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Tannins and Related Compounds. XLI.[1] Isolation and Characterization of Novel Ellagitannins, Punicacorteins A, B, C and D, and Punigluconin from the Bark of *Punica granatum* L.

TAKASHI TANAKA, GEN-ICHIRO NONAKA, and ITSUO NISHIOKA*

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

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A chemical examination of the bark of *Punica granatum* L. (Punicaceae) has led to the isolation of five new ellagitannins, punicacorteins A, B, C and D, and punigluconin, together with the known ellagitannins, casuariin (**6**) and casuarinin (**7**). Based on chemical and spectroscopic evidence, the structures of punicacorteins A, B, C and D were established as novel C-glycosidic ellagitannins, the former two possessing a unique tetraphenyl (gallagyl) ester group (**1** and **2**), and the latter two containing a galloyl group in place of the gallagyl group (**3** and **4**), while punigluconin was characterized as 2,3-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoylgluconic acid (**5**).

Keywords——*Punica granatum*; pomegranate; Punicaceae; punicacortein; punigluconin; C-glycosidic ellagitannin; gallagic acid; gluconic acid

In the preceding paper,[1] we reported on the revision of the structures of punicalin (**8**) and punicalagin (**9**), and on the structure elucidation of 2-*O*-galloylpunicalin, isolated from the bark of *Punica granatum* L. (Punicaceae). Further chemical examination of polyphenolic metabolites of this plant has now resulted in the isolation of novel C-glycosidic hydrolyzable tannins, designated as punicacorteins A (**3**), B (**4**), C (**1**) and D (**2**). In addition, we have isolated a new-type ellagitannin, punigluconin (**5**), which contains a gluconic acid core. This paper deals with the isolation and structure determination of these tannins.

A combination of Sephadex LH-20, MCI-gel CHP-20P and Avicel cellulose chromatographies with various solvent systems was applied to further separation of the fractions[1] which were previously obtained from the aqueous acetone extract of the fresh bark, to afford punicacorteins A (**3**), B (**4**), C (**1**) and D (**2**), and punigluconin (**5**), together with the known tannins, casuariin (**6**)[2] and casuarinin (**7**).[2]

Punicacortein C (**1**), a pale yellow amorphous powder, $[\alpha]_D$ $-37.7°$ (H_2O), $C_{48}H_{28}O_{30}\cdot H_2O$, gave positive ferric chloride and nitrous acid tests.[3] The carbon-13 nuclear

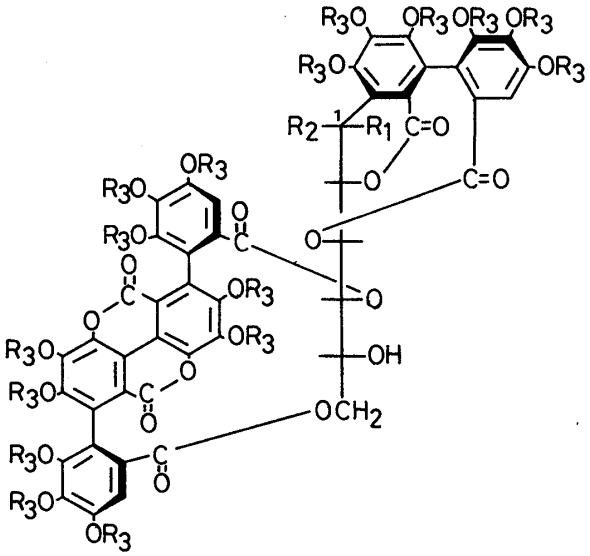
TABLE I. ^{13}C-NMR Chemical Shifts for Compounds **1**—**7** (δ Values)

	C_1	C_2	C_3	C_4	C_5	C_6
1[a]	74.4[c]	73.2[c]	73.2[c]	68.1[c]	68.1[c]	67.6[c]
2[a]	79.2[d]	74.9[d]	73.0[d]	70.0[d]	65.6[d]	68.2
3[a]	73.1	77.3	68.0	74.3	70.6	61.4
4[b]	72.4	77.3	68.4	75.7	70.1	66.9
6[a]	70.8	77.0	68.5[e]	77.0	67.7[e]	68.2
7[a]	71.0	76.8	66.7	73.9	69.6	64.4
5[a]	169.9	73.8[f]	73.0[f]	71.5[f]	70.7[f]	64.9

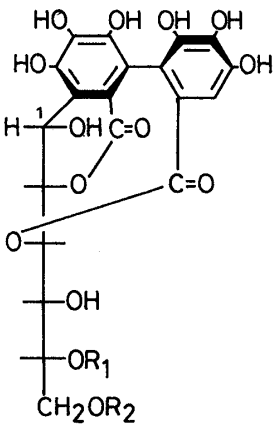
a) In acetone-d_6. *b*) In acetone-d_6+D_2O. *c*—*f*) Signals were not individually assigned.

