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ScienceDirect
Phytochemistry 69 (2008) 827–832
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Antioxidant phenylpropanoid glycosides from the leaves of *Wasabia japonica*

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Received 21 November 2006; received in revised form 15 June 2007
Available online 24 October 2007

Abstract

From the MeOH extract of the leaves of *W. japonica*, seven phenylpropanoid gentiobiosides (**1**–**7**) were isolated along with eight known phenylpropanoids (**8**–**15**). Structures of **1**–**7** were determined based on spectroscopic data and chemical evidence. The activity of compounds **1**–**15** to scavenge superoxide anion radicals was investigated using an electron spin resonance (ESR) method.

Keywords: *Wasabia japonica* Matsumura; Cruciferae; Electron spin resonance (ESR); Phenylpropanoid glycosides; Superoxide anion radicals

1. Introduction

Wasabi (the root of *Wasabia japonica* Matsumura, Cruciferae), also known as ''Japanese horseradish'', is used widely in Japanese cuisine as a pungent spice to garnish traditional dishes like sushi and sashimi. The pungency is derived from volatile allylisothiocyanates via the reaction with myrosinase (Kojima et al., 1973). Allylisothiocyanate derivatives are found in the root, stem and leaf of wasabi and horseradish plants (Eto et al., 1990). They are reported to have antimicrobial (Ono et al., 1998), antimutagenic (Kinae et al., 2000), antiplatelet (Morimitsu et al., 2000), anti-lung tumorigenesis (Yano et al., 2000), anti-gastric carcinogenesis (Tanida et al., 1991), and apoptosis-inducing (Watanabe et al., 2003b) activities. Although wasabi is known to exhibit such biological activities, the leaves of the plant are usually discarded and have been little studied chemically or biologically. In the present study, we have isolated seven new phenylpropanoid glycosides (**1**–**7**) along with eight known compounds (**8**–**15**) from the fresh wasabi leaves, and have measured their antioxidant activities by an electron spin resonance (ESR) method (Yun et al., 2003). We describe herein the isolation and structure elucidation of **1**–**7** using spectroscopic data analysis and chemical evidence as well as the antioxidant activities of **1**–**15**.

2. Results and discussion

Both the *n*-BuOH- and EtOAc-soluble portions of the MeOH extract of fresh *W. japonica* leaves exhibited superoxide anion radicals ($O_2^{\cdot-}$) scavenging activity of 82% at 1 mg/mL. The *n*-BuOH extract yielded seven phenylpropanoid glycosides (**1**–**7**) (Fig. 1) and the EtOAc extract afforded eight phenylpropanoids (**8**–**15**) by repeated column chromatography and preparative reversed-phase HPLC, as described in 3. Compounds **8**–**15** were identified as *trans*-*p*-hydroxycinnamic acid, *trans*-ferulic acid, *trans*-sinapic acid, 3,4-dimethoxy-*trans*-cinnamic acid, *trans*-ferulic acid methyl ester, *trans*-sinapic acid methyl ester, 3,4-dihydroxy-5-methoxy-*trans*-cinnamic acid, and 3,4-dihydroxy-5-methoxy-*trans*-cinnamic acid methyl ester, respectively, by comparison of their spectroscopic data with those of authentic samples.

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{33}H_{40}O_{19}$ from the $[M+H]^+$ ion peak at 741.2185 (calc. for

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doi:10.1016/j.phytochem.2007.08.021

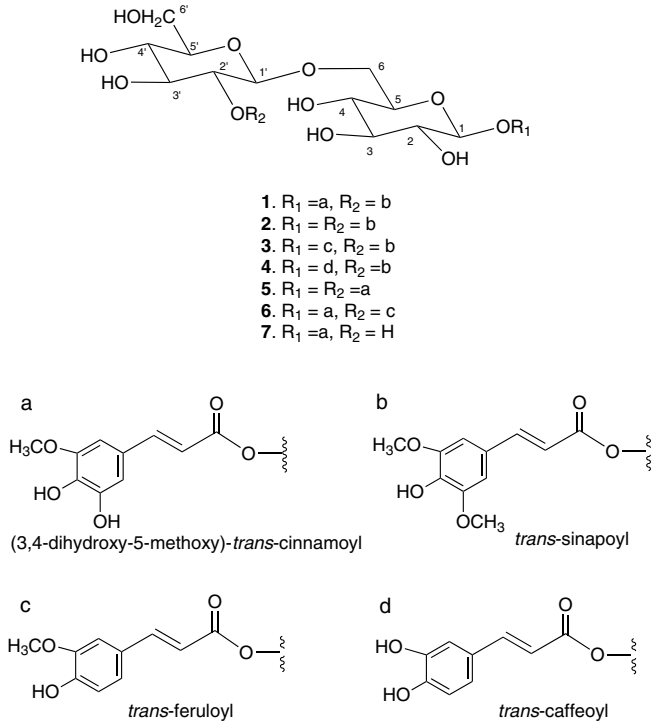

Fig. 1. Phenylpropanoid glycosides isolated from leaves of *Wasabia japonica*.

