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Tannins and Related Compounds. I.[1] Rhubarb (1)

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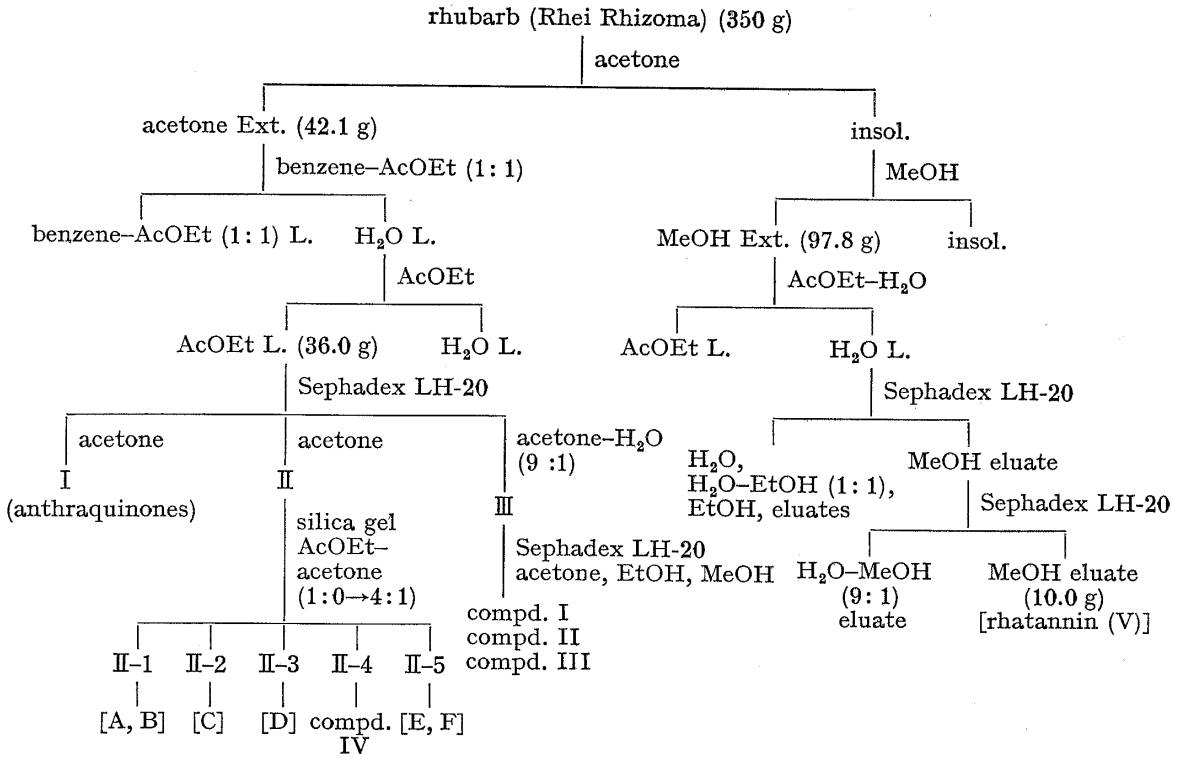
Three new tannin-related compounds (I, II and III), along with lindleyin (IV), (+)-catechin, 3-O-galloyl-(−)-epicatechin, gallic acid, 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside, 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside and 4-(4′-hydroxyphenyl)-2-butanone 4′-O-β-D-glucopyranoside, have been isolated from commercial rhubarb (Rhei Rhizoma). On the basis of spectral and chemical evidence, I, II and III were characterized as 3,3′-di-O-galloylprocyanidin B-2, 3-O-galloylprocyanidin B-1 and 1,2,6-tri-O-galloylglucose, respectively. The occurrence of IV in rhubarb is of great significance, since IV has been reported to have analgesic and anti-inflammatory activities almost equal to those of aspirin and phenylbutanone.

Tannins in rhubarb have been partially purified (designated as rhatannin (V)). Thiolysis degradation and enzymatic hydrolysis have shown that V is mainly composed of C_4 to C_8 linked 3-O-galloyl-(−)-epicatechin units in the extension part (upper part) with either 3-O-galloyl-(−)-epicatechin or (+)-catechin unit in the lower terminal part.

Keywords——rhubarb; polygonaceae; 3,3′-di-O-galloylprocyanidin B-2; 3-O-galloylprocyanidin B-1; 1,2,6-tri-O-galloylglucose; lindleyin; rhatannin

Recent extensive studies on the constituents of rhubarb (Rhei Rhizoma) have revealed the occurrence of a variety of phenolic compounds, *i.e.*, anthracene derivatives[2,3] (which are the active principles of the purgative effect), naphthalene derivatives,[4] stilbene glycosides,[5–8] and tannin-related compounds such as simple galloyl esters of glucose[9,10] and catechin derivatives.[5,11–13] Previously, we reported that intraperitoneal administration of the aqueous extract from rhubarb caused a remarkable decrease of urea-nitrogen concentration (BUN) in rat serum,[14] and later the BUN-decreasing activity, after fractionation of the extract mainly by dialysis and Sephadex LH-20 column chromatography, was found in the water-soluble, non-dialyzable fractions which presumably contained the high-molecular-weight phenolic compounds, although a little activity was still detected in the low-molecular-weight fractions.[15] Rhubarb has long been known to contain polyphenolic compounds, so-called "tannins", the nature of which, however, is not yet clear. Since it is not easy to separate individual components from the tannin fractions in rhubarb because they are composed of a complex mixture of heavily hydroxylated compounds, we initially began to examine the comparatively lower-molecular-weight compounds which would be expected to be active in decreasing BUN and also to be related to the tannins in rhubarb. This work resulted in the isolation of two new galloylprocyanidins and a new trigalloylglucose, together with lindleyin, the gallate of hydroxyphenylbutanone glucoside previously isolated from *Aeonium lindleyi* by a Spanish group.[16] This paper deals with the isolation and structure elucidation of these compounds and also presents evidence leading to the gross structures of rhubarb tannins.

