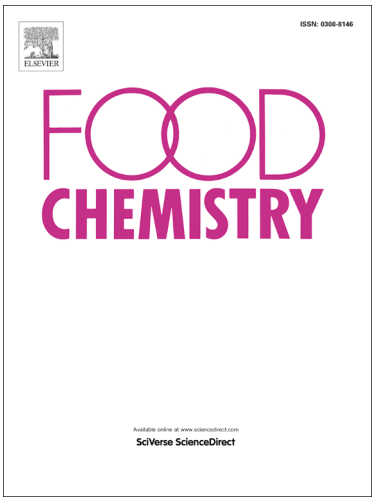

Analytical Methods

Oligomerization mechanism of tea catechins during tea roasting

Hitomi Morikawa, Keita Okuda, Yuji Kunihira, Aoi Inada, Chika Miyagi, Yosuke Matsuo, Yoshinori Saito, Takashi Tanaka

PII: S0308-8146(19)30238-9
DOI: https://doi.org/10.1016/j.foodchem.2019.01.163
Reference: FOCH 24258

To appear in: *Food Chemistry*

Received Date: 19 October 2018
Revised Date: 29 December 2018
Accepted Date: 24 January 2019

Please cite this article as: Morikawa, H., Okuda, K., Kunihira, Y., Inada, A., Miyagi, C., Matsuo, Y., Saito, Y., Tanaka, T., Oligomerization mechanism of tea catechins during tea roasting, *Food Chemistry* (2019), doi: https://doi.org/10.1016/j.foodchem.2019.01.163

Food Chemistry (Research paper)

Oligomerization mechanism of tea catechins during tea roasting

Hitomi Morikawa[a], Keita Okuda[a], Yuji Kunihira[a], Aoi Inada[a], Chika Miyagi[b], Yosuke Matsuo[a], Yoshinori Saito[a], Takashi Tanaka[a,*]

[a] *Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*

[b] *Department of Natural Product Chemistry, School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*

[*] Corresponding author: Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan, Tel: +81 95 8192433, E-mail address: t-tanaka@nagasaki-u.ac.jp (T. Tanaka)

Abstract: Roasting of green tea causes oligomerization of tea catechins, which decreases the astringency. The aim of this study was to elucidate the oligomerization mechanism. The ^{13}C NMR spectrum of the oligomer fraction showed signals arising from catechin and sugar residues. Heating of epigallocatechin-3-*O*-gallate with ^{13}C-labeled glucose (150°C for 2 h) suggested that condensation of sugars with catechin A-rings caused the oligomerization. The dimeric product obtained by heating for a shorter period (30 min) suggested cross-linking occurred between sugars and catechin A-rings. Furthermore, heating of phloroglucinol, a catechin A-ring mimic, with glucose, methylglyoxal, and dihydroxyacetone, confirmed that the basic mechanism included reaction of the catechin A-ring methine carbons with carbonyl carbons of glucose and their pyrolysis products.

Keywords: roasted tea; catechin; sugar; methylglyoxal; dihydroxyacetone; phloroglucinol; polyphenol

1. Introduction

Roasting is an important method for processing, cooking, and preserving foods, and is essential in the production of cocoa and coffee as it add characteristic aromas and flavors. However, chemical changes occur in phenolic substances during the roasting process and these changes are complex and not clearly understood (Taeye, Bodart, Caullet, & Collin, 2017; Moreira, et al., 2017). In Japan, roasted green tea is a popular processed tea, and it is served in traditional Japanese multi-course dinners for the elderly. Roasting adds characteristic flavor via caramelization and Maillard reactions and decreases the caffeine content by sublimation, which is beneficial to reduce the effect of tea on sleep. In addition, roasted green tea has a milder taste than non-roasted green tea, and this is thought to be caused by a decrease in the content of astringent tea catechins. A comparison of HPLC profiles of an original green tea product and a roasted green tea (Fig. 1) showed roasting resulted in epimerization at the catechin C-2 position (Suzuki, et al., 2003; Seto, Nakamura, Nanjo, & Hara, 1997) and generation of oligomeric products, which were detected as a broad hump on the baseline. Astringency is caused by interactions with salivary proteins (Haslam, 1996; Baxter, Lilley, Haslam, & Williamson, 1997); thus, the epimerization products (**1a**–**4a**) do not contribute to the decrease in astringency because the affinities of 2,3-*trans* epimerization products in human serum albumin are stronger than in the original 2,3-*cis* tea catechins (Ishii et al., 2010). Therefore, oligomerization of tea catechins is mainly responsible for the decrease in tea astringency with roasting; however, the chemical mechanisms for this are unknown. The aim of this study was to elucidate the oligomerization mechanism using model experiments and spectroscopic methods.

2. Materials and methods

2.1. Materials

Green tea leaves were supplied by Nagasaki Agriculture and Forestry Technical Development Center, Higashisonogi Tea Research Station (Nagasaki, Japan). 1-^{13}C-D-Glucose and U-$^{13}C_6$-D-glucose were purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). Epigallocatechin-3-*O*-gallate was isolated from commercial green tea according to a reported method (Nonaka, Kawahara, & Nishioka, 1983).

2.2. Analytical procedures

Ultraviolet (UV)–visible spectra were obtained using a JASCO V-560 spectrophotometer (Jasco Co., Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded in acetone-d_6, CD_3OD, and DMSO-d_6 (Kanto Chemical Co. Inc., Tokyo, Japan) at 27°C with a JEOL JNM-AL400 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 400 and 100 MHz for ^{1}H and ^{13}C nuclei, respectively. The coupling constants are expressed in hertz, and chemical shifts are presented on the delta (ppm) scale. Column chromatography was performed using Sephadex LH-20 (25–100 μm, GE Healthcare Bio-Science AB, Uppsala), Diaion HP20SS (Mitsubishi Chemical, Japan), MCI-gel CHP 20P (75–150 μm; Mitsubishi Chemical, Tokyo, Japan), Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical, Kasugai, Japan), and silica gel 60N (100–250 μm, Kanto Chemical Co., Tokyo, Japan) columns. Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2-mm thick, Merck KGaA, Darmstadt, Germany) with toluene:ethyl formate:formic acid (1:7:1, v/v/v) and $CHCl_3$:MeOH:water (14:6:1, v/v/v). Spots were detected under UV illumination after

spraying with 2% ethanolic $FeCl_3$ or a 5% sulfuric acid reagent and heating. Analytical HPLC was performed using a Cosmosil $5C_{18}$-AR II (Nacalai Tesque Inc., Kyoto, Japan) column (4.6 mm i.d. × 250 mm) with a gradient elution from 4%–30% CH_3CN (39 min) and 30%–75% CH_3CN (15 min) in 50 mM H_3PO_4. The mobile phase flow rate was 0.8 mL/min, and detection was achieved using a Jasco MD-910 photodiode array detector.

*2.3. Heating of (−)-epigallocatechin-3-O-gallate (**2**)*

An aqueous solution (200 mL) of (−)-epigallocatechin-3-*O*-gallate (**2**) (1.0 g) was lyophilized, and the resulting white powder was heated at 150°C for 60 min in an electric furnace (Yamato Scientific co., ltd., Tokyo, Japan). The reaction mixture was subjected to Sephadex LH-20 column chromatography (3 cm i.d. × 25 cm) with 0–100% MeOH containing 1% trifluoroacetic acid (10% stepwise, each 100 mL) to produce (−)-gallocatechin-3-*O*-gallate (142.5 mg), gallic acid (18.2 mg), and tricetinidin (12.2 mg) (Kuhnert N., Clifford M. N., & Radenac A-G., 2001; Coggon, Moss, Graham, & Sanderson, 1973) and recover **2** (756 mg).

