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HYDROLYSABLE TANNINS AND PROANTHOCYANIDINS FROM GREEN TEA*

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Key Word Index—*Thea sinensis*; Theaceae; green tea; ellagitannin; gallotannin; proanthocyanidin; polyphenol.

Abstract—Two hydrolysable tannins were isolated from green tea, and their structures were characterized by chemical and spectral means as 1,4,6-tri-*O*-galloyl-β-D-glucose and 1-*O*-galloyl-4,6-(−)-hexahydroxydiphenoyl-β-D-glucose. In addition, a new proanthocyanidin gallate was isolated, together with the known procyanidins B-2, B-4 and C-1. The structure of the proanthocyanidin was established as epigallocatechin-(4β → 8)-3-*O*-galloylepicatechin.

INTRODUCTION

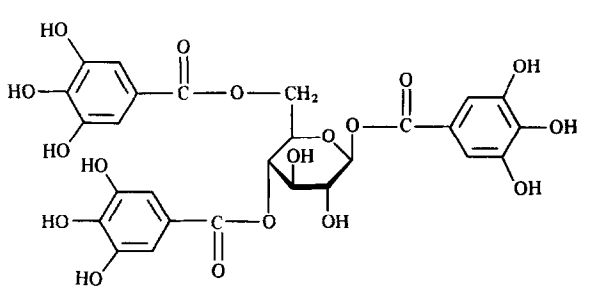
In a previous paper [1], we described the structural elucidation of four dimeric proanthocyanidin gallates which occur in green tea leaves (*Thea sinensis*). The concomitant isolation of two novel dimeric flavan-3-ol gallates (theasinensins A and B) in which two flavan units are linked through a carbon–carbon linkage at the B-ring was reported at the same time. Further chemical examination of polyphenolic constituents in green tea has resulted in the isolation of an additional proanthocyanidin gallate and two hydrolysable tannins, together with the known procyanidin dimers B-2 and B-4, and the trimer C-1. We now report herein the structures of these compounds.

RESULTS AND DISCUSSION

Initial extraction of commercial green tea (Sencha in Japanese, produced in Fukuoka, Japan) with 80% aqueous acetone followed by a combination of Sephadex LH-20 and high-porous polystyrene gel chromatography using a variety of solvent systems [2–4] gave two hydrolysable tannins **1** and **2**, and a proanthocyanidin **3**, together with the known procyanidins B-2 [3, 5, 6], B-4 [5, 6] and C-1 [3, 6] whose structures were confirmed by comparisons of physical and spectral data with those of authentic specimens.

The tannin **1**, obtained as colorless needles, mp 204–205°, gave a blue coloration with ethanolic ferric chloride solution. The ^{1}H NMR spectrum of **1** (in deutero-acetone) indicated the presence of three galloyl groups (δ7.12, 7.15 and 7.17, *s*, 2H each). Enzymatic hydrolysis of **1** with tannase afforded gallic acid and glucose. The allocations of the galloyl groups on the glucose nucleus were achieved by an exhaustive ^{1}H NMR analysis with the aid of extensive double resonance experiments. The spectrum showed a doublet (δ5.83, J = 8 Hz) and a triplet (δ5.26, J = 8 Hz) which were shifted somewhat downfield. The former doublet signal was easily assigned to the anomeric proton, while the latter triplet could be assigned to H-4 on the basis of the following evidence. When irradiated at the anomeric doublet, an upfield triplet (δ3.72, J = 8 Hz) changed into a doublet, thus permitting the assignment of this upfield triplet to H-2. Since this H-2 signal was shown not to be coupled with the above lowfield triplet by irradiation of each signal in turn, this triplet could be assigned to H-4. Accordingly, the locations of the two galloyl groups were determined to be at the C-1 and C-4 positions. The remaining galloyl group was concluded to be attached to the C-6 position on the basis of close similarities of C-6

*Part 22 in the series "Tannis and Related Compounds". For part 21 see Tanaka, T., Sueyasu, T., Nonaka, G. and Nishioka, I. *Chem. Pharm. Bull. Tokyo* (accepted).



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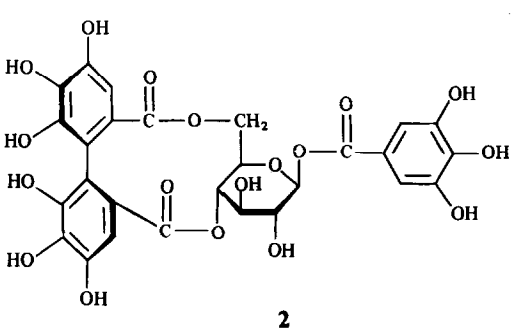


2

methylene chemical shifts (δ4.06, *d*, *J* = 14 Hz; δ4.87, *dd*, *J* = 3, 14 Hz) to those reported for 1,2,6-tri-*O*-galloyl-β-D-glucose [7]. The stereochemistry at the anomeric center was shown to have the β-configuration by the observation of a large coupling constant (*J* = 8 Hz) of the H-1, thus confirming the structure of **1** to be 1,4,6-tri-*O*-galloyl-β-D-glucose.

