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Caffeic Acid Derivatives in the Roots of Yacon (*Smallanthus sonchifolius*)

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Five caffeic acid derivatives were found in the roots of yacon, *Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson, Asteraceae, as the major water-soluble phenolic compounds. The structures of these compounds were determined by analysis of spectroscopic data. Two of these were chlorogenic acid (3-caffeoylquinic acid) and 3,5-dicaffeoylquinic acid, common phenolic compounds in plants of the family Asteraceae. Three were esters of caffeic acid with the hydroxy groups of aldaric acid, derived from hexose. The structure of the aldaric moiety was determined by hydrolysis and comparison of NMR spectra with those of standard aldaric acids. The compounds were novel caffeic acid esters of altraric acid: 2,4- or 3,5-dicaffeoylaltraric acid, 2,5-dicaffeoylaltraric acid, and 2,3,5- or 2,4,5-tricaffeoylaltraric acid.

KEYWORDS: Yacon; *Smallanthus sonchifolius*; Asteraceae; caffeic acid; altraric acid

INTRODUCTION

Yacon, *Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson, is a plant originally cultivated in South America, and the fresh root is eaten like a fruit in this area. Yacon was introduced to Japan in 1985 and has been gradually paid attention to due to its abundant content of fructooligosaccharide, which has some health-promoting effects such as improvement in the intestinal microflora balance, as a storage sugar in place of starch in its root (*1*). Yacon root is sometimes used for home cooking but has not been a common foodstuff because of decaying easily or rapid browning of the juice or injured tissues. The browning may be caused by condensation reaction of polyphenols with amino compounds (*2*) and enzymatic polymerization of polyphenols (*3*). Yacon juice contains 850 ppm polyphenol compounds (*4*), which generally have antioxidative activity, and chlorogenic acid was reported as a major antioxidant in yacon as well as tryptophan (*5*). In this study, we investigated on phenolic fraction of yacon roots and found three compounds containing altraric acid, which has rarely been reported previously.

MATERIALS AND METHODS

Reagents. Methanol-d_4 and D_2O were obtained from E. Merck (Darmstadt, Germany). D-Glucaric acid potassium salt was obtained from Nacalai Tesque (Kyoto, Japan), and D-talose was from Acros. Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan).

Plant Material and Isolation of Phenolic Compounds. Yacon tuberous roots were harvested at National Agricultural Research Center for Western Region in Japan and stored at 4 °C until use. A crude water extract of yacon root was analyzed by analytical HPLC on a 15 cm × 4.6 mm i.d. Cosmosil 5C18-MS packed column (Nacalai tesque, Kyoto, Japan) with MD-915 photodiode array detector set at 200−600 nm with a linear gradient of methanol (5−33.5%) in 5% acetic acid aqueous solution for 60 min at a flow rate of 1.0 mL/min. A whole yacon root (160 g) was homogenized in 0.2 M L-ascorbic acid aqueous solution (160 mL). The homogenate was roughly filtered with gauze and centrifuged at 1000*g* for 10 min. The supernatant was filtered with no. 5B filter paper (Kiriyama, Tokyo, Japan) and passed through a 19 cm × 2.6 cm i.d. column of Cosmosil 140 C_{18} (Nacalai tesque, Kyoto, Japan) for solid-phase extraction. The column was washed with water (1 L) to remove ascorbic acid, water-soluble sugars, proteins, and other common constituents and eluted with 30% methanol in water (500 mL). The eluate from solid-phase extraction was concentrated in vacuo and applied to a preparative HPLC using a 25 cm × 2 cm i.d. 5C18-MS Cosmosil packed column (Nacalai tesque, Kyoto, Japan) monitored with an 875-UV detector (Jasco, Tokyo, Japan). Phenolic compounds containing a caffeoyl moiety were eluted with a linear gradient of methanol (10−30%) in 2% acetic acid aqueous solution for 80 min at a flow rate of 8.0 mL/min and detected at 326 nm. After removal of organic solvent under reduced pressure, each fraction was lyophilized to give a colorless amorphous solid. The percentage yields of compound **1**−**5** were 6.4×10^{-3}, 4.5×10^{-3}, 4.6×10^{-3}, 2.0×10^{-3}, and 4.2×10^{-3} in fresh yacon root, respectively.

