Tannins and Related Compounds. LXXXIII.[1] Isolation and Structures of Hydrolyzable Tannins, Phillyraeoidins A—E from *Quercus phillyraeoides*

Gen-ichiro NONAKA, Shinji NAKAYAMA and Itsuo NISHIOKA*

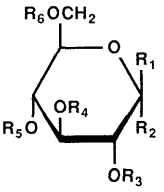
Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received January 21, 1989*

A chemical examination of the leaves of *Quercus phillyraeoides* A. GRAY (Fagaceae) has led to the isolation of five new tannins, phillyraeoidins A—E (27—31), together with twenty-six structurally known tannins and related compounds including gallotannins (1, 2), ellagitannins (3—14), proanthocyanidins (15—17), complex tannins (18—21) and simple phenol glucoside gallates (22—26). On the basis of spectroscopic and chemical evidence, the structures of phillyraeoidins A—D have been established as dimeric hydrolyzable tannins in which two glucose units are linked through a valoneayl ester bond, while phillyraeoidin E was characterized as 1,2,3,6-tetra-*O*-galloyl-4-valoneayl(lactone)-β-D-glucose.

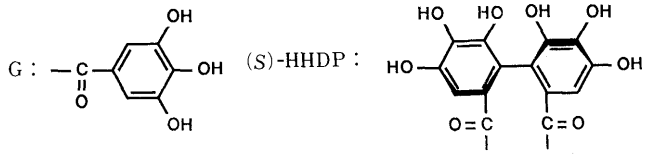
Keywords *Quercus phillyraeoides*; Fagaceae; dimeric hydrolyzable tannin; phillyraeoidin; valoneaic acid; gallotannin; ellagitannin; proanthocyanidin; complex tannin; phenol glucoside gallate

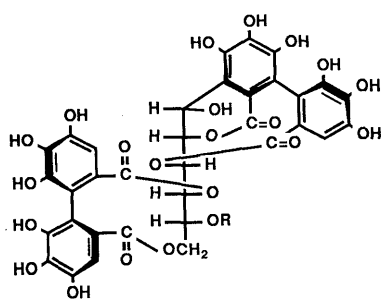
Our earlier work[1-12] showed that the Fagaceous plants elaborate an exceptionally complicated mixture of polyphenols consisting of condensed, hydrolyzable and complex tannins, and that the patterns of the phenolic components differ remarkably in each genus and species. For example, *Quercus stenophylla* MAKINO was found to contain more than seventy gallo- and ellagitannins based on a variety of polyalcohol cores such as D-glucopyranose,[7] quinic acid,[2] proto- and *scyllo*-quercitols[3] and a simple phenol glucoside (salidroside),[4] whereas tannins in *Q. mongolica* FISCHER var. *grosseserrata* (BLUME) REHD. *et* WILS. are composed mainly of galloyl shikimic acids,[11] *C*-glycosidic ellagitannins[12] and complex tannins.[12] As part of our systematic chemical studies on tannins in Fagaceous plants, we have examined *Quercus phillyraeoides* A. GRAY (Japanese name: ubamegashi), which is a relatively small evergreen tree distributed along the coast of the temperate Asia and sometimes planted in Japan as a hedge and ornamental purposes. As a result, we have isolated, together with twenty-six tannins and related compounds, five new hydrolyzable tannins named phillyraeoidins A, B, C, D and E, among which A—D are the first dimeric hydrolyzable tannins to be found in the Fagaceous plants. This paper presents details of the isolation and structural elucidation of these compounds.

Initial fractionation of the aqueous acetone extract of the fresh leaves of *Q. phillyraeoides* was achieved by Sephadex LH-20 chromatography with a solvent system of water–methanol–acetone.[13] Each fraction was repeatedly chromatographed on Sephadex LH-20 with ethanol and/or water–methanol and on various reverse-phase gels such as Fuji gel ODS G3, MCI-gel CHP 20P and Bondapak C_{18}/Porasil B with water containing increasing proportions of methanol to afford thirty-one compounds (**1**—**31**). Among them, compounds **1**—**26** were shown by physical and spectral comparisons to be known gallotannins [1,2,3-tri-*O*- (**1**)[14] and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucoses (**2**)[15]], ellagitannins [2,3-(*S*)-hexahydroxy diphenoyl (HHDP)-glucose (**3**),[16] 6-*O*-galloyl-2,3-(*S*)-HHDP-glucose (**4**),[17] 1-*O*-galloyl-4,6-(*S*)-HHDP-β-D-glucose (**5**),[18] 2-*O*-galloyl-4,6-(*S*)-HHDP-glucose (**6**),[19] pedunculagin (**7**),[20] 1(β)-*O*-galloyl pedunculagin (**8**),[21] eugeniin (**9**),[22] casuariin (**10**),[7] casuarinin (**11**),[7] castalagin (**12**),[23] vescalagin (**13**) and grandinin (**14**),[24]], proanthocyanidins [procyanidins B-1 (**15**) and B-3 (**16**)[25] and gallocatechin-(4α→8)-catechin

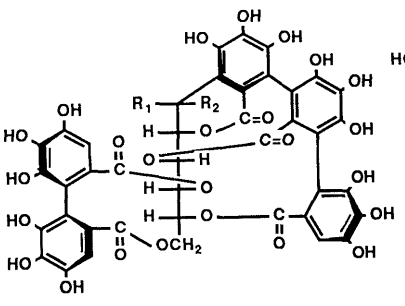


	R_1	R_2	R_3	R_4	R_5	R_6
1	OG	H	G	G	H	H
2	OG	H	G	G	G	G
3	(H, OH)		(*S*)-HHDP		H	H
4	(H, OH)		(*S*)-HHDP		H	G
5	OG	H	H	H	(*S*)-HHDP	
6	(H, OH)		G	H	(*S*)-HHDP	
7	(H, OH)		(*S*)-HHDP		(*S*)-HHDP	
8	OG	H	(*S*)-HHDP		(*S*)-HHDP	
9	OG	H	G	G	(*S*)-HHDP	

