Research Article

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Identifications of inhibitors of IgE production by human lymphocytes isolated from 'Cha Chuukanbohon Nou 6' tea leaves

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Abstract

BACKGROUND: Tea (*Camellia sinensis* L.) is consumed all over the world and in especially large quantities in Japan and China, where it has been used not only as a daily beverage but also for medicinal purposes for thousands of years. Tea has been found to exhibit various bioregulatory activities, including antiallergic, anticarcinogenic, antimetastatic, antioxidative, antihypertensive, antihypercholesterolemic, anti-dental caries and antibacterial effects, and to influence intestinal flora.

RESULTS: Cha Chuukanbohon Nou 6 is a tea cultivar improved by the National Institute of Vegetable and Tea Science (NIVTS) in Japan. On comparing chemical constituents of 11 varieties of tea leaves by high-performance liquid chromatography, we found two new major compounds in Cha Chuukanbohon Nou 6. Nuclear magnetic resonance spectroscopy revealed these compounds to be theogallin and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose. The two were similar in chemical structure to strictinin, an inhibitor of immunoglobulin (Ig) production. Thus their effects on the production of Igs by peripheral blood lymphocytes were tested. Both compounds, like strictinin, inhibited IgE production.

CONCLUSION: The results suggest Cha Chuukanbohon Nou 6 to be the basis of an antiallergic beverage.
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Keywords: Cha Chuukanbohon Nou 6; theogallin; 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose; inhibitor of IgE production

INTRODUCTION

Tea (*Camellia sinensis* L.) is consumed all over the world and in especially large quantities in Japan and China, where it has been used not only as a daily beverage but for medicinal purposes for thousands of years. Tea has been found to exhibit various bioregulatory activities, including anticarcinogenic,[1–6] antimetastatic,[7–11] antioxidative,[12–15] antihypertensive,[16] antihypercholesterolemic,[17–19] anti-dental caries[20,21] and antibacterial effects,[22] and to influence intestinal flora.[23] Catechins, a group of polyphenolic compounds, have been shown to be largely responsible for these activities. Catechins play a significant role in antiallergic responses. We have reported that *O*-methylated EGCGs (epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3″Me) and epigallocatechin-3-*O*- (4-*O*-methyl) gallate (EGCG4″Me)) had antiallergic effects,[24–27] and the Japanese tea cultivar 'Benifuuki' is rich in EGCG3″Me, which is not present in black tea.[28,29]

Strictinin, contained in the shoots of various tea cultivars in the first crop of the season, also has antiallergic properties.[30] An allergen-specific IgE antibody binds to mast cells in the early phase of an allergic reaction. Tachibana *et al*.,[30] screening for substances which inhibit IgE production using the human B cell line DND39, found that the hydrolysable tannins such as strictinin in green tea strongly inhibited the IgE class switch (IgE heavy chain germ transcript) of B cells. Strictinin is thought to obstruct an IL-4-induced class switch by inhibiting the tyrosine phosphorylation of STAT6.

Tea leaves contain various antiallergic compounds. In this study, we found two unknown compounds in Cha Chuukanbohon Nou 6, improved by the National Institute of Vegetable and Tea Science (NIVTS), on comparing the chemical constituents of 11 cultivars of tea leaves by high-performance liquid chromatography (HPLC). We have isolated and determined the structure of each compound and compared their antiallergic activities with those of strictinin.

MATERIALS AND METHODS

Chemicals

Methanol (MeOH), ethanol (EtOH), acetonitrile (MeCN), formic acid (HCOOH), trifluoroacetic acid (TFA), phosphoric acid (H_3PO_4) and caffeine (CAF) were purchased from Wako Pure Chemical

