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Content of Methylated Inositols in Familiar Edible Plants

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Supporting Information

ABSTRACT: Familiar plants contain large amounts of inositols; soybean, white clover, red clover, bush clover, locust tree, wisteria, and kudzu of the legume family contain pinitol (3-*O*-methyl-*chiro*-inositol) at approximately 200−600 mg/100 g fresh weight (FW). The contents of pinitol in other plants were 260 mg/100 g FW for sticky mouse-ear, 275 mg/100 g FW for chickweed, and 332 mg/100 g FW for ginkgo. *chiro*-Inositol of 191 and 156 mg/100 g FW was also found in dandelion and Japanese mallotus, respectively. Ononitol (4-*O*-methyl-*myo*-inositol) of 166 mg/100 g FW was found in sticky mouse-ear. Furthermore, young leaves of ginkgo contained sequoyitol (5-*O*-methyl-*myo*-inositol) of 287 mg/100 g FW. Hydroxyl radical scavenging activities of the methylated inositols were higher than those of the original inositols. Effective uses of these familiar edible plants are expected to promote good health.

KEYWORDS: *myo-inositol, chiro-inositol, pinitol, ononitol, sequoyitol, hydroxyl radical scavenging activity*

INTRODUCTION

Inositols (1,2,3,4,5,6-cyclohexane hexaol) have nine isomers with different configurations of six hydroxyl groups. Of these compounds, D-*chiro*-inositol and a compound methylated at the 3-position OH (pinitol) and methylated compounds of *myo*-inositol at the 4-position OH (ononitol) and 5-position OH (sequoyitol) are expected to have several physiological functions such as antihyperglycemic,[1] lipid-lowering,[2,3] antioxidant,[4] and hepatoprotective[5] effects. Kim et al.[6] determined the total *chiro*-inositol concentrations including pinitol, *chiro*-inositol, and their derivatives in 115 natural and food materials to identify economical sources for mass production of pinitol. Their results indicated that carob pod, *Bougainvillea*, soy whey, and soybean oligosaccharides are rich sources for mass production of pinitol. Furthermore, they demonstrated that pinitol isolated from soy whey and carob pod is beneficial for controlling blood glucose in animals with diabetes mellitus. Their natural and food materials, however, were restricted to common foods. On the other hand, petals of carnations[7] and ice plants[4] contain pinitol, and it was demonstrated that pinitol in the plants acts as an osmotic regulator and cryoprotectant in cells.[8] Thus, many plants are expected to contain inositols. In this study, we determined the content of several inositols for familiar edible plants to search for more easily available sources of inositols that can be used for the promotion of health. Furthermore, hydroxyl radical scavenging activities of methylated inositols were also estimated, in expectation of high activities as sugars with numerous hydroxyl groups.[9,10]

MATERIALS AND METHODS

Chemicals. Pinitol and *myo*-inositol were purchased from Sigma-Aldrich Co. LLC., Japan, and P-L Biochemicals, Inc., Milwaukee, WI, USA, respectively. *scyllo*-Inositol and *chiro*-inositol were obtained from Hokko Chemical Industry Co., Ltd., Kanagawa, Japan.

