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Tannins and Related Compounds. XLVIII.[1] Rhubarb. (7). Isolation and Characterization of New Dimeric and Trimeric Procyanidins

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Together with the known flavan-3-ols and procyanidins, a new procyanidin dimer (**11**) and several new trimers (**14**, **15**, **16**, **20**, **22**, **24** and **26**) have been isolated from high-quality rhubarb (長吉黄). On the basis of chemical and spectroscopic data, the structures of these compounds were characterized as procyanidin B-5 3,3′-di-*O*-gallate (**11**), procyanidin C-1 3′,3′′-di-*O*-gallate (**14**), procyanidin C-1 3,3′,3′′-tri-*O*-gallate (**15**), 3-*O*-galloylepicatechin-(4β→6)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin (**20**), 3-*O*-galloylepicatechin-(4β→6)-3-*O*-galloylepicatechin-(4β→6)-3-*O*-galloylepicatechin (**22**), 3-*O*-galloylepicatechin-(4β→6)-3-*O*-galloylepicatechin-(4β→8)-catechin (**26**), 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-catechin (**16**) and 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→6)-catechin (**24**).

Keywords——rhubarb; *Rheum* sp.; Polygonaceae; procyanidin; flavan-3-ol; condensed tannin; tannase; thiolytic degradation

In Europe, rhubarbs are used as a purgative and an appetite stimulant. However, in traditional Chinese medicine (Kampo), they are frequently contained in prescriptions used for a variety of diseases such as constipation, a blood-stasis syndrome, diarrhea, hypertension, mental and renal disorders, urticaria, *etc.* On the other hand, recent advances in phytochemistry, biochemistry and pharmacology have provided some scientific basis for these medicinal applications. Table I summarizes the biological activities and active components so far found in rhubarbs.

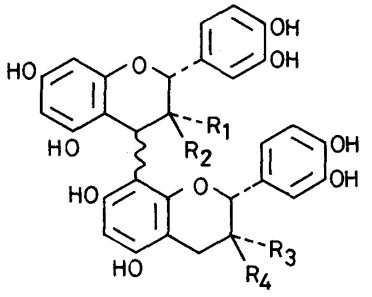
TABLE I. Biological Activities and Active Components in Rhubarbs[2]

Activity	Active component
Psychotropic activity[3]	RG-tannin
Improvement of nitrogen metabolism[4]	Rhatannins (i.p.)
Improvement of renal disorder[5]	?
Inhibition of angiotensin-converting enzyme (ACE)[6]	Rhatannins
Anti-inflammatory and analgestic activities[7]	Lindleyin
Purgative activity	Sennosides, rheinosides[8]
Anti-bacterial and anti-fungal activities	Rhein, aloe-emodin
Anti-tumor activity	Rhein, emodin

We previously isolated rhatannins[9] and RG-tannin[3] as the active components responsible for the improvement of nitrogen metabolism and the psychotropic activity, respectively, and we show them to be polymeric procyanidins bearing galloyl groups. Considerable attention has been focused on the chemistry of biologically active procyanidin polymers in rhubarbs, but little is known (except for the isolation of two procyanidin dimers[9]))

about the chemical nature of the lower-molecular procyanidins which also have the ability to combine with proteins, and might therefore have biological activity. We carried out an examination of rhubarb (commercial name: 長吉黄), which resulted in the isolation of new dimeric (**11**) and trimeric (**14**, **15**, **16**, **20**, **22**, **24** and **26**) procyanidins. We now wish to report details of the structural elucidation of these compounds.

Initial fractionation of the 80% aqueous acetone extract of rhubarb was achieved by chromatography over Sephadex LH-20 with increasing amounts of methanol in water to furnish six fractions. The fourth fraction contained a flavan-3-ol and dimeric and trimeric procyanidins, while the fifth fraction contained a flavan-3-ol gallate and dimeric procyanidin gallates. These fractions were separately subjected to chromatography over Sephadex LH-20 and MCI-gel CHP 20P with a variety of solvent systems to give (+)-catechin[10] and (−)-epicatechin 3-*O*-gallate[9,11,12] and various known procyanidins, *viz.*, procyanidins B-1 (**1**),[13,14] B-2 (**2**),[13,14] B-3 (**3**),[10] B-4 (**4**),[11] and B-7 (**9**),[14] epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (**13**),[14] procyanidin B-1 3-*O*-gallate (**5**),[9] procyanidin B-2 3′-*O*-gallate (**6**),[12] procyanidin B-2 3,3′-di-*O*-gallate (**7**),[9] and procyanidin B-4 3′-*O*-gallate (**8**).[12] From the sixth fraction, procyanidin B-7 3-*O*-gallate (**10**),[15] a dimeric procyanidin gallate (**11**) and a mixture of trimeric procyanidin gallates were obtained by repeated chromatography over Sephadex LH-20. The trimeric procyanidin fraction was further separated by a combination of Sephadex LH-20, MCI-gel CHP 20P and Bondapak C_{18}/Porasil B chromatographies with monitoring by high-performance liquid chromatography (HPLC) to furnish compounds **14**, **15**, **16**, **20**, **22**, **24** and **26**.