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Industries, Ltd (Osaka, Japan). For the analysis of components in tea leaves, catechins (epigallocatechin (EGC), catechin (C), epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)) and strictinin (ST) were purchased from Funakoshi Co., Ltd (Tokyo, Japan). EGCG3″Me purified to greater than 98% was kindly provided by Dr M Sano of Nagoya Women's University and Dr T Miyase of University of Shizuoka. For nuclear magnetic resonance (NMR) spectroscopy, acetone-d_6 was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan) and deuterium oxide was purchased from Wako Pure Chemical Industries, Ltd. Lymphocyte Separation Medium was acquired from Wako Pure Chemical Industries and RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Recombinant human IL-4 and anti-CD40 Ab were obtained from PeproTech Inc. (Rocky Hill, NJ, USA) and Becton Dickinson and Co. (Franklin Lakes, NJ, USA), respectively. Goat polyclonal anti-human IgE, IgM and IgG Abs (unlabeled and horseradish peroxidase (HRP)-conjugated) were purchased from BioSource Technical Service (Sunnyvale, CA, USA). Streptomycin (100 μg mL^{-1}) and penicillin (100 U mL^{-1}) were obtained from Wako Pure Chemical Industries.

Equipment

HPLC for measuring the chemical constituents of tea leaves was carried out with a class VP HPLC system, LC-10A pumps, a SIL-10Avp auto sampler, an SPD-M10Avp UV detector and a CTO-10ASvp column oven (Shimadzu Corporation, Kyoto, Japan). The isolation and purification of compounds were conducted using a Hitachi HPLC system, with an L-7150 pump, an L-7420 UV-visible detector and a D-2500 Chromato-Integrator (Hitachi, Ltd, Tokyo, Japan).

^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectra were recorded in acetone-d_6–deuterium oxide (7 : 1) at 25 °C with a Bruker AV600 (Bruker BioSpin GmbH, Rheinstetten, Germany). High-resolution time-of-flight mass spectrometry (HR-TOF-MS) was performed on an LCT Premier (Waters corporation, Milford, MA, USA). Electron spray ionization–mass spectrometry (ESI-MS) was carried out with an API3000 LC/MS/MS system (Applied Biosystems, Inc., Foster City, CA, USA).

Optical rotations were measured with a P-1030 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were obtained using a Hitachi U-2000 spectrophotometer. Circular dichroism (CD) spectra were recorded using a J-720 CD spectrophotometer (Jasco). Enzyme-linked immunosorbent assay (ELISA) was measured with a BenchMark Plus microplate reader (BioRad Laboratories Inc., Hercules, CA, USA).

Plant materials

Eleven varieties of tea leaves – Yabukita, Sayamakaori, Kanayamidori, Yutakamidori, Saemidori, Makinoharawase, Benifuuki, Seisintaipan, Ohba-oolong, Okuyutaka and Cha Chuukanbohon Nou 6 – were cultivated in the tea field of NIVTS. Freshly picked tea leaves were dried and stored in a refrigerator at −80 °C before analysis.

HPLC analysis of the chemical constituents in tea leaves

The chemical constituents of the tea leaves were analyzed according to a partially improved version of Horie's method.[31,32] Tea leaves were pulverized into a powder and milled using a Cyclon sample mill (UDY, Fort Collins, CO, USA) with a 1 mm mesh screen.

The sample (250 mg) was added to 20 mL of 2% H_3PO_4–EtOH (1 : 1) in a 25 mL measuring flask for 60 min at 30 °C in a water bath. The flask was then filled with distilled water (DW). To remove the residue, the solution was centrifuged at 1200 × *g* for 5 min at 4 °C, and the supernatant was diluted 10-fold with DW. The sample was filtered through a membrane filter (DISMIC-13HP-PTFE, pore size 0.45 μm; Advantec, Tokyo, Japan) before HPLC.

HPLC was carried out on a Wakopak Navi C18-5 column (4.6 mm i.d. × 150 mm, granule diameter; 5 μm, Wako Pure Chemical Industries). The oven temperature was 40 °C, detection wavelength was 280 nm for EGC, C, ST, CAF, EGCG, EC, EGCG3″Me and ECG, flow rate was 1.0 mL min^{-1} and the injection volume was 20 μL. HPLC was performed using a linear gradient system with mobile phase A (DW : MeCN : H_3PO_4 = 400 : 10 : 1) and mobile phase B (MeOH : mobile phase A = 1 : 2). The eluant was 100% A for 2 min, programmed to decrease to 20% mobile phase A over 27 min, maintained at 20% A for 10 min, and then returned to 100% A for 7 min.