The commercial rhubarb was treated as shown in Chart 1 to yield the ethyl acetate-soluble portion, which contains a complicated mixture of lower-molecular-weight phenolics. Chromatography of this fraction on a Sephadex LH-20 column eluted first with acetone and then with a mixture (9:1) of acetone and water, efficiently removed most of the anthraquinones which were mainly found in this fraction. Compounds I, II and III were isolated from the



A: (+)-catechin
B: 3-O-galloyl-(−)-epicatechin
C: gallic acid
D: 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside
E: 4-(4′-hydroxyphenyl)-2-butanone 4′-O-β-D-glucopyranoside
F: 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside

Chart 1

acetone–water (9 : 1) eluate by adsorption chromatography on Sephadex LH-20. On the other hand, compound IV was separated by silica gel column chromatography of the acetone eluate.

Compound I (I), an off-white amorphous powder, $[\alpha]_D$ −93.8° (acetone), $C_{44}H_{34}O_{20}\cdot 2H_2O$, which is strongly positive (a dark blue color) to the $FeCl_3$ reagent, exhibits astringency. Treatment of I with ethanolic HCl gave a deep red color which may be attributable to the formation of an anthocyanidin pigment,[17] thus suggesting I is a proanthocyanidin-related compound. The proton magnetic resonance (PMR) spectrum of I shows the presence of two galloyl groups (δ 6.99, 7.07), along with nine aromatic protons, three of which are observed at higher field (δ 5.93—6.12), and are ascribable to the C_6–, C_8–H on the A-ring of the flavan skeleton. The aliphatic proton signals at δ 4.98 (br. s), 5.55 (m) and 2.98 (2H, m) are similar to those due to the C_2–, C_3– and C_4–H of 3-O-galloylepicatechin, respectively. In the carbon-13 nuclear magnetic resonance (CMR) spectrum I exhibits four doublet signals (δ 69.1, 74.8, 75.5, 77.8) due to methine carbon atoms with oxygen functions.

Hydrolysis of I with tannase in 0.05 M acetate buffer (pH 5.0), followed by separation of the products by Sephadex LH-20 chromatography, yielded gallic acid and a hydrolysate (Ia), an off-white amorphous powder, $[\alpha]_D$ +32.2° (acetone), $C_{30}H_{26}O_{12}\cdot 1.5H_2O$. The PMR spectrum of Ia shows two pairs of signals (singlets at δ 4.95, 5.11 and multiplets at δ 4.00, 4.30) corresponding to the C_2– and C_3–H of the flavan frameworks, besides a broad singlet signal (δ 4.75) attributable to C_4–H of the upper flavan unit, indicating the presence of two epicatechin units in Ia. Finally, Ia was shown to be identical with procyanidin B-2 isolated from the seeds of *Areca catechu* by direct comparison.[18]