	R_1	R_2	R_3	R_4	~
1	OH	H	H	OH	—
2	OH	H	OH	H	—
3	H	OH	H	OH	---
4	H	OH	OH	H	---
5	OG	H	H	OH	—
6	OH	H	OG	H	—
7	OG	H	OG	H	—
8	H	OH	OG	H	---

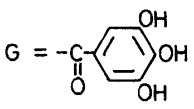
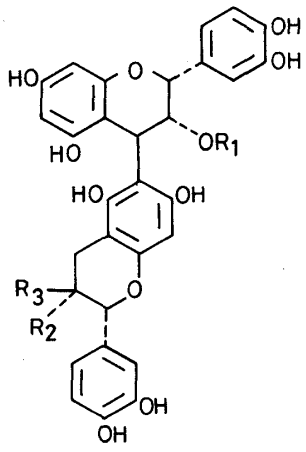
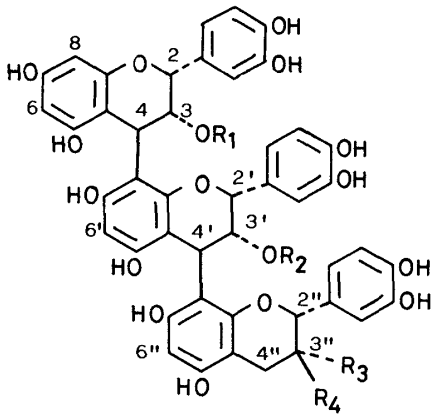


Chart 1



	R_1	R_2	R_3
9	H	H	OH
10	G	H	OH
11	G	OG	H
12	H	OH	H

Chart 2



	R_1	R_2	R_3	R_4
13	H	H	H	OH
14	H	G	OG	H
15	G	G	OG	H
16	G	G	H	OH
17	H	H	OH	H

Chart 3

Compound **11**, a tan amorphous powder, $[\alpha]_D$ +31.3° (acetone), $C_{44}H_{42}O_{24}\cdot 4H_2O$, gave an orange coloration (characteristic of procyanidins) with the anisaldehyde–sulfuric acid reagent. An intense blue coloration with the ferric chloride reagent and the appearance of a four-proton singlet at δ 7.06 in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum suggested the presence of two galloyl groups. The occurrence of two flavan frameworks in the molecule was deduced from the appearance of two pairs of signals due to the respective C-2 and C-3 in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. On enzymatic hydrolysis with tannase, **11** yielded gallic acid and an amorphous hydrolysate, the latter being identified as procyanidin B-5 (**12**).[13] Since signals due to flavan 3-H and 3′-H in the ^{1}H-NMR spectrum of **11** were shifted downfield as compared with those of **12**, two galloyl groups were concluded to be located at the C-3 and C-3′ positions. From these observations, **11** was characterized as procyanidin B-5 3,3′-di-*O*-gallate.

Compound **14**, a tan amorphous powder, $[\alpha]_D$ −5.5° (acetone), $C_{59}H_{46}O_{26}\cdot 4H_2O$, was also positive to the anisaldehyde–sulfuric acid reagent (an orange coloration) and to the ferric chloride test (a dark blue coloration). The ^{13}C-NMR spectrum showed six methine signals [δ 77.8, 76.9, 76.1, 73.0 (2C) and 69.1] ascribable to flavan C-2 and C-3, suggesting a triflavanoid constitution. The ^{1}H-NMR spectrum showed three flavan 2-H signals whose small coupling constants (J = *ca.* 0 Hz) suggested that compound **14** consists entirely of flavan-3-ol units with epicatechin stereochemistry [C(2), C(3): *cis*]. It also exhibited two two-proton singlets at δ 7.03 and 7.10 attributable to two galloyl ester groups. Enzymatic hydrolysis of **14** with tannase furnished gallic acid and a hydrolysate, which was shown to be identical with the known trimeric procyanidin, C-1 (**17**), by comparison of the physical and ^{1}H-NMR data with those of an authentic sample.[13,14]

In the ^{1}H-NMR spectrum of **14**, two of the three flavan 3-H signals were shifted downfield (δ 5.62 and 5.68) as compared with those of **17**. One (δ 5.62) was readily assignable to the lower terminal unit from the observation of the coupling with the neighboring 4″-H methylene protons, although the assignment of the remaining signals to either 3-H or 3′-H could not be made. These ^{1}H-NMR observations suggested that the two galloyl groups are situated at the C-3 positions in the lower terminal flavan unit and in one of the upper units. In order to confirm the locations of the galloyl groups, partial thiolytic degradation was attempted. On acid-catalyzed degradation with benzylmercaptan, **14** gave, together with the benzylthioethers (**18** and **19**) of (−)-epicatechin and 3-*O*-galloyl (−)-epicatechin derived from the upper units, a dimeric procyanidin gallate which was shown to be identical with procyanidin B-2 3,3′-di-*O*-gallate (**7**) by comparison with an authentic sample.[9] On the basis of these chemical and spectroscopic data, the structure of **14** was determined unambiguously as procyanidin C-1 3′,3″-di-*O*-gallate.

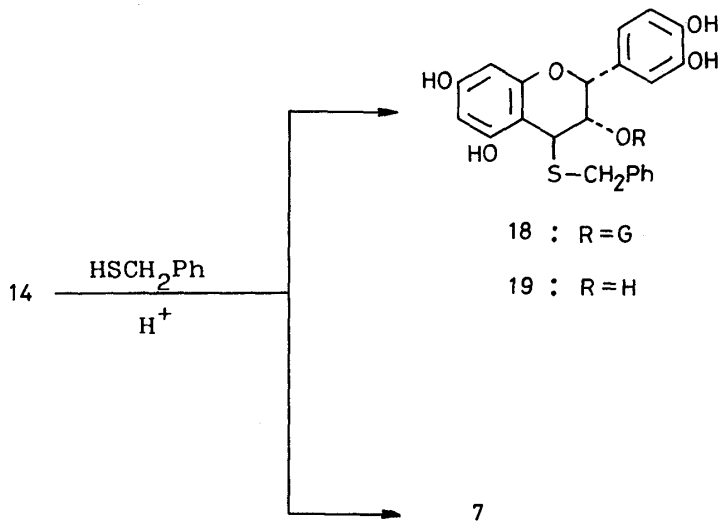


Chart 4

Compound **15**, a tan amorphous powder, $[\alpha]_D$ +13.4° (acetone), $C_{66}H_{50}O_{30}\cdot 3H_2O$, was readily recognized as a trimeric procyanidin from the three pairs of ^{13}C-NMR resonances due to flavan C-2 and C-3. The ^{1}H-NMR spectrum of **15** was similar to that of **14** except for the appearance of one additional galloyl signal (δ 6.97, 2H, s) and a downfield shift (δ 5.61) of the 3-H signal, suggesting that **15** is a procyanidin trimer having a galloyl group at each flavan C-3 position. Enzymatic hydrolysis of **15** with tannase to yield gallic acid and procyanidin C-1 (**17**) in a molar ratio of *ca.* 3 : 1 confirmed its constitution. The locations of the galloyl groups at the C-3, 3′ and 3″ positions were supported by the downfield shifts of the corresponding ^{13}C-NMR signals (δ 69.2, 72.9 and 74.9) as compared with those (δ 65.7, 71.5 and 72.7) observed in **17**. On the basis of these findings, **15** was characterized as procyanidin C-1 3,3′,3″-tri-*O*-gallate.