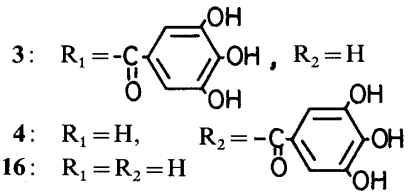
magnetic resonance (^{13}C-NMR) spectrum exhibited six carbonyl carbon signals (see Experimental), among which the chemical shifts (δ 158.4 and 159.0) of two clearly separated resonances (attributable to lactonic carbons) were consistent with those of a tetraphenyl (gallagyl) group.[4] The appearance of six aliphatic carbon signals (Table I) and the absence of an anomeric signal indicated that compound **1** possesses a C_6-polyalcohol core.

Acid-catalyzed degradation of compound **1** yielded a sugar, which was identified as glucose by gas-liquid chromatography (GLC) of its trimethylsilyl derivative, thus confirming that the configurations of the polyalcohol carbons except for the C_1-atom are the same as those of glucose.



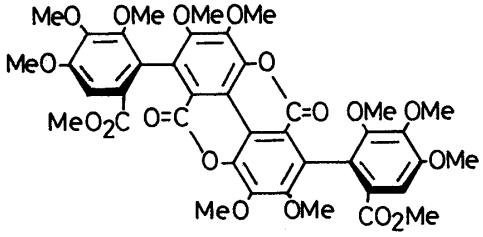
1: R_1=OH, R_2=R_3=H
2: R_1=R_3=H, R_2=OH
10: R_1=OH, R_2=H, R_3=Me





3: R_1=, R_2=H
4: R_1=H, R_2=
16: R_1=R_2=H

On methylation with dimethyl sulfate and potassium carbonate in dry acetone, compound **1** yielded the hexadecamethyl ether (**10**) [field desorption mass spectrum (FD-MS) *m/z*: 1308 $(M)^+$]. Subsequent alkaline methanolysis of the methyl ether (**10**) gave dimethyl decamethylgallagiate (**11**)[1] and a methanolysate (**12**) as major products. The measurement of the optical rotation, $[\alpha]_D$ −73.6°, of the gallagiate (**11**) confirmed the atrop-isomerism to be in the *S*,*S*-series. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the methanolysate showed an aromatic singlet at δ 7.37 and six methoxyl signals, together with ali-



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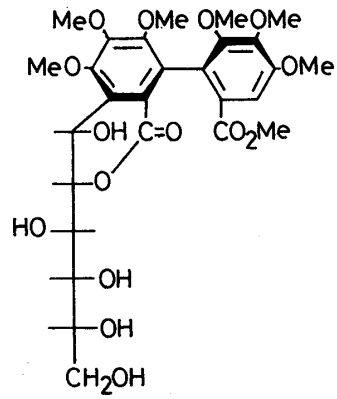

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TABLE II. ^{1}H-NMR Spectral Data for Compounds **1**—**6** and **20** (δ Values)[a]

	H-1	H-2	H-3	H-4	H-5	H-6
1[b]	5.49 (d, J=5)	4.86 (dd, J=2, 5)	5.17 (dd, J=2, 5)	4.32 (br d, J=5)	2.37 (br s)	3.78 (br s)
2[b]	4.80 (s)	4.64 (br s)	4.83 (br s)	4.47 (br d, J=4)	2.41 (br d)	3.72—3.92 (m)
3[b]	5.65 (d, J=5)	4.88 (dd, J=2, 5)	5.36 (br s)	4.16 (dd, J=3, 8)	5.00 (m)	3.93 (m)
4[c]	5.66 (d, J=5)	4.85 (dd, J=2, 5)	5.36 (br s)	3.60—4.00 (m)	3.60—4.00 (m)	4.32 (dd, J=6, 12) 4.58 (dd, J=2, 12)
6[b]	5.64 (d, J=5)	4.73 (dd, J=2, 5)	5.46 (t, J=3)	5.05 (dd, J=3, 8)	4.12 (br d, J=8)	3.86 (d, J=12) 4.67 (dd, J=3, 12)
5[b]	—	5.36 (d, J=4)	5.51 (dd, J=4, 8)	5.82 (br d, J=8)	4.45 (br s)	4.06 (d, J=13) 4.82 (dd, J=3, 13)
20[d]	—	3.88 (d, J=4)	4.16 (br d, J=8)	4.84 (dd, J=3, 8)	4.00 (br s)	3.74 (d, J=13) 4.51 (br d, J=13)

a) Measured at 100 MHz with TMS as an internal standard. J values are expressed in Hz. *b*) In acetone-d_6. *c*) In acetone-d_6+D_2O. *d*) In DMSO-d_6.

