# Tannins and Related Polyphenols of Euphorbiaceous Plants. IX.[1] Hydrolyzable Tannins with $^1C_4$ Glucose Core from *Phyllanthus flexuosus* MUELL. ARG.

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**Four new hydrolyzable tannins (phyllanthusiins A—D), a new polyphenol of related structure (phyllanthusiin E), and eight known polyphenols have been isolated from the leaves of *Phyllanthus flexuosus*. On the basis of the spectroscopic methods including $^1H-^{13}C$ long-range two-dimensional nuclear magnetic resonance techniques, phyllanthusiins A (9), B (12) and C (14), were characterized as oxidative metabolites of geraniin, each having a different new acyl group biogenetically formed *via* an oxidative cleavage of the dehydrohexahydroxydiphenoyl group, on the $^1C_4$ glucopyranose core. Phyllanthusiin D (15) was determined to be a condensate of geraniin with acetone, and phyllanthusiin E (16), was an oxidized congener of ellagic acid.**

**Keywords** *Phyllanthus flexuosus*; Euphorbiaceae; tannin; hydrolyzable tannin; phyllanthusiin A; phyllanthusiin B; phyllanthusiin C; phyllanthusiin D; phyllanthusiin E

Bergenin (**1**)[2] and some terpenoids, including several new triterpenes and a diterpene were previously isolated from the bark of *Phyllanthus flexuosus* (SIEB. *et* ZUCC.) MUELL. ARG. in our study on the chemical constituents of Euphorbiaceous plants.[3] Occurrence of **1**, which is structurally related to hydrolyzable tannins, in this species, and the previous finding of a wide distribution of geraniin (**3**)[4] (dehydroellagitannin) in Euphorbiaceous plants,[5] prompted us to investigate other polyphenols in the leaf of this plant, and we have isolated thirteen polyphenols, including four novel hydrolyzable tannins (phyllanthusiins A, B, C and D) and a new polyphenol of related structure (phyllanthusiin E).

The fresh leaves of *P. flexuosus* were extracted first with ether and acetone to remove triterpenes and other lipids, and then the residue was extracted with aqueous acetone and filtered. The *n*-BuOH soluble portion of the concentrated filtrate was chromatographed over columns of Diaion HP-20, Toyopearl HW-40 and Sephadex LH-20, successively, to afford phyllanthusiins A—E, and eight known polyphenols, bergenin (**1**), brevifolincarboxylic acid (**2**),[6] geraniin (**3**), corilagin (**4**),[4] chebulagic acid (**5**),[7] repandusinic acid A (**6**),[8] geraniinic acid B (**7**)[9] and putranjivain A (**8**).[10] The major components were corilagin (**4**) (0.57% per dried leaf), geraniin (**3**) (0.27%) and bergenin (**1**) (0.27%).

All of the new compounds, except for phyllanthusiin E, were shown to be ellagitannins by the characteristic coloration (brownish orange → greenish blue) with sodium nitrite and acetic acid reagent[11] on a thin-layer chromatography (TLC) plate.

Phyllanthusiin A (**9**), $[\alpha]_D$ $-94.5°$, was obtained as a pale yellow amorphous powder. Its molecular formula, $C_{41}H_{28}O_{27}$, was deduced from the $(M-H)^-$ ion peak at *m/z* 951 in the negative fast-atom bombardment mass spectrum (FAB-MS), and elemental analysis. The proton nuclear magnetic resonance ($^1$H-NMR) spectrum of **9** exhibited a 2H singlet ($\delta$ 7.14) and two 1H singlets ($\delta$ 6.66, 7.05) attributable to a galloyl group and a hexahydroxydiphenoyl (HHDP) group. The coupling pattern of the sugar proton signals (Table I) is characteristic of the $^1C_4$ glucopyranose residue, and is similar to that of geraniin (**3**) and geraniinic acid B (**7**). The $^{13}$C-NMR spectrum of **9** also

