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Tannins and Related Compounds. XLII.[1] Isolation and Characterization of Four New Hydrolyzable Tannins, Terflavins A and B, Tergallagin and Tercatain from the Leaves of *Terminalia catappa* 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 leaves of *Terminalia catappa* L. (Combretaceae) has led to the isolation and characterization of four new hydrolyzable tannins named terflavins A (**1**) and B (**2**), tergallagin (**3**) and tercatain (**4**), together with eight known tannins: punicalin (**5**), punicalagin (**6**), chebulagic acid (**7**), geraniin (**8**), granatin B (**9**), 1-desgalloyleugeniin (**10**), corilagin (**11**) and 2,3-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-D-glucose (**12**). Terflavins A (**1**) and B (**2**) possess novel structures in which a flavogallonyl (triphenyl) ester group is single bonded to the glucopyranose ring, and are presumed to be biosynthetic precursors of punicalagin (**6**) and punicalin (**5**), respectively, while tergallagin (**3**), which contains a gallagyl (tetraphenyl) group and a unique tergalloyl ester group attached to the glucose moiety, seems to be formed biosynthetically from punicalagin (**6**).

Keywords——*Terminalia catappa*; Combretaceae; terflavin A; terflavin B; tergallagin; tercatain; hydrolyzable tannin; tergallic acid; flavogallonic acid; biosynthesis

In the preceding papers,[1,2] we isolated a series of yellow-colored tannins, *viz*., punicalin, punicalagin, 2-*O*-galloylpunicalin and punicacorteins C and D, from the bark of *Punica granatum* L., and elucidated their structures as novel hydrolyzable tannins containing a polyhydroxytetraphenyl (gallagyl) ester group in the molecule. Tannins which possess this unique carboxylic acid (gallagic acid) ester are relatively rate in nature, and they have hitherto been foun only in the fruit of *Terminalia chebula* RETZ. (myrobalan),[3] and the pericarp[4] and the bark of *Punica granatum* L. In the course of a search for natural sources of tannins, we have found that the leaves of *Terminalia catappa* L. (Combretaceae) are rich in tannins of this class. The large-scale extraction of the leaves has led to the isolation and characterization of four new hydrolyzable tannins designated as terflavins A (**1**) and B (**2**), tergallagin (**3**) and tercatain (**4**), together with punicalin (**5**), punicalagin (**6**) and six known tannins (**7**—**12**). Among them, terflavins A (**1**) and B (**2**) seem to be of particular importance, since they may be intermediates in the biosynthesis of punicalin (**5**) and punicalagin (**6**). We now wish to describe the isolation, structural characterization and possible biosynthetic pathways of these tannins.

The aqueous acetone extract of the dried leaves of *T. catappa*, collected in Taiwan, was subjected to a combination of adsorption and partition (Sephadex LH-20 and MCI-gel CHP 20P) chromatographies to yield four new tannins, terflavins A (**1**) and B (**2**), tergallagin (**3**) and tercatain (**4**). Furthermore, these compounds were accompanied by eight known tannins, which were identified as punicalin (**5**),[1] punicalagin (**6**),[1] chebulagic acid (**7**),[5,6] geraniin (**8**),[7] granatin B (**9**),[3] 1-desgalloyleugeniin (**10**),[8] corilagin (**11**)[9] and 2,3-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-D-glucose (**12**),[10] by comparisons of their physical and spectral data with those of authentic samples.

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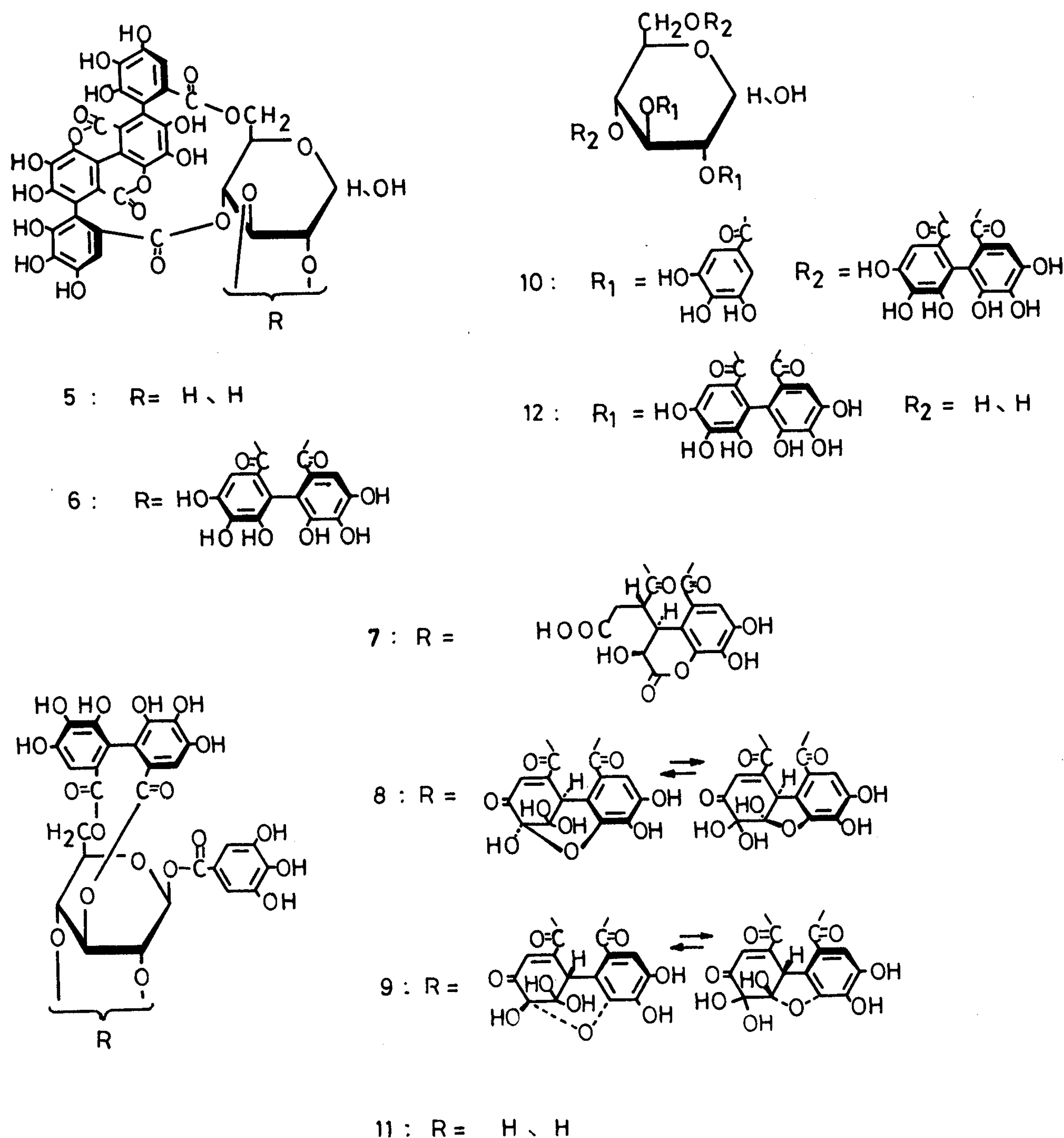