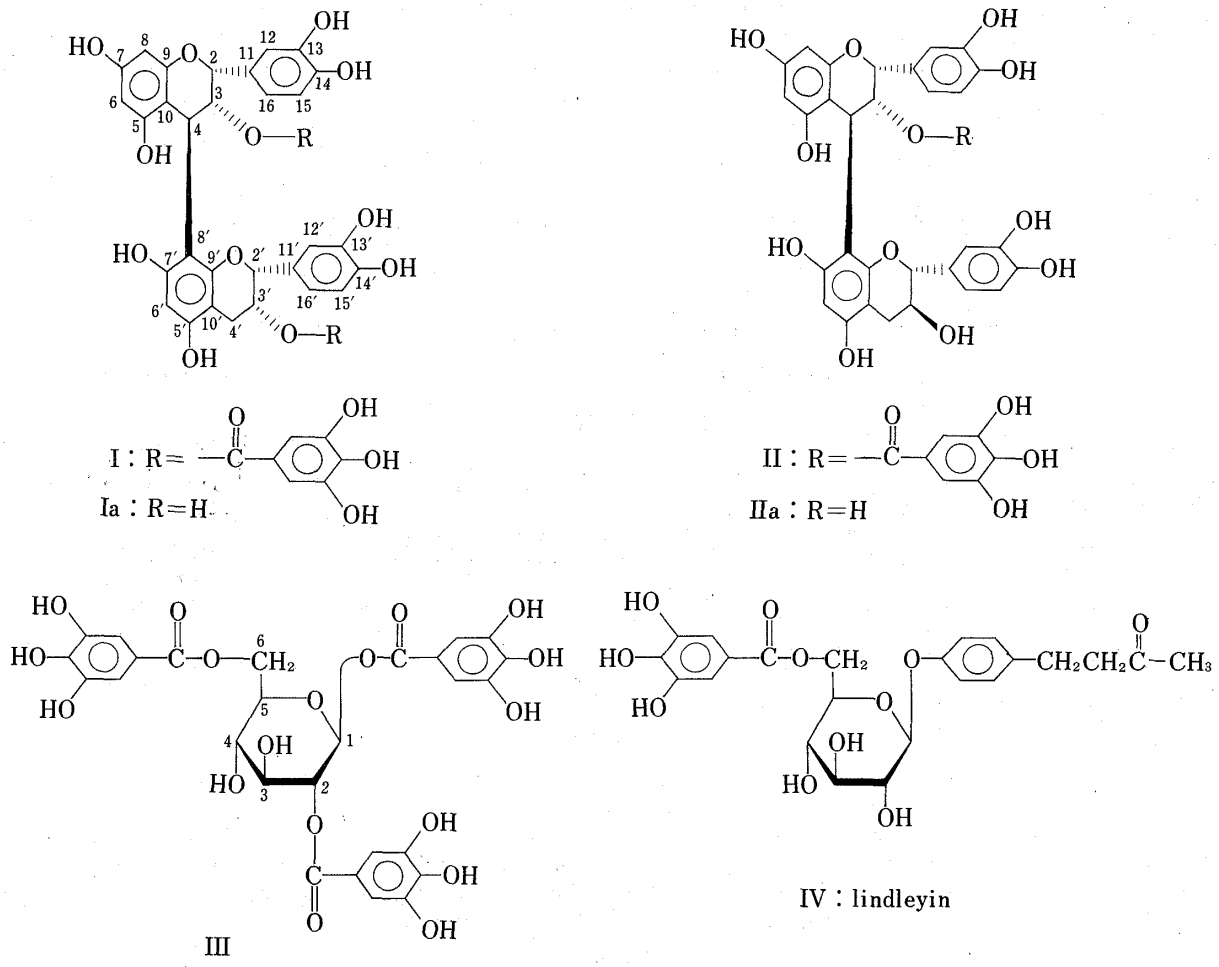


Fig. 1

Since two multiplet signals (δ 5.55) due to the protons geminal to galloyl groups, which on hydrolysis show up-field shifts (δ 4.00, 4.30), are observed on the PMR spectrum of I, two galloyl groups are located at the C_3 and C_3' positions.

Accordingly, compound I is characterized as 3,3′-di-O-galloylprocyanidin B-2.

Compound II (II), an off-white amorphous powder, $[\alpha]_D$ −21.2° (acetone), $C_{37}H_{30}O_{16}\cdot 3H_2O$, shows a blue spot with $FeCl_3$ reagent on thin-layer chromatography (TLC), and like I, gives rise to an anthocyan on acid treatment. The occurrence of two flavan units in II is easily deduced from the presence in the PMR spectrum of three aromatic signals at δ 5.80 (d, J=2 Hz), 5.93 (d, J=2 Hz) and 6.08 (s) ascribable to the C_6– and C_8–H on the flavan A-ring, which usually resonate at relatively higher field than the other aromatic protons. The singlet signal (δ 6.95, 2H) and ABX-type signals (δ 2.55, d.d, J=8, 16 Hz, C_{4a}'–H; 2.89, d.d, J=6, 16 Hz, C_{4e}'–H; 4.04, m, C_3'–H) suggest the presence of a galloyl group and a catechin moiety, respectively, the latter being in the lower unit of the dimer. Furthermore, the two signals at δ 5.46 (s) and 5.31 (m) due to the C_2– and C_3–H of the upper unit, which are notably deshielded, are analogous to those in I. This indicates that the upper half of the dimer is 3-O-galloylepicatechin-type.

II was hydrolyzed with tannase in aqueous solution and subsequently separated in the same way as for I to give an off-white amorphous hydrolysate (IIa), $[\alpha]_D$ +40.2° (acetone), along with gallic acid. The *Rf* value on TLC, $[\alpha]_D$ and the PMR spectrum of IIa coincided with those of procyanidin B-1.[18]

Thus, compound II is determined to be 3-O-galloylprocyanidin B-1.

TABLE I. PMR Spectral Data for I, Ia, II andIIa (δ Values)[a,b]

	C_2–H	$C_{2'}$–H	C_3–H	$C_{3'}$–H	C_4–H	$C_{4'}$–2H	C_6–H	C_8–H	$C_{6'}$–H	ArH (B,B′-ring)	Galloyl
I	5.65 (br. s)	4.98 (br. s)	5.55 (m)	5.55 (m)	4.78 (d, J=2)	2.98 (m)	5.93		6.12 (s)	6.56—7.04	6.99, 7.07
Ia	5.11 (br. s)	4.95 (br. s)	4.30 (m)	4.00 (m)	4.75 (br. s)	2.76—2.88 (m)		5.97—6.04		6.68—7.08	—
II	5.46 (br. s)	4.43 (d, J=6)	5.31 (m)	4.04 (m)	4.60 (d, J=2)	2.55 (d.d, J=8, 16) 2.89 (d.d, J=6, 16)	5.80 (d, J=2)	5.93 (d, J=2)	6.08 (s)	6.61—6.96	6.95
IIa	5.10 (br. s)	4.75 (d, J=8)	3.96 (m)	4.08 (m)	4.68 (d, J=2)	2.56 (d.d, J=7, 16) 2.81 (d.d, J=6, 16)	5.96 (d, J=2)	6.03 (d, J=2)	5.96 (s)	6.60—6.96	—

a) Measured in acetone-d_6 at 100 MHz with TMS as an internal standard. d, doublet; d.d, double doublets; t, triplet; m, multiplet.
b) J values are expressed in Hz.

TABLE II. CMR Spectral Data for I, II and IIa (δ Values)[a]

		I	II	IIa
C-ring	C_2	75.5	75.2	76.7
	C_3	74.8	74.5	72.4
	C_4	33.6	34.0	36.7
C′-ring	$C_{2'}$	77.8	81.6	81.9
	$C_{3'}$	69.1	67.3	67.7
	$C_{4'}$	—[b]	—[b]	—[b]
A,A′-ring	$C_{5,5',7,7',9,9'}$	154.5, 155.4(3C), 156.6, 156.9	153.9, 155.2(2C), 156.7(2C), 156.9	153.6, 154.9, 155.4, 157.3, 157.9(2C)
	$C_{6,6'}$	95.3, 95.9	95.1, 95.7	95.6, 96.0
	C_8	96.9	96.7	96.7
	$C_{8'}$	106.8	106.5	107.2
	$C_{10,10'}$	99.3, 101.9	100.7, 101.4	100.7(2C)
B,B′-ring	$C_{11,11'}$	130.4, 131.0	131.1, 131.4	131.8, 132.1
	$C_{12,12',15,15'}$	114.4, 114.7, 115.3, 115.5	114.5(2C), 115.4, 115.5	114.4, 115.0, 115.2, 115.4
	$C_{13,13',14,14'}$	144.5(2C), 145.0(2C)	144.8—145.7(4C)	144.8, 145.0(2C) 145.1
	$C_{16,16'}$	119.1(2C)	118.6, 119.3	119.1(2C)
Galloyl	C_1	120.1, 121.2	121.9	
	C_2	109.9(4C)	109.8(2C)	
	C_3	145.4(4C)	145.4(2C)	
	C_4	138.7(2C)	138.6	
	–COO–	166.2, 166.5	166.2	

a) Measured in acetone-d_6+D_2O at 25.05 MHz with TMS as an internal standard.
b) Overlapped with solvent peaks.