2.4. Roasting of green tea leaves and separation of oligomeric polyphenols

Green tea leaves (20 g) were heated at 180°C in an electric furnace for 30 min. After cooling, the leaves were extracted twice with acetone:H_2O (3:2, v/v, 300 mL) at r.t. The extract was concentrated using a rotary evaporator, and the resulting aqueous solution (150 mL) was partitioned with EtOAc (150 mL) twice to produce an EtOAc fraction (2.97 g). The aqueous layer was first concentrated to remove residual EtOAc and then subjected to Diaion HP20SS column chromatography (3 cm i.d. × 20 cm) with H_2O:MeOH (0–100%, 20% stepwise, each 100 mL). The eluate was monitored by TLC

and separated into two fractions (Fr.). Fraction 1 mainly contained sugars (3.1 g), and Fr. 2 contained catechins and caffeine (2.1 g). Fraction 2 was subjected to size-exclusion column chromatography using Sephadex LH-20 (2 cm i.d. × 55 cm) with 7 M urea:acetone (2:3, v/v, containing conc. HCl at 5 mL/L) (Yanagida, Shoji, & Shibusawa, 2003) to yield oligomers (241 mg) and a mixture of catechins, caffeine, and oligomers (1.6 g). The oligomers were detected at the origin in TLC analysis and as a broad hump on the baseline in HPLC analysis (Fig. S1).

*2.5. Heating of (−)-epigallocatechin-3-O-gallate (**2**) with sucrose*

(−)-Epigallocatechin-3-*O*-gallate (**2**) (1.0 g) and sucrose (1.0 g) were dissolved in H_2O (10 mL) and heated on a hot plate until most of the water evaporated. The resulting paste was heated at 150°C for 2 h in an electric furnace, and the products were separated by Sephadex LH-20 column chromatography (3 cm i.d. × 12 cm) with 0–100% MeOH (20% stepwise, each 100 mL) and then MeOH:H_2O:acetone (60:20:20 and 0:1:1 v/v/v steps, each 100 mL) to produce two fractions. The first fraction was purified by Diaion HP20SS column chromatography (3 cm i.d. × 12 cm) with 0–100% MeOH (10% stepwise, each 100 mL) to produce the pure oligomers (307 mg). The second fraction (1.16 g) also contained oligomers as the major constituents as well as gallic acid and minor impurities (Fig. S2).

*2.6. Heating of (−)-epigallocatechin-3-O-gallate (**2**) with glucose*

(−)-Epigallocatechin-3-*O*-gallate (**2**) (510 mg) and D-glucose (386 mg) were dissolved in H_2O:acetone (4:1, v/v, 2.5 mL) in a petri dish and concentrated by heating at 75°C. The resulting paste was heated at 150°C for 2 h in the electric furnace, and the

products were separated by Sephadex LH-20 column chromatography (2 cm i.d. × 25 cm) with 60%–100% MeOH (20% stepwise, each 100 mL) and then MeOH–H_2O–acetone (90:5:5, 80:10:10, 60:20:20, and 0:1:1 v/v/v steps, each 100 mL) to give five fractions: Fr. 1 (172 mg), Fr. 2 (186 mg), Fr. 3 (290.4 mg), Fr. 4 (77 mg), and Fr. 5 (192 mg). HPLC of Fr. 5 showed that it only contained oligomeric products.

*2.7. Heating of (−)-epigallocatechin-3-O-gallate (**2**) with ^{13}C-labeled glucose*

Similarly, **2** (510 mg) was heated with a 9:1 mixture of glucose and 1-^{13}C-D-glucose (total of 386 mg) to yield the oligomers (182 mg). Experiments using **2** (510 mg) and a 9:1 mixture of glucose and U-^{13}C6-D-glucose (total of 386 mg) afforded the oligomers (190 mg).

*2.8. Monomeric and dimeric products of **2** produced by heating with glucose*

An aqueous solution (400 mL) of **2** (2.0 g) and D-glucose (1.6 g) was lyophilized, and the resulting white powder was heated at 150°C for 30 min. The reaction mixture was subjected to MCI-gel CHP20P column chromatography (3 cm i.d. × 20 cm) with 0–100% MeOH (10% stepwise, each 100 mL) to give five fractions: Fr. 1 (773 mg), Fr. 2 (246 mg), Fr. 3 (654 mg), Fr. 4 (402 mg), and Fr. 5 (139 mg). Separation of Fr. 1 using the Sephadex LH-20 column (2 cm i.d. × 20 cm) with 0–100 % MeOH in H_2O (10% stepwise, each 100 mL) gave **5** (32.2 mg), **6** (95.7 mg), and **7** (12.3 mg). Fraction 3 was subjected to Sephadex LH-20 column chromatography (3 cm i.d. × 20 cm) with 0–100% MeOH (10% stepwise, each 100 mL) to produce **2** (93.8 mg) and **2a** (71.9 mg). Fractions 4 and 5 mainly contained oligomeric products, and separation of Fr. 4 using the Sephadex LH-20 column (3 cm i.d. × 20 cm) with 20–100% MeOH (10%

stepwise, each 100 mL) and subsequent purification using Chromatorex ODS column chromatography (3 cm i.d. × 25 cm) with 0–50% MeOH (5% stepwise, each 100 mL) furnished **8** (10.4 mg).

*2.8.1. 8-C-Glucosyl-(−)-epigallocatechin-3-O-gallate (**5**)*

Brown amorphous powder, $[\alpha]_D$ −195.4 (*c* 0.10, MeOH). FAB-MS *m/z*: 621 $[M+H]^+$, 643 $[M+Na]^+$. HR-FABMS *m/z:* 621.1465 (Calcd for $C_{28}H_{29}O_{16}$: 621.1456), 643.1293 (Calcd for $C_{28}H_{28}NaO_{16}$: 643.1275). UV λ_{max} nm (log ε): 211 (4.88), 276 (4.02). IR ν_{max} cm^{-1}: 3388, 1691, 1613, 1537, 1453, 1448. ^{1}H NMR (acetone-d_6 + D_2O) δ: 2.96 (2H, m, H-4), 3.42–3.88 (m, glc-2-6), 4.98 (1H, d, 9.8 Hz, glc-1), 5.06 (1H, br s, H-2), 5.34 (1H, m, H-3), 5.98 (1H, s, H-6), 6.75 (2H, s, B-2,6), 6.99 (2H, s, galloyl-H). ^{13}C NMR (acetone-d_6 + D_2O) δ: 26.3 (C-4), 61.6 (glc-6), 69.4 (C-3), 70.8 (glc-4), 73.5 (glc-2), 76.2 (glc-1), 77.9 (C-2), 78.7 (glc-3), 81.6 (glc-5), 96.8 (C-6), 98.4 (C-4a), 103.5 (C-8), 106.3 (B-ring-2,6), 109.8 (galloyl-2,6), 121.1 (galloyl-1), 130.6 (B-ring-1), 132.7 (B-ring-4), 138.7 (galloyl-4), 145.6 (galloyl-3,5), 146.0 (B-ring-3,5), 154.0 (C-8a), 156.3 (C-7), 157.0 (C-5), 166.4 (galloyl-7).

*2.8.2. 6-C-Glucosyl-(−)-epigallocatechin-3-O-gallate (**6**)*

Brown amorphous powder, $[\alpha]_D$ −66.6 (*c* 0.10, MeOH). FAB-MS *m/z*: 621 $[M+H]^+$, 643 $[M+Na]^+$. HR-FABMS *m/z*: 621.1451 (Calcd for $C_{28}H_{29}O_{16}$: 621.1456), 643.1279 (Calcd for $C_{28}H_{28}NaO_{16}$: 643.1275). UV λ_{max} nm (log ε): 211 (4.83), 276 (3.99). IR ν_{max} cm^{-1}: 3393, 1681, 1613, 1536, 1453, 1337. ^{1}H NMR (acetone-d_6 + D_2O) δ: 2.86 (1H, dd, 3, 18 Hz, H-4), 3.00 (1H, dd, 3, 18 Hz, H-4), 3.48–3.82 (m, glc-2-6), 4.83 (1H, d, 9.6 Hz, glc-1), 5.01 (1H, br s, H-2), 5.43 (1H, m, H-3), 6.06 (1H, s, H-8), 6.61 (2H, s, B-2,6), 6.99 (2H, s, galloyl-H). ^{13}C NMR (acetone-d_6 + D_2O) δ: 26.5 (C-4), 61.3 (glc-6), 69.5 (C-3), 70.1 (glc-4), 74.6 (glc-2), 76.8 (glc-1), 78.0 (C-2), 79.0 (glc-3),

81.8 (glc-5), 96.6 (C-8), 100.3 (C-4a), 105.4 (C-6), 106.5 (B-ring-2,6), 109.8 (galloyl-2,6), 121.4 (galloyl-1), 130.3 (B-ring-1), 133.0 (B-ring-4), 138.8 (galloyl-4), 145.8 (galloyl-3,5), 146.0 (B-ring-3,5), 155.3 (C-7), 155.6 (C-5), 156.0 (C-8a), 166.5 (galloyl-7).