Terflavin A (**1**), a pale-yellow crystalline powder (H_2O), mp 258 °C (dec.), $[\alpha]_D$ +240.6° (MeOH), $C_{48}H_{30}O_{30} \cdot 4H_2O$, and terflavin B (**2**), a pale-yellow crystalline powder (H_2O–acetone), mp 232 °C (dec.). $[\alpha]_D$ +178.2° (MeOH), $C_{34}H_{24}O_{22} \cdot 9/2H_2O$, are structurally related in that upon acid hydrolysis (2% H_2SO_4, 90 °C, 5 h), **1** yielded **2**, together with ellagic acid. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of terflavin A (**1**) exhibits in the aromatic field a two-proton singlet signal (δ 6.80) due to a galloyl group and four one-proton singlets, of which two are attributable to a 4,4′,5,5′,6,6′-hexahydroxydiphenoyl ester group, while the spectrum of terflavin B (**2**) shows the presence of a galloyl group (δ 6.71, 2H, s) and two singlets (δ 7.31 and 7.50), the chemical shifts of the latter being similar to those (δ 7.23 and 7.49) found in terflavin A (**1**). Acid hydrolysis of terflavin B (**2**) under more drastic conditions (5% H_2SO_4, 100 °C, 18 h) liberated glucose, gallic acid and a carboxylic acid (**13**). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of the acid (**13**) shows eighteen aromatic signals corresponding to three aromatic rings, and signals ascribable to δ-lactones (δ 157.4 and 159.2) and a carboxylic acid (δ 167.5). Furthermore, the observation of two aromatic singlets (δ 7.29 and 7.52) in the ^{1}H-NMR spectrum suggests the acid (**13**) to be

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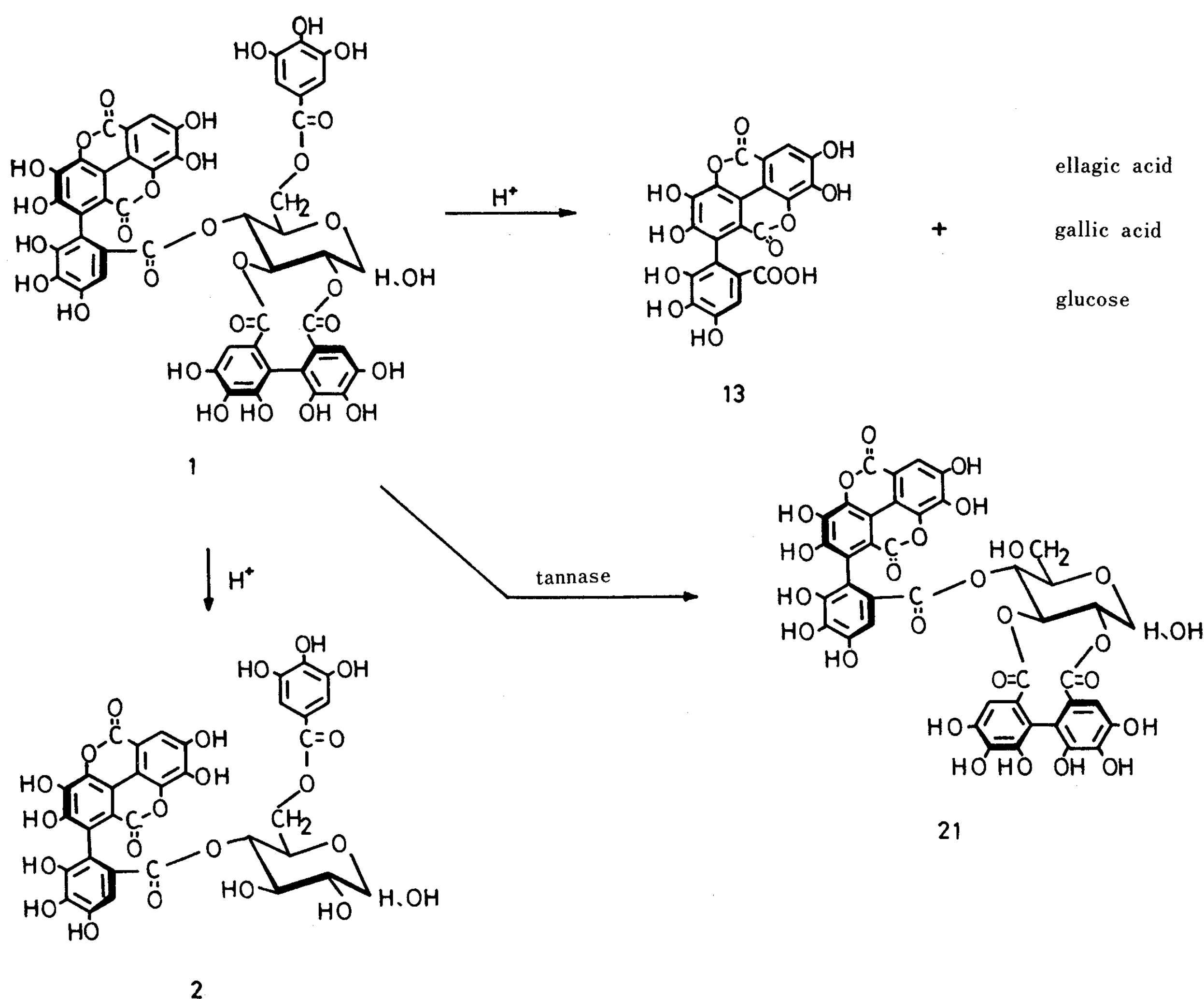

Chart 1

flavogallonic acid, and this was confirmed by comparison of its infrared (IR) and ^{1}H-NMR spectra with those of an authentic sample.[11]

The ^{1}H-NMR spectra of terflavins A (**1**) and B (**2**) show duplicated signals due to the glucose moiety, indicating that each anomeric center is not acylated. Methylation of terflavin A (**1**) with dimethyl sulfate and potassium carbonate in dry acetone gave two crystalline methyl ethers (**14**) and (**15**), which were assigned as α- and β-anomers, respectively, on the basis of ^{1}H-NMR analyses ($J_{1,2}=4$ Hz in **14** and $J_{1,2}=8$ Hz in **15**). In the ^{1}H-NMR spectrum of **15**, glucose H-2, H-3 and H-4 appear as clearly separated triplets (δ 5.01, 5.63 and 5.33, respectively) having large coupling constants ($J_{1,2}=8$ Hz, $J_{2,3}=J_{3,4}=J_{4,5}=9$ Hz). These findings indicate that the glucopyranose ring adopts the $^4C_1$ conformation.

On alkaline methanolysis, the methyl ethers (**14**) and (**15**) furnished methyl 3,4,5-trimethoxybenzoate (**16**), dimethyl hexamethoxydiphenoate (**17**) and methyl heptamethylflavogallonate (**18**). The sign of the specific optical rotation [$-24.3°$ ($CHCl_3$)] of **17** establishes the atropisomerism to be in the *S*-series,[12] while similarities of the circular dichroism (CD) curves (Fig. 1) of **18** and dimethyl (*S*,*S*)-decamethylgallagiate (**19**)[1] indicate that the chirality of the flavogallonyl group is in the *S*-series.