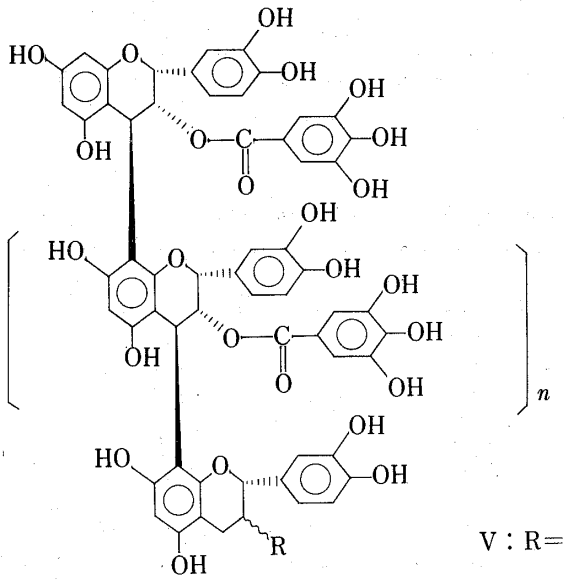
Compound III (III), colorless needles, mp 208.5—210.5°C, $[\alpha]_D$ −98.2° (MeOH), $C_{27}H_{24}O_{18}\cdot 1.5H_2O$, does not form an anthocyan pigment on treatment with acid. The infrared (IR) spectrum of III exhibits strong absorptions due to hydroxyl (3400 cm^{-1}) and ester carbonyl (1700 cm^{-1}) functions. The PMR and CMR spectra are suggestive of the presence of three galloyl groups (δ 7.08, 7.10, 7.16, each 2H) and one mole of hexose moiety (δ 6.40 (t), 70.8 (d),

73.7 (d), 74.8 (d), 75.7 (d), 93.4 (d)) in III. This was subsequently confirmed chemically by hydrolysis of III with tannase in aqueous solution, which yielded gallic acid and glucose.

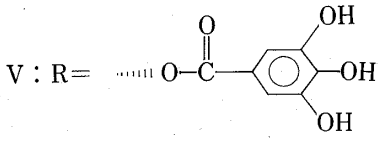
The locations of the three galloyl groups in the glucose moiety were determined to be the C_1, C_2 and C_6 positions by analysis of the PMR spectrum of III, which was notable for the deshielding of the corresponding C_1–H (δ 5.98, d, J=8 Hz), C_2–H (δ 5.28, t, J=9 Hz) and C_6–H (δ 4.41, d.d, J=4, 12 Hz; δ 4.67, br.d, J=12 Hz), being assignable to the protons geminal to galloyl groups. The assignment of these C_1– and C_2–H was carried out by the spin–decoupling technique.

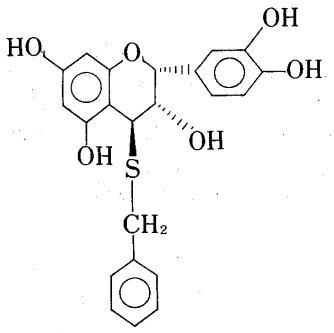
Therefore, compound III is characterized as 1,2,6-tri-O-galloylglucose.

Compound IV (IV), colorless needless, mp 206—207°C, $[\alpha]_D$ −18.6° (MeOH), $C_{23}H_{26}O_{11}$, shows hydroxyl (3350 cm^{-1}), carbonyl (1705 cm^{-1}) and ester (1685 cm^{-1}) absorption in the IR spectrum. Its PMR spectrum exhibits signals due to an acetyl group (δ 2.08), four methyl-

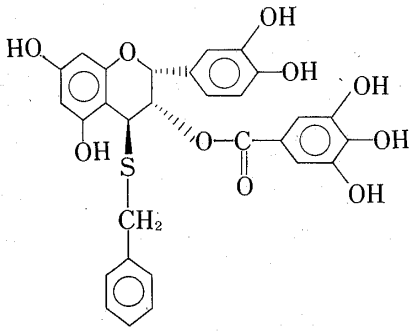


V : R=

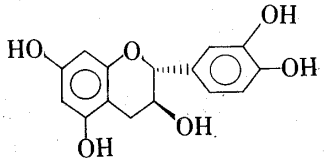




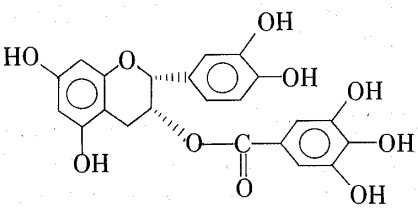
VI



VII



VIII : (+)-catechin



IX : 3-O-galloyl-(−)-epicatechin

Fig. 2

ene protons (δ 2.68, m) and A_2B_2-type aromatic protons (δ 6.89, 2H, d, J=8 Hz; δ 7.05, 2H, d, J=8 Hz), along with a galloyl group (δ 7.00) and sugar protons (δ 3.20—4.86).

