[Chem. Pharm. Bull. 34(8)3208—3222(1986)]

Tannins and Related Compounds. XLV.[1] Rhubarb. (5). Isolation and Characterization of Flavan-3-ol and Procyanidin Glucosides

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(Received February 3, 1986)

A chemical examination of high-quality commercial rhubarb (Choukichio: 長吉黄) has led to the isolation and characterization of eight flavan-3-ol glucosides and three proanthocyanidin glucosides, together with several known compounds, *i.e.*, (+)-catechin 5-*O*-*β*-D-glucopyranoside (**1**), (+)-catechin 7-*O*-*β*-D-glucopyranoside (**2**), procyanidin B-2 8-*C*-*β*-D-glucopyranoside (**3**) and procyanidin B-2 6-*C*-*β*-D-glucopyranoside (**4**). On the basis of chemical and spectroscopic evidence, the former compounds were characterized as (+)-catechin 3′-*O*-*β*-D-glucopyranoside (**5**), (+)-catechin 4′-*O*-*β*-D-glucopyranoside (**6**), (+)-catechin 7,3′-di-*O*-*β*-D-glucopyranoside (**7**), (+)-catechin 5,3′-di-*O*-*β*-D-glucopyranoside (**8**), (+)-catechin 3′,4′-di-*O*-*β*-D-glucopyranoside (**9**), (+)-catechin 5,4′-di-*O*-*β*-D-glucopyranoside (**10**), (+)-catechin 8-*C*-*β*-D-glucopyranoside (**11**), (+)-catechin 6-*C*-*β*-D-glucopyranoside (**12**), procyanidin B-3 7-*O*-*β*-D-glucopyranoside (**13**), procyanidin B-1 8-*C*-*β*-D-glucopyranoside (**14**) and procyanidin B-1 6-*C*-*β*-D-glucopyranoside (**15**).

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

Rhubarb, one of the crude drugs used most frequently in traditional Chinese prescriptions for a variety of diseases, is imported in large amounts from China under various commercial names. Although rhubarb itself has been produced since ancient times from several species of *Rheum* plants such as *R. palmatum, R. officinale, R. tanguticum, etc.*, it is difficult, because of the uncertainly regarding production methods, geographical sources, *etc.* in China, to establish the exact plant origin a particular sample of rhubarb even with the aid of modern morphological and chemical methods; the components in commercial rhubarbs may be heterogeneous (in both qualitative and quantitative senses). Under these circumstances, we have been investigating, for the purpose of the quality evaluation of rhubarbs by chemical means, polyphenolic constituents in various rhubarbs classified according to their commercial names. We have so far reported the structural elucidation of dimeric and polymeric proanthocyanidin gallates, the major condensed tannins contained in a high-quality rhubarb[2] (commercial name: 雅黄 Gao) and of galloyl and (hydroxy)cinnamoyl esters with glucose and fructose from Batei-Daio (馬蹄大黄)[3] and Imo-Daio (芋大黄),[3] together with a variety of phenolics such as anthraquinones,[4] naphthalenes,[5] chromones,[6] chromanone,[6] stilbenes[7] and flavan-3-ols.[8] In this paper, we wish to present details of the isolation and structure determination of flavan-3-ol and procyanidin glucosides, which were isolated from another high-quality rhubarb (commercial name: 長吉黄 Choukichio).

The aqueous acetone extract of rhubarb purchased from a market in Hong Kong was directly subjected to chromatography over Sephadex LH-20 with H_2O containing increasing amounts of methanol (1:0—0:1) to give six fractions. From the fractions eluted earlier, compounds **1**—**15** were isolated by a combination of Sephadex LH-20, MCI-gel CHP 20P, Fuji-gel ODS G3 and Bondapak C_{18} chromatographies (Chart 1).

Among these compounds, **1**—**4** were identified as (+)-catechin 5-*O*-*β*-D-glucopyranoside

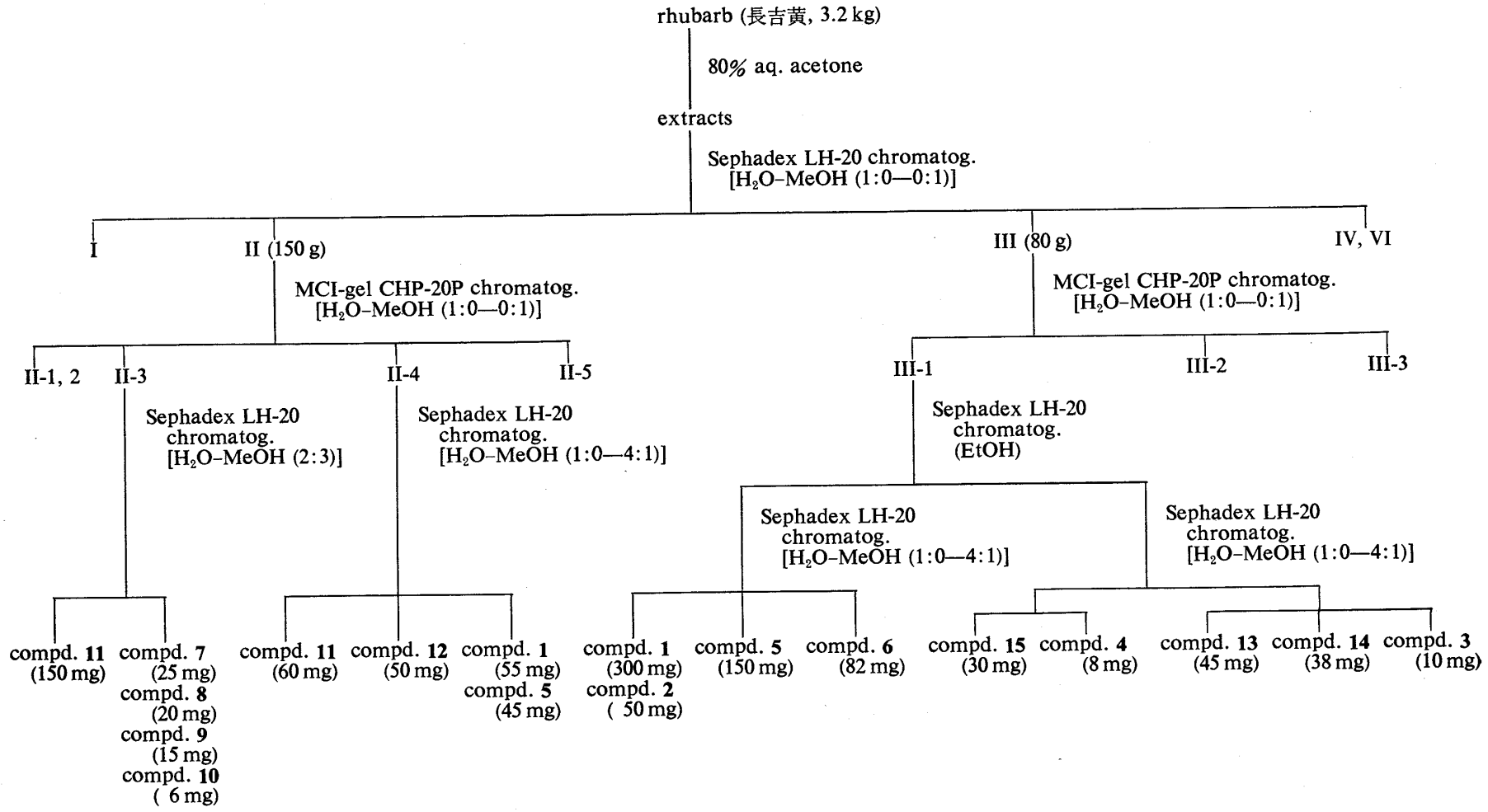


Chart 1

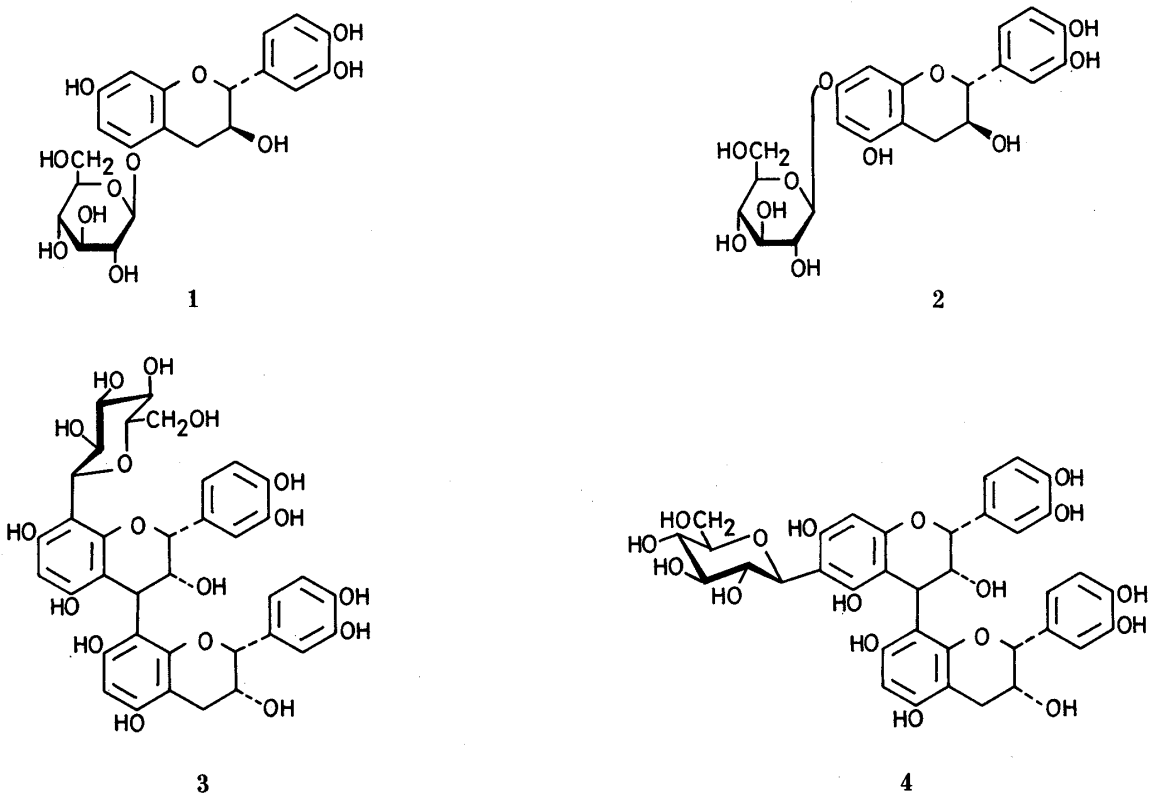
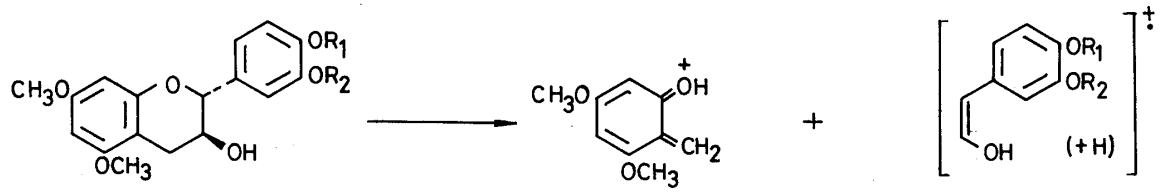


Chart 2

(**1**),[8] (+)-catechin 7-*O*-β-D-glucopyranoside (**2**),[9] procyanidin B-2 8-*C*-β-D-glucopyranoside (**3**),[10] and procyanidin B-2 6-*C*-β-D-glucopyranoside (**4**)[10] by comparisons of their physical and spectral data with those of authentic samples.