Quantification was carried out using the external standard method. The chemical constituents were quantified using data acquisition and the processing system of the LC workstation (Class VP system, Shimadzu Corporation).

Purification of compound 1

Extracts of Cha Chuukanbohon Nou 6 leaves (48 g) were obtained with DW (400 mL × 3), 50% MeOH (400 mL × 3) and MeOH (400 mL × 3). The DW extract and 50% MeOH extract were mixed and evaporated to remove MeOH. The concentrated solution was subjected to Sep-Pak® C18 Vac 20 mL cartridge (Waters Corporation) column chromatography by stepwise elution with DW (20 mL), 10% MeCN (20 mL), 15% MeCN (20 mL) and 30% MeCN (20 mL). After evaporation of the DW and 10% MeCN extracts to remove MeCN, the concentrated fraction was subjected to reversed-phase HPLC using an Inertsil ODS-3 column (2.0 mm i.d. × 25 cm, GL Sciences Inc., Tokyo, Japan). The detection wavelength was 280 nm, flow rate was 12 mL min^{-1} and the mobile phase was DW-MeOH-TFA (95 : 5:0.1, v/v/v). The peak fraction with a retention time of 32–40 min was collected and evaporated to remove MeOH. The concentrated fraction was again subjected to reversed-phase HPLC using an Inertsil ODS-3 column (2.0 mm i.d. × 25 cm). The detection wavelength was 280 nm and flow rate was 12 mL min^{-1}. Mobile phase A was DW and mobile phase B was DW–MeOH (60 : 40, v/v). The eluant was 100% A for 10 min, then 100% B for 17 min, and this fraction was evaporated and lyophilized to give Compound **1** (24.6 mg).

Purification of compound 2

Extracts of Cha Chuukanbohon Nou 6 leaves (10.4 g) were obtained with DW (200 mL × 3), 50% MeOH (200 mL × 3) and MeOH (200 mL × 3). The DW extract was evaporated to reduce the solution's volume. The concentrated solution was subjected to Sep-Pak® C18 Vac 20 mL cartridge column chromatography by stepwise elution with 5% MeCN (20 mL), 15% MeCN (20 mL) and 50% MeCN (20 mL). After evaporation of the 50% MeCN fraction, the concentrated fraction was subjected to reversed-phase HPLC using an Inertsil ODS-3 column (2.0 mm i.d. × 25 cm). The detection wavelength was 280 nm, flow rate was 10 mL min^{-1} and the mobile phase was DW–MeCN–HCOOH (84 : 16:0.1, v/v/v). The 23 min peak fraction was collected and evaporated to remove MeCN. The concentrated fraction was

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Table 1. The chemical constituents in tea leaves (g kg^{-1})

Cultivar	Compound **1**	EGC	C	ST	CAF	EGCG	Compound **2**	EC	EGCG3″Me	ECG
Yabukita	1.2	36.8	4.9	14.0	29.5	61.2	0.0	13.5	0.0	13.9
Sayamakaori	0.9	24.0	3.5	22.3	29.3	54.5	0.0	9.6	0.6	15.2
Kanayamidori	1.3	47.3	3.2	9.1	33.0	66.8	0.0	15.0	2.5	16.4
Yutakamidori	1.2	42.7	2.8	10.0	31.2	67.7	0.0	16.1	1.2	17.8
Saemidori	1.0	30.3	2.3	9.8	24.6	42.8	0.0	10.8	0.4	11.5
Makimoharawase	1.5	42.2	2.0	8.2	29.8	68.1	0.0	18.0	1.1	23.0
Benifuuki	1.6	36.2	3.9	14.1	39.9	81.4	0.0	14.2	7.4	25.7
Seisintaipan	1.7	33.8	3.6	9.6	38.3	79.7	0.0	12.1	0.3	17.3
Ohba-oolong	2.6	27.6	4.9	11.0	38.0	62.3	0.0	7.8	3.1	10.2
Okuyutaka	1.8	32.3	2.0	12.2	27.2	53.4	0.0	13.4	0.4	14.0
Cha Chuukanbohon Nou 6	6.7	17.6	0.4	11.0	27.6	48.8	87.6	7.8	2.3	24.4