IV, when incubated with tannase in 0.02 M acetate buffer, furnished gallic acid and a hydrolysate (IVa), colorless prisms, mp 115—116°C, $C_{16}H_{22}O_7$, whose PMR spectrum was identical with that of the glucoside of 4-(4′-hydroxyphenyl)-2-butanone, which is known to occur in rhubarb.[6]

Next, the galloyl group was concluded to be linked to the C_6 position in the glucose moiety on the basis of the PMR spectrum of IV, since two-proton signals (δ 4.21, d.d, J=6, 12 Hz; δ 4.48, br.d, J=12 Hz) which shifted downfield can be assigned to C_6–H in glucose.

Consequently, compound IV is 4-(4′-hydroxyphenyl)-2-butanone 4′-O-β-D-(6″-O-galloyl)-glucopyranoside, and it was confirmed by comparison of the melting points and $[\alpha]_D$ to be identical with lindleyin which has been isolated from *Aeonium lindleyi* by Gonzalez *et al.*[16]

It is noteworthy that lindleyin has been reported to have analgesic and anti-inflammatory activities almost equal to those of aspirin and phenylbutanone, and also to have very low toxicity;[19] thus, the anti-inflammatory activity in rhubarb is presumably due to lindleyin.

The MeOH extract, after removal of the ethyl acetate-soluble portion, is highly astringent, and was treated as shown in Chart 1 to give a tannin fraction designated as rhatannin.

Rhatannin (V), a brown amorphous powder, yielded cyanidin and gallic acid when treated with ethanolic HCl, and is presumed to be a proanthocyanidin polymer on the basis of its chromatographic properties. The IR spectrum of V shows strong absorption bands due to ester (1680—1700 cm^{-1}) and hydroxyl (3200—3400 cm^{-1}) groups. V was subjected to thiolysis with toluene-α-thiol in the presence of acetic acid[17] to afford (+)-catechin (VIII) and 3-O-galloyl-(−)-epicatechin (IX), which were derived from the lower terminal unit of the polymer, in addition to 4-benzylthioethers of 3-O-galloyl-(−)-epicatechin (VII) and a small amount of (−)-epicatechin (VI) formed from the extension unit (upper unit). From these spectral and chemical findings, V is suggested to be a mixture of proanthocyanidin polymers composed of 3-O-galloyl-(−)-epicatechin and (−)-epicatechin units in the chain extension part, and of (+)-catechin and 3-O-galloyl-(−)-epicatechin units in the chain termination part. Furthermore, judging from the yields of these thiolysis products and the occurrence of 4,8′-linked proanthocyanidin dimers in rhubarb, V would be expected to consist predominantly of C_4 to C_8 linked 3-O-galloyl-(−)-epicatechin polymers having either 3-O-galloyl-(−)-epicatechin or (+)-catechin unit in the lower terminal portion. Work is continuing to separate individual components and to estimate the molecular weight distribution of the tannins in rhubarb. Biological tests of the low-molecular-weight compounds isolated are also under way.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 digital polarimeter (cell length: 0.5 dm). IR spectra were obtained with a JASCO IR-G spectrometer. PMR and CMR spectra were measured with JEOL PS-100 and JEOL FX-100 spectrometers at 100 MHz with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Abbreviations used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad. Column chromatography was performed with Kieselgel 60 (70—230 mesh, Merck), polyamide C-200 (Wako) and Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemicals). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck, 0.20 mm thick), precoated polyamide 11 F_{254} plates (Merck, 0.15 mm thick) and precoated Avicel SF cellulose plates (Funakoshi) using $FeCl_3$ reagent, anisaldehyde-H_2SO_4 reagent and 10% H_2SO_4 as detectors. The ratio of solvent and reagent is given in v/v in each case.

Isolation of Compounds I, II and III——Commercial rhubarb (雅黄, 350 g) was finely powdered and successively extracted at room temperature with acetone, MeOH and H_2O. The acetone extract (42.1 g) was partitioned between H_2O (500 ml) and benzene–AcOEt (1: 1) (200 ml×4), and the aqueous layer was extracted with AcOEt (300 ml×5). The AcOEt layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a brown oily residue (36.0 g) which was chromatographed over Sephadex LH-20 (2.5×45 cm) with acetone and then with a mixture of acetone-H_2O (9: 1). The acetone–H_2O (9: 1) eluate was repeatedly chromatographed over Sephadex LH-20 using acetone, EtOH

and MeOH as eluents to afford compounds I (530 mg), II (270 mg) and III (128 mg). The above acetone eluate, after removal of most of the anthraquinone glycosides which were eluted first with acetone, was subjected to silica gel column chromatography with a mixture of AcOEt and acetone (1: 0—4: 1) to yield compound IV (471 mg), together with (+)-catechin (1.27 g), 3-O-galloyl-(−)-epicatechin (44 mg), 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside (709 mg), 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside (57 mg), gallic acid (9 mg) and 4-(4′-hydroxyphenyl)-2-butanone 4′-O-β-D-glucopyranoside (95 mg).

Compound I (I)——An off-white amorphous powder, $[\alpha]_D^{31}$ −93.8° (c=1.0, acetone). *Anal.* Calcd for $C_{44}H_{34}O_{20}\cdot 2H_2O$: 57.79; H, 4.45. Found: C, 57.51; H, 4.17. PMR: Table I. CMR: Table II.