Compound **5**, a white amorphous powder, $[\alpha]_D$ −39.3° (MeOH), $C_{21}H_{24}O_{11}\cdot H_2O$, and compound **6**, a white amorphous powder, $[\alpha]_D$ −34.0° (MeOH), $C_{21}H_{24}O_{11}\cdot H_2O$, gave orange colorations characteristic of flavan-3-ol derivatives on treatment with the anisaldehyde–sulfuric acid reagent.[11] The field-desorption mass spectra (FD-MS) of **5** and **6** showed the same ion peaks at *m/z* 491, 475 and 453 due to $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$, respectively, together with a prominent peak at *m/z* 163 suggestive of the presence of a hexosyl moiety. Enzymatic hydrolyses of **5** and **6** with crude hesperidinase gave, in each case, D-glucose and an aglycone which was shown to be identical with (+)-catechin. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **5** and **6** were similar to those of (+)-catechin except for the presence of sugar signals. The differences in the ^{1}H- and ^{13}C-chemical shifts for the B-ring in the catechin nucleus suggested that the glucosyl moiety was bound to either the C-3′ or C-4′ hydroxyl group.

Methylation of **5** and **6** with dimethyl sulfate and potassium carbonate in dry acetone, followed by enzymatic hydrolysis with crude hesperidinase, gave (+)-catechin trimethyl ethers (**5b** and **6b**, respectively). The electron-impact mass spectra (EI-MS) of **5b** and **6b** exhibited a fragment ion peak at *m/z* 167 formed by a retro-Diels–Alder fission of the C-ring (Chart 3), indicating that the methoxyl groups are present at the C-5, 7 and 3′ or C-5, 7 and 4′ positions. The locations of the methoxyl groups in **5b** were confirmed to be the C-5, 7 and 4′ positions by ^{1}H-NMR nuclear Overhauser effect (NOE) measurements on the diacetate **5c**. Irradiation of the methoxyl region (δ 3.77—3.82) resulted in 21% enhancement of the integral intensity of the H-5′ signal. In the case of **6b**, the locations of the methoxyl groups were concluded to be the C-5, 7 and 3′ positions from a similar NOE experiment: when the methoxyl region (δ 3.74—3.85) was irradiated, the integral intensity of the H-2′ signal was

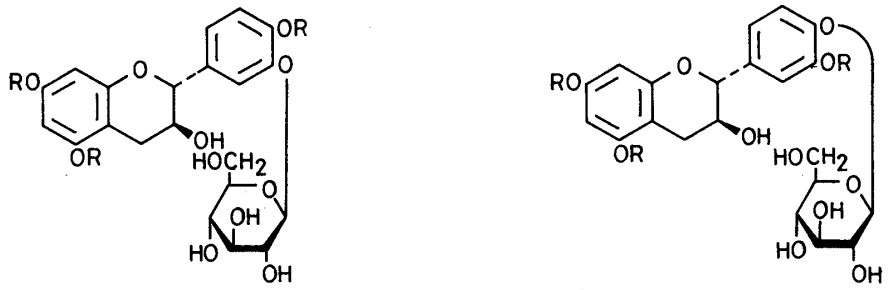


	R_1	R_2
5b	CH_3	H
6b	H	CH_3
9b	H	H

m/z 167

m/z 167 (**5b**, **6b**)
m/z 152 (**9b**)

Chart 3



5: R=H
5a: R=CH_3

6: R=H
6a: R=CH_3

Chart 4

enhanced by 20%. Thus, the glucosyl moieties in **5** and **6** were concluded to be bound to the C-3′ and C-4′ hydroxyls of the (+)-catechin nuclei, respectively. The anomeric configurations in **5** and **6** were determined to be β from the coupling constants (d, J=8 Hz, in each case) of the anomeric proton signals. On the basis of these chemical and spectral data, **5** and **6** were characterized as (+)-catechin 3′-*O*-β-D-glucopyranoside and (+)-catechin 4′-*O*-β-D-glucopyranoside, respectively.

Compound **7**, a white amorphous powder, $[\alpha]_D$ −67.4° (MeOH), $C_{27}H_{34}O_{16}\cdot 3/2H_2O$, compound **8**, a white amorphous powder, $[\alpha]_D$ −48.2° (MeOH), $C_{27}H_{34}O_{16}\cdot 3/2H_2O$ and compound **9**, a white amorphous powder, $[\alpha]_D$ −54.4° (MeOH), $C_{27}H_{34}O_{16}\cdot 3/2H_2O$, gave mutually related ^{1}H-NMR spectra which showed signals arising from a catechin skeleton and two anomeric protons in each case. The FD-MS of **7**, **8** and **9** exhibited a peak at *m/z* 637 due to $[M+Na]^+$, together with prominent peaks at *m/z* 452 and 290 formed by loss of one and two hexosyl groups from the molecular ion, respectively, suggesting that two hexosyl moieties are present in each molecule. Enzymatic hydrolyses of **7**, **8** and **9** with crude hesperidinase yielded glucose and an aglycone which was identified as (+)-catechin.

The ^{1}H-NMR spectrum of **7** showed signals of H-6 and H-8 at δ 6.33 (d, J=2 Hz) and 6.14 (d, J=2 Hz), respectively; these chemical shifts are in good accord with those (δ 6.34, 6.15, each d, J=2 Hz) of the known (+)-catechin glucoside (**2**). It also exhibited signals due to H-2′, H-5′ and H-6′ at δ 7.23 (d, J=2 Hz), 6.87 (d, J=8 Hz) and 7.00 (dd, J=2, 8 Hz), respectively, the chemical shifts being almost identical with those [δ 7.24 (d, J=2 Hz), 6.84 (d, J=8 Hz), 7.01 (dd, J=2, 8 Hz)] of **5**. These observations suggested the occurrence of two glucosyl moieties at the C-7 and C-3′ hydroxyls in **7**. In the case of **8**, the ^{1}H-NMR spectrum resembled that of **7** except for the chemical shifts (Table I) of H-4, H-6 and H-8, which were similar to those of **1** rather than those of **2**. From these observations, two glucosyl moieties were presumed to be located at the C-5 and C-3′ hydroxyl groups. In the ^{1}H-NMR spectrum of **9**, the chemical shifts (δ 5.90, 6.04) of H-6 and H-8 were in good accord with those (δ 5.90, 6.04) found in (+)-catechin, suggesting that the glucosyl residues were bound to neither the

TABLE I. ^{1}H-NMR Spectral for Compounds **1**, **2**, **5**—**11** and **12**, and (+)-Catechin

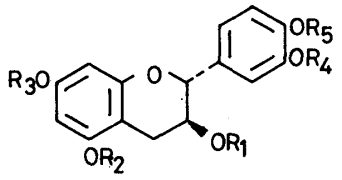
	H-2	H-3	H-4		H-6	H-8	H-2′	H-5′	H-6′	Anom.-H
	(δ values, J values in Hz)									
Catechin[a]	4.57	4.02	2.50	2.96	5.90	6.04	6.90	6.80	6.72	
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	
1[b]	4.64	4.06	2.61	3.08	6.35	6.06	6.92	6.87	6.76	4.95
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
2[b]	4.67	4.08	2.57	2.91	6.34	6.15	6.92	6.86	6.75	4.92
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
5[b]	4.60	4.02	2.52	2.94	5.88	6.04	7.24	6.84	7.01	4.90
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
6[b]	4.62	4.02	2.52	2.91	5.88	6.04	6.93	7.16	6.83	4.83
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
7[b]	4.72	—[c]	2.57	2.90	6.33	6.14	7.23	6.87	7.00	4.90 (2H)
	(d, J=8)		(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
8[b]	4.62	—[c]	2.56	3.04	6.35	6.03	7.24	6.84	7.00	4.89 (2H)
	(d, J=8)		(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
9[b]	4.65	—[c]	2.52	2.94	5.90	6.04	7.32	7.26	7.08	4.98, 5.00
	(d, J=8)		(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
10[b]	4.64	—[c]	2.56	3.06	6.36	6.06	6.96	7.12	6.78	4.90, 4.94
	(d, J=8)		(dd, J=8, 16)	(dd, J=6, 16)	(d, J=2)	(d, J=2)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=8)
11[b]	4.74	—[c]	2.54	2.82	6.08	—	7.10	6.78	6.85	4.81
	(d, J=8)		(dd, J=8, 16)	(dd, J=6, 16)	(s)		(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=10)
12[b]	4.60	4.02	2.53	2.88	—	5.96	6.59	6.82	6.71	4.88
	(d, J=8)	(m)	(dd, J=8, 16)	(dd, J=6, 16)		(s)	(d, J=2)	(d, J=8)	(dd, J=2, 8)	(d, J=10)

Anom. = anomeric. *a*) Measured in acetone-d_6. *b*) Measured in acetone-d_6 + D_2O. *c*) Overlapped with DOH.