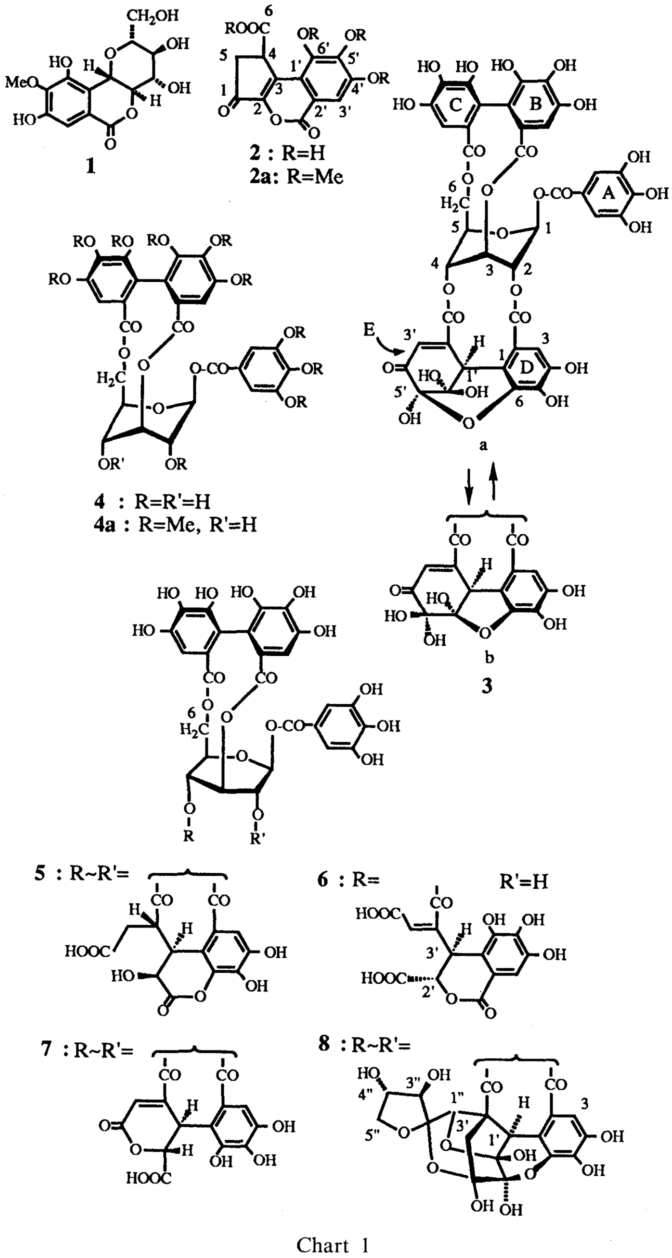


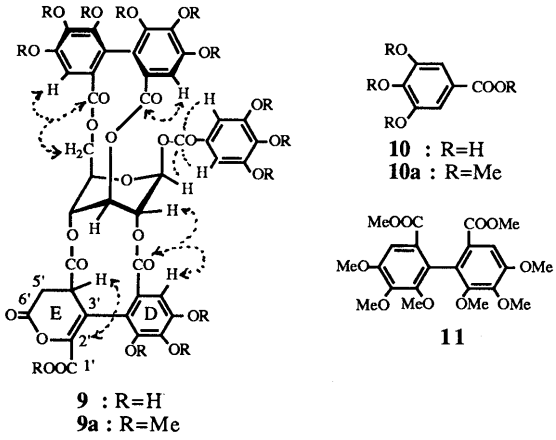
Chart 1

showed a close resemblance to that of **3**, except for the difference of the signals due to the dehydrohexahydroxydiphenoyl (DHHDP) group (rings D, E) in **3** (Table II), indicating that **9** has a corilagin moiety in the molecule. This partial structure was substantiated by production of corilagin (**4**) upon partial hydrolysis of **9** in hot water.

Since the hydroxyl groups of the glucose residue in **9** are all acylated, as indicated by the chemical shifts of the glucose proton signals (Table I), phyllanthusiin A (**9**) was sssumed to be an ellagitannin in which only an acyl group at O-2/O-4 differs from that in **3**. The $^1$H-NMR spectrum of **9** showed, besides the signals due to the corilagin moiety, mutually coupled methine and methylene proton signals [$\delta$ 3.46 (br d, $J=7.0$ Hz), 3.09 (br d, $J=16.5$ Hz), 3.20 (dd, $J=7.0$, 16.5 Hz)], and a 1H aromatic singlet ($\delta$ 7.17), which are attributable to the O-2/O-4 acyl group. The $^{13}$C-NMR spectrum of **9** exhibited fourteen carbon resonances (four carbonyl carbons, eight $sp^2$ carbons and two $sp^3$ carbons) due to the O-2/O-4 acyl moiety (Table II). Among the carbonyl carbons, two are assignable to a lactone ($\delta$ 162.15) and a carboxyl ($\delta$ 169.26) carbon, based on the comparison with the E-ring carbons of geraniinic acid B (**7**).[12] The chemical shifts of six $sp^2$ carbon resonances in **9** are similar to those of the D-ring of **7**, while two $sp^2$ carbons are those of a tetrasubstituted double bond. These NMR data imply that phyllanthusiin A is a regioisomer of **7** concerning the double bond in the O-2/O-4 acyl group.

Methylation of **9** with diazomethane afforded a tridecamethyl derivative (**9a**) [electron impact mass spectrum (EI-MS) $m/z$ 1134 (M$^+$)], which, upon methanolysis with sodium methoxide in methanol, yielded methyl tri-$O$-methylgallate (**10a**) and dimethyl hexamethoxydiphenate (**11**). However, the product derived from the O-2/O-4 acyl group was not detected as a discernible spot on the TLC plate.

The linking mode of the O-2/O-4 acyl group with glucose in **9** was determined from the $^1$H–$^{13}$C long-range shift correlation spectrum (COSY) ($J_{CH}=7$ Hz), which showed a clear correlation between the H-2 ($\delta$ 5.42) of the glucose



**9** : R=H
**9a** : R=Me

**10** : R=H
**10a** : R=Me

**11**

Chart 2

TABLE I. $^1$H-NMR Data for **3**, **9**, **12**, **14** and **15** [500 MHz, Acetone-$d_6$–$D_2O$; $J$ (Hz) in Parenthesis]

| | | **3a**[a] | **3b**[a] | **9** | **12** | **14**[b] | **15** |
|---|---|---|---|---|---|---|---|
| Glucose | H-1 | 6.60 (br s) | 6.60 (br s) | 6.30 (br s) | 6.44 (br s) | 6.34 (br s) | 6.55 (br s) |
| | H-2 | 5.60 (br s) | 5.60 (br s) | 5.42 (br s) | 5.32 (br s) | 5.48 (br s) | 5.54 (br s) |
| | H-3 | 5.50 (br s) | 5.60 (br s) | 5.64 (br s) | 6.12 (br s) | 5.53 (br s) | 5.49 (br s) |
| | H-4 | 5.56 (br s) | 5.46 (br s) | 5.23 (br s) | 5.15 (br s) | 5.36 (br s) | 5.41 (br s) |
| | H-5 | 4.81 (m) | 4.81 (m) | 4.55 (br t)<br>(8.5) | 4.76 (br t)<br>(8.5) | 5.03 (br t)<br>(8.5) | 4.82 (br t)<br>(7.5) |
| | H-6 | 4.93 (t)<br>(11.0) | 4.78 (m) | 4.79 (t)<br>(11.5) | 4.75 (t)<br>(11.5) | 4.82 (t)<br>(11.5) | 4.75 (t)<br>(7.5) |
| | | 4.33 (dd)<br>(8.0, 11.0) | 4.45 (dd)<br>(6.0, 9.0) | 4.31 (dd)<br>(8.5, 11.5) | 4.32 (dd)<br>(8.5, 11.5) | 4.32 (dd)<br>(8.5, 11.5) | 4.38 (dd)<br>(7.5, 10.5) |
| Galloyl (ring-A) | H-2, 6 | 7.22 (s) | 7.20 (s) | 7.14 (s) | 7.14 (s) | 7.05 (s) | 7.15 (s) |
| HHDP (ring-B/C) | H-3 (3′) | 7.13 (s)<br>6.71 (s) | 7.08 (s)<br>6.69 (s) | 7.05 (s)<br>6.66 (s) | 7.05 (s)<br>6.58 (s) | 6.84 (s)<br>6.64 (s) | 7.05 (s)<br>6.63 (s) |
| Ring-D | H-3 | 7.25 (s) | 7.28 (s) | 7.17 (s) | 7.12 (s) | 7.09 (s) | 7.20 (s) |
| Ring-E | H-1′ | 5.16 (s) | 4.72 (d)<br>(1.5) | | | 4.57 (s) | 4.89 (d)<br>(1.5) |
| | H-2′ | | | | 5.52 (d)<br>(2.0) | | |
| | H-3′ | 6.56 (s) | 6.26 (d)<br>(1.5) | | 4.43 (d)<br>(2.0) | 2.35 (dd)<br>(6.5, 10.5)<br>2.23 (br t)<br>(10.5) | 6.28 (d)<br>(1.5) |
| | H-4′ | | | 3.46 (d)<br>(7.0) | | 4.58 (br dd)<br>(6.5, 10.5) | |
| | H-5′ | | | 3.20 (dd)<br>(7.0, 16.5)<br>3.09 (br d)<br>(16.5) | 3.26 (d)<br>(17.0)<br>3.16 (d)<br>(17.0) | | |
| | H-7′ | | | | | | 3.46 (d)<br>(15.5)<br>2.95 (d)<br>(15.5) |
| | H-9′ | | | | | | 2.17 (3H, s) |

