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Phenylpropanoid derivatives from edible canna, *Canna edulis*

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Abstract

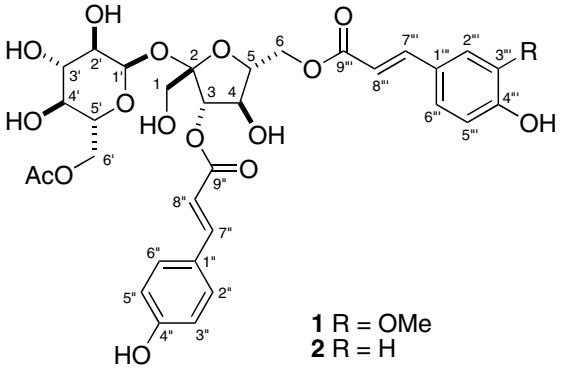
Two phenylpropanoid sucrose esters were isolated from dry rhizomes of *Canna edulis* Ker Gawl., along with a known phenylpropanoid sucrose ester and four known phenylpropanoids. On the basis of analysis of spectroscopic data and chemical evidence, these two phenylpropanoid sucrose esters were shown to be 3-*O*-*p*-coumaroyl-6-*O*-feruloyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside and 3,6-di-*O*-*p*-coumaroyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside.

Keywords: *Canna edulis*; Cannaceae; Phenylpropanoid sucrose ester; Rosmarinic acid; Caffeic acid derivatives

1. Introduction

The rhizome of edible canna (*Canna edulis* Ker Gawl., Cannaceae), which is also called Sagu, Biri, or arrowroot (Piyachomkwan et al., 2002), contains about 10% starch that is reportedly more digestible than that from other sources (Pérez et al., 1997). Therefore, this plant is cultivated in tropical regions as a source of starch. However, there has been no thorough search for other useful substances that may be present in this plant rhizome.

Thus, we examined the rhizome of edible canna and isolated two phenylpropanoid sucrose esters (**1** and **2**) together with a known phenylpropanoid sucrose ester and four known phenylpropanoids, i.e. caffeic acid, rosmarinic acid, caffeoyl-4′-hydroxyphenyllactic acid and salvianolic acid B. The structures of **1** and **2** were determined to be 3-*O*-*p*-coumaroyl-6-*O*-feruloyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside (**1**) and 3,6-di-*O*-*p*-coumaroyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside (**2**), on the basis of chemical evidence and spectroscopic data including 2D-NMR spectral analysis.



2. Results and discussion

The ethyl acetate-soluble residue of the methanolic extract derived from dry rhizomes of edible canna was subjected to silica gel column chromatography. The fractions obtained were further separated by ODS or Sephadex LH-20 column chromatography, followed by reversed-phase HPLC to give two phenylpropanoid sucrose esters **1** and **2**, caffeic acid, and caffeoyl-4′-hydroxyphenyllactic acid.

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The *n*-butanol-soluble residue of the methanolic extract was subjected to HP-20 column chromatography, followed by Sephadex LH-20 or silica gel column chromatography, and finally purified by reversed-phase HPLC to give a phenylpropanoid sucrose ester, 6-*O*-*p*-coumaroyl-β-D-fructofuranosyl α-D-glucopyranoside, along with two phenylpropanoids, rosmarinic acid and salvianolic acid B. Such caffeic acid derivatives are known to have antioxidant activity (Petersen and Simmonds, 2003).