TABLE II. ^{13}C-NMR Spectral Data for Compounds **1**, **2**, **5**—**9**, **11** and **12**, and (+)-Catechin

	Catechin[a]	**1**[b]	**2**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[b]	**11**[b]	**12**[b]
C-2	82.3	82.0	82.6	82.2	82.1	82.0	82.4	82.0	81.1	81.7
C-3	68.0	67.6	68.2	67.9	68.1	67.6	67.9	68.4	67.5	67.9
C-4	27.5	27.8	28.5	28.5	28.7	27.8	28.8	28.8	27.6	28.4
C-4a	100.2	103.1	104.1	100.5	100.4	103.3	103.1	100.5	100.6	101.9
C-5	156.7	157.0	157.3	156.9	157.1	156.8	157.7	157.0	156.0[c]	155.3[c]
C-6	95.0	97.8	97.3	95.2	95.2	97.4	97.8	95.2	96.8	105.0
C-7	157.1	156.8	158.4	157.3	157.5	157.8	157.5	157.3	156.4[c]	155.7[c]
C-8	95.9	96.6	98.0	96.3	96.2	96.5	96.9	96.4	103.9	96.2
C-8a	156.3	155.9	156.7	156.4	156.4	156.1	156.2	156.3	154.4	155.3
C-1′	131.5	131.2	132.0	131.7	135.8	131.6	131.7	135.4	131.8	131.7
C-2′	115.0	115.4	116.0	117.1	115.8	116.5	116.7	118.4	114.9	115.1
C-3′	145.2	145.2[c]	146.0	147.3	145.9	147.1	147.7	147.8	145.2[c]	145.6
C-4′	145.2	145.3[c]	146.0	145.7	147.7	145.7	145.8	147.8	145.3[c]	145.6
C-5′	115.4	116.3	116.9	116.6	118.1	116.7	117.4	119.0	115.8	115.8
C-6′	119.6	120.1	120.6	123.3	119.8	123.1	123.4	123.2	119.3	131.7
Sugar										
C-1		101.4	102.0	102.8	103.6	101.4, 102.6	102.0, 103.0	102.6, 102.8	75.6	76.8
C-2		73.8	74.5	74.0	74.2	73.9 (2C)	74.2 (2C)	74.2 (2C)	72.8	74.5
C-3		77.0	77.6	77.3	77.6	77.1 (2C)	77.5 (2C)	77.5 (2C)	78.9	79.0
C-4		70.4	71.1	70.6	70.8	70.4 (2C)	71.0, 70.8	70.6 (2C)	70.8	70.2
C-5		76.7	77.5	76.7	76.9	77.1, 76.6	77.5, 77.0	76.9 (2C)	81.4	82.3
C-6		61.7	62.4	61.8	62.1	61.8, 61.7	62.3, 62.1	61.8 (2C)	61.9	61.4

a) Measured in acetone-d_6. *b*) Measured in acetone-d_6 + D_2O. *c*) Assignments with the superscript *c*) may be interchanged in each column.



	R_1	R_2	R_3	R_4	R_5
7b	H	CH_3	H	H	CH_3
7c	Ac	CH_3	Ac	Ac	CH_3
8b	H	H	CH_3	H	CH_3
9b	H	CH_3	CH_3	H	H

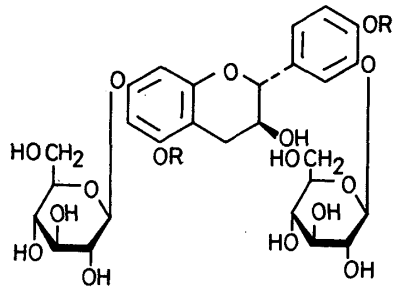
Chart 5

C-5 nor the C-7 hydroxyl group. Thus, the locations of the two glucosyl moieties in **9** were presumed to be the C-3′ and C-4′ hydroxyl groups.

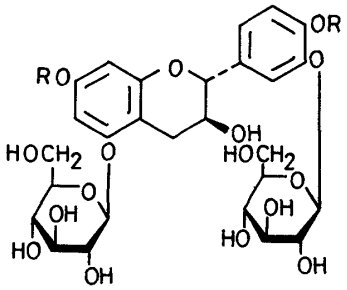
The locations of the glucosyl moieties in **7**, **8** and **9** were determined unambiguously as follows; **7**, **8** and **9** were methylated with diazomethane to give the corresponding dimethyl ethers **7a**, **8a**, and **9a**, which, on enzymatic hydrolyses with crude hesperidinase, yielded glucose and aglycones **7b**, **8b** and **9b**, respectively. Compounds **7b**, **8b** and **9b** possessed two methoxyl groups as revealed by ^{1}H-NMR spectral and EI-MS (M^+ at *m/z* 318) analyses. The locations of the methoxyl groups in **7b** were concluded to be the C-5 and C-4′ positions by NOE experiments on the triacetate **7c**; irradiation of the methoxyl signal at δ 3.77 resulted in 27% enhancement of the integral intensity of the H-6 signal (δ 6.22), no NOE enhancement of H-8 being observed, while upon irradiation of another methoxyl signal at δ 3.82, the integral intensity of the H-5′ signal (δ 6.92, d, J=8 Hz) increased by 25%. On the other hand, **8b** was shown to be 7,4′-di-*O*-methyl (+)-catechin by spectral comparisons with an authentic sample[12] isolated from the bark of *Cinnamomum cassia*. Compound **9b** showed fragment ion peaks at *m/z* 167 and 152 (Chart 3) in the EI-MS, suggesting that the methoxyl groups were present at both the C-5 and C-7 positions. To confirm the structure of **9b**, 5,7-di-*O*-methyl (+)-catechin was prepared according to the procedure reported by Hathway and Seakins.[13]

Spectral comparison revealed that **9b** was identical with the synthetic sample thus obtained. From these chemical data, the positions of the glucosyl moieties in **7**, **8** and **9** were concluded to be C-7 and C-3′, C-5 and C-3′, and C-3′ and C-4′, respectively. The anomeric configurations in **7**, **8** and **9** were concluded to be β from the coupling constant values (d, $J=$ 8 Hz, in each case) of the anomeric proton signals. On the basis of the above-mentioned evidence, **7**, **8** and **9** were concluded to be (+)-catechin 7,3′-di-*O*-β-D-glucopyranoside, (+)-catechin 5,3′-di-*O*-β-D-glucopyranoside and (+)-catechin 3′,4′-di-*O*-β-D-glucopyranoside, respectively.

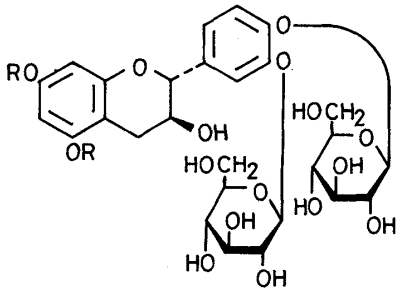
Compound **10**, a white amorphous powder, $[\alpha]_D$ −46.8° (MeOH), $C_{27}H_{34}O_{16}\cdot 7/2H_2O$, was obtained in a small quantity. The FD-MS showed the same $[M+Na]^+$ peak at *m/z* 637 as those of **7**, **8** and **9**, suggestive of the presence of two glucose moieties. On enzymatic hydrolysis, **10** gave glucose and (+)-catechin. The ^{1}H-NMR spectrum of **10** resembled that of **8** except for the B-ring signals, whose chemical shifts were rather similar to those of **6** (Table I), indicating the glucosyl moieties to be at the C-5 and C-4′ positions. The modes of the sugar linkages in **10** were confirmed to be β based on the coupling constants of the anomeric proton signals. From these chemical and spectral data, **10** was assigned as (+)-catechin 5,4′-di-*O*-β-D-glucopyranoside.



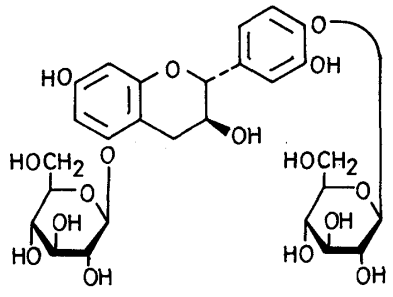
7 : R=H
7a : R=CH_3



8 : R=H
8a : R=CH_3



9 : R=H
9a : R=CH_3



10

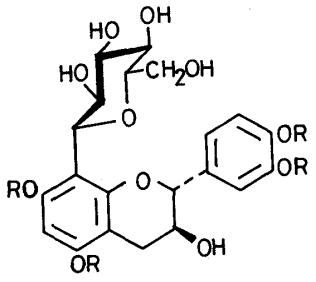
Chart 6

Compound **11**, a white amorphous powder, $[\alpha]_D$ −60.2° (MeOH), $C_{21}H_{24}O_{11}\cdot H_2O$, and compound **12**, a white amorphous powder, $[\alpha]_D$ +34.8° (MeOH), $C_{21}H_{24}O_{11}\cdot 2H_2O$, showed almost the same *Rf* values and coloring reactions on thin-layer chromatography (TLC) as those of **1**, **2**, **5** and **6**. The ^{1}H-NMR spectra of **11** and **12** showed the occurrence of a catechin and a sugar moiety in each molecule. The observation of one aromatic singlet (δ 6.08 in **11**; δ 5.96 in **12**), assignable to H-6 or H-8, suggested that the C-8 or C-6 position was substituted. The ^{13}C-NMR spectra showed, in addition to fifteen carbon signals arising from the catechin nucleus, six aliphatic signals (Table II), whose chemical shifts were consistent with those of the

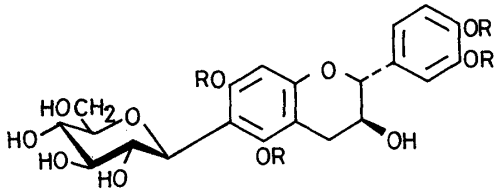
sugar moiety in *C*-glucosides such as mangiferin,[14] aloesin,[15] *etc.* The occurrence of the *C*-glucoside moiety was further confirmed by oxidative degradation with ferric chloride, which liberated arabinose and glucose.

On ordinary phenol methylation, **11** and **12** yielded tetramethyl ethers **11a** and **12a**, respectively, which showed the same molecular ion peak at *m/z* 508 in the FD-MS. In the ^{13}C-NMR spectrum of **11a**, signals due to C-6, C-4a and C-8 were observed at δ 89.6, 103.1 and 111.5, respectively, the chemical shifts being in agreement with those[16] (C-6, δ 88.6; C-4a, δ 102.5; C-8, δ 112.2) found in the C-8 substituted catechin derivative, gambiriin A-1 nonamethyl ether.[17] In the case of **12a**, the carbon resonances due to C-6, C-4a and C-8 appeared at δ 120.8, 107.4 and 97.3, respectively, consistent with C-6 substitution (*i.e.* gambiriin A-3 nonamethyl ether[17]; C-6, δ 117.7; C-4a, δ 108.4; C-8, δ 96.1). Furthermore, the ^{1}H-NMR spectra of **11** and **12** showed signals due to 2-H at δ 4.74 and 4.60, respectively, and the chemical shift of the latter was almost identical with that of (+)-catechin (δ 4.57), indicating that the glucosyl substituent did not influence 2-H. On the other hand, the chemical shift of 2-H in **11** was observed at lower field than that of (+)-catechin, indicating that the substituent is situated close to C-2.[18] Based on these observations, the glucosyl moieties in **11** and **12** were concluded to be attached to the C-8 and C-6 positions, respectively, of the catechin moiety. The β-linkage of the glucosyl moiety in each case was confirmed by examinations of the ^{1}H-NMR spectra of **11** and **12**, in which sugar anomeric proton signals appeared at δ 4.84 and 4.88 with large coupling constants (J = 10 Hz in each case).