*2.8.3 6-C-Substituted product of (−)-epigallocatechin-3-O-gallate (**7**)*

Brown amorphous powder, $[\alpha]_D$ −136.8 (c 0.12, MeOH), UV (MeOH) λ_{max} (log ε): 268 (4.04), IR ν_{max} cm^{-1}: 3404, 1693, 1619, 1537, 1455, 1339. HR-ESI-MS $[M+H]^+$ *m/z*: 765.1873 $[M+H]^+$ (Calcd for $C_{34}H_{37}O_{20}$, 765.1873). ^{1}H NMR (acetone-d_6 + D_2O) δ: 2.98 (2H, m, H-4), 5.07 (1H, br s, H-2), 5.33 (1H, br, s, H-3), 6.75 (2H, s, B-ring-2,6), 6.98 (2H, s, galloyl-2,6), C-6-sugar unit: 3.01 (1H, d, *J* = 16.5 Hz,, H-1), 3.20 (1H, d, *J* = 16.5 Hz, H-1), 3.63 (2H, m, H-6), 3.89 (1H, m, H-5), 4.05 (1H, d, *J* = 8.3 Hz, H-3), 4.16 (1H, t, *J* = 8.3 Hz, H-4), C-8-glucosyl: 3.45 (1H, m, H-5), 3.66 (m, H-4), 3.67 (m, H-3), 3.83 (m, H-2), 3.84 (2H, m, H-6). ^{13}C NMR (acetone-d_6 + D_2O) δ: 26.0 (C-4), 69.2 (C-3), 78.1 (C-2), 94.8 (C-4a), 104.5 (C-6), 104.7 (C-8), 106.9 (B-ring-2,6), 109.8 (galloyl-2,6), 121.0 (galloyl-1), 130.4 (B-ring-1), 132.7 (B-ring-4), 138.8 (galloyl-4), 145.5 (B-ring-3,5), 146.0 (galloyl-3,5), 151.6 (C-7), 153.3 (C-5), 157.9 (C-8a), 166.4 (galloyl-7); C-6-sugar unit: 34.8 (C-1), 64.3 (C-6), 76.6 (C-4), 80.7 (C-3), 83.9 (C-5), 116.9 (C-2); C-8-glucosyl: 61.4 (C-6), 70.6 (C-4), 73.8 (C-3), 75.4 (C-1), 78.6 (C-2), 81.6 (C-5).

*2.8.4. Dimeric product (**8**)*

Brown amorphous powder, $[\alpha]_D$ −168.6 (*c* 0.10, MeOH), UV (MeOH) λ_{max} (log ε): 211 (5.02), 269 (4.41), IR ν_{max} cm^{-1}: 3438, 1697, 1620, 1537, 1445. HR-ESI-MS $[M+Na]^+$ *m/z*: 1065.1955 $[M+Na]^+$ (Calcd for $C_{50}H_{42}NaO_{25}$: 1065.1913). ^{1}H NMR (acetone-d_6 + D_2O) δ: 2.98, 3.09 (each 2H, m, H-4, 4'), 5.06, 5.12 (each 1H, br s, H-2,

2'), 5.51 (2H, br, s, H-3, 3'), 6.13, 6.51 (each 1H, s, A-ring-H), 6.67, 6.68 (each 2H, s, B-ring-2,6), 7.00, 7.12 (each 2H, s, galloyl-2,6), sugar unit: 3.68 (1H, ddd, *J* = 2.8, 7.1, 9.2, 3.79, H-5), 3.79 (1H, dd, *J* = 7.1, 11.4 Hz,, H-6), 4.01 (1H, dd, *J* = 2.8, 11.4 Hz,, H-6), 4.63 (1H, dd, *J* = 3.0, 9.2 Hz, H-4), 5.39 (1H, dd, *J* = 1.6, 3.0 Hz, H-3), 6.52 (1H, d, *J* = 1.6 Hz, H-2). ^{13}C NMR (acetone-d_6 + D_2O) δ: 27.1, 27.3 (C-4, 4'), 69.1, 69.5 (C-3, 3'), 78.1, 79.1 (C-2, 2'), 91.1, 98.2 (A-ring methine), 99.1, 101.9 (C-4a, 4a'), 102.9, 111.2 (A-ring C-6 or C-8), 106.5 (B-ring-2,6), 109.8, 109.9 (galloyl-2,6), 121.0, 121.3 (galloyl-1), 129.9, 130.2 (B-ring-1), 133.0, 133.2 (B-ring-4), 138.8, 139.0 (galloyl-4), 145.7, 145.8 (galloyl-3,5), 146.1, 146.2 (B-ring-3,5), 155.5 (C-8a), 155.3, 157.0 (C-5), 154.1, 156.6 (C-7), 166.1 (galloyl-7); C-6-sugar unit: 38.1 (C-3), 64.3 (C-6), 74.0 (C-4), 74.4 (C-5), 101.7 (C-2), 148.2 (C-1).

2.9. Reaction of phloroglucinol and glucose

Phloroglucinol (6.3 g) and D-glucose (4.5 g) were dissolved in dimethyl formamide (5 mL) and heated at 80°C for 24 h. The mixture was poured into H_2O (100 mL), and the resulting phloroglucinol precipitate (2.0 g) was removed by filtration. The filtrate was applied to a Chromatorex ODS column (3 cm i.d. × 25 cm) with 0–100% MeOH (10% stepwise, each 100 mL) to yield Fr. 1 and **10** (227 mg). Fraction 1 was separated using Sephadex LH-20 column chromatography (4 cm i.d. × 30 cm) with 0–100% MeOH (20% stepwise, each 200 mL) to give glucose, Fr. 1-1, and Fr. 1-2, and recover phloroglucinol (1.7 g). Fraction 1-1 was purified via silica gel column chromatography ($CHCl_3$:MeOH:H_2O, 70:30:5, and 60:40:10 v/v/v steps) to yield **9** (378.7 mg). Similar silica gel column chromatography of Fr. 1-2 yielded an inseparable mixture of stereoisomers of **11** (346.7 mg). Treatment of **11** (40 mg) with Ac_2O (0.8

mL) in pyridine (0.5 mL) at r.t. for 10 h and subsequent silica gel column chromatography with hexane:acetone (3:2, v/v) afforded an acetate of one of the isomers of **11** (47 mg).

*2.9.1. Phloroglucinol-C-β-D-glucoside (**9**)*

White amorphous powder. $[\alpha]_D$ +37.9 (*c* 0.1, MeOH). FAB-MS *m/z*: 289 $[M+H]^+$. HR-FABMS *m/z*: 289.0923 (Calcd for $C_{12}H_{17}O_8$: 289.0918). IR ν_{max} cm^{-1}: 3373, 1620, 1452, 1148, 1040. ^1H NMR (400 MHz, CD_3OD) δ: 3.37 (1H, m, glc-5), 3.46 (2H, m, glc-6), 3.74 (1H, dd, *J* = 12.1, 4.8 Hz glc-4), 3.83 (1H, dd, *J* = 9.8, 4.6 Hz, glc-2), 3.86 (1H, brd, *J* = 4.6 Hz, glc-3), 4.79 (1H, d, *J* = 9.8 Hz, glc-1), 5.86 (2H, s, H-3, 4). ^{13}C NMR (100 MHz, CD_3OD) δ: 62.4 (glc-6), 71.4 (glc-4), 73.8 (glc-2), 76.8 (glc-1), 79.9 (glc-3), 82.4 (glc-5), 96.4 (C-3, 5), 104.3 (C-1), 158.9 (C-2, 6), 159.6 (C-4).