Compound **1**, a light yellow amorphous solid with a molecular formula of $C_{33}H_{38}O_{17}$, exhibited a UV absorption maximum at 319 nm, which implied the presence of a highly conjugated double bond system. The IR spectrum showed the presence of hydroxyl groups (3388 cm^{-1}) and aromatic rings (1630, 1603 and 1515 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of one 1,4-disubstituted aromatic ring [δ 7.52 (2H, *d*, $J = 8.7$ Hz), 6.81 (2H, *d*, $J = 8.7$ Hz)], one 1,2,4-trisubstituted aromatic ring [δ 7.20 (1H, *d*, $J = 1.8$ Hz), 7.09 (1H, *dd*, $J = 8.0$, 1.8 Hz) and 6.83 (1H, *d*, $J = 8.0$ Hz)], one acetyl group [δ 2.08 (3H, s)] and two sets of trans olefinic protons at δ 7.72 and 6.42, and δ 7.65 and 6.38, with coupling constants of 16.0 and 15.9 Hz, respectively. A characteristic doublet signal with a small coupling constant ($J = 3.9$ Hz) at δ 5.47 in the ^1H NMR spectrum was ascribed to the anomeric proton in the α-glucopyranose unit (Binkley et al., 1969). The ^{13}C NMR spectrum had signals caused by two pairs of double bonds, two carbonyl carbons, two aromatic rings (δ 161.4–111.7), two hexoses (δ 104.9–65.4), and one acetyl group (δ 172.9 and 20.9). The sugar moiety was identified as sucrose by direct comparison of the alkaline hydrolysate with an authentic sample of sucrose on TLC. Thus, the basic structure of **1** was concluded to be a bisphenylpropanoyl ester of sucrose with an acetyl

Table 1
Chemical shifts (δ) of ^1H NMR signals of compounds **1** and **2**, and sucrose[a]

	1	**2**	Sucrose[b]
Fructose			
1	3.65 (1H, *d*, 12.2)	3.64 (1H, *d*, 12.2)	3.64 (1H, *d*, 12.3)
	3.60 (1H, *d*, 12.2)	3.59 (1H, *d*, 12.2)	3.60 (1H, *d*, 12.3)
3	5.49 (1H, *d*, 8.2)	5.49 (1H, *d*, 8.1)	4.09 (1H, *d*, 8.2)
4	4.48 (1H, *t*, 8.2)	4.48 (1H, *t*, 8.1)	4.02 (1H, *t*, 8.2)
5	4.15 (1H, [c])	4.15 (1H, [c])	3.75 (1H, [c])
6	4.52 (2H, [c])	4.52 (2H, [c])	3.74 (2H, [c])
Glucose			
1′	5.47 (1H, *d*, 3.9)	5.48 (1H, *d*, 3.7)	5.39 (1H, *d*, 3.8)
2′	3.44 (1H, *dd*, 9.8, 3.9)	3.40 (1H, *dd*, 9.7, 3.7)	3.44 (1H, *dd*, 9.8, 3.8)
3′	3.63 (1H, [c])	3.62 (1H, [c])	3.71 (1H, [c])
4′	3.25 (1H, *dd*, 9.8, 9.1)	3.26 (1H, *dd*, 9.7, 9.2)	3.33 (1H, 9.8, 9.4)
5′	4.16 (1H, [c])	4.15 (1H, *dd*, 9.7, 3.8)	3.80 (1H, [c])
6′	4.16 (1H, [c])	4.15 (1H, [c])	3.71 (1H, [c])
	4.52 (1H, [c])	4.53 (1H, [c])	3.80 (1H, [c])
3-Coumaroyl			
2″, 6″	7.52 (2H, *d*, 8.7)	7.52 (2H, *d*, 8.6)	
3″, 5″	6.81 (2H, *d*, 8.7)	6.81 (2H, *d*, 8.6)	
7″	7.72 (1H, *d*, 16.0)	7.72 (1H, *d*, 15.9)	
8″	6.42 (1H, *d*, 16.0)	6.42 (1H, *d*, 15.9)	
6-Feruloyl			
2‴	7.20 (1H, *d*, 1.8)		
5‴	6.83 (1H, *d*, 8.0)		
6‴	7.09 (1H, *dd*, 8.0, 1.8)		
7‴	7.65 (1H, *d*, 15.9)		
8‴	6.38 (1H, *d*, 15.9)		
OMe	3.90 (3H, *s*)		
6-Coumaroyl			
2‴, 6‴		7.47 (2H, *d*, 8.7)	
3‴, 5‴		6.80 (2H, *d*, 8.7)	
7‴		7.66 (1H, *d*, 15.9)	
8‴		6.36 (1H, *d*, 15.9)	
6′-Acetyl	2.08 (3H, *s*)	2.08 (3H, *s*)	