again subjected to reversed-phase HPLC using an Inertsil ODS-3 column (2.0 mm i.d. × 25 cm). The detection wavelength was 280 nm, flow rate was 12 mL min^{-1}, and the mobile phase was DW–MeCN–HCOOH (87 : 13:0.1, v/v/v). The 46 min peak fraction was collected and evaporated to remove MeCN. To remove HCOOH, the concentrated fraction was subjected to Sep-Pak® C18 Vac 20 mL cartridge column chromatography by stepwise elution with DW (25 mL) and MeOH (25 mL). The MeOH fraction was evaporated and lyophilized to give Compound **2** (9.8 mg).

Assay for Ig production by human blood lymphocytes

Cell isolation and cell culture

To isolate peripheral blood leukocytes (PBL), blood obtained from healthy human subjects was mixed with an equal volume of sterile phosphate-buffered saline (PBS), layered on to Lymphocyte Separation Medium and centrifuged. The cells at the interface were then collected, washed and resuspended in RPMI 1640 medium. PBL at the optimal concentration (2×10^6 cells 200 µL^{-1}) were costimulated with 80 U mL of human rIL-4 and 2 µg mL^{-1} of anti-CD40 in RPMI 1640 medium containing 100 µg mL^{-1} of streptomycin, 100 U mL^{-1} of penicillin, and 10% FBS from Invitrogen to induce IgE synthesis. Cell cultures were performed in 96-well plates and treated with increasingly higher concentrations of test samples or left untreated. Cells were incubated at 37 °C in a 5% CO_2 incubator. Supernatants were harvested 14 days post culture, and Ig levels were assayed by ELISA.

ELISA

IgM, IgG and IgE levels were measured as described previously.[33] Micro-titer plates were coated overnight at 4 °C with anti-human IgE Ab, anti-human IgG and anti-human IgM, respectively. The

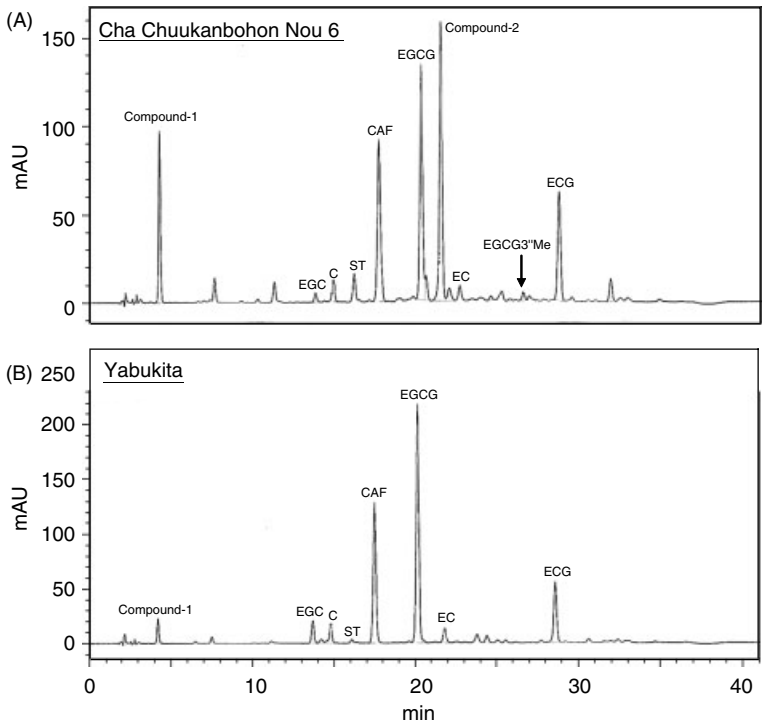

Figure 1. HPLC of Yabukita and Cha Chuukanbohon Nou 6. EGC, epigallocatechin; C, catechin; ST, strictinin; CAF, caffeine; EGCG, epigallocatechin gallate; EC, epicatechin; EGCG3″Me, epigallocatechin-3-*O*-(3-*O*-methyl) gallate; ECG, epicatechin gallate.