I-Acetate——A solution of I in acetic anhydride and pyridine was allowed to stand overnight at room temperature, and the mixture was worked up in the usual manner to furnish I-acetate, a white powder (EtOH), mp 135.5—141°C, $[\alpha]_D^{18}$ −62.9° (c=0.58, $CHCl_3$). *Anal.* Calcd for $C_{72}H_{62}O_{34}$: C, 58.77; H, 4.25. Found: C, 58.53; H, 4.24. IR ν_{max}^{KBr} cm^{-1}: 1775 ($OCOCH_3$), 1730 (–COO–). PMR ($CDCl_3$): 1.64—2.32 ($OCOCH_3$), 3.04 (2H, m, C_4'–2H), 4.50 (1H, d, J=3 Hz, C_4–H), 4.76 (1H, br. s, C_2'–H), 5.32 (1H, m, C_3'–H), 5.56 (1H, m, C_3–H), 5.68 (1H, br. s, C_2–H), 6.20 (1H, d, J=2 Hz, C_6–H), 6.28 (1H, d, J=2 Hz, C_8–H), 6.68 (1H, s, C_6'–H), 6.90—7.36 (6H, m, ArH of B,B′-ring), 7.54, 7.68 (each 2H, s, galloyl H).

Hydrolysis of I with Tannase——I (100 mg) in 0.05 M acetate buffer (pH 5.0, 5 ml) was incubated at 37°C with tannase for 1 h. The reaction mixture was passed through a column of Sephadex LH-20, and the column, after being washed with H_2O, was eluted with 80% aq. MeOH to afford gallic acid (44 mg) and an off-white amorphous hydrolysate (Ia), $[\alpha]_D^{31}$ +32.2° (c=1.0, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{12}\cdot 1.5H_2O$: C, 59.18; H, 4.68. Found: C, 59.50; H, 4.58. PMR: Table I. By direct comparison of the *Rf*-value on TLC. $[\alpha]_D$ and the PMR spectrum, Ia was shown to be identical with procyanidin B-2 which was isolated from the seeds of *Areca catechu*.[18]

Compound II (II)——An off-white amorphous powder, $[\alpha]_D^{31}$ −21.2° (c=1.0, acetone). *Anal.* Calcd for $C_{37}H_{30}O_{16}\cdot 3H_2O$: C, 56.27; H, 4.81. Found: C, 56.63; H, 4.62. PMR: Table I. CMR: Table II.

II-Acetate——II was acetylated in the same way as I to give a white powder (EtOH), mp 132—138°C, $[\alpha]_D^{16}$ +20.0° (c=0.35, MeOH). *Anal.* Calcd for $C_{61}H_{54}O_{28}$: C, 59.31; H, 4.40. Found: C, 59.22; H, 4.56. IR ν_{max}^{KBr} cm^{-1}: 1770 ($OCOCH_3$), 1730 sh. (–COO–). PMR ($CDCl_3$): 1.63—2.36 ($OCOCH_3$), 2.50—3.36 (2H, m, C_4'–2H), 4.35 (1H, d, J=10 Hz, C_2'–H), 4.51 (1H, d, J=2 Hz, C_4–H), 5.26 (1H, m, C_3'–H), 5.40 (1H, m, C_3–H), 5.55 (1H, br. s, C_2–H), 6.05 (1H, d, J=2 Hz, C_6–H), 6.32 (1H, d, J=2 Hz, C_8–H), 6.68 (1H, s, C_6'–H), 6.88—7.40 (6H, m, ArH or B,B′-ring), 7.50 (2H, s, galloyl H).

Hydrolysis of II with Tannase——An aqueous solution of II was treated with tannase at 37°C. After 30 min incubation, the solvent was evaporated off under reduced pressure, and the residue was chromatographed over Sephadex LH-20 using EtOH as an eluent to furnish gallic acid and a hydrolysate (IIa), an off-white amorphous powder, $[\alpha]_D$ +40.2° (c=0.8, acetone). PMR: Table I. IIa was shown to be identical with procyanidin B-1[18] by direct comparison (TLC, $[\alpha]_D$, PMR and CMR spectra).

Compound III (III)——Colorless needles, mp 208.5—210.5°C, $[\alpha]_D^{19}$ −98.2° (c=0.55, MeOH). *Anal.* Calcd for $C_{27}H_{24}O_{18}\cdot 1.5H_2O$: C, 50.09; H, 3.81. Found: C, 50.23; H, 3.90. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (–COO–). PMR (acetone-d_6): 4.41 (1H, d.d, J=4, 12 Hz, glu. C_6–H), 4.67 (1H, br. d, J=12 Hz, glu. C_6–H), 5.28 (1H, t, J=9 Hz, glu. C_2–H), 5.98 (1H, d, J=8 Hz, glu. C_1–H), 7.08, 7.10, 7.16 (each 2H, s, galloyl H). CMR (acetone-d_6): 64.0 (t, glu. C_6), 70.8 (d, glu. C_4), 73.7 (d, glu. C_2), 74.8 (d, glu. C_3), 75.7 (d, glu. C_5), 93.4 (d, glu. C_1), 109.7, 109.9 (d, 6×galloyl C_2), 119.3, 120.5, 120.8 (s, galloyl C_1), 138.8, 139.0, 139.5 (s, galloyl C_4), 145 (s, 6×galloyl C_3), 165.3, 166.6, 166.9 (s, –COO–).

Hydrolysis of III with Tannase——An aqueous solution (1 ml) of III (5 mg) was hydrolyzed with tannase at 37°. After 30 min the solvent was evoporated off, and the residue was checked by chromatography on Avicel SF cellulose and silica gel plates with *n*-BuOH–pyridine–H_2O (6: 4: 3) (solv. 1) and the lower layer of $CHCl_3$–MeOH–H_2O (7: 3: 1) (solv. 2). Glucose (solv. 1: *Rf* 0.35) and gallic acid (solv. 2: *Rf* 0.3) were identified.