The assignment of the carboxylic acid esters was achieved in the following ways. An attempt to hydrolyze partially the methyl ether (**14**) with methanolic sodium methoxide (0.5%, 5 min) yielded a hydrolysate (**20**) almost quantitatively. The electron-impact mass

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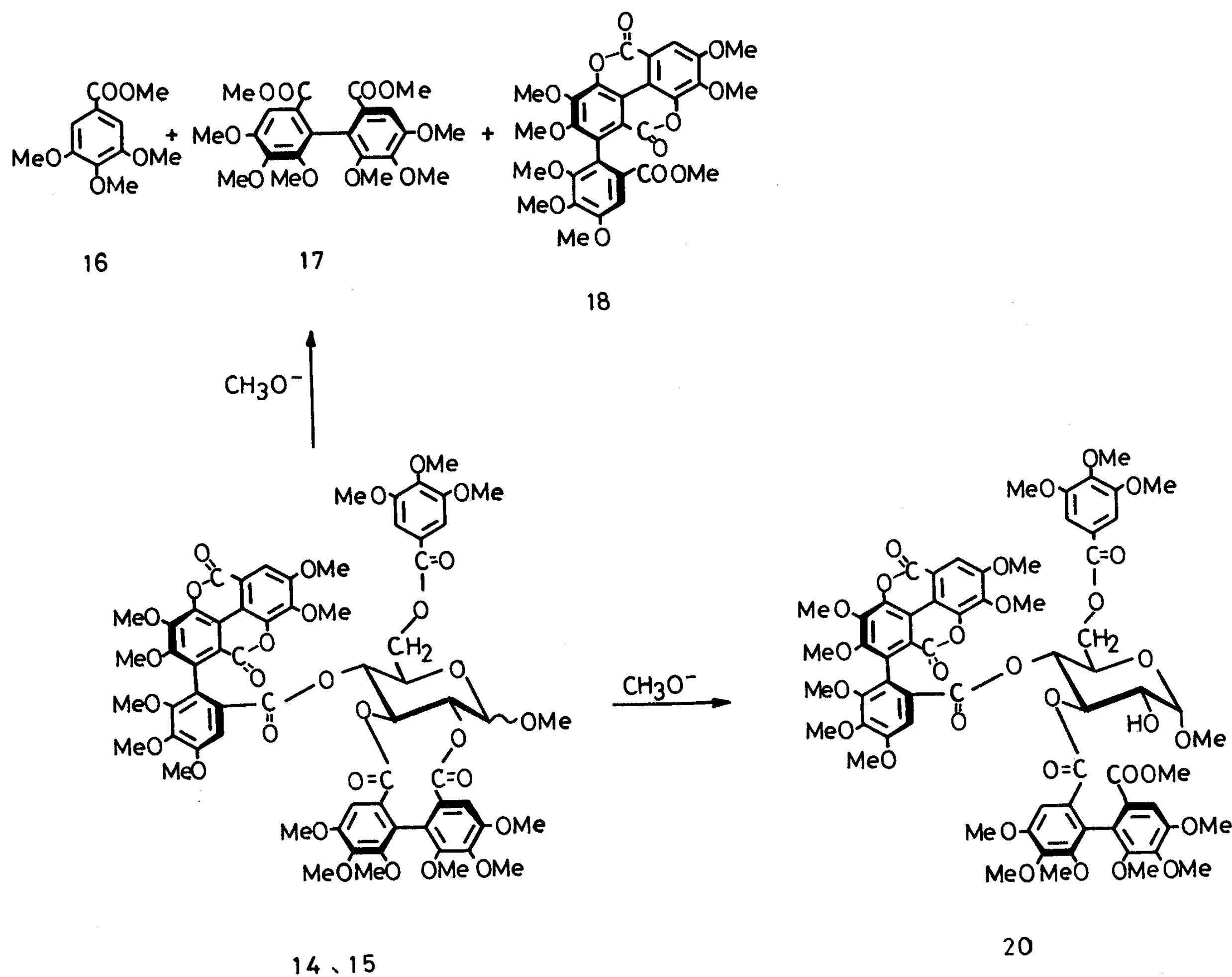

Chart 2

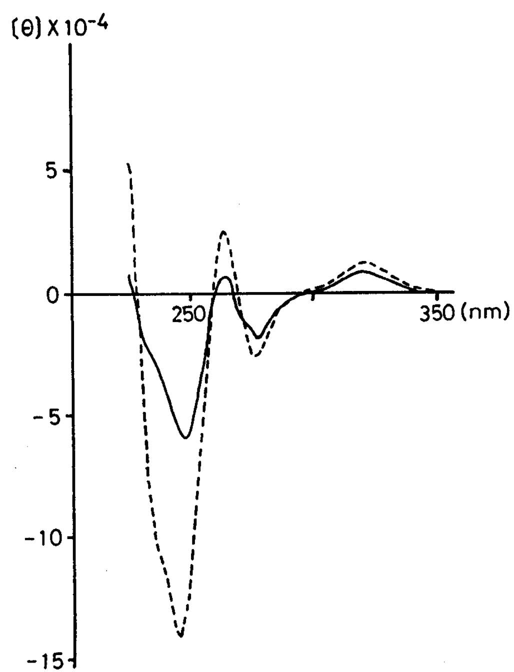

Fig. 1. CD Spectra of **18** and **19** (in CH_3CN)
——, **18**, ----, **19**.

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spectrum (EI-MS) of the hydrolysate (**20**) reveals the M^+ ion peak at *m/z* 1356, which is 32 mass units (corresponding to 1 mol of methanol) more than that in the case of the starting compound (**14**). In the ^{1}H-NMR spectrum of **20**, the glucose H-2 signal, which is observed at δ 5.12 in the spectrum of **14**, is shifted considerably upfield (overlapped with the methoxyl signals at δ 3.40—4.10). Furthermore, the presence of trimethoxybenzoyl, hexamethoxydiphenoyl and heptamethylflavogallonyl ester groups in **20** is confirmed by the aromatic proton resonances. Based on these observations coupled with the above-mentioned facts that the glucopyranose ring preferentially takes the $^4C_1$ conformation and that the hexahydroxydiphenoyl group has *S*-configuration,[13] one of the esters of the hexahydroxydiphenoyl group is considered to be selectively hydrolyzed, and the locations of this carboxylic acid can therefore be identified as the C-2 and C-3 positions. In contrast, partial hydrolysis of terflavin A (**1**) with tannase gave gallic acid and a hydrolysate (**21**), whose ^{1}H- and ^{13}C-NMR spectra show remarkable upfield shifts of the glucose C-6 methylene signals (δ 3.56 and 61.4) as compared with those of terflavin A (**1**). Thus, the galloyl group is confirmed to be located at the C-6 position. Consequently, the position of the remaining flavogallonyl ester group is concluded to be at C-4, and hence the structures of terflavins A and B are represented by the formulae **1** and **2**, respectively.

