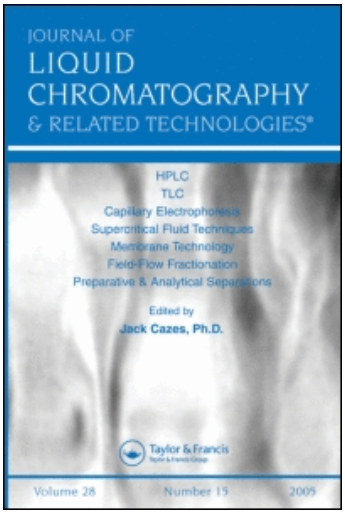

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High Performance Liquid Chromatographic Determination of Ginkgotoxin and Ginkgotoxin-5′-Glucoside in *Ginkgo Biloba* Seeds

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Abstract: A method for simultaneous determination of ginkgotoxin (4′-O-methylpyridoxin), ginkgotoxin-5′-glucoside, and vitamin B_6 compounds in Ginkgo biloba seeds, has been developed by using high performance liquid chromatography (HPLC) with fluorescent detection. Satisfactory separation was accomplished on a C_{18} reversed-phase column by gradient elution with potassium phosphate containing sodium 1-pentanesulfonate (pH 2.5)-acetonitrile within 20 min. The linearity of the calibration curves for ginkgotoxin and ginkgotoxin-5′-glucoside ranged from 0.1 pmol to 10 pmol per injection, and the detection limit was 0.025 pmol (SN = 5). Hot water extracts of raw, canned, and plastic packaged Ginkgo biloba seeds obtained in Japan were analyzed by using this method.

Keywords: Ginkgotoxin, Ginkgotoxin-5′-glucoside, Ginkgo biloba seeds, HPLC, Fluorescence detection

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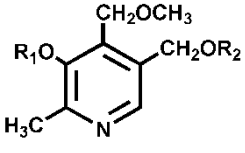
INTRODUCTION

Ginkgo biloba seeds have been used as food and medicine in China and Japan. It is known that Ginkgo biloba seeds contain a component that causes poisoning characterized by convulsions and loss of consciousness, and that over consumption of seeds leads to toxicity, especially in children under six years old.[1,2] Isolation and structural analysis of the toxic component from Ginkgo biloba seeds by Wada et al. revealed that the neurotoxic antivitamin B_6, ginkgotoxin (4′-O-methylpyridoxine, 3-hydroxy-5-hydroxy-methyl-4-methoxymethyl-2-methylpyridine, **1** in Figure 1) is responsible for this poisoning.[3,4] On the other hand, Scott et al. first reported the presence of ginkgotoxin-5′-glucoside (3-hydroxy-5-hydroxymethyl-4-methoxymethyl-2-methylpyridine 5′-O-β-D-glucopyranoside, 2) in Ginkgo biloba seeds in 2000.[5] The structure of ginkgotoxin-5′-glucoside was confirmed by analysis of mass spectrum using LC/ESI-MS, and the liberation of ginkgotoxin by enzymatic hydrolysis with β-glucosidase; however, direct determination of ginkgotoxin-5′-glucoside in Ginkgo biloba seeds was not performed because of the unavailability of an authentic specimen of ginkgotoxin-5′-glucoside. The content of ginkgotoxin- 5′-glucoside was, therefore, estimated from total and free ginkgotoxin contents obtained with or without enzymatic hydrolysis.[5,6] Information on the content of ginkgotoxin-5′-glucoside in Ginkgo biloba seeds and products such as canned seeds is important from a toxicological point of view, since the presence of ginkgotoxin-5′-glucoside may affect the potential toxicity of Ginkgo biloba seeds, although little is known about the toxicity and bioavailability of ginkgotoxin-5′-glucoside in experimental animals and humans.

Separation and quantification of vitamin B_6 compounds and also ginkgotoxin are carried out, mainly by high performance liquid chromatography (HPLC) coupled with appropriate detection systems because of the structural characteristics of the compounds.[7–9] It is generally accepted that HPLC with a fluorescent detection system is useful as a sensitive assay.



1 : R_1 = H R_2 = H
2 : R_1 = H R_2 = β-O-Glucosyl
3 : R_1 = β-O-Glucosyl R_2 = H

Figure 1. Structures of ginkgotoxin and ginkgotoxin glucosides. 1: Ginkgotoxin, 2: Ginkgotoxin-5′-glucoside, 3: Ginkgotoxin-3-glucoside.