Plants. Plants were collected from the University of Tsukuba in the spring of 2010 as follows: Fabaceae (*Glycine max*, *Trifolium repens*, *Trifolium pratense*, *Pueraria lobata*, *Wisteria floribunda*, *Robinia pseudoacacia*, *Lespedeza bicolor*, *Vicia angustifolia*), Asteraceae (*Artemisia indica*, *Taraxacum officinale*, *Gnaphalium affine*, *Cirsium japonicum*), Plantaginaceae (*Veronica persica*), Lamiaceae (*Lamium purpureum*, *Lamium amplexicaule*), Araliaceae (*Aralia cordata*), Caryophyllaceae (*Cerastium glomeratum*, *Stellaria media*), Rosaceae (*Cerasus* × *yedoensis*), Theaceae (*Camellia sinensis*, *Camellia japonica*), Amaryllidaceae (*Allium macrostemon*), Aquifoliaceae (*Ilex latifolia*), Rubiaceae (*Galium spurium*), Saururaceae (*Houttuynia cordata*), Ginkgoaceae (*Ginkgo biloba*), Cercidiphyllaceae (*Cercidiphyllum japonicum*), Vitaceae (*Cayratia japonica*), Caprifoliaceae (*Lonicera japonica*), Euphorbiaceae (*Mallotus japonicus*), Moraceae (*Morus alba*), and Ebenaceae (*Diospyros kaki*). Furthermore, fresh vegetables were purchased from a supermarket as follows: green bean, green pea, green broad bean, green soybean, flower of florists' daisy (*Chrysanthemum morifolium*), stem and leaf of crown daisy (*Glebionis coronaria*), red shiso (*Perilla frutescens purpurea*), spinach, Chinese cabbage, Chinese colza, broccoli, strawberry, ice plant, and bell pepper. The moisture content of fresh plants or vegetables was determined by heating at 60 °C for 3 h in an oven. The content in 10 g of each plant was measured twice and averaged.

Anion Exchange HPLC. Inositols and sugars were analyzed with a Dionex DX-500 high-performance anion exchange chromatography-pulsed amperometric detection (HPAE-PAD) system (Dionex Corp., Sunnyvale, CA, USA) equipped with a Dionex CarboPac MA1 column (250 mm × 4 mm i.d.) and an MA1 guard column (50 mm × 4 mm i.d.). Elution was performed with an aqueous NaOH solution of 100 mM for 10 min, from 100 to 500 mM in 10 min (a linear gradient), and a final hold at 500 mM for 10 min (or 50 min) at 25 °C at a flow rate of 0.4 mL/min.[7]

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NMR Analysis. NMR spectra were measured with a JNM-A400 spectrometer (JEOL, Japan). The NMR spectra (^{1}H, ^{13}C, DEPT, COSY, CHSHF, HMBC) of isolated inositols were measured with D_2O and 3-(trimethylsilyl)propionic acid sodium salt as an internal standard.

Extraction and Separation of Inositols from Plants. Ten grams of each plant was homogenized in 100 mL of 80% EtOH for 1 min with a Polytron (Kinematica AG, Switzerland) at room temperature. After filtration of the mixture with a filter paper under suction, the residue was re-extracted twice with 100 mL of 80% EtOH. Filtrates were combined and concentrated to dryness with a rotary evaporator under vacuo. The dried filtrate was dissolved in 20 mL of water and extracted three times with 10 mL of *n*-pentane to remove chlorophyll. The water solution was concentrated to remove *n*-pentane and made up to 50 mL with water. One milliliter of the solution was passed through an ODS cartridge (⌀ 1 × 1 cm, BondElut C18, Varian) equilibrated with water after washing thoroughly with MeOH. The pass-through fraction was charged to a Dowex 1 × 4 column ($^-$OH type, ⌀ 0.6 × 3.5 cm) connected to an ODS cartridge. The cartridge was washed with 3 mL of water. The eluate was successively passed through the Dowex small column. After removal of the ODS cartridge, the Dowex column was further washed with 4 mL of water. Eluates (total 8 mL) were combined and made up to 10 mL with water. Twenty-five microliters of the solution containing inositols was analyzed by HPAE-PAD. Extraction and separation of inositols from each plant and HPAE-PAD analysis were carried out twice and the contents of inositols averaged.