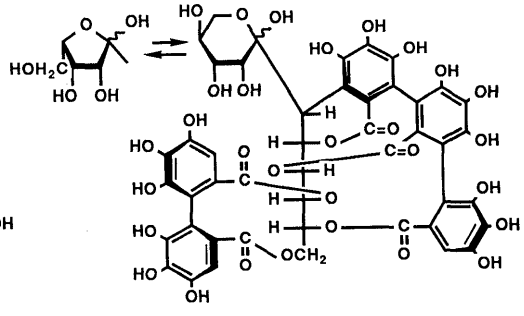




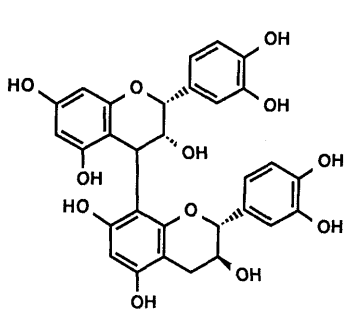
10 : R=H
11 : R=G



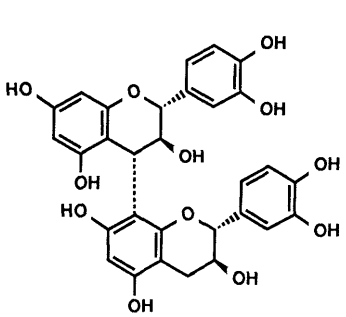
12 : R_1=OH, R_2=H
13 : R_1=H, R_2=OH



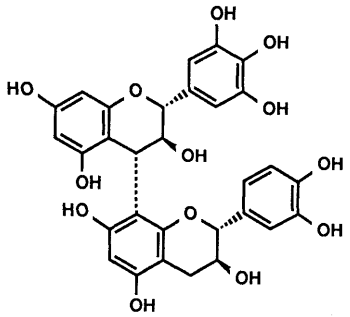
14



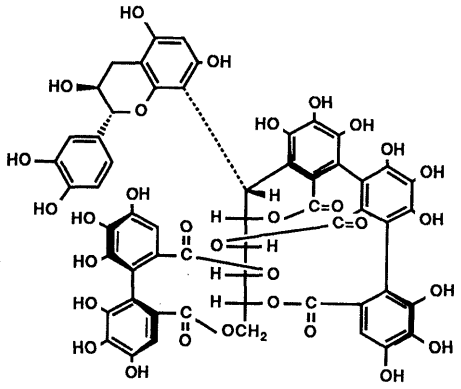
15



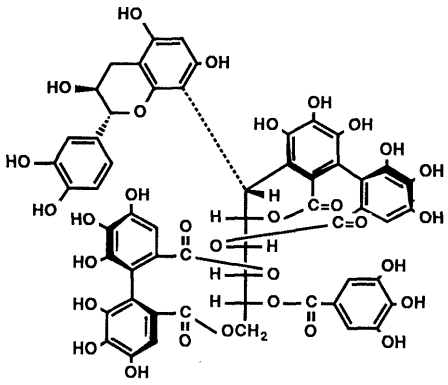
16



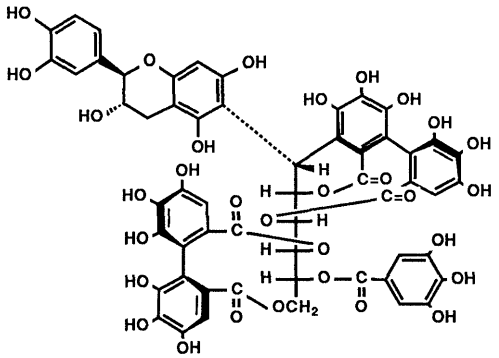
17



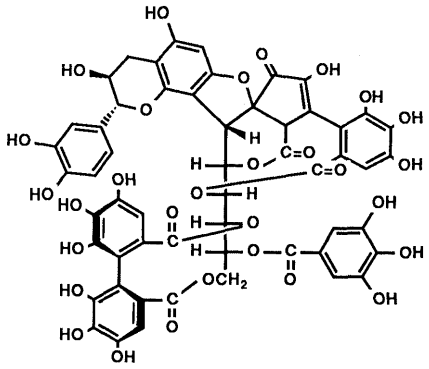
18



19



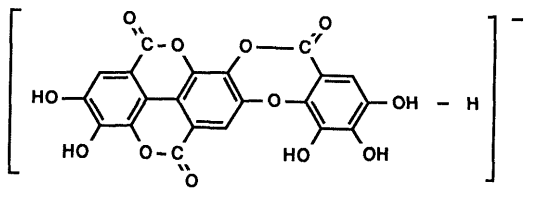
20



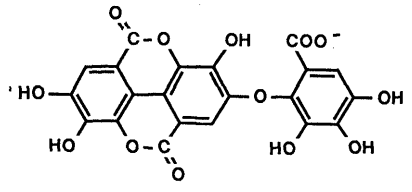
21

(**17**)[26]], complex tannins [acutissimin A (**18**),[10] stenophyllanins A (**19**) and B (**20**)[7] and stenophyllinin A (**21**)[27]] and simple phenol glucoside gallates [6′′-*O*-galloyl salidroside (**22**),[4] gallic acid methyl ester 3-*O*-β-D-(6′-*O*-galloyl)-glucoside (**23**),[14] *p*-hydroxyphenethylalcohol 4′-*O*-β-D-(6′′-*O*-galloyl)-glucoside (**24**),[14] esculin 6′-*O*-gallate (**25**)[14] and 1-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-β-D-glucose (**26**)[14]].

Phillyraeoidin E (**27**) gave a dark blue coloration with the ferric chloride reagent. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited well-defined sugar signals (δ 5.55, dd, J=8, 9 Hz, H-2; δ 5.79, t, J=9 Hz, H-4; δ 5.91, t, J=9 Hz, H-3; δ 6.19, d, J=8 Hz, H-1), the coupling patterns and chemical shifts being in good agreement with those of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**2**).[15] The aromatic signals consist of four two-proton singlets (δ 6.76, 6.97, 7.00, 7.04) attributable to galloyl groups and three



m/z 451



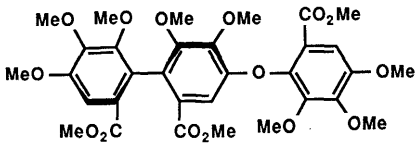
m/z 469

one-proton singlets (δ7.02, 7.13, 7.59), which are considered to be due to a valoneayl group. The relatively lowfield shifts of two of the one-proton signals suggested that the valoneayl group forms dilactone rings. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum showed seven carboxyl carbon signals, of which two are shifted upfield (δ 160.6, 161.1), being consistent with the presence of a valoneayl dilactone.[28] Further support for this was obtained from the negative fast atom bombardment mass spectrum, (FAB-MS), which exhibited an intense $(M-H)^-$ ion peak at *m/z* 1239, together with fragment peaks at *m/z* 451 and 469 arising from the fission of the ester linkage.