The tannin **2**, obtained as a pale brown amorphous solid, responded to the nitrous acid test [8] for ellagitannins. Its ^{1}H NMR spectrum showed the presence of one galloyl (δ7.20, *s*, 2H) and one hexahydroxydiphenoyl (δ6.62 and 6.76, *s*, 1H each) groups. A sugar anomeric proton appeared in the low field as a doublet (δ5.76, *J* = 8 Hz), indicating that the anomeric center is acylated. A lowfield double doublet (δ5.22, *J* = 6, 14 Hz, 1H) and a triplet (δ4.92, *J* = 9 Hz, 1H) were considered to be ascribable to the protons geminal to the ester groups. Treatment of **2** with tannase yielded a partial hydrolysate, which was shown by comparison of the spectral data to be identical with 4,6-(−)-hexahydroxydiphenoyl glucose obtained by similar tannase hydrolysis of 1,2,3-tri-*O*-galloyl-4,6-(−)-hexahydroxydiphenoyl-β-D-glucose (eugeniin) [9]. On the basis of these chemical and spectroscopic evidence, the structure of **2** was concluded to be 1-*O*-galloyl-4,6-(−)-hexahydroxydiphenoyl-β-D-glucose [10].

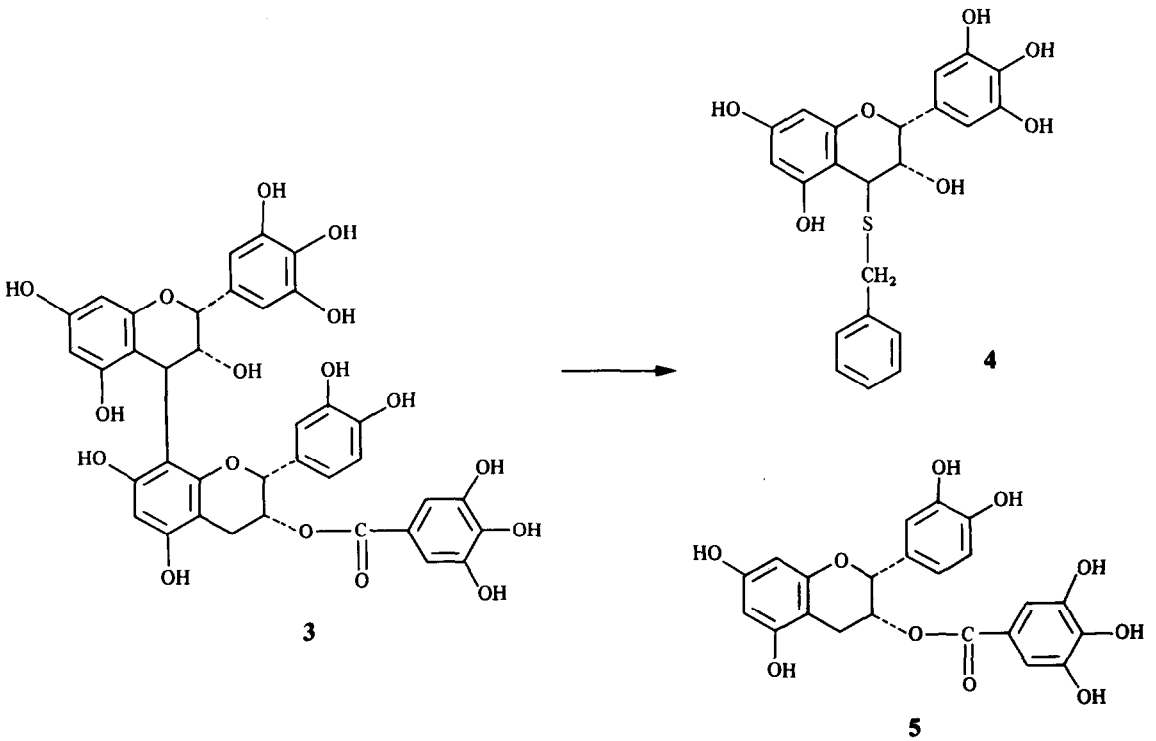
A proanthocyanidin **3**, which formed deep red pigments on treatment with mineral acid, contained one galloyl group as shown by the ^{1}H NMR spectrum (δ7.08, *s*, 2H). The occurrence of two flavan units in the molecule was deduced from the appearance of each couple of signals due to the respective C-2, C-3 and C-4 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum closely resembled that of prodelphinidin B-23-*O*-gallate [11] except for the ABX-type aromatic signals, indicating that **3** possesses the C-rings with the same stereochemistry as that of proanthocyanidin B-2 and that the point of the interflavanoid linkage is C-4–C-8. Acid-catalysed degradation of **3** with benzylmercaptan [6] afforded the 4-benzylthioflavan-3-ol **4** and (−)-epicatechin 3-*O*-gallate **5**. The structure of **4** was characterized by analysis of its ^{1}H NMR spectrum, which showed the occurrence of a 3′,4′,5′-tri-hydroxylated B-ring (δ6.56, *s*, 2H), an epicatechin-type C-ring (C-2, C-3: *cis*) (δ5.21, *s*, 1H, H-2) and a phloroglucinol-type A-ring (δ5.91, *d*, *J* = 2 Hz, H-6; δ6.03, *d*, *J* = 2 Hz, H-8). Based upon these observations, the structure of **3** was characterized as epigallocatechin-(4β → 8)-3-*O*-galloylepicatechin.