The aim of this study was to develop an HPLC method for the quantification of ginkgotoxin and its 5′-glucoside in raw, canned, and plastic-packaged Ginkgo biloba seeds using chemically synthesized authentic specimens.

EXPERIMENTAL

Materials

Ginkgotoxin and 5′-O-(β-D-glucopyranosyl)pyridoxine (pyridoxine-5′-glucoside) were chemically synthesized as reported previously.[10,11] 4-Pyridoxic acid and pyridoxal hydrochloride were purchased from Sigma (St. Louis, MO, USA). Pyridoxine hydrochloride, sodium 1-pentanesulfonate and 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose were obtained from Wako Pure Chemical (Osaka, Japan). β-D-Glucosidase (from sweet almond) was obtained from Oriental Yeast Co., Ltd (Tokyo, Japan). Bond Elut C_{18} cartridges were purchased from Varian (Harbor City, CA, USA). Reversed-phase column chromatography for purification of ginkgotoxine- 5′-glucoside was carried out using a column packed with Bond Elut C_{18} resins. All other reagents were of analytical grade.

Synthesis of Ginkgotoxine-5′-Glucoside

Acetic anhydride (0.8 mL) was added dropwise to a solution of ginkgotoxin (1.90 g), triethylamine (1.6 mL), and dimethylaminopyridine (0.38 g) in dichloromethane (60 mL), under ice-cooling. The mixture was stirred under ice-cooling for 20 min, and then the solvent was evaporated in vacuo. The residue was diluted with saturated sodium hydrogen carbonate and extracted with ethyl acetate. The organic layer was washed with saturated sodium hydrogen carbonate and dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel, using chloroform/methanol (50/1, v/v) as an eluent, to give 3-acetoxy-5-hydroxymethyl-4-methoxymethyl-2-methylpyridine (ginkgotoxin-3-acetate) (1.16 g) as a pale yellow oily product. IR (neat):3300 (OH), 1775 (C═O). ^{1}H-NMR ($CDCl_3$):2.34 (3H, s, CH_3CO-), 2.36 (3H, s, 2-CH_3), 3.33 (3H, s, 4-$CH_2OC\underline{H_3}$), 4.45 (2H, s, 4-$C\underline{H_2}OCH_3$), 4.64 (2H, s, 5-$C\underline{H_2}OH$), 8.31 (1H, s, 6-H).

A mixture of ginkgotoxin-3-acetate (1.82 g), 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (3.99 g),[12] silver carbonate (2.68 g), and Molecular Sieves 3A (14 g) in dry dichloromethane (50 mL) was refluxed for 3 days in the dark. After removal of insoluble materials by filtration, the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel using benzene/acetone (5/1) and

recrystallization from ethyl acetate/hexane, to give ginkgotoxin-5′-glucoside pentaacetate (2.0 g) as colorless prisms. mp: 126–127°C. IR (nujol):1750 (C═O). ^{1}H-NMR ($CDCl_3$):1.96, 1.98, 1.99, 2.08, 2.33 (each 3H, s, CH_3CO-), 2.39 (3H, s, 2-CH_3), 3.26 (3H, s, 4-$CH_2OC\underline{H}_3$), 3.67 (1H, ddd, J = 2.3, 4.6, 10.0 Hz, Glc H-5), 4.14 (1H, dd, J = 2.3, 12.0 Hz, Glc H-6), 4.24 (1H, dd, J = 4.6, 12.0 Hz, Glc H-6), 4.36, 4.40 (2H, AB, J = 12.0 Hz, 4-$C\underline{H}_2O$-), 4.54 (1H, d, J = 8.0 Hz, Glc H-1), 4.69, 4.97 (2H, AB, J = 12.6 Hz, 5-$C\underline{H}_2O$-), 4.98-5.09 (3H, m, Glc H-2, H-3, H-4), 8.31 (1H, s, 6-OH).

