Long-Chain Alkyl Ferulates in Three Varieties of *Ipomoea batatas* (L.) Lam.

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Chemical components from the roots of *Ipomoea batatas* (L.) Lam. (cv. Simon) with white skin and two other varieties (cvs. Kokei 14 and Narutokintoki) with violet color have been examined to obtain a mixture of long-chain alkyl ferulates, assigned by chemical and spectral methods. The alkyl alcohol parts of the esters were shown to consist of hexadecyl, heptadecyl, and octadecyl components on the basis of MS and GC/MS analysis.

The roots of *Ipomoea batatas* (L.) Lam. (cv. Simon), known as Batata Simão Brazil, were found to be the ethnic food and medicine in Minas Gerais, Brazil, by Simon S. Cheng (Yang, 1974). Since 1973, when the roots were imported to Japan, the plants have been cultivated for food, including health foods. People suffering from anemia, hypertension, diabetes, and hemorrhaging reported improvements in their illnesses using this plant medicinally (Yang, 1974, 1975). On the other hand, the root of *I. batatas* (L.) Lam., known as Fan shu in China (Jiang shu xin yi xue yuan bian, 1977) and Kansho or Bansho in Japan (Akamatsu, 1970), is one of the crude drugs useful for melena, jaundice, gonorrhea, and constipation. Thus, the aim of our present work was to compare the components in the three varieties of *I. batats* (L.) Lam. and to isolate and identify some of them. In this connection, chlorogenic acid, isochlorogenic acids, neochlorogenic acid, caffeic acid, and others have been found in *I. batatas* (L.) Lam. in the United States (Walter et al., 1979) and Japan (Hayase and Kato, 1984).

MATERIALS AND METHODS

The roots of *I. batatas* (L.) Lam. (cv. Simon) produced in Sukumo-shi, Ehime-ken, Japan, were peeled and dried to obtain 0.2 kg of skin and 1.8 kg of flesh. The roots of two varieties of *I. batatas* (L.) Lam. (cvs. Kokei 14 and Narutokintoki) produced in Kagoshima-shi, Kagashima-ken, and Naruto-shi, Tokushima-ken, Japan, respectively, were treated the same as Simon, and 13.4 g of skin and 49.8 g of flesh from Kokei 14 and 9.5 g of skin and 29.3 g of flesh from Narutokintoki were used for experiments.

Extraction. The skin (0.2 kg) and flesh (1.8 kg) of Simon were cut, sliced, and then extracted with chloroform, 0.2 and 1.5 L, respectively, for 20 h three times. All extracts from each part of the roots were combined and concentrated in vacuo. The weights of the final concentrates of viscous brown materials from skin and flesh were 0.4 and 9 g, respectively. Each macerate was extracted with methanol, 0.2 and 1.5 L, respectively, for 20 h three times. All methanol extracts from each part of the roots were treated the same as the chloroform extracts. The weights of methanol extracts for skin and flesh were 31.2 and 238 g, respectively. Skin (13.4 g) and flesh (49.8 g) from Kokei 14 and skin (9.5 g) and flesh (29.3 g) from Narutokintoki were extracted with chloroform, 0.1 and 0.12 L and 0.08 and 0.1 L, respectively, for 20 h three times, to obtain the final concentrate of viscous brown material. The weights of each extract for skin and flesh were 0.26 and 0.36 g and 0.09 g and 0.06 g, respectively.

Detection of the Compounds from Each Extract on TLC. The compounds from each extract were detected by quenching under 254 nm and coloring by spraying 10% H_2SO_4 followed by heating on silica gel TLC (Merck silica gel GF 254) (chloroform).

Separation of Two Compounds from the Extracts of Simon. As shown on Table I, R_f values for the compounds detected in the chloroform extract from skin of Simon were almost the same as the ones from flesh. Both extracts were then combined and chromatographed on a silica gel column with a gradient system of chloroform and methanol. The eluate with chloroform–methanol (9:1) gave a white powder, which consisted of two compounds detected by TLC. Two compounds were separated by column chromatography under the same conditions as the first one.

Spectral Analysis of Esters. The UV and IR spectra were recorded with Hitachi 220A and Hitachi 270-30 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were recorded with a Varian XL-200, and mass spectra and GC/MS were recorded with a Hitachi M 80. For GC/MS, on OV 101 column, 0.3 × 100 cm, was employed with helium as the carrier gas and the temperature was programmed from 150 to 290 °C at 6 °C/min (2-min initial hold, no final hold). The mass spectra were recorded in electron ionization mode at 20 eV,and the ion source temperature was 250 °C. The scan repetition rate was 4 s over the mass range 50–500 amu.