Compound **20**, a tan amorphous powder, $[\alpha]_D$ −40.7° (acetone), $C_{66}H_{50}O_{30}\cdot 3H_2O$, and compound **22**, a tan amorphous powder, $[\alpha]_D$ +51.0° (acetone), $C_{66}H_{50}O_{30}\cdot 2H_2O$, were concluded to be procyanidin gallates from the chromatographic properties and color reactions, which were similar to those of **15**. The presence of three galloyl groups in each molecule was evident from the appearance of the corresponding aromatic singlets [δ 7.04 (2H, s) and 7.08 (4H, s) in **20**; δ 6.98, 7.04 and 7.08 (each 2H, s) in **22**]. Tannase hydrolyses of **20** and **22** yielded gallic acid and hydrolysates in a molar ratio of *ca.* 3 : 1, and the hydrolysates were identified as epicatechin-(4$\beta\rightarrow$6)-epicatechin-(4$\beta\rightarrow$8)-epicatechin (**21**) and epicatechin-(4$\beta\rightarrow$6)-epicatechin-(4$\beta\rightarrow$6)-epicatechin (**23**), respectively, by comparisons with authentic samples.[16] The locations of the galloyl groups in **20** and **22** were determined in both cases to be at the C-3, 3′ and 3″ positions from the downfield shifts of all of the 3-H and C-3 signals in the ^{1}H- and ^{13}C-NMR spectra: δ 5.44 (2H, br s, 3 and 3′-H) and 5.66 (1H, br s, 3″-H); δ 69.2 and 75.4 (2C) in **20**; δ 5.57 (3H, br s, 3,3′ and 3″-H); δ 69.4 and 75.4 (2C) in **22**. On the basis of these findings, **20** and **22** were concluded to be 3-*O*-galloylepicatechin-(4$\beta\rightarrow$6)-3-*O*-galloylepicatechin-(4$\beta\rightarrow$8)-3-*O*-galloylepicatechin[17] and 3-*O*-galloylepicatechin-(4$\beta\rightarrow$6)-3-*O*-galloylepicatechin-(4$\beta\rightarrow$6)-3-*O*-galloylepicatechin, respectively.

Compound **26**, a pale brown powder, mp 204—206 °C, $[\alpha]_D$ +66.1° (acetone), $C_{59}H_{46}O_{26}\cdot 7/2H_2O$, gave a ^{1}H-NMR spectrum which showed a four-proton aromatic singlet (δ 7.04) corresponding to two galloyl groups. In the aliphatic region, the signal pattern was complicated by conformational isomerism, and the spectrum provided no further information on the structure. Tannase hydrolysis of **26** gave gallic acid and a hydrolysate (**27**). The ^{13}C-NMR spectrum of **27** exhibited flavan C-2 signals at δ 81.6 and 77.0 (2C), the chemical shift of