Instrumental Analyses. NMR [^{1}H, ^{13}C, double quantum filtered correlated spectroscopy (DQF-COSY), heteronuclear single quantum coherence spectroscopy (HSQC), and heteronuclear multiple bond correlation spectroscopy (HMBC)] spectra were recorded on Bruker

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **2**, **3**, and **5** (800.13 MHz, CD_3OD)

	compound **2**		compound **3**		compound **5**	
atom	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
altraric acid						
2 (5)	5.253 d	1.8	5.493 d	2.5	5.678 d	1.8
3 (4)	4.774 dd	1.8, 9.8	4.196 dd	2.5, 10.0	5.661 dd	1.8, 9.9
4 (3)	5.493 d	9.8	4.582 dd	10.0, 2.8	4.838 dd	9.9, 1.8
5 (2)	4.535 br s		5.499 d	2.8	5.288 d	1.8
2 (5)-*O*-caffeoyl						
2	7.078 d	2.1	7.089 d	2.0	7.086 d	1.9
5	6.782 d	8.3	6.786 d	8.1	6.783 d	8.1
6	6.962 dd	2.1, 8.3	6.987 dd	2.0, 8.1	6.972 dd	1.9, 8.1
7	7.643 d	15.9	7.776 d	15.9	7.656 d	15.9
8	6.351 d	15.9	6.412 d	15.9	6.368 d	15.9
3 (4)-*O*-caffeoyl						
2					7.022 d	1.9
5					6.744 d	8.1
6					6.912 dd	1.9, 8.1
7					7.530 d	15.9
8					6.220 d	15.9
4 (3)-*O*-caffeoyl						
2	7.024 d	2.0				
5	6.758 d	8.3				
6	6.920 dd	2.0, 8.3				
7	7.529 d	15.8				
8	6.231 d	15.8				
5 (2)-*O*-caffeoyl						
2			7.075 d	2.0	7.074 d	1.9
5			6.785 d	8.2	6.778 d	8.2
6			6.973 dd	2.0, 8.2	6.968 dd	1.9, 8.2
7			7.677 d	15.9	7.644 d	15.8
8			6.389 d	15.9	6.363 d	15.8

DRX 300, DRX 600, and Avance 800 spectrometers (Bruker, Karlsruhe, Germany), operating at 300.13, 600.13, and 800.13 MHz, respectively, for ^{1}H and 201.21 MHz for ^{13}C on Avance 800. Tetramethylsilane (TMS) and 2-methyl-2-propanol were used for methanol-d_4 and D_2O solutions, respectively, as internal standards. Mass spectra were obtained on a SX-102 spectrometer (Jeol, Tokyo, Japan) by fast atom bombardment ionization in negative mode. High-resolution Fourier transformation ion cyclotron resonance (FTICR) mass spectra were obtained on an Apex II 70e spectrometer (Bruker Daltonics) by electrospray ionization. UV spectra were recorded on a UV-3100 spectrophotometer (Shimadzu, Kyoto, Japan).

Hydrolysis of Compound 5. One milligram of compound **5** was dissolved in a 0.1 M solution of K_2CO_3/D_2O (0.6 mL) and heated at 80 °C for 60 min in a water bath. The ^{1}H NMR spectrum of the reaction mixture was recorded on the DRX 600 spectrometer.

Synthesis of D-Altraric Acid. D-Altraric acid was synthesized by a conventional method for synthesis of aldaric acids. Fifteen milligrams of D-talose was dissolved in concentrated nitric acid (100 μL) in a flask and heated at 90 °C for 30 min in an oil bath. After heating, excessive nitric acid was removed with an aspirator and appropriate addition of a small amount of D_2O. Subsequently K_2CO_3 (20 mg) was added, and the mixture was heated at 80 °C for 60 min in the oil bath to cleave the lactone ring. The ^{1}H NMR spectrum of the total product was recorded on the DRX 300 spectrometer.

Spectroscopic Data of 2,4- or 3,5-Dicaffeoylaltraric Acid (2). UV (ethanol) λ_{max} ϵ 334 nm (2.90×10^4), 246 (1.51×10^4), 219 (2.16×10^4); fast atom bombardment mass spectrometry (FAB-MS) (glycerol) m/z 533 $[M - H]^-$, 371 $[M - C_9H_7O_3$ (1 caffeoyl group)$]^-$, 209 $[M - C_{18}H_{13}O_6$ (2 caffeoyl groups)$]^-$; high-resolution FTICR-MS (methanol/H_2O = 1/1) calcd for $C_{24}H_{22}O_{14}Na$ ($[M + Na]^+$) 557.09063, found 557.09018. NMR assignments are shown in **Tables 1** and **2**.

