*d*, *J* = 7.1 Hz, 1H, H-1″), 4.22 (*m*, 2H, H-6″), 5.00–5.34 (*m*, 4H, sugar protons), 6.87–6.96 (*m*, 3H, H-2′,5′ and 6′).

Acid hydrolysis of compound **3**. Compound **3** (30 mg) was refluxed with 0.1 N HCl (15 ml) for 30 min, the mixture was neutralized with $Ba(OH)_2$ and filtered. The filtrate was evapd and the residue was examined by Avicel TLC in comparison with the authentic sample of glucose.

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