Tergallagin (**3**), a yellow crystalline powder (H_2O), mp 268 °C (dec.), $[\alpha]_D$ −249.0° (MeOH), $C_{55}H_{30}O_{34} \cdot 7H_2O$, shows in the ^{1}H- and ^{13}C-NMR spectra carbohydrate resonances almost identical with those found in punicalagin (**6**), but their spectra differ in the aromatic field. The observation of two δ-lactone signals (δ 157.6 and 158.2) similar to those of punicalin (**5**) and punicalagin (**6**), as well as the characteristic yellow coloration of tergallagin (**3**), is consistent with the presence of a tetraphenyl (gallagyl) ester group. Together with four ester carbon signals (δ 167.4, 168.0, 168.4 and 169.4), an additional carboxyl resonance appears at δ 163.1; this finding is consistent with the ^{1}H-NMR data, which show the presence of five aromatic protons, one proton more than in the case of punicalagin (**6**).

When hydrolyzed in 1 N sulfuric acid, tergallagin (**3**) afforded punicalin (**5**) and a phenolcarboxylic acid (**22**). The molecular formula of $C_{21}H_{10}O_{13} \cdot H_2O$ for the acid (**22**), determined by elemental analysis and fast atom bombardment mass spectrometry (FAB-MS), corresponds to a trimeric gallic acid structure possessing two lactone functions. Since the ^{1}H-NMR spectrum of the acid shows three aromatic singlets at δ 6.84 (1H) and 7.42 (2H),[14] the

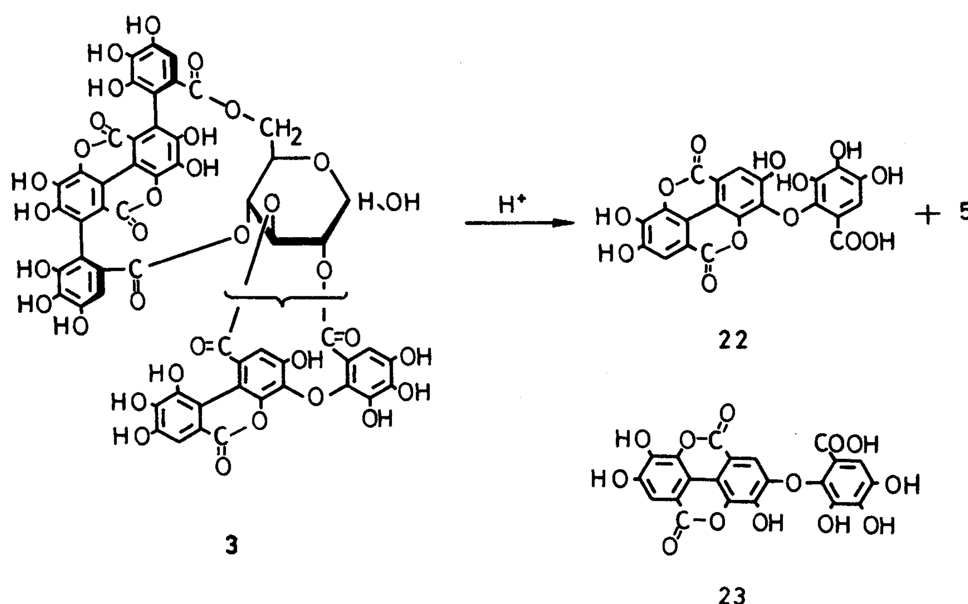

Chart 3

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acid was considered to be either valoneaic acid dilactone (**23**)[15] or its structural isomer (**22**). Comparison of the physical and spectral data of the acid with those of **23** showed a clear difference, so the structure of the acid (designated as tergallic acid dilactone) was concluded to be **22**.

Judging from close similarities in the chemical shifts of the glucose proton signals between tergallagin (**3**) and punicalagin (**6**), two of the three carboxyl groups in tergallic acid should be linked to the glucose C-2 and C-3 positions. The locations of the esterification in tergallic acid were determined as follows. Methylation of tergallagin (**3**) in the same way as described above afforded two octadecamethyl ethers (**24**) and (**25**), which were characterized as α- and β-anomers, respectively, by ^{1}H-NMR analysis ($J_{1,2}=4$ Hz in **24** and $J_{1,2}=6$ Hz in **25**). The appearance of the M^+ ion peak at *m/z* 1486 in the EI-MS of **25** confirms the presence of one lactone ring in the tergallic acid moiety. On subsequent alkaline methanolysis, each methyl ether yielded dimethyl (*S*,*S*)-decamethylgallagiate (**19**) and an acid ester (**26**). The ^{1}H-NMR spectrum of the acid ester (**26**) exhibits signals due to one phenolic hydroxyl group and ten methoxyls including three carboxylic acid methyl esters. The molecular mass of **26** was determined to be 646 by EI-MS, with a substantial fragmentation to the ion at *m/z* 614 (base ion peak) which results from the loss of one methanol molecule. The fact that **26** easily releases a methanol molecule in the EI-MS suggests that the phenolic hydroxyl group is located at a position where the formation of the lactone is easy. Furthermore, measurement of the specific optical rotation [0° ($CHCl_3$)] of this compound confirmed that there is no atropisomerism in the biphenyl moiety, indicating that the lactone involves a biphenyl linkage. Thus, two possible structures (**26**) and (**26′**) were considered for this compound. To