Compound IV (IV)——Colorless needles, mp 206—207°C, $[\alpha]_D^{19}$ −18.6° (c=0.8, MeOH). *Anal.* Calcd for $C_{23}H_{26}O_{11}$: C, 57.74; H, 5.48. Found: C, 57.51; H, 5.49. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1705 ($COCH_3$), 1685 (–COO–). PMR (DMSO-d_6): 2.08 (3H, s, $COCH_3$), 2.68 (4H, m, $-CH_2CH_2-$), 4.21 (1H, d.d, J=6, 12 Hz, glu. C_6–H), 4.48 (1H, br. d, J=12 Hz, glu. C_6–H), 4.84 (1H, d, J=7 Hz, glu. C_1–H), 6.89 (2H, d, J=8 Hz, ArH), 7.05 (2H, d, J=8 Hz, ArH), 7.00 (2H, s, galloyl H). CMR (DMSO-d_6): 28.1 (q, C_1), 29.6 (t, C_3), 43.9 (t, C_4), 63.0 (t, glu. C_6), 69.7 (d, glu. C_4), 72.8 (d, glu. C_2), 73.5 (d, glu. C_5), 76.0 (d, glu. C_3), 100.1 (d, glu. C_1), 108.4 (2C) (d, 2×galloyl C_2), 115.7 (2C) (d, 2×C_2'), 119.1 (s, galloyl C_1), 128.6 (2C) (d, 2×C_3'), 134.0 (s, C_1'), 137.9 (s, galloyl C_4), 145.0 (2C) (s, 2×galloyl C_3), 154.9 (s, C_4'), 165.3 (s, –COO–), 207.7 (s, C_2).

Hydrolysis of IV with Tannase——A suspension of IV (82 mg) in 0.02 M acetate buffer (pH 5.0, 5 ml) was incubated at 37°C with tannase for 4 h. The reaction mixture was applied to a Sephadex LH-20 column and eluted with H_2O to afford gallic acid (14 mg) and a hydrolysate (45 mg) (IVa). IVa: Colorless needles, mp 115—116°C. *Anal.* Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.79; H, 6.80. PMR (acetone-d_6): 2.08 (3H, s, $COCH_3$), 2.75 (4H, m, $-CH_2CH_2-$), 4.89 (1H, d, J=7 Hz, glu. C_1–H), 6.95 (2H, d, J=8 Hz, ArH), 7.13 (2H, d, J=8 Hz, ArH). The PMR spectrum of IIa coincided with that of the sample isolated from rhubarb.[6]

Isolation of Rhatannin (V)——The MeOH extract (97.8 g) of rhubarb was suspended in H_2O (500 mg) and the suspension was extracted with AcOEt (500 ml×6). The aqueous layer was concentrated to a small volume and applied to a column (4.8×54 cm) of Sephadex LH-20, which was eluted successively with H_2O (4 l), H_2O–EtOH (1: 1) (3 l), EtOH (2 l) and MeOH (8 l). The MeOH eluate (11.9 g) was again loaded on a Sephadex LH-20 column with H_2O–MeOH (1: 9), and subsequent elution with MeOH provided V as a brown amorphous powder (10.0 g). *Anal.* Calcd for $(C_{22}H_{16}O_{10}\cdot 0.5H_2O)_n$: C, 58.79; H, 3.81. Found: C, 58.54; H, 4.24. IR ν_{max}^{KBr} cm^{-1}: 3200—3400 (OH), 1680—1700 (–COO–).

Thiolysis of V——1)[17]: A solution of V (1.0 g) in EtOH (40 ml) containing toluene-α-thiol (8 ml) and acetic acid (5 ml) was refluxed for 28 h under an N_2 atmosphere. The reaction mixture was concentrated below 40°C to give an oily residue which was chromatographed over Sephadex LH-20 (1.6×55 cm) using $CHCl_3$–MeOH (2: 1). Three fractions (Fr. I, II and III) were obtained on the basis of TLC analysis. Fr. I (166 mg) was further purified on a Sephadex LH-20 column (1.3×22 cm) with acetone as an eluent to give pure VI (59 mg), an off-white amorphous powder, $[\alpha]_D^{19}$ −28.0° (c=1.0, acetone). PMR (acetone-d_6): 3.97 (1H, d, J=2 Hz, C_3–H), 4.03 (2H, s, $-CH_2-S-$), 4.13 (1H, d, J=2 Hz, C_4–H), 5.29 (1H, s, C_2–H), 5.92 (1H, d, J=2 Hz, C_6–H), 6.05 (1H, d, J=2 Hz, C_8–H), 6.72—7.60 (8H, m, ArH). Fr. II (440 mg) was rechromatographed over Sephadex LH-20 with acetone to afford VII (300 mg) and VIII (20 mg). VII: an off-white amorphous powder. *Anal.* Calcd for $C_{29}H_{24}O_{10}S\cdot 0.5H_2O$: C, 60.77; H, 4.47. Found: C, 60.73; H, 4.39. PMR (acetone-d_6+D_2O): 4.16 (2H, s, $-CH_2-S-$), 4.33 (1H, d, J=2 Hz, C_4–H), 5.39 (1H, m, C_3–H), 5.58 (1H, br. s, C_2–H), 6.03 (1H, d, J=2 Hz, C_6–H), 6.08 (1H, d, J=2 Hz, C_8–H), 6.78—7.60 (8H, m, ArH), 6.99 (2H, s, galloyl H). CMR (acetone-d_6+D_2O): 37.0 (t, $-SCH_2-$), 40.8 (d, C_4), 72.7 (d, C_3), 74.0 (d, C_2), 95.4 (d, C_6), 96.9 (d, C_8), 98.6 (s, C_{10}), 109.7 (2C) (d, 2×galloyl C_2), 114.7, 115.5 (d, C_{12}, C_{15}), 118.8 (d, C_{16}), 120.9 (s, galloyl C_1), 127.4 (d, phenyl C_4), 128.9 (2C) (d, 2×phenyl C_2, C_3), 130.4 (s, C_{11}), 138.8 (s, galloyl C_4), 139.5 (s, phenyl C_1), 145.3, 145.6 (s, C_{13}, C_{14}), 145.6 (2C) (s, 2×galloyl C_3), 156.6, 157.8, 158.8 (s, C_5, C_7, C_9), 165.8 (s, –COO–). Hydrolysis of VII with tannase in an aqueous solution afforded VI and gallic acid as determined by TLC analysis. VIII was crystallized from H_2O to yield colorless needles, mp 177°C, $[\alpha]_D^{21}$ +12.3° (c=0.36, acetone), which were identical with (+)-catechin by direct comparison (mp, $[\alpha]_D$, PMR spectrum).[5] Fr. III (113 mg) was again chromatographed over Sephadex LH-20 using acetone to furnish IX (58 mg). IX: could not be crystallized. $[\alpha]_D^{21}$ −160.6° (c=0.22, acetone). PMR (acetone-d_6): 2.95—3.02 (2H, m, C_4–2H), 5.13 (1H, br. s, C_2–H), 5.55 (1H, m, C_3–H), 6.04 (1H, d, J=2 Hz, C_6–H), 6.08 (1H, d, J=2 Hz, C_8–H), 6.72—7.16 (3H, m, ArH), 7.04 (2H, s, galloyl H). CMR (acetone-d_6): 26.4 (t, C_4), 69.7 (d, C_3), 77.8 (d, C_2), 95.3 (d, C_6), 96.3 (d, C_8), 98.5 (s, C_{10}), 109.7 (2C) (d, 2×galloyl C_2), 114.6, 115.4 (d, C_{12},C_{15}), 118.7 (d, C_{16}), 121.0 (s, galloyl C_1), 130.7 (s, C_{11}), 138.7 (s, galloyl C_4), 145.0 (2C) (s, C_{13}, C_{14}), 145.5 (2C) (s, 2×galloyl C_3), 156.4, 156.9, 157.0 (s, C_5, C_7, C_9), 166.3 (s, –COO–). IX was identical with 3-O-galloyl-(−)-epicatechin isolated from rhubarb.[10–12]