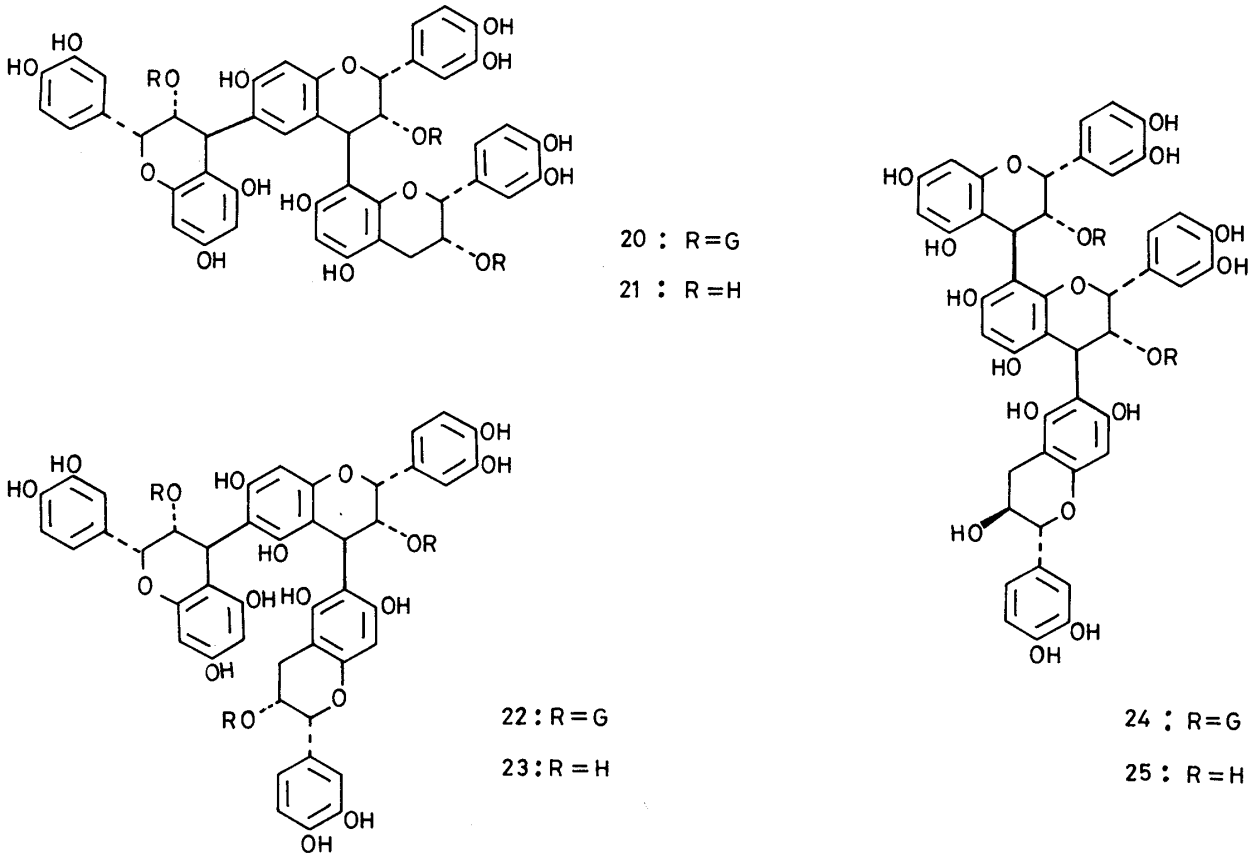


Chart 5

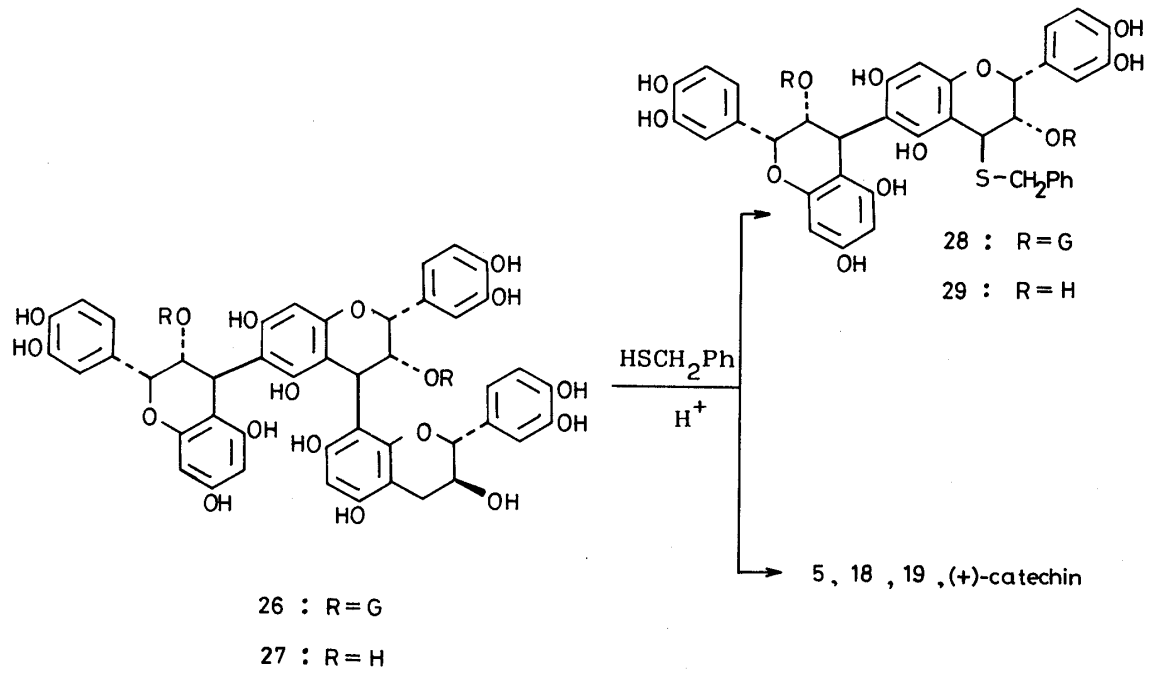


Chart 6

the former being consistent with the presence of one catechin unit in the molecule. To confirm the locations of the interflavanoid linkages as well as the locations of the galloyl groups, partial thiolytic degradation was attempted. Treatment with acetic acid in the presence of

benzylmercaptan furnished, in addition to 3-*O*-galloyl (−)-epicatechin 4-*β*-benzylthioether (**19**) and (+)-catechin, a dimeric procyanidin benzylthioether (**28**) and procyanidin B-1 3-*O*-gallate (**5**).[9] Compound **28** contained two galloyl groups as revealed by two two-proton singlets (δ 7.00 and 7.04, each 2H, s) in the ^{1}H-NMR spectrum, and furnished procyanidin B-5 4′-benzylthioether (**29**)[16] on tannase hydrolysis. On the basis of these chemical and spectral data, the structure of **26** was established unequivocally as 3-*O*-galloylepicatechin-(4*β*→6)-3-*O*-galloylepicatechin-(4*β*→8)-catechin.

Compound **16**, a tan amorphous powder, $[\alpha]_D$ +19.4° (acetone), $C_{59}H_{46}O_{26}\cdot 3H_2O$, showed a four-proton singlet at δ 6.99 attributed to two galloyl groups in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **16** was related to those of **15** and **20** in respect of the appearance of three sets of flavan C-2 and C-3 signals, and the chemical shifts (δ 75.8, 2C) of two of the C-2 signals were similar to those observed in **15** and **20**, suggesting the presence of two 3-*O*-galloylepicatechin units. On the other hand, the low-field shift (δ 80.9) of the remaining C-2 signal was consistent with the occurrence of a catechin unit. The presence of the catechin unit was supported by the observation of the ^{1}H-NMR signal at δ 4.98 (d, J= 7 Hz) due to flavan 2-H. Tannase hydrolysis of **16** furnished gallic acid and a hydrolysate, the latter being identified as epicatechin-(4*β*→8)-epicatechin-(4*β*→8)-catechin (**13**).[14] Since the C-3″ signal appeared at δ 67.0 in the ^{13}C-NMR spectrum of **16** being consistent with that of C-3″ in **13**, a galloyl group was concluded to be absent at this position. Based on these observations, **16** was characterized as 3-*O*-galloylepicatechin-(4*β*→8)-3-*O*-galloylepicatechin-(4*β*→8)-catechin.