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Table 2. ^{13}C- and ^{1}H-NMR spectra data for compound **2** measured in acetone-d_6–deuterium oxide (7 : 1) (150 MHz for ^{13}C, 600 MHz for ^{1}H)

	2	
Position	δ_C	δ_H
Glucose (glc)		
1	93.1	5.96 (d = 8.4)
2	73.5	5.37 (t = 9.0)
3	72.2	4.23 (t = 9.6)
4	71.8	5.03 (t = 9.6, 10.2)
5	72.4	4.28 (dd = 6.6, 10.2)
6	62.8	3.85 (d = 13.2)
		5.23 (dd = 6.6, 13.2)
Galloyl-1 (gall-1)		
1	118.7	
2, 6	109.4	7.095 (s)
3, 5	145.3	
4	139.2	
7	164.8	
Galloyl-2 (gall-2)		
1	119.7	
2, 6	109.5	7.102 (s)
3, 5	145.2	
4	138.6	
7	165.9	
HHDP		
1	115.5	
2	125.6[a]	
3	107.5	6.76 (s)
4	144.4	
5	135.9	
6	143.6	
7	167.7	
1′	115.0	
2′	125.1[a]	
3′	107.1	6.63 (s)
4′	144.4	
5′	135.5	
6′	143.7	
7′	168.1	

[a] Signals were interchangeable on the same column.

wells were blocked with 0.5% bovine serum albumin in PBS for 1 h at 37 °C, 50 μL of culture supernatant was plated in triplicate wells, and the plates were incubated for 2 h at 37 °C. After the plates were washed, HRP-labeled anti-human Ig Abs were added for detection, followed by the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) substrate. The reaction was stopped with 0.1% oxalic acid and read at a wavelength of 415 nm using a BenchMark Plus microplate reader.

Statistical analysis

Statistical analyses were performed using Dunnett's test by SPSS14.0 (SPSS Japan Inc., Japan). All data are presented as means ± SD of triplicate experiments, with $P < 0.05$ and $P < 0.01$ considered significant.

RESULTS AND DISCUSSION

Reversed-phase HPLC analysis for tea leaves

From the result of reversed-phase HPLC analyses, eight compounds were identified as EGC, C, ST, CAF, EGCG, EC, EGCG3″Me and ECG in tea leaves (Table 1). Comparing the chromatogram of Yabukita with that of Cha Chuukanbohon Nou 6, compound **1** was more abundant by almost five times in Cha Chuukanbohon Nou 6 than Yabukita, and compound **2** was not detected in the other tea leaves (Fig. 1). For determining the structures of compounds **1** and **2**, these compounds were isolated from Cha Chuukanbohon Nou 6.

Chemical structure of compounds 1 and 2

Repeated preparative HPLC led to the isolation of compounds **1** and **2** from the DW and 50% MeOH extracts of Cha Chuukanbohon Nou 6 leaves. Compound **1** was identified as theogallin through a comparison of its spectroscopic and physical data with those previously reported (Fig. 3).[34–36] Compound **2** was isolated first from the leaves of *Camellia oleifera*[37] and later from walnuts (the seeds of *Juglans regia* L.)[38] and green tea prepared from the leaves of *C. taliensis*.[39] However, no spectroscopic data for compound **2** had been presented. Compound **2** was obtained as a white amorphous powder. Its molecular formula was deduced to be $C_{34}H_{26}O_{22}$ on the basis of the negative HR-TOF-MS (*m/z* 785.0799 ($[M - H]^-$), calculated 785.0837) together with the ^{13}C NMR spectral data. The ^{1}H- and ^{13}C-NMR spectra of compound **2** was characteristic of an ellagitannin (Table 2), as revealed by its close similarity to that of 1,3-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose, which has the same molecular formula.[40] The spectrum exhibited two two-proton singlets (δ 7.095 and 7.102) and two one-proton singlets (δ 6.63 and 6.76) in the aromatic region. The presence of a β-D-glucopyranose residue taking the $^{4}C_1$ conformation was evident from the coupling patterns of the aliphatic proton signals. The aromatic proton signals of compound **2** were accounted for by the presence of two galloyl groups and one hexahydroxydiphenoyl (HHDP) group.