Potassium hydroxide, 2 M (2 mL) was added to a solution of ginkgotoxin-5′-glucoside pentaacetate (222 mg) in methanol (4 mL). The mixture was stirred at room temperature overnight in the dark. The pH was adjusted to 8–9 with acetic acid, and the solvent was evaporated in vacuo. The residue was dissolved in a minimal amount of water and was applied to a reversed-phase column (240 × 15 mm) equilibrated with water. After washing the column with water, elution was accomplished with 3% methanol. The eluate was lyophilized to give ginkgotoxin- 5′-glucoside as white crystals (115 mg). mp: 128–133°C. IR (nujol):3400 (OH). ^{1}H-NMR (D_2O-DMSO-d_6):2.32 (3H, s, 2-CH_3), 2.92-3.11 (4H, m, Glc H-2, H-3, H-4, H-5), 3.23 (3H, s, 4-$CH_2O\underline{CH}_3$), 3.42 (1H, dd, J = 5.7, 11.7 Hz, Glc H-6), 3.65 (1H, dd, J = 1.7, 11.7 Hz, Glc H-6), 4.13 (1H, d, J = 7.4 Hz, Glc H-1), 4.52, 4.55 (2H, AB, J = 11.4 Hz, 4-$C\underline{H}_2O$-), 4.54, 4.78 (2H, AB, J = 12.0 Hz, 5-$C\underline{H}_2O$-), 7.92 (1H, s, 6-H). UV λmax (5 mM potassium phosphate, pH 2.5) nm:291. ESI-MS m/z:346 $[M + H]^+$.

Synthesis of Ginkgotoxin-3-Glucoside (3 in Figure 1)

A mixture of ginkgotoxin (1.83 g), 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (4.93 g), silver carbonate (3.31 g) and Molecular Sieves 3A (14 g) in dry dichloromethane (50 mL) was refluxed for 2 d in the dark. After removal of insoluble materials by filtration, the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel using chloroform/methanol (70/1) and recrystallization from ethyl acetate/hexane to give ginkgotoxin-3-O-β-D-glucoside tetraacetate (1.3 g) as colorless prisms. mp: 145–146°C. IR (nujol):1770, 1740 (C═O). ^{1}H-NMR ($CDCl_3$):1.98, 1.99, 2.01, 2.12 (each 3H, s, CH_3CO-), 2.49 (3H, s, 2-CH_3), 3.41 (3H, s, 4-$CH_2OC\underline{H}_3$), 3.53 (1H, ddd, J = 2.3, 4.6, 10.0 Hz, Glc H-5), 3.98 (1H, dd, J = 2.3, 12.0 Hz, Glc H-6), 4.21 (1H, dd, J = 4.6, 12.0 Hz, Glc H-6), 4.56, 4.63 (2H, AB, J = 12.6 Hz, 4-or 5-$C\underline{H}_2O$-), 4.57, 4.65 (2H, AB, J = 10.9 Hz, 4- or 5-$C\underline{H}_2O$-), 4.76 (1H, d, J = 8.0 Hz, Glc H-1), 5.12-5.36 (3H, m, Glc H-2, H-3, H-4), 8.32 (1H, s, 6-OH).

Potassium hydroxide, 2 M, (2 mL) was added to a solution of ginkgotoxin-3-O-β-D-glucoside tetraacetate (274 mg) in methanol (4 mL). The mixture was stirred at room temperature overnight, and then worked up in a manner similar to that described above. Lyophilization of the eluate afforded

ginkgotoxin-3-glucoside as white crystals (140 mg). mp: 145–150°C. IR (nujol):3350 (OH). ^{1}H-NMR (D_2O-DMSO-d_6): 2.46 (3H, s, 2-CH_3), 2.95 (1H, m, Glc H-5), 3.07-3.28 (3H, m, Glc H-2, H-3, H-4), 3.22 (3H, s, 4-$CH_2O\underline{CH_3}$), 3.36 (1H, dd, J = 5.7, 11.5 Hz, Glc H-6), 3.54 (1H, dd, J = 1.7, 11.5 Hz, Glc H-6), 4.43 (1H, d, J = 7.4 Hz, Glc H-1), 4.51, 4.61 (2H, AB, J = 10.9 Hz, 4-$C\underline{H_2}O$-), 4.53 (2H, s, 5-$C\underline{H_2}O$-), 8.23 (1H, s, 6-H). UV λmax (5 mM potassium phosphate, pH 2.5) nm:278. ESI-MS m/z:346 $[M + H]^+$.