$C_{33}H_{41}O_{19}$, 741.2242) in the high-resolution ESI MS (HR ESI MS). The IR spectrum showed absorptions for hydroxyl (3416 cm^{-1}) and conjugated carbonyl (1704 cm^{-1}) groups and aromatic rings (1633 and 1518 cm^{-1}). The UV absorption maxima occurred at 239 and 329 nm, implying the presence of conjugated double bond systems in the molecule. Analysis of the ^1H NMR spectrum (Table 1) showed signals for four aromatic ring protons (δ 6.70, 6.71 and 6.93 × 2), four olefinic protons (δ 6.27, 6.54, 7.60 and 7.69), three methoxy groups (δ 3.86 × 3), and 14 sugar-derived protons (δ 3.28–5.54). The ^1H NMR spectrum of **1** indicates the presence of two 1,3,4,5-tetrasubstituted aromatic rings [δ 6.70 (1H, *d*, $J = 1.6$ Hz), 6.71 (1H, *d*, $J = 1.6$ Hz), and 6.93 (2H, *s*)]. The ^{13}C NMR spectrum (Table 2) exhibited signals for two ester carbonyl carbons (δ 167.4 and 168.5), 14 aromatic and olefinic carbons (δ 105.1–149.7), three methoxy groups (δ 56.7 and 56.8 × 2), and 12 sugar-derived carbons (δ 62.6 69.2, 71.2, 71.6, 73.9, 75.2, 76.2, 77.9, 78.0, 78.7, 95.6, and 102.6). Moreover, the four large J values of 15.8 Hz each suggested the presence of two pairs of *trans*-olefinic protons (Cuendet et al., 2001). Acid hydrolysis of **1** gave D-glucose as the sugar component, which was confirmed by comparing the HPLC and optical rotation data with those of an authentic sample, as described in 3 (Watanabe et al., 2003a). Analysis of the HMQC and HMBC spectra of **1** established the presence of a (3″,4″-dihydroxy-5″-methoxy)-cinnamoyl group, a sinapoyl group, and two glucoses. An HMBC NMR correlation between the H-1 (δ 5.54) signal of one of the glucose and the C-9″ (δ 167.4) resonance of the 3″,4″-dihydroxy-5″-methoxy-cinnamoyl group and the characteristic chemical shift of the anomeric carbon (δ 95.6) suggested that this glucose unit has an *O*-glucosidic linkage at C-9″ of the acyl group. The second glucose unit was found to be linked to the hydroxyl group at C-6 by observation of an HMBC correlation between the H-1′ (δ 4.70) and C-6 (δ 69.2) resonances. In the ^1H NMR spectrum of **1** in CD_3OD, the signal for H-2′ of the glucose unit was shifted to a lower field (δ 4.80), which was considered to be caused by the aromatic acid ester bond. The HMBC experiment for **1** revealed a correlation between the ester carbonyl carbon C-9‴ (δ 168.5) and the H-2′ proton, confirming that the sinapoyl group is located at the C-2′ hydroxyl group. Thus, the structure of **1** was determined as 1-(3″,4″-dihydroxy-5″-methoxy)-*O*-*trans*-cinnamoyl-2′-*O*-*trans*-sinapoyl gentiobiose.

Compound **2**, a yellow amorphous solid, gave the molecular formula $C_{34}H_{42}O_{19}$, as determined by its HRESIMS (*m*/*z* 755.2362 $[M+H]^+$). As described in Section 3, the IR, UV, ^1H and ^{13}C NMR spectroscopic features of **2** were generally similar to those of **1**, implying that **2** also consists of two sinapoyl groups and two sugars. Acidic hydrolysis and HPLC analysis of **2** were used to deduce that the two sugars are D-glucose units. In the HMBC studies, two sinapoyl groups were shown to be connected to the OH-1 and OH-2′ of glucose, since correlations were observed between H-1 (δ 5.56) and a carbonyl carbon (δ 167.4), and between H-2′ (δ 4.80) and a carbonyl carbon (δ 168.5). Thus, the HMBC spectrum of **2** provided evidence that, like **1**, there were linkages between the two sinapoyl groups and the two glucoses. On analysis of all of the available data, the structure of **2** was elucidated as 1,2′-di-*O*-*trans*-sinapoyl gentiobiose.

Compound **3** was obtained as a yellow amorphous solid. The molecular formula was determined to be $C_{33}H_{40}O_{18}$ from the $[M+Na]^+$ ion peak at *m*/*z* 747.2128 in the HRESIMS (Calc. for $C_{33}H_{40}O_{18}Na$, 747.2112). The IR, UV, and ^1H and ^{13}C NMR spectra of **3** were very similar to those of **1** and **2**, suggesting that **3** consists of two glucoses, a feruloyl group, and a sinapoyl group. Long-range correlations were observed between the H-1 (δ 5.55) and C-9″ (δ 167.4), and between the H-2′ (δ 4.89) and C-9‴ (δ 168.5) in their HMBC spectra, showing that the feruloyl group was located at C-1 and the sinapoyl group at C-2′. Therefore, the structure of **3** was proposed as 1-*O*-*trans*-feruloyl-2′-*O*-*trans*-sinapoyl gentiobiose.

Compound **4**, a yellow amorphous solid, gave the molecular formula $C_{32}H_{38}O_{18}$ by HRESIMS (*m*/*z* 733.2011, $[M+Na]^+$). Its IR, UV, and ^1H and ^{13}C NMR data were also generally similar to those of **1**–**3** and suggested the presence of two glucoses, a caffeoyl group, and a sinapoyl group. From the HMBC spectrum of **4**, the position of the caffeic acid was deduced as C-1 of glucose, and the location of the sinapoyl group was found to be at C-2′ from the long-range correlations between H-1 (δ 5.54) and H-2′ (δ 4.80) and the carbonyl carbons of caffeic acid