*a*) 400 MHz. *b*) Measured in MeOH-$d_4$.

TABLE II. $^{13}$C-NMR Data for **3**, **9**, **12**, **14** and **15** (126 MHz, Acetone-$d_6$–$D_2O$)

| | **3a**[a] | **3b**[a] | **9** | **12** | **14**[b] | **15** |
|---|---|---|---|---|---|---|
| Glucose | | | | | | |
| C-1 | 90.79 | 91.79 | 91.73 | 91.80 | 92.64 | 91.92 |
| C-2 | 69.92 | 70.48 | 69.65 | 70.62 | 68.32 | 70.38 |
| C-3 | 63.29 | 62.38 | 63.23 | 61.52 | 63.03 | 62.38 |
| C-4 | 65.96 | 66.87 | 66.48 | 67.77 | 66.24 | 66.66 |
| C-5 | 72.62 | 73.30 | 73.31 | 73.61 | 73.39 | 73.14 |
| C-6 | 63.73 | 63.73 | 64.00 | 63.96 | 64.45 | 63.89 |
| Galloyl (ring-A) | | | | | | |
| C-1 | 120.30 | 120.30 | 120.44 | 120.19 | 120.03 | 120.12 |
| C-2, 6 | 110.99 | 110.78 | 110.78 | 110.55 | 110.92 | 110.63 |
| C-3, 5 | 145.87 | 145.87 | 145.95 | 145.84 | 146.43 | 146.05 |
| C-4 | 139.67 | 139.67 | 139.63 | 139.62 | 140.73 | 140.00 |
| C-7 | 164.65 | 164.77 | 164.55 | 164.88 | 166.10 | 165.27 |
| HHDP (ring-B) | | | | | | |
| C-1 | 117.21 | 116.86 | 116.88 | 116.65 | 117.37 | 117.29 |
| C-2 | 124.76 | 124.61 | 124.82[c] | 124.45[c] | 124.37[c] | 124.34[c] |
| C-3 | 110.61 | 110.17 | 110.40 | 110.35 | 110.44 | 110.31 |
| C-4 | 144.57 | 144.18 | 144.67 | 144.52 | 145.26 | 144.70 |
| C-5 | 137.73 | 137.73 | 137.67 | 137.64 | 138.61 | 137.82 |
| C-6 | 145.07 | 145.00 | 144.98[d] | 144.85[d] | 145.49[d] | 145.03[d] |
| C-7 | 166.09 | 166.09 | 166.28 | 166.25 | 167.51 | 166.37 |
| (ring-C) | | | | | | |
| C-1 | 115.16 | 115.01 | 115.20 | 115.20 | 116.27 | 115.33 |
| C-2 | 125.73 | 125.52 | 125.71[c] | 125.71[c] | 125.50[c] | 125.35[c] |
| C-3 | 108.03 | 108.17 | 107.95 | 107.95 | 108.03 | 107.85 |
| C-4 | 145.40 | 145.40 | 145.44 | 145.44 | 145.56 | 145.46 |
| C-5 | 136.47 | 136.46 | 136.46 | 136.46 | 137.56 | 136.45 |
| C-6 | 144.96 | 145.16 | 145.16[d] | 145.16[d] | 146.09[d] | 145.26[d] |
| C-7 | 168.29 | 168.15 | 168.43 | 168.42 | 170.08 | 168.67 |
| 2,4-Acyl (ring-D) | | | | | | |
| C-1 | 115.16 | 120.12 | 117.74 | 116.77 | 119.18 | 120.07 |
| C-2 | 119.59 | 117.07 | 120.54[e] | 117.93 | 117.50 | 120.07 |
| C-3 | 113.46 | 113.46 | 111.17 | 112.81 | 111.84 | 113.41 |
| C-4 | 145.72 | 147.60 | 144.32[f] | 147.28 | 147.60 | 147.70 |
| C-5 | 138.82 | 137.27 | 138.67 | 135.54 | 136.42 | 137.42 |
| C-6 | 143.40 | 147.16 | 145.57[f] | 150.81 | 150.03 | 147.02 |
| C-7 | 165.36 | 165.18 | 165.21 | 165.20 | 165.46 | 164.82 |
| (ring-E) | | | | | | |
| C-1′ | 46.21 | 52.02 | 169.26 | 172.68[e] | 63.88 | 51.95 |
| C-2′ | 154.55 | 149.21 | 142.23 | 81.57 | 78.82 | 145.49 |
| C-3′ | 128.63 | 124.99 | 120.86[e] | 56.73 | 46.83 | 126.93 |
| C-4′ | 191.69 | 194.99 | 46.89 | 77.55 | 75.58 | 197.77 |
| C-5′ | 96.22 | 92.35 | 31.06 | 43.10 | 117.30 | 80.92 |
| C-6′ | 92.47 | 92.35 | 162.15 | 172.71[e] | 174.18 | 109.75 |
| C-7′ | 165.53 | 165.68 | 166.33 | 171.76 | | 49.99 |
| C-8′ | | | | | | 206.42 |
| C-9′ | | | | | | 31.99 |
| C-10′ | | | | | | 165.54 |