Methylation of Alkyl Ferulates. The mixture of alkyl ferulates (5 mg) dissolved in methanol (3 mL) was treated with excess diazomethane in ether, which was prepared by reaction between *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide in ether and 5% NaOH in a mixture of ethanol and water (10:1), and left to stand at room temperature overnight. The solvent was removed, and the residue was analyzed optically.

Hydrolysis of Alkyl Ferulates. The mixture of alkyl ferulates (10 mg) dissolved in dioxane (1 mL) was refluxed with 10% HCl (1 mL) for 2 h, and then the reaction mixture was treated with ether, into which the acidic product was extracted and concentrated and the resultant product compared to ferulic (Tokyo Kasei Kogyo Co. Ltd.) and isoferulic acids (Hiramoto and Watanabe, 1939) by TLC.

Preparation of Octadecyl Ferulate. Ferulic acid (100 mg) was refluxed with $SOCl_2$ (0.5 mL) in CH_2Cl_2 for 2 h, into which octadecanol (100 mg) in CH_2Cl_2 was added, and the resultant mixture refluxed for 2 h. After the solvent was removed, the residue was purified by silica gel column chromatography with chloroform–methanol (10:1). The eluate was compared by TLC and optical spectra to that of natural alkyl ferulates.

Detection of Alkyl Ferulates from Kokei 14 and Narutokintoki. To detect alkyl ferulates from chloroform extracts of skin and flesh of Kokei 14 and Narutokintoki, TLC data of each extract were compared with those of alkyl ferulates obtained from Simon. The R_f value of the compound from each extract was identical with that of one of the alkyl ferulates giving the reddish color reaction with 10% sulfuric acid on TLC. Then, two extracts from the flesh of Kokei 14 and Narutokintoki were separately thin-layer chromatographed along with alkyl ferulates. One spot from each extract corresponding to the mixture of alkyl ferulates was scraped off, eluted with chloroform, and concentrated. Each residue was applied by EI-MS.

Isolation of Chlorogenic Acid. One of the zones for the methanol extract from Simon corresponding to chlorogenic acid

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Table I. Comparison of the Components in Chloroform Extracts from the Roots of *I. batatas* (L.) Lam. Simon, Kokei 14, and Narutokintoki on TLC[a]

		R_f (×100)																
Simon	skin	98		74		44	43	32	28	24	23		14	12	10	5	3	0
	flesh	98	87	74	59	44	43	32	28	24	23		14	12	10	5	3	0
Kokei 14	skin	98		74	53		43	34			23		15			5		0
	flesh	98		74	53		43				23		15		10	5		0
Narutokintoki	skin	98	87	74	59	44	43	34			23	17	15	10	8	5	3	0
	flesh	98		74	63	44	43				23	17	15	10		5	3	0
alkyl ferulates							43											
β-sitosterol											23							

[a] TLC conditions, see text.

Table II. ^{1}H and ^{13}C NMR Data of Long-Chain Alkyl Ferulates

C no.	^{1}H NMR (200 MHz), δ	^{13}C NMR (50 MHz), δ
1		127.02 (s)
2	7.06 (1 H, d, J = 2.0 Hz)	114.72 (d)
3		147.92 (s)
4		146.78 (s)
5	6.94 (1 H, d, J = 8.0 Hz)	115.65 (d)
6	7.09 (1 H, dd, J = 8.0, 2.0 Hz)	123.02 (d)
7	7.65 (1 H, d, J = 16.0 Hz)	144.63 (d)
8	6.32 (1 H, d, J = 16.0 Hz)	109.30 (d)
9		167.39 (s)
OCH_3	3.94 (3 H, s)	55.92 (q)
$COOCH_2$	4.21 (2 H, t, J = 7.0 Hz)	64.61 (t)
$COOCH_2CH_2$	1.70 (2 H, t, J = 7.0 Hz)	31.93 (t)
$CH_2CH_2CH_2$	1.26 (s)	22.69, 26.01, 28.79, 29.32, 29.56 (each t)
CH_2CH_3	0.88 (3 H, t, J = 7.0 Hz)	14.12 (q)

developed on TLC plate (silica gel, 1-butanol saturated with water) was scraped off and then extracted with 1-butanol saturated with water. After evaporation of the solvent, the residue was methylated with diazomethane in ether at room temperature followed by evaporation of the solvent. The residue was then acetylated by acetic anhydride–pyridine at room temperature overnight. The reaction product was purified by preparative TLC using chloroform–methanol (25:1) on silica gel G. The authentic chlorogenic acid (5 mg) (Wako Pure Chemical Industries Ltd.) in methanol (3 mL) was methylated with diazomethane in ether followed by acetylation with acetic anhydride–pyridine. Both of the products were then compared by TLC (chloroform–methanol (25:1)) and MS.