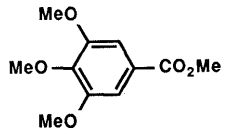
When treated with tannase, **27** furnished gallic acid and a partial hydrolysate (**27a**). The ^{1}H-NMR spectrum (in acetone-d_6) of **27a** was duplicated owing to the existence of α- and β-anomers. The observation of only the valoneayl signals [δ 7.16 (1/2 H), 7.18 (1/2 H), 7.24 (1H), 7.63 (1/2 H), 7.64 (1/2 H), each s] in the aromatic field, as well as the negative FAB-MS data [*m/z* 631 $(M-H)^-$], was consistent with the valoneayl (dilactone) glucose structure. In the two-dimensional ^{1}H–^{1}H-shift correlation (^{1}H–^{1}H COSY) spectrum (in pyridine-d_5) of **27a**, the lowfield triplets [δ 5.77 (1/2 H), 5.78 (1/2 H)], attributed to the proton geminal to the valoneayl group, were readily assignable to H-4 of the glucose moiety. Thus, the location of the valoneayl group was concluded to be at the C-4 position. Based on these findings, the structure of phillyraeoidin E was concluded to be represented by the formula **27**.

Phillyraeoidin A (**28**), the major compound (0.4% of the fresh leaves), was isolated from the most polar tannin fraction. The ^{1}H-NMR spectrum revealed the presence of seven galloyl groups (see Experimental) and one valoneayl group (δ6.24, 6.56, 6.97, each 1H, s). In the aliphatic region, two anomeric proton signals (δ 6.09, 6.17, each d, J=8 Hz) were observed, together with sugar signals all having large coupling constants. These observations indicated the presence of two β-glucopyranose moieties with $^4C_1$ conformation. Furthermore, the observation of a prominent $(M-H)^-$ peak at *m/z* 1875 in the negative FAB-MS supported the dimeric hydrolyzable tannin structure.

Methylation of **28** with dimethyl sulfate and potassium carbonate in dry acetone afforded the nonacosamethyl ether (**28a**). On subsequent alkaline methanolysis, **28a** liberated trimethyl octa-*O*-methyl valoneate (**28b**) and methyl tri-*O*-methyl gallate (**28c**). The measurement [$-15.9°$ ($CHCl_3$)] of the specific optical rotation of **28b** established the atropisomerism of the biphenyl bond to be in the *S*-series.[29]



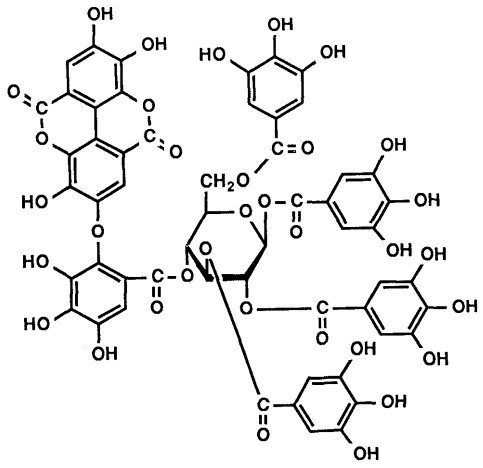
28b



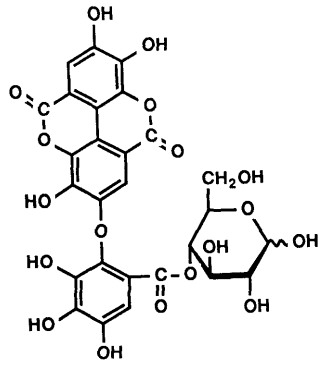
28c

In order to allocate the galloyl and valoneayl groups in **28**, the following experiments were carried out. On tannase hydrolysis, **28**, gave gallic acid and a hydrolysate (**28d**), whose ^{1}H-NMR spectrum exhibited signals (δ 6.11, 6.75, 7.15) due to a valoneayl group and no galloyl peaks. Methylation of **28d** with dimethyl sulfate and potassium carbonate, followed by methyl iodide and silver oxide in dimethyl formamide (DMF), yielded the permethyl derivative, which was treated successively with sodium methoxide in methanol and methanolic hydrochloride. The methylated sugars thus obtained were analyzed by gas-liquid chromatography (GLC) to detect methyl 2,3,6-tri-*O*-methyl- and methyl 2,3-di-*O*-methyl-α-D-glucopyranosides. On the basis of these results, the valoneayl group was determined to be located at the C-4 and C-4/C-6 positions in the glucose moieties. This conclusion is consistent with the co-occurrence of phillyraeoidin E (**27**) and also with the result of hot water treatment of **28d**, which led to the formation of 4-*O*-galloylglucose (**28e**)[30] through the facile cleavage of the aromatic ether linkage.[31] Since all of the glucose signals were observed shifted downfield in the ^{1}H-NMR spectrum of **28**, the galloyl groups could be concluded to be located at the remaining glucose hydroxyl groups (the possibility of the presence of a depsidically linked galloyl group is excluded). Accordingly, the structure of phillyraeoidin A is shown by the formula **28**.

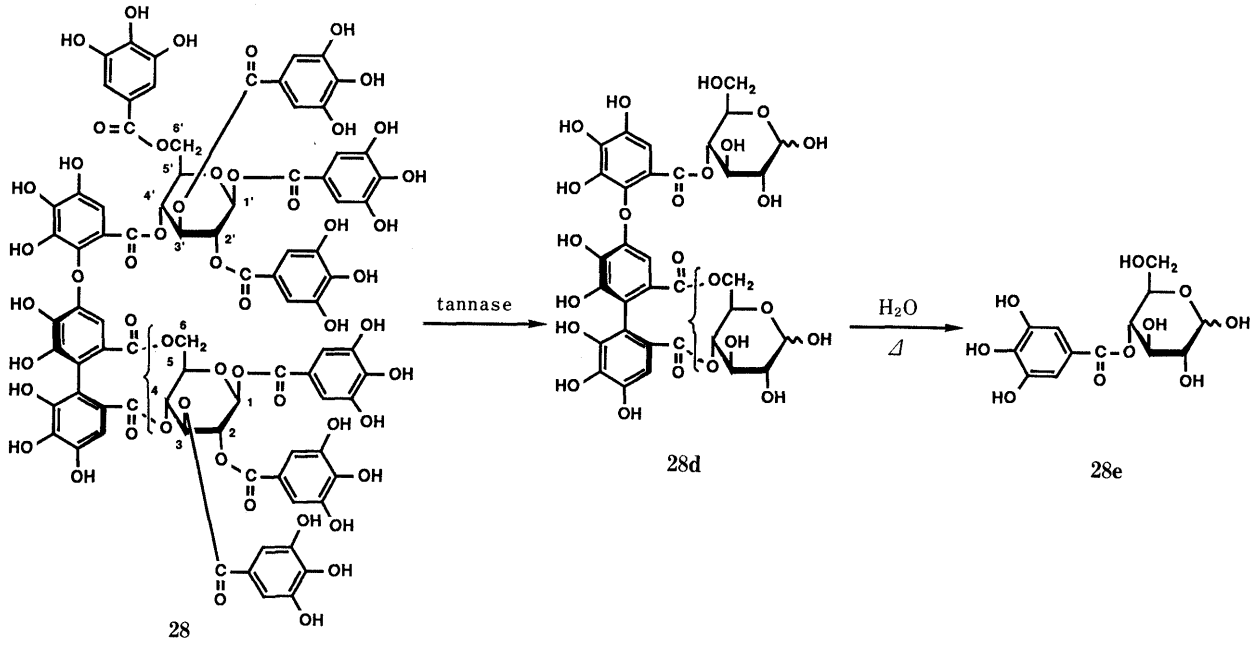
The negative FAB-MS of phillyraeoidin B (**29**) exhibited the $[M-H]^-$ peak at *m/z* 1723, which corresponded to the loss of one galloyl group from the molecule of **28**. The ^{1}H-NMR spectrum was duplicated, revealing a mixture of α- and β-anomers. The upfield shift (δ 5.09, d, J=8 Hz, β-H; δ 5.54, d, J=4 Hz, α-H) of one of the anomeric proton signals indicated the absence of a galloyl group at the sugar C-1 position.