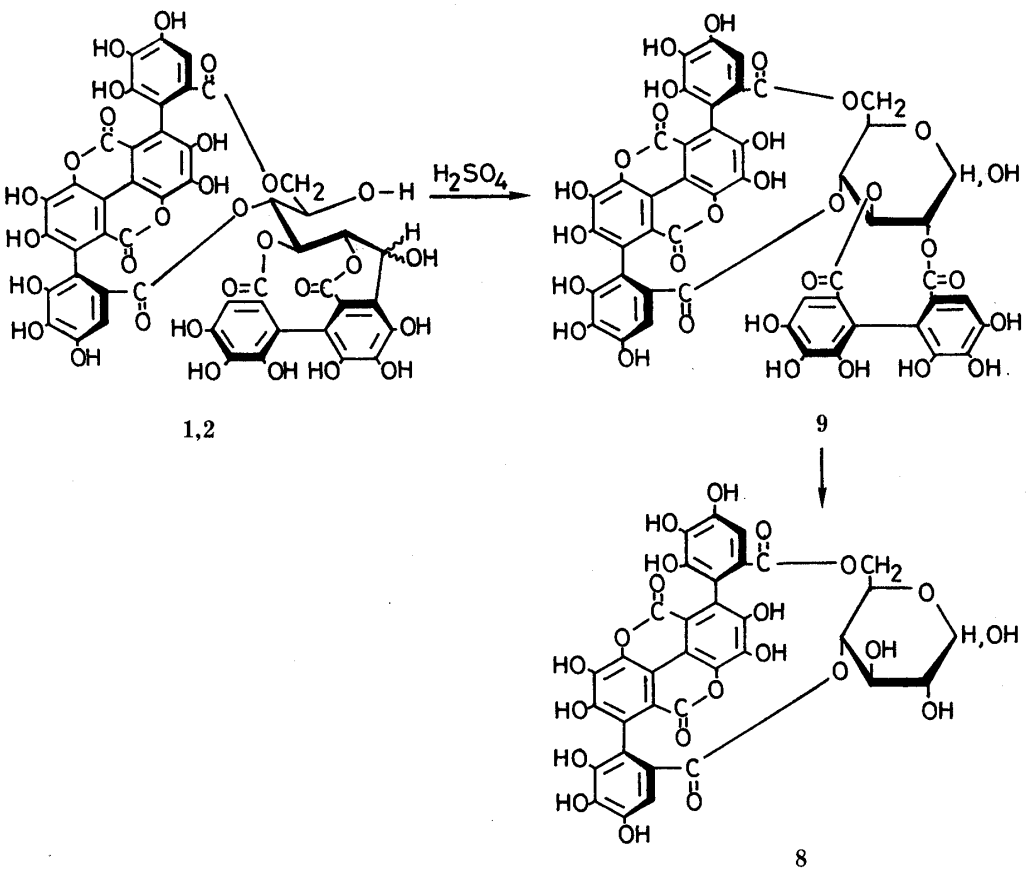


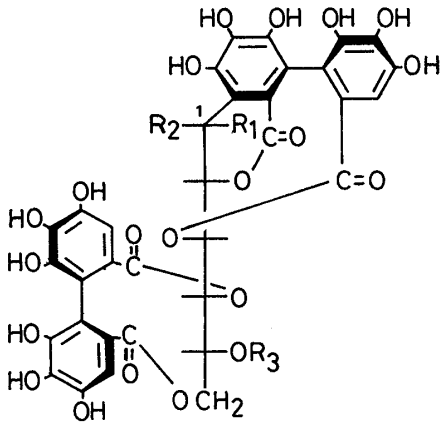
Chart 1

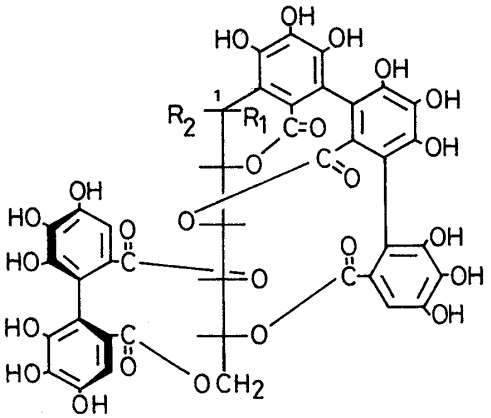
phatic multiplets arising from the polyalcohol moiety. Thus, the structure of the methanolysate was considered to be **12**,[2a] and comparison of the physical and ^{1}H-NMR data with those of a sample prepared from casuarinin (**7**) confirmed its structure.