Compound **24**, a tan amorphous powder, $[\alpha]_D$ +81.2° (acetone), $C_{59}H_{46}O_{26}\cdot 4H_2O$, provided a ^{1}H-NMR spectrum similar to that of **16** except for the chemical shift (δ 4.58, d, J= 8 Hz) of 2″-H, which rather resembled that of 2′-H of the C(4)–C(6) linked dimer, procyanidin B-7 (**9**). On enzymatic hydrolysis with tannase, **24** yielded gallic acid and epicatechin-(4*β*→8)-epicatechin-(4*β*→6)-catechin (**25**).[16] Since the ^{13}C-NMR chemical shift (δ 68.2) for C-3″ was almost identical with that (δ 68.0) in **25**, the galloyl groups were concluded to be located at the C-3 and C-3′ positions, thus establishing the structure as 3-*O*-galloylepicatechin-(4*β*→8)-3-*O*-galloylepicatechin-(4*β*→6)-catechin.

This is the first example of the isolation of trimeric procyanidin gallates from a natural source. Since the dimeric and trimeric procyanidin gallates obtained in this study possess strong astringency [that is, ability to precipitate proteins (enzymes)], they may be expected to show some biological and pharmacological activities.

Experimental

The instruments and chromatographic conditions used throughout this work are the same as described in the preceding paper.[1]

Isolation——Details of the fractionation of the 80% aqueous acetone extract of commercial rhubarb (commercial name: 長吉黄) (3.2 kg) were described in the preceding paper, and the following fraction numbers correspond to those appearing in that paper.[1]

Fraction IV-1 was chromatographed over Sephadex LH-20 (solvent: EtOH) to give three fractions (IV-1a—IV-1c). Fraction IV-1a consisted mainly of (+)-catechin, and crystallization from H_2O furnished a pure sample[10] (*ca.* 32 g). Fraction IV-1b, consisting of a mixture of dimeric procyanidins, was separated by MCI-gel CHP 20P [solvent: H_2O–MeOH (1:0—7:3)] and Sephadex LH-20 (solvent: 60% aqueous MeOH) chromatographies to afford procyanidins B-1 (**1**)[13,14] (2.1 g), B-2 (**2**)[13,14] (300 mg), B-3 (**3**)[10] (300 mg) and B-4 (**4**)[11] (32 mg). Chromatographies of fraction IV-1c over Sephadex LH-20 (solvents: acetone and 60% aqueous MeOH) gave procyanidin B-7 (**9**)[14] (98 mg) and epicatechin-(4*β*→8)-epicatechin-(4*β*→8)-catechin (**13**)[14] (82 mg). The previously obtained fraction V-3 was chromatographed over Sephadex LH-20 (solvent: 80% aqueous MeOH), and crystallization from H_2O furnished (−)-epicatechin 3-*O*-gallate[9,11,12] (6.9 g). Fraction V-4 consisted of a mixture of dimeric procyanidin gallates and was further fractionated by chromatography over Sephadex LH-20 (solvent: 80% aqueous MeOH) to give three fractions (V-4a—V-4c). Fraction V-4a was rechromatographed over MCI-gel CHP 20P [solvent: H_2O–MeOH (4:1—1:1)] and Sephadex LH-20 (solvent: EtOH) to furnish procyanidin B-1 3-*O*-gallate (**5**)[9] (2.6 g) and procyanidin B-2 3′-*O*-

gallate (**6**)[12] (1.2 g). Chromatographies of fractions V-4b and V-4c over MCI-gel CHP 20P [solvent: H_2O–MeOH (4 : 1—1 : 1)] and Sephadex LH-20 (solvent: EtOH) afforded procyanidin B-4 3′-*O*-gallate (**8**)[12] (440 mg) and procyanidin B-2 3,3′-di-*O*-gallate (**7**)[9] (9.8 g), respectively. Fraction VI (400 g) was chromatographed over Sephadex LH-20 [solvent: EtOH–H_2O–acetone (1 : 0 : 0—48 : 32 : 20)] to give two further fractions (VI-1 and VI-2). Chromatography of fraction VI-1 over Sephadex LH-20 (solvent: 80% aqueous MeOH) furnished procyanidin B-7 3-*O*-gallate (**10**)[15] (150 mg) and compound **11** (2.9 g). Fraction VI-2, containing a mixture of trimeric procyanidin gallates, was separated by Sephadex LH-20 chromatography [solvent: H_2O–MeOH (1 : 4—0 : 1)] to give six fractions (VI-2a—VI-2f). Fractions VI-2a—VI-2e were separately purified by chromatography over MCI-gel CHP 20P [solvent: H_2O–MeOH (7 : 3—0 : 1)] to afford compounds **16** (830 mg), **14** (330 mg), **15** (2.9 g), **24** (780 mg) and **26** (600 mg), respectively. Fraction VI-2f was further separated by chromatographies over MCI-gel CHP 20P [solvent: H_2O–MeOH (7 : 3—0 : 1)] and Bondapak C_{18}/Porasil B [solvent: H_2O–MeOH (4 : 1—1 : 1)] to furnish compounds **20** (520 mg) and **22** (120 mg).