Spectroscopic Data of 2,5-Dicaffeoylaltraric acid (3). UV (ethanol) λ_{max} ϵ 331 nm (3.05×10^4), 245 (1.22×10^4), 219 (2.64×10^4); FAB-MS (glycerol) m/z 533 $[M - H]^-$, 371 $[M - C_9H_7O_3$ (1 caffeoyl group)$]^-$, 209 $[M - C_{18}H_{13}O_6$ (2 caffeoyl groups)$]^-$; high-resolution FTICR-MS (methanol/H_2O = 1/1) calcd for $C_{24}H_{22}O_{14}Na$ ($[M + Na]^+$) 557.09063, found 557.09018. NMR assignments are shown in **Tables 1** and **2**.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **2**, **3**, and **5** (200.21 MHz, CD_3OD)

	δ (ppm)		
atom	compound **2**	compound **3**	compound **5**
altraric acid			
1 (6)	171.78	172.52	170.39
2 (5)	73.39	75.46	72.71
3 (4)	71.50	72.27	72.71
4 (3)	75.00	71.76	69.98
5 (2)	69.47	73.77	73.13
6 (1)	174.50	171.35	171.61
2 (5)-*O*-caffeoyl			
1	127.69	127.88	127.84
2	115.25	115.31	115.29
3	146.82	146.88	146.82
4	149.80	149.76	149.80
5	116.53	116.59	116.53
6	123.31	123.20	123.35
7	148.09	147.89	147.21
8	114.38	114.68	114.27
9	168.43	168.60	168.37
3 (4)-*O*-caffeoyl			
1			127.59
2			115.29
3			146.84
4			149.90
5			116.53
6			123.37
7			148.40
8			113.96
9			167.43
4 (3)-*O*-caffeoyl			
1	127.87		
2	115.27		
3	146.82		
4	149.80		
5	116.53		
6	123.26		
7	147.97		
8	114.41		
9	167.77		
5 (2)-*O*-caffeoyl			
1		127.92	127.69
2		115.34	115.29
3		146.88	146.87
4		149.76	149.92
5		116.59	116.56
6		123.16	123.44
7		147.85	148.37
8		114.73	114.15
9		168.60	168.09

Spectroscopic Data of 2,3,5- or 2,4,5-Tricaffeoylaltraric acid (5). UV λ_{max} ϵ 333 nm (4.19×10^4), 246 (2.33×10^4), 219 (3.36×10^4); FAB-MS (glycerol) m/z 695 $[M - H]^-$, 533 $[M - C_9H_7O_3$ (1 caffeoyl group)$]^-$, 371 $[M - C_{18}H_{13}O_6$ (2 caffeoyl groups)$]^-$, 209 $[M - C_{27}H_{19}O_9$ (3 caffeoyl groups)$]^-$; high-resolution FTICR-MS (methanol/H_2O = 1/1) calcd for $C_{33}H_{28}O_{17}Na$ ($[M + Na]^+$) 719.12231, found 719.12187. NMR assignments are shown in **Tables 1** and **2**.

RESULTS AND DISCUSSION

The results of analytical HPLC with photodiode array detection showed almost all ingredients had absorption maxima around 326 nm, and the chromatogram at 326 nm is shown in **Figure 1**. Five phenolic compounds **1**−**5** were isolated by preparative HPLC. Compounds **1** and **4** were identified from NMR data (data not shown) as chlorogenic acid (3-caffeoylquinic acid) and 3,5-dicaffeoylquinic acid, respectively. The former has already been reported as a major antioxidant in yacon (*5*).

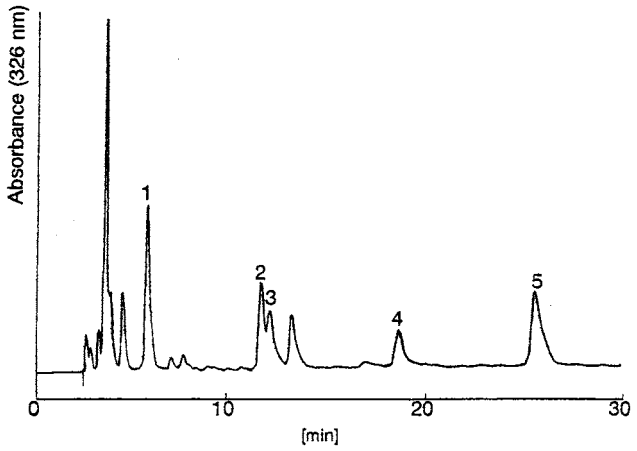
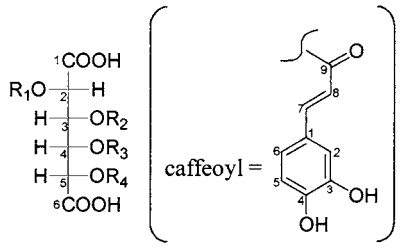