*2.9.2. Dimeric product **10***

Yellow amorphous powder. $[\alpha]_D$ −24.4 (*c* 0.1, MeOH). FAB-MS *m/z*: 397 $[M+H]^+$. HR-FABMS *m/z*: 397.1145 (Calcd for $C_{18}H_{21}O_{10}$: 397.1134). UV λ_{max} nm (log ε): 216 (4.69). IR ν_{max} cm^{-1}: 3373, 1620, 1452, 1148, 1040. ^1H NMR (400 MHz, CD_3OD) δ: 3.48 (1H, dd, *J* = 5.8, 13.6 Hz, sug-6), 3.62 (1H, dd, *J* = 3.4, 13.6 Hz, sug-6), 3.51 (1H, dd, *J* = 2.3, 7.3 Hz, sug-4), 3.59 (1H, ddd, *J* = 3.4, 5.8, 7.3 Hz, sug-5), 3.74 (1H, dd, *J* = 2.3, 3.2 Hz, sug-3), 3.82 (1H, dd, *J* = 3.2, 6.5 Hz, sug-2), 4.53 (1H, d, *J* =.5 Hz, sug-1), 6.07 (1H, d, *J* = 2.3 Hz, H-5'), 6.08 (1H, d, *J* = 2.3 Hz, H-3'), 6.11 (1H, d, *J* = 2.3 Hz, H-3'), 6.12 (1H, d, *J* = 2.3 Hz, H-3'). ^{13}C NMR (100 MHz, CD_3OD) δ: 33.5 (glc-1), 64.5 (glc-6), 70.1 (glc-3), 73.0 (glc-5), 75.3 (glc-4), 79.4 (glc-2), 99.4 (C-3), 98.9 (C-5), 96.3 (C-3'), 96.7 (C-7'), 103.9 (2C, C-1, C-1'), 156.1 (C-2'), 156.2 (C-6'), 156.9 (C-2), 157.1 (C-6), 158.4 (C-4'), 158.5 (C-4).

*2.9.3 Acetate of bisphloroglucinolyl glucose (**11**)*

White amorphous powder; $[\alpha]_D$ +112.2 (*c* 0.11, MeOH); UV λ_{max} nm (log ε): 280 (0.15), 206 (1.80); IR ν_{max} cm^{-1}: 3453, 1748, 1211; FAB-MS (positive, matrix; *m*-nitrobenzyl alcohol) *m/z*: 775 $[M+H]^+$; HR-FAB-MS *m/z*: 775.2088 $[M+H]^+$ (Calcd for $C_{36}H_{39}O_{19}$: 775.2086); ^{1}H NMR (acetone-d_6, 500 MHz) δ: 6.89, 6.85 (each 1H, d, *J* = 2.2 Hz, B-3,5), 6.48 (1H, d, *J* = 2.0 Hz, A-3), 6.30 (1H, d, *J* = 2.0 Hz, A-5), 5.54 (1H, dd, *J* = 3.5, 8.2 Hz, glc-4), 5.27 (1H, dd, *J* = 1.3, 3.5 Hz, glc-3), 5.08 (1H, ddd, J = 3.4, 4.2, 8.2 Hz, glc-5), 5.03 (1H, dd, *J* = 1.3, 5.9 Hz, glc-2), 4.48 (1H, d, *J* = 5.9 Hz, glc-1), 4.23 (1H, dd, J = 3.4, 12.4 Hz, glc-6), 4.20 (1H, dd, *J* = 4.2, 12.4 Hz, glc-6), 2.39, 2.26, 2.25, 2.13, 2.07, 2.02, 1.99, 1.98, 1.97 (each 3H, s, CH_3). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 170.8, 170.6, 169.9, 169.8, 169.5, 168.8, 168.3, 167.9, 167.7 (COO), 161.3 (A-2), 151.6 (A-4), 150.2, 149.9, 148.9 (B-2,4,6), 146.6 (A-6), 122.0 (B-1), 117.9 (A-1), 115.5, 113.3 (B-3,5), 107.8 (A-5), 101.1 (A-3), 88.8 (glc-2), 71.2 (glc-3), 69.8 (glc-4), 68.2 (glc-5), 61.3 (glc-6), 40.2 (glc-1), 21.1 (2C), 20.9, 20.8, 20.7, 20.6, 20.4, 20.3, 20.0 (CH_3).

2.10. Reaction of phloroglucinol and dihydroxyacetone dimer

Phloroglucinol (1.26 g) and dihydroxyacetone dimer (0.45 g) were dissolved in acetone (5 mL), and then the acetone was removed using a rotary evaporator. The resulting syrup was heated at 130°C for 1 h, and then separated by silica gel column chromatography with $CHCl_3$:MeOH:H_2O (90:10:1, 85:15:1, 80:20:2, 75:25:3, 70:30:5, and 50:50:0 v/v/v steps) to produce **12** (356 mg), **13** (42 mg), phloroglucinol (348 mg), and an oligomer fraction (768 mg). A similar experiment using phloroglucinol (1.26 g) and methylglyoxal (0.36 g) yielded **12** (686 mg) and recovered phloroglucinol (445

mg).

*2.10.1 Phloroglucinol dimer (**12**)*

Pale brown amorphous powder; UV λ_{max} nm (log ε): 208 (4.47), 215 (4.61), 223 (4.05), 272 (0.62); IR ν_{max} cm^{-1}: 3242, 1624, 1487; FAB-MS (positive, matrix: glycerol) *m/z*: 289 $[M+H]^+$; HR-FAB-MS *m/z*: 289.0710 $[M+H]^+$ (Calcd for $C_{15}H_{13}O_6$: 289.0712); ^{1}H NMR (acetone-d_6, 500 MHz) δ: 5.99, 5.98 (each 2H, d, *J* = 2.0 Hz, H-3,5,3',5'), 4.59 (1H, s, H-7), 1.77 (3H, s, H-9). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 160.5, 160.2, 153.5 (each 2C, C-2,4,6,2',4',6'), 124.7 (C-8), 106.3 (2C, C-1, 1'), 96.8, 91.2 (each 2C, C-2, 4, 2', 4'), 30.6 (C-7), 24.6 (C-9).

*2.10.2. Phloroglucinol dimer (**13**)*

Pale brown powder; UV λ_{max} nm (log ε): 272 (2.20); IR ν_{max} cm^{-1}: 3360, 1631, 1514, 1466, 1257, 1133, 928, 822, 452; ESI-MS (positive, matrix: glycerol) *m/z*: 289 $[M+H]^+$; HR-FAB-MS *m/z*: 289.0713 $[M+H]^+$ (Calcd for $C_{15}H_{13}O_6$: 289.0712); ^{1}H NMR (acetone-d_6, 500 MHz) δ: 6.34 (1H, d, *J* = 7.9 Hz, H-5a), 6.01 (1H, d, *J* = 2.0 Hz, H-9), 5.98 (1H, d, *J* = 2.0 Hz, H-7), 5.85 (1H, d, *J* = 2.0 Hz, H-2), 5.71 (1H, d, *J* = 2.0 Hz, H-4), 4.08 (1H, m, H-10b), 3.59 (1H, dd, *J* = 2.0, 15.1 Hz, H-11), 2.53 (1H, dd, *J* = 6.3, 15.1 Hz, H-11); ^{13}C NMR (acetone-d_6, 125 MHz) δ: 162.0 (C-6a), 159.5 (C-3), 157.3 (C-8), 156.3 (C-10), 155.6 (C-1), 155.2 (C-4a), 106.2 (C-5a), 105.4 (C-11a), 105.0 (C-10a), 98.2 (C-7), 96.6 (C-2), 89.7 (C-4), 40.8 (C-10b), 18.6 (C-11).

3. Results and discussion

3.1. Pyrolysis of (−)-epigallocatechin-3-*O*-gallate

Despite the high popularity of roasted green tea in Japan, only a few chemical studies have been published, and these studies have shown isomerization and

oligomerization of tea catechins occur on roasting (Nakagawa, 1967; Hara & Kubota, 1969; Anan, Amano, & Nakagawa, 1981). However, the structures of the oligomers have not been studied. In this study, we examined pyrolysis of epigallocatechin-3-*O*-gallate (**2**) as a preliminary experiment. This catechin was selected because catechins with pyrogallol-type B-rings and galloyl esters account for over 70% and 60% of total tea catechins, respectively (Anan et al., 1981). Green tea leaves are typically roasted at 150–180°C (Nakagawa, 1967; Hara et al., 1969); therefore, a lyophilized powder of **2** was heated at 150°C for 60 min to afford (−)-gallocatechin-3-*O*-gallate, gallic acid, and an anthocyanidin together with recovered **2**. The anthocyanidin was identified as tricetinidin, which was produced by elimination of gallic acid and subsequent oxidation. In this experiment, little or no oligomeric products were produced.