Ginkgo biloba Seeds

All Ginkgo biloba seeds (raw, canned, and plastic-packaged) were collected in Japan. Two of the 4 kinds of raw seeds used (Raw A and Raw B) were commercially available as food, and the other two kinds were seeds that had fallen from different Ginkgo trees (Raw C and Raw D). Two kinds of canned seeds were used, one that was labeled as boiled (Canned A) and one that was labeled as dry-packed (Canned B). The sample packaged in plastic was boiled seeds (Plastic). All canned and packaged products had constituents such as citric acid, sugar, and sodium chloride.

All seeds were lyophilized, pulverized in a small mortar with a pestle, and stored at −25°C until analysis.

Apparatus and Chromatographic Conditions

Melting points (mp) were determined with a Mitamura micro hot-stage apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Jeol JNM-ECA 500 spectrometer at 500 MHz, with tetramethylsilane as an internal standard. Infrared (IR) spectra were obtained using a Jasco FT/IR300 spectrometer and are expressed in cm^{-1}. UV spectra were measured with a Shimadzu UV2200 spectrophotometer.

The HPLC apparatus consisted of a Shimadzu LC-10Avp system equipped with an RF-10A spectrofluorometer or an SPD-10A spectrophotometer (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on an Inertsil ODS-3 (150 × 4.6 mm in i.d., 5 μm, GL Sciences, Tokyo, Japan) column using a gradient elution mode, at a flow rate of 1.0 mL/min. The column temperature was ambient. Mobile phase A was 5 mM potassium phosphate containing 5 mM sodium pentanesulfonate adjusted to pH 2.5 with phosphoric acid, and mobile phase B was acetonitrile. The gradient program was as follows: a linear gradient from 4% of mobile phase B to 8% of mobile phase B over a period of 20 min. For the determination of all vitamin B_6 compounds except ginkgotoxin-3-glucoside, fluorescence measurement was carried out at an emission wavelength of 395 nm with an excitation wavelength of 295 nm. For the identification of

ginkgotoxin-3-glucoside in Ginkgo biloba seeds, a UV detector, which was set at a wavelength of 278 nm, was used because ginkgotoxin-3-glucoside is a nonfluorescent compound.

Extraction Procedure

Twenty five mg of lyophilized powder of Ginkgo biloba seeds was suspended in 2 mL of water, and the solution was heated at 70°C for 30 min in the dark with occasional shaking. After cooling, the solution was centrifuged, filtered through a 0.45-μm membrane, appropriately diluted with water, and then subjected to HPLC analysis.

In some experiments, 1 mL extracts were treated with β-glucosidase (32 units) at 37°C for 1 h to hydrolyze ginkgotoxin-5′-glucoside present, and were then analyzed by the HPLC method.

RESULTS AND DISCUSSION

Separation of Ginkgotoxin, Ginkgotoxin-5′-glucoside and Vitamin B_6 Compounds

Authentic specimens of ginkgotoxin-5′- and 3-glucosides were synthesized using Koenigs-Knorr reaction in order to separate and determine them in Ginkgo biloba seeds. Their structures were confirmed by ^{1}H- and ^{13}C-NMR and MS spectra data. The chemical structures of ginkgotoxin and its glucosides are shown in Figure 1.

Several formats for HPLC separation of vitamin B_6 compounds have been published. For simultaneous analysis of ginkgotoxin, ginkgotoxin-5′-gluocoside, and vitamin B_6 compounds, ion-pair reversed-phase chromatography combined with fluorometric quantification appears to be appropriate in terms of sensitivity and resolution. Various combinations of organic modifier, pH of buffer solution, and ion-pair reagent, were examined on an Inertsil ODS-3 column. Since the use of acetonitrile gradient elution provided sharp and symmetrical peaks, as well as more efficient resolution of all of the compounds within a shorter period of time, acetonitrile was employed as an organic modifier. In the lower pH region (<3.75), which has been widely selected for separation of the compounds, pH had little effect on the retention time and the peak shape of each compound, as suggested by previous findings.[8] When the ion pair reagents sodium pentanesulfonate and sodium heptanesulfonate were tested, a higher resolution of all of the compounds than that in the absence of the reagents was observed. There was no significant difference between the effects of the two reagents on resolution, although the retention time was affected. These results indicated that the optimum mobile phase consists of a mixture of 5 mM potassium

phosphate (pH 2.5), 5 mM sodium pentanesulfonate and acetonitrile from 4 to 8.5%.