In the ^{1}H-NMR spectrum of compound **1**, the chemical shift (δ 2.36) of the C_5-methine signal indicated that the C_5-hydroxyl group is not acylated, and the abnormal upfield shift of the signal might be interpreted in terms of a strong shielding effect caused by the gallagyl ester group. Thus, the gallagyl ester was considered to be located at the C_4- and C_6-positions of the polyalcohol moiety.

Further support for the structure of compound **1** was obtained unexpectedly by acid treatment of **1**, which yielded punicalin (**8**) and punicalagin (**9**) through the cleavage of a carbon–carbon linkage and subsequent ring closure as shown in Chart 1. On the basis of the evidence described above, the structure of punicacortein C was concluded to be represented by the formula **1**.

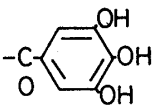
Punicacortein D (**2**), a yellow amorphous powder, $[\alpha]_D$ $-96.1°$ (H_2O), $C_{48}H_{28}O_{30}\cdot 5H_2O$, exhibited the same $(M+H)^+$ ion peak (*m/z*: 1085) as that of compound **1** in the fast atom bombardment mass spectrum (FAB-MS). The ^{1}H-NMR spectrum was closely related to that of **1**, but differed in the chemical shifts of aliphatic signals (Table I). In the ^{1}H-NMR spectrum, the C_4-, C_5- and C_6-proton resonances were similar to those found in **1**, while the C_1-proton signal appeared considerably upfield (δ 0.7), and its coupling constant was smaller than that of **1** (Table II). These findings were consistent with those in the cases of stachyurin (**13**)[2a] and vescalagin (**15**)[5] which are the epimers (at the C_1-position) of casuarinin (**7**) and castalagin (**14**),[6] respectively (Table III).



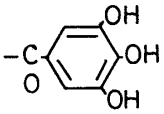


6: R_1=OH, R_2=R_3=H

14: R_1=OH, R_2=H
15: R_1=H, R_2=OH



7: R_1=OH, R_2=H, R_3=



13: R_1=H, R_2=OH, R_3=

Epimerization[7] at the C_1-position by refluxing compound **1** in aqueous solution (38 h) successfully gave compound **2** in 17% yield, together with punicalagin (**9**). On the other hand, when heated in 1% sulfuric acid (90 °C, 9 h), compound **2** afforded punicalin (**8**), punicalagin (**9**) and a trace amount of compound **1**.

From these spectral and chemical data, punicacortein D was characterized as an epimer of punicacortein C (**1**), the structure represented by the formula **2**.

TABLE III. ^{1}H-NMR Chemical Shifts and Coupling Constants for H-1 of Compounds **1**, **2**, **7**, **13**, **14**, and **15**

	1[a]	2[a]	7[a]	13[a]	14[b]	15[b]
Chemical shifts (δ values)	5.49	4.80	5.67	4.93	5.38	4.75
Coupling constants (Hz)	5	0	5	2	4.8	1.8

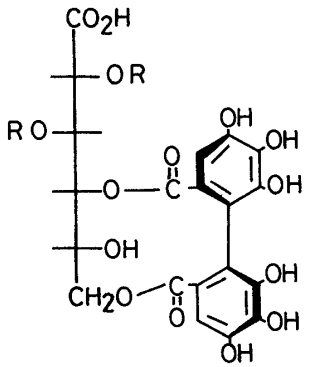
a) Measured in acetone-d_6. *b*) Measured in CD_3OD.

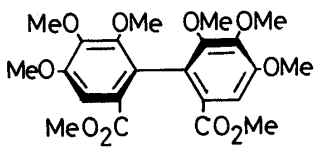
Punicacortein A (**3**), a tan amorphous powder, $[\alpha]_D$ $-73.8°$ (MeOH), $C_{27}H_{22}O_{18}\cdot 1/2H_2O$, and punicacortein B (**4**), a tan amorphous powder, $[\alpha]_D$ $+11.9°$ (MeOH), $C_{27}H_{22}O_{18}\cdot 1/2H_2O$, showed the same $(M+H)^+$ ion peak (*m/z*: 635) in the FAB-MS. The ^{13}C-NMR spectra of these compounds were closely related to each other, showing the presence of a galloyl ester group and a C_6-polyalcohol moiety (Table I). In the ^{1}H-NMR spectra, the chemical shifts and the coupling patterns of three methine signals assignable to the C_1-, C_2- and C_3-protons were almost identical with those of casuariin (**6**) (Table II).

On enzymatic hydrolysis with tannase, punicacorteins A (**3**) and B (**4**) gave gallic acid and a hydrolysate, the latter being identified as the product (**16**) prepared by partial acid hydrolysis of casuariin (**6**).