2): V (1.0 g) was refluxed in EtOH (25 ml) containing toluene-α-thiol (8 ml) and 1 N HCl (5 ml), under an N_2 atmosphere for 8 h. The reaction mixture was diluted with H_2O and extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. The oily residue was chromatographed over Sephadex LH-20 (22×3.5 cm) with acetone as an eluent. The eluates were grouped into three fractions (Fr. I, II and III) based upon TLC analysis using the bottom layer of $CHCl_3$–MeOH–H_2O (7: 3: 1) as a developing solvent. Fr. I (217 mg) was purified by Sephadex LH-20 column chromatography to afford VI (26 mg). Fr. II (388 mg) and Fr. III (416 mg), which contained VII, were further chromatographed over Sephadex LH-20 with $CHCl_3$–MeOH (4: 1) as an eluent to furnish VII (553 mg). The presence of (+)-catechin or 3-O-galloyl-(−)-epicatechin could not be detected in either fraction on TLC.

Hydrolysis of V with Tannase——V (1.0 g) in 0.05 M acetate buffer (pH 5.0) was incubated with tannase at 38°C for 6 h. The mixture was extracted twice with AcOEt. The AcOEt layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield colorless needles (109 mg), which were shown to be identical with gallic acid. The aqueous layer was concentrated below 40°C to 1/2 volume, and allowed to stand overnight at room temperature. Brown precipitates which appeared were filtered off and the filtrate was subjected to Sephadex LH-20 chromatography (2.7×35 cm) with H_2O and then MeOH as eluents. The H_2O eluate afforded gallic acid (69 mg). The MeOH eluate gave a brown powder (520 mg), which lacks carbonyl absorption bands in the IR spectrum.

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References and Notes

1) This work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1979.
2) H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto, and M. Tsukui, *Chem. Pharm. Bull.*, **22**, 823 (1974).

3) H. Okabe, K. Matsuo, and I. Nishioka, *Chem. Pharm. Bull.*, **21**, 1254 (1973).
4) M. Tsuboi, M. Minami, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **25**, 2708 (1977).
5) G. Nonaka, M. Minami, and I. Nishioka, *Chem. Pharm. Bull.*, **25**, 2300 (1977).
6) T. Murakami and K. Tanaka, *Yakugaku Zasshi*, **93**, 733 (1973).
7) L. Csupor, *Arch. Pharm.*, **303**, 681 (1970).
8) M. Hemegerg and P. Horak, *Acta. Polon. Pharm.*, **16**, 189 (1959).
9) F. Tutin and H.W.B. Cleweer, *J. Chem. Soc.*, **99**, 946 (1911).
10) W. Mayer, G. Schultz, S. Wrede, and G. Schilling, *Ann. Chem.*, **1975**, 946.
11) E. Gilson, *Bull. Acad. R. Med. Belg.*, **16**, 827 (1902).
12) H. Friedrich and Höhle, *Arch. Pharm.*, **299**, 857 (1966).
13) L.T. Pashinina and T.K. Chumbalov, *Khim. Prir. Soedin*, **3**, 646 (1967).
14) T. Nagasawa, S. Shibutani, and H. Oura, *Yakugaku Zasshi*, **99**, 71 (1979).
15) T. Nagasawa, S. Shibutani, H. Oura, Y. Shoyama, and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 1736 (1980).
16) A.G. González, C.G. Francisco, R. Freire, R. Hernández, J.A. Salazar, and E. Suarez, *Phytochemistry*, **15**, 344 (1976).
17) R.S. Thompson, D. Jacques, E. Haslam, and R.J.N. Tanner, *J. Chem. Soc., Perkin I*, **1972**, 1387.
18) The isolation of procyanidins B-1 and B-2[17] from the seeds of *Areca catechu* was orally reported at the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April, 1981.
19) V. Darias, A.G. González, J.N. Boada, M. Feria, and F. Martorrell, *Farmaco.*, **33**, 460 (1978).