The position of each acyl group on the glucose core was determined from the HMBC data for compound **2**, which showed connectivity between each aromatic proton and glucose proton via a carbonyl carbon signal. The glucose H-4 signal at δ 5.03 showed connectivity with the signal at δ 6.76 (HHDP H-3) via correlations with a common ester carbonyl carbon at δ 167.7 (HHDP C-7). One of the other ester carbonyl carbon signals of the HHDP group at δ 168.1 (HHDP C-7′) was correlated with glucose H-6 (δ 3.85 and 5.23) and HHDP H-3′ (δ 6.63) via three-bond couplings, establishing the position of the HHDP group at O-4/O-6 of the glucose (Fig. 2). The H-2 and H-6 signals of the two galloyl groups at δ 7.095 (galloyl-1) and 7.102 (galloyl-2) showed connectivity with the signal at δ 5.96 (glucose H-1) and 5.37 (glucose H-2) via correlations with a common ester carbonyl carbon at δ 164.8 (galloyl-1 C-7) and 165.9 (galloyl-2 C-7), respectively. These data indicated that two galloyl groups are located at O-1 and O-2 of glucose (Fig. 2). The atropisomerism at the chiral HHDP group in compound **2** was determined to be *S*-series by the strong positive Cotton effects at 238 nm in the CD spectrum.[41] Therefore, compound **2** was established as 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose (Fig. 3).

Compound **2***: 1,2-di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl-β-D-glucopyranose*

White amorphous powder; $[\alpha]_D + 197.9^\circ$ ($c = 0.1$, MeOH); CD (MeOH) $[\theta]$ (nm) 5.7×10^4 (236), -1.8×10^4 (263), 7.3×10^3

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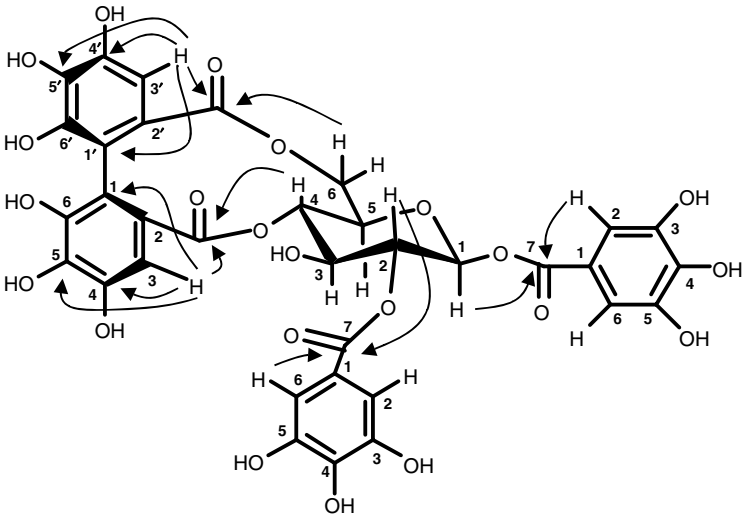

Figure 2. HMBC correlations important for the structural determination of compound **2**.