The location of the galloyl group was determined on the basis of the following evidence. Comparison of the ^{1}H-NMR spectra of compounds **3** and **16** clearly showed that a multiplet signal appeared downfield (δ 4.99 in **3**; δ 3.74 in **16**), and the assignment of the multiplet to the C_5-proton was achieved by a spin-decoupling experiment. Thus, the galloyl group was considered to be attached to the C_5-position. On the other hand, the downfield shifts (δ 4.32 and 4.58) of the C_6-methylene signals in the spectrum of **4** indicated that the galloyl group is located at the C_6-position. These ^{1}H-NMR observations were consistent with the ^{13}C-NMR chemical shifts of the C_6-signals appearing upfield (δ 61.4) in **3** and considerably downfield (δ 66.9) in **4**. Based on these observations, punicacorteins A and B were assigned the structures **3** and **4**, respectively.

Punigluconin (**5**), a tan amorphous powder, $[\alpha]_D$ $+45.5°$ (MeOH), $C_{34}H_{26}O_{23}\cdot 2H_2O$, showed an $(M+H)^+$ ion peak at *m/z* 803 in the FAB-MS. The occurrence of two galloyl and a hexahydroxydiphenoyl ester group was easily deduced from the two two-proton aromatic





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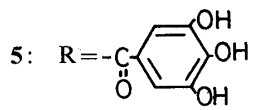

5: R =

20: R = H

singlets (δ 7.12 and 7.19) and a pair of one-proton singlets (δ 6.54 and 6.92) in the ^{1}H-NMR spectrum. In the ^{13}C-NMR spectrum (Table I), the appearance of five sp^3-carbon signals and a carboxyl signal suggested the presence of an aldohexonic acid moiety in the molecule. This was supported by ^{1}H-NMR examination, which showed six well-separated aliphatic signals (Table II). The aldohexonic acid was characterized as gluconic acid by acid hydrolysis of **5** with dilute sulfuric acid.

Methylation of **5** with dimethyl sulfate and potassium carbonate in dry acetone afforded two methyl ethers (**17**) and (**18**), which exhibited the same M^+ ion peak at *m/z* 984 in the electron-impact mass spectra (EI-MS). The production of the two methyl ethers was probably due to epimerization at the C_2-position, since the ^{1}H-NMR spectra showed different coupling constants ($J=8$ Hz in **17**; $J=4$ Hz in **18**) of the C_2-proton signals. On alkaline methanolysis, the methyl ethers (**17**) and (**18**) gave methyl trimethoxybenzoate and dimethyl (*S*)-hexamethoxydiphenoate (**19**), thus confirming the component acid moieties, and the chirality of the hexahydroxydiphenoyl group.

Enzymatic hydrolysis of **5** with tannase gave a hydrolysate (**20**) and gallic acid. In the ^{1}H-NMR spectrum of **20** (Table II), signals due to the C_2- and C_3-protons were remarkably shifted to upper field as compared with those observed in **5**, whereas the signals ascribable to C_4- and one of the C_6-methylene protons appeared downfield. From these observations, the location of the (*S*)-hexahydroxydiphenoyl ester group was confirmed to be at the 4,6-positions. Consequently, punigluconin was characterized as 2,3-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoylgluconic acid (**5**), and represents the first reported example of a hydrolyzable tannin containing a gluconic acid core.

It is interesting from the viewpoint of plant physiology that the bark of *Punica granatum* L. contains ellagitannins in which (dehydro)hexahydroxydiphenoyl ester group(s) is located exclusively at the 2,3- and/or 4,6-positions in a carbohydrate moiety, whereas in leaf tannins[8] the ester group(s) is attached to the 3,6-, 2,4- and/or 1,6-positions.

Experimental

Details of the instruments and chromatographic conditions used throughout this work were the same as described in the previous paper[1] except in the following respects. High-performance liquid chromatography (HPLC) was carried out on a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system and a UV-8 model II spectrometer, and a TSK-410 column (4 mm i.d. × 300 mm) [mobile phase: CH_3CN–50 mM NaH_2PO_4 (13 : 87, v/v)]. Analytical GLC was conducted over 1.5% SE-30 (4 mm i.d. × 2 m) with N_2 as the carrier gas.

Isolation of Compounds 1—7——The fractionation of the aqueous acetone extracts of the fresh bark of *Punica granatum* L. (5 kg) was described in the preceding paper,[1] and fractions described here correspond to those of the previous work. Fraction III-b was subjected to cellulose chromatography with 2% AcOH, followed by purification on an MCI-gel CHP-20P column with H_2O–MeOH (1 : 0—3 : 1, v/v) to afford punigluconin (**5**) (700 mg). Fraction IV-a was chromatographed over MCI-gel CHP-20P with H_2O–MeOH (1 : 0—7 : 3, v/v) to afford punicacorteins A (**3**) (139 mg) and B (**4**) (135 mg). After removal of the crystals of 2-*O*-galloylpunicalin by filtration, fr. IV-b was chromatographed over MCI-gel CHP-20P using H_2O–MeOH (4 : 1—2 : 3, v/v) to yield casuariin (**6**)[2b] (4.7 g). Successive chromatography of fr. IV-c over Sephadex LH-20 with 40% aqueous MeOH, MCI-gel CHP-20P with H_2O–MeOH (1 : 0—3 : 2, v/v), and Sephadex LH-20 with EtOH afforded punicacorteins C (**1**) (5.5 g) and D (**2**) (310 mg). Fraction VI was subjected to MCI-gel CHP-20P chromatography with H_2O–MeOH (4 : 1—3 : 2, v/v) to give casuarinin (**7**)[2b] (16 g).