3.2. Catechin oligomer from roasted green tea leaves

Next, the commercial green tea leaves were heated at 180°C for 30 min, and oligomeric polyphenols were obtained via solvent partitioning, adsorption column chromatography, and size-exclusion chromatography (Yanagida, Shoji, & Shibusawa, 2003). Using HPLC analysis, the oligomeric products were detected as a broad hump on the baseline, and the UV absorption was similar to that of **2** (Fig. S1). The ^{13}C NMR spectrum showed broad signals, which were closely related to those of **2** (Fig. 2A). However, the A-ring methine signals in the δ 95–100 range were much smaller than those of **2** (Fig. 2C). These signals shifted to lower field probably in the δ 105–115 range, which suggests that oligomerization reactions occur at the A-ring methine carbons. In addition, broad signals in the δ 60–80 range suggest sugars participate in the

catechin oligomerization upon roasting.

3.3. Catechin oligomer production from 2 and sucrose

Because the most abundant sugar in tea leaves is sucrose, **2** was heated with sucrose at 150°C for 2 h, and the resulting oligomers were separated (Fig. S2). The ^{13}C NMR spectrum (Fig. 2B) was similar to that of the oligomer fraction obtained from roasted green tea leaves except for the absence of the catechol-type B-ring originating from **3** and **4**. These results confirmed the cross-linking of **2** with the sugars.

3.4. Catechin oligomer produced from 2 and ^{13}C-labeled glucose

Next, the oligomers generated upon heating (150°C for 2 h) of **2** with glucose were examined because pyrolysis of sucrose upon roasting produces glucose and fructose as major products (Sˇimkovic, Sˇurina, & Vricˇan, 2003). The ^{13}C NMR spectrum of the oligomeric polyphenols obtained upon heating of **2** with glucose (Fig. 3 A) was similar to those of oligomers of roasted green tea leaves and oligomers prepared from **2** and sucrose (Fig. 2). The co-oligomerization was clearly confirmed by experiments using 1-^{13}C-labeled glucose (Fig. 3B) and U-^{13}C6-labeled glucose (Fig. 3C). The spectrum of the oligomer obtained upon heating with 1-^{13}C-labeled glucose exhibited large signals at δ 100 and δ 33 (Fig. 3B, black arrows), which were attributed to the carbons of the glucose anomeric carbon. The signal at δ 100 may be explained by formation of the *O*-glycosidation linkage at the anomeric position. In the spectrum of the oligomer obtained upon heating of the U-^{13}C6-labeled glucose, signals at δ 115 and from δ 60 to 84 (Fig. 3C, black arrows) were enhanced in addition to the δ 100 and δ 33 signals. The large signals between δ 60 and 84 were attributed to glucose C-2 to C-6

carbons. Enhancement of the signals at δ 115 and 33 upon incorporation of the ^{13}C was likely related to the oligomerization mechanism.

3.5. Monomeric and dimeric products of 2 produced upon heating with glucose

To understand the oligomerization mechanisms, lyophilized powder of a mixture of **2** and D-glucose was heated at 150°C for a shorter period (30 min), and the products were separated by column chromatography to yield four products. Products **5** and **6** were a pair of isomers of glucose C-glucosides according to FAB-MS, which had a $[M+H]^+$ peak at *m/z* 621. The ^1H and ^{13}C NMR spectra showed signals arising from **2** and glucopyranose moieties; however, the A-ring proton signals were observed as one proton singlet signal in each spectrum [**5**: 5.98 (s, H-6), **6**: 6.06 (s, H-8)], and the glucose C-1 of **5** and **6** resonated at δ 76.2 and δ 76.8, respectively (Fig. S3). These data confirmed that glucose was attached to the A-ring C-8 or C-6 of **2** via C-glycosidic linkages. The HMBC spectrum of **6** showed the correlation of the A-ring C-8a with the A-ring H-8 and C-ring H-2, indicating that the glucose of **6** was located at the C-6 position (Fig. S3). Thus, we concluded the glucose in **5** was located at C-8. From these spectroscopic data, **5** and **6** were determined to be epigallocatechin-3-*O*-gallate 8-C-glucoside and 6-C-glucoside, respectively (Fig. 4).

The ^1H and ^{13}C NMR spectra of the other monomeric product, **7**, showed a set of signals of **2** and two sets of signals for the sugar moiety. The absence of the A-ring methine proton signal in the ^1H NMR spectrum and the molecular formula of $C_{34}H_{37}O_{20}$, which was confirmed by HR-ESI-MS ($[M+H]^+$ *m/z* 765.1873, Calcd for $C_{34}H_{37}O_{20}$: 765.1873), both indicated that **7** was a disubstituted analog of **5** and **6**. ^1H and ^{13}C signals arising from one of the two sugar units were similar to the signals of

C-glycosidic glucopyranose moieties of **5** and **6**. Another sugar unit showed signals that were assignable to two methylenes (δ 34.8, C-1‴; and 61.4, C-6‴), three oxygenated methines (δ 76.6, C-4‴; 80.7, C-3‴; and 83.9, C-5‴), and an acetal quaternary carbon (δ 116.9, C-2‴). The HMBC correlations (Fig. 5) and unsaturation index of this molecule (17) suggested the presence of a spiroketal structure involving an ether linkage with an A-ring phenolic hydroxy group. The location of the hydroxy group participating in the acetal ring formation was determined by observation of hydrogen–deuterium exchange shifts of the A-ring carbons (Pfeffer, Valentine, & Parrish, 1979). The ^{13}C NMR spectra of **7** measured in acetone-d_6 + H_2O and acetone-d_6 + D_2O were carefully compared, and a distinct chemical shift difference was observed for C-7 (Δδ +0.123). By contrast, the shifts for C-5 (Δδ −0.008) and C-8a (Δδ +0.025) were minimal (Fig. S4). This observation indicated the presence of a free hydroxy group at the A-ring C-7. Because the sugar units originated from D-glucose, the configuration of the sugar carbons was self-evident except for the spiroketal carbon. We deduced the spiroketal was in the *S*-configuration from the NOE between the C-1 and C-3 protons. Based on these results, the structure of **7** was determined (Fig. 4). The chemical shifts of the spiroketal carbon (C-2‴, δ 116.9) and benzylic methylene carbon (C1‴, δ 34.8) coincided with the values of enhanced carbon signals (δ 115 and 33) in the spectra of the oligomers obtained upon condensation with the ^{13}C-labeled glucoses (Fig. 3). Therefore, a similar spiroketal structure may be present in the oligomeric products.

According to the $[M+Na]^+$ peak at *m/z* 1065.1955 in the HR-FAB-MS (Calcd for $C_{50}H_{42}NaO_{25}$: 1065.1913), product **8** is a dimeric product. The ^{1}H and ^{13}C NMR spectra showed that a sugar residue connected two epigallocatechin gallate moieties. The two A-ring proton singlet signals at δ 6.13 and 6.51 indicated that the two A-ring

methine carbons (C-6 or C-8) were attached to the sugar residue. The sugar residue was composed of six carbons: a trisubstituted double bond (δ 148.2, C-1; δ 101.7, C-2), a non-oxygenated methine carbon (δ 38.1, C-3), two oxygenated methines (δ 74.0, C-4; δ 74.4, C-5), and an oxygenated methylene carbon (δ 64.3, C-6). Formation of a pyran was deduced from the unsaturation index (30), and ^{1}H-^{1}H COSY and HMBC correlations of the sugar protons with the A-rings of epigallocatechin units showed a dimeric structure (Fig. 4). It was acceptable that the 4-, 5-, and 6-positions of the sugar moiety retained the configuration of the D-glucose, and the 3,4-*cis* configuration was suggested by the small $J_{2,3}$ value (3.0 Hz). However, we could not determine where the sugar residue attached to catechin C-6 or C-8 because of the lack of the HMBC correlations from the catechin C-ring H-2 to the A-ring C-8a; therefore, the structure in Fig. 4 is a tentative one. A plausible production mechanism of **8** is proposed in Scheme S1, which explains the catechin cross-linking with the sugar. Similar reactions may contribute to oligomerization of **2** in roasted tea, although it was difficult to identify the signals assignable to the sugar moiety of **8** in the ^{13}C NMR spectra of the oligomers (Fig. 3).