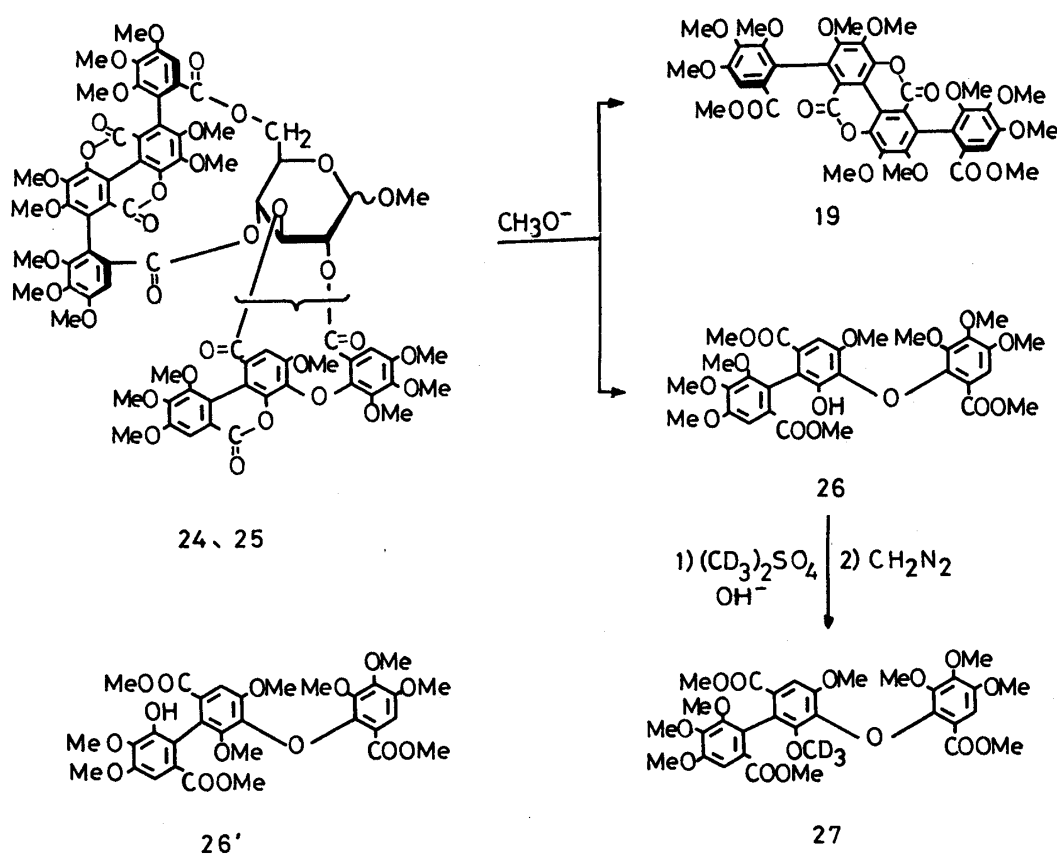

Chart 4

determine the position of the phenolic hydroxyl group in this compound, the deuteromethyl derivative (**27**), prepared by methylation of the compound with hexadeutero-dimethyl sulfate

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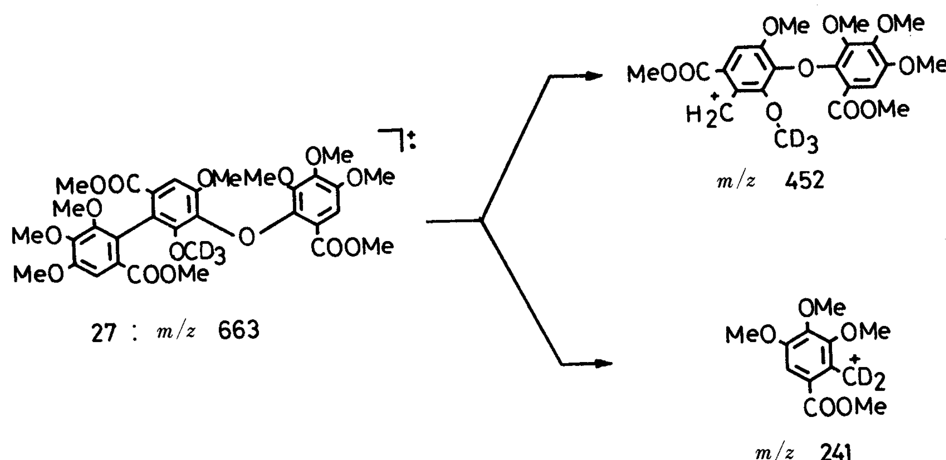
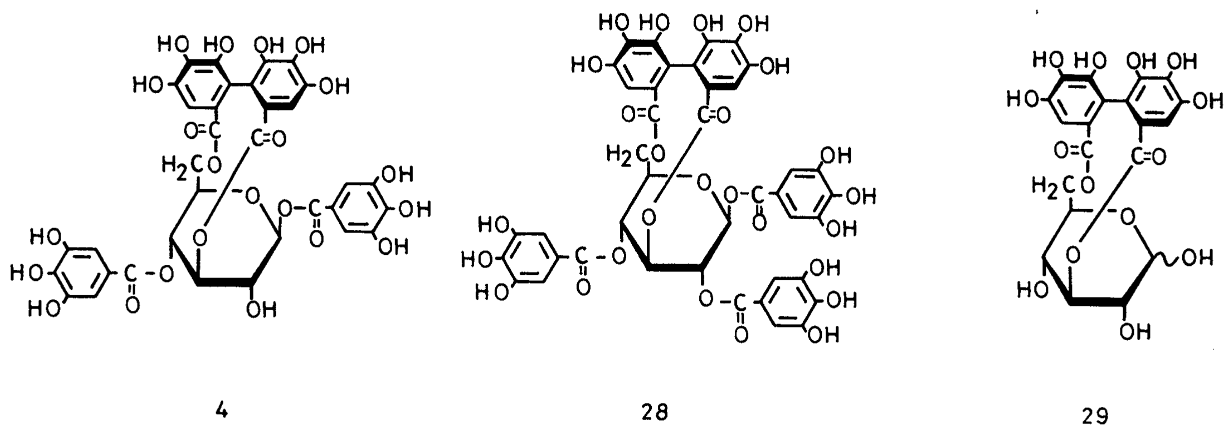


Chart 5. Mass Fragmentation Pattern of **27**

in an alkaline medium, followed by diazomethane treatment, was subjected to EI-MS analysis. Together with a prominent M^+ ion peak at *m/z* 663 (100%), two characteristic fragment peaks appear at *m/z* 241 (24%) and 452 (2.3%),[16] which originate from the cleavage of the biphenyl moiety (Chart 5), the fragmentation being consistent with Mayer's observations.[16] Thus, the structure **26** was assigned for the foregoing ester, and tergallagin was therefore assigned the structure **3**.

Tercatain (**4**) was obtained as a white amorphous powder, $[\alpha]_D$ $-71.1°$ (MeOH), $C_{34}H_{26}O_{22} \cdot H_2O$. The ^{1}H-NMR spectrum indicated the presence of two galloyl groups (δ 7.18 and 7.21, each 2H, s) and one hexahydroxydiphenoyl ester group (δ 6.78 and 6.88, each 1H, s). The chemical shifts and coupling patterns of the carbohydrate moiety were well correlated with those found in punicafolin (1,2,4-tri-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-β-D-glucose) (**28**), which was previously obtained from the leaves of *Punica granatum* L.,[17] except



for the remarkable upfield shift (δ 4.27) of the C-2 proton signal in **4**. Enzymatic hydrolysis of tercatain (**4**) with tannase afforded gallic acid and a hydrolysate which was found to be identical with 3,6-(*R*)-hexahydroxydiphenoyl-D-glucose (**29**)[17] by direct spectral comparisons. Therefore, the structure of tercatain is considered to be 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-D-glucose. Further support for this structure was obtained by comparison of the ^{13}C-NMR chemical shifts of the glucose moieties of corilagin (**11**) and punicafolin (**28**). The similar chemical shifts for the anomeric signals in tercatain (δ 94.5) and corilagin (δ 94.1), and the observation of the high-field shift (δ 91.9) in punicafolin suggest that the neighboring C-2 hydroxyl is not acylated in tercatain. On the other hand, the chemical

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shifts (δ 64.6 and 64.7) for C-4 in tercatain and punicafolin are almost the same, in contrast with the high-field shift (δ 62.0) in corilagin (**11**), indicating the presence of acyl groups at C-4 and the adjacent C-3 position in tercatain.

The configuration at the anomeric center was concluded to be β from the close similarities in the ^{13}C-NMR chemical shifts of anomeric resonances in tercatain and corilagin (**11**). On the basis of these chemical and spectroscopic data, tercatain was identified as 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-β-D-glucose (**4**).