Compound 11——A tan amorphous powder, $[\alpha]_D^{28}$ +31.3° (c=1.16, acetone). *Anal.* Calcd for $C_{44}H_{42}O_{24}\cdot 4H_2O$: C, 55.35; H, 4.43. Found: C, 55.38; H, 4.45. ^{1}H-NMR (acetone-d_6) δ: 4.00—4.16 (2H, m, 4′-H), 4.66 (1H, s, 4-H), 5.15 (2H, br s, 2 and 2′-H), 5.44 (1H, s, 3-H), 5.60 (1H, m, 3′-H), 6.04, 6.16 (each 1H, d, J=2 Hz, 6 and 8-H), 6.17 (1H, s, 8′-H), 6.7—7.1 (6H, m, B- and B′-ring-H), 7.06 (4H, s, 2 × galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 34.5 (C-4), 69.9 (C-3′), 75.2 (C-3 and 2′), 73.9 (C-2), 95.3, 96.3, 97.0 (C-6, 8 and 8′), 99.3, 100.0 (C-4a and 4a′), 107.1 (C-6′), 109.9 (4C) (galloyl C-2), 114.8 (2C) (B-ring C-2 and 2′), 115.7 (2C) (B-ring C-5 and 5′), 118.9 (2C) (B-ring C-6 and 6′), 120.4, 121.3 (galloyl C-1), 130.7, 130.9 (B-ring C-1 and 1′), 138.9, 139.5 (galloyl C-4), 145.3 (4C) (galloyl C-3), 145.8 (4C) (B-ring C-3, 4, 3′ and 4′), 155.3, 155.9, 157.0, 157.7, 158.3 (C-5, 7, 8a, 5′, 7′ and 8a′), 166.7, 168.1 (–COO–).

Compound 14——A tan amorphous powder, $[\alpha]_D^{28}$ −5.5° (c=0.64, acetone). *Anal.* Calcd for $C_{59}H_{46}O_{26}\cdot 4H_2O$: C, 57.01; H, 4.38. Found: C, 56.95; H, 4.44. ^{1}H-NMR (acetone-d_6) δ: 2.92—3.12 (2H, m, 4″-H), 4.06 (1H, br s, 3-H), 4.95 (2H, s, 4 and 4′-H), 5.28 (2H, s, 2′ and 2″-H), 5.62 (1H, m, 3″-H), 5.68 (2H, s, 2 and3′-H), 6.95—6.16 (4H, m, A-ring H), 6.56—7.20 (9H, m, B-ring H), 7.03, 7.10 (each 2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.7 (C-4″), 33.9 (C-4′), 36.4 (C-4), 69.1 (C-3″), 73.0 (C-3 and 3′), 76.1, 76.9 (C-2′ and 2″), 77.8 (C-2), 95.7, 96.4, 97.1 (C-6, 8, 6′ and 6″), 99.2 (C-4a″), 101.5, 102.4 (C-4a and 4a′), 106.9, 107.2 (C-8′ and 8″), 110.3 (4C) (galloyl C-2), 114.7, 115.0, 115.7 (B-ring C-2, 5, 2′, 5′, 2″ and 5″), 119.1, 119.3 (B-ring C-6, 6′ and 6″), 121.4, 121.7 (galloyl C-1), 130.9, 131.3, 132.3 (B-ring C-1, 1′ and 1″), 145.4, 145.6 (galloyl C-3), 154.2, 154.7, 155.4, 155.7, 156.0, 157.4, 157.8 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 165.8, 166.5 (–COO–).

Partial Thiolysis of 14——A mixture of **14** (130 mg), benzylmercaptan (3 ml) and acetic acid (2 ml) in ethanol (13 ml) was refluxed for 4 h with stirring. The reaction mixture was concentrated under reduced pressure to give an oily residue, which was chromatographed over Sephadex LH-20. Elution with acetone removed excess reagent. Subsequent elution with 95% aqueous acetone gave a mixture of benzylthioethers. Further elution with 93% aqueous acetone afforded a dimeric procyanidin gallate (31 mg), which was shown to be identical with procyanidin B-2 3,3′-di-*O*-gallate (**7**) by comparison of the physical and ^{1}H-NMR data with those of an authentic sample.[9] A mixture of the benzylthioethers was separated by chromatography over Sephadex LH-20 (solvent: 80% aqueous MeOH) to furnish (−)-epicatechin 4-β-benzylthioether (**18**)[9,13] (3 mg) and 3-*O*-galloyl (−)-epicatechin 4-β-benzylthioether (**19**)[9] (10 mg).

Compound 15——A tan amorphous powder, $[\alpha]_D^{28}$ +13.4° (c=0.93, acetone). *Anal.* Calcd for $C_{66}H_{50}O_{30}\cdot 3H_2O$: C, 57.56; H, 4.10. Found: C, 57.98; H, 4.60. ^{1}H-NMR (acetone-d_6) δ: 2.96—3.16 (2H, m, 4″-H), 4.90 (2H, br s, 4 and 4′-H), 5.34 (1H, s, 2″-H), 5.38 (1H, br s, 2′-H), 5.61 (1H, br s, 3-H), 5.68 (3H, m, 2, 3′ and 3″-H), 5.90—6.18 (4H, m, A-ring H), 6.44—7.20 (9H, m, B-ring H), 6.97, 7.04, 7.08 (each 2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.7 (C-4″), 33.6, 34.0 (C-4 and 4′), 69.2 (C-3″), 72.9 (C-3), 74.9 (C-3′), 75.7, 76.1 (C-2 and 2′), 77.6 (C-2″), 95.7, 96.3, 97.0 (C-6, 8, 6′ and 6″), 99.2 (C-4a″), 101.9, 102.4 (C-4a and 4a′), 106.4, 106.9 (C-8′ and 8″), 110.2 (6C) (galloyl C-2), 114.6, 114.9 (B-ring C-2, 2′ and 2″), 115.5, 115.7 (B-ring C-5, 5′ and 5″), 119.3 (B-ring C-6, 6′ and 6″), 121.3, 121.6 (galloyl C-1), 130.9, 131.2 (B-ring C-1, 1′ and 1″), 138.5, 139.0 (galloyl C-4), 144.5, 144.7, 144.9, 145.1 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.4, 145.6, 145.7 (galloyl C-3), 153.9, 155.3, 155.6, 155.9, 156.1, 156.8, 157.0 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 165.4, 166.7 (2C) (–COO–).