Isolation and Identification of Inositols. Several inositols were isolated from the remaining solutions of the above crude extracts and used in the preparation of samples for the HPAE-PAD analyses. The extract solutions were charged to a Wakosil ODS (C18, Tokyo, Japan) column (⌀ 2 × 10 cm) and washed with water. The pass-through fractions were concentrated and further separated with a Dowex 1 × 4 column ($^-$OH type, ⌀2 × 10 cm). Water elutes were fractionated into 5 g each and eluates of each fraction detected by spotting on a TLC plate, spraying 40% sulfuric acid to spots, and heating the plates at 105 °C. Fractions containing inositols were concentrated and lyophilized. Chemical structures of isolates were identified by comparison with NMR spectra of authentic samples or the literature.[11−21]

Determination of Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activities of inositols were determined by adding them to a generating/detecting system and observing their competition with the detector.[9] Hydroxyl radicals were generated from ascorbate and detected by their ability to hydroxylate salicylic acid.[9,10,22] The reaction mixture contained 150 mM K-Pi buffer (pH 7.4), 0.26 mM ascorbic acid, 0.15 mM Fe(III)−EDTA, 0.6 mM H_2O_2, 2 mM sodium salicylate, and 20 mM inositol or other compound in a total volume of 2 mL. After incubation for 60 min at 25 °C, hydroxylated salicylate was extracted and spectrophotometrically determined by the following methods. To the reaction mixture were added 0.5 mL of 2.0 M HCl saturated NaCl and 2 mL of diethyl ether, and then the solution was mixed with a Vortex mixer for 30 s. After removal of the upper ether layer, hydroxylated salicylate was re-extracted with 2 mL of diethyl ether. The combined ether layer was evaporated to dryness at 50 °C with N_2 gas. To the residues dissolved in 0.25 mL of deionized water were added 0.125 mL of 10% (w/v) trichloroacetic acid dissolved in 0.5 M HCl, 0.25 mL of 10% (w/v) sodium tungstate, and 0.25 mL of 0.5% (w/v) sodium nitrite. After standing for 5 min, 0.5 mL of 0.5 M KOH and 1.375 mL of deionized water was added to the solution, and absorbances were measured at 510 nm. Assays for each compound were carried out four times, and the inhibitory activities on salicylate hydroxylation by hydroxyl radicals were averaged.

RESULTS AND DISCUSSION

Separation and Determination of Inositols by HPAE-PAD. Chair conformations of *myo*-inositol, *chiro*-inositol, and *scyllo*-inositol including ononitol, sequoyitol, and pinitol are indicated in Figure 1. Inositols contain six hydrogen atoms. In the *scyllo*-inositol (c), the bonds of hydrogen atoms are all axial, whereas those of the hydrogen atoms at the 2-position in *myo*-inositol (a) and at the 1- and 6-positions in *chiro*-inositol (b) are equatorial. Ononitol and pinitol are methylated compounds consisting of *myo*-inositol at the OH of the 4-position and *chiro*-inositol at the OH of the 3-position. The five compounds having similar structures were well separated by HPAE-PAD with a Dionex CarboPac MA1 column (Figure 2). 5-*O*-Methyl-*myo*-inositol (sequoyitol) and *β*-D-methyl-glucose (d), however, had the same retention times as ononitol and *scyllo*-inositols,

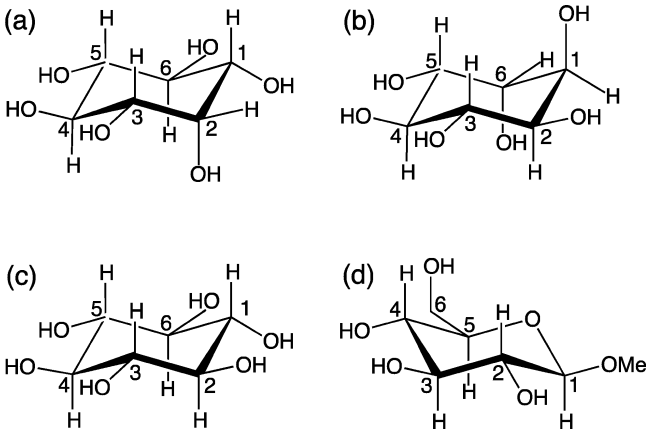

Figure 1. Structures of *myo*-inositol (a), *chiro*-inositol (b), *scyllo*-inositol (c), and *β*-D-methyl-glucose (d). O-methylated compounds at the 4-and 5-positions in (a) and at the 3-position in (b) are ononitol, sequoyitol, and pinitol, respectively.