Identification of the Compound from the Second Eluate. The white powder from the second eluate was compared to authentic β-sitosterol (Nakarai Chemicals Ltd.) by mixed melting point data and thin-layer cochromatography.

RESULTS

R_f values of compounds from each extract are shown on Table I.

First eluted mixture: white amorphous crystals; yield 0.003%; UV (λ_{max}, nm) (MeOH) 327, 300 (sh), 236, 218, 205; IR (ν_{max}, cm^{-1}) (KBr) 3450, 2960, 2930, 2855, 1720, 1640, 1610, 1520, 1430, 1270, 1180; ^{1}H and ^{13}C NMR and mass spectra, Tables II and III.

Optical spectral data for methyl ether derivatives of the mixture are shown on Tables IV and V.

The R_f value for the acidic product after hydrolysis was 0.44, and R_f values for commercial ferulic acid and synthetic isoferulic acid (Hiramoto and Watanabe, 1939) were 0.44 and 0.65, respectively, on silica gel TLC developed with chloroform–methanol (10:1). ^{1}H NMR of the acidic product was identical with that of ferulic acid. The R_f value for the product from esterification of ferulic acid chloride with octadecyl alcohol was the same as for the mixture of alkyl ferulates obtained from the chloroform extract of Simon on TLC. The compound was also identical with natural alkyl ferulates by ^{1}H NMR, except for the signal intensity of methylene protons from the alkyl groups and the m/z 446 ion for octadecyl ferulates (MS).

Alkyl ferulates were detected in Kokei 14 and Narutokintoki by comparison of the TLC (Table I) and MS data (Table VI) with that for the mixture of alkyl ferulates from Simon.

Table III. MS Data of Long-Chain Alkyl Ferulates from Simon

ion	EI-MS: m/z (rel intens)	formula	high-resolut MS: m/z
M^+	418 (40.1)	$C_{26}H_{42}O_4$	418.308 for 418.308
M^+	432 (4.4)	$C_{27}H_{42}O_4$	432.321 for 432.323
M^+	446 (32.5)	$C_{28}H_{46}O_4$	446.339 for 446.339
fragment	194 (100)	$C_{10}H_{10}O_4$	194.056 for 194.057
	177 (61.1)	$C_{10}H_9O_4$	177.059 for 177.055

Table IV. ^{1}H NMR Data of Methyl Ethers of Long-Chain Alkyl Ferulates

C no.	^{1}H NMR (200 MHz), δ
2	7.06 (1 H, d, J = 2.0 Hz)
5	6.94 (1 H, d, J = 8.0 Hz)
6	7.09 (1 H, dd, J = 8.0, 2.0 Hz)
7	7.65 (1 H, d, J = 16.0 Hz)
8	6.32 (1 H, d, J = 16.0 Hz)
OCH_3	3.94 (6 H, s, ×2)
$COOCH_2$	4.21 (2 H, t, J = 7.0 Hz)
$COOCH_2CH_2$	1.70 (2 H, t, J = 7.0 Hz)
$CH_2CH_2CH_2$	1.26 (s)
CH_2CH_3	0.88 (3 H, t, J = 7.0 Hz)

Table V. MS Data of Methyl Ethers of Long-Chain Alkyl Ferulates

GC–MS retention time, min	M^+, m/z	high-resolut MS: m/z
27.2	432	$C_{27}H_{44}O_4$ (432.322 for 432.324)
27.8	446	$C_{28}H_{46}O_4$ (446.343 for 446.339)
28.9	460	$C_{29}H_{48}O_4$ (460.356 for 460.355)

Table VI. Ratio of Long-Chain Alkyl Ferulates from *I. batatas* (L.) Lam. Simon, Kokei 14, and Narutokintoki Calculated by Relative Intensity on MS

	ratio of esters from rel intens, %		
M^+, m/z	Simon	Kokei 14	Narutokintoki
418	52.1	31.7	30.5
432	5.7	1.6	0.0
446	42.2	66.7	69.5

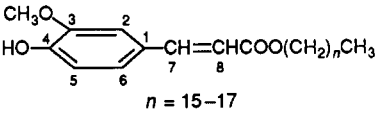
The compound corresponding to chlorogenic acid on preparative TLC was obtained, methylated, and acetylated. The product gave R_f 0.23 by TLC (chloroform–methanol (25:1) on silica gel G) and molecular ion m/z 536 by MS. The product of methylation followed by acetylation of chlorogenic acid gave R_f 0.23 by TLC and molecular ion m/z 536 by MS.