The structural features of terflavins A (**1**) and B (**2**), combined with the fact that punicalagin (**6**) and punicalin (**5**) co-exist with these compounds in this plant source, suggest that the gallagyl group is formed biosynthetically by an oxidative carbon-to-carbon condensation of a galloyl group and a flavogallonyl group, and that **1** and **2** are therefore key intermediates in the biosynthesis of **6** and **5**, respectively. The tergalloyl group, on the other hand, is presumed to be biosynthesized by an oxidative carbon-to-oxygen coupling of a luteoyl (originally hexahydroxydiphenoyl) group and a galloyl group. Considering that the hexahydroxydiphenoyl ester attached to the glucopyranose C-2 position in terflavin A (**1**) and punicalagin (**6**) is more susceptible to hydrolysis than the ester at the C-3 position, it seems more likely that the orientation of the tergalloyl group would be one in which the galloyl residue is linked at the C-2 position and the luteoyl one at C-3.

Finally, it should be noted that the ellagitannins of *Terminalia catappa* L., which bear a galloyl group at the anomeric position, all possess a 3,6-bridged (*R*)-hexahydroxydiphenoyl ester group, whereas tannins having a free anomeric center contain 2,3- and/or 4,6-positioned acyl group(s) such as (*S*)-hexahydroxydiphenoyl and (*S*,*S*)-gallagyl group(s), which is another characteristic feature of the metabolism of tannins in this plant.

Experimental

Details of the instruments and chromatographic conditions used throughout this work are the same as described in the previous paper.[1]

Isolation of Tannins——The air-dried leaves (1.6 kg) of *Terminalia catappa* (collected in Taiwan) were extracted with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure (*ca.* 40 °C), and the resulting precipitates, consisting mainly of chlorophylls, were removed by filtration. The filtrate was further concentrated and applied to a column of Sephadex LH-20. Elution with H_2O containing increasing amounts of MeOH gave six fractions; frs. I (13.5 g), II (2.3 g), III (15.5 g), IV (51.4 g), V (27 g) and VI (15.2 g). Fraction I was rechromatographed over Sephadex LH-20 with the same solvent system to afford 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (**12**) (1 g), punicalin (**5**) (880 mg) and terflavin B (**2**) (63 mg). Fractions, II, III and IV were separately chromatographed over MCI-gel CHP 20P with H_2O containing increasing proportions of MeOH, followed by purification on a Sephadex LH-20 column with 80% aqueous MeOH to furnish gallic acid (900 mg), corilagin (**11**) (800 mg) and punicalagin (**6**) (7.7 g), respectively. Fraction V was repeatedly chromatographed over MCI-gel CHP 20P with H_2O–MeOH (1 : 0—3 : 2, v/v), Sephadex LH-20 with 80% aqueous MeOH and with EtOH, and Avicel micro-crystalline cellulose with 2% AcOH to give terflavin A (**1**) (930 mg), tercatain (**4**) (287 mg), geraniin (**8**) (295 mg), granatin B (**9**) (451 mg) and chebulagic acid (**7**) (88 mg). Fraction VI was chromatographed over Sephadex LH-20 with MeOH, followed by purification on an MCI-gel CHP 20P column with H_2O–MeOH (1 : 0—3 : 2, v/v), to afford 1-desgalloyleugeniin (**10**) (312 mg) and tergallagin (**3**) (638 mg).

Terflavin A (1)——A pale-yellow crystalline powder (H_2O), mp 258 °C (dec.). $[\alpha]_D^{27}$ +240.6 ° (c=1.0, MeOH). *Anal.* Calcd for $C_{48}H_{30}O_{30} \cdot 4H_2O$: C, 49.75; H, 3.31. Found: C, 49.49; H, 3.14. ^{1}H-NMR (acetone-d_6 + D_2O) ppm: 6.42, 6.59 (each 1H, s, HHDP[18]–H), 6.80 (2H, s, galloyl H), 7.23, 7.49 (each 1H, s, flavogallonyl H). ^{13}C-NMR (acetone-d_6 + D_2O) ppm: 62.7, 67.8, 68.2, 68.4, 73.1, 75.2, 77.6, 77.8, 91.5, 95.2 (glucose C), 110.0 (galloyl C-2 and C-6), 12618, 127.0 (HHDP C-2 and C-2′), 158.4, 160.1 (δ-lactone), 165.6, 166.6, 168.7, 169.0, 169.1 (COO).

Partial Acid Hydrolysis of 1——A solution of terflavin A (**1**) (45 mg) in 2% H_2SO_4 (20 ml) was heated at 90 °C for 5 h, then allowed to cool. The resulting precipitates were collected by filtration and recrystallized from pyridine to give pale brown needles. This product was identified as ellagic acid by IR and TLC comparisons with an authentic sample. The filtrate was concentrated under reduced pressure, and the residue was applied to a column of MCI-gel CHP 20P with H_2O–MeOH (1 : 0—3 : 1, v/v) to give terflavin B (**2**) (17 mg).

Methylation of 1——A mixture of terflavin A (**1**) (230 mg), dimethyl sulfate (1.7 ml) and anhydrous potassium carbonate (2 g) in dry acetone (25 ml) was refluxed for 10 h with stirring. After removal of inorganic salts by filtration,

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the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (9:1, v/v) furnished the methyl ether (**14**) as colorless needles (MeOH), mp 170—172 °C, $[\alpha]_D^{18}$ +169.0° (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{65}H_{64}O_{30}$: C, 58.91; H, 4.87. Found: C, 58.60; H, 4.97. EI-MS m/z: 1324 (M^+, 100%), 662 (24%). ^{1}H-NMR ($CDCl_3$) ppm: 4.94 (1H, d, J=4 Hz, H-1), 5.12 (1H, dd, J=4, 10 Hz, H-2), 5.52 (2H, m, H-3 and H-4), 6.46, 6.74 (each 1H, s, HMDP[18]-H), 6.95 (2H, s, galloyl H), 7.39, 7.53 (each 1H, s, flavogallonyl H). Further elution with the same solvent system afforded the methyl ether (**15**) as colorless needles (MeOH), mp 181—183 °C, $[\alpha]_D^{18}$ +158.7° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{65}H_{64}O_{30}$: C, 58.91; H, 4.87. Found: C, 58.66; H, 5.08. EI-MS m/z: 1324 (M^+, 100%), 662 (23%). ^{1}H-NMR ($CDCl_3$) ppm: 4.64 (1H, d, J=8 Hz, H-1), 4.96 (1H, d, J=13 Hz, H-6), 5.01 (1H, t, J=8 Hz, H-2), 5.33 (1H, t, J=9 Hz, H-4), 5.63 (1H, t, J=9 Hz, H-3), 6.46, 6.77 (each 1H, s, HMDP-H), 6.90 (2H, s, galloyl H), 7.34, 7.53 (each 1H, s, flavogallonyl H).