It has generally been believed that green tea leaf polyphenols are composed exclusively of so-called condensed tannins [12, 13], and our successful isolation of hydrolysable tannins from tea leaf is, therefore, of great value. The co-existence in tea leaf of ellagi- and gallotannins which are both 1,4,6-triacylated glucoses, suggests that biogenetically the hexahydroxydiphenoyl group in **1** is formed by oxidative coupling of two 4,6-positioned galloyl groups [14, 15]. Finally, it should be noted that in the course of a chemical examination of tannins in black tea, we have also isolated the hydrolysable tannins **1** and **2** in fairly good yields.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively, and chemical shifts are given in the δ (ppm) scale with TMS as int. standard. TLC was carried out over silica gel and Avicel SF cellulose, and spots were visualized by spraying $FeCl_3$, $NaNO_3$–HOAc or anisaldehyde–H_2SO_4 (for phenolics) and aniline hydrogen phthalate (for sugars) reagents. Green tea (a Yame variety produced in Yame, Fukuoka, Japan) was purchased from the market in Fukuoka, Japan.

Isolation of compounds. Green tea (2.0 kg) was extracted × 3 at



room temp. with 80% aq. Me_2CO. Evapn of Me_2CO *in vacuo* gave an aq. soln which was extracted with Et_2O to remove chlorophylls and caffeine. The aq. layer, after concn *in vacuo* to *ca* 1 l., was analysed by CC on Sephadex LH-20. Elution first with H_2O and then with H_2O containing an increasing amount of MeOH yielded five fractions which contained proanthocyanidins and hydrolysable tannins. The first two fractions were separately purified by CC over Sephadex LH-20 with 80% aq. MeOH to yield procyanidins B-2 [3] and B-4 [5]. The third fraction was CC on cellulose with 2% HOAc to give compound **1** (0.01 g) and procyanidin C-1 (0.02 g) [3]. The last 2 fractions were separately purified by CC over high-porous polystyrene gel (Diaion HP-20) [2–4] with H_2O containing an increasing amount of MeOH to give compounds **2** (0.06 g) and **3** (0.1 g).

1,4,6-*Tri*-O-*galloyl*-β-D-*glucose* (**1**). Colorless needles (H_2O), mp 204–205°, $[\alpha]_D^{24}$ +49.7° (Me_2CO; *c* 0.48). ^1H NMR (Me_2CO-d_6): δ3.72 (1H, *t*, *J* = 8 Hz, H-2), 4.06 (1H, *d*, *J* = 14 Hz, H-6), 4.87 (1H, *dd*, *J* = 3, 14 Hz, H-6), 5.26 (1H, *t*, *J* = 8 Hz, H-4), 5.83 (1H, *d*, *J* = 8 Hz, H-1), 7.12, 7.15, 7.17 (each 2H, *s*, galloyl H). (Found: C, 48.1; H, 3.8. $C_{27}H_{24}O_{18} \cdot 2H_2O$ requires: C, 48.2; H, 4.2%.)

Enzymatic hydrolysis of **1**. A soln of **1** (2 mg) in H_2O was incubated with tannase for 30 min at 37°. The products were directly analysed by cellulose TLC using 2% HOAc for phenolic acids, and *n*-BuOH–pyridine–H_2O (6:4:3) for sugars. Gallic acid (R_f 0.51) and glucose (R_f 0.40) were identified by co-chromatography with authentic samples.