[a] *J*-values are given in Hz in parentheses.
[b] Data given for reference.
[c] Multiplicity was not determined due to overlapping of the signals.

group. An HMBC experiment determined the locations of the bonds between the *p*-coumaroyl, feruloyl and sucrose units, and of the acetyl group. The *p*-coumaroyl moiety was shown to be esterified with 3-OH of fructose, since a correlation was observed between 3-H (δ 5.49) and the carbonyl carbon of the *p*-coumaroyl moiety (δ 168.3), and that the feruloyl moiety was esterified with the 6-OH of fructose, since an HMBC spectral correlation was observed between 6-H_2 (δ 4.52), one of the methylene protons of fructose, and the carbonyl carbon (δ 168.8) of the feruloyl moiety. The methylene protons at 6 (δ 4.16 and 4.52) of glucose showed a cross-peak with the acetyl carbonyl carbon (δ 172.9), which showed that the acetyl group was linked to 6′-OH of glucose. In the ^{1}H NMR spectra of **1** in CD_3OD, the shift of the signals of fructose 3-H and 6-H_2 to lower field was considered to be caused by the aromatic acid ester bonds, while that of the signals of glucose 6′-H_2 was thought to be due to O-acetylation. Thus, the structure of compound **1** was determined to be 3-*O*-*p*-coumaroyl-6-*O*-feruloyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside.

Compound **2**, a light yellow amorphous solid with a molecular formula of $C_{32}H_{36}O_{16}$, had a UV spectral absorption maximum at 316 nm, which implied the presence of a conjugated double bond system. The IR absorption spectrum showed the presence of hydroxyl groups (3375 cm^{-1}) and aromatic rings (1630, 1603 and 1514 cm^{-1}). As shown in Section 3, the UV, IR, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) spectral features of **2** are generally similar to those of **1**, implying that **2** is also a phenylpropanoid sucrose ester. The ^{1}H NMR and ^{13}C NMR spectral data showed that **2** contained two *p*-coumaroyl groups, two hexoses and one acetyl group. The sugar moiety in **2** was demonstrated to be sucrose, as in **1**. In the ^{1}H NMR spectrum, the coupling constant between the olefinic proton signals at δ 7.72 and 6.42, and that between the signals at δ 7.66 and 6.36 were both 15.9 Hz, showing that these two pairs of olefinic protons represented two trans double bonds. The number of protons on the aromatic rings in the ^{1}H NMR spectrum [δ 7.52 (2H), 7.47 (2H), 6.81 (2H), and 6.80 (2H)] and the coupling constants showed that **2** had two 1,4-disubstituted aromatic rings. In HMBC studies, two *p*-coumaroyl groups were shown to be connected to 3-OH and 6-OH of fructose, since correlations were observed between 3-H (δ 5.49) and a carbonyl carbon (δ 168.3), and between 6-H_2 (δ 4.52) and a carbonyl carbon (δ 168.8). The acetyl group was positioned at 6′-OH on the basis of the cross-peak observed between 6′-H_2 (δ 4.53 and 4.15) and the carbonyl carbon (δ 172.9) of the acetyl group. Accordingly, the structure of **2** was determined to be 3,6-di-*O*-*p*-coumaroyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside.

Table 2
Chemical shifts (δ) of ^{13}C-NMR signals of compounds **1** and **2**, and sucrose

	1	**2**	Sucrose[a]
Fructose			
1	65.4	65.4	64.0
2	104.9	104.9	105.3
3	79.0	79.0	79.3
4	74.4	74.4	75.7
5	81.2	81.2	83.8
6	65.7	65.7	63.3
Glucose			
1′	92.6	92.6	93.6
2′	73.1	73.1	73.2
3′	74.9	74.9	74.6
4′	71.9	71.9	71.3
5′	72.1	72.1	74.4
6′	65.7	65.7	62.2
3-Coumaroyl			
1″	127.1	127.1	
2″, 6″	131.4	131.4	
3″, 5″	116.8	116.8	
4″	161.4	161.4	
7″	147.5	147.5	
8″	114.5	114.5	
9″	168.3	168.3	
6-Feruloyl			
1‴	127.7		
2‴	111.7		
3‴	149.2		
4‴	150.7		
5‴	116.5		
6‴	124.1		
7‴	147.0		
8‴	115.2		
9‴	168.8		
OMe	56.5		
6-Coumaroyl			
1‴		127.1	
2‴, 6‴		131.2	
3‴, 5‴		116.8	
4‴		161.4	
7‴		146.8	
8‴		114.9	
9‴		168.8	
6′-Acetyl			
Me	20.9	20.9	
C=O	172.9	172.9	

[a] Data given for reference.