*a*) 100 MHz. *b*) Measured in MeOH-$d_4$. *c*—*f*) Values in the same column are interchangeable.

core and the D-ring proton ($\delta$ 7.17), by three-bond couplings through the ester carbonyl carbon ($\delta$ 165.21). The other long-range couplings are shown in the formula **9**.

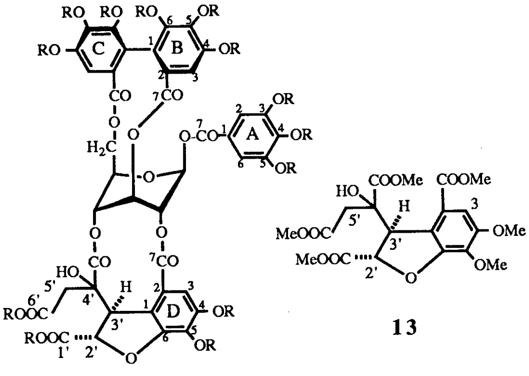
Based on these data, the structure of phyllanthusiin A, except for the absolute configuration at C-4′ of the E-ring, was established as **9**.

Phyllanthusiin B (**12**), $[\alpha]_D$ −43°, and phyllanthusiin C (**14**), $[\alpha]_D$ −49°, have been shown by $^1$H-NMR spectral analysis to be ellagitannins possessing structures analogous to **9** excluding the E-ring. A 2H singlet and three 1H singlets, ascribable to a galloyl group, an HHDP group and an aromatic D-ring proton, and the sugar proton signals characteristic of the $^1C_4$ glucopyranose core were exhibited (see Table I).

A spectral feature of the aliphatic acyl moiety distinguishing between **9** and **12** is the presence of the signals of an isolated methylene group [$\delta$ 3.16 and 3.26 (each d, $J = 17.0$ Hz)] and mutually coupled methine proton [$\delta$ 4.43 and 5.52 (each d, $J = 2.0$ Hz)] in the latter. The $^{13}$C-NMR spectrum of **12** in the aliphatic region disclosed a signal at $\delta$ 77.55 which is due to a quaternary carbon bearing an oxygen function, besides the glucose, methylene and methine carbon signals. Among its seven carbonyl carbon resonances ($\delta$ 164.88—172.68), two ($\delta$ 172.68, 171.76) were assigned to the carboxyl carbons in the aliphatic acyl moiety. In the $^1$H–$^{13}$C long-range COSY of **12**, one of the carboxyl carbon was correlated through two- and three-bond couplings with both of the methine protons ($\delta$ 5.52, 4.43). The methine proton at $\delta$ 4.43 (H-3′) was also correlated with the D-ring C-1 ($\delta$ 116.77) and C-6 ($\delta$ 150.81) signals, as illustrated in Fig. 1. The other methine proton at $\delta$ 5.52 (H-2′) similarly showed cross peaks with the methine ($\delta$ 56.73, C-3′) and quaternary ($\delta$ 77.55, C-4′) carbon signals and the D-ring C-6 signal. The methylene protons were correlated with the quaternary and carboxyl carbons. Based on these observations and on the negative FAB-MS data of **12** [*m/z* 969 $(M-H)^-$], the structure of the acyl group at O-2/O-4 of phyllanthusiin B was assigned as shown in the formula **12**. The positions of each acyl group on the glucose core were also established by the correlations through three-bond long-range couplings among ester carbonyl carbons and acyl protons, as indicated in Fig. 1.

The structure (**12**) thus assigned for phyllanthusiin B was further substantiated as follows. Methylation of **12** with dimethyl sulfate and potassium carbonate in dry acetone afforded a tridecamethyl derivative (**12a**), *m/z* 1153 $(M+H)^+$. The $^1$H-NMR spectrum of **12a** showed a 1H singlet at $\delta$ 4.43, which disappeared upon addition of $D_2O$. The presence of an aliphatic tertiary hydroxyl group in **12** was thus confirmed. Methanolysis of **12a** gave **10a**, **11** and a hexamethyl derivative (**13**), which showed an $(M+H)^+$ ion peak at *m/z* 457 in FAB-MS. The $^1$H-NMR spectrum of **13** exhibited signals of an aromatic proton ($\delta$ 7.08, 1H, s), and of two methine ($\delta$ 4.31, 5.50, each d, $J=1.5$ Hz) and a methylene ($\delta$ 2.55, 2.89, each d, $J=16.5$ Hz) protons, which are consistent with the structure **13**. The small coupling constant between the methine protons indicated their *trans* relationship.[13] The absolute configuration at the benzylic position (C-3′), as in the formula **13**, was assinged based on the application of the aromatic quadrant rule[14] as follows. The circular dichroism (CD) spectrum of **13** showed three Cotton effects at 228, 264 and 300 nm, corresponding to the absorption maxima in the ultraviolet (UV) spectrum (Fig. 2). The Cotton effects at 264 and 300 nm are analogous to those ascribable to $A_{1g} \rightarrow B_{1u}$ and $A_{1g} \rightarrow B_{2u}$ transitions around 240 and 280 nm of codeine, galanthamine, *etc.*[14] The bathochromic shifts of the Cotton effects in **13** from those of the alkaloids are attributable to the difference of aromatic substituents. According to the quadrant projection proposed for the above alkaloids,[14] the negative Cotton effect at 264 nm and positive Cotton effect at 300 nm in **13** indicate the absolute configuration at the benzylic asymmetric center to be as shown in the formula **13**.