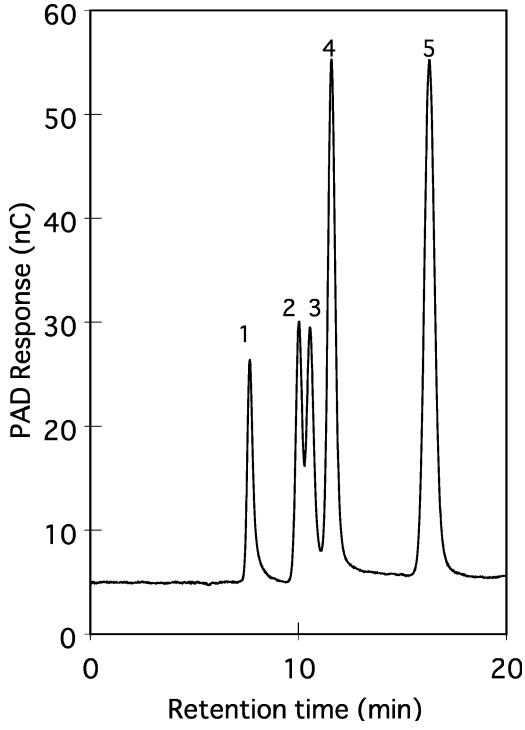

Figure 2. Separation of inositols by HPAE-PAD. Peaks: 1, ononitol or sequoyitol (retention time, 7.7 min); 2, pinitol (10.0 min); 3, *myo*-inositol (10.6 min); 4, *scyllo*-inositol or *β*-D-methyl-glucose (11.6 min); 5, *chiro*-inositol (16.3 min). Additionally, retention time of glucose was 37.4 min. See the details of the separation conditions under Materials and Methods.

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Table 1. Content of Inositols in Familiar Plants[a]

plant	content of inositols (mg/100 g FW)					moisture (g/100 g FW)
	ononitol	pinitol	*myo*-inositol	*scyllo*-inositol	*chiro*-inositol	
soybean (*Glycine max*)						
young leaf	12.6	269.7	–	1.8	4.8	82.47
young bean	–	46.6	48.0	–	6.1	68.29
white clover (*Trifolium repens*)						
leaf	63.6	286.9	70.2	(30.1)[b]	–	83.64
stem	50.1	421.1	–	(110.3)[b]	–	92.04
red clover (*Trifolium pratense*)						
leaf	59.4	387.7	126.2	(18.9)[b]	–	77.12
stem	46.4	458.1	22.6	(65.1)[b]	–	88.33
bush clover (*Lespedeza bicolor*)						
leaf	39.0	452.5	26.7	–	19.0	77.23
locust tree (*Robinia pseudoacacia*)						
young leaf	350.0	625.1	–	–	–	74.63
flower	95.8	269.1	–	–	–	87.28
wisteria (*Wisteria nutt*)						
young leaf	34.8	259.0	26.5	–	–	78.51
flower	–	214.8	15.1	–	–	86.69
kudzu (*Pueraria lobata*)						
young vine	–	212.1	–	–	–	85.97
dandelion (*Taraxacum officinale*)						
young leaf	–	–	80.9	–	141.3	84.66
flower	–	–	173.8	–	191.3	84.40
sticky mouse-ear (*Cerastium glomeratum*)						
stem and leaf	166.2	260.0	22.3	2.5	7.0	86.48
chickweed (*Stellaria media*)						
stem and leaf	11.3	274.7	–	–	6.8	89.86
ginkgo (*Ginkgo biloba*)						
young leaf	(287.0)[c]	332.0	49.1	–	–	80.42
Japanese mallotus (*Mallotus japonicus*)						
young leaf	–	–	19.2	–	156.4	74.97