1-O-*galloyl*-4,6-(−)-*hexahydroxydiphenoyl*-β-D-*glucose* (**2**). A pale brown amorphous solid, $[\alpha]_D^{24}$ −64.1° (Me_2CO; *c* 1.1). ^1H NMR (Me_2CO-d_6): δ3.6–4.2 (3H, *m*, H-2, H-3, H-6), 4.92 (1H, *t*, *J* = 9 Hz, H-4), 5.22 (1H, *dd*, *J* = 6, 14 Hz, H-6), 5.76 (1H, *d*, *J* = 8 Hz, H-1), 6.61, 6.76 (each 1H, *s*, hexahydroxydiphenoyl H), 7.20 (2H, *s*, galloyl H). ^{13}C NMR (Me_2CO-d_6): δ63.7 (*t*, C-6), 72.7, 73.1, 74.5, 75.5 (each *d*, glucosyl C), 95.8 (*d*, C-1), 107.9, 108.3, 115.7, 116.1, 126.3, 126.7, 136.1, 136.4 (hexahydroxydiphenoyl C), 110.3, 120.5, 139.5, 145.1 (galloyl C), 165.5, 168.3, 168.4 (ester C).

Enzymatic hydrolysis of **2**. A soln of **2** (120 mg) in H_2O (5 ml) was shaken with tannase at room temp. for 10 min. The solvent was concd *in vacuo* and the residue was treated with EtOH. The EtOH soluble portion was subjected to CC over Sephadex LH-20. Elution with MeOH yielded gallic acid and 4,6-(−)-hexahydroxydiphenoylglucose (30 mg), colorless needles, mp 218° (dec), $[\alpha]_D^{24}$ +39.7° (Me_2CO; *c* 0.7). (Found: C, 44.8; H, 4.5. $C_{20}H_{18}O_{14} \cdot 3H_2O$ requires: C, 44.8; H, 4.5%.)

Epigallocatechin-(4β → 8)-3-*O*-*galloylepicatechin* (**3**). An off-white amorphous powder, $[\alpha]_D^{24}$ −71.8° (Me_2CO; *c* 1.0). ^1H NMR (Me_2CO-d_6): δ2.8–3.2 (2H, *m*, H-4'), 4.04 (1H, *m*, H-3), 4.86 (1H, *br s*, H-4), 5.17 (1H, *s*, H-2), 5.23 (1H, *s*, H-2'), 5.60 (1H, *m*, H-3'), 5.98–6.08 (3H, *m*, H-6, H-8, H-6'), 6.51 (2H, *s*, B-ring H), 6.66–7.12 (3H, *m*, B'-ring H), 7.09 (2H, *s*, galloyl H). ^{13}C NMR (Me_2CO-d_6): δ26.5 (*t*, C-4'), 36.3 (*t*, C-4), 69.3 (*d*, C-3'), 72.8 (*d*, C-3), 76.8 (*d*, C-2), 77.8 (*d*, C-2'), 95.5, 96.1, 97.1 (each *d*, C-6, C-8, C-6'), 99.2, 102.3 (each *s*, C-4a, C-4a'), 110.2, 121.3, 139.1 (galloyl C), 166.8 (ester C). (Found: C, 57.0; H, 4.2. $C_{37}H_{30}O_{17} \cdot 2H_2O$ requires: C, 56.8; H, 4.4%.)

Acid-catalysed degradation of **3**. Compound **3** (50 mg) was heated for 15 hr under reflux in EtOH (7 ml) containing benzylmercaptan (2 ml) and HOAc (1 ml). Removal of solvents *in vacuo* left on oily residue which was purified by CC over Sephadex LH-20. Elution with $CHCl_3$–MeOH (4:1) furnished (−)-epicatechin 3-*O*-gallate (6 mg) (**5**) as colorless needles, mp 227–228°, $[\alpha]_D^{23}$ −174.5° (Me_2CO; *c* 0.9), and 4-benzylthioepigallocatechin (12 mg; **4**) as an off-white amorphous powder, $[\alpha]_D^{16}$ −155.3° (Me_2CO; *c* 1.0). ^1H NMR (Me_2CO-d_6): δ3.9–4.2 (4H, *m*, H-3, H-4, CH_2), 5.21 (1H, *s*, H-2), 5.91 (1H, *d*, *J* = 2 Hz, H-6), 6.03 (1H, *d*, *J* = 2 Hz, H-8), 6.56 (2H, *s*, B-ring H), 7.2–7.5 (5H, *m*, aromatic H).

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