The (*S*)-configuration at the HHDP moiety in **12** was determined from the CD spectrum, which showed a negative Cotton effect at 240 nm ($[\theta]-2.8\times10^4$), similar to that of geraniin (**3**),[15] although the amplitude is smaller because of overlapping with the Cotton effect arising from the O-2/O-4 acyl group, mentioned above. Based on these data, the structure of phyllanthusiin B, except for the absolute



**12** : R=H
**12a** : R=Me

**13**

Chart 3

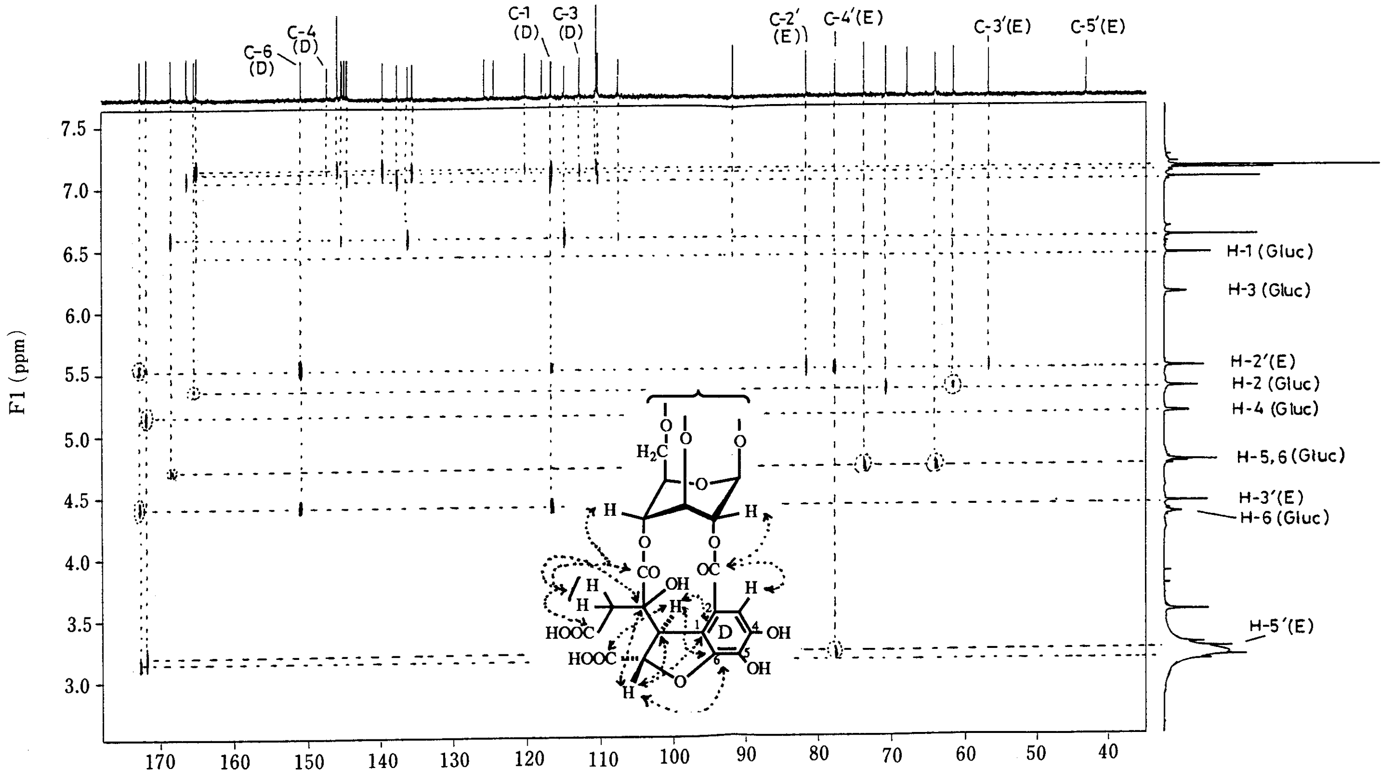


Fig. 1. $^1$H–$^{13}$C Long-Range COSY of Phyllanthusiin B (**12**) ($J_{CH}=8$ Hz)

configuration at C-4′, is represented by **12**.

The structure of phyllanthusiin C was determined to be **14** based on the following evidence. The $^1$H-NMR spectrum of **14** exhibited in the aliphatic region AA'X signals [$\delta$ 2.23 (t, $J$=10.5 Hz), 2.35 (dd, $J$=6.5, 10.5 Hz) and 4.58 (dd, $J$=6.5, 10.5 Hz)] and a 1H singlet ($\delta$ 4.57), besides the $^1C_4$ glucopyranose signals. The $^1$H–$^{13}$C heteronuclear shift correlation (HETCOR) spectrum showed that the E-ring of **14** consists of five carbons [$\delta$ 46.83 (methylene C), 63.88 (methine C), 75.58 (methine C), 78.82 and 117.30 (quaternary carbons)]. One ($\delta$ 117.30) of the quaternary carbons can be assigned to a *gem*-diol or a hemiacetal carbon based on its chemical shift, which is analogous to that of C-6′ in geraniin (**3a**) (see Table II). In addition, the carbon signals ascribable to C-4 ($\delta$ 147.60) and C-6 ($\delta$ 150.03) of the D-ring in **14** shifted downfield by *ca.* 2.6—4.5 ppm relative to the corresponding signals of geraniinic acid B (**7**) and phyllanthusiin A (**9**), possessing the hydroxyl group at C-6 of the D-ring. These shifts, which are analogous to those of phyllanthusiin B (**12**), suggest the presence of an ether bond at the D-ring C-6.