Figure 1. Chromatogram of a crude water extract of yacon root.



2: [R_1=R_3=caffeoyl, R_2=R_4=H] or [R_2=R_4=caffeoyl, R_1=R_3=H]
3: R_1=R_4=caffeoyl, R_2=R_3=H
5: [R_1=R_2=R_4=caffeoyl, R_3=H] or [R_1=R_3=R_4=caffeoyl, R_3=H]

Figure 2. Structure of three caffeic acid esters of altraric acid from yacon.

Chlorogenic acid and 3,5-dicaffeoylquinic acid were found in other asteraceous plants such as burdock (*Arctium lappa* L.) (*6*) and garland (*Chrysanthemum coronarium* L.) (*7*), and they are also widely distributed among plants of other families, for example, coffee (*Coffea canephora*) (*8*) and moroheiya (*Corchorus olitorius* L.) (*9*). Caffeic acid derivatives including these compounds have antioxidative activity resulting in useful functionalities such as protection against ultraviolet rays (*10*) and suppression of melanogenesis (*11*). Compounds **2** and **3** were deduced to be dicaffeoyl esters of hexaric acid, i.e., aldaric acid derived from hexose, and compound **5**, a tricaffeoyl ester of hexaric acid, from their FAB-MS and 1D NMR spectra. **Figure 2** shows the structures of compounds **2**, **3**, and **5**.

Compound **5** showed signals of three caffeoyl groups at low field and four aliphatic protons at 4.8−5.7 ppm on the ^{1}H NMR spectrum. Each of the four aliphatic protons was shown to be bound to each of the four adjoining carbons by DQF-COSY, and both ends of the carbon chain were revealed to be attached to carboxyl groups by HSQC and HMBC. This moiety was therefore determined to be hexaric acid, which was supported by MS data. Binding positions of caffeic acids in compound **5** were considered to be 2,3,5-*O* or 2,4,5-*O* of the aldaric acid from the DQF-COSY, HSQC, and HMBC data. It was possible to make relative sequential assignment of protons and carbons in acyclic aldaric acid in NMR spectra but difficult to assign absolute sequence as indicated in **Tables 1** and **2**. It is because the proton-proton coupling constants of the acyclic compounds do not give enough information connected with dihedral angles since there are many possible conformations in those compounds. This ambiguity makes the complete assignment difficult.

Hexaric acid has six diastereomers. To identify the hexaric acid of compound **5**, it was hydrolyzed to caffeic acid and hexaric acid. The ^{1}H NMR spectra of the hydrolysates revealed

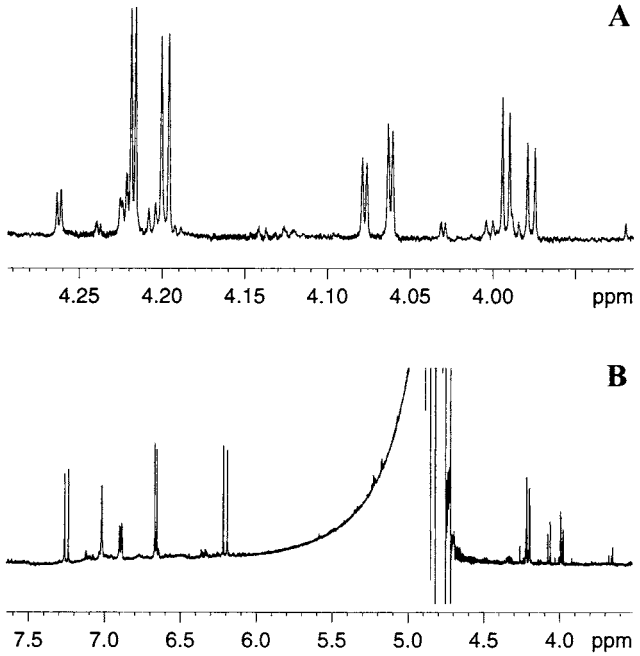


Figure 3. ^{1}H NMR spectrum of hydrolyzed tricaffeoylaltraric acid. (**A**) Aldaric acid moiety; (**B**) total spectrum (600.13 MHz, in D_2O).