Compound 20——A tan amorphous powder, $[\alpha]_D^{28}$ −40.7° (c=0.88, acetone). *Anal.* Calcd for $C_{66}H_{50}O_{30}\cdot 3H_2O$: C, 57.56; H, 4.10. Found: C, 57.71; H, 4.34. ^{1}H-NMR (acetone-d_6) δ: 2.70—3.18 (2H, m, 4″-H), 4.60 (1H, s, 4-H), 4.69 (1H, s, 4′-H), 5.19 (1H, s, 2-H), 5.40 (2H, br s, 2′ and 2″-H), 5.44 (2H, br s, 3 and 3′-H), 5.66 (1H, br s, 3″-H), 5.92 (1H, s, 6″-H), 6.05, 6.10 (each 1H, d, J=2 Hz, 6 and 8-H), 6.20 (1H, s, 8′-H), 6.6—7.2 (9H, m, B-ring H), 7.04 (2H, s, galloyl-H), 7.08 (4H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.8 (C-4″), 34.5 (2C) (C-4 and 4′), 69.2 (C-3″), 75.4 (C-2, 2′, 3 and 3′), 78.0 (C-2″), 95.3, 96.6, 97.2 (C-6, 8, 8′ and 6″), 99.9 (C-4a′ and 4a″), 103.3 (C-4a), 106.3, 107.0 (C-6′ and 8″), 110.0 (6C) (galloyl C-2), 115.0, 115.5, 115.8 (B-ring C-2, 2′, 2″, 5, 5′ and 5″), 119.1, 119.8 (B-ring C-6, 6′ and 6″), 120.7, 121.4 (galloyl C-1), 130.3, 130.8, 131.2 (B-ring C-1, 1′ and 1″), 139.1, 139.5 (3C in total galloyl C-4), 144.9, 145. 1, 145.3, 145. 5 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.9 (6C) (galloyl C-3), 155.1, 155.3, 155.5, 155.7, 155.9, 157.1, 157.9, 158.7 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 166.8 (3C) (–COO–).

Compound 22——A tan amorphous powder, $[\alpha]_D^{28}$ +51.0° (c=0.58, acetone), *Anal.* Calcd for $C_{66}H_{50}O_{32}\cdot 2H_2O$: C, 58.32; H, 4.01. Found: C, 58.41; H, 4.49. ^{1}H-NMR (acetone-d_6) δ: 2.95—3.16 (2H, m, 4″-H), 4.70 (1H, s, 4-H),

4.88 (1H, s, 4′-H), 5.16 (1H, br s, 2′-H), 5.28 (2H, s, 2 and 2″-H), 5.57 (3H, br s, 3, 3′ and 3″-H), 5.80—6.20 (4H, m, A-ring H), 6.52—7.16 (9H, m, B-ring H), 6.98, 7.04, 7.08 (each 2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.1 (C-4″), 34.5, 35.0 (C-4 and 4′), 69.4 (C-3″), 75.4 (C-2, 2′, 3 and 3′), 78.0 (C-2″), 95.7, 96.4, 96.6 (C-6, 8, 8′ and 8″), 101.2 (C-4a, 4a′ and 4a″), 107.1, 108.5 (C-6′ and 6″), 110.1 (6C) (galloyl C-2), 114.8, 115.1, 115.6, 115.9 (B-ring C-2, 2′, 2″, 5, 5′ and 5″), 119.0, 119.4 (B-ring C-6, 6′ and 6″), 120.3, 121.5 (galloyl C-1), 130.5, 131.0 (B-ring C-1, 1′ and 1″), 139.0, 139.6 (galloyl C-3), 144.9, 145.0, 145.4, 145.9 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.8 (6C) (galloyl C-3), 154.1, 157.0, 158.2, 159.1 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 166.6 (3C) (–COO–).

Compound 26——A pale brown powder, mp 204—205 °C, $[\alpha]_D^{28}$ +66.1° (c=0.66, acetone), *Anal.* Calcd for $C_{59}H_{46}O_{26}\cdot 7/2H_2O$: C, 57.42; H, 4.33. Found: C, 57.59; H, 4.17. ^{1}H-NMR (acetone-d_6) δ: 2.6—3.1 (2H, m, 4″-H), 3.96 (3H, m, 3, 3′ and 3″-H), 4.52 (1H, br s, 4-H), 4.66 (2H, m, 4′ and 2″-H), 4.89 (1H, m, 2-H), 4.96 (1H, m, 2′-H), 5.96—6.24 (4H, m, A-ring H), 6.56—7.18 (9H, m, B-ring H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.8 (C-4″), 37.1 (C-4 and 4′), 67.5 (C-3″), 72.3 (C-3 and 3′), 77.0 (C-2 and 2′), 81.6 (C-2″), 95.5, 95.9, 96.6 (C-6, 8, 8′ and 6″), 101.9 (C-4a″), 103.1 (C-4a and 4a′), 106.7, 107.1 (C-6′ and 8″), 110.0 (4C) (galloyl C-2), 114.9 (B-ring C-2, 2′ and 2″), 115.7 (B-ring C-5, 5′ and 5″), 119.1, 119.3 (B-ring C-6, 6′ and 6″), 120.7 (4C) (galloyl C-1), 130.7, 131.3 (B-ring C-1, 1′ and 1″), 139.4 (2C) (galloyl C-4), 145.1, 145.3 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.8 (2C), 145.9 (2C) (galloyl C-3), 155.7, 157.1, 158.0, 158.8 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 168.0 (2C) (–COO–).