Typical chromatograms obtained using authentic specimens were recorded at an excitation wavelength of 295 nm and an emission wavelength of 395 nm (Figure 2a). All of the compounds were completely separated from each other without noticeable peak asymmetry within 20 min under the present HPLC conditions, the retention of vitamin B_6 species increased in the following order: pyridoxic acid < pyridoxal < pyridoxine < pyridoxine-5′-glucoside < ginkgotoxin-5′-glucoside < ginkgotoxin. The chromatographic behavior of ginkgotoxin-3-glucoside absent in Ginkgo biloba seeds,[5] was also examined using a UV detector with a wavelength set at 278 nm. The peaks due to ginkgotoxin-3-glucoside and pyridoxine-5′-glucoside could not be resolved successfully under the present HPLC conditions (Figure 2b). The poor resolution would not lead to serious error in determining pyridoxine-5′-glucoside, since ginkgotoxin-3-glucoside did not fluoresce at detector wavelengths used in this method.

The calibration curves were constructed by plotting the peak area of each compound against the amounts of the corresponding compound. A good linear relationship to each compound was obtained over the range of 0.1–10 pmol with linear correlation coefficients of more than 0.999 for all compounds. The detection limit of ginkgotoxin and ginkgotoxin-5′-glucoside was estimated to be ca. 0.025 pmol/injection (signal-to-noise ratio = 5).

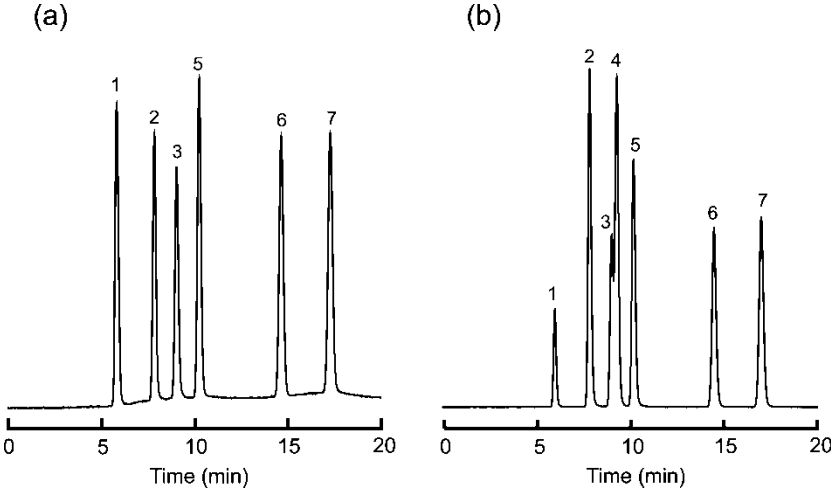


Figure 2. Typical high performance liquid chromatograms of the standard compounds. (a) Fluorescent detection. (b) UV detection. 1: Pyridoxic acid, 2: Pyridoxal, 3: Pyridoxine-5′-glucoside, 4: Ginkgotoxin-3-glucoside, 5: Pyridoxine, 6: Ginkgotoxin-5′-gluocoside, 7: Ginkgotoxin.

Extraction Efficiency

Several extraction procedures for ginkgotoxin have been reported.[3,5,6] Extraction of ginkgotoxin and ginkgotoxin-5′-glucoside from Ginkgo biloba seeds was performed with hot water (70°C) in this study. The stability of ginkgotoxin and ginkgotoxin-5′-glucoside was initially examined using the standard solution (10 μM) at 70°C in the dark. Unfavorable side reaction and degradation were not observed in the case of heating for 2 h at 70°C. Other vitamin B_6 compounds were also stable under the same conditions. Extraction efficiency of ginkgotoxin and ginkgotoxin-5′-glucoside from Ginkgo biloba seeds was periodically monitored by the HPLC method. Changes in the recoveries of ginkgotoxin and ginkgotoxin-5′-glucoside from Ginkgo biloba seeds, in which ginkgotoxin or ginkgotoxin-5′-glucoside exist, in response to changes in the heating time are shown in Figure 3. Maximum and consistent recoveries of ginkgotoxin and ginkgotoxin-5′-glucoside were obtained in the heating time range of 15 to 120 min. Extraction with hot water (70°C) does not create any disadvantages in determining them. Thirty minutes was a sufficient time for extraction of ginkgotoxin and ginkgotoxin-5′-glucoside from powdered Ginkgo biloba seeds.