Punicacortein C (1)——A yellow amorphous powder, $[\alpha]_D^{28}$ $-37.7°$ ($c=1.2$, H_2O). *Anal.* Calcd for $C_{48}H_{28}O_{30}\cdot H_2O$: C, 52.28; H, 2.74. Found: C, 52.47; H, 2.78. ^{1}H-NMR (acetone-d_6): 6.34, 6.66, 7.30 (each 1H, s, aromatic H), Table II. ^{13}C-NMR (acetone-d_6): 158.4, 159.0 (δ-lactone), 164.7, 167.4, 167.8, 168.5 (CO_2), Table I.

Acid-Catalyzed Degradation of 1——A solution of **1** (15 mg) in 2 N H_2SO_4 (3 ml) was refluxed for 24 h. The reaction mixture was neutralized with $BaCO_3$, and the resulting inorganic salts were filtered off. The filtrate was concentrated to dryness by evaporation under reduced pressure, and the residue was trimethylsilylated with *N*-trimethylsilylimidazole. GLC analysis showed peaks (t_R 10.82, 17.14 min; column temperarture 155 °C; flow rate 60 ml/min) corresponding to the trimethylsilyl derivatives of glucose.

Methylation of 1——A mixture of **1** (300 mg), anhydrous potassium carbonate (1 g) and dimethyl sulfate (0.9 ml)

in dry acetone (25 ml) was heated under reflux for 4 h. The inorganic salts were filtered off, and the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (9 : 1, v/v) gave the hexadecamethyl ether (**10**) (89 mg) as a pale yellow amorphous powder, $[\alpha]_D^{24}$ −77.7° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{64}H_{60}O_{30}\cdot 2H_2O$: C, 57.14; H, 4.80. Found: C, 57.25; H, 4.75. FD-MS m/z: 1309 $[M+H]^+$, 1308 $[M]^+$. ^{1}H-NMR ($CDCl_3$): 2.23 (1H, br s, H-5), 4.43 (1H, dd, J=5, 2 Hz, H-4), 4.87 (1H, dd, J=5, 2 Hz, H-2), 5.16 (1H, dd, J=5, 2 Hz, H-3), 5.40 (1H, d, J=5 Hz, H-1), 6.51, 6.95, 7.50 (each 1H, s, aromatic H).

Alkaline Methanolysis of 10——A 3% methanolic solution of NaOMe (1.5 ml) was added dropwise to a solution of **10** (50 mg) in MeOH (7.5 ml), and the mixture was stirred at room temperature for 7 d. The resulting precipitates were collected by filtration and crystallized from MeOH to give dimethyl (*S,S*)-decamethylgallagiate (**11**) (5 mg) as yellow prisms, mp 275—276 °C, $[\alpha]_D^{24}$ −73.6° (c=0.1, $CHCl_3$). The filtrate was neutralized with Amberlite IR-120B (H^+ form) resin and the solution was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–EtOH (20 : 3, v/v) yielded the methanolysate (**12**) as a colorless syrup, $[\alpha]_D^{26}$ −50.0° (c=1.4, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{34}O_{15}$: C, 54.17; H, 5.73. Found: C, 54.39; H, 6.02. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1720. ^{1}H-NMR ($CDCl_3$): 5.64 (1H, br s, H-1), 7.37 (1H, s, aromatic H). This compound was identified by ^{1}H-NMR and IR comparisons with an authentic sample prepared from pentadecamethyl casuarinin in a similar way.

Conversion of 1 to 8 and 9——A solution of **1** (200 mg) in 1% H_2SO_4 (10 ml) was refluxed for 12 h. After cooling, the reaction mixture was subjected to Sephadex LH-20 chromatography. Elution with MeOH–H_2O (3 : 2, v/v) afforded punicalin (**8**) (28 mg). Subsequent elution with MeOH–H_2O (4 : 1, v/v) gave punicalagin (**9**) (15 mg).

Punicacortein D (2)——A yellow amorphous powder, $[\alpha]_D^{24}$ −96.1° (c=0.6, H_2O). *Anal.* Calcd for $C_{48}H_{28}O_{30}\cdot 5H_2O$: C, 49.07; H, 3.26. Found: C, 48.80; H, 3.26. FAB-MS m/z: 1085 $[M+H]^+$. ^{1}H-NMR (acetone-d_6): 6.33, 6.76, 7.29 (each 1H, s, aromatic H), Table II. ^{13}C-NMR (acetone-d_6): 158.3, 158.4 (δ-lactone), 165.2, 167.6, 168.4 (×2) (CO_2), Table I.

Epimerization of 1——A solution of **1** (95 mg) in H_2O was refluxed for 38 h. The solvent was evaporated off under reduced pressure, and the residue was chromatographed on a column of MCI-gel CHP-20P. Elution with 15% MeOH afforded punicacortein D (**2**) (17 mg). Further elution with 20% and 25% MeOH gave unreacted **1** (22 mg) and punicalagin (**9**) (9 mg), respectively.

Conversion of 2 to 9 and 8——A solution of **2** (5 mg) in 1% H_2SO_4 (1.5 ml) was heated (90 °C) for 9 h. The reaction mixture was analyzed by HPLC. The chromatogram showed peaks (t_R 3.0, 3.4 min; t_R 3.6 min; t_R 4.0 min; t_R 5.4, 7.7 min) corresponding to punicalin (**8**), punicacortein D (**2**), punicacortein C (**1**) and punicalagin (**9**), respectively.

Punicacortein A (3)——A tan amorphous powder, $[\alpha]_D^{28}$ −73.8° (c=0.6, MeOH), *Anal.* Calcd for $C_{27}H_{22}O_{18}\cdot 1/2H_2O$: C, 50.40; H, 3.60. Found: C, 50.08; H, 3.84. FAB-MS m/z: 635 $[M+H]^+$. ^{1}H-NMR (acetone-d_6): 6.41 (1H, s, aromatic H), 7.07 (2H, s, galloyl-H), Table II. ^{13}C-NMR (acetone-d_6): 110.7 (galloyl-$C_{2,6}$), 165.3, 166.7, 169.3 (CO_2), Table I.

Enzymatic Hydrolysis of 3 with Tannase——A solution of **3** (40 mg) in H_2O (10 ml) was incubated with tannase at room temperature for 3 h. Evaporation of the solvent afforded a residue, which was treated with EtOH. The EtOH-soluble portions was applied to a column of Sephadex LH-20 with EtOH to give gallic acid (8 mg) and the hydrolysate (**12**) (20 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +55.2° (c=0.5, MeOH). *Anal.* Calcd for $C_{20}H_{18}O_{14}\cdot 1/2H_2O$: C, 48.89; H, 3.90. Found: C, 49.08; H, 4.14. ^{1}H-NMR (acetone-d_6 + D_2O): 3.61 (2H, s, H-6), 3.74 (2H, m, H-4, 5), 4.81 (1H, dd, J=5, 2 Hz, H-2), 5.28 (1H, br s, H-3), 5.64 (1H, d, J=5 Hz, H-1), 6.47 (1H, s, aromatic H). ^{13}C-NMR (acetone-d_6 + D_2O): 63.8 (C_6), 67.8 (C_3), 71.8 (C_5), 72.3 (C_1), 75.3 (C_4), 77.5 (C_2), 166.0, 171.5 (CO_2).

Punicacortein B (4)——A tan amorphous powder, $[\alpha]_D^{28}$ +11.9° (c=0.5, MeOH). *Anal.* Calcd for $C_{27}H_{22}O_{18}\cdot 1/2H_2O$: C, 50.40; H, 3.60. Found: C, 50.24; H, 3.72. FAB-MS m/z: 635 $[M+H]^+$. ^{1}H-NMR (acetone-d_6 + D_2O): 6.45 (1H, s, aromatic H), 7.14 (2H, s, galloyl-H), Table II. ^{13}C-NMR (acetone-d_6 + D_2O): 110.1 (galloyl-$C_{2,6}$), 165.0, 167.3, 171.5 (CO_2), Table I. Hydrolysis with tannase in the same way as described above gave gallic acid and the hydrolysate (**12**).

Acid Hydrolysis of 6——A solution of **6** (500 mg) in 1 N H_2SO_4 (15 ml) was heated (90 °C) for 6.5 h. After removal of the resulting precipitates by filtration, the filtrate was neutralized with Na_2CO_3, and subjected to Sephadex LH-20 chromatography. Elution with H_2O furnished the hydrolysate (**12**) (27 mg).

Punigluconin (5)——A tan amorphous powder, $[\alpha]_D^{25}$ +45.5° (c=0.7, MeOH). *Anal.* Calcd for $C_{34}H_{26}O_{23}\cdot 2H_2O$: C, 48.70; H, 3.61. Found: 48.32; H, 3.60. FAB-MS m/z: 803 $[M+H]^+$. ^{1}H-NMR (acetone-d_6): 6.54, 6.92 (each 1H, s, HHDP-H), 7.12, 7.19 (each 2H, s, galloyl-H), Table II. ^{13}C-NMR (acetone-d_6): 107.4, 109.3 (HHDP-$C_{3,3'}$), 110.2, 110.7 (galloyl-$C_{2,6}$), 115.0, 116.0 (HHDP-$C_{1,1'}$), 120.9 (galloyl-C_1), 125.8, 127.2 (HHDP-$C_{2,2'}$), 135.8, 136.7 (HHDP-$C_{5,5'}$), 139.2 (galloyl-C_4), 144.5, 145.2, 145.8, 146.0 (HHDP-$C_{4,4',6,6'}$, galloyl-$C_{3,5}$), 165.9, 166.2, 167.6, 169.0 (CO_2), Table I.

Acid Hydrolysis of 5——A solution of **5** (10 mg) in 1 N H_2SO_4 (2 ml) was heated under reflux for 8 h. After cooling, the resulting pale yellow needles (2 mg) were collected by filtration, and the product was identified as ellagic acid by infrared (IR) comparison. The filtrate was extracted with AcOEt, and the organic layer was examined by thin-layer chromatography (TLC), which showed a spot corresponding to gallic acid. The remaining aqueous layer was

neutralized with $BaCO_3$, and the inorganic salts were filtered off. The filtrate was concentrated to dryness, and the residue was trimethylsilylated with *N*-trimethylsilylimidazole. GLC analysis showed peaks (t_R 3.95, 4.65 and 5.72 min; column temperature 180 °C; flow rate 50 ml/min) corresponding to the trimethylsilyl derivatives of gluconic acid.

Methylation of 5——A mixture of **5** (120 mg), dimethyl sulfate (1 ml) and anhydrous potassium carbonate (1.5 g) in dry acetone (20 ml) was refluxed for 3 h, and the reaction mixture was treated as described above to give two methyl ethers (**17**) (17 mg) and (**18**) (13 mg). **17**: A white amorphous powder, $[\alpha]_D^{22}$ +21.2° (c=0.4, $CHCl_3$). EI-MS *m/z*: 984 $[M]^+$. ^{1}H-NMR ($CDCl_3$): 4.26 (1H, d, J=13 Hz, H-6), 4.40 (1H, d, J=8 Hz, H-5), 4.99 (1H, dd, J=3, 13 Hz, H-6), 5.60 (2H, br d, J=8 Hz, H-2, H-3 or 4), 6.04 (1H, t, J=8 Hz, H-4 or 3), 6.57, 6.70 (each 1H, s, aromatic H), 7.15, 7.34 (each 2H, s galloyl-H). **18**: A white amorphous powder, $[\alpha]_D^{22}$ −5.5° (c=0.4, $CHCl_3$). EI-MS *m/z*: 984 $[M]^+$. ^{1}H-NMR ($CDCl_3$): 4.22 (1H, d, J=13 Hz, H-6), 4.40 (1H, m, H-5), 4.82 (1H, dd, J=3, 13 Hz, H-3), 5.61 (1H, d, J=4 Hz, H-2), 5.70—5.88 (2H, m, H-3, 4), 6.70, 6.88 (each 1H, s, aromatic H), 7.19, 7.29 (each 2H, s, galloyl-H).

Alkaline Methanolysis of 17 and 18——A mixture (20 mg) of the methyl ethers **17** and **18** was treated with 2% methanolic NaOMe (5 ml) at room temperature for 48 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) resins, and the solution was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (19 : 1, v/v) yielded methyl trimethoxybenzoate as colorless prisms (MeOH) (2 mg), mp 80—81 °C, and dimethyl (*S*)-hexamethoxydiphenoate (**19**) as a colorless syrup (6.1 mg), $[\alpha]_D^{22}$ −26.7° (c=0.8, $CHCl_3$).

Enzymatic Hydrolysis of 5 with Tannase——A solution of **5** (70 mg) in H_2O (10 ml) was shaken with tannase at room temperature for 30 min. The reaction mixture was worked up as described above to give gallic acid (18 mg) and 4,6-(*S*)-hexahydroxydiphenoylgluconic acid (**20**) (23 mg) as a tan amorphous powder, $[\alpha]_D^{20}$ +50.8° (c=0.6, MeOH). *Anal.* Calcd for $C_{30}H_{18}O_{15} \cdot H_2O$: C, 46.52; H, 3.90. Found: C, 46.82; H, 3.87. FAB-MS *m/z*: 521 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6): 6.28, 6.63 (each 1H, HHDP-H), Table II.

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