The second eluted compound (yield 0.007%) was identical with authentic β-sitosterol by mixed melting point data and R_f value by TLC. β-Sitosterol was detected in Kokei 14 and Narutokintoki by TLC.

DISCUSSION

A number of compounds were detected in chloroform extracts from three varieties of *I. batatas* on TLC by detection with quenching and coloring by spraying 10% H_2SO_4 followed by heating (Table I). There were some different components among the three varieties and between their skin and flesh. The components of each extract from skin and flesh for Simon were almost the same on silica gel TLC. Then, each extract was combined and column chromatographed.

The white powder was obtained from the first eluate with chloroform–methanol (9:1). Since the powder consisted of two compounds detected on TLC, another column chromatography was carried out for their separation under the same conditions. The first eluate gave the white powder after solvent removal, which had one spot on TLC detected by quenching at 254 nm and coloring in pink by spraying 10% H_2SO_4 and heating. The absorption of the fraction was similar to that of ferulic acid/isoferuric acid by UV spectral analysis. The IR spectrum suggested the presence of aromatic, aliphatic, and ester groups. The signals for the feruly/isoferulyl group and also for alkyl groups on ^1H NMR were assigned as shown on Table II. To confirm the presence of a free hydroxy group on the aromatic ring, the powder was methylated to give a product showing another methoxy signal on ^1H NMR (Table IV) and appeared with three peaks on GC/MS (Table V). Along with these results, the feruly/isoferulyl group and the long-chain alkyl groups were assigned for acidic and alcoholic parts of esters, respectively. The bond for the ethylene chain connected with an aromatic group was determined only as a trans form by ^1H NMR (J = 16.0 Hz) (Table II). Fragment peak m/z 194 appeared as a basic ion corresponding to the ferulic acid/isoferulic acid on MS. Molecular ions (molecular formula) m/z 418 ($C_{26}H_{42}O_4$), 432 ($C_{27}H_{44}O_4$), and 446 ($C_{28}H_{46}O_4$) were equal to 193, the feruloic–isoferuloic part, plus 225, 239, and 253, calculated for hexadecyl, heptadecyl, and octadecyl, respectively (Table III). To determine the acidic group for esters, the powder was hydrolyzed under acidic conditions to obtain the acidic product, which was identified with purchased ferulic acid (R_f 0.44), but not with isoferulic acid (R_f 0.65) obtained by methylation of caffeic acid (Hiramoto and Watanabe, 1939) by comparison on TLC. The peaks detected on ^{13}C NMR were consistent with these assignments (Table II). Molecular ions m/z 432, 446, and 460 for three peaks detected in order on GC/MS for methylation products corresponded to 417, 431, and 445, which were assigned as molecular ions of the original mixture minus 1, plus 15 for CH_3. Their molecular formulas gave $C_{27}H_{44}O_4$, $C_{28}H_{46}O_4$, and $C_{29}H_{48}O_4$ by high-resolution MS, respectively (Table V). Then, the mixture was determined to consist of hexadecyl, heptadecyl, and octadecyl ferulates. A mixture of alkyl ferulates was identical with octadecyl ferulate, which was prepared by reaction between ferulic acid chloride and octadecanol and identified by thin-layer cochromatography and optical spectra. Alkyl ferulates were also detected in Kokei 14 and Narutokintoki by TLC and EI-MS (Table VI). Heptadecyl ferulate was in a relatively smaller amount compared to hexadecyl and octadecyl ferulates; it was not detected in Narutokintoki by EI-MS.



The developed zone from the methanol extract of the skin of Simon, identical with the developed spot of the authentic chlorogenic acid by preparative TLC, was scraped off, extracted, methylated, and acetylated. The product proved to be identical with the compound prepared by methylation followed by acetylation of authentic chlorogenic acid on TLC and MS.

The white powder obtained from the second eluate was confirmed to be β-sitosterol by mixed melting point data and thin-layer cochromatography with the authentic compound. β-Sitosterol, common in the plants, was found in Kokei 14 and Narutokintoki.