The $^1$H–$^{13}$C long-range COSY ($J_{CH}$=8 Hz) of **14** showed correlations of the methylene proton with all carbons of the E-ring by two- and three-bond long range couplings. It also showed correlations of a methine proton at $\delta$ 4.57 with the D-ring C-1 and C-6 signals, and with a carbonyl carbon signal at 174.18. The binding modes of each acyl group, except for the E-ring, on the glucose core were also established, by the connectivities among the acyl protons and glucose protons through three-bond couplings with the ester carbonyl carbon signals, as illustrated in Fig. 3. Since the hydroxyl groups of glucose residue are all acylated as indicated by the chemical shifts of the sugar proton signals (Table I), the E-ring should be on O-4 of the glucose core. The structure **12** thus assigned is consistent with the negative FAB-MS data [$m/z$ 925 (M−H)$^-$].

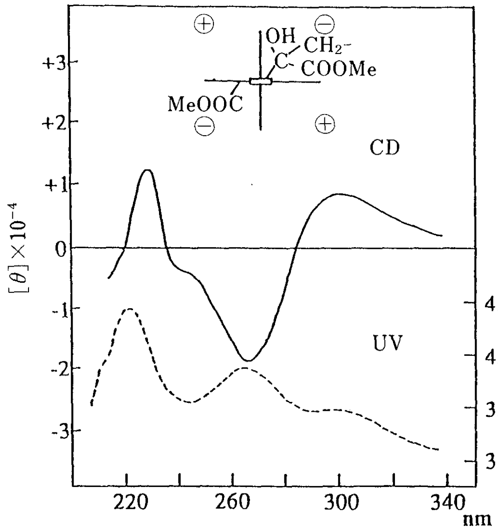


Fig. 2. CD Spectrum of **13** in MeOH

Methylation of **14** with diazomethane gave a dodecamethyl derivative (**14a**), whose $^1$H-NMR spectrum showed the presence of a tertiary and a secondary hydroxyl group, as a singlet ($\delta$ 4.74) and a doublet ($\delta$ 4.18) that disappeared upon addition of $D_2O$. On the other hand, methylation of **14** with dimethyl sulfate and potassium carbonate afforded two partially degraded products, which were identified as deca-*O*-methylcorilagin (**4a**)$^{16)}$ and methyl tri-*O*-methylbrevifolincarboxylate (**2a**). The formation of **2a** can be rationalized in terms of a series of reactions including a $\beta$-elimination, keto-enol tautomerization and transesterification, as depicted in Chart 4.

Phyllanthusiin D (**15**), $[\alpha]_D$ −93°, was obtained as colorless fine needles, mp 245—247 °C. The $^1$H-NMR spectrum of **15** is closely similar to that of geraniin (**3**), except for the extra signals due to an isolated methylene [$\delta$ 2.96, 3.46 (each 1H, d, $J$=15.5 Hz)] and a methyl [$\delta$ 2.17 (3H, s)] group in the former. Crystalline geraniin readily equilibrated between five- and six-membered hemiacetal forms (**3a** ⇄ **3b**) of the DHHDP moiety in an aqueous acetone solution, to give a duplicated signal for each proton in the $^1$H-NMR spectrum. However, such a phenomenon was not observed for **15**, and the spectrum of **15** was similar to that of the five-membered form (**3b**) of geraniin (Table I). The $^{13}$C-NMR spectrum of **15**, which was assigned by HETCOR and $^1$H–$^{13}$C long-range COSY ($J_{CH}$=7 Hz), also showed close similarity to that of **3b**, although the chemical shifts of the C-2, C-4 and C-5 signals of the E-ring in **15**