Partial Thiolysis of 26——A mixture of **26** (200 mg), benzylmercaptan (3 ml) and acetic acid (3.5 ml) in ethanol (18 ml) was refluxed for 6 h with stirring. The reaction mixture was worked-up as for **14**, and chromatographed over Sephadex LH-20. From the 95% aqueous acetone eluate, 3-*O*-galloyl(−)-epicatechin 4-*β*-benzylthioether (**19**) and (+)-catechin were obtained. The 93% aqueous acetone eluate was further separated by chromatography over Sephadex LH-20 (solvent: 80% aqueous MeOH) to furnish procyanidin B-1 3′-*O*-gallate (**5**)[9] (7 mg) and the benzylthioether (**28**) (41 mg) as a tan amorphous powder, $[\alpha]_D^{26}$ −35.5° (c=1.02, acetone). *Anal.* Calcd for $C_{52}H_{40}O_{20}S\cdot 5/2H_2O$: C, 58.34; H, 4.32. Found: C, 58.60; H, 4.63. ^{1}H-NMR (acetone-d_6) δ: 4.16 (2H, s, –SCH_2–), 4.34 (1H, s, 4′-H), 4.65 (1H, s, 4-H), 5.15 (1H, s, 2-H), 5.15 (1H, s, 2-H), 5.39 (1H, s, 2′-H), 5.44 (1H, s, 3′-H), 5.54 (1H, s, 3-H), 6.00, 6.14 (each 1H, d, J=2 Hz, 6 and 8-H), 6.16 (1H, s, 8′-H), 6.70—7.10 (6H, m, B-ring H), 7.00, 7.04 (each 2H, s, galloyl-H), 7.2—7.6 (5H, m, aromatic-H).

Compound 16——A tan amorphous powder, $[\alpha]_D^{28}$ +19.4° (c=0.95, acetone). *Anal.* Calcd for $C_{59}H_{46}O_{26}\cdot 3H_2O$: C, 57.84; H, 4.29. Found: C, 57.69; H, 4.21. ^{1}H-NMR (acetone-d_6) δ: 2.60—2.98 (2H, m, 4″-H), 4.16 (1H, m, 3″-H), 4.75 (1H, s, 4-H), 4.86 (1H, br s, 4′-H), 4.98 (1H, d, J=7 Hz, 2″-H), 5.26 (1H, br s, 2′-H), 5.56 (1H, s, 3′-H), 5.64 (2H, br s, 2 and 3-H), 5.85—6.18 (4H, m, A-ring H), 6.40—7.10 (9H, m, B-ring H), 6.99 (4H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O): 30.8 (C-4″), 33.6, 34.5 (C-4 and 4′), 67.0 (C-3″), 72.8 (C-3′), 74.9 (C-3), 75.8 (2C) (C-2 and 2′), 80.9 (C-2″), 95.7, 96.3, 96.9 (C-6, 8, 6′ and 6″), 100.3, 101.2, 102.4 (C-4a, 4a′ and 4a″), 106.4 (C-8′ and 8″), 110.2 (4C) (galloyl C-2), 114.3, 115.0, 115.5, 115.8 (B-ring C-2, 2′, 2″, 5, 5′ and 5″), 119.1, 119.3 (B-ring C-6, 6′ and 6″), 121.2, 121.5 (galloyl C-1), 130.8, 131.2, 132.0 (B-ring C-1, 1′ and 1″), 138.8, 139.0 (galloyl C-4), 144.7, 144.9, 145.0, 145.2 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.5 (2C), 145.7 (2C) (galloyl C-3), 153.3, 155.2, 155.6, 155.7, 156.3, 156.8, 157.1 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 165.6, 167.0 (–COO–).

Compound 24——A tan amorphous powder, $[\alpha]_D^{28}$ +81.2° (c=1.09, acetone). *Anal.* Calcd for $C_{59}H_{46}O_{26}\cdot 4H_2O$: C, 57.01; H, 4.38. Found: C, 56.97; H, 4.31. ^{1}H-NMR (acetone-d_6) δ: 2.4—3.2 (2H, m, 4″-H), 4.06 (1H, m, 3″-H), 4.58 (1H, d, J=8 Hz, 2″-H), 4.68 (1H, s, 4-H), 5.88 (1H, s, 4′-H), 5.25 (1H, br s, 2′-H), 5.50—5.76 (3H, m, 2, 3 and 3′-H), 5.90—6.28 (4H, m, A-ring H), 6.56—7.20 (9H, m, B-ring H), 7.05, 7.13 (each 2H, s, galloyl-H), ^{13}C-NMR (acetone-d_6 + D_2O) δ: 30.8 (C-4″), 34.6, 34.9 (C-4 and 4′), 68.2 (C-3″), 74.8, 75.4 (C-2, 2′, 3 and 3′), 82.4 (C-2″), 95.7, 96.6, 97.1 (C-6, 8, 6′ and 8″), 101.8, 102.3 (C-4a, 4a′ and 4a″), 106.8 (C-8′ and 6″), 110.1 (4C) (galloyl C-2), 114.5, 115.0, 115.8 (B-ring C-2, 2′, 2″, 5, 5′ and 5″), 119.3, 120.2 (B-ring C-6, 6′ and 6″), 120.7, 121.2 (galloyl C-1), 130.7, 131.2, 131.6 (B-ring C-1, 1′ and 1″), 138.6, 139.0 (galloyl C-4), 144.9, 145.0, 145.3, 145.5 (B-ring C-3, 4, 3′, 4′, 3″ and 4″), 145.8 (4C) (galloyl C-3), 155.0, 155.2, 155.7, 156.4, 157.0 (C-5, 7, 8a, 5′, 7′, 8a′, 5″, 7″ and 8a″), 167.0, 167.6 (–COO–).

General Procedures for Tannase Hydrolysis——An aqueous solution of each sample (30—160 mg) was shaken with tannase at room temperature for 1—2 h. The reaction mixture was concentrated under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was subjected to chromatography over Sephadex LH-20. Elution with 70% aqueous MeOH gave gallic acid. Further elution with the same solvent afforded the hydrolysate. Each hydrolysate was characterized by physical and spectral comparisons with an authentic sample.

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