27



27a



28 28d 28e

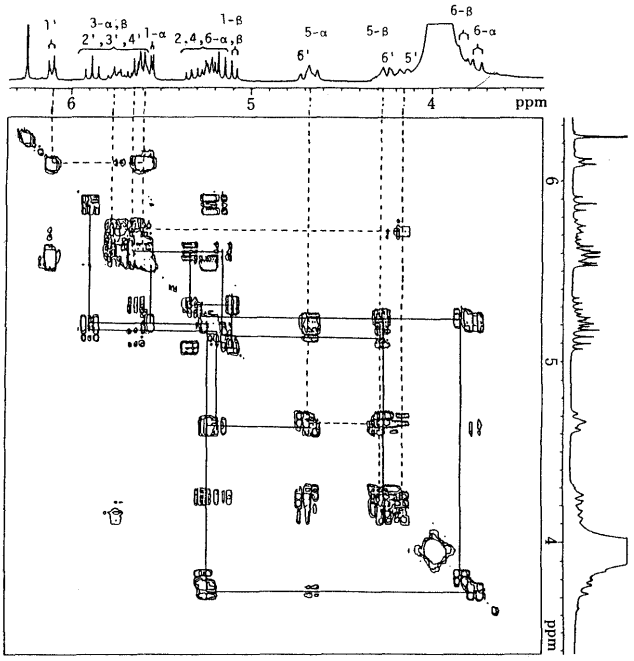


Fig. 1. ^1H–^1H COSY Spectrum of **29** (in Acetone-d_6 + D_2O)

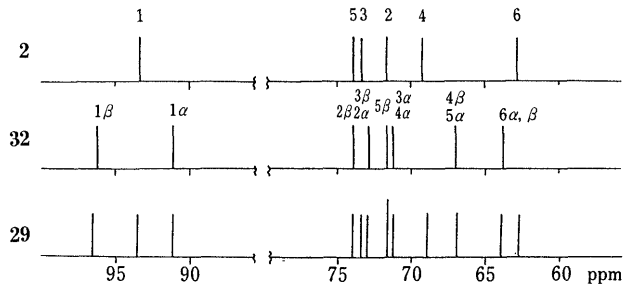
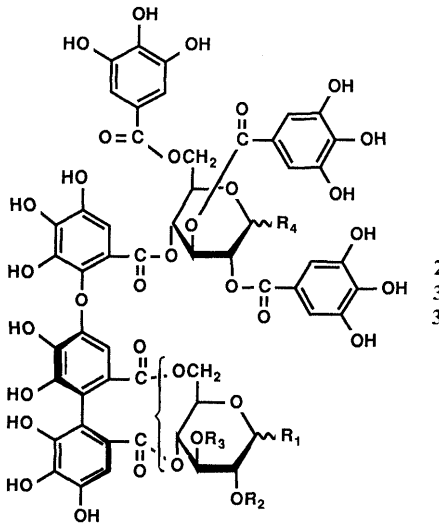


Fig. 2. ^{13}C-NMR Spectra of **2**, **32** and **29** (in Acetone-d_6 + D_2O)

When hydrolyzed with tannase, **29** yielded gallic acid and a hydrolysate, which was found to be identical with **28d**, thus confirming the location and the atropisomerism



	R_1	R_2	R_3	R_4
29	OH	G	G	β-OG
30	β-OG	H	H	β-OG
31	β-OG	H	H	OH

of the valoneayl group to be the same as those of **28**.

The ^{13}C-NMR spectrum of **29** was consistent with the structure of 1-desgalloyl phillyraeoidin A. The lack of the C-1 galloyl group was also indicated by the appearance of two split anomeric signals at δ 91.9 and 96.5 arising from α- and β-anomers, respectively, and the chemical shifts of the sugar signals were in good agreement with those of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**2**) plus 1-desgalloyl eugeniin (**32**) (Fig. 2). Furthermore, all the glucose signals (except for H-1), assigned on the basis the of ^1H–^1H COSY spectrum, were found to be shifted downfield. From these findings, the structure of phillyraeoidin B was concluded to be represented by the formula **29**.

The negative FAB-MS of phillyraeoidins C (**30**) and D (**31**) showed the $(M-H)^-$ peaks at *m/z* 1571 and 1419, in agreement with the losses of two and three galloyl groups, respectively, from **28**. On tannase hydrolysis, both compounds yielded **28d**, together with gallic acid. In the ^1H-NMR spectrum of **30**, two glucose signals due to methines free of the galloyl group were observed at upper field (*ca.* δ 3.8). The assignments of these signals were based on the

spin-decoupling experiment (Fig. 3). When the anomeric doublet at δ 5.68 was irradiated, one of the upfield signals was changed into a doublet. Next, irradiation of a triplet at δ 4.93 caused a change of the upper field signal. Since it is evident from the result of tannase hydrolysis that the valoneayl group is located at the C-4′ and C-4/C-6 positions of the glucose moieties, the relatively lowfield signal at δ 4.93 was assigned to H-4, and the two upfield signals therefore to H-2 and H-3. The absence of the galloyl groups at the C-2 and C-3 positions was also supported by the ^{13}C-NMR spectrum of **30**, which showed close resemblance with the combined spectra of **2** and **5**. On the basis of these findings, phillyraeoidin C was characterized as 2,3-des-galloyl phillyraeoidin A (**30**).

The ^{1}H- and ^{13}C-NMR spectra of **31** were similar to those of **30**, but were more complicated owing to the existence of α- and β-anomers. The absence of the galloyl group at the C-1′ position was indicated by sugar resonances, analogous to those of 2,3,4,6-tetra-*O*-galloyl-D-glucose (**32**). The rest of the sugar signals, on the other hand, were found to correspond well with those of **5**. Further chemical work could not be done because of the small amount of the sample, but from the above-mentioned results, we concluded the structure of phillyraeoidin D to be represented by the formula **31**.