[a]Underlined values were identified by NMR analyses. –, undetected. [b]In clover, β-D-methyl-glucose was detected instead of *scyllo*-inositol. [c]In gingko, sequoyitol (5-*O*-methyl-*myo*-inositol) was detected instead of ononitol.

respectively (Figure 2), under our HPLC conditions. By PAD, we were able to determine the sugars and cyclitols in the amounts of 10 pmol, without modifications.[7]

Identification of Inositols by NMR. In Table 1, the compounds with the values underlined were all identified by ^{1}H and ^{13}C NMR analyses after the isolations, which were completed by using Wakosil ODS and Dowex 1 × 4 columns as indicated under Materials and Methods. The yields of most isolates in Table 1 were >40%. The ^{1}H NMR spectra in Figure 3 are distinguishable from each other for identifications. The H-2 position in *myo*-inositol, the H-1 and H-6 positions in *chiro*-inositol, and those in their methylated compounds were equatorial (Figure 1). Signals of these protons were detected at approximately 4.0 ppm (Figure 3A–E). Proton signals of methoxyl groups were observed at 3.6 ppm in methylated compounds (Figure 3B,C,E). Methylations of the hydroxyl groups in *myo*-inositol and *chiro*-inositol changed the chemical shifts of the axial protons, especially H-4 of B, H-5 of C, and H-3 of E showed low frequencies. By comparing the signal patterns of the ^{1}H NMR spectra in pinitol, ononitol, and sequoyitol, we were able to identify each of them easily. Furthermore, the spectrum of β-D-methyl-glucose is similar to that of the methylated inositols, but the existences of anomeric proton of H-1 (about 4.4 ppm) and 2 protons of H-6 are remarkably different from the inositols (Figure 3E). On the other hand, the signals of protons and carbon atoms were each observed as one signal (3.35 and 76.4 ppm, respectively) in authentic *scyllo*-inositol, the structure of which is symmetric (Figure 1).

^{1}H NMR and ^{13}C NMR Spectral Data. The spectra of *myo*-inositol, D-*chiro*-inositol, D-pinitol, and β-D-methyl-glucose were in agreement with those of the authentic samples and data in the literature.[11−21] The NMR spectral data of methylated inositols were assigned as follows:

Ononitol: ^{1}H NMR (400 MHz, D_2O) δ 4.05 (1H, t, J = 2.8 Hz, H-2), 3.67 (1H, t, J = 5.6 Hz, H-6), 3.63 (1H, t, J = 5.6 Hz, H-3), 3.60 (3H, s, 4-OMe), 3.49 (1H, dd, J = 10.0, 2.8 Hz, H-1), 3.39 (1H, t, J = 9.2 Hz, H-4), 3.36 (1H, t, J = 9.2 Hz, H-5); ^{13}C NMR (100 MHz, D_2O) δ 85.3 (C-4), 76.5 (C-5), 75.3 (C-6), 75.1 (C-2), 73.8 (C-1), 73.4 (C-3), 62.6 (OMe).[12,15]

Sequoyitol: ^{1}H NMR (400 MHz, D_2O) δ 4.05 (1H, t, J = 2.8 Hz, H-2), 3.71 (2H, t, J = 10.0 Hz, H-4, H-6), 3.60 (3H, s, 5-OMe), 3.55 (2H, dd, J = 10.0, 2.8 Hz, H-1, H-3), 3.07 (1H, t, J = 9.6 Hz, H-5); ^{13}C NMR (100 MHz, D_2O) δ 87.0 (C-5), 74.8 (C-2), 74.5 (C-4, C-6), 73.9 (C-1, C-3), 62.4 (OMe).[14]

D-Pinitol: ^{1}H NMR (400 MHz, D_2O) δ 4.01 (2H, m, H-1, H-6), 3.82 (1H, dd, J = 10.0, 2.0 Hz, H-5), 3.77 (1H, dd, J = 10.0, 2.0 Hz, H-2), 3.66 (1H, t, J = 10.0 Hz, H-4), 3.60 (3H, s, 3-OMe), 3.35 (1H, t, J = 10.0 Hz, H-3); ^{13}C NMR (100 MHz, D_2O) δ 85.6 (C-3), 74.9 (C-4), 74.5 (C-1 or C-6), 74.3 (C-6 or C-1), 73.4 (C-2), 72.6 (C-5), 62.5 (OMe).[13]