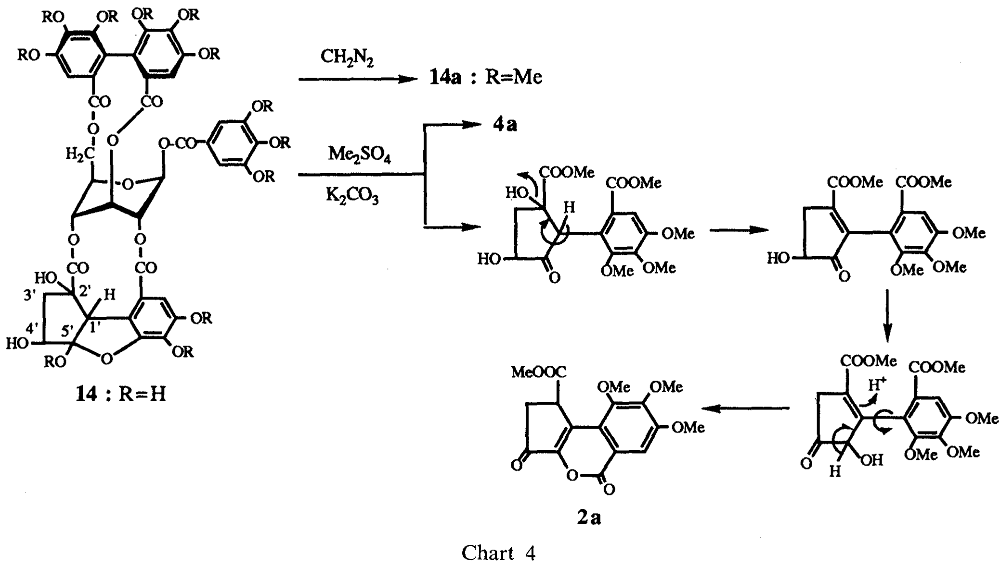


Chart 4

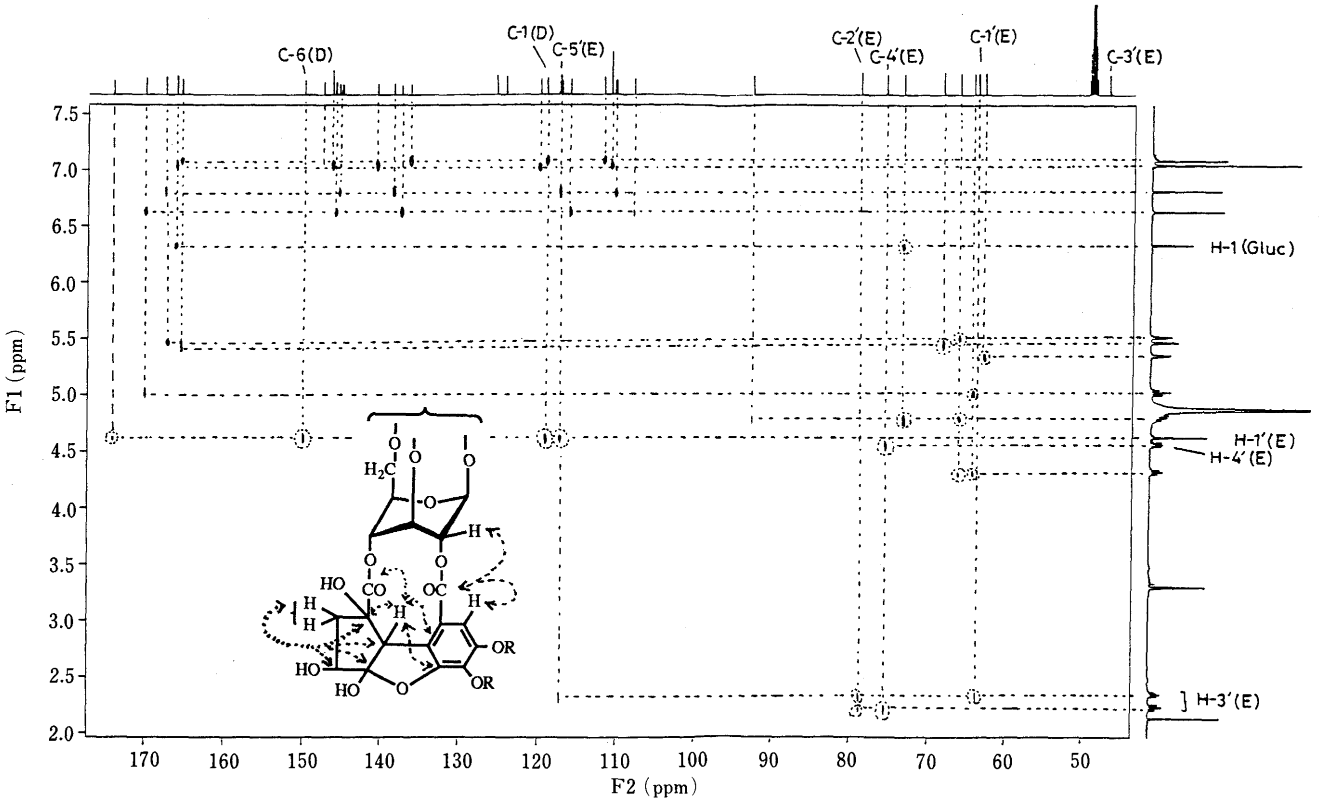


Fig. 3. $^{1}$H–$^{13}$C Long-Range COSY of Phyllanthusiin C (**14**) (in MeOH-$d_4$, $J_{CH}=8$ Hz)

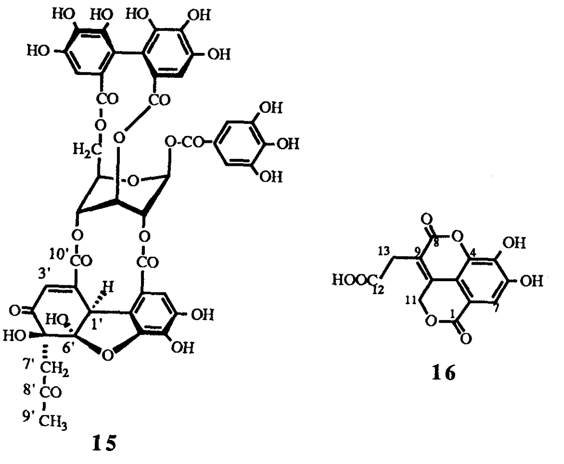


Chart 5

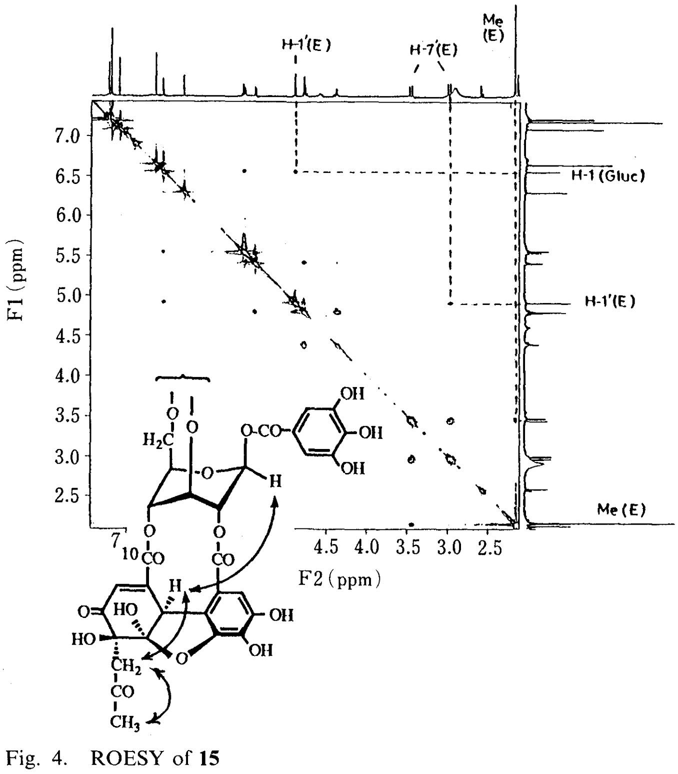


Fig. 4. ROESY of **15**

were significantly different from those of the latter (see Table II). Three additional carbon resonances [$\delta$ 31.99, 49.99 and 206.42], attributable to a methyl, a methylene and a ketonic carbonyl carbon, respectively, were also seen. This methylene group is located at C-5′ of the E-ring, as indicated by its correlation with $\alpha,\beta$-unsaturated ketone carbonyl carbon through three-bond coupling in the $^{1}$H–$^{13}$C long-range COSY. Methylation of **15** with dimethyl sulfate and potassium carbonate afforded deca-*O*-methylcorilagin (**4a**) to prove the presence of the corilagin moiety in **15**. Based on these observations, phyllanthusiin D was characterized as a condensate of geraniin with acetone, as shown in the formula **15**, which is consistent with the negative FAB-MS data [*m*/*z* 991 $(M-H)^-$].