In this study, a mixture of hexadecyl, heptadecyl, and octadecyl ferulates was found in the roots of three different varities of *I. batatas* L. (Lam.), although some different compounds were detected by TLC. Hexacosyl ferulate was already reported in oats as an antioxidant (Daniels and Martin, 1967). Therefore, alkyl ferulates from *I. batatas* would have antioxidative activity. It is worthwhile noting that ferulic acid, a component part of alkyl ferulates, is the monomethyl ether of caffeic acid and caffeic acid is a component part for chlorogenic acids known as antioxidants (Hayase and Kato, 1984). One of them, chlorogenic acid, the most active antioxidant, was also detected in Simon. Distribution of hexadecyl ferulate in alkyl ferulates was more than octadecyl ferulate in Simon, but in reverse in Kokei 14 and Narutokintoki. These alkyl ferulates and chlorogenic acids may have effect the body as antioxidants when the roots are taken in the body as food and ethnic drugs. Studies identifying the structures of the rest of compounds detected in the three varieties by TLC are in progress.

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Registry No. Hexadecyl ferulate, 64190-80-3; heptadecyl ferulate, 123641-45-2; octadecyl ferulate, 64190-81-4; chlorogenic acid, 327-97-9; β-sitosterol, 83-46-5.

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Chromium Concentration in Plants: Effects of Soil Chromium Concentration and Tissue Contamination by Soil

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The effect of chromium (Cr) concentration of soil on the Cr concentration in plants was investigated. Tissue samples of a number of different plant species were collected from high-Cr and low-Cr soils found in eastern and western United States. Sample contamination by soil, estimated by titanium (Ti) analysis, was an important factor contributing to the total Cr concentration of most plant tissues analyzed. Plant samples taken from plants growing on high-Cr soils contained higher concentrations of Cr than similar plants growing on low-Cr soils. However, some of this Cr was apparently due to plant contamination by soil. The method used to quantitate contamination was not sensitive enough to be able to determine whether the concentration of Cr absorbed by plants was influenced by the total amount of Cr in the soil. However, it is clear that plant samples taken from the field are contaminated with foreign material that may invalidate certain analytical values.

Chromium (Cr) is important to animal and human nutrition because it is required for normal carbohydrate metabolism (Mertz, 1969; Anderson, 1981). Because part of the human population may be deficient in Cr, a number of studies were initiated to investigate the chemistry of Cr in soil and its uptake by plants (Desmet et al., 1975; Cary et al., 1977a,b; Lahouti and Peterson, 1979; Ramachandran et al., 1980). These studies provided evidence that Cr uptake and translocation by plant cells is very low (i.e., Cr concentration associated with the root is greater than in the leaf, which in turn is greater than in the fruit). Evidence favored plant uptake of Cr(VI) over Cr(III) from soils, perhaps because Cr(VI) is mobile in soil while Cr(III) is not (Cary et al. 1977a,b; Lahouti and Peterson, 1978). Bartlett and Kimble (1976) reported that, in soils, Cr(VI) is reduced to Cr(III) by organic matter. However, in 1979 Bartlett and James reported that Cr(III) is also oxidized to Cr(VI) in soil under conditions that may exist in the field. Lyon et al. (1970) reported that some plants growing on some soils containing relatively high Cr concentrations have elevated Cr concentrations but the portion of Cr due to tissue contamination by soil was not recognized. The objectives of this study were to determine the concentration of Cr in a wide variety of plants growing on soils containing a low or a high, naturally occurring, concentration of Cr, to estimate the apparent contamination by soil of carefully collected plant samples, and to investigate whether a relationship between Cr absorption by plants and soil Cr concentration exists.

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METHODS AND MATERIALS

Sample sites were located and soils were described in cooperation with soil scientists of the USDA's Soil Conservation Service (Table I). Plant and soil samples were also collected by the Soil Conservation Service. The sites included serpentine areas of Maryland, North Carolina, and northern California. The predominant minerology or parent material of the soils sampled was dunite, granite, ash, mixed ash, montmorilonite, mica shist, olivine, serpentine, and some mixed materials with no known dominant mineral. Surface to 15-cm soil samples were taken at all plant sampling sites, dried in clean cloth bags, and ground with a porcelain mortar to pass a 100-mesh polypropylene sieve. After mixing, 0.2-g subsamples of soil were analyzed for titanium (Ti) and Cr by the ICP method of Cary et al. (1986). Plant samples were placed in clean cloth bags, dried at 70 °C to constant weight, and then separated into various plant parts and ground using a micro Wiley mill fitted with a 20-mesh stainless steel screen. Some, but not all, plants were washed with deionized water, then shaken, and put in the cloth bag. Titanium was determined in plants as in soils, and Cr was determined by the AA method of Cary and Olson (1975). Of the plant samples, about 10% was reanalyzed with use of the AA method of Cary and Rutzke (1983) as a quality control mea-