In order to establish the absolute stereostructures of **11** and **12**, an attempt was made to prepare (+)-catechin *C*-glucosides by acid-catalyzed condensation[19] of (+)-catechin and D-glucose. Treatment of the mixture in dry dioxane in the presence of *p*-toluenesulfonic acid, followed by separation on Sephadex LH-20 (H_2O) afforded two products which were identical with **11** and **12** in respect of their specific rotation values and ^{1}H-NMR data. On the basis of the chemical and spectral evidence described above, **11** and **12** were determined to be (+)-catechin 8-*C*-β-D-glucopyranoside and (+)-catechin 6-*C*-β-D-glucopyranoside, respectively.



11: R = H
11a: R = CH_3



12: R = H
12a: R = CH_3

Chart 7

Compound **13**, a tan amorphous powder, $[\alpha]_D$ −197.5° (MeOH), $C_{36}H_{36}O_{17}\cdot H_2O$, showed a peak at *m/z* 741 due to $[M+H]^+$ in the fast atom bombardment mass spectrum (FAB-MS). The ^{1}H-NMR spectrum of **13** was complicated by conformational isomerism, and provided no information on the structure. However, the ^{13}C-NMR spectrum showed signals arising from the major conformer, and its signal pattern was closely related to that of procyanidin B-3. It also showed six carbon signals at δ 61.8, 70.7, 74.1, 76.5, 77.0 and 100.9 due to a sugar moiety, the chemical shifts being consistent with those of methyl-β-D-glucoside. Enzymatic hydrolysis with crude hesperidinase yielded glucose and an aglycone, which was found to be identical with procyanidin B-3. On acid-catalyzed degradation with benzylmercaptan, **13** gave (+)-catechin and two benzylthioethers **13a** and **13b**, the structures of

the latter two compounds being confirmed by ^{1}H-NMR examination and by their conversion with Raney nickel to **2**. From these chemical and spectral data, **13** was characterized as procyanidin B-3 7-*O*-*β*-D-glucopyranoside.

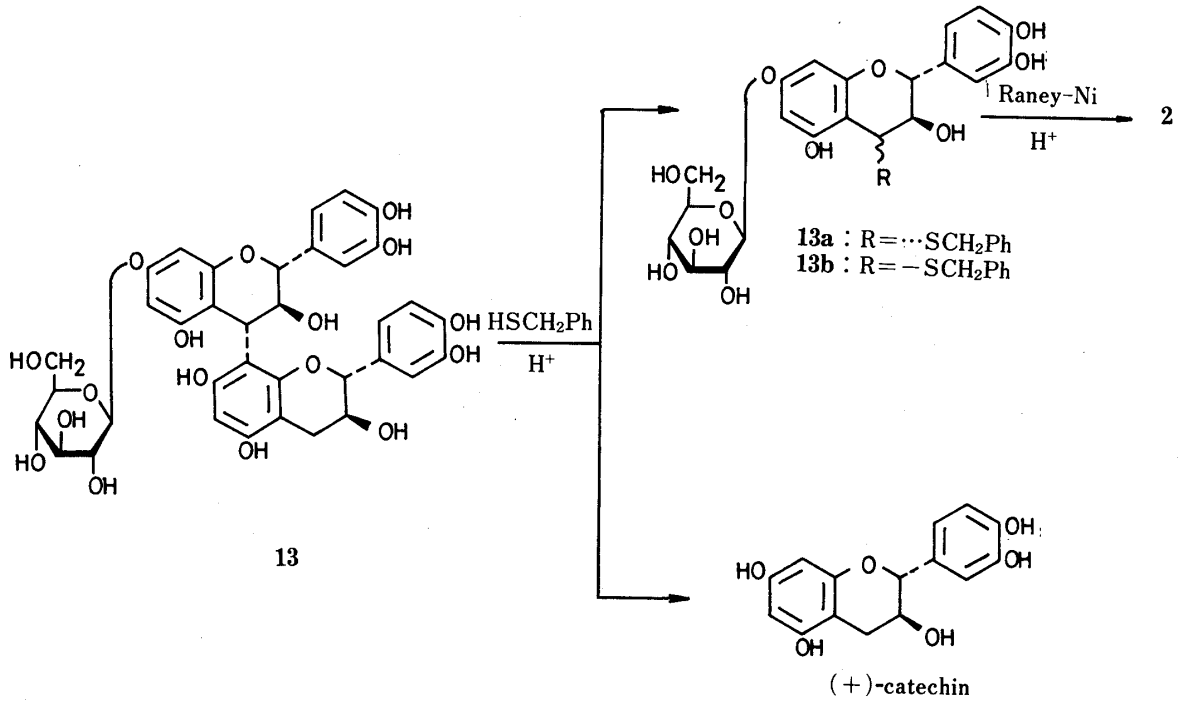


Chart 8

Compound **14**, a tan amorphous powder, $[\alpha]_d$ +33.7° (MeOH), $C_{36}H_{36}O_{17}\cdot 3/2H_2O$, and compound **15**, a tan amorphous powder, $[\alpha]_D$ +45.1° (MeOH), $C_{36}H_{36}O_{17}\cdot H_2O$, showed in the FAB-MS a peak at *m/z* 741 due to $[M+H]^+$, which was identical with that observed in **13**. The ^{13}C-NMR spectra of **14** and **15** exhibited two sets of C-2 signals (δ 76.9 and 81.3 in **14**; δ 77.0 and 81.9 in **15**), indicative of the presence of epicatechin and catechin moieties in each molecule. It also showed six aliphatic carbon signals analogous to those observed in **11** and **12**, consistent with the presence of a *C*-glucosyl moiety in each molecule. Thiolytic degradation of **14** gave (+)-catechin and the thioether **14a**, while that of **15** afforded (+)-catechin and **15a**. Compounds **14a** and **15a** were identified as 8-*C*-*β*-D-glucopyranosyl (−)-epicatechin 4-*β*-benzylthioether and 6-*C*-*β*-D-glucopyranosyl (−)-epicatechin 4-*β*-benzylthioether, respectively, which were previously obtained by similar thiolytic degradation of **3** and **4**.[10]

The ^{1}H-NMR spectra of **14** and **15** were closely related to that of procyanidin B-1. In particular, the chemical shift of 2-H in the catechin moiety (δ 4.75 in **14**: δ 4.70 in **15**) was consistent with that (δ 4.76) of procyanidin B-1 rather than that (δ 4.52) of procyanidin B-7,[18a] which is the C(4)–C(6) linked dimer. Thus, the location of the interflavonoid linkage was concluded to be C(4)–C(8) in each case. On the basis of these observations, **14** and **15** were assigned as procyanidin B-1 8-*C*-*β*-D-glucopyranoside and procyanidin B-1 6-*C*-*β*-D-glucopyranoside, respectively.

This is the first example of the isolation of flavan-3-ol diglucosides and *C*-glucosides from a natural source. We previously reported the isolation of (+)-catechin 5-*O*-*β*-D-glucopyranoside from Gao (雅黃), but the occurrence of a homologous series of glucosides of (+)-catechin and procyanidins in the high-quality rhubarb, Choukichio (長吉黃), is in marked contrast to the cases of other commercial rhubarbs. It would be of interest to know the plant of origin of this rhubarb.

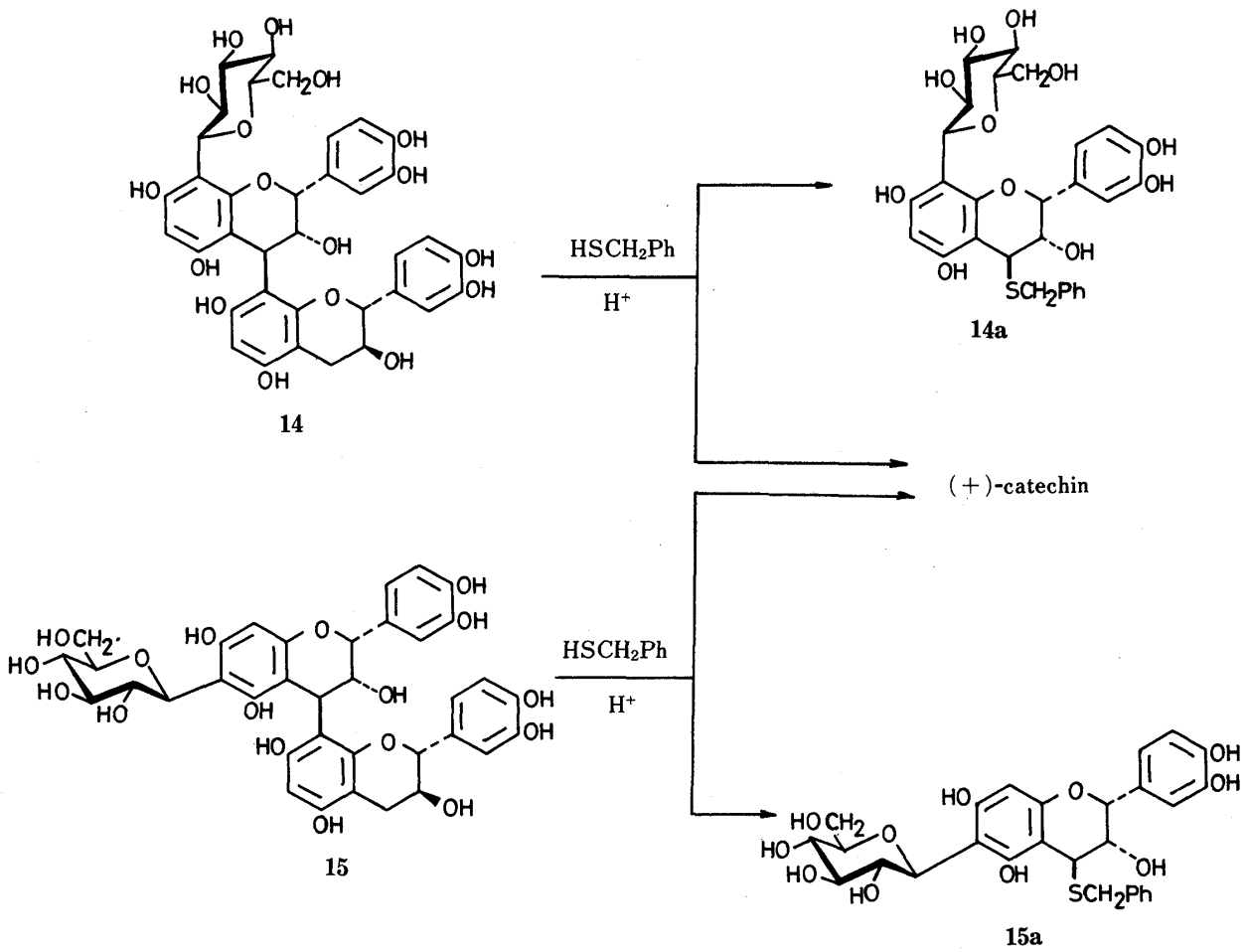


Chart 9

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. The EI-MS were obtained with a JEOL JMS D-300 instrument, and FD- and FAB-MS with a JEOL JMS DX-300 instrument. The ^{1}H- and ^{13}C-NMR spectra were measured with JEOL PS-100 and FX-100 spectrometers, respectively, using tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Column chromatography was performed with Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.), Fuji-gel ODS-G3 (43—65 μ, Fuji Gel Hanbai Co., Ltd.) and Bondapak C_{18}/Porasil B (35—75 μ, Waters Associates, Inc.). TLC was conducted on precoated Kieselgel 60 F_{254} (0.20 mm, Merck) and Avicel SF cellulose plates (Funakoshi), and spots were detected by ultraviolet (UV) illumination and by spraying 2% ethanolic ferric chloride, 10% sulfuric acid, anisaldehyde–sulfuric acid and aniline–hydrogen phthalate reagents. High peformance liquid chromatography (HPLC) was conducted on a Toyo Soda apparatus equipped with an SP-8700 solvent delivery system and a UV-8 model II spectrophotometer.

Isolation of Compounds 1—15——Rhubarb (長吉黄) (3.2 kg) purchased at a market in Hong Kong was powdered and extracted five times with 80% aqueous acetone at room temperature. The extracts, after removal of the solvent by evaporation, were subjected to chromatography over Sephadex LH-20 with H_2O containing increasing amounts of MeOH (1 : 0—0 : 1) to give six fractions (I—VI). Fractions I, II (150 g) and III (80 g) consisted of lower-molecular-weight phenolics. Fractions II and III were separately rechromatographed over MCI-gel CHP 20P [solvent: H_2O–MeOH (1 : 0—0 : 1)] to afford further five fractions (II-1—II-5) and three fractions (III-1—III-3), respectively. Fraction II-3 was subsequently chromatographed over Sephadex LH-20 [solvent: H_2O–MeOH (2 : 3)] to afford two fractions (II-3a, II-3b). Fraction II-3a was purified by chromatography over MCI-gel CHP 20P [solvent: H_2O–MeOH (1 : 0—4 : 1)] to furnish **11** (150 mg). Fraction II-3b, consisting of a mixture of flavanoid diglucosides, was separated by chromatography over Bondapak C_{18} [solvent: H_2O–MeOH (9 : 1)] to give **7** (25 mg), **8** (20 mg), **9** (15 mg) and **10** (6 mg). Fraction II-4 was further fractionated by Sephadex LH-20 chromatography [solvent: H_2O–MeOH (1 : 0—4 : 1)] into three fractions (II-4a—II-4c). Fractions II-4a and II-4b were separately purified by chromatography

over Bondapak C_{18} [solvent: H_2O–MeOH (9 : 1)] to give **11** (60 mg) and **12** (50 mg), respectively. Fraction II-4c was further chromatographed over MCI-gel CHP 20P [solvent: H_2O–MeOH (1 : 0—4 : 1)] and Bondapak C_{18} [solvent: H_2O–MeOH (9 : 1)] to afford (+)-catechin 5-*O*-*β*-D-glucopyranoside (**1**) (55 mg) and **5** (45 mg). Fraction III-1 was fractionated further by chromatography over Sephadex LH-20 (EtOH) to give two fractions (III-1a and III-1b). Repeated chromatography of fraction III-1a over Sephadex LH-20 [solvent: H_2O–MeOH (1 : 0—4 : 1)] and Fuji-gel ODS-G3 [solvent: H_2O–MeOH (9 : 1)] gave **1** (300 mg), **6** (82 mg) and **5** (150 mg), and (+)-catechin 7-*O*-*β*-D-glucopyranoside (**2**) (50 mg). Fraction III-1b, consisting of a mixture of procyanidin glucosides, was repeatedly chromatographed over Sephadex LH-20 [solvents: H_2O–MeOH (1 : 0—4 : 1) and EtOH] and Bondapak C_{18} [solvent: H_2O–MeOH (9 : 1)] to yield **13** (45 mg), **14** (38 mg), and **15** (30 mg), as well as procyanidin B-2 8-*C*-*β*-D-glucopyranoside (**3**) (10 mg) and procyanidin B-2 6-*C*-*β*-D-glucopyranoside (**4**) (8 mg).

Compound 5——A white amorphous powder, $[\alpha]_D^{23}$ −39.3° (*c* = 0.6, MeOH). *Anal.* Calcd for $C_{21}H_{24}O_{11} \cdot H_2O$: C, 53.61; H, 5.57. Found: C, 53.46; H, 5.82. FD-MS *m/z*: 491 $[M+K]^+$, 475 $[M+Na]^+$, 453 $[M+H]^+$, 290 $[M+H-glc.]^+$, 163. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Enzymatic Hydrolysis of 5——An aqueous solution of **5** (20 mg) was incubated with crude hesperidinase at room temperature for 2 h. The solution was concentrated to dryness under reduced pressure, and the residue was treated with EtOH. The EtOH-soluble portion was subjected to chromatography over Sephadex LH-20. Elution with EtOH afforded D-glucose [*Rf* 0.35; solvent, *n*-BuOH–pyridine–H_2O (6 : 4 : 3)], $[\alpha]_D^{24}$ +48.4° (*c* = 0.39, H_2O). Further elution with EtOH furnished an aglycone (12 mg) as colorless needles, mp 150—151 °C, $[\alpha]_D^{24}$ +10.8° (*c* = 0.97, acetone). This product was shown to be identical wih (+)-catechin by physical and spectral comparisons.

Methylation of 5——A mixture of **5** (20 mg), dimethyl sulfate (0.2 ml) and anhydrous potassium carbonate (300 mg) in dry acetone (6 ml) was refluxed for 2.5 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated. The residue was chromatographed over silica gel using $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1) to yield the trimethyl ether (**5a**) (18 mg) as colorless needles (dil. MeOH), mp 167—168 °C, $[\alpha]_D^{26}$ −53.5° [*c* = 0.61, acetone–H_2O (1 : 1)]. *Anal.* Calcd for $C_{21}H_{30}O_{11} \cdot 1/2H_2O$: C, 57.25; H, 6.21. Found: C, 57.20; H, 6.29. FD-MS *m/z*: 494 $[M]^+$, 332 $[M+H-glc.]^+$, 163. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.42 (1H, dd, *J* = 8, 16 Hz, 4-H), 2.70 (1H, dd, *J* = 5, 16 Hz, 4-H), 3.1—3.9 (6H, m, sugar-H), 3.68, 3.72, 3.74 (each 3H, s, OCH_3), 4.00 (1H, m, 3-H), 4.68 (1H, d, *J* = 8 Hz, 2-H), 4.88 (1H, d, *J* = 8 Hz, anomeric-H), 6.05, 6.12 (each 1H, d, *J* = 2 Hz, 6, 8-H), 6.92—7.18 (3H, m, B-ring-H). ^{13}C-NMR (DMSO-d_6 + D_2O): 27.0 (C-4), 55.0, 55.3, 55.6 (OCH_3), 60.1 (glc. C-6), 65.5 (C-3), 69.2 (glc. C-4), 72.8 (glc. C-2), 76.5 (glc. C-5), 76.7 (glc. C-3), 80.6 (C-2), 91.1 (C-6), 93.0 (C-8), 99.7 (C-4a), 101.3 (glc. C-1), 112.1 (C-2′), 113.9 (C-5′), 131.6 (C-1′), 145.8, 148.3 (C-3′, 4′), 154.8 (C-8a), 158.1, 159.0 (C-5, 7).

Enzymatic Hydrolysis of 5a——A mixture of **5a** (15 mg) and crude hesperidinase in DMSO–H_2O (1 : 1) (5 ml) was incubated at 37 °C overnight. The reaction mixture was diluted with H_2O, and the resulting white precipitate was collected by filtration. The white powder was treated with MeOH, and the MeOH-soluble portion was crystallized from dil. MeOH to give the aglycone **5b** (9 mg), colorless needles, mp 154—155 °C, $[\alpha]_D^{26}$ −2.9° (*c* = 0.54, acetone). *Anal.* Calcd for $C_{18}H_{20}O_6$: C, 65.04; H, 6.06. Found: C, 65.13; H, 6.30. EI-MS *m/z*: 332 $[M]^+$, 167. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.40 (1H, dd, *J* = 8, 16 Hz, 4-H), 2.69 (1H, dd, *J* = 5, 16 Hz, 4-H), 3.69, 3.74, 3.75 (each 3H, s, OCH_3), 3.90 (1H, m, 3-H), 4.62 (1H, d, *J* = 2 Hz, 6, 8-H), 6.70—6.95 (3H, m, B-ring-H).

Acetylation of 5b——**5b** (10 mg) was treated with acetic anhydride (0.5 ml) and dry pyridine (0.5 ml) at room temperature for 2 h. Excess reagents were removed by blowing N_2, and the residue was purified by silica gel chromatography [solvent: *n*-hexane–AcOEt (5 : 1)] to afford the diacetate **5c** (6 mg), colorless needles (benzene), mp 112—113 °C, $[\alpha]_D^{22}$ −5.2° (*c* = 0.27, acetone). *Anal.* Calcd for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.36; H, 5.81. EI-MS *m/z*: 416 $[M]^+$, 356 $[M-AcOH]^+$, 314, 208, 167, 166. ^{1}H-NMR ($CDCl_3$): 1.96, 2.28 (each 3H, s, OAc), 2.65 (1H, dd, *J* = 8, 16 Hz, 4-H), 2.90 (1H, dd, *J* = 6, 16 Hz, 4-H), 3.77 (6H, s, OCH_3), 3.82 (3H, s, OCH_3), 5.02 (1H, d, *J* = 7 Hz, 2-H), 5.30 (1H, m, 3-H), 6.09, 6.16 (each 1H, d, *J* = 2 Hz, 6, 8-H), 6.91 (1H, d, *J* = 8 Hz, 5′-H), 7.01 (1H, d, *J* = 2 Hz, 2′-H), 7.21 (1H, dd, *J* = 2, 8 Hz, 6′-H).

Compound 6——A white amorphous powder, $[\alpha]_D^{22}$ −34.0° (*c* = 0.59, MeOH). *Anal.* Calcd for $C_{21}H_{24}O_{11} \cdot H_2O$: C, 53.61; H, 5.57. Found: C, 53.43; H, 5.51. FD-MS *m/z*: 491 $[M+K]^+$, 475 $[M+Na]^+$, 453 $[M+H]^+$, 290 $[M+H-glc.]^+$, 163. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Enzymatic Hydrolysis of 6——An aqueous solution of **6** (20 mg) was shaken with crude hesperidinase at room temperature for 2 h. The reaction mixture was treated in the same way as described above to furnish (+)-catechin and D-glucose [*Rf* 0.35; solvent, *n*-BuOH–pyridine–H_2O (6 : 4 : 3)], $[\alpha]_D^{24}$ +46.2° (*c* = 0.4, H_2O).

Methylation of 6——A mixture of **6** (50 mg), dimethyl sulfate (0.35 ml) and anhydrous potassium carbonate (500 mg) in dry acetone (15 ml) was refluxed for 4 h. The reaction mixture was worked up in the same way as described for **5** to afford the trimethyl ether **6a** (34 mg) as colorless needles ($CHCl_3$–MeOH), mp 199—200 °C, $[\alpha]_D^{22}$ −49.7° (*c* = 0.37, MeOH). *Anal.* Calcd for $C_{24}H_{30}O_{11} \cdot H_2O$: C, 56.24; H, 6.29. Found: C, 55.88; H, 6.38. FD-MS *m/z*: 517 $[M+Na]^+$, 494 $[M]^+$, 331 $[M-glc.]^+$, 163. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.42 (1H, dd, *J* = 8, 16 Hz, 4-H), 2.75 (1H, dd, *J* = 6, 16 Hz, 4-H), 3.1—3.9 (6H, m, sugar-H), 3.76 (3H, s, OCH_3), 3.74 (6H, s, OCH_3), 3.96 (1H, m, 3-H), 4.68 (1H, d, *J* = 8 Hz, 2-H), 4.90 (1H, d, *J* = 7 Hz, anomeric-H), 6.04, 6.24 (each 1H, d, *J* = 2 Hz, 6, 8-H), 6.86 (1H, dd, *J* = 2, 8 Hz, 6′-H), 6.98 (1H, d, *J* = 2 Hz, 2′-H), 7.07 (1H, d, *J* = 8 Hz, 5′-H). ^{13}C-NMR (DMSO-d_6 + D_2O): 27.7 (C-4), 55.1, 55.3, 55.6 (OCH_3), 60.5 (glc. C-6), 65.6 (C-3), 69.5 (glc. C-4), 73.0 (glc. C-2), 76.4 (glc. C-5), 76.7 (glc. C-3), 80.9

(C-2), 91.2 (C-6), 93.0 (C-8), 100.0 (C-4a), 101. 4(glc. C-1), 111.6 (C-2′), 114.9 (C-5′), 119.5 (C-6′), 132.9 (C-1′), 146.0 (C-3′), 148.4 (C-4′), 154.9 (C-8a), 158.1, 159.1 (C-5, 7).

Enzymatic Hydrolysis of 6a——**6a** (25 mg) in DMSO–H_2O (1 : 1) (10 ml) was shaken with crude hesperidinase at room temperature for 4 h. The reaction mixture was treated in the same way as described for **5a**. The products were purified by chromatography over silica gel [solvent: benzene–acetone (6 : 1)] to give the aglycone **6b** (14 mg) as colorless needles (*n*-hexane–AcOEt), mp 153—154 °C, $[\alpha]_D^{24}$ −9.9° (*c*=0.41, MeOH). *Anal.* Calcd for $C_{18}H_{20}O_{16}$: C, 65.04; H, 6.06. Found: C, 64.98; H, 6.05. EI-MS *m/z*: 332 $[M]^+$, 167. ^{1}H-NMR (acetone-d_6 + D_2O): 2.52 (1H, dd, *J*= 8, 16 Hz, 4-H), 2.92 (1H, dd, *J*=6, 16 Hz, 4-H), 3.74 (3H, s, OCH_3), 3.85 (6H, s, OCH_3), 4.04 (1H, m, 3-H), 4.64 (1H, d, *J*=8 Hz, 2-H), 6.04, 6.15 (each 1H, d, *J*=2 Hz, 6, 8-H), 6.80 (1H, d, *J*=8 Hz, 5′-H), 6.90 (1H, dd, *J*=2, 8 Hz, 6′-H), 7.02 (1H, d, *J*=2 Hz, 2′-H).

Compound 7——A white amorphous powder, $[\alpha]_D^{29}$ −67.4° (*c*=0.63, MeOH). *Anal.* Calcd for $C_{27}H_{34}O_{16}\cdot 3/2H_2O$: C, 50.54; H, 5.81. Found: C, 50.52; H, 5.81. FD-MS *m/z*: 637 $[M+Na]^+$, 615 $[M+H]^+$, 452 $[M+H-glc.]^+$, 290 $[M+2H-2\times glc.]^+$. ^{1}H-NMR: Table I. ^{14}C-NMR: Table II.

Enzymatic Hydrolysis of 7——An aqueous solution of **7** (7 mg) was treated with crude hesperidinase at room temperature for 2 h. The reaction mixture was worked up as described for **5** to afford D-glucose and (+)-catechin.

Methylation of 7——A solution of **7** (15 mg) in MeOH was treated with an ethereal solution of CH_2N_2 at room temperture for 2 h. The solvent was evaporated off, and the residue was purified by silica gel chromatography [solvent: $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2—10 : 3 : 0.3)] to furnish the dimethyl ether **7a** (7 mg) as a white amorphous powder, $[\alpha]_D^{24}$ −46.1° (*c*=0.8, MeOH). FD-MS *m/z*: 643 $[M+H]^+$, 481 $[M+2H-glc.]^+$, 319 $[M+3H-2\times glc.]^+$. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.3—2.8 (2H, m, 4-H), 3.1—3.9 (12H, m, sugar-H), 3.74, 3.76 (each 3H, s, OCH_3), 4.00 (1H, m, 3-H), 4.68 (1H, d, *J*=8 Hz, 2-H), 4.84, 4.70 (each 1H, d, *J*=8 Hz, anomeric-H), 6.16, 6.27 (each 1H, d, *J*= 2 Hz, 6, 8-H), 6.92—7.08 (3H, m, B-ring-H).

Enzymatic Hydrolysis of 7a——An aqueous solution of **7a** (7 mg) was treated with crude hesperidinase overnight at room temperature. The reaction mixture was worked up as described for **5** to yield the aglycone **7b** (2.5 mg) as a white amorphous powder, $[\alpha]_D^{19}$ −3.9° (*c*=0.41, MeOH). EI-MS *m/z*: 318 $(M)^+$, 166, 153. ^{1}H-NMR (acetone-d_6): 2.50 (1H, dd, *J*=8, 16 Hz, 4-H), 2.89 (1H, dd, *J*=6, 16 Hz, 4-H), 3.76, 3.84 (each 3H, s, OCH_3), 4.00 (1H, m, 3-H), 4.64 (1H, d, *J*=8 Hz, 2-H), 6.00, 6.10 (each 1H, d, *J*=2 Hz, 6, 8-H), 6.90—7.10 (3H, m, B-ring-H).

Acetylation of 7b——**7b** (2 mg) was acetylated with acetic anhydride (0.5 ml) and dry pyridine (0.5 ml) at room temperature for 2 h. Work-up as described for **5b** gave the triacetate **7c** (1.5 mg) as a white amorphous powder. EI-MS *m/z*: 444 $[M]^+$, 384 $[M-AcOH]^+$, 342, 300, 250, 208, 195, 166, 153. ^{1}H-NMR ($CDCl_3$): 1.98 (3H, s, OAc), 2.30 (6H, s, 2 × OAc), 2.68 (1H, dd, *J*=8,16 Hz, 4-H), 2.91 (1H, dd, *J*=6, 16 Hz, 4-H), 3.77, 3.82 (each 3H, s, OCH_3), 5.04 (1H, d, *J*=8 Hz, 2-H), 5.30 (1H, m, 3-H), 6.22, 6.36 (each 1H, d, *J*=2 Hz, 6, 8-H), 6.92 (1H, d, *J*=8 Hz, 5′-H), 7.02 (1H, d, *J*=2 Hz, 2′-H), 7.16 (1H, dd, *J*=2, 8 Hz, 6′-H).

Compound 8——A white amorphous powder, $[\alpha]_D^{29}$ −48.2° (*c*=0.53, MeOH). *Anal.* Calcd for $C_{27}H_{34}O_{16}\cdot 3/2H_2O$: C, 50.54; H, 5.81. Found: C, 50.32; H, 5.69. FD-MS *m/z*: 637 $[M+Na]^+$, 452 $[M+H-glc.]^+$, 290 $[M+2H-2\times glc.]^+$, 163. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Enzymatic Hydrolysis of 8——**8** (5 mg) was hydrolyzed with crude hesperidinase in the same way as before to afford (+)-catechin and glucose.

Methylation of 8——**8** (13 mg) was methylated with an ethereal solution of CH_2N_2 in the same manner as described for **7**. Chromatography of the reaction mixture over silica gel [solvent: $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2—10 : 3 : 0.3)] yielded the dimethyl ether **8a** (6 mg) as a white amorphous powder, $[\alpha]_D^{24}$ −39.0° (*c*=0.66, MeOH). FD-MS *m/z*: 642 $[M]^+$, 480 $[M+H-glc.]^+$, 318 $[M+2H-2\times glc.]^+$, 163. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.2—2.8 (2H, m, 4-H), 3.1—3.9 (12H, m, sugar-H), 3.96 (1H, m, 3-H), 4.68 (1H, d, *J*=8 Hz, 2-H), 4.80, 4.93 (each 1H, d, *J*=8 Hz, anomeric-H), 6.11, 6.32 (each 1H, d, *J*=2 Hz, 6, 8-H), 6.92—7.12 (3H, m, B-ring-H).

Enzymatic Hydrolysis of 8a——**8a** (6 mg) was treated with crude hesperidinase overnight at room temperature. The reaction mixture was worked up as described for **7a** to give the aglycone **8b** (2.5 mg), which was shown to be identical with (+)-catechin 7,4′-di-*O*-methylate by direct comparison with an authentic sample.

Compound 9——A white amorphous powder, $[\alpha]_D^{29}$ −48.2° (*c*=0.53, MeOH). *Anal.* Calcd for $C_{27}H_{34}O_{16}\cdot 3/2H_2O$: C, 50.54; H, 5.81. Found: C, 50.57; H, 5.58. FD-MS *m/z*: 637 $[M+Na]^+$, 452 $[M+H-glc.]^+$, 290 $[M+2H-2\times glc.]^+$, 163. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Enzymatic Hydrolysis of 9——**9** (5 mg) was hydrolyzed with crude hesperidinase in the same manner as described above to yield (+)-catechin and glucose.

Methylation of 9——**9** (10 mg) was methylated in the same way as described for **7**. Similar work-up furnished the dimethyl ether **9a** (4.5 mg) as a white amorphous powder, $[\alpha]_D^{24}$ −37.4° (*c*=0.5, MeOH). FD-MS *m/z*: 642 $[M]^+$, 480 $[M+H-glc]^+$, 318 $[M+2H-2\times glc.]^+$, 163. ^{1}H-NMR (DMSO-d_6 + D_2O): 2.4—2.8 (2H, m, 4-H), 3.1—3.9 (12H, m, sugar-H), 3.67, 3.74 (each 3H, s, OCH_3), 4.00 (1H, m, 3-H), 4.71 (1H, d, 2-H), 4.81, 4.83 (each 1H, d, *J*=8 Hz, anomeric-H), 6.07, 6.15 (each 1H, d, *J*=2 Hz, 6, 8-H), 6.92—7.21 (3H, m, B-ring-H).

Enzymatic Hydrolysis of 9a——**9a** (4 mg) was treated with crude hesperidinase overnight at room temperature. Work-up as described for **7a** gave the aglycone **9b** (1 mg), which was shown to be identical with synthetic 5,7-di-*O*-methyl (+)-catechin by direct comparison.

Preparation of 5,7-di-*O*-Methyl (+)-Catechin——A mixture of 4 M sodium metaborate (3.5 ml), dimethyl sulfate (0.7 ml) and 2.5 N sodium hydroxide (2.8 ml) was added dropwise to an aqueous solution of (+)-catechin (1 g) with stirring at room temperature during 15 min. The reaction mixture was diluted with ice-water and acidified with 5% HCl. The acidic solution was extracted three times with AcOEt. The AcOEt layer, after removal of the solvent, was subjected to chromatography over MCI-gel CHP 20P [solvent: H_2O–MeOH (3:2—0:1)] to afford 5,7-di-*O*-methyl (+)-catechin (430 mg) as colorless needles (dil. MeOH), mp 218—219 °C, $[\alpha]_D^{24}$ −2.0° (c=1.0, acetone). EI-MS m/z: 318 $[M]^+$, 167, 152. ^{1}H-NMR (acetone-d_6 + D_2O): 2.50 (1H, dd, J=8, 16 Hz, 4-H), 2.88 (1H, dd, J=6, 16 Hz, 4-H), 3.72, 3.77 (each 3H, s, OCH_3), 4.00 (1H, m, 3-H), 4.59 (1H, d, J=8 Hz, 2-H), 6.02, 6.12 (each 1H, d, J=2 Hz, 6, 8-H), 6.68—6.94 (3H, m, B-ring-H).

Compound 10——A white amorphous powder, $[\alpha]_D^{18}$ −46.8° (c=0.19, MeOH). *Anal.* Calcd for $C_{27}H_{34}O_{16}\cdot 7/2H_2O$: C, 47.86; H, 6.10. Found: C, 48.00; H, 6.09. FD-MS m/z: 637 $[M+Na]^+$, 615 $[M+H]^+$, 475 $[M+Na+2H-glc.]^+$, 453 $[M+2H-glc.]^+$, 290 $[M+2H-2\times glc.]^+$, 163. ^{1}H-NMR: Table I.

Enzymatic Hyrolysis of 10——An aqueous solution of **10** (2 mg) was treated with crude hesperidinase at room temperature for 2 h. The solvent was evaporated off under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was directly analyzed by TLC to detect catechin [Rf 0.71, solvent: benzene–ethyl formate–formic acid (2:7:1)] and glucose [Rf 0.35, solvent: n-BuOH–pyridine–H_2O (6:4:3)].

Compound 11——A white amorphous powder, $[\alpha]_D^{23}$ −60.2° (c=0.64, MeOH). *Anal.* Calcd for $C_{21}H_{24}O_{11}\cdot H_2O$: C, 53.61; H, 5.57. Found: C, 53.61; H, 5.67. FD-MS m/z: 453 $[M+H]^+$, 434 $[M-H_2O]^+$. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Degradation of 11——A mixture of **11** (10 mg) and 10% aqueous $FeCl_3$ (1.5 ml) was heated on a water bath for 5 h. The reaction mixture was diluted with water, neutralized with Amberlite MB-3, and concentrated to dryness. The residue was analyzed by Avicel cellulose TLC [n-BuOH–pyridine–H_2O (6:4:3)] to detect glucose (Rf 0.35) and arabinose (Rf 0.50).

Methylation of 11——A mixture of **11** (50 mg), dimethyl sulfate (0.35 ml) and anhydrous potassium carbonate (500 mg) in dry acetone (10 ml) was refluxed for 4.5 h. The reaction mixture was treated in the same manner as described for **5**. The products were purified by chromatography over silica gel [solvent: AcOEt–acetone–H_2O (10:3:0.3)] to give the tetramethyl ether **11a** (34 mg) as a white amorphous powder, $[\alpha]_D^{23}$ −64.9° (c=0.76, MeOH). *Anal.* Calcd for $C_{25}H_{32}O_{11}\cdot 3/2H_2O$: C, 56.07; H, 6.59. Found: C, 55.60; H, 6.46. FD-MS m/z: 508 $[M]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.54 (1H, dd, J=8, 16 Hz, 4-H), 2.79 (1H, dd, J=5, 16 Hz, 4-H), 3.77, 3.80, 3.82 (12H in total, 4 × OCH_3), 3.2—4.4 (7H, m, sugar-H and 3-H), 4.83 (2H, d, J=7 Hz, 2-H and anomeric-H), 6.29 (1H, s, 6-H), 6.82—7.16 (3H, m, B-ring-H). ^{13}C-NMR (acetone-d_6 + D_2O): 27.8 (C-4), 55.8, 56.0, 56.3, 56.9 (OCH_3), 63.3 (glc. C-6), 67.4 (C-3), 71.5 (glc. C-4), 72.0 (glc. C-2), 74.8 (glc. C-1), 79.5 (glc. C-5), 81.4 (C-2), 89.6 (C-6), 103.1 (C-4a), 111.5 (C-8), 112.3 (C-2′, 5′), 120.1 (C-6′), 132.9 (C-1′), 149.6, 149.8 (C-3′, 4′), 155.2 (C-8a), 159.0, 159.2 (C-5, 7).

Compound 12——A white amorphous powder, $[\alpha]_D^{23}$ +34.8° (c=0.61, MeOH). *Anal.* Calcd for $C_{21}H_{24}O_{11}\cdot 2H_2O$: C, 51.64; H, 5.78. Found: C, 51.69; H, 5.38. FD-MS m/z: 452 $[M]^+$, 434 $[M-H_2O]^+$, 290. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Degradation of 12——A mixture of **12** (10 mg) and 10% aqueous $FeCl_3$ (1.5 ml) was heated on a water bath for 5 h. The reaction mixture was worked up as described for **11**, and glucose and arabinose were detected by TLC analysis [Rf 0.35 (glucose), 0.50 (arabinose), solvent: n-BuOH–pyridine–H_2O (6:4:3)].

Methylation of 12——A mixture of **12** (23 mg), dimethyl sulfate (0.16 ml) and anhydrous potassium carbonate (230 mg) in dry acetone (6 ml) was refluxed for 6 h with stirring. The reaction mixture was worked up in the same manner as described for **11** to furnish the tetramethyl ether **12a** (15 mg), as colorless needles, mp 135—136 °C, $[\alpha]_D^{26}$ +34.3° (c=0.64, acetone). *Anal.* Calcd for $C_{25}H_{32}O_{11}\cdot 1/2H_2O$: C, 58.02; H, 6.43. Found: C, 58.15; H, 6.54. FD-MS m/z: 508 $[M]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.69 (1H, dd, J=8, 16 Hz, 4-H), 2.9—3.1 (1H, m, 4-H), 3.3—4.0 (6H, m, sugar-H), 4.10 (1H, m, 3-H), 4.5—4.9 (2H, m, 2-H and anomeric-H), 6.28 (1H, s, 8-H), 6.9—7.1 (3H, m, B-ring-H). ^{13}C-NMR (acetone-d_6 + D_2O): 28.7 (C-4), 56.2 (4C) (OCH_3), 62.0 (glc. C-6), 67.7 (C-3), 71.5 (glc. C-4), 80.0 (glc. C-1), 81.7 (glc. C-5), 82.6 (C-2), 97.3 (C-8), 107.4 (C-4a), 112.3 (C-2′, 5′), 120.8 (C-6, 6′), 132.6 (C-1′), 149.9 (C-3′, 4′), 156.4 (C-8a), 159.8 (C-5, 7).

Condensation of (+)-Catechin and D-Glucose——A mixture of (+)-catechin (870 mg) and D-glucose in dry dioxane (20 ml) containing p-toluenesulfonic acid (70 mg) was heated at 80 °C for 10 h with stirring. The solvent was evaporated off under reduced pressure, and the residue was chromatographed over Sephadex LH-20 (solvent: H_2O). Rechromatography over Sephadex LH-20 (solvent: H_2O) gave **11** (28 mg) and **12** (23 mg).

Compound 13——A tan amorphous powder, $[\alpha]_D^{19}$ −197.5° (c=0.69, MeOH), *Anal.* Calcd for $C_{36}H_{36}O_{17}\cdot H_2O$: C, 56.99; H, 5.05. Found: C, 57.12; H, 5.07. FAB-MS m/z: 741 $[M+H]^+$. The ^{1}H-NMR spectrum was complicated by rotational isomerism. ^{13}C-NMR (acetone-d_6 + D_2O): 28.1 (C-4′), 38.1 (C-4), 61.8 (glc. C-6), 68.2 (C-3′), 70.7 (glc. C-4), 73.6 (C-3), 74.1 (glc. C-2), 76.5 (glc. C-5), 77.0 (glc. C-3), 81.7 (C-2), 83.1 (C-2′), 100.9 (glc. C-1).

Enzymatic Hydrolysis of 13——An aqueous solution of **13** (10 mg) was incubated with crude hesperidinase overnight at 37 °C. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was treated with EtOH. The EtOH-soluble portion was subjected to chromatography over Sephadex LH-20. Elution with EtOH afforded glucose and unreacted **13**. Further elution with EtOH gave an aglycone. Analysis by HPLC and TLC

showed it to be procyanidin B-3 [t_R 8.0 min, column: Nucleosil C_{18}, solvent 5% CH_3CN–H_2O, flow rate: 0.75 ml/min; *Rf* 0.48, solvent: benzene–ethyl formate–formic acid (1 : 7 : 1)].

Thiolytic Degradation of 13——A mixture of **13** (25 mg), benzyl mercaptan (1 ml) and acetic acid (2 ml) in EtOH (5 ml) was refluxed for 4.5 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 EtOH afforded benzylthioethers. Further elution with EtOH yielded (+)-catechin. The benzylthioethers were seperated by chromatography over Sephadex LH-20 [solvent: $CHCl_3$–EtOH (3 : 1)] to give **13a** (2 mg) and **13b** (1.5 mg). **13a**: A white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): 3.2—4.0 (8H, m, sugar-H and $-SCH_2-$), 4.14 (1H, d, J=5 Hz, 4-H), 4.32 (1H, dd, J=5, 8 Hz, 3-H), 4.56 (1H, d, J=8 Hz, 2-H), 4.89 (1H, d, J=7 Hz, anomeric-H), 6.12, 6.41 (each 1H, d, J=2 Hz, 6, 8-H), 6.7—7.0 (3H, m, B-ring-H), 7.2—7.4 (5H, m, aromatic-H). **13b**: A white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): 3.5—4.2 (8H, m, sugar-H and $-SCH_2-$), 4.38 (1H, dd, J=4, 8 Hz, 3-H), 4.70 (1H, d, J=4 Hz, 4-H), 4.95 (1H, d, J=9 Hz, 2-H), 4.92 (1H, d, J=7 Hz, anomeric-H), 5.95, 6.41 (each 1H, d, J=2 Hz, 6, 8-H), 6.8—7.0 (3H, m, B-ring-H), 7.2—7.6 (5H, m, aromatic-H).