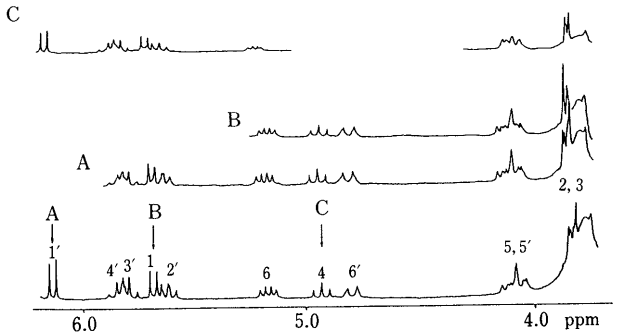


Fig. 3. ^{1}H-NMR Spin-Decoupling of **30** (in Acetone-d_6 + D_2O)

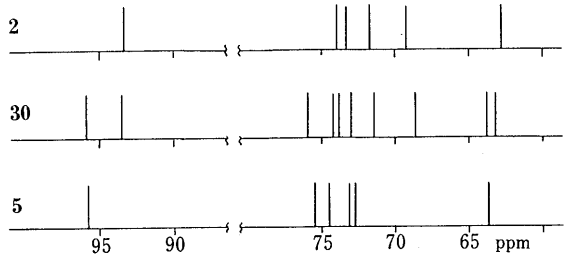
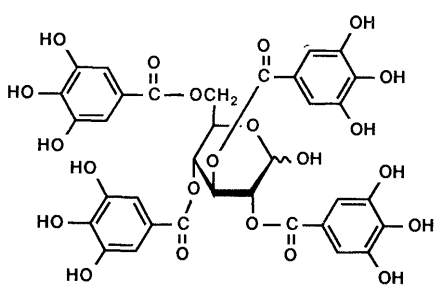


Fig. 4. ^{13}C-NMR Spectra of **2**, **30** and **5** (in Acetone-d_6 + D_2O)



32

Although we have so far examined one *Castanopsis*, two *Castanea* and eight *Quercus* species, this is the first isolation of dimeric hydrolyzable tannins from Fagaceous plants, and the occurrence of these compounds as major metabolites in *Q. phillyraeoides* is interesting from the viewpoint of chemotaxonomy.

Experimental

Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FD-MS (for methyl ethers) were taken with a JEOL JMS DX-300 instrument equipped with a JMA 3500 data system and FAB-MS (for phenolics) with a JEOL JMS-HX 100/JMA 3500 data system using dimethyl sulfoxide/glycerin as a matrix. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL PS-100, JEOL FX-100, GX-270 and GX-400 spectrometers using tetramethylsilane as an internal standard, and chemical shifts are given on the δ-scale. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.), Bondapak C_{18}/Porasil B (37—75 μ, Waters Associates, Inc.), Fuji gel ODS-G3 (43—65 μ, Fuji gel Hanbai Co., Ltd.) and Kieselgel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck) and precoated cellulose F_{254} plates (0.1 mm, Merck) with solvent systems of benzene–ethyl formate–formic acid (Kieselgel: 2:7:1, 1:7:1, 1:5:2. cellulose: 2% acetic acid) (for phenolics) and benzene–acetone (for methyl ethers), and spots were located by ultraviolet illumination (Manasul light 2536 Å) and by spraying the ferric chloride reagent or 10% sulfuric acid followed by heating. Analytical GLC for methyl sugars was performed with a Shimadzu GC 4BM PF instrument over 1% neopentyl glycol succinate polyester (carrier gas: nitrogen).

Extraction and Isolation The fresh leaves (7.7 kg) of *Q. phillyraeoides*, collected at Matsuyama City, Ehime Prefecture, were extracted four times with 80% aqueous acetone at room temperature. The combined extracts were concentrated under reduced pressure, and the resulting precipitates, consisting mainly of chlorophylls and waxes, were removed by filtration. The filtrate was subjected to Sephadex LH-20 chromatography. Elution with water containing increasing proportions of methanol and finally with a mixture of water and acetone afforded three fractions. The first fraction was rechromatographed on Sephadex LH-20 with ethanol containing increasing amounts of water to give 2,3-(*S*)-HHDP[32]-D-glucose (**3**) (191 mg), vescalagin (**13**) (198 mg) and grandinin (**14**) (483 mg). Similar chromatography of the second fraction on Sephadex LH-20 furnished further five fractions (II-1—5). Repeated chromatography of fraction II-1—3 on MCI-gel CHP 20P, Fuji gel ODS G3 and Bondapak C_{18}/Porasil B with a mixture of water and methanol yielded 6-*O*-galloyl-2,3-(*S*)-HHDP-D-glucose (**4**) (43 mg), 1-*O*-galloyl-4,6-(*S*)-HHDP-β-D-glucose (**5**) (310 mg), 2-*O*-galloyl-4,6-(*S*)-HHDP-D-glucose (**6**) (40 mg), procyanidins B-1 (**15**) (767 mg) and B-3 (**16**) (264 mg), gallocatechin-(4α→8)-catechin (**17**) (121 mg), 6″-*O*-galloyl salidroside (**22**) (365 mg), gallic acid methyl ester 3-*O*-β-D-(6′-*O*-galloyl)-glucose (**23**) (32 mg), *p*-hydroxyphenethylalcohol 4′-*O*-β-D-(6″-*O*-galloyl)-glucoside (**24**) (44 mg), esculin 6′-*O*-gallate (**25**) (22 mg) and 1-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-β-D-glucose (**26**) (16 mg). Fraction II-4 was rechromatographed on Sephadex LH-20 with water containing increasing amounts of methanol to give casuariin (**10**) (1.43 g) and phillyraeoidin E (**27**) (129 mg). Chromatography of fraction II-5 over MCI-gel CHP 20P with water and methanol furnished castalagin (**12**) (1.94 g), acutissimin A (**18**) (3.28 g), stenophyllanin B (**21**) (190 mg), stenophyllinin A (**21**) (220 mg) and phillyraeoidin D (**31**) (90 mg). The final fraction was rechromatographed on Sephadex LH-20 and then with Fuji gel ODS-G3 with water and methanol to afford 1,2,3-tri-*O*- (**1**) (221 mg) and 1,2,3,4,6-penta-*O*-galloylglucoses (**2**) (43 mg), pedunculagin (**7**) (358 mg), 1(β)-*O*-galloyl pedunculagin (**8**) (152 mg), eugeniin (**9**) (134 mg), casuarinin (**11**) (125 mg), stenophyllanin A (**19**) (46 mg) and phillyraeoidins A (**28**) (30.0 g), B (**29**) (3.30 g) and C (**30**) (163 mg).