The phenylpropanoid sucrose ester 6-*O*-*p*-coumaroyl-β-D-fructofuranosyl α-D-glucopyranoside was a colorless amorphous solid that was identified by comparison of its spectral profiles with those in the literature (Wang et al., 2003).

The phenylpropanoids caffeic acid, rosmarinic acid, caffeoyl-4′-hydroxyphenyllactic acid and salvianolic acid B were obtained as light yellow amorphous powders and identified by comparison of their spectroscopic and physical data with the respective data described in the literature (Ai and Li, 1988; Lu and Foo, 1999).

In this study, five known compounds and two new phenylpropanoid sugar esters were isolated from rhizomes of edible canna. The two new phenylpropanoid sugar esters are rather unusual in that the sugar moiety is sucrose. The occurrence of phenylpropanoid esters with sucrose as the sugar component has previously been seen in plants in the Brassicaceae (Linscheid et al., 1980), Polygonaceae (Fukuyama et al., 1983), Polygalaceae (Hamburger and Hostettmann, 1985), Liliaceae (Shimomura et al., 1988), Rosaseae (Yoshinari et al., 1990), Sparganiaceae (Shirota et al., 1996), Iridaceae (Lin et al., 1998) and Compositae families (Wang et al., 2003). This is the first report on the isolation of phenylpropanoid esters of sucrose from a plant of the family Cannaceae.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-360 digital polarimeter. ESI-MS spectra were obtained on a Micromass LCT spectrometer, and UV and IR absorption spectra were obtained on a Hitachi U-2100 spectrophotometer and a JASCO FT/IR-620 spectrometer, respectively. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker DRX-500 spectrometer in CD_3OD. Standard pulse sequences and parameters were used for the experiments. ^{1}H chemical shifts in CD_3OD were referenced to residual CD_2HOD (3.31 ppm); ^{13}C chemical shifts were referenced to the solvent (CD_3OD, 49.0 ppm). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Art. 5715; Merck) and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% ethanolic solution of phosphomolybdic acid (Wako Pure Chemical Industries) followed by heating at 110 °C. HPLC was performed using a JASCO PU-980 pump (flow speed: 5 ml/min), a JASCO UV-975 detector (254 nm), and a Mightysil RP-18 column (250 × 20 mm i.d., particle size 5 μm), or using two Shimadzu LC-10AT pumps (flow speed: 1 ml/min), a SPD-10A detector (254 nm), and a Mightysil RP-18 column (250 × 4.6 mm i.d., particle size 5 μm).

3.2. Plant materials

Fresh rhizomes of cultivated *C. edulis* Ker Gawl. (10 kg) were obtained from Tanegashima Experimental Station, National Institute of Health Sciences, in Kagoshima prefecture, in 2001 and air-dried in the shade. A voucher specimen has been deposited at Tanegashima Experimental Station, National Institute of Health Sciences.

4. Extraction and isolation

Dry rhizomes of *C. edulis* Ker Gawl. (3.5 kg) were cut into thin slices and extracted with MeOH (15 l × 3) at room temperature for one week. After filtration and removal of the solvent by evaporation in vacuo, a residue (84.6 g) was obtained, which was suspended in H_2O (1 l). The suspension was then treated with *n*-hexane (1 l × 3), EtOAc (1 l × 3), and *n*-BuOH (1 l × 3), successively, to give, after removal of the solvent, *n*-hexane-soluble (14.1 g), EtOAc-soluble (5.8 g), and *n*-BuOH-soluble (8.4 g) residues. The *n*-hexane-soluble residue was not analyzed further in the present study.