Methanolysis of 14 and 15——A mixture (168 mg) of the methyl ethers (**14** and **15**) in 0.5% methanolic MeONa was stirred at room temperature for 20 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) resin, and the solution was chromatographed over silica gel. Elution with n-hexane–acetone (8:2, v/v) afforded methyl trimethoxybenzoate (**16**) as colorless prisms (MeOH) (5 mg), mp 81—82 °C, dimethyl (S)-4,4′,5,5′,6,6′-hexahydroxydiphenoate (**17**) as a colorless syrup (36 mg), $[\alpha]_D^{18}$ −24.3° (c=0.98, $CHCl_3$), and methyl (S)-heptamethylflavogallonate (**18**) as colorless needles (MeOH) (5 mg), mp 207—208 °C, $[\alpha]_D^{17}$ −21.8° (c=0.32, $CHCl_3$). EI-MS m/z: 582 (M^+), 371, 239. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1715, 1592. CD (c=2.7×10^{-3}, acetonitrile) [θ] (nm): −2.18×10^4 (230), −6.18×10^4 (248), +0.72×10^4 (264), −1.82×10^4 (276), +1.19×10^4 (321).

Partial Methanolysis of 14——The methyl ether (**14**) (20 mg) was treated with 0.5% methanolic MeONa (10 ml) for 5 min. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) resin, and the solution was concentrated to dryness. The residue was applied to a column of silica gel with benzene–acetone (17:3, v/v) to give the methanolysate (**20**) as a pale yellow amorphous powder (19 mg), $[\alpha]_D^{18}$ +140.1° (c=0.7, $CHCl_3$). *Anal.* Calcd for $C_{66}H_{68}O_{31}$: C, 58.41; H, 5.05. Found: C, 58.35; H, 5.36. EI-MS m/z: 1356 (M^+), 168, 551. ^{1}H-NMR ($CDCl_3$) ppm: 2.53 (1H, d, J=8 Hz, OH), 4.73 (1H, d, J=4 Hz, H-1), 4.82 (1H, br d, J=13 Hz, H-6), 5.28 (1H, t, J=10 Hz, H-4), 5.42 (1H, t, J=10 Hz, H-3), 6.89 (2H, s, galloyl H), 7.05, 7.22, 7.29, 7.50 (each 1H, s, HHMD-H and flavogallonyl H).

Enzymatic Hydrolysis of 1 with Tannase——A solution of terflavin A (**1**) (100 mg) in H_2O was incubated with tannase at room temperature for 2 h. Evaporation of the solvent afforded a gum, which was treated with EtOH. The EtOH-soluble portion was applied to a Sephadex LH-20 column with MeOH–H_2O–acetone (14:3:3, v/v) to give gallic acid (12 mg) and the hydrolysate (**21**) as a pale yellow amorphous powder, $[\alpha]_D^{22}$ +32.6° (c=1.2, MeOH). *Anal.* Calcd for $C_{41}H_{26}O_{26}\cdot 3H_2O$: C, 49.81; H, 3.26. Found: C, 49.85; H, 3.48. ^{1}H-NMR (acetone-d_6+D_2O) ppm: 3.56 (2H, br s, H-6), 6.38, 6.40, 6.54 (each s, HHDP-H), 7.30, 7.31, 7.55 (each s, flavogallonyl H). ^{13}C-NMR (acetone-d_6+D_2O) ppm: 61.4 (C-6), 68.0, 68.3, 70.7, 75.1, 75.8, 77.4, 91.0, 94.5 (glucose C), 158.8, 160.8 (δ-lactone), 166.1, 169.1, 169.2 (COO).

Terflavin B (2)——A pale-yellow crystalline powder (H_2O–acetone), mp 232 °C (dec.), $[\alpha]_D^{19}$ +178.2° (c=0.9, MeOH). *Anal.* Calcd for $C_{34}H_{24}O_{22}\cdot 9/2H_2O$: C, 47.18; H, 3.84. Found: C, 46.99; H, 3.65. FAB-MS m/z: 785 [$(M+H)^+$, 2%], 453 (5%). ^{1}H-NMR (acetone-d_6+D_2O) ppm: 6.71 (2H, s, galloyl H), 7.31, 7.50 (each 1H, s, flavogallonyl H). ^{13}C-NMR (acetone-d_6+D_2O) ppm: 62.2, 67.9, 70.7, 71.9, 73.0, 93.1 (α-glucose C), 62.2, 70.5, 72.3, 74.8, 75.6, 97.6 (β-glucose C), 109.1 (galloyl C-2 and C-6), 159.3, 161.3 (δ-lactone), 166.7, 167.1 (COO).

Acid Hydrolysis of 2——A solution of terflavin B (**2**) (20 mg) in 5% H_2SO_4 (3 ml) was heated under reflux for 18 h. After cooling, the reaction mixture was subjected to Sephadex LH-20 chromatography. Elution with H_2O gave a fraction containing a sugar and H_2SO_4. After neutralization with barium carbonate and removal of the inorganic salts by filtration, the filtrate was analyzed by TLC on cellulose (n-BuOH–pyridine–H_2O=6:4:3, v/v), which showed a spot (Rf 0.40) corresponding to that of glucose. Further elution with H_2O–MeOH (4:1, v/v) and (2:3, v/v) gave gallic acid (3 mg) and flavogallonic acid (**13**) (7 mg), respectively. **13**: mp >290 °C. IR ν_{max}^{KBr} cm^{-1}: 1715 (COO). ^{1}H-NMR (CD_3OD) ppm: 7.29, 7.52 (each 1H, s). ^{13}C-NMR (DMSO-d_6) ppm: 106.6, 109.6 (×2), 111.6, 112.8, 117.9, 120.2, 125.2, 135.2, 136.3, 136.7, 139.1, 139.9, 143.2, 143.7 (×2), 147.0, 147.7 (aromatic C), 157.4, 159.2 (δ-lactone), 167.5 (COOH).

Tergallagin (3)——A yellow crystalline powder (H_2O), mp 268 °C (dec.), $[\alpha]_D^{20}$ −249.0° (c=0.8, MeOH). *Anal.* Calcd for $C_{55}H_{30}O_{34}\cdot 7H_2O$: C, 48.54; H, 3.26. Found: C, 48.21; H, 3.13. FAB-MS m/z: 1235 [$(M+H)^+$, 0.2%]. ^{1}H-NMR (acetone-d_6) ppm: 2.17 (1H, m, H-5), 3.27 (1H, t, J=10 Hz, H-6), 4.22 (1H, t, J=10 Hz, H-6), 4.60—5.35 (4H, m), 6.54, 6.56, 6.63, 6.86, 6.94, 7.01 (each s, aromatic H). ^{13}C-NMR (acetone-d_6) ppm: 64.3, 66.9, 71.0, 74.8, 76.3, 90.1 (α-glucose C), 64.3, 71.0, 72.5, 77.1, 78.9, 94.5 (β-glucose C), 157.6, 158.2, 163. 1(δ-lactone), 167.4, 168.0, 168.4, 169.4 (COO).