3.6. Reaction of phloroglucinol with glucose and its pyrolysis products

In addition to the low-field shifts of the A-ring methine carbon signals in the ^{13}C NMR spectra of the oligomeric products (Figs. 2 and 3), the results of the abovementioned chemical examinations indicated that the A-rings were responsible for the catechin oligomerization. To investigate the reaction mechanism in more detail, we selected phloroglucinol as a simple A-ring mimic and examined the reactions with glucose. Heating of phloroglucinol with glucose afforded C-glycoside **9** (Onodera,

Yamamoto, Abe, & Ueno, 1994) and two new dimeric products, **10** and **11** (Fig. 6). The structure of product **10** was determined by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments (Fig. S5). Dimer **11** was obtained as an inseparable mixture of stereoisomers. The major component of this mixture was purified as an acetate, and its structure was confirmed (Fig. S6). In the ^{13}C NMR spectrum of **10**, the C-1 of the glucose moiety resonated at δ 33.5, and the chemical shift was similar to one of the enhanced carbon signals of the oligomers obtained in the experiments using the ^{13}C-labeled glucoses (Fig. 3). The structures of **10** and **11** suggested another mechanism for the cross-linking of **2** with the sugars in addition to that suggested by the production of **8** (Scheme S2).

Reactions with the pyrolysis products of the sugars should also be considered because heating of sucrose, the most abundant sugar in tea leaves, is known to afford glucose and fructose, and further degradation of the monosaccharides generates methyl glyoxal (MG) and dihydroxyacetone (DHA) (Kabyemela, Adschiri, Malaluan, & Ara, 1997). Both MG and DHA are also generated in the Maillard reaction (Totlani & Peterson, 2006). In addition, MG is produced from DHA. In this study, phloroglucinol was heated separately with MG and the DHA dimer, and **12** was obtained as a major product in the reaction with MG. Products **12** and **13** were obtained from the reaction with DHA. The structures were determined by spectroscopic methods and computer calculations (Figs. S7 and S8). Production of **12** via both reactions supported the conversion of DHA to MG upon heating. These reactions also afforded oligomeric products. The ^{13}C NMR spectrum of the oligomeric products obtained by heating phloroglucinol with 2-^{13}C-labeled DHA (Fig. S9) showed broad signals at δ 124 (enhanced by incorporation of ^{13}C), δ 48, and δ 24, which coincided with the C-8 (δ

124.5), C-7 (δ 49.6), and C-9 (δ 24.7) signals of **12**. This indicates that the oligomerization mechanism of phloroglucinol with DHA is similar to the production mechanism of **12** (Scheme S3). Furthermore, the broad signal observed in the δ 105–115 range is assignable to aromatic carbons where the MG unit connected. In the ^{13}C NMR spectrum of the oligomer obtained from the roasted tea (Fig. 2), signals for the catechin C6 and C8 carbon that were involved in the oligomerization reaction were likely present in a similar region.

4. Conclusions

In this study, we showed that roasting green tea leaves causes epimerization of the catechin C-ring and co-oligomerization of the catechin with sugar or its pyrolysis products. The co-oligomerization occurs at the catechin A-ring, which is shown by low-field shifts of the A-ring methine carbon signals from their original locations at δ 95–98 to δ 105–115. Structures of the products obtained via model reactions using **2** and its A-ring mimic, phloroglucinol, suggest that the oligomerization of catechins does not proceed uniformly; however, the basic mechanism involves cross-linking at the A-ring C6 and/or C8 with the carbonyl carbons of sugar or its pyrolysis products. The roasting process decreases the astringency caused by tea catechins and the astringency of the oligomer fraction obtained from roasted tea is milder than that of the original green tea catechins. A similar reaction should occur during roasting of cacao and other catechin- and proanthocyanidin-containing foods. Evaluation of the biological functions of the oligomers is now in progress.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science KAKENHI (Grant Nos. 17K08338 and 16K07741). The authors are grateful to N. Tsuda, K. Chifuku, and H. Iwata at the Center for Industry, University and Government Cooperation, Nagasaki University, for recording the NMR and MS data. We thank Gabrielle David, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/

References

Anan, T., Amano, I, & Nakagawa, M. (1981). Changes in contents of some ingredients during heating of green tea. *Journal of Japanese Society of Food Science and Technology*, *28*, 74–78.

Baxter, N. J., Lilley, T. H., Haslam, E., & Williamson, M. P. (1997). Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry*, *36*, 5566–5577.

Coggon, P., Moss, G. A., Graham, H. N., & Sanderson, G. W. (1973). Biochemistry of tea fermentation. Oxidative degallation and epimerization of the tea flavanol gallates. *Journal of Agricultural and Food Chemistry, 21*, 727–733.

De Taeye, C., Bodart, M., Caullet, G., & Sonia Collin, S. (2017). Roasting conditions

for preserving cocoa flavan-3-ol monomers and oligomers: interesting behavior of Criollo clones. *Journal of the Science of Food and Agriculture*, *97*, 4001–4008.

Hara, T. & Kubota, E. (1969) Effects of roasting condition on the qualities of roasted green tea. *Journal of Japanese Society of Food Science and Technology*, *16*, 145–149.

Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: possible modes of action, *Journal of Natural Products*, *59*, 205–215.

Ishii, T., Minoda, K., Bae, M.-J., Mori, T., Uekusa, Y., Ichikawa, T., Aihara, Y., Furuta, T., Wakimoto, T., Kan, T., & Nakayama, T. (2010). Binding affinity of tea catechins for HSA: Characterization by high-performance affinity chromatography with immobilized albumin column, *Molecular Nutrition & Food Research*, *54*, 816–822.

Kabyemela, B. M., Adschiri, T., Malaluan, R. M., & Arai, K. (1997). Kinetics of glucose epimerization and decomposition in subcritical and supercritical water, *Industrial & Engineering Chemistry Research*, *36*, 1552–1558.

Kuhnert, N., Clifford, M. N., & Radenac, A-G. (2001). Boron trifluoride–etherate mediated synthesis of 3-desoxyanthocyanidins including a total synthesis of tricetanidin from black tea. *Tetrahedron Letters*, *42*, 9261–9263 (2001)

Moreira, A. S. P., Nunes, F. M., Simões, C., Maciel, E., Domingues, P., Domingues, M. R. M., & Coimbra, M. A. (2017). Transglycosylation reactions, a main mechanism of phenolics incorporation in coffee melanoidins: Inhibition by Maillard reaction, *Food Chemistry*, *227*. 422–431.

Nakagawa, M. (1967). The nature and the origin of polyphenols in Hoji-cha (roasted green tea), *Agricultural and Biological Chemistry, 31*, 1283–1287.

Nonaka, G., Kawahara, O., & Nishioka, I. (1983) Tannins and related compounds. XV.

A new class of dimeric flavan-3-ol gallates, theasinensins A and B, and proanthocyanidin gallates from green tea leaf. (1), *Chemical & Pharmaceutical Bulletin*, *31*, 3906–3914.

Onodera, J., Yamamoto, T., Abe, K., & Ueno, N. (1994). Cosmetics containing phloroglucinol derivatives, Jpn. Kokai Tokkyo Koho, JP 06092835 A 19940405.

Pfeffer, P. E., Valentine, K, M., & Parrish, F. W. (1979). Deuterium-induced differential isotope shift carbon-13 NMR. 1. Resonance reassignments of mono- and disaccharides. *Journal of the American Chemical Society*, 101, 1265-1274.

S˘imkovic, I., S˘urina, I., & Vric˘an, M. (2003). Primary reactions of sucrose thermal degradation, *Journal of Analytical and Applied Pyrolysis*, *70*, 493–504.

Seto, R., Nakamura, H., Nanjo, F., & Hara, Y. (1997). Preparation of epimers of tea catechins by heat treatment, *Bioscience, Biotechnology, and Biochemistry*, *61*, 1434–1439.

Suzuki, M., Sano, M., Yoshida, R., Degawa, M., Miyase, T., & Maeda-Yamamoto, M. (2003). Epimerization of tea catechins and *O*-methylated derivatives of (-)-epigallocatechin-3-*O*-gallate: relationship between epimerization and chemical structure, *Journal of Agricultural and Food Chemistry, 51*, 510–514.

Totlani, V. M. & Peterson, D. G. (2006). Epicatechin carbonyl-trapping reactions in aqueous Maillard systems: identification and structural elucidation, *Journal of Agricultural and Food Chemistry, 54*, 7311–7318.

