Tannins and Related Compounds. LV. 1) Isolation and Characterization of Acutissimins A and B, Novel Tannins from Quercus and Castanea Species

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Two novel and closely related tannins, designated as acutissimins A (1) and B (2), have been isolated from various Fagaceous plants: Quercus acutissima, Q. miyagii, Q. stenophylla, Q. mongolica var. grosseserrata and Castanea crenata. Degradative and synthetic studies combined with 1H- and 13C-NMR spectrometry and various mass spectral measurements have permitted the assignments of the structures, in which (++)-catechin (4) and the C-glycosylated ellagitannin, castalagin (3), are connected through a carbon–carbon linkage.

Keywords—Quercus sp.; Castanea crenata; Fagaceae; acutissimin A; acutissimhib B; tannin; flavan-3-ol; C-glycosylated ellagitannin

In previous papers, we demonstrated that the bark of Quercus stenophylla MAKINO (Fagaceae) produces a homologous series of unusual tannins (stenophyllanins A, B and C2) and stenophyna A and 1B) in which a flavan-3-ol (catechin) unit, one of the component units of condensed tannins, is connected through a carbon–carbon linkage to a hydrolyzable tannin. Taking the structural features into account, these tannins can not be classified into either condensed or hydrolyzable tannins, and we therefore believe that the recognition of these tannins as a new class is an interesting extension of the phytochemistry of tannins. In a continuing systematic chemical examination of the polyphenolic constituents in various Fagaceous plants, we have now isolated two new tannins, acutissimins A (1) and B (2), which are structurally related to the above compounds, from Quercus acutissima CARRUTH. (Japanese name: Kunugi), Q. miyagii KOIDZ. (Okinawa-urajirogashi), Q. stenophylla MAKINO (Urajirogashi), Q. mongolica FISCHER var. grosseserrata (BL.) REHD. et WILS. (Mizunara) and Castanea crenata SIEB. et ZUCC. (Kuri), and in this paper we present a detailed account of the structural determination of these compounds.

Typical procedures for the isolation of acutissimins A (1) and B (2) from each plant material are as follows. Initially, the aqueous acetone extract was subjected to Sephadex LH-20 chromatography. Stepwise elution with water containing increasing amounts of methanol effected fairly good fractionation. Earlier fractions contained simple phenolic glycosides and lower-molecular-weight tannins, while acutissimins were less mobile with this solvent system and were almost invariably eluted in the final fractions. The acutissimin-containing fractions were subsequently chromatographed with a mixture of methanol–water on reversed-phase gels such as MCI-gel CHP-20P, Bondapak C18 Porasil B and/or Fuji-gel ODS-G3 to yield pure samples.

Acutissimin A (1), obtained as an off-white amorphous powder, [α]D 74.0° (acetone), gave a reddish pink coloration on treatment with the anisaldehyde–sulfuric acid reagent,4) suggesting the presence of a flavan-3-ol framework in the molecule, while a brown coloration
in the nitrous acid test\textsuperscript{5) was consistent with an ellagitannin. The proton nuclear magnetic resonance (\textsuperscript{1}H-NMR) spectrum showed three one-proton aromatic singlets at \(\delta\) 6.56, 6.76 and 7.08, two of which were attributable to the protons of a hexahydroxydiphenoyl ester group. Other aromatic resonances appeared as an ABX-type signal at \(\delta\) 6.76 (d, \(J=8\) Hz), 6.90 (br s) and 6.94 (br d, \(J=8\) Hz), and as a high-field singlet at \(\delta\) 6.32, the chemical shifts suggesting the presence of catechol and phloroglucinol rings, respectively. In the aliphatic proton region, the

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
 & 1 & 2 & 10 \\
\hline
\textbf{Catechin moiety} & & & \\
2-H & 5.48 (br s) & 4.58 (d, \(J=8\)) & 4.82 (d, \(J=8\)) \\
3-H & 4.56 (m) & - & -\textsuperscript{b)} \\
4-H & 2.40 (br d, \(J=16\)) & 2.3–3.0 (m) & 2.50–2.84 (m) \\
 & 2.90 (br d, \(J=16\)) & & \\
6-H & 6.32 (s) & - & 6.03 (s) \\
8-H & - & 6.10 (s) & - \\
2'-H & 6.90 (br s) & 6.96 (br s) & 6.82 (br s) \\
5'-H & 6.76 (d, \(J=8\)) & 6.76 (d, \(J=8\)) & 6.78 (d, \(J=8\)) \\
6'-H & 6.94 (br d, \(J=8\)) & 6.88 (br d, \(J=8\)) & 6.70 (br d, \(J=8\)) \\
\textbf{Polyalcohol moiety} & & & \\
1'-'H & 4.84 (s) & 4.72 (s) & 4.60 (s) \\
2'-'H & 5.20 (s) & 5.12 (s) & 5.33 (s) \\
3'-'H & 4.76 (d, \(J=8\)) & 4.87 (d, \(J=8\)) & 4.64 (d, \(J=8\)) \\
4'-'H & 5.28 (t, \(J=8\)) & 5.28 (t, \(J=8\)) & - \\
5'-'H & 5.60 (d, \(J=8\)) & 5.68 (d, \(J=8\)) & 5.16 (m) \\
6'-'H & 4.12 (d, \(J=12\)) & 3.98 (d, \(J=12\)) & -\textsuperscript{b) \\
 & 4.60 (d, \(J=12\)) & - & - \\
\textbf{Biphenoyl H} & 6.56 (s) & 6.64 (s) & - \\
 & 6.76 (s) & 6.80 (s) & \\
\textbf{Triphenoyl H} & 7.08 (s) & 7.10 (s) & 6.70 (s) \\
\hline
\end{tabular}
\caption{\textsuperscript{1}H-NMR Data for Acutissimins A (1) and B (2) and the Hydrolysate (10)\textsuperscript{a)}

\textsuperscript{a)} Measured at 100 MHz in acetone-\(d_4\) + D\(_2\)O; \(J\)-values are expressed in Hz. \textsuperscript{b)} Overlapped with an HOD or H\(_2\)O signal which lies in the range of \(\delta 4.0–4.5\).}
appearance of a pair of broad doublets at $\delta 2.40$ ($J = 16$ Hz) and $2.90$ ($J = 16$ Hz) assignable to the C-4 benzylic methylene was characteristic of a flavan-3-ol derivative. In addition, the pattern of the low-field signals due to a polyalcohol methylene and methines bearing acyl groups was closely analogous to that found in a C-glycosylated ellagitannin, castalagin (3).$^6$)

These $^1$H-NMR observations suggested that a 5,7,3',4'-tetrahydroxy-flavan-3-ol is attached to a C-glycosylated ellagitannin at the C-6 or C-8 position. The carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) data were in good agreement with these observations, but were more informative. Almost the same chemical shifts for flavan C-ring carbons as those in (+)-

![Chart 2](image)

Fig. 1. $^{13}$C-NMR Spectra of Acutissimins A (1) and B (2), (+)-Catechin (4) and Castalagin (3) (in Acetone-$d_6 + D_2O$)

These $^1$H-NMR observations suggested that a 5,7,3',4'-tetrahydroxy-flavan-3-ol is attached to a C-glycosylated ellagitannin at the C-6 or C-8 position. The carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) data were in good agreement with these observations, but were more informative. Almost the same chemical shifts for flavan C-ring carbons as those in (+)-
catechin (4) (Fig. 1) implied the 2,3-trans configuration of the flavan unit,7) while the
difference in the chemical shifts of the A-ring carbons, especially in the low-field shift (δ 106.9)
of the C-6 or C-8 carbon, clearly indicated the location of a substituent at the A-ring. Other
signals, except those arising from polyalcohol carbons, were almost in line with those of 3 plus
4 (Fig. 1). These observations coupled with the significant upfield shift (δ 37.9) of the
polyalcohol C-1 atom as compared with that in 3 suggested that acutissimin A (1) is a
condensation product of 3 and 4. This was supported by the appearance of the [M + H]+ peak
at m/z 1207 in the fast atom bombardment mass spectrum (FAB-MS) of 1.

On treatment with mineral acids, 1 yielded a complex mixture of degradation products,
and no information about the structure was obtained. However, oxidative degradation of 1
with 10% ferric chloride afforded glucose and arabinose, thus confirming that the polyalcohol
carbons, except for the C-1 atom, possess the same configuration as those of glucose. On the
other hand, refluxing of 1 in ethanol containing acetic acid (20%) for a long period,2) followed
by repeated chromatography over Sephadex LH-20, yielded, among many uncharacterized
products, a crystalline compound, mp 143 °C, [α]D +16.7° (acetone), which was shown to be
identical with (+)-catechin (4).

The 1H-NMR spectrum of 1 showed a broad singlet at δ 4.84 due to the polyalcohol C-1
proton, the coupling pattern suggesting that the dihedral angle between the C-1 and C-2
protons is close to 90°. Inspection of a Dreiding model revealed that when the C-1 proton
occupied the β-position, the dihedral angle was about 85° (Fig. 2), which is consistent with the
1H-NMR data. Furthermore, in a comparison of the 1H-NMR coupling constant of the C-1
proton in 1 with those in 3 and its C-1 epimer, vescalagin (5),6) the J-value of 1 was similar to
that (J = 1 Hz) of 5 rather than that (J = 5 Hz) of 3, thus indicating that the C-1 atom has the
same configuration as that of 5.

The differentiation of C-6 and C-8 substituted catechin derivatives by 13C-NMR
spectroscopy of their methyl ethers has been described repeatedly.2,8) To determine the point
of the linkage between the catechin and C-glycosylated ellagitannin moieties, this method was
applied. Methylation of 1 with dimethyl sulfate and anhydrous potassium carbonate in dry
acetone furnished the nonadecamethyl ether (1a), the field-desorption mass spectrum (FD-
MS) of which exhibited a prominent M+ peak at m/z 1472, consistent with the proposed
structure. The 13C-NMR spectrum of 1a aided by an off-resonance technique showed an
unsubstituted A-ring carbon signal at δ 89.5 (d), the chemical shift being in good agreement
with those of C-8 substituted catechin derivatives8) [e.g., gambiriin A1 nonamethyl ether (6): δ 88.6]
rather than those of the C-6 substituted alternatives [e.g., gambiriin A3 nonamethyl ether (7): δ 96.1] (Fig. 3). Based on these observations, the ellagitannin was concluded to be
connected through a carbon–carbon linkage to the C-8 position of the catechin nucleus.

The chiralities of the hexahydroxydiphenoyl and nonahydroxytriphenoyl ester groups
were determined as follows. Alkaline methanalysis of the methyl ether (1a) with sodium
methoxide in methanol yielded optically active dimethyl hexamethoxydiphenoate (8) ([α]D
−31.6° (CHCl3)), together with the methanolysate (9) [FD-MS m/z 1118 (M)+]. The sign of
the specific optical rotation of 8 thus established unequivocally the atropisomerism to be in
the S-series.\(^\text{10)}\) On the other hand, upon enzymatic hydrolysis with tannase, 1 liberated ellagic
acid and an amorphous compound (10), whose molecular mass \([m/z\ 903\ (M-H)^-\text{ in the}
\text{negative FAB-MS}]\] confirmed its deshexahydroxydiphenoyl structure. The circular dichroism
(CD) spectrum (Fig. 4) of 10 showed an intense positive Cotton effect at 237 nm and a
negative one at 263 nm, both corresponding well to those found in castalin (11), whose
triphenoyl ester moiety had been established to possess the S,S-configuration.\(^\text{6b)}\) Thus, the
atropisomerism in the triphenoyl group was concluded to be in the S,S-series.

Unequivocal structural assignment of 1 was successfully achieved by condensation of 3 and 4. Refluxing of the mixture in dry acetone containing p-toluenesulfonic acid, followed by repeated chromatography over Sephadex LH-20 with ethanol and 60% aqueous methanol, afforded, together with a large amount of unreacted 3, a condensation product, which was found to be identical with 1.

Acutissimin B (2), obtained as an off-white amorphous powder, [α]D -5.5° (acetone), showed chromatographic properties and color reactions similar to those of 1. The FAB-MS with the [M+H]+ peak at m/z 1207 indicated the same molecular mass as that of 1. The 1H- and 13C-NMR spectra were also closely correlated with those of 1 (Table I and Fig. 1): the almost identical signal patterns in the aromatic fields showed that similar functional groups exist in the molecule, while the chemical shifts for the polyalcohol carbons confirmed the presence of the same substitution system in the polyalcohol moiety. The 1H-NMR coupling patterns of the flavan C-2 and C-4 protons clearly indicated the presence of a catechin moiety. In addition, the appearance of a singlet at δ 4.72 due to the polyalcohol C-1 proton confirmed the configuration of the C-1 atom to be the same as that of 1.

On methylation in the same way as described for 1, 2 formed the nonadecamethyl ether (2a). Subsequent alkaline methanolysis of 2a with sodium methoxide in methanol yielded a methanolysate (12) and (-)-dimethyl hexamethoxydiphenoate (8) ([α]D -31.0° (CHCl3)), thus establishing unambiguously the atropomerism of the hexahydroxydiphenoyl ester group to be in the S-series. The spectral data of 12 including FD-MS [m/z 1118 (M)+] were consistent with its deshexahydroxydiphenoyl structure (similar to that of 9).

The 13C-NMR spectrum of 2a aided by an off-resonance experiment showed an
unsubstituted flavan A-ring singlet at \( \delta 96.8 \), the chemical shift being in good agreement with that (\( \delta 96.1 \)) of the C-6 substituted catechin derivative, gambirin \( \text{A}_3 \) nonamethyl ether (7).\(^9\) Furthermore, the \(^1\)H-NMR chemical shift for the flavan C-2 proton supported the C-6 substitution in the catechin moiety\(^{11}\): the chemical shift (\( \delta 4.58 \)) for the C-2 proton was almost identical with that (\( \delta 4.54 \)) found in (+)-catechin (4), whereas in the case of 1, the C-2 proton signal appeared considerably downfield (\( \delta 5.48 \)), indicating that in 2 there is no magnetic through-space interaction between the C-2 proton and the substituent, and that the substituent is therefore located at the remote C-6 position.

The atropisomerism of the triphenoyl ester group was determined to be in the S,S-series from the close similarities of the CD spectra of 2 and 1 (Fig. 5). The stronger intensities of the Cotton effects at 230–235 and 260 nm in 2 and 1 than those observed in 10 and 11 also supported the view that all the biphenyl and triphenyl chiralities are in the S-series.

From the chemical and spectral data described above, the whole structure was concluded to be represented by the formula 2.

Several Fagaceous plants contain both hydrolyzable and condensed tannins, though their contents and compositions differ remarkably among the species and also even in different parts of the plants. Considering that acutissimins A (1) and B (2) occur almost invariably in association with (+)-catechin (4) and castalagin (3), they are likely to be biosynthesized by condensation of these compounds.

**Experimental**

The following instruments were used to obtain physical and spectral data. A Yanagimoto micro-melting point apparatus (melting points), a JASCO DIP-4 digital polarimeter (optical rotations), a Hitachi 100-50 type spectrophotometer, JEOL D-300 and DX-300 spectrometers (FD- and FAB-MS), JEOL PS-100 and FX-100 spectrometers (\(^1\)H (100 MHz)- and \(^13\)C (25.05 MHz)-NMR spectra), and a JASCO J-20 apparatus (CD spectra). Column chromatography was performed using Sephadex LH-20 (25–100 µm, Pharmacia Fine Chemicals), MCI-gel CHP-20P (75–150 µm, Mitsubishi Chemical Industries Ltd.), Bondapak C\(_18\) Porasil B (Waters Associates), Fuji-gel ODS-G3 (43–65 µm, 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 (0.20 mm, Merck) and precoated cellulose \( F_{254} \) plates (0.10 mm, Merck), and spots were detected by their blue fluorescence under ultraviolet (UV) light and with
ferric chloride, anisaldehyde–sulfuric acid or 10% sulfuric acid reagent spray. Analytical gas-liquid chromatography (GLC) for sugars was carried out over 1.5%, SE-30 (2 m x 4 mm) with nitrogen as the carrier gas.

Isolation of Acutissimins A (1) and B (2) — Due to significant differences in the polyphenolic constituents from plant to plant, general procedures for the isolation of acutissimins were not available, and typical isolation procedures (from Quercus acutissima Carruth.) are described herein.

The fresh bark (6.1 kg) of Q. acutissima was chopped into small pieces and extracted at room temperature with acetone–water (4:1). Concentration of the extract under reduced pressure afforded an aqueous solution, which was deposited a resinous precipitate. After filtration, the filtrate was subjected to Sephadex LH-20 chromatography. Elution with water containing increasing proportions of methanol and finally with water–acetone (1:1) furnished three fractions. Fraction (fr.) I contained relatively lower-molecular-weight polyphenols. Fraction II was chromatographed over Sephadex LH-20 with a solvent system of ethanol–water–acetone [12] to yield five further fractions; frs. II-1 (5.5 g), II-2 (5.1 g), II-3 (0.3 g), II-4 (7.3 g) and II-5 (32 g). The final fraction II-5 was repeatedly chromatographed over reversed-phase gels; MCI-gel CHP-20P, Bondapak C18 Porasil B and Fuji-gel, with water containing increasing amounts of methanol, to yield acutissimin A (1) (2.0 g). Similarly, fraction III was subjected to rechromatography over Sephadex LH-20 in water containing increasing amounts of methanol to afford four fractions; frs. III-1 (5 g), III-2 (8 g), III-3 (12 g) and III-4 (2.4 g). Repeated chromatography of fraction III-1 over the above reversed gels gave acutissimin B (2) (0.48 g).

Acutissimin A (1) — An off-white amorphous powder, [α] D 29  = 74.0° (c = 1.2, acetone). Anal. Calcd for C46H30O11·0.5H2O: C, 51.85; H, 3.73. Found: C, 51.84; H, 4.13. FAB-MS m/z: 1207 [M]+. UV λ max (log e): 275 sh (4.93), 225 sh (4.46). 13C-NMR (acetone-d6) ppm: 23.8 (C-4), 37.9 (C-1”), 65.7 (C-6”), 67.6 (C-3), 71.0 (x x), 72.2, 77.6 (C-2”, C-3”, C-4” and C-5”), 80.0 (C-2), 96.8 (C-6), 98.3 (C-4a), 106.9 (C-8), 107.1, 107.4, 108.9 (unsubstituted biphenyl and triphenyl C), 131.5 (C-1’), 152.5, 155.9, 157.1 (C-5, C-7 and C-8a), 165.9, 167.3 (x x), 169.4 (-COO–). CD (c = 1.6 x 10–3, MeOH) [θ] (nm): –5.29 x 103 (260), 5.24, +1.13 x 104 (233). Oxidative Degradation of 1 — A solution of 1 (14 mg) in 10% ferric chloride (2 ml) was heated under reflux for 5 d. The dark blue reaction mixture was neutralized with Amberlite MB-3 resin and the solvent was evaporated off under reduced pressure. The residue was passed through a Sep-pak (ODS) column, and the eluent was concentrated to dryness to yield a colorless syrup, which was treated with N-trimethylsilylimidazole. The trimethylsilyl derivatives were analyzed by GLC (flow rate: 40 ml/min), and peaks corresponding to glucose [tR: 14.7 and 23.6 min (column temp.: 150 °C)] and arabinose [tR: 7.6 and 8.8 min (column temp.: 130 °C)] were confirmed.

Acid-Catalyzed Degradation of 1 — A solution of 1 (1.01 g) in ethanol (6 ml) containing acetic acid (1.5 ml) was heated under reflux for 5 d. The solvent was evaporated off under reduced pressure, and the residue was repeatedly chromatographed over Sephadex LH-20 with ethanol to yield (+)-catechin (4) as colorless needles (1.2 mg), mp 143 °C, [α] D 29  = +16.7° (c = 0.12, acetone).

Methylolation of 1 — A mixture of 1 (0.3 g), dimethyl sulfate (2 ml) and anhydrous potassium carbonate (2.5 g) in dry acetone was heated under reflux for 3 h. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure, and subjected to silica gel chromatography. Stepwise elution with benzene containing increasing proportions of acetone furnished the nonadecamethyl ether (la) as a white amorphous powder (0.19 g), [α] D 29  = -140.0° (c = 0.54, CHCl3). Anal. Calcd for C56H54O11·1/2H2O: C, 70.66; H, 5.24. Found: C, 60.69; H, 5.35. FAB-MS m/z: 1472 (M)+. 1H-NMR (CDCl3) ppm: 5.70 (1H, d, J = 8.8 Hz, 5”-H), 6.26 (1H, s, 6-H), 6.60–7.16 (4H, m, aromatic H). 13C-NMR (CDCl3) ppm: 27.7 (C-4), 38.0 (C-1”), 65.6 (C-6”), 68.0 (C-3”), 69.4 (C-7”), 71.0 (C-2”), 72.2, 77.6 (C-2”, C-3”, C-4” and C-5”), 80.0 (C-2), 96.8 (C-6), 98.3 (C-4a), 106.9 (C-8), 107.1, 107.4, 108.9 (unsubstituted biphenyl and triphenyl C), 131.5 (C-1’), 152.5, 155.9, 157.1 (C-5, C-7 and C-8a), 165.9, 167.3 (x x), 169.4 (-COO–). CD (c = 1.6 x 10–3, MeOH) [θ] (nm): –5.29 x 103 (260), 5.24, +1.13 x 104 (233).

Alkaline Methanolysis of la — A solution of la (0.13 g) in methanol (4 ml) was treated with sodium methoxide (0.25 g) in methanol (12 ml) at room temperature for 3 d. After neutralization with Amberlite IR-120B (H+ form) resins, the reaction products were separated by silica gel chromatography with benzene-acetone (4:1—1:1) to give S-dimethyl hexamethoxydiphenoate (8) as a colorless syrup, [α] D 29  = -31.6° (c = 0.55, CHCl3), and the methanolysate (9). A white amorphous powder, [α] D 29  = -32.7° (c = 0.55, CHCl3). Anal. Calcd for C56H54O31·1/2H2O: C, 59.62; H, 5.63. Found: C, 59.62; H, 5.70. FD-MS m/z: 1118 (M)+. 1H-NMR (CDCl3) ppm: 2.20–3.04 (2H, m, 4-H), 4.48 (1H, s, 3-H), 6.04 (1H, s, 6-H), 6.60–7.16 (4H, m, aromatic H). 13C-NMR (CDCl3) ppm: 26.8 (C-4), 37.5 (C-1’), 63.6 (C-6’), 67.3 (C-3’), 70.6, 74.4, 77.0, 79.3 (C-2’’, C-3’’, C-4’’ and C-5’’), 81.6 (C-2’), 89.0 (C-3’), 89.0 (C-6’), 102.1 (C-4a), 165.2 (C-5’), 170.5 (C-6’’). Tannase Hydrolysis of 1 — A solution of 1 (0.13 g) in water was incubated overnight with tannase at 37 °C. The solvent was evaporated off under reduced pressure, and the residue was treated with ethanol. The ethanol-soluble portion was subjected to chromatography over Sephadex LH-20 with water containing increasing amounts of methanol and then over Fuji-gel with water–methanol (7:3) to yield ellagic acid, the hydrolysat (10) (8 mg) and the starting material (1) (55 mg). A: An off-white amorphous powder, [α] D 29  = -7.3° (c = 0.9, MeOH). Anal. Calcd for C21H16O14·1/2H2O: C, 52.50; H, 4.31. Found: C, 50.10; H, 4.06. Negative FAB-MS m/z: 903 (M–H)– UV λ max (log e): 275 sh (4.89), 227 sh (4.44).

Preparation of 1 — A mixture of (+)-catechin (4) (2.9 g) and castalagin (3) (2.9 g) in dry dioxyne (150 ml) containing p-toluensulfonic acid (0.13 g) was heated under reflux for 24 h. The solvent was evaporated off under reduced pressure, and the residue was repeatedly chromatographed over Sephadex LH-20 with 60% aqueous
methanol and ethanol to give a condensation product (0.14 g), which was shown to be identical with acutissimin A (1) by $[\alpha]_D$ and $^1$H- and $^{13}$C-NMR spectral comparisons.

**Acutissimin B (2)**—An off-white amorphous powder, $[\alpha]_{D32} - 5.5$ (c = 0.84, acetone). Anal. Calcd for $C_{56}H_{38}O_{31}$·$\frac{7}{2}H_2O$: C, 52.96; H, 3.57. Found: C, 53.23; H, 3.01. FAB-MS m/z: 1207 (M + H$^+$). UV $\lambda_{max}$ nm (log ε): 275 sh (4.89), 225 sh (4.40). $^{13}$C-NMR (acetone-d$_6$ + D$_2$O) ppm: 29.4 (C-4), 38.0 (C-1''), 65.8 (C-6''), 70.2, 71.3, 71.8, 78.2 (C-2'', C-3'', C-4'' and C-5''), 82.2 (C-2), 96.2 (C-8), 101.2 (C-4a), 107.5 (C-6), 107.2, 108.8, 110.0 (unsubstituted biphenoyl and triphenoyl C), 115.5, 115.9 (C-2' and C-5'), 120.3 (C-6'), 131.4 (C-1'), 154.9 ($\times$ 2), 156.2 (C-5, C-7 and C-8a), 165.9, 167.1, 167.4, 167.7, 169.4 (–COO–). CD (c = 1.5 × 10$^{-5}$, MeOH) $[\alpha]$ (nm): $-5.58 \times 10^4$ (260), 0 (251), $+1.74 \times 10^5$ (235).

**Methylation of 2** A mixture of 2 (0.15 g), dimethyl sulfate (1.5 ml) and anhydrous potassium carbonate (1.5 g) in dry acetone (10 ml) was refluxed for 3 h with stirring. After cooling, the inorganic precipitate was filtered off, and the filtrate was concentrated to dryness under reduced pressure to give a residue, which was purified by silica gel chromatography. Elution with benzene-acetone (2:1) yielded the nonadecamethyl ether (2a) as a white amorphous powder, $[\alpha]_{D29} - 25.4$ (c = 0.55, CHCl$_3$). Anal. Calcd for $C_{75}H_{76}O_{31}$·H$_2$O: C, 60.39; H, 5.27. Found: C, 60.46; H, 5.39. FD-MS m/z: 1472 (M$^+$). $^1$H-NMR (CDCl$_3$) ppm: 4.68 (1H, br s, 1''-H), 4.80 (1H, d, J= 8 Hz, 2-H), 4.88 (1H, d, J=8 Hz, 3''-H), 5.06 (1H, t, J=8 Hz, 5''-H), 6.28 (1H, s, 8-H), 6.74, 6.88, 7.16 (each 1H, s, aromatic H), 7.92–7.04 (3H, m, 2'-H, 5'-H and 6'-H). $^1$C-NMR (CDCl$_3$) ppm: 28.8 (C-4), 37.9 (C-1''), 65.3 (C-6''), 68.2 (C-3), 70.1, 70.3, 71.1, 77.0 (C-2'', C-3'', C-4'' and C-5''), 82.3 (C-2), 96.8 (C-8), 106.1 (C-4a), 108.4, 107.8 (unsubstituted biphenyl C), 110.0, 112.7, 111.3 (C-2', C-5' and unsubstituted triphenoyl C), 113.7 (C-6), 120.3 (C-6'), 155.3, 157.6, 159.0 (C-5', C-7 and C-8a), 163.9, 164.0, 165.0, 166.1, 167.7 (–COO–).

**Alkaline Methanolysis of 2a**—A solution of 2a (40 mg) in methanol (8 ml) containing sodium methoxide (0.05 g) was stirred at room temperature for 7 d. The reaction mixture was neutralized with Amberlifer IR-120B (H$^+$ form), and the solvent was evaporated off under reduced pressure. The residue was chromatographed over silica gel with benzene-acetone (4 : 1) to yield S-dimethyl hexamethoxydiphenoate (8) as a colorless syrup ($[\alpha]_{D24} - 31.0$ (c = 0.1, CHCl$_3$), and the methanolysate (12) (22 mg). 12: A white amorphous powder, $[\alpha]_{D24} + 52.4$ (c = 0.41, CHCl$_3$). Anal. Calcd for $C_{56}H_{62}O_{24}$·$\frac{1}{2}H_2O$: C, 59.62; H, 5.63. Found: C, 59.63; H, 5.94. FD-MS m/z: 1118 (M$^+$). $^1$H-NMR (CDCl$_3$) ppm: 6.22 (1H, s, 8-H), 6.84–7.04 (3H, m, 2'-H, 5'-H and 6'-H), 7.16 (1H, s, aromatic H).

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**References and Notes**


13) The $^1$H- and $^{13}$C-NMR spectra were duplicated owing to rotational isomerism, caused probably by steric interaction between the catechin B-ring and the castalagin moiety.