Acid Hydrolysis of 3——A solution of tergallagin (**3**) (36 mg) in 1 N H_2SO_4 (3 ml) was heated at 90 °C for 3 h, then allowed to cool. The resulting precipitates were collected by filtration, and recrystallized from H_2O to give tergallic acid dilactone (**22**) as a white crystalline powder (5 mg), mp 245—247 °C (dec.). *Anal.* Calcd for $C_{21}H_{10}O_{13}\cdot H_2O$: C, 51.65; H, 2.48. Found: C, 51.46; H, 2.51. FAB-MS m/z: 471 [$(M+H)^+$, 2%]. IR ν_{max}^{KBr} cm^{-1}: 1740 (sh.), 1720 (COO), 1608. ^{1}H-NMR (DMSO-d_6) ppm: 6.84 (1H, s, aromatic H), 7.42 (2H, s, aromatic H). The filtrate was applied to a Sephadex LH-20 column with MeOH–H_2O (4:1, v/v) to furnish punicalin (**5**) (9 mg).

Methylation of 3——A mixture of tergallagin (**3**) (200 mg), dimethyl sulfate (1.5 ml) and anhydrous potassium

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carbonate (1 g) in dry acetone (20 ml) was refluxed for 7 h. The reaction mixture was worked up in the same way as described for terflavin A to give the methyl ether (**24**) as a white crystalline powder (MeOH), mp 241—243 °C, $[\alpha]_D^{18}$ $-206.5°$ (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{73}H_{66}O_{34} \cdot 1/2H_2O$: C, 58.60; H, 4.51. Found: C, 58.60; H, 4.68. EI-MS m/z: 1486 (M^+, 2%). ^{1}H-NMR ($CDCl_3$) ppm: 2.61 (1H, dd, J=3, 10 Hz, H-5), 2.93 (1H, d, J=12 Hz, H-6), 4.62 (1H, d, J=6 Hz, H-1), 4.97 (1H, dd, J=6, 10 Hz, H-2), 5.28 (1H, dd, J=8, 10 Hz, H-3), 6.72, 6.78, 6.83, 7.13, 7.44 (each 1H, s, aromatic H), and the methyl ether (**25**) as a white crystalline powder (MeOH), mp 261—263 °C, $[\alpha]_D^{18}$ $-171.3°$ (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{73}H_{66}O_{34} \cdot 1/2H_2O$: C, 58.60; H, 4.51. Found: C, 58.45; H, 4.69. EI-MS m/z: 1486 (M^+, 2%). ^{1}H-NMR ($CDCl_3$) ppm: 4.72 (1H, d, J=4H, H-1), 5.03 (1H, dd, J=4, 9 Hz, H-2), 5.49 (1H, t, J=9 Hz, H-3), 6.67, 6.75, 6.86, 7.12, 7.34 (each 1H, s, aromatic H).

Methanolysis of 24 and 25——A mixture (100 mg) of **24** and **25** in MeOH (15 ml) was treated with 3% methanolic MeONa (3 ml) at room temperature for 20 h. The reaction mixture was worked up as described above to give dimethyl (S,S)-decamethylgallagiate (**19**) as yellow prisms (MeOH) (10 mg), mp 276—278 °C, $[\alpha]_D^{18}$ $-74.0°$ (c=0.1, $CHCl_3$). CD (c=3.0×10^{-3}, acetonitrile) $[\theta]$ (nm): -9.70×10^4 (235), -14.1×10^4 (245), $+2.58 \times 10^4$ (264), -2.70×10^4 (276), $+1.25 \times 10^4$ (322), and the methanolysate (**26**) as a colorless syrup (17 mg), $[\alpha]_D^{19}$ 0° (c=1.2, $CHCl_3$). EI-MS m/z: 646 (M^+, 22%), 614 [$(M-MeOH)^+$, 100%), 583 (77%). ^{1}H-NMR ($CDCl_3$) ppm: 3,56, 3.58, 3.82, 3.83, 3.85, 3,86, 3.91 (OCH_3), 6.99, 7.20, 7.36 (each 1H, s, aromatic H), 7.68 (1H, s, OH).

Deuteromethylation of 26——A mixture of **26** (15 mg), 10% aqueous NaOH (2 ml), MeOH (1 ml) and hexadeuterodimethyl sulfate (0.5 ml) was heated at 90 °C for 2 h. The reaction mixture was acidified with 1 N HCl and extracted with ether. The organic layer was dried over Na_2SO_4 and evaporated to give a residue, which was treated with ethereal diazomethane for 30 min. Purification of the product over silica gel (benzene–acetone: 24 : 1, v/v) afforded **27** as a colorless syrup (13 mg), $[\alpha]_D^{19}$ 0° (c=1.2, $CHCl_3$). EI-MS m/z: 663 (M^+, 100%), 632 (3%), 452 (2%), 241 (24%). ^{1}H-NMR ($CDCl_3$) ppm: 3.58, 3.62, 3.75, 3.80, 3.87, 3.91, 3.92 (OCH_3), 7.16, 7.32, 7.37 (each 1H, s, aromatic H). ^{13}C-NMR ($CDCl_3$) ppm: 108.2, 108.8, 109.6, 117.0, 123.9, 125.1, 126.4, 127.1, 143.9, 145.3, 145.5, 146.1, 146.5, 148.5, 149.7, 151.3, 152.1 (aromatic C), 166.1, 166.8 (COO).

Tercatain (4)——A white amorphous powder, $[\alpha]_D^{19}$ $-71.1°$ (c=1.0, MeOH). *Anal.* Calcd for $C_{34}H_{26}O_{22} \cdot H_2O$: C, 50.76; H, 3.51. Found: C, 50.95; H, 3.49. ^{1}H-NMR (acetone-d_6) ppm: 4.20—4.40 (2H, m, H-2, H-5), 4.57 (1H, dd, J=4, 13 Hz, H-6), 4.68 (1H, dd, J=6, 13 Hz, H-6), 5.00 (1H, br s, H-3), 5.80 (1H, d, J=3 Hz, H-4), 6.31 (1H, d, J=4 Hz, H-1), 6.78, 6.88 (each 1H, s, HHDP-H), 7.18, 7.21 (each 2H, s, galloyl H). ^{13}C-NMR (acetone-d_6) ppm: 64.6 (C-4, C-6), 70.4 (C-2), 72.3 (C-3), 74.9 (C-5), 94.5 (C-1), 108.6, 109.8 (HHDP C-3 and C-3′), 110.3, 110.5 (galloyl C-2 and C-6), 115.4, 116.3 (HHDP C-1 and C-1′), 120.6, 120.9 (galloyl C-1), 125.4 (HHDP C-2 and C-2′), 136.6, 137.0 (HHDP C-5 and C-5′), 139.2, 139.4 (galloyl C-4), 144.8, 145.3, 145.9 (galloyl C-3 and C-5, HHDP C-4, C-4′, C-6 and C-6′), 165.4, 166.8, 168.4 (COO).

Enzymatic Hydrolysis of 4 with Tannase——A solution of tercatain (**4**) (19 mg) in H_2O–MeOH (1 : 1, v/v) was shaken with tannase at room temperature for 25 min. The reaction mixture was worked up as described above to give gallic acid (5 mg) and 3,6-(R)-hexahydroxydiphenoyl-D-glucose (**29**) as a tan amorphous powder (6.5 mg), $[\alpha]_D^{22}$ $-21.3°$ (c=0.3, EtOH). ^{1}H-NMR (acetone-d_6) ppm: 6.76, 6.77, 6.78, 6.79 (each s, HHDP-H).

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