Yanagida, A., Shoji, T., & Shibusawa, Y. (2003). Separation of proanthocyanidins by degree of polymerization by means of size-exclusion chromatography and related techniques. *Journal of Biochemical and Biophysical Methods*, *56*, 311–322.

Figure Captions

Fig. 1. HPLC profiles (max abs) of extracts of green tea leaves and roasted green tea leaves: A, Extract of green tea leaves; B, extract of roasted green tea leaves (0.02 g/mL in 60% EtOH); and C, vertical axis expansion of B. Compounds: **1**, (−)-epigallocatechin; **2**, (−)-epigallocatechin-3-*O*-galalte; **3**, (−)-epicatechin; **4**, (−)-epicatechin-3-*O*-galalte; **1a**, (−)-gallocatechin; **2a**, (−)-gallocatechin-3-*O*-galalte; **3a**, (−)-catechin; **4a**, (−)-catechin-3-*O*-galalte; GA, gallic acid; and caf, caffeine.

Fig. 2 ^{13}C-NMR spectra (in DMSO-d_6) of oligomeric polyphenols obtained from roasted green tea leaves (180°C) (A), oligomer obtained upon heating of **2** with sucrose (B), and **2** (C). Labeling: A, A-ring; B, B-ring; C, C-ring; G, galloyl; and Cat-B, catechol-type B-ring.

Fig. 3. ^{13}C-NMR spectra of the oligomer fraction obtained upon heating of **2** with glucose (A), 1-^{13}C-labeled glucose (B), and U-^{13}C6-labeled glucose (C). The black arrows indicate signals enhanced by incorporation of ^{13}C.

Fig. 4. Structures of **5**–**8** produced upon heating of **2** with glucose.

Fig. 5. Selected ^{1}H-^{1}H COSY and HMBC correlations of **7**.

Fig. 6. Products generated from phloroglucinol with glucose (**9**–**11**), methyl glyoxal (**12**), and dihydroxyacetone (**12** and **13**).

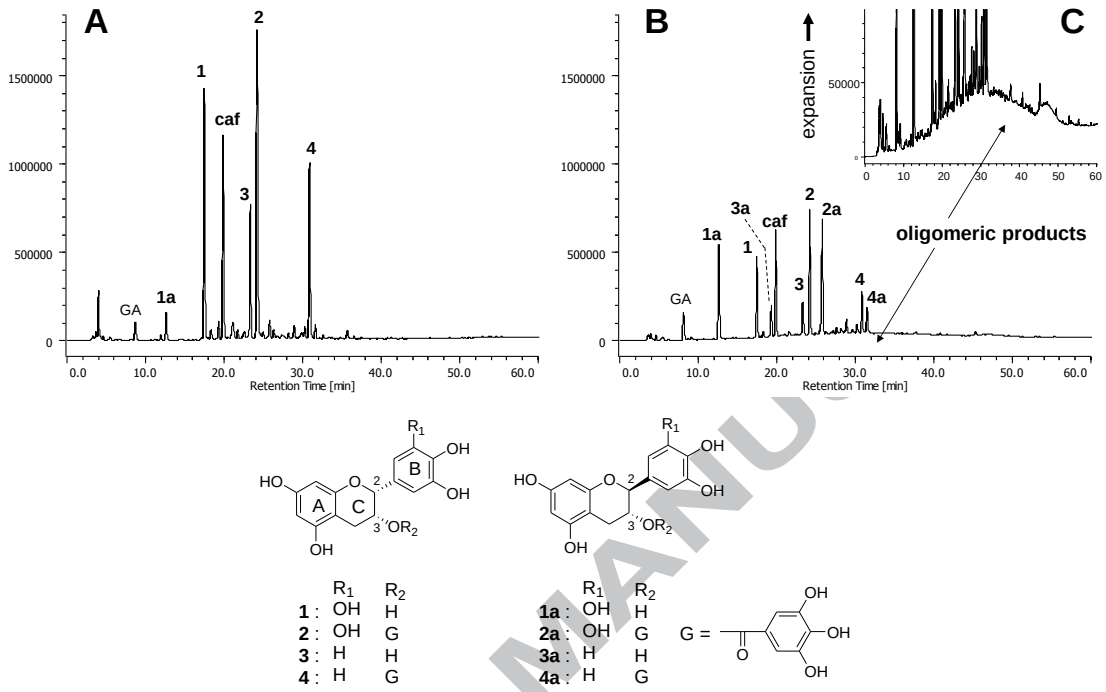

Fig. 1. HPLC profiles (max abs) of extracts of green tea leaves and roasted green tea leaves.

A: Extract of green tea leaves, B: extract of roasted green tea leaves (0.02 g/mL 60% EtOH). C: vertical axis expansion of B. **1**: (-)-epigallocatechin, **2**: (-)-epigallocatechin-3-*O*-galalte, **3**: (-)-epicatechin, **4**: (-)-epicatechin-3-*O*-galalte, **1a**: (-)-gallocatechin, **2a**: (-)-gallocatechin-3-*O*-galalte, **3a**: (-)-catechin, **4a**: (-)-catechin-3-*O*-galalte, GA: gallic acid, caf: caffeine.

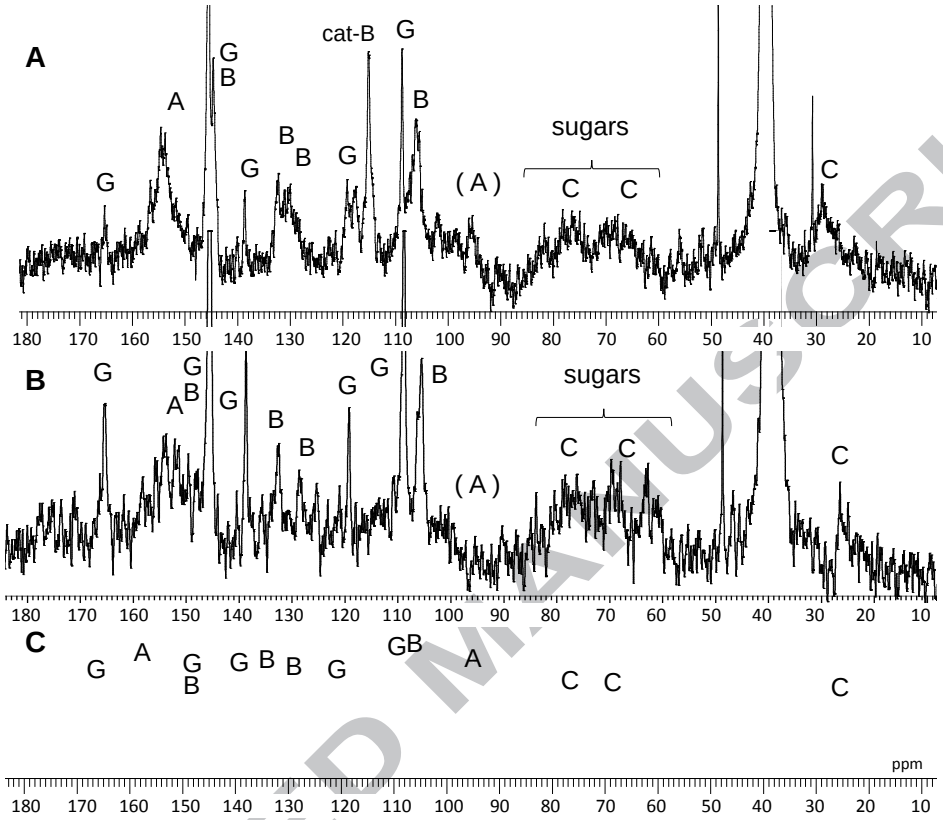

Fig. 2 ^{13}C-NMR spectra (in DMSO-d_6) of oligomeric polyphenols obtained from roasted green tea leaves (180°C) (A), oligomer obtained upon heating of **2** with sucrose (B), and **2** (C). A: A-ring, B: B-ring, C: C-ring, G: galloyl, Cat-B: catechol-type B-ring.