Phillyraeoidin E (27) A pale brown amorphous powder, $[\alpha]_D^{28}$ +33.9° (c = 0.89, acetone). *Anal.* Calcd for $C_{25}H_{36}O_{34}\cdot 13/2\,H_2O$: C, 48.64; H, 3.64. Found: C, 48.40; H, 3.64. Negative FAB-MS *m/z*: 1239 $(M-H)^-$. ^{1}H-NMR (acetone-d_6 + D_2O, 270 MHz): 3.95 (1H, dd, J=3, 13 Hz, H-6), 4.38 (2H, m, H-5, 6), 5.55 (1H, dd, J=8, 9 Hz, H-2), 5.79 (1H, t, J=9 Hz, H-4), 5.91 (1H, t, J=9 Hz, H-3), 6.19 (1H, d, J=8 Hz, H-1), 7.02, 7.13, 7.59 (each 1H, s, valoneayl H), 6.76, 6.97, 7.00, 7.04 (each 2H, s, galloyl H). ^{13}C-NMR (acetone-d_6 + D_2O, 25.05 MHz): 62.2 (C-6), 68.9 (C-4), 71.8

(C-2), 73.4 (C-3, 5), 93.3 (C-1), 160.6, 161.1 (δ-lactone), 164.5, 165.4, 166.3 (×2C), 166.6 (–COO–).

Tannase Hydrolysis of 27 A solution of **27** (46 mg) in water (5 ml) was shaken with tannase for 1 h at room temperature. The reaction mixture was directly applied to a column of Fuji gel ODS-G3, and elution with water containing increasing proportions of methanol afforded gallic acid and a hydrolysate (**27a**) (17 mg) as a pale brown amorphous powder, $[\alpha]_D^{20}$ +45.4° (c=0.4, acetone). Negative FAB-MS m/z: 631 $(M-H)^-$. ^{1}H-NMR (pyridine-d_5, 270 MHz): 4.07 (dd, J=8.9 Hz, H-2α), 4.11 (dd, J=3, 9 Hz, H-2β), 4.34 (t, J=2, 9 Hz, H-3β), 4.58 (m, H-5α), 4.79 (t, J=9 Hz, H-3α), 5.24 (d, J=8 Hz, H-1β), 5.75 (d, J=3 Hz, H-1α), 5.77 (t, J=9 Hz, H-4α), 5.78 (t, J=9 Hz, H-4β), 7.84, 7.86, 8.04, 8.10, 8.11 (3H in total, valoneayl H).

Phillyraeoidin A (28) A pale brown amorphous powder, $[\alpha]_D^{31}$ +75.8° (c=1.1, acetone). *Anal.* Calcd for $C_{82}H_{60}O_{52}\cdot 4H_2O$: C, 50.52; H, 3.52. Found: C, 50.58; H, 3.76. Negative FAB-MS m/z: 1875 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O, 400 MHz): 3.94 (1H, d, J=13 Hz, H-6), 4.28 (1H, dd, J=5, 8 Hz, H-5), 4.25 (1H, d, J=13 Hz, H-6′), 5.13 (1H, t, J=8 Hz, H-4), 5.17 (1H, dd, J=5, 13 Hz, H-6), 5.29 (1H, t, J=8 Hz, H-3′), 5.31 (1H, t, J=8 Hz, H-2′), 5.35 (1H, t, J=8 Hz, H-2), 5.37 (1H, t, J=8 Hz, H-4′), 5.44 (1H, t, J=8 Hz, H-3), 6.09 (1H, d, J=8 Hz, H-1′), 6.17 (1H, d, J=8 Hz, H-1), 6.24, 6.56, 6.97 (each 1H, s, valoneayl H), 6.96, 7.05, 7.06, 7.08, 7.10, 7.12, 7.22 (each 2H, s, galloyl H). ^{13}C-NMR (acetone-d_6+D_2O, 25.05 MHz): 62.4, 63.3 (C-6,6′), 68.9, 70.1, 71.7, 72.8, 73.4 (C-2—C-5, C-2′—C5′), 93.4, 93.6 (C-1,1′), 105.1, 107.6, 110.3, 113.8, 115.4, 117.2, 119.2, 119.8, 119.9, 120.9, 125.1, 126.0, 136.3, 136.5, 139.2, 139.6, 140.1, 140.8, 143.5, 144.6, 145.3, 145.8, 146.0, 146.9 (aromatic H), 165.1, 165.4, 166.3, 166.9, 168.1, 168.3 (–COO–).

Methylation of 28 A mixture of **28** (3.64 g), dimethyl sulfate (4 ml), anhydrous potassium carbonate (6.0 g) in dry acetone (35 ml) was heated under reflux for 6 h. The inorganic compounds were removed by filtration, and the filtrate, after concentration, was applied to a silica gel column. Elution with benzene containing increasing proportions of acetone yielded the nonacosamethyl ether (**28a**) (1.72 g) as a white amorphous powder, $[\alpha]_D^{31}$ +59.8° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{111}H_{118}O_{52}$: C, 58.37; H, 5.21. Found: C, 58.02; H, 5.19. ^{1}H-NMR ($CDCl_3$, 100 MHz): 3.5—4.1 (OMe), 6.45, 6.70 (each 1H, s, valoneayl H), 7.1—7.45 (galloyl and valoneayl H).

Alkaline Methanolysis of 28a A solution of **28a** (400 mg) in methanolic 2% sodium hydroxide (5 ml) was kept for 12 h at room temperature. The reaction mixture was neutralized with Amberlite IRA 120B (H^+ form) and subjected to silica gel chromatography. Elution with a solvent system of benzene–acetone furnished trimethyl octa-*O*-methyl valoneate (**28a**) (6 mg) as a colorless syrup, $[\alpha]_D^{28}$ −15.9° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$, 100 MHz): 3.48, 3.57, 3.60, 3.67, 3.78 (×2), 3.93 (×2), 3.94, 3.98, 4.08 (OMe), 6.92, 7.30, 7.35 (each 1H, s valoneayl H), and methyl trimethyl gallate (**28c**) (15 mg) as colorless needles, mp 80—81 °C.