Determination of Ginkgotoxin and Ginkgotoxin-5′- Glucoside

The reliability of the method was assessed by repeated analysis of ginkgotoxin and ginkgotoxin-5′-glucoside in a Ginkgo biloba seed on the same day (intra-assay) or on four different days (inter-assay). The intra-assay and inter-assay coefficients of variation (CV) of ginkgotoxin and ginkgotoxin-5′-glucoside assays were less than 2.0% (n = 6) and 3.2% (n = 4), respectively.

Figure 4 shows typical chromatograms obtained from the extracts of a raw seed and a canned seed. The peak of ginkgotoxin-5′-glucoside in Figure 4a

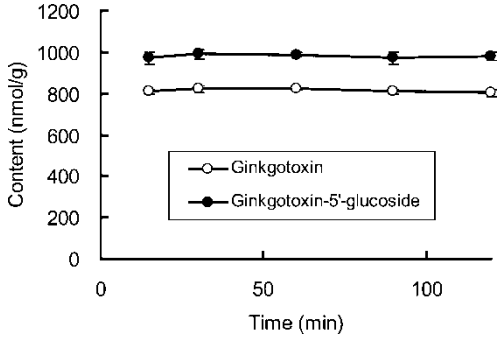


Figure 3. Extraction of ginkgotoxin and ginkgotoxin-5′-glucoside from *Ginkgo biloba* seeds. Values are the mean ± SD of quadruplicate experiments.

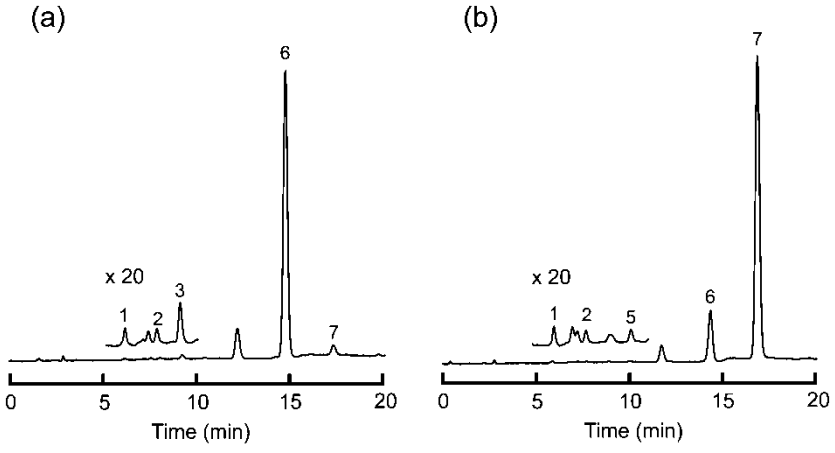


Figure 4. High performance liquid chromatograms of ginkgotoxin and ginkgotoxin-5′-glucoside in a canned seed (a), and a raw seed (b). Peak identity is the same as in Figure 2.

was identified on the basis of the retention time, and further identification was performed by enzymatic hydrolysis with β-glucosidase. Addition of β-glucosidase to a seed extract caused disappearance of the peak and an increase in ginkgotoxin concentration. Ginkgotoxin-3-glucoside was not detected in any Ginkgo biloba seeds as reported previously.

The results obtained from raw, canned, and plastic packaged seeds are summarized in Tables 1 and 2. Either ginkgotoxin or ginkgotoxin-5′-glucoside was the predominant component in all of the Ginkgo biloba seeds, accounting for more than ca. 93% of the total amounts of vitamin B_6 compounds. A marked difference in the ginkgotoxin and ginkgotoxin-5′-glucoside profiles was observed between the raw seeds and the canned/plastic packaged seeds. The level of ginkgotoxin in raw seeds (Raw A, B, C, and D) was significantly higher than that of ginkgotoxin-5′-glucoside, accounting for 936–1177 nmol/g. The proportion of ginkgotoxin-5′-glucoside to total vitamin B_6 ranged from 1.2 to 15.6%. On the other hand, ginkgotoxin-5′-glucoside was present in canned and plastic packaged seeds (canned A, B, and plastic) at high levels of 997–1668 nmol/g. No significant proportion of ginkgotoxin was observed in contrast to the high levels in raw seeds. Of particular interest is the noticeable difference in the form of ginkgotoxin in Ginkgo biloba seeds, and our results are similar to previous findings of Scott et al.,[5,6] that ginkgotoxin-5′-glucoside is present at high concentrations in canned and vacuum packaged white nuts. The same tendency for pyridoxine as that for ginkgotoxin was found. Pyridoxine-5′-glucoside, which was absent or present in only trace amounts in raw seeds, was detected at levels of 32.6–45.7 nmol/g.

Table 1. Concentrations of ginkgotoxin, ginkgotoxin-5′-glucoside and vitamin B_6 compounds in raw *Ginkgo biloba* seeds

	Raw A	Raw B	Raw C	Raw D
Vitamin B_6	nmol/g (%)	nmol/g (%)	nmol/g (%)	nmol/g (%)
Pyridoxic acid	7.85 ± 0.7 (0.62)[a]	6.28 ± 1.1 (0.44)	8.48 ± 3.5 (0.80)	7.95 ± 4.4 (0.81)
Pyridoxal	6.31 ± 3.4 (0.50)	9.61 ± 4.0 (0.67)	9.02 ± 4.1 (0.85)	8.82 ± 3.8 (0.90)
Pyridoxine-5′-glucoside	n.d.[b] (0)	1.25 ± 1.7 (0.09)	n.d. (0)	n.d. (0)
Pyridoxine	59.8 ± 11.4 (4.71)	15.0 ± 11.8 (1.05)	51.3 ± 11.8 (4.84)	20.2 ± 5.4 (2.05)
Ginkgotoxin-5′-glucoside	33.4 ± 29.2 (2.63)	223 ± 94.5 (15.6)	22.6 ± 20.8 (2.13)	12.0 ± 4.4 (1.22)
Ginkgotoxin	1161 ± 184 (91.4)	1177 ± 61.2 (82.2)	967 ± 320 (91.1)	936 ± 113 (95.0)

[a]Each value is the mean ± SD ($n = 5$). Values in parentheses are percentages of the total vitamin B_6 compounds.
[b]Not detected.

Table 2. Concentrations of ginkgotoxin, ginkgotoxin-5′-glucoside and vitamin B_6 compounds in canned and plastic-packaged *Ginkgo biloba* seeds

	Canned A	Canned B	Plastic
Vitamin B_6	nmol/g (%)	nmol/g (%)	nmol/g (%)
Pyridoxic acid	2.63 ± 0.4 (0.25)[a]	7.48 ± 0.9 (0.45)	3.84 ± 0.4 (0.22)
Pyridoxal	2.12 ± 0.4 (0.20)	5.07 ± 0.8 (0.30)	8.08 ± 0.7 (0.46)
Pyridoxine-5′-glucoside	33.6 ± 0.8 (3.17)	45.7 ± 5.6 (2.72)	32.6 ± 1.1 (1.86)
Pyridoxine	1.69 ± 0.14 (0.16)	n.d.[b] (0)	0.85 ± 1.4 (0.05)
Ginkgotoxin-5′-glucoside	997 ± 76.8 (94.2)	1586 ± 305 (94.4)	1668 ± 116 (95.0)
Ginkgotoxin	21.3 ± 0.90 (2.02)	35.2 ± 5.0 (2.09)	42.2 ± 5.5 (2.41)

[a]Each value is the mean ± SD ($n = 5$). Values in parentheses are percentages of the total vitamin B_6 compounds.

[b]Not detected.

CONCLUSIONS

Ginkgotoxin-5′-glucoside was chemically synthesized for analysis of the profiles of ginkgotoxin and ginkgotoxin-5′-glucoside in various Ginkgo biloba seeds. The proposed HPLC method is suitable for simultaneous determination of ginkgotoxin, ginkgotoxin-5′-glucoside, and vitamin B_6 compounds in Ginkgo biloba seeds. Further studies on the distributions of ginkgotoxin and ginkgotoxin-5′-glucoside in various kinds of seeds using this method are in progress.

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