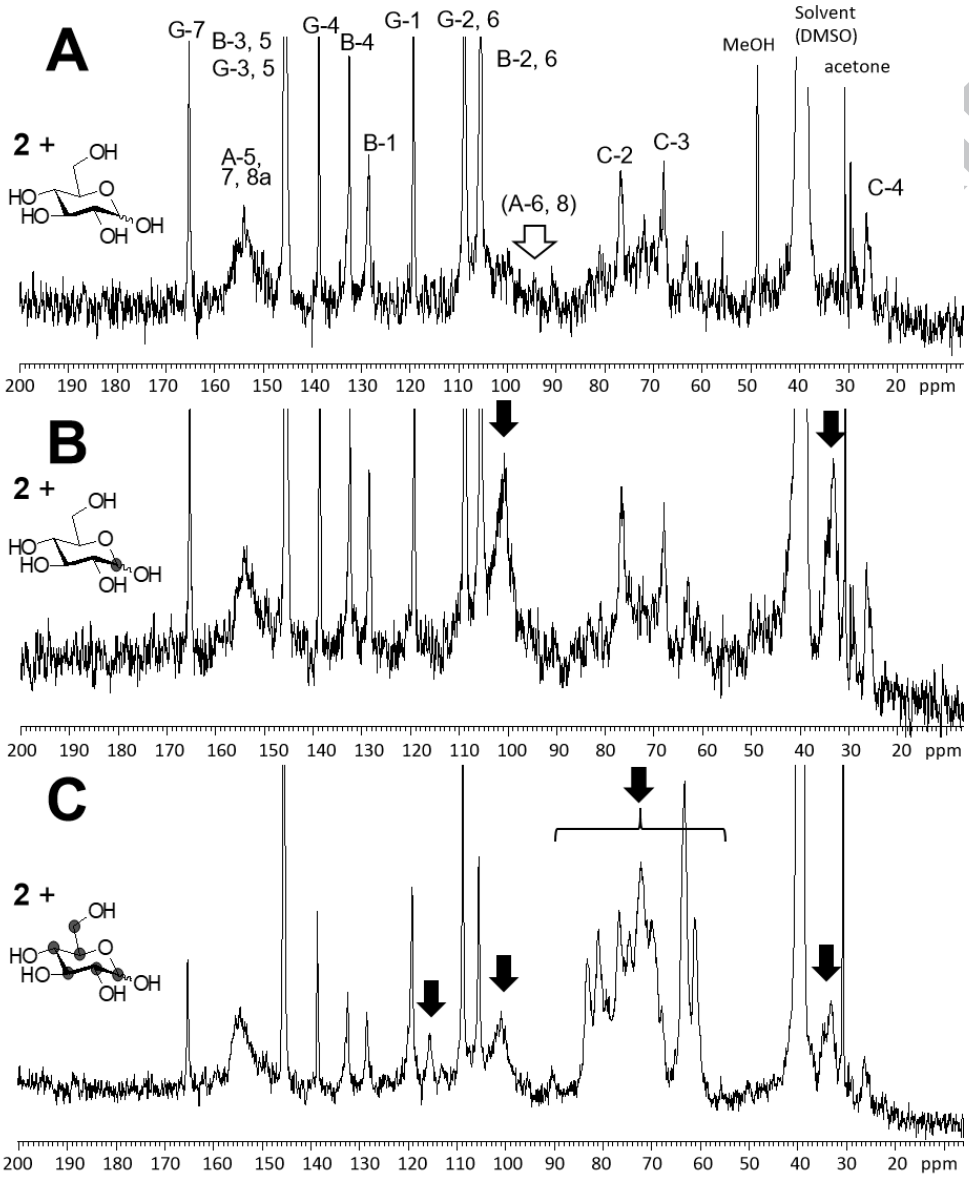

Fig. 3. ^{13}C-NMR spectra of oligomer fractions obtained upon heating of **2** with glucose (A), 1-^{13}C-labeled glucose (B) and U-^{13}C6-labeled glucose (C). The black arrows indicate signals enhanced by incorporation of ^{13}C.

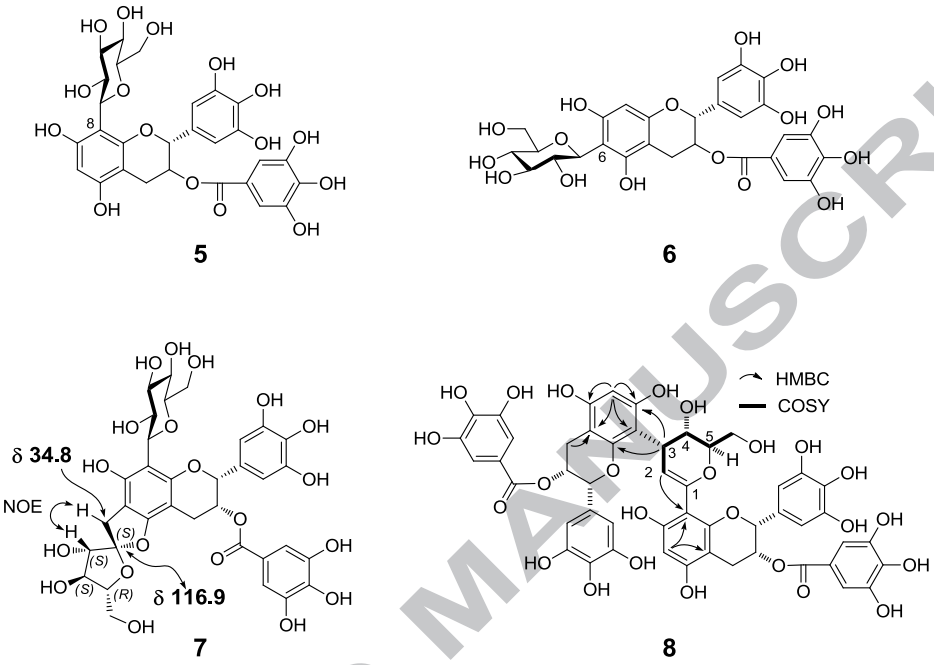

Fig. 4. Structures of **5**–**8** produced upon heating **2** with glucose.

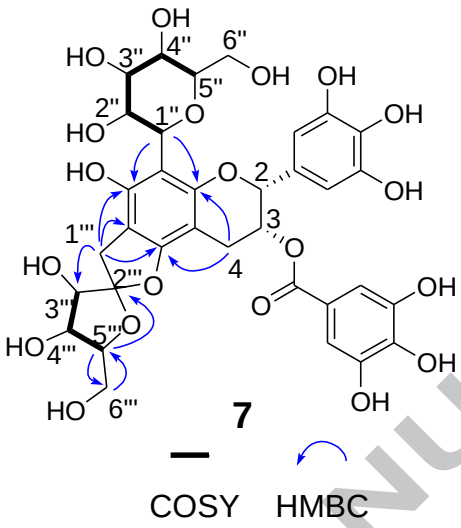

Fig. 5. Selected ^{1}H-^{1}H COSY and HMBC correlations of **7**.

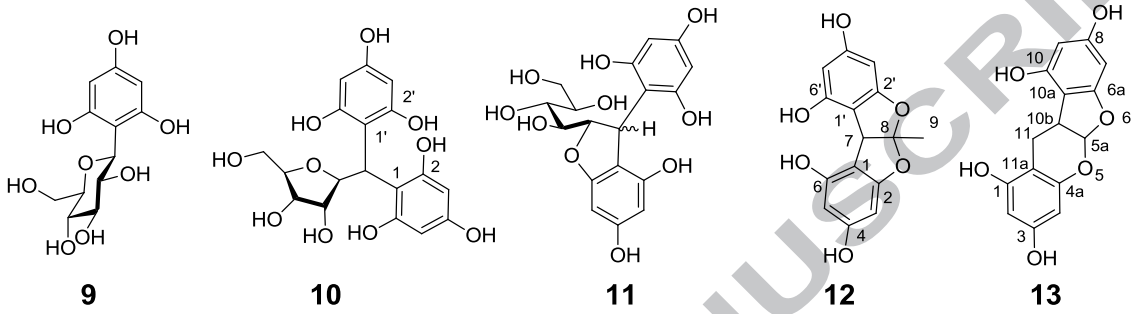

Fig. 6. Products generated from phloroglucinol with glucose (**9**-**11**), with methyl glyoxal (**12**), and with dihydroxyacetone (**12** and **13**).

Research Highlights

- Roasting of green tea decrease astringency by catechin oligomerization.
- Spectroscopic studies suggested the presence of sugar units in the oligomers.
- Model experiments using ^{13}C-labeled glucose confirmed the participation of sugars.
- Model experiments were performed using tea catechin and phloroglucinol.
- Reactions of carbonyl groups with catechin A-rings causes the oligomerization.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: