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Studies on Rhubarb (Rhei Rhizoma). VI.[1] Isolation and Characterization of Stilbenes

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Nineteen stilbene derivatives (I—XIX), of which five are novel *cis*-stilbenes, have been obtained from a rhubarb of low quality (commercial name: 芋大黄, Imo-Daio). Based on chemical and spectroscopic evidence, these compounds have been characterized as rhapontigenin (I), rhaponticin (II), rhapontigenin 3′-*O*-*β*-D-glucopyranoside (III), rhaponticin 6″-*O*-gallate (IV), rhaponticin 2″-*O*-gallate (V), rhaponticin 2″-*O*-*p*-coumarate (VI), piceatannol (VII), piceatannol 3-*O*-*β*-D-glucopyranoside (VIII), piceatannol 3′-*O*-*β*-D-glucopyranoside (IX), piceatannol 3′-*O*-*β*-D-xylopyranoside (X), piceatannol 3-*O*-*β*-D-(6″-*O*-galloyl) glucopyranoside (XI), desoxyrhaponticin (XII), desoxyrhaponticin 6″-*O*-gallate (XIII), 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(6″-*O*-galloyl) glucopyranoside (XIV), *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-*β*-D-glucopyranoside (XV), *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-*β*-D-(6″-*O*-galloyl) glucopyranoside (XVI), *cis*-3,5-dihydroxy-4′-methoxystilbene 3-*O*-*β*-D-glucopyranoside (XVII), *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-*β*-D-(2″-*O*-galloyl) glucopyranoside (XVIII) and *cis*-3,3′,5-trihydroxy-4′-methoxystilbene (XIX).

Keywords——rhubarb; Polygonaceae; *trans*-stilbene; *cis*-stilbene; gallic acid ester; *p*-coumaric acid ester; rhaponticin; desoxyrhaponticin; piceatannol; 3,4′,5-trihydroxystilbene

Rhubarb produces a variety of secondary phenolic metabolites, *i.e.*, anthraquinones,[2] naphthalenes,[3] stilbenes,[4] chromones,[1] flavonoids,[5] and tannins and related compounds.[6] Among these compounds, stilbenes are considered to be of particular chemotaxonomical importance, since according to "The Japanese Pharmacopoeia X,"[7] the chemical evaluation of rhubarbs, that is, the distinction between high-quality and low-quality rhubarb, is based on the presence or absence of the stilbene glucoside, rhaponticin, which is believed to occur in rhubarbs of low quality. Previously, we demonstrated the existence in representatives of high-quality rhubarbs (commercial names: 信州大黄, Shinshu Daio and 雅黄, Gao) of large amounts of 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-glucopyranoside and its gallate, whose structures are closely related to that of rhaponticin, and hence we stated at that time that it might be difficult to evaluate rhubarbs by using thin-layer chromatographic techniques as described in "The Japanese Pharmacopoeia X." As a continuation of our chemical studies on rhubarb, we have now undertaken the analysis of stilbene derivatives occurring in large quantities in a low-quality rhubarb (commercial name: 芋大黄, Imo-Daio), from which rhaponticin,[8] desoxyrhaponticin[9] and piceid[4a] have previously been isolated. We have isolated and characterized nineteen stilbenes, including rhaponticin and desoxyrhaponticin, and this paper presents a detailed account of the structural determination of these compounds.

The aqueous acetone extract of Imo-Daio was directly subjected to chromatography over Sephadex LH-20 dextran gel with H_2O containing increasing amounts of MeOH. Subsequent chromatographies of each effluent over Sephadex LH-20, MCI-gel CHP-20 and silica gel yielded compounds I—XIX (Chart 1). These compounds did not include piceid, 3,4′,5-trihydroxystilbene 3-*O*-*β*-D-glucopyranoside, which had previously been reported to occur in

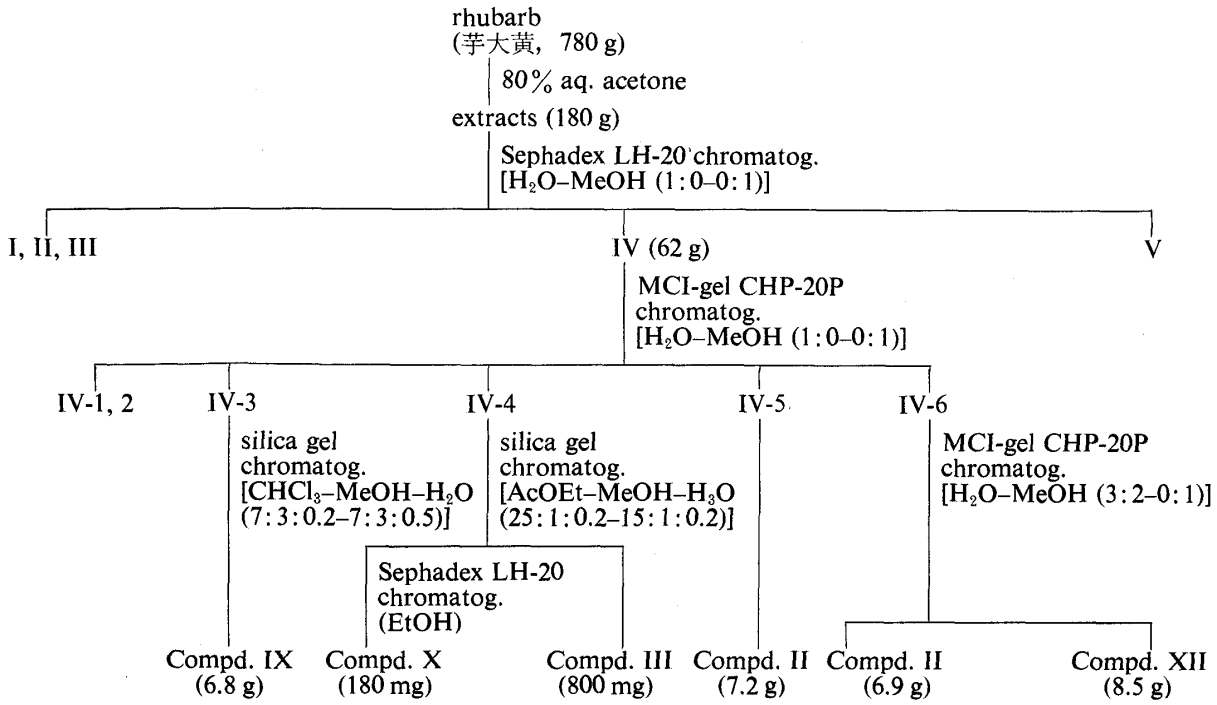


Chart 1-1

this plant material.[4a)]

Compound I, colorless plates (dil. MeOH), mp 196—197 °C, showed bluish-purple fluorescence on ultraviolet (UV) irradiation. The infrared (IR) spectrum showed a hydroxyl absorption (ν_{max}^{KBr} cm^{-1}: 3250), while the UV spectrum suggested the presence of a highly conjugated system (λ_{max}^{MeOH} nm: 302, 322). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed, together with signals due to aromatic methoxyl (δ 3.84) and *trans*-olefinic protons (δ 6.84, 7.02, each 1H, d, J=16 Hz), the occurrence of two independent aromatic rings, each with three protons. One ring showed ABX-type signals (δ 6.88, 1H, d, J=8 Hz; δ 7.02, 1H, dd, J=2, 8 Hz; δ 7.09, 1H, d, J=2 Hz) which were assignable to protons on a 1,3,4-trisubstituted aromatic ring, while the other showed AX_2-type signals (δ 6.29, 1H, t, J=2 Hz; δ 6.56, 2H, d, J=2 Hz) assignable to protons on a 1,3,5-trisubstituted system. From these spectral data, I was presumed to be rhapontigenin, and this was confirmed by comparison of its physical and spectral data with those described in the literature.[9a)]

Compounds II, colorless needles (dil. acetone), mp 246—248 °C, $[\alpha]_D$ −56.3° (acetone–H_2O) and III, colorless needles (dil. acetone), mp 246—248 °C, $[\alpha]_D$ −70.0° (acetone–H_2O), $C_{21}H_{24}O_9 \cdot 1/2H_2O$, showed UV absorption bands similar to those of I. The ^{1}H-NMR spectra of II and III were related to that of I, showing the presence in each molecule of a methoxyl group and a *trans* olefinic group, and two aromatic rings with 1,3,4-(catechol-type)- and 1,3,5-(resorcinol-type)-trisubstitution systems. The ^{1}H-NMR spectra also exhibited a sugar anomeric proton doublet (J=7 Hz) in both cases (δ 4.85 in II and δ 4.99 in III). Other sugar signals appeared in the range of δ 3.2—3.9. The presence of a glucose moiety in each molecule was deduced from an examination of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, which displayed signal patterns analogous to that of methyl glucoside. Considering that the ^{1}H- and ^{13}C-NMR spectra of II exhibited an unsymmetrical signal pattern of the resorcinol ring, and that II was obtained as the major constituent of this drug, this compound was assumed to be rhaponticin, that is, rhapontigenin 3-*O*-β-D-glucopyranoside, previously isolated from *Rheum rhaponticum*.[8)] This conclusion was confirmed by comparison of the

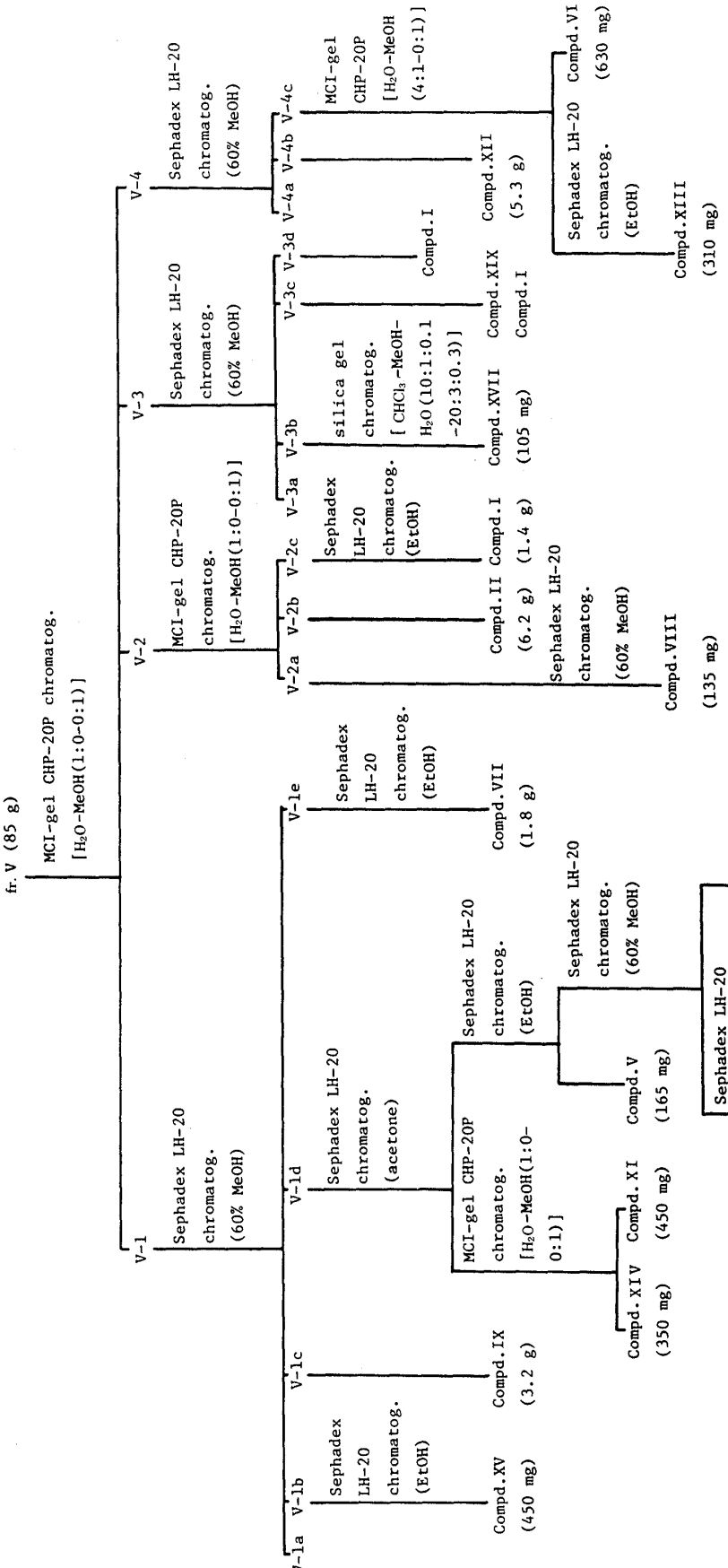


Chart 1-2

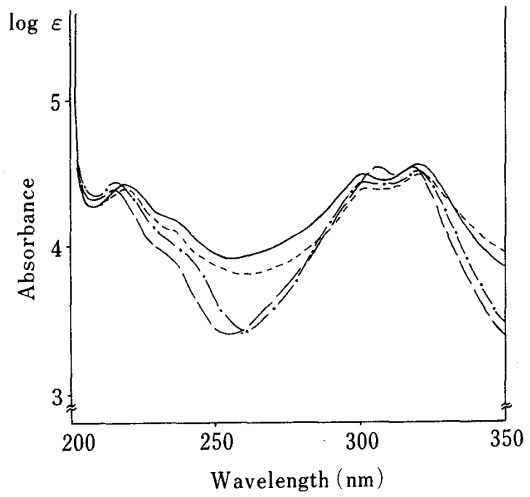


Fig. 1. UV Spectra of Compounds I—III and XII (MeOH)
——, I; – – – –, II; —·—, III; ——, XII.

physical and spectral data with those of an authentic sample.[4a] Compound III, on the other hand, showed a symmetrical signal pattern of the resorcinol ring in the ^{1}H- and ^{13}C-NMR spectra, suggesting that the sugar was attached to the catechol-type ring. The aglycone and the sugar were confirmed to be rhapontigenin and D-glucose, respectively, by acid hydrolysis of III. The configuration at the anomeric center was concluded to be β on the basis of the above-mentioned ^{1}H-NMR coupling constant value. From these chemical and spectroscopic data, III was characterized as rhapontigenin 3′-*O*-β-D-glucopyranoside.

Compounds IV, colorless granules (dil. MeOH), mp 189—190 °C, $[\alpha]_D$ −109.1° (acetone), $C_{28}H_{28}O_{13}\cdot H_2O$, and V, colorless granules ($CHCl_3$–MeOH), mp 243—244 °C, $[\alpha]_D$ +15.9° (acetone), $C_{28}H_{28}O_{13}\cdot 1/2H_2O$, showed blue colorations with ferric chloride reagent. The field desorption mass spectra (FD-MS) of IV and V exhibited the same intense molecular ion peak at *m/z* 572, together with a minor peak at *m/z* 153 suggestive of the presence of a galloyl group. The ^{1}H- and ^{13}C-NMR spectra of IV and V were quite similar, except for the low-field sugar signals. The presence of a galloyl group in both compounds was shown by the appearance of a two-proton aromatic singlet (δ 7.16 in IV and δ 7.17 in V) in the ^{1}H-NMR spectra. On tannase hydrolysis, both afforded gallic acid and a hydrolysate which was identified as rhaponticin (II). The location of the galloyl group in IV was concluded to be at the C-6 position in the glucosyl moiety, since in the ^{1}H-NMR spectrum, two proton signals (δ 4.42, 1H, dd, J=4, 12 Hz; δ 4.66, 1H, dd, J=2, 12 Hz) which were assignable to the C-6 methylene protons of the glucosyl moiety in view of the large coupling constant, were shifted downfield. This conclusion was also supported by the deshielding of the C-6 carbon signal in the ^{13}C-NMR spectrum (Table I). On the basis of these observations, IV was assigned as rhaponticin 6″-*O*-gallate. The position of the galloyl group in V was determined to be the C-2 hydroxyl group of the glucose residue by ^{1}H-NMR spectroscopy; a methine proton attached to the carbon bearing the galloyl group appeared downfield as a triplet (δ 6.14, J=8 Hz), and this signal was assigned to the C-2 proton based on the observation that this signal was coupled with the anomeric proton signal (δ 5.77, d, J=8 Hz) as revealed by spin-decoupling experiments. From these observations, V was characterized as rhaponticin 2″-*O*-gallate.

Compound VI, colorless granules (dil. MeOH), mp 229—231 °C, $[\alpha]_D$ −9.8° (acetone), $C_{30}H_{30}O_{11}\cdot 1/2H_2O$, gave a UV spectrum similar to those of the above compounds. The ^{1}H-NMR spectrum was similar to that of V, except that an aromatic singlet due to the galloyl group was absent, and *trans*-coupled olefinic (δ 6.43, d, J=16 Hz; δ 7.64, d, J=16 Hz) and A_2B_2-type aromatic (δ 6.80, 2H, d, J=8 Hz; δ 7.77, 2H, d, J=8 Hz) signals were observed instead. Alkaline hydrolysis of VI with sodium methoxide in methanol afforded methyl *p*-coumarate and a hydrolysate which was found to be identical with rhaponticin. The location of the *p*-coumaroyl moiety in VI was determined in the following ways; in the ^{1}H-NMR

Table I. ^{13}C-NMR Data for Compounds II—VI

	II[a)]	III[a)]	IV[b)]	V[b)]	VI[a)]
C_1	139.1	139.0	140.6	140.6	139.0
C_2	105.0	104.4	107.2	106.7	105.0
C_3	158.4	158.2	159.9	159.6	158.3
C_4	102.8	101.7	103.9	104.0	102.8
C_5	158.0	158.2	159.1	159.2	158.1
C_6	107.0	104.4	108.0	108.5	107.5
$C_{1'}$	129.8	129.9	131.4	131.3	129.8
$C_{2'}$	112.7	112.8	113.5	113.3	112.7
$C_{3'}$	146.3	146.5	147.4	147.3	146.3
$C_{4'}$	147.6	148.6	148.5	148.5	147.7
$C_{5'}$	112.0	112.3	112.6	112.6	112.0
$C_{6'}$	118.6	121.0	119.8	119.6	118.8
C_α	126.0	126.9	127.1	126.8	125.8
C_β	128.5	127.7	129.6	129.6	128.8
$-OCH_3$	55.5	55.6	56.3	56.3	55.5
Glucose					
C_1	100.4	100.2	101.7	100.1	98.5
C_2	73.1	73.1	74.4	75.5	73.4
C_3	76.8	76.9	77.4	74.7	72.8
C_4	69.6	69.7	70.8	71.2	69.8
C_5	76.3	76.6	74.8	77.5	77.0
C_6	60.5	60.6	64.3	62.1	60.5
Galloyl					
C_1			121.3	121.3	
C_2			109.9 (2C)	110.0 (2C)	
C_3			146.0 (2C)	146.0 (2C)	
C_4			139.0	139.1	
–COO–			167.2	166.5	
p-Coumaroyl					
C_1					125.1
C_2					115.8 (2C)
C_3					130.3 (2C)
C_4					159.5
–CH=CH–					114.1
					145.1
–COO–					165.8

a) Measured in DMSO-d_6 + D_2O.
b) Measured in acetone-d_6 + D_2O.

spectrum of VI, the chemical shift of the sugar signal (δ 6.09, 1H, t, J=8 Hz), which appeared downfield as a result of acylation, was in good agreement with that observed in V (δ 6.14, 1H, t, J=8 Hz). Furthermore, in the ^{13}C-NMR spectrum, the signal pattern of the sugar moiety was similar to that found in V (Table I). Thus, the *p*-coumaroyl group was concluded to be attached to the C-2 hydroxyl of the glucose moiety. Based on these observations, VI was determined as rhaponticin 2″-*O*-*p*-coumarate.

Compound VII, colorless plates (dil. MeOH), mp 233—235 °C, showed a blue coloration with ferric chloride reagent. The UV (λ_{max}^{MeOH} nm: 303, 323) and IR (ν_{max}^{KBr} cm^{-1}: 3340, 1600, 1520) spectra were similar to those of I, suggesting the presence of a stilbene skeleton. The ^{1}H-NMR spectrum of VII closely resembled that of I, except for the absence of an aromatic methoxyl singlet. From these data, VII was presumed to be 3,3′,4′,5-tetrahydroxystilbene (piceatannol), and this conclusion was confirmed by comparison of its physical and spectral data with those described in the literature.[10)]

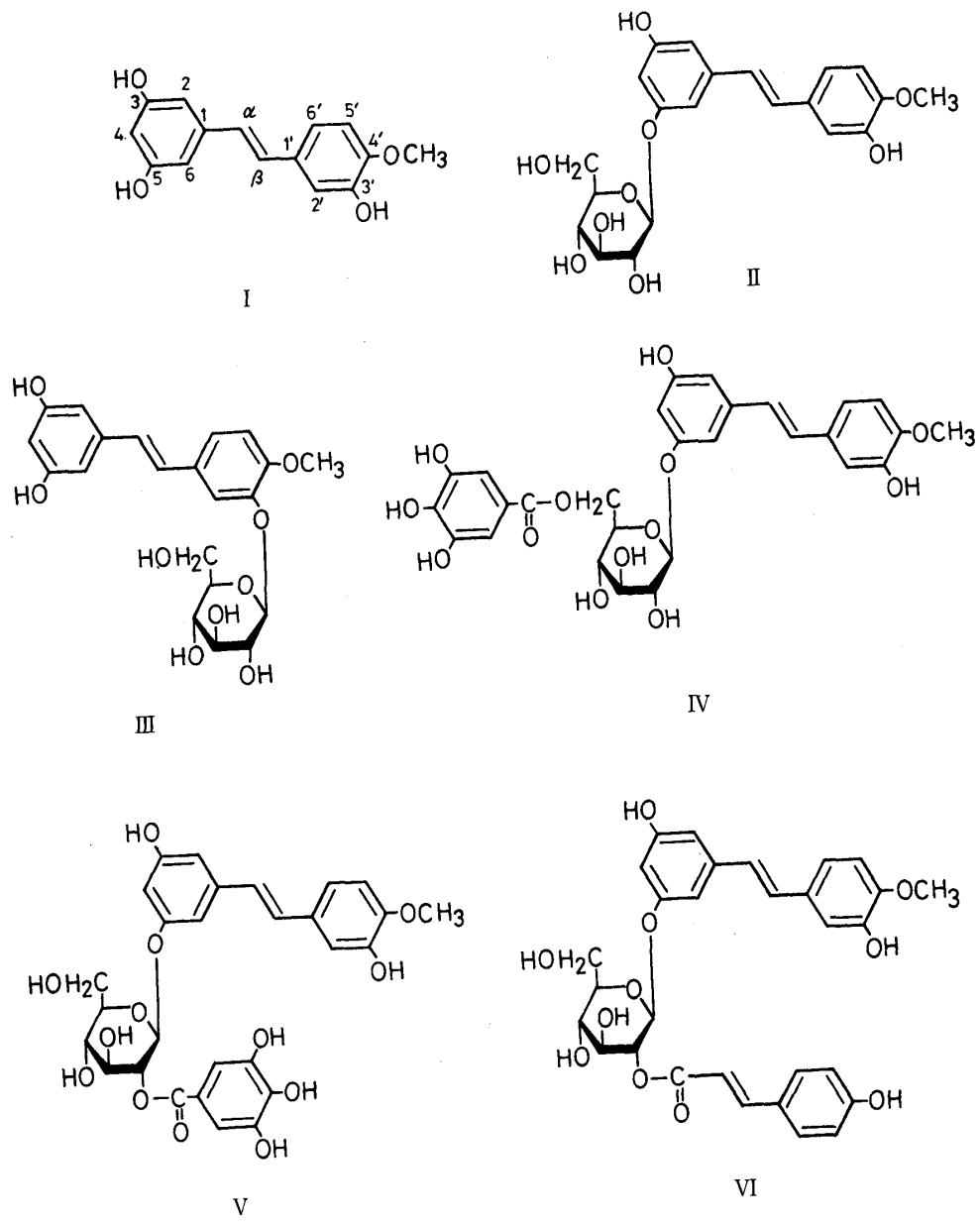


Chart 2

Compounds VIII, an off-white amorphous powder, $[\alpha]_D$ −59.8° (MeOH), $C_{20}H_{22}O_9 \cdot 1/2H_2O$, IX, colorless needles (dil. MeOH), mp 229—230 °C, $[\alpha]_D$ −40.4° (MeOH), $C_{20}H_{22}O_9$, and X, an off-white amorphous powder, $[\alpha]_D$ +1.7° (MeOH), $C_{19}H_{20}O_8 \cdot 1/2H_2O$, gave almost the same UV spectra as that of VII. In the FD-MS, both VIII and IX showed a peak at *m/z* 406 due to the molecular ion, together with a prominent peak at *m/z* 163 suggestive of the presence of a hexosyl moiety, whereas X exhibited a molecular ion peak at *m/z* 376 and a prominent peak at *m/z* 133, the latter suggesting the presence of a pentosyl moiety. Acid hydrolysis of VIII and IX with 5% sulfuric acid gave D-glucose and an aglycone identical with piceatannol (VII). The ^{1}H- and ^{13}C-NMR spectra of VIII were similar to those of II except for the absence of a methoxyl signal, indicating the structure of VIII to be piceatannol 3-*O*-*β*-D-glucopyranoside. Compounds IX and X showed mutually related ^{1}H-NMR spectra which were, apart from the lack of a methoxyl singlet, similar to that of III, showing the symmetry of the resorcinol ring. These observations suggested that the sugar in each compound was bound to either the C-3′ or C-4′ hydroxyl group of piceatannol. The location of the glucosyl moiety in IX was determined as follows; IX was methylated with

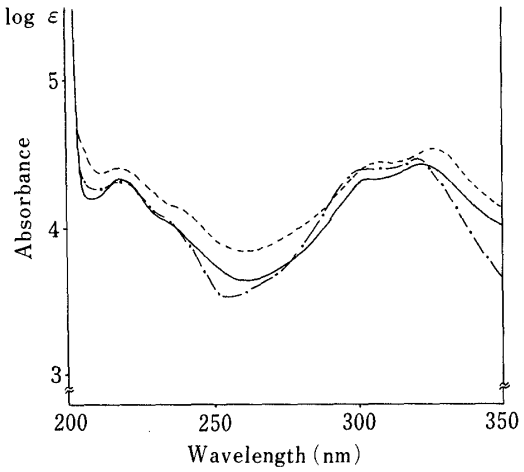


Fig. 2. UV Spectra of Compounds VII—IX (MeOH)
——, VII; – – – –, VIII; — — —, IX.

TABLE II. ^{13}C-NMR Data for Compounds VIII—XI

	VIII[a)]	IX[a)]	X[a)]	XI[b)]
C_1	139.3	139.1	139.1	140.4
C_2	106.9	104.3	104.4	105.7
C_3	158.7	158.2	158.2	159.2
C_4	102.6	102.1	102.2	104.1
C_5	158.0	158.2	158.2	159.2
C_6	104.9	104.3	104.4	105.7
$C_{1'}$	128.5	128.8	128.8	130.6
$C_{2'}$	115.6	115.8	116.2	117.2
$C_{3'}$	145.0[c)]	146.3	146.7	148.2
$C_{4'}$	145.3[c)]	145.4	144.9	146.3
$C_{5'}$	113.0	114.0	114.7	117.2
$C_{6'}$	118.9	122.0	121.6	123.2
C_α	125.1	126.2	126.2	127.6
C_β	128.9	127.8	127.9	128.7
Glucose				
C_1	100.4	101.7		102.7
C_2	73.1	73.1		74.4
C_3	76.8	77.1		76.9
C_4	69.6	69.9		70.6
C_5	76.3	75.7		75.0
C_6	60.5	60.7		63.8
Xylose				
C_1			101.7	
C_2			72.9	
C_3			75.5	
C_4			69.2	
C_5			65.5	
Galloyl				
C_1				121.2
C_2				110.0 (2C)
C_3				146.0 (2C)
C_4				139.0
–COO–				167.2

a) Measured in DMSO-d_6 + D_2O.
b) Measured in acetone-d_6 + D_2O.
c) Assignments may be interchanged.

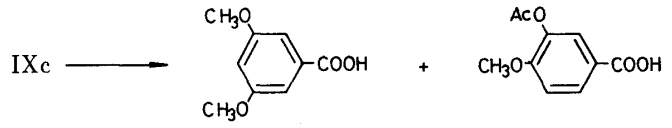


Chart 3

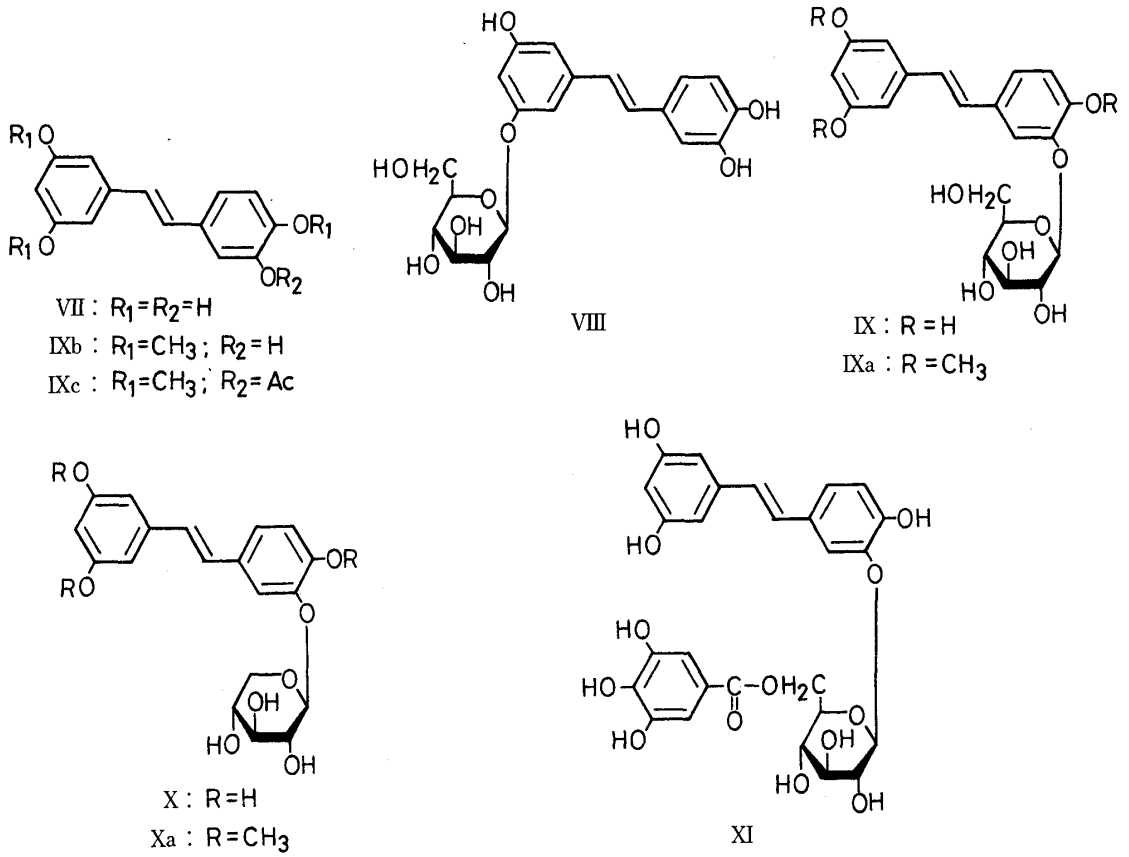


Chart 4

dimethyl sulfate and potassium carbonate in dry acetone to give a trimethyl ether (IXa). Acid hydrolysis of IXa yielded glucose and an aglycone (IXb). Acetylation of IXb followed by oxidation with potassium permanganate in slightly alkaline solution gave 3,5-dimethoxybenzoic acid and acetylisovanillic acid. Thus, the glucosyl moiety in IX was concluded to be bound to the C-3′ hydroxyl of piceatannol. In the case of X, the component sugar was identified as D-xylose by acid hydrolysis of the trimethyl ether (Xa) prepared in the same way as described for IX, and the sugar residue was concluded to be located at the C-3′ position based on the fact that Xa gave IXb on acid hydrolysis. The anomeric configurations in IX and X were determined to be β from the coupling constant value (d, $J=7$ Hz) of the anomeric proton signal. On the basis of the chemical and spectral evidence described above, IX and X were assigned as piceatannol 3′-*O*-β-D-glucopyranoside and piceatannol 3′-*O*-β-D-xylopyranoside, respectively.

Compound XI, an off-white amorphous powder, $[\alpha]_D$ $-89.4°$ (acetone), $C_{27}H_{26}O_{13}\cdot H_2O$, was positive to the ferric chloride reagent (a blue coloration). The FD-MS exhibited a prominent peak at *m/z* 170 corresponding to gallic acid, and a molecular ion peak at *m/z* 558. The presence of the galloyl group in XI was also apparent from the ^{1}H- and ^{13}C-NMR spectra [δ 7.20, 2H, s; δ 110.0 (2C), 121.2, 139.0, 146.0 (2C), 167.2]. Enzymatic hydrolysis of XI with tannase gave gallic acid and a hydrolysate which was found to be identical with IX by direct comparisons. The location of the galloyl group in XI was concluded

Table III. ^{13}C-NMR Data for Compounds XII—XIV

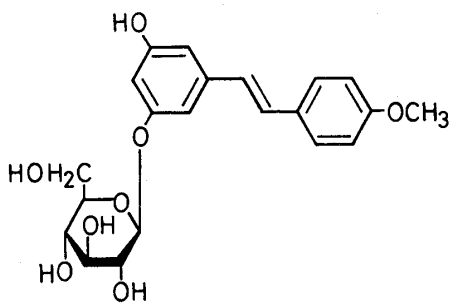
	XII[a)]	XIII[b)]	XIV[b)]
C_1	139.2	140.6	140.3
C_2	105.0	107.1	105.7
C_3	158.7	159.8	159.2
C_4	102.8	103.9	102.8
C_5	158.1	159.0	159.2
C_6	107.1	107.9	105.7
$C_{1'}$	129.4	130.6	132.4
$C_{2'}$	127.8	128.6	128.4
$C_{3'}$	114.1	114.6	117.5
$C_{4'}$	158.9	160.2	157.8
$C_{5'}$	114.1	114.6	117.5
$C_{6'}$	127.8	128.6	128.4
C_α	126.1	126.9	128.1
C_β	128.2	129.3	128.4
$-OCH_3$	55.1	55.6	—
Glucose			
C_1	100.4	101.6	101.4
C_2	73.1	74.2	74.2
C_3	76.8	77.2	77.4
C_4	69.9	70.7	71.2
C_5	76.3	74.8	74.9
C_6	60.6	64.4	64.7
Galloyl			
C_1		121.1	121.2
C_2		109.9 (2C)	110.0 (2C)
C_3		146.0 (2C)	146.0 (2C)
C_4		139.1	139.1
–COO–		167.4	167.2

a) Measured in DMSO-d_6 + D_2O.
b) Measured in acetone-d_6 + D_2O.

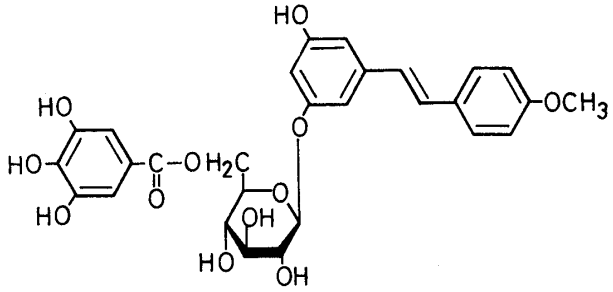
to be at the C-6 hydroxyl of the glucose moiety, since in the ^{1}H-NMR spectrum two proton signals having a large coupling constant (δ 4.50, 1H, dd, J=4, 12 Hz; δ 4.59, 1H, dd, J=2, 12 Hz), which were assignable to the C-6 methylene protons of the glucose moiety, were shifted downfield. On the basis of these findings, XI was determined as piceatannol 3′-*O*-β-D-(6′′-*O*-galloyl) glucopyranoside.

Compound XII, colorless needles (dil. acetone), mp 227—228 °C, $[\alpha]_D$ −59.5° (acetone–H_2O), was concluded to possess a stilbene skeleton on the basis of the UV absorption bands (λ_{max}^{MeOH} nm: 306, 319). The ^{1}H-NMR spectrum of XII resembled that of II except for the appearance of A_2B_2-type aromatic signals at δ 6.93 and 7.51 (each 2H, d, J=8 Hz) instead of ABX-type signals. From these spectral data, XII was presumed to be desoxyrhaponticin, and this was confirmed by comparison of the physical and spectral data with those described in the literature.[9)]

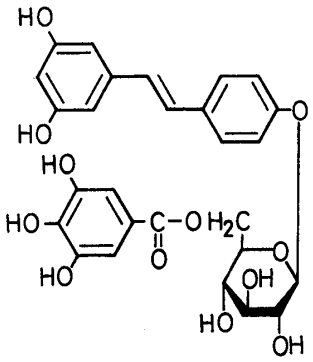
Compound XIII, an off-white amorphous powder, $[\alpha]_D$ −101.6° (acetone), $C_{28}H_{28}O_{12} \cdot 1/2H_2O$, gave a blue spot with ferric chloride reagent on thin-layer chromatography (TLC), and on tannase hydrolysis liberated gallic acid and a hydrolysate which was found to be identical with desoxyrhaponticin. The ^{1}H-NMR spectrum of XIII showed deshielded sugar C-6 methylene signals (δ 4.42, 1H, dd, J=4, 12 Hz; δ 4.67, 1H, dd, J=2, 12 Hz) analogous to those found in IV and XI, thus indicating that the galloyl group was linked to the C-6 hydroxyl of the glucose moiety. Consequently, XIII was characterized as desoxyrhaponticin 6′′-*O*-gallate.



XII



XIII



XIV

Chart 5

Compound XIV, colorless needles (H_2O), mp 256—257 °C, $[\alpha]_D$ −88.2° (acetone), showed a blue coloration with ferric chloride reagent. The ^{1}H-NMR spectrum of XIV differed from that of XIII in the following two aspects; the absence of a methoxyl singlet and the appearance of AX_2-type aromatic signals, the latter indicating a symmetrical nature of the resorcinol ring. Thus, XIV was presumed to be 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(6″-*O*-galloyl) glucopyranoside which had previously been isolated from high-quality rhubarbs.[4b] This conclusion was confirmed by a direct comparison of XIV with an authentic sample.

Compound XV, an off-white amorphous powder, $[\alpha]_D$ −33.6° (acetone), $C_{21}H_{24}O_9 \cdot 1/2H_2O$, had the same *Rf* value as rhaponticin (II) on silica gel TLC, and could not be obtained pure by normal-phase column chromatography. However, application of reversed-type chromatography using Sephadex LH-20 (60% aq. MeOH) effected the isolation of this compound from the mixture. The FD-MS showed a molecular ion peak at *m/z* 420 and a fragment peak at *m/z* 163 derived from a hexosyl cation; these peaks were identical with those observed in II. The ^{13}C-NMR spectrum was also correlated with that of II, showing the presence of similar functional groups. However, the IR spectrum of XV showed no *trans*-olefinic absorption in the range of 920—980 cm^{-1}, and the intensities of the UV absorption maxima were decreased as compared with those of compounds I—XIV. These UV data implied the occurrence of a stronger steric interaction in XV than in I—XIV. Furthermore, in the ^{1}H-NMR spectrum of XV, two olefinic proton signals appeared at comparatively high field as a pair of doublets (δ 6.30, d, J = 12 Hz; δ 6.46, d, J = 12 Hz), whose coupling constant suggested the presence of a *cis*-olefinic group. Based on these observations, XV was assumed to be a *cis*-isomer of rhaponticin (II). In order to establish the structure of XV, an attempt was made to transform II to XV. A solution of II in dioxane–water was irradiated with a low-pressure mercury lamp[11] to give an equilibrium mixture of *cis*- and *trans*-forms. These were separated by reverse-phase chromatography as described above, yielding a pure *cis*-stilbene. Spectral comparison revealed that XV was identical with the synthetic sample thus obtained.

TABLE IV. ^{13}C-NMR Data for Compounds XV—XVII

	XV[a]	XVI[b]	XVII[a]
C_1	138.7	140.2	138.8
C_2	107.2	108.5	107.1
C_3	158.3	159.5	158.2
C_4	102.5	104.0	102.5
C_5	157.7	158.7	157.7
C_6	109.2	111.0	109.2
$C_{1'}$	129.3	130.9	128.9
$C_{2'}$	111.7	112.2	129.8
$C_{3'}$	145.7	146.6	113.6
$C_{4'}$	146.8	147.8	158.2
$C_{5'}$	115.5	116.4	113.6
$C_{6'}$	120.0	121.5	129.8
C_α	128.2	129.3	128.5
C_β	129.9	130.9	129.6
$-OCH_3$	55.4	56.2	54.9
Glucose			
C_1	100.5	102.0	100.4
C_2	72.9	74.1	72.8
C_3	76.4	77.2	76.3
C_4	69.9	70.6	69.1
C_5	76.2	74.6	76.1
C_6	60.1	64.1	60.1
Galloyl			
C_1		121.2	
C_2		110.0 (2C)	
C_3		146.0 (2C)	
C_4		139.0	
–COO–		167.3	

a) Measured in DMSO-d_6+D_2O.
b) Measured in acetone-d_6+D_2O.

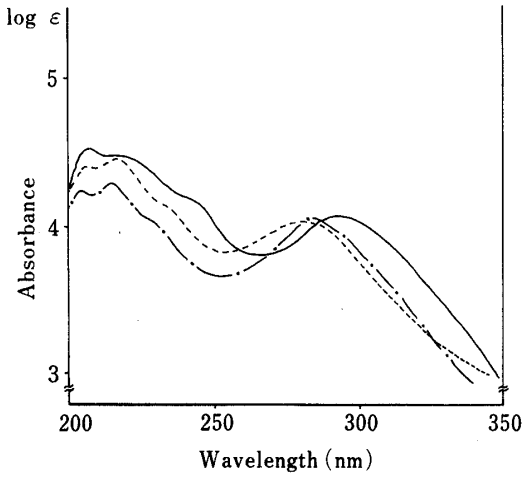


Fig. 3. UV Spectra of Compounds XV—XVII (MeOH)
——, XV; – – – –, XVI; —·—, XVII.

On the basis of the above-mentioned chemical and spectral data, XV was characterized as *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-*β*-D-glucopyranoside.

Compounds XVI, an off-white amorphous powder, $[\alpha]_D$ −47.4° (acetone), $C_{28}H_{28}O_{13}\cdot 3/2H_2O$, and XVII, colorless needles (H_2O), mp 147—148 °C, $[\alpha]_D$ −33.4° (acetone), $C_{21}H_{24}O_8$, each gave UV (Fig. 3) and IR spectra consistent with a *cis*-stilbene structure. The ^{1}H-NMR spectra of XVI and XVII were similar to that of XV, but differed in the fol-

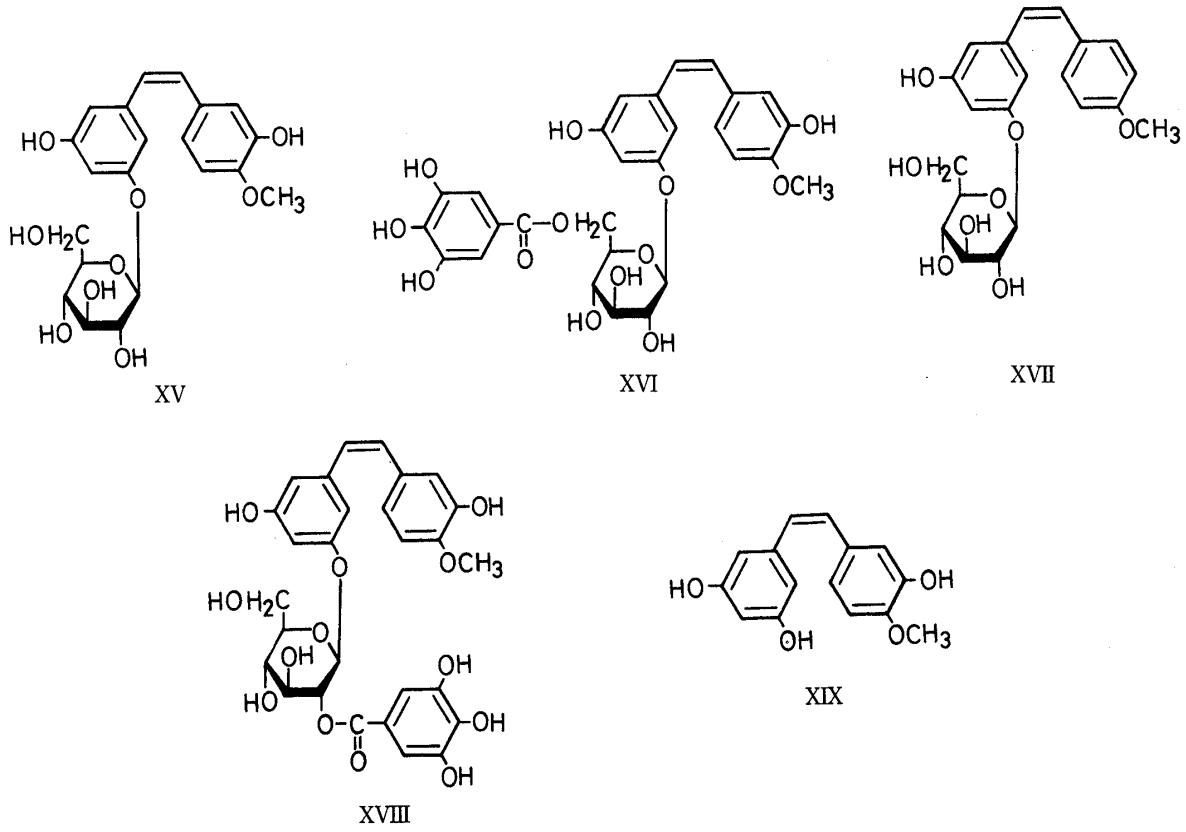


Chart 6

lowing aspects; in the spectrum of XVI, a galloyl singlet (δ 7.14, 2H) and deshielded glucose C-6 methylene signals (δ 4.32, 1H, dd, $J=4$, 12 Hz; δ 4.49, 1H, dd, $J=2$, 12 Hz) were observed, while in XVII, A_2B_2-type aromatic signals (δ 6.82, 7.18, each 2H, d, $J=8$ Hz) appeared in place of ABX-type signals. These findings suggested that XVI and XVII were *cis*-isomers of IV and XII, respectively. Further structural confirmation was obtained by the formation of XVI from IV and of XVII from XII by similar photoisomerizations, thus permitting assignment of the structures *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-β-D-(6′′-*O*-galloyl) glucopyranoside and *cis*-3,5-dihydroxy-4′-methoxystilbene 3-*O*-β-D-glucopyranoside for XVI and XVII, respectively.

Neither XVIII nor XIX could be isolated pure; they were slightly contaminated with V and I, respectively. However, examination of the ^{1}H-NMR spectra implied that they were *cis*-isomers of V and I; in the case of XVIII, the spectrum exhibited aromatic and olefinic signal patterns closely related to those of XVI, and sugar proton signals which were similar to those of V rather than those of XVI. On the other hand, in XIX, the spectrum revealed the absence of sugar and galloyl signals, and almost the same signal patterns of the olefinic and aromatic protons as in XVIII, suggesting that XIX is the aglycone of XVIII, that is, a *cis*-isomer of I. To confirm the structures of these compounds, a similar photoisomerization reaction was applied to V and I. This resulted in the formation of equilibrium mixtures of *cis*- and *trans*-forms, whose ^{1}H-NMR spectra were consistent with the structures of XVIII and XIX. On the basis of these findings, XVIII and XIX were respectively concluded to be *cis*-3,3′,5-trihydroxy-4′-methoxystilbene 3-*O*-β-D-(2′′-*O*-galloyl)-glucopyranoside and *cis*-3,3′,5-trihydroxy-4′-methoxystilbene.

Although the existence of *cis*-stilbenes in nature has long been recognized,[11] there has been no previous report on their isolation, and this is the first example of the isolation of *cis*-stilbenes from a natural source.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. UV and IR spectra were obtained with Shimadzu MPS-2000 and JASCO DS-301 spectrometers, and EI- and FD-MS with JEOL D-300 and JEOL DX-300 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100 and FX-100 spectrometers, respectively, with 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.) and MCI-gel CHP-20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.). TLC was conducted on precoated Keiselgel 60 F_{254} plates (0.20 mm, Merck) and precoated Avicel SF cellulose plates (Funakoshi), and spots were detected by UV illumination and by spraying $FeCl_3$, 10% H_2SO_4 and aniline-hydrogenphthalate reagents.

Isolation of Compounds I—XIX——The pulverized rhubarb (commercial name: 芋大黄, Imo-Daio) (780 g) was extracted three times with 80% aqueous acetone at room temperature. The extracts, after removal of the solvent, were subjected to chromatography over Sephadex LH-20 with H_2O containing increasing amounts of MeOH (1 : 0–0 : 1) to give five fractions (fractions I—V). Fractions IV (62 g) and V (85 g) consisted of a large amount of stilbene derivatives, which exhibited bluish-purple fluorescent spots on UV illumination of the TLC plates. Thus, these fractions were separately rechromatographed over MCI-gel CHP-20P [solvent: H_2O–MeOH (1 : 1–0 : 1)] to afford a further six (fractions IV-1—IV-6) and four (V-1—V-4) fractions, respectively. Fraction IV-3 was purified by silica gel chromatography [solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.2–7 : 3 : 0.5)] to furnish compound IX (6.8 g). Further chromatography of fraction IV-4 over silica gel [solvent: AcOEt–MeOH–H_2O (25 : 1 : 0.2–15 : 1 : 0.2)] gave compound III (800 mg). Compound X obtained from fraction IV-4 was not pure, and was purified by chromatography over Sephadex LH-20 with EtOH as an eluent (yield: 180 mg). Fraction IV-5 consisted mainly of compound II, and recrystallization from aqueous acetone furnished a pure sample (7.2 g). Repeated chromatography of fraction IV-6 over MCI-gel CHP-20P [solvent: H_2O–MeOH (3 : 2–0 : 1)] yielded compounds II (6.9 g) and XII (8.5 g). Fraction V-1 was subsequently chromatographed over Sephadex LH-20 (solvent: 60% MeOH) to give a further five fractions (fractions V-1a—V-1e). Purification of fractions V-1b and V-1e over Sephadex LH-20 (solvent: EtOH) yielded compounds XV (450 mg) and VII (1.8 g), respectively. Fraction V-1d, consisting of a mixture of stilbene gallates, was repeatedly chromatographed over Sephadex LH-20 (solvent: acetone, EtOH, 60% MeOH) and MCI-gel CHP-20P [solvent: H_2O–MeOH (1 : 0–0 : 1)] to furnish compounds IV (480 mg), V (165 mg), XI (450 mg) and XIV (350 mg). Fraction V-1d also contained compound XVIII, but several attempts to isolate XVIII in pure form were unsuccessful due to contamination by compound V. Fraction V-2 contained compounds I and VIII, together with a large amount of compound II, and these components were separated by chromatography over MCI-gel CHP-20P [solvent: H_2O–MeOH (4 : 1–0 : 1)] and over Sephadex LH-20 (solvent: EtOH, 60% MeOH) to give compounds I (1.4 g) and VIII (135 mg) and additional compound II. Fraction V-3 was further fractionated by Sephadex LH-20 chromatography (solvent: 60% MeOH) into four fractions (fractions V-3a—V-3d). Repeated chromatography of fraction V-3b over MCI-gel CHP-20P [solvent: H_2O–MeOH (4 : 1–0 : 1)] gave compound XVII (105 mg). Fraction V-3c contained compounds XIX and I, but XIX could not be isolated. Fraction V-4 was fractionated further by chromatography over Sephadex LH-20 (60% MeOH) to give three fractions (V-4a—V-4c). Repeated chromatography of fraction V-4c over MCI-gel CHP-20P [solvent: H_2O–MeOH (4 : 1–0 : 1)] and Sephadex LH-20 (solvent: acetone, EtOH) furnished compounds XIII (310 mg) and VI (630 mg).

Compound I (Rhapontigenin)——Colorless plates (dil. MeOH), mp 196—197 °C. UV λ_{max}^{MeOH} nm (log ε): 220 (4.41), 302 (4.49), 322 (4.53). IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1610, 1590, 1510 (C=C). ^{1}H-NMR (acetone-d_6): 3.84 (3H, s, OCH_3), 6.29 (1H, t, J=2 Hz, C_4–H), 6.56 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.84, 7.02 (each 1H, d, J=16 Hz, olefinic H), 6.88 (1H, d, J=8 Hz, $C_{5'}$–H), 7.02 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.09 (1H, d, J=2 Hz, $C_{2'}$–H), 7.60 (1H, OH, disappeared on addition of D_2O), 8.23 (2H, OH, disappeared on addition of D_2O).

Compound II (Rhaponticin)——Colorless needles (dil. acetone), mp 246—248 °C, $[\alpha]_D^{24}$ −56.3° [c=0.88, acetone–H_2O (1 : 1)]. UV λ_{max}^{MeOH} nm (log ε): 219 (4.40), 302 (4.38), 324 (4.48). IR ν_{max}^{KBr} cm^{-1}: 3450, 3320 (OH), 1610, 1580, 1510 (C=C). ^{1}H-NMR (DMSO-d_6): 3.2—3.9 (6H, m, sugar-H), 3.78 (3H, s, OCH_3), 4.85 (1H, d, J=7 Hz, anomeric H), 6.37 (1H, br s, C_4–H), 6.60, 6.75 (each 1H, br s, $C_{2,6}$–H), 6.84, 7.04 (each 1H, d, J=16 Hz, olefinic H), 6.83 (1H, d, J=8 Hz, $C_{5'}$–H), 6.99 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.03 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table I.

Compound III——Colorless needles (dil. acetone), mp 246—248 °C, $[\alpha]_D^{16}$ −70.0° [c=0.4, acetone–H_2O (1 : 1)], *Anal.* Calcd for $C_{21}H_{24}O_9 \cdot 1/2H_2O$: C, 58.73; H, 5.87. Found: C, 58.44; H, 5.68. FD-MS (m/z): 420 $[M]^+$, 258 $[M-glc.]^+$, 163. UV λ_{max}^{MeOH} nm (log ε): 217 (4.40), 303 (4.43), 330 (4.51). ^{1}H-NMR (DMSO-d_6 + D_2O): 3.2—3.9 (6H, m, sugar-H), 3.78 (3H, s, OCH_3), 4.99 (1H, d, J=7 Hz, anomeric H), 6.16 (1H, t, J=2 Hz, C_4–H), 6.44 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.84, 7.02 (each 1H, d, J=16 Hz, olefinic H), 6.95 (1H, d, J=8 Hz, $C_{5'}$–H), 7.13 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.39 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table I.

Acid Hydrolysis of III——A solution of III (150 mg) in 5% H_2SO_4–50% aqueous acetone (15 ml) was refluxed for 4 h. After neutralization with 5% KOH–MeOH, the solution was concentrated to dryness under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was subjected to chromatography over

Sephadex LH-20. Elution with EtOH afforded a sugar [*Rf* 0.34, solvent: *n*-BuOH–pyridine–H_2O (6:4:3)], $[\alpha]_D^{21}$ +50.2° (*c*=0.88, H_2O), identical with D-glucose. Further elution with EtOH furnished an aglycone (38 mg), which was shown to be identical with rhapontigenin (I) by direct comparisons.

Compound IV——Colorless granules (dil. EtOH), mp 189—190 °C, $[\alpha]_D^{16}$ −109.1° (*c*=0.72, acetone), *Anal.* Calcd for $C_{28}H_{28}O_{13}\cdot H_2O$: C, 56.95; H, 5.12. Found: C, 56.72; H, 5.05. FD-MS (*m/z*): 572 $[M]^+$, 420 $[M-galloyl]^+$, 258, 153. ^{1}H-NMR (acetone-d_6+D_2O): 3.4—4.0 (4H, m, sugar-H), 4.42 (1H, dd, *J*=4, 12 Hz, $C_{6''}$–H), 4.66 (1H, dd, *J*=2, 12 Hz, $C_{6''}$–H), 5.45 (1H, d, *J*=7 Hz, anomeric H), 6.56 (1H, t, *J*=2 Hz, C_4–H), 6.72, 6.75 (each 1H, d, *J*=2 Hz, $C_{2,6}$–H), 6.84, 7.00 (each 1H, d, *J*=16 Hz, olefinic H), 6.72 (1H, d, *J*=8 Hz, $C_{5'}$–H), 7.11 (1H, dd, *J*=2, 8 Hz, $C_{6'}$–H), 7.15 (1H, d, *J*=2 Hz, $C_{2'}$–H), 7.16 (2H, s, galloyl H). ^{13}C-NMR: Table I.

Tannase Hydrolysis of IV——An aqueous solution of IV (100 mg) was treated with tannase at room temperature for 1 h. The solution was concentrated to dryness under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was chromatographed over MCI-gel CHP-20P [solvent: H_2O–MeOH (4:1–2:3)] to afford gallic acid and a hydrolysate (28 mg), which was identical with rhaponticin (II).

Compound V——Colorless needles ($CHCl_3$–MeOH), mp 243—244 °C, $[\alpha]_D^{16}$ +15.9° (*c*=0.7, acetone), *Anal.* Calcd for $C_{28}H_{28}O_{13}\cdot 1/2H_2O$: C, 57.83; H, 5.03. Found: C, 57.97; H, 4.90. FD-MS (*m/z*): 572 $[M]^+$, 420 $[M-galloyl]^+$, 258, 153. ^{1}H-NMR (acetone-d_6+D_2O): 3.5—4.1 (5H, m, sugar-H), 3.84 (3H, s, OCH_3), 5.17 (1H, t, *J*=8 Hz, $C_{2''}$–H), 5.26 (1H, d, *J*=8 Hz, anomeric H), 6.43 (1H, t, *J*=2 Hz, C_4–H), 6.67, 6.69 (each 1H, d, *J*=2 Hz, $C_{2,6}$–H), 6.80, 7.00 (each 1H, d, *J*=16 Hz, olefinic H), 6.88 (1H, d, *J*=8 Hz, $C_{5'}$–H), 7.00 (1H, dd, *J*=2, 8 Hz, $C_{6'}$–H), 7.08 (1H, d, *J*=2 Hz, $C_{2'}$–H), 7.17 (2H, s, galloyl H). ^{1}H-NMR (pyridine-d_5): 3.71 (3H, s, OCH_3), 4.0—4.6 (5H, m, sugar-H), 5.77 (1H, d, *J*=8 Hz, anomeric H), 6.14 (1H, t, *J*=8 Hz, $C_{2''}$–H), 6.93 (1H, d, *J*=8 Hz, $C_{5'}$–H), 7.07 (1H, t, *J*=2 Hz, C_4–H), 7.14 (1H, d, *J*=2 Hz, C_6–H), 7.24, 7.41 (each 1H, d, *J*=16 Hz, olefinic H), 7.93 (2H, s, galloyl H).

Tannase Hydrolysis of V——An aqueous solution of V (80 mg) was shaken with tannase at room temperature for 1 h. The reaction mixture was treated in the same way as described above to furnish gallic acid and a hydrolysate (32 mg), which was found to be identical with rhaponticin (II) by direct comparisons.

Compound VI——Colorless granules (dil. MeOH), mp 229—231 °C, $[\alpha]_D^{16}$ −9.8° (*c*=0.64, acetone), *Anal.* Calcd for $C_{30}H_{30}O_{11}\cdot 1/2H_2O$: C, 62.60; H, 5.43. Found: C, 62.43; H, 5.06. FD-MS (*m/z*): 566 $[M]^+$, 420 $[M-p\text{-coumaroyl}]^+$, 283. UV λ_{max}^{MeOH} nm (log ε): 220 (4.32), 302 (sh) (4.43), 315 (4.49). ^{1}H-NMR (DMSO-d_6): 3.2—3.9 (5H, m, sugar-H), 3.79 (3H, s, OCH_3), 4.93 (1H, t, *J*=8 Hz, $C_{2''}$–H), 5.18 (1H, d, *J*=8 Hz, anomeric H), 6.29 (1H, br s, C_4–H), 6.43, 7.64 (each 1H, d, *J*=16 Hz, *p*-coumaroyl olefinic-H), 6.60, 6.71 (each 1H, br s, $C_{2,6}$–H), 6.80, 7.77 (each 2H, d, *J*=8 Hz, *p*-coumaroyl $C_{3,5}$– and $C_{2,6}$–H), 6.82, 7.02 (each 1H, d, *J*=16 Hz, olefinic H), 6.84 (1H, d, *J*=8 Hz, $C_{6'}$–H), 6.99 (1H, dd, *J*=2, 8 Hz, $C_{5'}$–H), 7.03 (1H, d, *J*=2 Hz, $C_{2'}$–H). ^{1}H-NMR (pyridine-d_5): 3.72 (3H, s, OCH_3), 4.0—4.6 (5H, m, sugar-H), 5.81 (1H, d, *J*=8 Hz, anomeric H), 6.09 (1H, t, *J*=8 Hz, $C_{2''}$–H), 6.68, 8.04 (each 1H, d, *J*=16 Hz, *p*-coumaroyl olefinic-H), 6.82 (1H, d, *J*=8 Hz, $C_{5'}$–H). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of VI——A solution of VI (100 mg) in 1% NaOMe–MeOH (10 ml) was left to stand at room temperature for 5 h. The reaction mixture was neutralized with Amberlite IR-120 (H^+ form), and the hydrolysates were separated by chromatography over Sephadex LH-20 (solvent: EtOH) to give methyl *p*-coumarate (16 mg), colorless needles (dil. MeOH), mp 142—143 °C, and a hydrolysate (62 mg) which was identified as rhaponticin (II) by comparison of the physical and spectral data.

Compound VII (Piceatannol)——Colorless plates (dil. MeOH), mp 233—235 °C. UV λ_{max}^{MeOH} nm (log ε): 220 (4.41), 303 (4.36), 323 (4.48). IR ν_{max}^{KBr} cm^{-1}: 3520, 3340 (OH), 1600, 1520 (C=C). ^{1}H-NMR (acetone-d_6): 6.28 (1H, t, *J*=2 Hz, C_4–H), 6.55 (2H, d, *J*=2 Hz, $C_{2,6}$–H), 6.80, 7.00 (each 1H, *J*=16 Hz, olefinic H), 6.80 (1H, d, *J*=8 Hz, $C_{5'}$–H), 6.92 (1H, dd, *J*=2, 8 Hz, $C_{6'}$–H), 7.08 (1H, d, *J*=2 Hz, $C_{2'}$–H), 7.88, 8.00, 8.12 (4H in total, OH, disappeared on addition of D_2O).

Compound VIII——An off-white amorphous powder, $[\alpha]_D^{23}$ −59.8° (*c*=0.5, MeOH), *Anal.* Calcd for $C_{20}H_{22}O_9\cdot 1/2H_2O$: C, 57.83; H, 5.58. Found: C, 57.94; H, 5.42. FD-MS (*m/z*): 406 $[M]^+$, 244 $[M-glc.]^+$, 163. UV λ_{max}^{MeOH} nm (log ε): 219 (4.53), 303 (4.45), 326 (4.54). ^{1}H-NMR (DMSO-d_6): 3.1—3.9 (6H, m, sugar-H), 4.82 (1H, d, *J*=7 Hz, anomeric H), 6.34 (1H, br s, C_4–H), 6.57, 6.72 (each 1H, br s, $C_{2,6}$–H), 6.71 (1H, d, *J*=8 Hz, $C_{5'}$–H), 6.74, 6.98 (each 1H, d, *J*=16 Hz, olefinic H), 6.86 (1H, dd, *J*=2, 8 Hz, $C_{6'}$–H), 6.98 (1H, d, *J*=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table II.

Acid Hydrolysis of VIII——A solution of VIII (70 mg) in 5% H_2SO_4–50% aqueous acetone (6 ml) was refluxed for 4 h. The acetone was removed by evaporation under reduced pressure, and the aqueous solution was subjected to MCI-gel CHP-20P chromatography. Elution with H_2O afforded a sugar fraction which still contained H_2SO_4, and it was therefore neutralized with Amberlite IRA-400 (OH^- form). The sugar was further purified by chromatography over Sephadex LH-20 (solvent: EtOH) to furnish D-glucose [*Rf* 0.34, solvent: *n*-BuOH–pyridine–H_2O (6:4:3)], $[\alpha]_D^{21}$ +46.4° (*c*=0.69, H_2O). The above column was eluted subsequently with H_2O–MeOH (4:1–0:1) to give an aglycone (26 mg) identical with piceatannol (VII).

Compound IX——Colorless needles (dil. MeOH), mp 229—230 °C, $[\alpha]_D^{23}$ −40.4° (*c*=0.56, MeOH), *Anal.* Calcd for $C_{20}H_{22}O_9$: C, 59.10; H, 5.46. Found: C, 58.83; H, 5.31. FD-MS (*m/z*): 406 $[M]^+$, 244 $[M-glc.]^+$, 163. UV λ_{max}^{MeOH} nm (log ε): 217 (4.38), 302 (4.41), 320 (4.48). IR ν_{max}^{KBr} cm^{-1}: 3480, 3350 (OH), 1590, 1520 (C=C). ^{1}H-NMR (DMSO-d_6): 3.1—3.9 (6H, m, sugar-H), 4.76 (1H, d, *J*=7 Hz, anomeric H), 6.15 (1H, t, *J*=2 Hz, C_4–H), 6.41 (2H, d,

J=2 Hz, $C_{2,6}$–H), 6.79 (1H, d, J=8 Hz, $C_{5'}$–H), 6.80, 6.94 (each 1H, d, J=16 Hz, olefinic H), 7.07 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.45 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table II.

Acid Hydrolysis of IX——A solution of IX (250 mg) in 5% H_2SO_4–50% aqueous acetone (12 ml) was refluxed for 5 h. The reaction mixture was worked up as above to afford an aglycone identical with piceatannol (VII), and D-glucose [*Rf* 0.34, solvent: *n*-BuOH–pyridine–H_2O (6 : 4 : 3)], $[\alpha]_D^{21}$ +50.1° (c=0.63, H_2O).

Methylation of IX——A mixture of IX (1 g), dimethyl sulfate (3.5 ml) and anhydrous potassium carbonate (5 g) in dry acetone (70 ml) was refluxed for 1 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated to a syrup, which was chromatographed over silica gel using $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1) to yield the trimethyl ether (IXa) (420 mg), colorless needles (dil. MeOH), mp 175—176 °C, $[\alpha]_D^{23}$ −50.7° (c=0.57, MeOH), *Anal.* Calcd for $C_{23}H_{28}O_9 \cdot 1/2H_2O$: C, 60.38; H, 6.39. Found: C, 60.17; H, 6.18. FD-MS (m/z): 448 $[M]^+$, 286 $[M-glc.]^+$. ^{1}H-NMR (acetone-d_6): 3.5—4.0 (6H, m, sugar-H), 3.80 (6H, s, 2 × OCH_3), 3.83 (3H, s, OCH_3), 4.99 (1H, d, J=7 Hz, anomeric H), 6.37 (1H, t, J=2 Hz, C_4–H), 6.70 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.94 (1H, d, J=8 Hz, $C_{5'}$–H), 6.98, 7.15 (each 1H, d, J=16 Hz, olefinic H), 7.14 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.48 (1H, d, J=2 Hz, $C_{2'}$–H).

Acid Hydrolysis of IXa——A solution of IXa (480 mg) in 5% H_2SO_4–50% aqueous acetone (20 ml) was refluxed for 5 h. The reaction mixure was neutralized with Amberlite IRA-400 (OH^- form), and subjected to chromatography over silica gel (solvent: *n*-hexane) to afford the aglycone (IXc) (250 mg), colorless prisms (benzene), mp 92—94 °C, *Anal.* Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.35; H, 6.27. EI-MS (m/z): 286 $[M]^+$, 225, 78. ^{1}H-NMR (acetone-d_6): 3.80 (6H, s, 2 × OCH_3), 3.84 (3H, s, OCH_3), 6.37 (1H, t, J=2 Hz, C_4–H), 6.72 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.89 (1H, d, J=8 Hz, $C_{5'}$–H), 6.93, 7.14 (each 1H, d, J=16 Hz, olefinic H), 7.00 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.11 (1H, d, J=2 Hz, $C_{2'}$–H), 7.56 (1H, OH, disappeared on addition of D_2O).

Acetylation of IXb——IXb (220 mg) was treated overnight with acetic anhydride (2 ml) and dry pyridine (2 ml), and the reaction mixture was worked up as usual to give the monoacetate (IXc) (210 mg), colorless prisms (MeOH), mp 93—95 °C, *Anal.* Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.41; H, 6.01. EI-MS (m/z): 328 $[M]^+$, 286, 225. ^{1}H-NMR (acetone-d_6): 2.16 (3H, s, $OCOCH_3$), 3.80 (6H, s, 2 × OCH_3), 3.82 (3H, s, OCH_3), 6.39 (1H, t, J=2 Hz, C_4–H), 6.73 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.99, 7.19 (each 1H, d, J=16 Hz, olefinic H), 7.04 (1H, d, J=8 Hz, $C_{5'}$–H), 7.38 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.32 (1H, d, J=2 Hz, $C_{2'}$–H).

Oxidation of IXc——A mixture of IXc (200 mg) and potassium permanganate (1 g) in 0.05 M Na_2HPO_4–35% aqueous acetone was stirred at room temperature for 3 h. The reaction mixture was acidified with 1 N H_2SO_4, and $NaHSO_3$ was added until a colorless suspension was obtained. The acetone was removed by evaporation under reduced pressure, and the aqueous solution was extracted three times with AcOEt. The AcOEt layer, after removal of the solvent, was subjected to chromatography over MCI-gel CHP-20P [H_2O–MeOH (4 : 1–0 : 1)] to afford 3,5-dimethoxybenzoic acid (40 mg), colorless needles (dil. EtOH), mp 185—188 °C, IR ν_{max}^{KBr} cm^{-1}: 2500—3000 (COOH), 1690 (COOH), 1600 (C=C), and acetylisovanilic acid (36 mg), colorless needles (dil. EtOH), mp 217—219 °C, IR ν_{max}^{KBr} cm^{-1}: 2550—3000 (COOH), 1760 ($OCOCH_3$), 1680 (COOH), 1610, 1580, 1510 (C=C).

Compound X——An off-white amorphous powder, $[\alpha]_D^{23}$ +1.7° (c=0.96, MeOH), *Anal.* Calcd for $C_{19}H_{20}O_8 \cdot 1/2H_2O$: C, 59.22; H, 5.49. Found: C, 59.29; H, 5.35. FD-MS (m/z): 376 $[M]^+$, 244 $[M-xyl.]^+$, 133. UV λ_{max}^{MeOH} nm (log ε): 217 (4.46), 305 (4.45), 322 (4.49). ^{1}H-NMR (DMSO-d_6): 3.1—3.9 (5H, m, sugar-H), 4.82 (1H, d, J=7 Hz, anomeric H), 6.14 (1H, t, J=2 Hz, C_4–H), 6.41 (2H, d, J=2 Hz, $C_{2,6}$–H) 6.77, 6.96 (each 1H, d, J=16 Hz, olefinic H), 6.80 (1H, d, J=8 Hz, $C_{5'}$–H), 7.11 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.22 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table II.

Methylation of X——A mixture of X (100 mg), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (1 g) in dry acetone (20 ml) was refluxed for 1.5 h. The reaction mixture was worked up in the same way as described for IX. The reaction products were chromatographed over silica gel [solvent: benzene–acetone (1 : 1–1 : 2)] to afford the trimethyl ether (Xa) (62 mg), colorless needles (dil. MeOH), mp 168—170 °C, $[\alpha]_D^{23}$ −9.1° (c=0.49, MeOH), *Anal.* Calcd for $C_{22}H_{26}O_8 \cdot 1/2H_2O$: C, 61.82; H, 6.37. Found: C, 61.88; H, 6.54. FD-MS (m/z): 418 $[M]^+$, 285 $[M-xyl.]^+$, 133. ^{1}H-NMR (acetone-d_6): 3.3—4.0 (5H, m, sugar-H), 3.80 (6H, s, 2 × OCH_3), 3.84 (3H, s, OCH_3), 5.12 (1H, d, J=7 Hz, anomeric H), 6.38 (1H, t, J=2 Hz, C_4–H), 6.72 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.98, 7.19 (each 1H, d, J=16 Hz, olefinic H), 6.98 (1H, d, J=8 Hz, $C_{5'}$–H), 7.11 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.38 (1H, d, J=2 Hz, $C_{2'}$–H).

Acid Hydrolysis of Xa——A solution of Xa (40 mg) in 5% H_2SO_4–50% aqueous acetone (5 ml) was refluxed for 5 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and the products were chromatographed over MCI-gel CHP-20P [solvent: H_2O–MeOH (4 : 1–0 : 1)] to give an aglycone and a sugar. The aglycone was further purified by silica gel chromatography (solvent: *n*-hexane) to furnish colorless prisms (benzene) (10 mg); this product was identical with compound IXb. The sugar, purified by chromatography over Sephadex LH-20 (solvent: EtOH), was identified as D-xylose [*Rf* 0.48, solvent: *n*-BuOH–pyridine–H_2O (6 : 4 : 3)], $[\alpha]_D^{21}$ +23.9° (c=0.63, H_2O).

Compound XI——An off-white amorphous powder, $[\alpha]_D^{16}$ −89.4° (c=0.83, acetone), *Anal.* Calcd for $C_{27}H_{26}O_{13} \cdot H_2O$: C, 56.25; H, 4.90. Found: C, 56.69; H, 4.74. FD-MS (m/z): 558 $[M]^+$, 406 $[M-glc.]^+$, 244, 170. ^{1}H-NMR (acetone-d_6): 3.4—3.9 (4H, m, sugar-H), 4.50 (1H, dd, J=4, 12 Hz, $C_{6''}$–H), 4.59 (1H, dd, J=2, 12 Hz, $C_{6''}$–H), 4.90 (1H, d, J=7 Hz, anomeric H), 6.28 (1H, t, J=2 Hz, C_4–H), 6.55 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.82, 7.00 (each 1H, d, J=16 Hz, olefinic H), 6.86 (1H, d, J=8 Hz, $C_{5'}$–H), 7.20 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 7.20 (2H, s, galloyl H), 7.36 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR: Table II.

Tannase Hydrolysis of XI——An aqueous solution of XI (80 mg) was incubated with tannase at room temperature for 30 min. The reaction mixture was worked up as described for IV to yield gallic acid and a hydrolysate (23 mg), which was identical with IX.

Compound XII——Colorless needles (dil. acetone), mp 227—228 °C, $[\alpha]_D^{16}$ −59.5° [c=0.37, acetone–H_2O (1 : 1)]. UV λ_{max}^{MeOH} nm (log ε): 215 (4.40), 306 (4.52), 319 (4.52). IR ν_{max}^{KBr} cm^{-1}: 3300—3400 (OH), 1590, 1510 (C=C). ^{1}H-NMR (DMSO-d_6): 3.2—3.9 (6H, m, sugar-H), 3.76 (3H, s, OCH_3), 4.83 (1H, d, J=7 Hz, anomeric H), 6.38 (1H, br s, C_4–H), 6.62, 6.78 (each 1H, br s, $C_{2,6}$–H), 6.90, 7.12 (each 1H, d, J=16 Hz, olefinic H), 6.93, 7.51 (each 2H, d, J=8 Hz, $C_{3',5'}$- and $C_{2',6'}$–H). ^{13}C-NMR: Table III.

Compound XIII——An off-white amorphous powder, $[\alpha]_D^{16}$ −101.6° (c=0.68, acetone), *Anal.* Calcd for $C_{28}H_{28}O_{12}\cdot 1/2H_2O$: C, 59.46; H, 5.22. Found: C, 59.65; H, 5.08. FD-MS (m/z): 579 $[M+Na]^+$, 565 $[M]^+$, 405, 278. ^{1}H-NMR (acetone-d_6): 3.5—3.9 (4H, m, sugar-H), 3.79 (3H, s, OCH_3), 4.42 (1H, dd, J=4, 12 Hz, $C_{6''}$–H), 4.67 (1H, dd, J=2, 12 Hz, $C_{6''}$–H), 5.04 (1H, d, J=7 Hz, anomeric H), 6.56 (1H, t, J=2 Hz, C_4–H), 6.52, 6.58 (each 1H, d, J=2 Hz, $C_{2,6}$–H), 6.84, 7.08 (each 1H, d, J=16 Hz, olefinic H), 6.88, 7.40 (each 2H, d, J=8 Hz, $C_{3',5'}$– and $C_{2',6'}$–H), 7.16 (2H, s, galloyl H). ^{13}C-NMR: Table III.

Tannase Hydrolysis of XIII——A solution of XIII (80 mg) in H_2O was shaken with tannase at room temperature for 1 h. The reaction mixture was treated in the same manner as described for IV to furnish gallic acid and a hydrolysate (33 mg), which was identical with desoxyrhaponticin (XII).

Compound XIV——Colorless needles (H_2O), mp 256—257 °C, $[\alpha]_D^{16}$ −88.2° (c=0.66, acetone). ^{1}H-NMR (acetone-d_6+D_2O): 3.5—4.0 (4H, m, sugar-H), 4.48 (1H, dd, J=8, 12 Hz, $C_{6''}$–H), 4.68 (1H, dd, J=2, 12 Hz, $C_{6''}$–H), 5.03 (1H, d, J=7 Hz, anomeric H), 6.30 (1H, t, J=2 Hz, C_4–H), 6.58 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.87, 7.04 (each 1H, d, J=16 Hz, olefinic H), 7.06, 7.44 (each 2H, d, J=8 Hz, $C_{3',5'}$– and $C_{2',6'}$–H), 7.13 (2H, s, galloyl H). ^{13}C-NMR: Table III.

Compound XV——An off-white amorphous powder, $[\alpha]_D^{19}$ −33.6° (c=0.78, acetone), *Anal.* Calcd for $C_{21}H_{24}O_9\cdot 1/2H_2O$: C, 58.73; H, 5.87. Found: C, 59.15; H, 5.86. FD-MS (m/z): 443 $[M+Na]^+$, 420 $[M]^+$, 404 $[M-OH]^+$, 163. UV λ_{max}^{MeOH} nm (log ε): 216 (4.46), 288 (4.08). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1610, 1590, 1510 (C=C). ^{1}H-NMR (DMSO-d_6): 3.1—3.8 (6H, m, sugar-H), 3.74 (3H, s, OCH_3), 4.67 (1H, d, J=8 Hz, anomeric H), 6.30, 6.46 (each 1H, d, J=12 Hz, olefinic H), 6.28—6.44 (3H, m, $C_{2,4,6}$–H), 6.66 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 6.70 (1H, d, J=2 Hz, $C_{2'}$–H), 6.82 (1H, d, J=8 Hz, $C_{5'}$–H). ^{13}C-NMR: Table IV.

Photoisomerization of II——A solution of II (1.06 g) in 50% aqueous dioxane (505 ml) was irradiated with a low-pressure mercury lamp (30 W) for 5 h with stirring. The solution was concentrated to dryness under reduced pressure, and the residue was treated with dil. MeOH. The insoluble portion consisted mainly of unreacted II. The soluble portion was subjected to Sephadex LH-20 chromatography with 60% aqueous MeOH to furnish a *cis*-isomer (450 mg), which was shown to be identical with compound XV by comparisons of the physical and spectral data.

Compound XVI——An off-white amorphous powder, $[\alpha]_D^{19}$ −47.4° (c=0.78, acetone), *Anal.* Calcd for $C_{28}H_{28}O_{13}\cdot 3/2H_2O$: C, 56.09; H, 5.21. Found: C, 55.92; H, 5.54. FD-MS (m/z): 595 $[M+Na]^+$, 572 $[M]^+$, 420 [M−galloyl]$^+$, 258. UV λ_{max}^{MeOH} nm (log ε): 217 (4.46), 280 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (Ar–COO–), 1610, 1590, 1510 (C=C). ^{1}H-NMR (acetone-d_6): 3.4—3.9 (4H, m, sugar-H), 3.80 (3H, s, OCH_3), 4.32 (1H, dd, J=4, 12 Hz, $C_{6''}$–H), 4.49 (1H, dd, J=2, 12 Hz, $C_{6''}$–H), 4.74 (1H, d, J=7 Hz, anomeric H), 6.26, 6.43 (each 1H, d, J=12 Hz, olefinic H), 6.44 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.54 (1H, t, J=2 Hz, C_4–H), 6.67 (1H, dd, J=2, 8 Hz, $C_{6'}$–H), 6.80 (1H, d, J=2 Hz, $C_{2'}$–H), 6.82 (1H, d, J=8 Hz, $C_{5'}$–H), 7.14 (2H, s, galloyl H). ^{13}C-NMR: Table IV.

Photoisomerization of IV——A solution of IV (122 mg) in 50% aqueous dioxane (43 ml) was irradiated with a low-pressure mercury lamp (30 W) for 5 h. The reaction mixture was worked up in the same way as described above to give a *cis*-isomer (20 mg), which was identical with compound XVI.

Compound XVII——Colorless needles (H_2O), mp 147—148 °C, $[\alpha]_D^{19}$ −33.4° (c=0.78, acetone), *Anal.* Calcd for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98. Found: C, 62.83; H, 6.07. FD-MS (m/z): 443 $[M+K]^+$, 404 $[M]^+$, 163. UV λ_{max}^{MeOH} nm (log ε): 215 (4.30), 284 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1605, 1590, 1510 (C=C). ^{1}H-NMR (DMSO-d_6): 3.1—3.7 (6H, m, sugar-H), 3.72 (3H, s, OCH_3), 4.60 (1H, d, J=8 Hz, anomeric H), 6.32 (2H, d, J=2 Hz, $C_{2,6}$–H), 6.36, 6.52 (each 1H, d, J=12 Hz, olefinic H), 6.38 (1H, t, J=2 Hz, C_4–H), 6.82, 7.18 (each 2H, d, J=8 Hz, $C_{3',5'}$– and $C_{2',6'}$–H). ^{13}C-NMR: Table IV.

Photoisomerization of XII——A solution of XII (614 mg) in 50% aqueous dioxane (304 ml) was irradiated with a low-pressure mercury lamp for 5 h. Work-up as above gave a *cis*-isomer (200 mg), which was identical with compound XVII.

Photoisomerization of V and I——A solution of V (50 mg) in 50% aqueous dioxane (18 ml) was irradiated with a low-pressure mercury lamp (30 W) for 5 h. Evaporation of the solvent under reduced pressure gave a mixture of compounds XVIII and V, which was directly subjected to ^{1}H-NMR examination. Similarly, a solution of I (50 mg) in 50% aqueous dioxane (39 ml) was treated as described for V to afford a mixture of compounds XIX and I.

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