The ethyl acetate-soluble residue was subjected to silica gel (150 g) column chromatography (CC) using a $CHCl_3$–MeOH gradient system (1:0–1:1) to give five fractions (frs. 1a–5a). Fr. 3a (0.69 g) gave caffeic acid (36.5 mg) when subjected to ODS CC (2.5 cm × 45 cm) eluting with H_2O–MeOH (1:4) followed by ODS-HPLC eluting with H_2O–MeCN–HOAc (85:15:1). Fr. 4a (0.80 g) gave compounds **1** (4.9 mg), **2** (4.1 mg), and caffeoyl-4′-hydroxyphenyllactic acid (4.4 mg) when purified by Sephadex LH-20 chromatography eluting with a mixture of H_2O–MeOH (1:1, 1:4 and 0:1) followed by ODS-HPLC eluting with H_2O–MeCN (70:30) for **1** and **2** and with H_2O–MeCN–HOAc (75:25:1) for caffeoyl-4′-hydroxyphenyllactic acid.

The *n*-butanol-soluble residue was subjected to HP-20 column (7.5 cm × 45 cm) chromatography eluting with increasing concentrations of MeOH in H_2O to give four fractions (frs. 1b–4b). Chromatography of fr. 1b (0.27 g) over Sephadex LH-20 (2.5 cm × 35 cm) eluting with a mixture of H_2O–MeOH (1:4) and purification by ODS-HPLC with H_2O–MeCN–HOAc (65:35:1) gave rosmarinic acid (9.3 mg). Fr. 2b (1.26 g) was subjected to silica gel CC eluted with a $CHCl_3$–MeOH gradient (1:0–1:1) to give six fractions (frs. 1c–6c). Separation of fr. 5c (181.3 mg) by ODS-HPLC with H_2O–MeCN–HOAc (85:15:1) afforded 6-*O*-*p*-coumaroyl-β-D-fructofuranosyl α-D-glucopyranoside (2.5 mg). Fr. 6c (191.3 mg) was further separated by ODS-HPLC with H_2O–MeCN–HOAc (70:30:1) to give salvianolic acid B (4.3 mg).

3-*O*-*p*-Coumaroyl-6-*O*-feruloyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside (**1**) was obtained as a light yellow amorphous solid. In 0.00014% (w/w) yield of the dry rhizome. $[\alpha]_D^{23}$ +23.8 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (nm) 319 (log ε 4.84); IR (neat) ν_{max} (cm^{-1}) 3388, 1702, 1630, 1603, 1515; for ^{1}H NMR (CD_3OD, 500 MHz); ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1 and 2; HR ESI-MS *m/z* 729.2057 $[M + Na]^+$ (calcd. for $C_{33}H_{38}O_{17}Na$, 729.2007).

3,6-*O*-Di-*p*-coumaroyl-β-D-fructofuranosyl 6-*O*-acetyl-α-D-glucopyranoside (**2**) was obtained as a light yellow amorphous solid. In 0.00012% (w/w) yield of the dry rhizome. $[\alpha]_D^{23}$ +23.4 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (nm) 316 (log ε 4.76); IR (neat) ν_{max} (cm^{-1}) 3375,

1700, 1630, 1603, 1514; ^{1}H NMR (CD_3OD, 500 MHz); ^{13}C-NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1 and 2; HR ESI-MS m/z 699.1916 $[M+Na]^+$ (calcd. for $C_{32}H_{36}O_{16}Na$, 699.1901).

4.1 Alkaline hydrolysis of **1** and **2**

Each phenylpropanoid sugar ester (ca. 0.5 mg) was dissolved in 3% KOH–MeOH (1 ml) and kept at room temperature for 2 h. The mixture was neutralized with 1 N HCl and subjected to Sephadex LH-20 column chromatography eluting with MeOH. The sugar-containing fractions were analyzed by TLC (solvent: acetone–*n*-BuOH–HOAc (5:4:1)). The spots were detected by treating the plate with an ethanolic solution of 10% phosphomolybdic acid reagent followed by heating, and compared with reference sugars on a silica gel plate ($R_f = 0.20$).

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