Desulfurization of 13a and 13b——A mixture of **13a** and **13b** (3 mg) and acetic acid (1 ml) in EtOH (4 ml) was treated at room temperature with an EtOH slurry of Raney-nickel (W-4) for 30 min. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Sephadex LH-20 chromatography (solvent: EtOH) to yield **2** (1.5 mg).

Methylation of 13——A solution of **13** (10 mg) in MeOH was treated with an ethereal solution of CH_2N_2 at room temperature for 5 h. The solvent was evaporated off, and the residue was chroamtographed over silica gel [solvents: AcOEt–acetone–H_2O (10 : 3 : 0.3) and $CHCl_3$–MeOH (20 : 1)] to afford the heptamethyl ether (6 mg) as a white amorphous powder, $[\alpha]_D^{25}$ −143.7° (c=0.7, MeOH). *Anal.* Calcd for $C_{43}H_{50}O_{17}\cdot 5/2H_2O$: C, 58.43; H, 6.27. Found: C, 58.45; H, 6.62. FD-MS *m/z*: 877 $[M+K]^+$, 861 $[M+Na]^+$, 838 $[M]^+$, 442. The ^{1}H-NMR spectrum was complicated by rotational isomerism.

Compound 14——A tan amorphous powder, $[\alpha]_D^{19}$ +33.7° (c=0.88, MeOH). *Anal.* Calcd for $C_{36}H_{36}O_{17}\cdot 3/2H_2O$: C, 56.32; H, 5.12. Found: C, 56:62; H, 5.22. FAB-MS *m/z*: 741 $[M+H]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.4—3.0 (2H, m, 4′-H), 3.5—4.2 (8H, m, sugar-H, 3-H and 3′-H), 4.70 (1H, br s, 4-H), 4.7—5.0 (2H, m, 2′-H and anomeric-H), 5.24 (1H, br s, 2-H), 5.9—6.1 (2H, m, 6, 6′-H), 6.6—7.2 (6H, m, B-ring-H). ^{13}C-NMR (acetone-d_6 + D_2O): 29.5 (C-4′), 36.4 (C-4), 61.5 (glc. C-6), 67.7 (C-3′), 70.5 (glc. C-4), 72.7 (glc. C-2), 73.6 (C-3), 76.5 (glc. C-1), 76.9 (C-2), 78.6 (glc. C-3), 81.3 (C-2′), 81.6 (glc. C-5), 96.8 (2C) (C-6, 6′), 100.6, 101.7 (C-4a, 4a′), 103.1 (C-8), 107.4 (C-8′), 114.6, 115.2, 115.7, 115.9 (B-ring C-2, 5, 2′, 5′), 118.4, 119.2 (B-ring C-6, 6′), 132.1, 132.4 (B-ring C-1, 1′), 144.9, 145.1, 145.3 (2C) (B-ring C-3, 4, 3′, 4′), 153.6, 155.1 (2C), 156.7, 157.9 (C-5, 7, 8a, 5′, 7′, 8a′).

Thiolytic Degradation of 14——A mixture of **14** (20 mg), benzyl mercaptan (1 ml) and acetic acid (2 ml) in EtOH (5 ml) was refluxed for 5 h with stirring. The reaction mixture was treated as described for **13** to furnish (+)-catechin and the benzylthioether (**14a**) (4 mg) as a white amorphous powder, $[\alpha]_D^{17}$ −19.9° (c=0.51, MeOH). FAB-MS *m/z*: 613 $[M+K]^+$, 597 $[M+Na]^+$, 575 $[M+H]^+$, 451 $[M-SCH_2\ Ph]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 3.4—4.0 (6H, m, sugar-H), 3.91 (1H, d, J=2 Hz, 4-H), 4.05 (2H, s, $-SCH_2-$), 4.15 (1H, d, J=2 Hz, 3-H), 4.91 (1H, d, J=10 Hz, anomeric-H), 5.39 (1H, br s, 2-H), 6.09 (1H, s, 6-H), 6.64 (1H, dd, J=2, 8 Hz, 6′-H), 6.80 (1H, d, J=8 Hz, 5′-H), 7.19 (1H, d, J=2 Hz, 2′-H), 7.2—7.5 (5H, m, aromatic-H). **14a** was identified as 8-*C*-β-D-glucopyranosyl (−)-epicatechin 4-β-benzylthioether by comparison of the spectral and physical data with those of an authentic sample.[10]

Compound 15——A tan amorphous powder, $[\alpha]_D^{19}$ +45.1° (c=0.79, MeOH). FAB-MS *m/z*: 741 $[M+H]^+$. *Anal.* Calcd for $C_{36}H_{36}O_{17}\cdot H_2O$: C, 56.99; H, 5.02. Found: C, 57.22; H, 5.26. ^{1}H-NMR (acetone-d_6 + D_2O): 2.54 (1H, dd, J=8, 16 Hz, 4′-H), 2.78 (1H, dd, J=6, 16 Hz, 4′-H), 3.5—3.9 (6H, m, sugar-H), 3.96 (1H, br s, 3-H), 4.10 (1H, m, 3′-H), 4.66 (1H, s, 4-H), 4.70 (1H, d, J=8 Hz, 2′-H), 4.86 (1H, d, J=10 Hz, anomeric-H), 5.16 (1H, s, 2-H), 6.00 (1H, s, 6′-H), 6.04 (1H, s, 8-H), 6.6—7.0 (6H, m, B-ring-H). ^{13}C-NMR (acetone-d_6 + D_2O): 27.7 (C-4′), 36.5 (C-4), 61.5 (glc. C-6), 67.7 (C-3′), 70.5 (glc. C-4), 72.6 (glc. C-2), 74.4 (C-3), 76.6 (glc. C-1), 77.0 (C-2), 78.9 (glc. C-3) 81.9 (2C) (glc. C-5 and C-2′), 96.3, 96.9 (C-8, 6′), 100.9 (C-4a, 4a′), 104.2 (C-6), 107.6 (C-8′), 114.9, 115.2, 115.5, 115.9 (B-ring C-2, 5, 2′, 5′), 119.2 (2C) (B-ring C-6, 6′), 131.9, 132.3 (B-ring C-1, 1′), 145.1 (2C), 145.3 (2C) (B-ring C-3, 4, 3′, 4′), 155.1, 155.4, 156.7 (C-5, 7, 8a, 5′, 7′, 8a′).

Thiolytic Degradation of 15——**15** (20 mg) was degraded in the same way as described for **14** to give (+)-catechin and the benzylthioether **15a** (3 mg) as a white amorphous powder, $[\alpha]_D^{19}$ +10.5° (c=0.39, MeOH). FAB-MS *m/z*: 597 $[M+Na]^+$, 575 $[M+H]^+$, 451 $[M-SCH_2\ Ph]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 3.4—3.6 (6H, m, sugar-H), 3.96 (1H, d, J=4 Hz, 4-H), 4.03 (2H, s, $-SCH_2-$), 4.13 (1H, d, J=4 Hz, 3-H), 4.89 (1H, d, J=10 Hz, anomeric-H), 5.29 (1H, br s, 2-H), 6.00 (1H, s, 8-H), 6.71 (1H, dd, J=2, 8 Hz, 6′-H), 6.83 (1H, d, J=8 Hz, 5′-H), 7.02 (1H, d, J=2 Hz, 2′-H), 7.2—7.5 (5H, m, aromatic-H). **15a** was identified as 6-*C*-β-D-glucopyranosyl (−)-epicatechin 4-β-benzylthioether by comparison with an authentic sample.[10]

Acknowledgement The authors wish to thank Mr. T. Tanaka of Tanabe Seiyaku Co., Ltd. for providing crude hesperidinase. Thanks are also due to Mr. Y. Tanaka, Miss K. Soeda and Mr. K. Isobe for the measurements of EI-MS, FD-MS, FAB-MS, and ^{1}H- and ^{13}C-NMR spectra, and to the staff of the Central Analysis Room of this university for elemental analysis. This work was supported by a grant (No. 60771862) from the Ministry of

Education, Science and Culture, Japan.

References and Notes

1) Part XLIV: H. Nishimura, G. Nonaka, and I. Nishioka, *Phytochemistry*, "submitted." This paper also forms Part VIII of "Studies on Rhubarb (Rhei Rhizoma)." For Part VII, see G. Nonaka, I. Nishioka, M. Nishizawa, and T. Yamagishi, *J. Med. Pharm. Soc. for WAKAN-YAKU*, **2**, 39 (1985).
2) G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981); G. Nonaka and I. Nishioka, *ibid.*, **31**, 1652 (1983).
3) Y. Kashiwada, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 3461 (1984).
4) H. Okabe, K. Matsuo, and I. Nishioka, *Chem. Pharm. Bull.*, **21**, 1254 (1973).
5) M. Tsuboi, M. Minami, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **25**, 2708 (1977).
6) Y. Kashiwada, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 3493 (1984).
7) A. Yagi, K. Koizumi, and I. Nishioka, *Shoyakugaku Zasshi*, **25**, 52 (1971); G. Nonaka, M. Minami, and I. Nishioka, *Chem. Pharm. Bull.*, **25**, 2300 (1977); Y. Kashiwada, G. Nonaka, and I. Nishioka, *ibid.*, **32**, 3501 (1984).
8) G. Nonaka, E. Ezaki, K. Hayashi, and I. Nishioka, *Phytochemistry*, **22**, 1659 (1983).
9) M. Takani, M. Nakano, and K. Takahashi, *Chem. Pharm. Bull.*, **25**, 3388 (1977).
10) S. Morimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 643 (1986).
11) G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 3906 (1983).
12) S. Morimoto, G. Nonaka, I. Nishioka, N. Ezaki, and N. Takizawa, *Chem. Pharm. Bull.*, **33**, 2281 (1985).
13) D. E. Hathway and J. W. T. Seakins, *J. Chem. Soc.*, **1957**, 1562.
14) K. R. Markham and J. W. Wallace, *Phytochemistry*, **19**, 415 (1980).
15) G. Speranza, P. Gramatica, G. Dada, and P. Manitto, *Phytochemistry*, **24**, 1571 (1985).
16) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 4268 (1982).
17) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 3148 (1980).
18) *a*) G. Nonaka, F.-L. Hsu, and I. Nishioka, *J. Chem. Soc., Chem. Commun.*, **1981**, 781; *b*) G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 4277 (1982).
19) G. Nonaka, H. Nishimura, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1985**, 163.