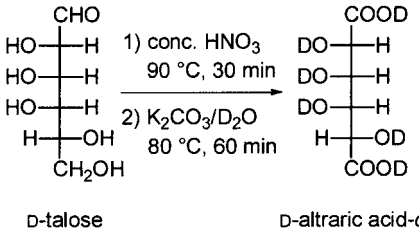


Figure 4. Synthesis of D-altraric acid from D-talose.

the asymmetric structure of the hexaric acid; that is, the four non-hydroxyl protons were magnetically unequivalent (**Figure 3**). Among the six diastereomers of hexaric acid, asymmetrical ones are glucaric acid and altraric acid. Thus the spectrum of the aldaric part of the hydrolysates was compared with those of glucaric acid and altraric acid. Glucaric acid is commercially available, but altraric acid is not. Altraric acid was synthesized from D-talose (**Figure 4**). The ^{1}H NMR spectrum of the aldaric part of the hydrolyzed tricaffeoylhexaric acid coincided with that of altraric acid but not with that of glucaric acid (**Figure 5**). Thus, compound **5** was identified as 2,3,5- or 2,4,5-tricaffeoylaltraric acid.

Compounds **2** and **3** were identified as dicaffeoylaltraric acid in the same way. The binding positions of caffeic acid were elucidated to be 2,4-*O* or 3,5-*O* for compound **2** and 2,5-*O* for compound **3**. As for compound **2**, the correct binding pattern of caffeoyl moieties could not be chosen because of the ambiguity of its acyclic conformation as in the case of compound **5**. As for compound **3**, the positions of substitutions, 2 and 5, were symmetrical in its chain structure. Compounds **2** and **3** were, therefore, identified as 2,4- or 3,5-dicaffeoylaltraric acid and 2,5-dicaffeoylaltraric acid, respectively.

Yacon contains abundant caffeic acid derivatives in the roots, in which the rare caffeic acid esters with altraric acid found here are the major polyphenol compounds together with chlorogenic acid and 3,5-dicaffeoylquinic acid. Only a few analogous compounds of caffeoylaltraric acids have been reported, such as four isomers of caffeoylglucaric acid from leaves of *Cestrum euanthes* (*12*) and 2- or 5-caffeoylglucaric

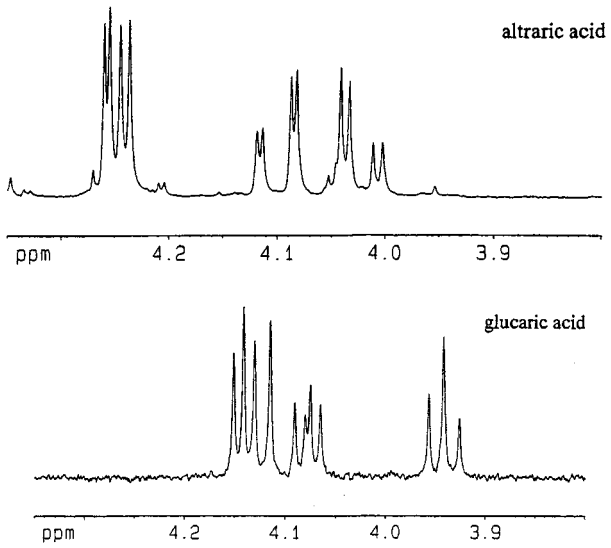


Figure 5. ^{1}H NMR spectra of altraric acid and glucaric acid (300.13 MHz, in D_2O).

acid from cotyledons of tomato (*Lycopersion esculentum*) (*13*). There are some reports of other phenylpropanoid esters of aldaric acid, such as 2-*p*-coumaroylgalactaric acid, 2-feruloylgalactaric acid, 2- or 5-*p*-coumaroylglucaric acid, 2- or 5-feruloylglucaric acid, and 2,4- or 3,5-diferuloylglucaric acid isolated from peel of citrus (*Citrus sinensis*) (*14*, *15*). However, there has been no report of acylated altraric acid. This work shows that yacon is not only a healthful food but also an important source of these rare caffeic acid esters of altraric acid.

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