Tannase Hydrolysis of 28 A solution of **28** (300 mg) in water (20 ml) was treated with tannase for 1 h at room temperature. The reaction products were separated by Fuji gel ODS-G3 chromatography with water and methanol to yield gallic acid and a hydrolysate (**28d**) (93 mg) as a pale brown amorphous powder, $[\alpha]_D^{31}$ −19.9° [c=0.14, H_2O–MeOH (1 : 1]. *Anal.* Calcd for $C_{33}H_{32}O_{24}\cdot 3H_2O$: C, 45.73; H, 4.41. Found: C, 45.72; H, 4.20. FAB-MS m/z: 831 $(M+H)^+$, 853 $(M+Na)^+$. ^{1}H-NMR (pyridine-d_5, 270 MHz): 5.16 (d, J=7 Hz, H-1β), 5.86 (t, J=9 Hz, H-4), 7.07, 7.08, 7.13, 7.15, 7.35, 7.39, 7.82, 7.85, 7.87 (valoneayl H). ^{1}H-NMR (acetone-d_6+D_2O, 100 MHz): 6.11, 6.75, 7.15 (each 1H, valoneayl H). ^{13}C-NMR (acetone-d_6+D_2O, 25.05 MHz): 61.0, 64.5, 67.1, 69.8, 71.6, 72.0, 72.5, 72.8, 73.1, 73.9, 74.8, 75.5 (C-2—C-5, C-2′—C5′), 92.9, 93.3, 96.7, 97.9 (C-1,1′), 104.3, 108.1, 110.2, 114.8, 114.9, 116.0, 117.7, 125.4, 125.8, 136.3, 136.5, 136.8, 140.0, 140.9, 143.3, 145.1, 145.3, 147.0 (aromatic C), 166.6, 169.4 (–COO–).

Permethylation of 28d, Followed by Methanolysis A mixture of **28d** (18 mg), dimethyl sulfate (0.6 ml) and anhydrous potassium carbonate (1.0 g) in dry acetone (6 ml) was refluxed with stirring for 4 h. After cooling, the inorganic salts were removed by filtration, and the filtrate, after concentration, was passed through a silica gel column. Without further purification, the product was stirred for 1.5 h with methyl iodide (1 ml) and freshly prepared silver oxide (0.5 g) in dimethyl formamide (0.5 ml) at room temperature. The insolubles were filtered off, and the filtrate was concentrated to dryness by blowing nitrogen gas. The residue was suspended in water and shaken with ether. The organic layer was washed with water, dried over sodium sulfate and concentrated to give the permethyl derivative, which was treated for 2 h with 1% methanolic sodium hydroxide under reflux. The reaction mixture was passed through a column of Dowex 50W-×8 (H^+ form). The eluate, after concentration, was heated under reflux for 20 min with 1 N methanolic hydrochloride. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and analyzed by TLC[Kieselgel $60F_{254}$, solvent: ethyl acetate–methanol (50 : 1)] and GLC (N_2 flow rate: 60 ml/min) to detect methyl 2,3-dimethyl-[*Rf* 0.27, t_R 3.65 min (column temperature 152 °C)] and methyl 2,3,6-trimethyl-α-D-glucosides [*Rf* 0.40, t_R 3.90 min (column temperature 129 °C)].

Hot Water Treatment of 28d A solution of **28d** (100 mg) in water (5 ml) was heated under reflux for 21 h. The reaction mixture were separated by Fuji gel ODS-G3 with water and methanol to furnish 4-*O*-galloylglucose (**28e**) (2 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +15.3° (c=0.17, acetone). ^{1}H-NMR (acetone-d_6+D_2O, 100 MHz): 3.29 (t, J=8 Hz, β-H-2), 3.74 (t, J=9 Hz, β-H-3), 4.01 (t, J=9 Hz, α-H-3), 4.60 (d, J=8 Hz, β-H-1), 4.91 (t, J=9 Hz, β-H-4), 4.92 (dd, J=9, 10 Hz, α-H-4), 5.19 (d, J=4 Hz, α-H-1), 7.14 (2H, s, galloyl H).

Phillyraeoidin B (29) A pale brown amorphous powder, $[\alpha]_D^{21}$ +103.4° (c=0.9, acetone). *Anal.* Calcd for $C_{75}H_{56}O_{48}\cdot 5\ H_2O$: 49.62; H, 3.66. Found: C, 49.55; H, 3.88. Negative FAB-MS m/z: 1723 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O, 270 MHz): 3.75 (d, J=13 Hz, H-6α), 3.83 (d, J=13.5 Hz, H-6β), 4.15 (m, H-5′), 4.25 (m, H-5β, H-6′), 4.7 (m, H-5α,6′), 5.09 (d, J=8 Hz, H-1β), 5.14 (t, J=10 Hz, H-4β), 5.17 (t, J=10 Hz, H-4α), 5.23 (dd, J=7, 13 Hz, H-6α), 5.25 (dd, J=7, 14 Hz, H-6β), 5.30 (dd, J=8, 9 Hz, H-2β), 5.54 (d, J=4 Hz, H-1α), 5.58 (t, J=8 Hz, H-2′), 5.62 (t, J=9 Hz, H-3β), 5.64 (t, J=9 Hz, H-3′), 5.75 (t, J=10 Hz, H-4′), 5.88 (t, J=10 Hz, H-3α), 6.11 (d, J=8 Hz, H-1′), 6.20, 6.21, 6.51, 6.52 (each s, valoneayl H), 6.95—7.13 (galloyl and valoneayl H). ^{13}C-NMR (acetone-d_6): 62.7 (C-6′), 63.9 (C-6), 66.9 (C-4), 68.9 (C-4′), 71.2, 71.6, 73.0, 73.4, 74.0 (C-2, 3, 5, 2′,3′, 5′), 91.1 (C-1α), 93.5 (C-1′), 96.5 (C-1β).

Phillyraeoidin C (30) A pale brown amorphous powder, $[\alpha]_D^{21}$ +17.7 °C (c=0.8, acetone). *Anal.* Calcd for $C_{68}H_{52}O_{44}\cdot 3\ H_2O$: C, 50.19; H, 3.59. Found: C, 50.07; H, 3.72. Negative FAB-MS m/z: 1571 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O, 270 MHz): 4.79 (1H, d, J=13 Hz, H-6′), 4.93 (1H, t-like, H-4), 5.17 (1H, dd, J=4, 13 Hz, H-6), 5.61 (1H, dd, J=8, 9 Hz, H-2′), 5.68 (1H, d, J=8 Hz, H-1), 5.78 (1H, t, J=9 Hz, H-3′), 5.83 (1H, t, J=9 Hz, H-4′), 6.12 (d, J=8 Hz, H-1′), 6.19, 6.79, 6.98 (each 1H, s, valoneayl H), 7.00, 7.07 (×2), 7.17, 7.26 (each s, galloyl H). ^{13}C-NMR (acetone-d_6, 25.05 MHz): 63.2, 63.8 (C-6, 6′), 68.6, 71.4, 73.0, 73.8, 74.2, 75.9 (C-2,2′, 3,3′, 4,4′, 5,5′), 93.5 (C-1′), 95.9 (C-1), 164.6, 165.0, 165.5, 165.7, 166.7 (×2), 168.2 168.4 (–COO–).

Phillyraeoidin D (31) A pale brown amorphous powder, $[\alpha]_D^{18}$ +59.4° (c=0.1, acetone). *Anal.* Calcd for $C_{61}H_{48}O_{40}\cdot 4\ H_2O$: C 49.07; H, 3.78. Found: C, 48.82; H, 3.89. Negative FAB-MS m/z: 1419 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O, 270 MHz): 5.14 (d, J=8 Hz, H-1′β), 5.52 (d, J=4 Hz, H-1′α), 5.68 (1H, d, J=8 Hz, H-1), 6.07 (1H, t, J=9 Hz, H-3′), 6.13, 6.20, 6.77, 6.79, 6.95, 7.00 (valoneayl H), 7.02—7.30 (galloyl H). ^{13}C-NMR (acetone-d_6+D_2O, 25.05 MHz): 63.3, 64.0 (C-6,6′), 67.7, 69.4, 71.7, 72.1, 72.8, 73.6, 75.5 (C-2,2′, 3,3′, 4,4′, 5,5′), 90.9, 95.9 (C-1,1′), 165.3, 166.0, 166.4, 166.6, 167.0, 167.1, 167.4 (–COO–).

Tannase Hydrolysis of 29, 30 and 31 Each sample (30 mg) in water (3 ml) was shaken with tannase at room temperature for 3 h. The product was purified on Fuji gel ODS-G3 eluting with water containing increasing proportions of methanol to give gallic acid and a hydrolysate, the ^{1}H-NMR spectrum and the specific optical rotation of which were identical with those of **28d**.

Acknowledgements We thank Dr. K. Mihashi, Faculty of Pharmaceutical Sciences, Fukuoka University, for measurements of 400 MHz, ^{1}H-NMR spectra, Mr. T. Tanaka, Sankyo Co., Ltd., for the provision of tannase, Mr. Y. Tanaka and Miss Y. Soeda for ^{1}H- and ^{13}C-NMR measurements, and Mr. R. Isobe for MS measurements.

References and Notes

1) Part LXXXII: R. Saijo, G. Nonaka and I. Nishioka, *Phytochemistry*, "in press."
2) H. Nisimura, G. Nonaka and I. Nishioka, *Phytochemistry*, **23**, 2621 (1984).
3) H. Nishimura, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 1741 (1984); *idem*, *ibid.*, **32**, 1750 (1984); *idem*, *Phytochemistry*, **25**, 2599 (1986).
4) G. Nonaka, H. Nishimura and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 2061 (1982); H. Nishimura, G. Nonaka and I. Nishioka, *ibid.*, **32**, 1735 (1984).
5) G. Nonaka, K. Ishimaru, T. Tanaka and I. Nishioka, *Chem. Pharm.*

Bull., **32**, 483 (1984); H. Feng, G. Nonaka and I. Nishioka, *Phytochemistry*, **27**, 1185 (1988).
6) G. Nonaka, M. Ageta and I. Nishioka, *Chem. Pharm. Bull.*, **33**, 96 (1985); M. Ageta, K. Ishimaru, G. Nonaka and I. Nishioka, *ibid.*, **36**, 870 (1988); M. Ageta, G. Nonaka and I. Nishioka, *ibid.*, **36**, 1646 (1988).
7) G. Nonaka, H. Nishimura and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1985**, 163.
8) H. Nishimura, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 3223 (1986).
9) K. Ishimaru, G. Nonaka and I. Nishioka, *Phytochemistry*, **26**, 1147 (1987); *idem*, *ibid.*, **26**, 1167 (1987).
10) K. Ishimaru, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 602 (1987).
11) K. Ishimaru, G. Nonaka and I. Nishioka, *Phytochemistry*, **26**, 1501 (1987).
12) G. Nonaka, K. Ishimaru, K. Mihashi, Y. Iwase, M. Ageta and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 857 (1988); K. Ishimaru, M. Ishimatsu, G. Nonaka, K. Mihashi, Y. Iwase and I. Nishioka, *ibid.*, **36**, 3312 (1988); K. Ishimaru, M. Ishimatsu, G. Nonaka, K. Mihashi, Y. Iwase and I. Nishioka, *ibid.*, **36**, 3319 (1988).
13) G. Nonaka, I. Nishioka, T. Nagasawa and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981).
14) Unpublished data.
15) M. Nishizawa, Y. Yamagishi, G. Nonaka and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2963.
16) M. Seikel and W. E. Hillis, *Phytochemistry*, **9**, 1115 (1970).
17) Y. Kashiwada, H. Nishimura, G. Nonaka and I. Nishioka, Abstracts of Papers, 29th Annual Meeting of the Japanese Society of Pharmacognosy, Sapporo, September 1982, p. 48.
18) G. Nonaka, R. Sakai and I. Nishioka, *Phytochemistry*, **23**, 1753 (1984).
19) K. V. Rao, *Lloydia*, **40**, 169 (1977).
20) O. T. Schmidt, L. Würtele and A. Harréus, *Justus Liebigs Ann. Chem.*, **690**, 150 (1965).
21) R. K. Gupta, S. M. Al-Shafi, K. Laiden and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2525.
22) G. Nonaka, M. Harada and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 685 (1980).
23) G. Nonaka, I. Ishimaru, M. Watanabe, I. Nishioka, T. Yamauchi and A. S. C. Wan, *Chem. Pharm. Bull.*, **35**, 217 (1987).
24) K. Ishimaru, G. Nonaka, I. Nishioka and K. Mihashi, Abstracts of Papers, 33rd Annual Meeting of the Japanese Society of Pharmacognosy, Saitama, October 1986, p. 31.
25) K. Weinges, K. Göritz and F. Nader, *Justus Liebigs Ann. Chem.*, **715**, 164 (1968); R. S. Thompson, D. Jacques, E. Haslam and R. J. N. Tanner, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1387.
26) L. Y. Foo and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, **1978**, 1186.
27) K. Ishimaru, S. Nakayama, H. Nishimura, G. Nonaka and I. Nishioka, Abstracts of Papers, 33rd Annual Meeting of the Japanese Society of Pharmacognosy, Saitama, October 1986, p. 32.
28) T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 650 (1986).
29) Y. Ikeya, H. Taguchi, I. Yoshioka and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).
30) The structure of **28c** was established as follows. The ^{1}H-NMR spectrum of **28e** exhibited a pair of galloyl singlets at δ 7.12 and 7.14 corresponding to two protons in total, together with lowfield signals at δ 4.91 (t, J=9 Hz) and 4.92 (dd, J=9, 10 Hz). The assignments of these lowfield signals to the C-4 proton could be achieved by analysis of the ^{1}H–^{1}H COSY spectrum, which clearly showed the correlations of all the signals even in a duplicated form. Based of these results, **28e** was concluded to be 4-*O*-galloylglucose.
31) W. Mayer, R. Fikentsher, J. Schmidt and O. T. Schmidt, *Chem. Ber.*, **93**, 2763 (1960).
32) HHDP=hexahydroxydiphenoyl.