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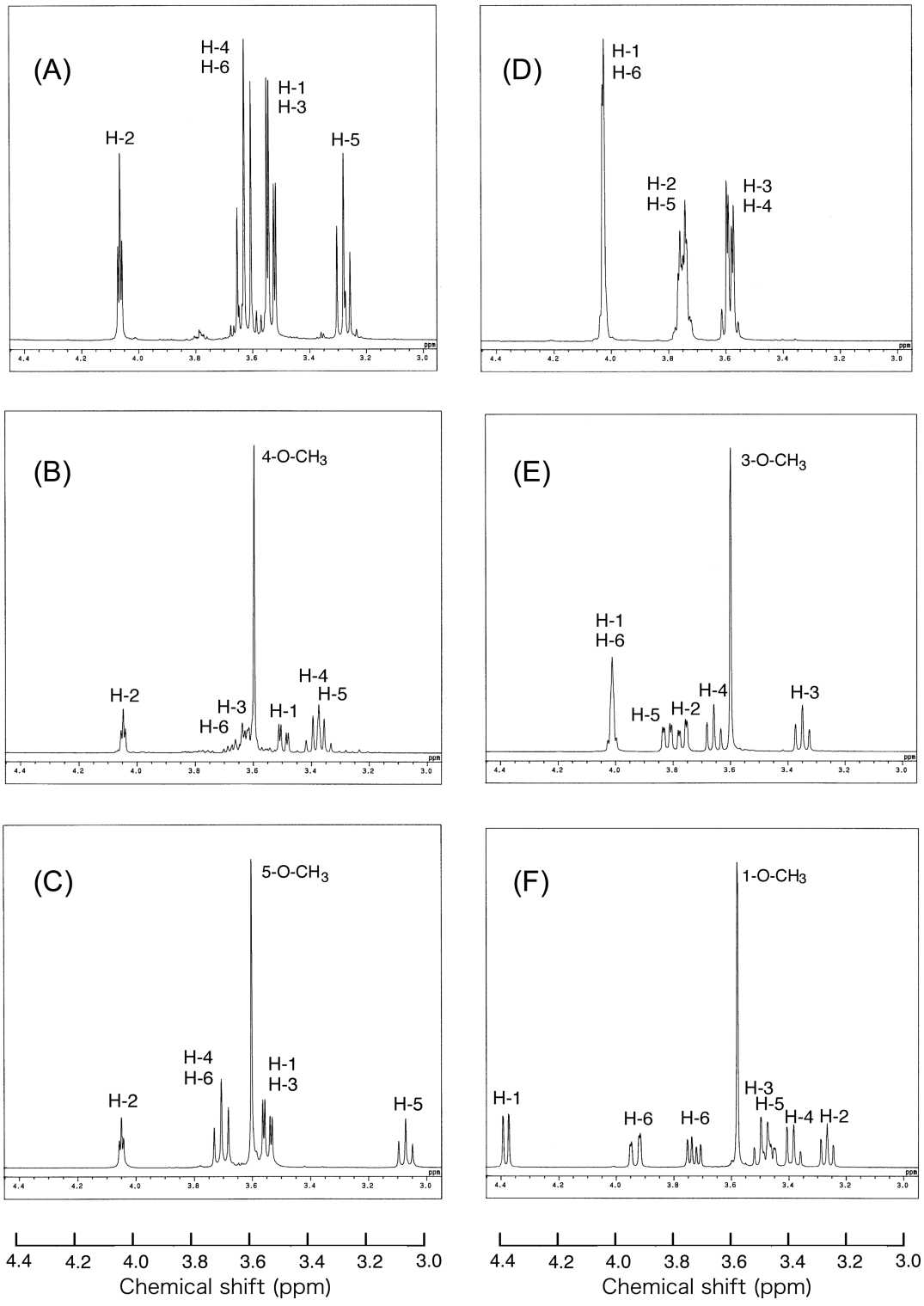