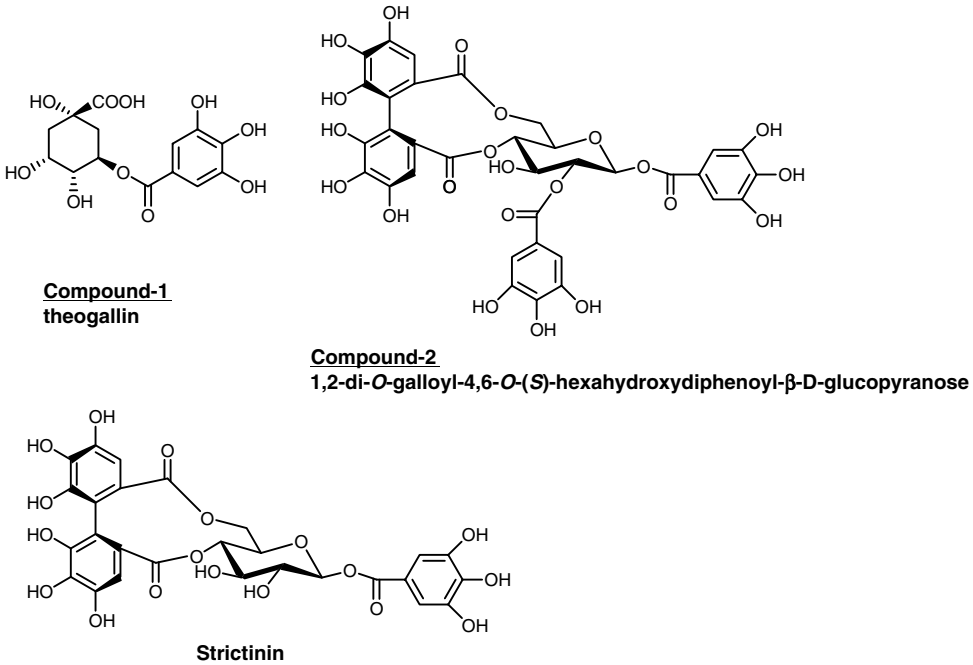

Figure 3. Chemical structures of compound **1**, compound **2** and strictinin.

(283); ^{1}H and ^{13}C NMR, see Table 1; HR-TOF-MS (negative ions) *m/z* 785.0799 $[M - H]^-$ (calculated for $C_{34}H_{25}O_{22}$, 785.0837).

Effects of compounds 1 and 2 on Ig production by PBL

The effects of strictinin, compound **1** and compound **2** on the production of Igs by PBL were examined. As shown in Fig. 4, strictinin and compound **1** significantly inhibited IgE production compared to vehicle at 1, 100 and 1000 nmol L^{-1} at 0.1, 1, 10, 100 and 1000 nmol L^{-1}, respectively. Compound **2** inhibited IgE production at 1, 10, 100 and 1000 nmol L^{-1} in a dose-dependent manner. Strictinin and compound **1** significantly inhibited IgG production compared to vehicle at 1000 nmol L^{-1} and at 0.1, 1, 10, 100 and 1000 nmol L^{-1}, respectively. Furthermore, all compounds showed a significant reduction in IgM production at 1000 nmol L^{-1}.

In this study, we measured chemical constituents of 11 cultivars of tea leaves by HPLC and found that Cha Chuukanbohon Nou 6 had two unknown major compounds. Compound **1** was almost five times more abundant in Cha Chuukanbohon Nou 6 than in Yabukita and compound **2** was not detected in other tea leaves. We isolated these compounds and using NMR spectroscopy, and identified them as theogallin and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose, respectively. Compound **2** was similar in chemical structure to strictinin, known as an inhibitor of IgE production,[30] and the structure of compound **1** has a galloyl group, which is present in the structure of strictinin. Therefore, the effects of the three compounds on the Igs production by human PBL were tested. Both theogallin and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose inhibited IgE production – similarly to stric-

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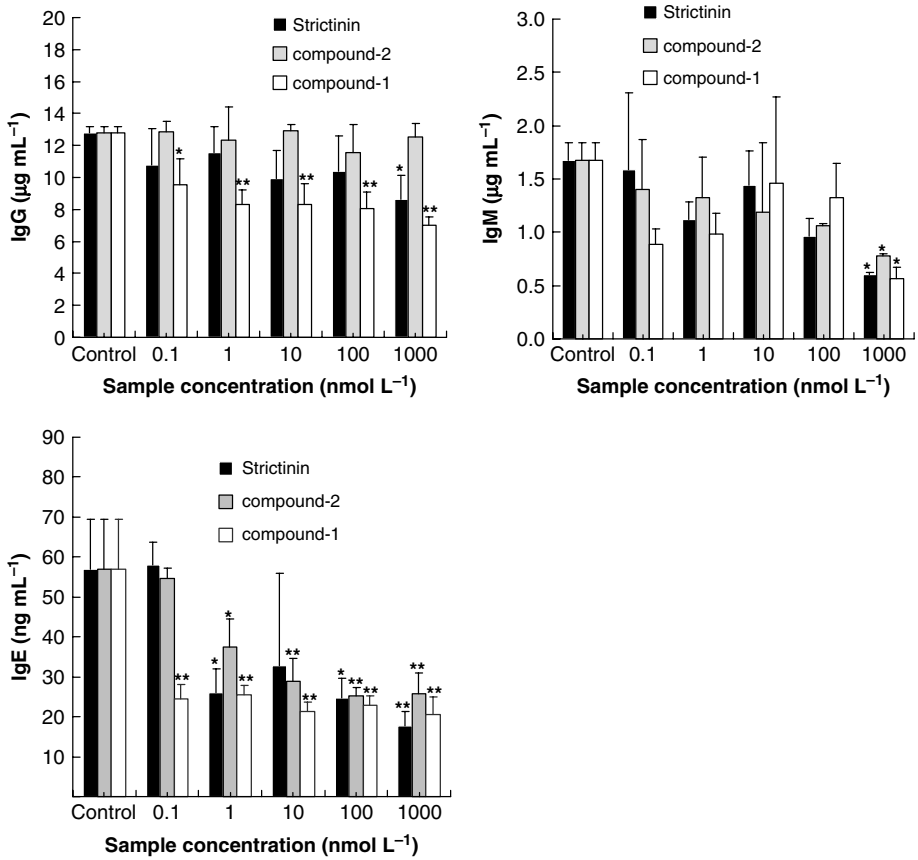

Figure 4. The effects of strictinin, compounds **1** and **2** on Ig production by PBL. Results are means ± SD ($n = 3$). $^{*}P < 0.05$, $^{**P} < 0.01$, compared with no-additive control.

tinin. Although 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose has been found in walnuts (the seeds of *Juglans regia* L.), in the leaves of *Camellia oleifera* and in the leaves of *C. taliensis*, this is the first time its been found in a cultivar originally improved in Japan.

IgE plays a critical role in the pathogenesis of allergic disease. The production of IgE is remarkably increased in hay fever sufferers and atopic patients.[42,43] Therefore, decreasing IgE production is effective in inhibiting allergic disease. In this study, we showed that theogallin and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose, including in Cha Chuukanbohon Nou 6 tea leaves, inhibited IgE production, like strictinin. To our knowledge, this is the first report that demonstrates the effects of these compounds on IgE production. Theogallin inhibited IgE production at 0.1 nmol L^{-1} but 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose and strictinin did not inhibit IgE production at the same concentrations; therefore, theogallin is a stronger inhibitor of IgE production than 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose and strictinin. We expect that theogallin inhibits IgE production dose-dependently less than 0.1 nmol L^{-1}.

IgG is the most abundant Ig and it constitutes 70–75% of serum Igs in humans. There are four IgG subclasses (IgG1, IgG2, IgG3 and IgG4) in humans; IgG1 is the most abundant, at about 66%, while IgG2, IgG3 and IgG4 constitute about 23%, 7% and 4%, respectively. It is important to decrease IgG1 production for inhibiting allergic disease.[44] Among IgG, the increase and decrease of IgG1 is closely linked to pathogenesis and improvement of allergic disease, as is IgE.[45–47] It was reported that oral injection of peanut seed skin procyanidin A1 decreased IgE and IgG1 levels in serum of mice immunized intraperitoneally with allergen ovalbumin,[48] and retinoic acid inhibited IgE and IgG1 production of LPS-induced murine spleen B cells.[49] In this study, theogallin inhibited IgG production at 0.1, 1, 10, 100 and 1000 nmol L^{-1} dose-dependently. We expect that theogallin inhibits IgG1 production, which is included in IgG subclasses.

These compounds are potentially new antiallergic agents which inhibit the production of IgE. The results suggest Cha Chuukanbohon Nou 6 to be the basis of an antiallergic beverage.

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