Figure 3. [1]H NMR spectra of several isolates: (A) *myo*-inositol from young leaf of dandelion; (B) ononitol from stem and leaf of sticky mouse-ear; (C) sequoyitol from young leaf of ginkgo; (D) *chiro*-inositol from young leaf of Japanese mallotus; (E) pinitol from stem and leaf of chickweed; (F) *β*-D-methyl-glucose from stem of white clover. As compared with these spectra, only one signal was observed at 3.35 ppm in the spectrum of authentic *scyllo*-inositol.

Content of Inositols in the Familiar Plants. The content of inositols in 45 kinds of edible plants from 23 families was investigated. Table 1 shows that 12 familiar plants contain large amounts of *myo*-inositol, *chiro*-inositol, and their methylated compounds. Soybean, white, red and bush clovers, locust tree, wisteria, and kudzu, which are all Fabaceae plants, contained pinitol of 200−600 mg/100 g fresh weight. Similar amounts of pinitol were found in sticky mouse-ear, chickweed, and ginkgo, whereas dandelion and Japanese mallotus contained large amounts of nonmethylated type *chiro*-inositol. Kim et al.[6] reported that total *chiro*-inositol contents in 100 g dry weight of carob pod, *Bougainvillea*, soy whey, and soybean oligosacchar-

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ides were 4.0, 2.0, 2.0, and 1.74 g, respectively. A comparison with moisture contents showed 100 g dry weights of pinitol for young leaves of soybean, stems of white clover, stems of red clover, young leaves of locust tree, sticky mouse-ear, and chickweed were 1.54, 5.29, 3.93, 2.46, 1.92, and 2.71 g, respectively. On the other hand, peaks of ononitol were detected in Fabaceae plants and sticky mouse-ear, especially in the young leaves of locust trees. In ginkgo a peak of ononitol was detected, but this compound was identified as sequoyitol by NMR analysis after isolation. Similarly, in the peaks of *scyllo*-inositol detected in white and red clovers by HPAE-PAD, *β*-D-methyl-glucose was detected. In plants the accumulation of pinitol is commonly thought to be beneficial for stress adaptation and stabilization of membranes against osmotic alternations.[13] Furthermore, the plants used in this study were collected in the spring, suggesting that plants accumulate large amounts of inositols as cryoprotectants to protect themselves against the winter cold.[8] Furthermore, Adams et al. reported an apparent correlation in the postharvest longevity of flowers and content of inositols, particularly D-pinitol.[7] Pinitol content of flower petals from carnation was 7.2 mg/g FW.[7] It is presumed that pinitol is biosynthesized from *myo*-inositol via ononitol or sequoyitol.[15,23,24] In dandelion and Japanese mallotus (Table 1), it is suggested that some *myo*-inositol remains without methylation and that ononitol has already been epimerized and demethylated to be *chiro*-inositol, whereas pinitol in ginkgo, which belongs to gymnosperm, seems to be synthesized via sequoyitol.[15,23,24] In the plants not shown in Table 1, 4.9 mg/100 g FW of ononitol, 19.0 mg/100 g FW of pinitol, and 3.4 mg/100 g FW of *myo*-inositol in ice plant (*Mesembryanthemum crystallinum*) and 40.8 mg/100 g FW of pinitol and 27.9 mg/100 g FW of *myo*-inositol in *Artemisia indica* were detected. Furthermore, 160−210 mg/100 g FW of *myo*-inositol was detected in the leaves of *Cercidiphyllum japonicum*, *Lonicera japonica*, *Morus alba*, and *Diospyros kaki*. Furthermore, dandelion and Japanese mallotus (Table 1) contained 191.3 (or 141.3) mg/100 g FW and 156.4 mg/100 g FW of *chiro*-inositol, respectively. These high contents are compared to the other plants in Table 1. The sprouts of mung bean are reported to contain 5.79 mg/g DW or less of *chiro*-inositol,[25] which is not more than half the content of dandelion or almost the same as Japanese mallotus.

Hydroxyl Radical Scavenging Activity of Inositols. Table 2 shows the inhibitory effects of *myo*-inositol, *scyllo*-inositol, *chiro*-inositol, their methylated inositols, and several other compounds on salicylate hydroxylation by hydroxyl radicals. Inhibition with *myo*-inositol and *scyllo*-inositol was approximately 55%. Inhibition by *chiro*-inositol, mannitol, sorbitol, and glucose was about 65%. Furthermore, inhibitory activities of pinitol, ononitol, sequoyitol, and *β*-D-methyl-glucose were about 75%, which are higher than those of their native compounds by 10−20%. These results indicate that the methylated compounds have high activities for hydroxyl radical scavenging. On the other hand, substantial amounts ranging from 2.5 to 6.5 mM of *myo*-inositol, *chiro*-inositol, and *scyllo*-inositol were detected in mouse blood plasma after oral administration of 1 g/kg BW of three inositols.[26] This suggests that inositols can function as strong hydroxyl radical scavengers in the human body.

Applications in Our Daily Lives. This study showed that plants containing large amounts of inositols are commonly grown as well-known plants (Table 1). Boiled young soybeans are very often eaten in Japan, so people can ingest pinitol in their daily lives. However, the young leaves of soybean can be easily used for fried foods and soups. Young vines of kudzu are grown in spring to summer. Other Fabaceae plants such as clovers and locust trees are found everywhere and can be easily used. Furthermore, dandelion, sticky mouse-ear, and chickweed are weeds that are useful for human health. Leaves of ginkgo tree, dandelion, and kaki are already used as herbal teas in Japan. Other than eating plants, extracts from plants with boiled water are useful for cooking. Kim et al.[6] isolated pinitol from soy whey and carob pod and indicated that oral administration of soy pinitol and carob pinitol (10 mg/kg) decreased blood glucose by 15% at 2−6 h in streptozotocin-induced diabetic rats. This study demonstrated that pinitol can be beneficial in controlling blood glucose in an animal model of diabetes mellitus. On the other hand, considerable amounts of *myo*-inositol, *chiro*-inositol, and their methylated compounds, which are effective hydroxyl radical scavengers (Table 2), may be incorporated into the blood.[26] These results indicate that inositols play very important roles for protecting humans from stresses. Although we did not examine the physiological functions of inositols from the plants in this study, isolates from our plants likely have the same functions as those from soy whey and carob pod. Ingesting these plants is expected to provide good effects for human health. The familiar edible plants in this study may be more easily available and economical sources of inositols.

Table 2. Inhibitory Effect of Various Compounds on Salicylate Hydroxylation by Hydroxyl Radicals

compound	inhibition[a] (%)
myo-inositol	54.5 ± 3.8
scyllo-inositol	56.2 ± 1.5
chiro-inositol	66.0 ± 1.0
pinitol (3-*O*-methyl-*chiro*-inositol)	75.6 ± 1.3
ononitol (4-*O*-methyl-*myo*-inositol)	74.9 ± 1.7
sequoyitol (5-*O*-methyl-*myo*-inositol)	76.4 ± 1.2
mannitol	65.8 ± 2.0
sorbitol	66.9 ± 1.3
glucose	64.3 ± 2.4
β-D-methyl-glucose	75.7 ± 1.2
proline	22.3 ± 3.3
glycinebetaine	0.5 ± 3.4

[a]Inhibition (%) is expressed as $(1 - P/C) \times 100$, where P is A_{510} in the salicylate hydroxylation reaction with inositols or other compounds and C is A_{510} in the control reaction without those compounds; values are means ± SD.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra (^{1}H, DEPT, and COSY) of several isolates in Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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