The structure **15** was further substantiated by production of a condensate identical with phyllanthusiin D upon refluxing **3** in dry acetone containing trifluoroacetic acid for a week. The $\alpha$-configuration of the acetylmethylene group at C-5′ of the E-ring was determined with the aid of frame rotating nuclear Overhauser enhancement spectroscopy (ROESY), which showed clear nuclear Overhauser effects

(NOE's) between the methine proton ($\delta$ 4.89, H-1′) and one of the methylene protons ($\delta$ 2.96, H-7′), and also between the anomeric proton of the glucose core and H-1′ of the E-ring (Fig. 4).

Phyllanthusiin D obtained in the present experiment may be an artefact, since it was isolated from the plant materials soaked in acetone at the initial stage of the extraction, and it can be prepared *in vitro* by condensation of geraniin (**3**) with acetone in a weakly acidic medium. It was not produced when **3** was kept at room temperature for several weeks in aqueous acetone, which is generally employed as the extraction solvent for tannins. Upon refluxing geraniin (**3**) in an aqueous acetone, slow production of corilagin (**4**) after 3 d, without formation of phyllanthusiin D, was observed by high-performance liquid chromatography (HPLC). On the other hand, phyllanthusiin D (**15**) was recently isolated from the aqueous acetone homogenate of cell suspension cultures of *Geranium thunbergii*, which did not produce geraniin (**3**).[17] Therefore, phyllanthusiin D may be a metabolite producible under certain conditions.

Phyllanthusiin E (**16**) was obtained as a brown amorphous powder, and showed a pseudomolecular ion peak at *m/z* 291 $(M-H)^-$ in the negative FAB-MS. The $^1$H-NMR spectrum of **16** exhibited a 1H aromatic singlet ($\delta$ 7.38) and two 2H singlets [$\delta$ 3.47 and 5.54 (each 2H, s)]. The presence of two lactonic carbonyl carbons and a carboxyl carbon in **16** was indicated by its $^{13}$C-NMR spectrum [$\delta$ 163.25, 163.80 and 173.05]. The remaining carbon signals were those due to two $sp^3$, and eight $sp^2$ carbons, three of which bear an oxygen function (see Experimental). Based on these data and the $^1$H–$^{13}$C long-range COSY, the structure **16** was assigned for phyllanthusiin E.

It is noteworthy that the hydrolyzable tannins isolated in the present study could be products of metabolic modifications at the highly reactive DHHDP moiety in geraniin (**3**), and that they indicate the presence of a biogenetic system metabolizing the DHHDP group to yield several oxidized products in *Phyllanthus flexuosus*, and possibly in several other species of plants containing dehydroellagitannins.

## Experimental

Optical rotations were measured on a JASCO DIP-4 digital polarimeter, UV spectra on a Hitachi 200-10 spectrophotometer and CD spectra on a JASCO J-500A equipped with a DP data processor. $^1$H (500 MHz) and $^{13}$C (126 MHz)-NMR spectra were recorded on a Varian VXR 500 instrument, and chemical shifts are given in $\delta$ (ppm) relative to $(Me)_4Si$. Analytical TLC and preparative TLC were conducted with Kieselgel $PF_{254}$ (Merck) and spots were visualized by UV irradiation (254 nm) or by spraying $NaNO_2$–AcOH or $FeCl_3$ reagents. Column chromatography was performed with Diaion HP-20, MCI-Gel CHP-20P (Mitsubishi Kasei Co., Ltd.), Toyopearl HW-40 (coarse and fine grade) (Tosoh Corp.) and Sephadex LH-20 (Pharmacia Fine Chemicals). Solvents were evaporated under reduced pressure below 40 °C. Light petroleum refers to the fraction of bp 40—80 °C.

**Isolation** The fresh leaves (2.16 kg) of *P. flexuosus*, collected at Sekigahara, Gifu Prefecture, in summer 1986 and 1987, were extracted with ether and acetone (each 10 l × 6, 7 d) at room temperature, and filtered. The residue was then extracted with 70% aqueous acetone and filtered. The filtrate was concentrated and further extracted with ether (700 ml × 6), $CH_2Cl_2$ (700 ml × 6) and *n*-BuOH (2 l × 8). A part (200 g) of the *n*-BuOH extract (431 g) was submitted to column chromatography over Diaion HP-20 (9 cm × 60 cm), developing with $H_2O$ containing increasing amounts of MeOH in a stepwise gradient mode, to give the 10% MeOH eluate (fraction 1; 13.24 g), 20% MeOH eluate (fraction 2; 16.43 g), 40% MeOH eluate (fraction 3; 33.38 g), MeOH eluate (fraction 4; 24.76 g) and 70% aqueous acetone eluate (fraction 5; 0.13 g). Fraction 1 was further chromatographed over Toyopearl HW-40 (coarse) (5.0 cm i.d. × 50 cm) developing with 40% MeOH and 60% MeOH to yield bergenin (**1**) (2.4 g), brevifolincarboxylic acid (**2**) (1.1 g), repandusinic acid A (**6**) (888 mg) and phyllanthusiin B (**12**) (87 mg). Fraction 2 was further purified by column chromatography over Toyopearl HW-40 (coarse) (20% MeOH → 30% → 40% → 60% → 70%) to give **1** (3.7 g), **2** (678 mg), **4** (4.6 g), geraniinic acid B (**7**) (93 mg) and repandusinic acid A (**6**) (830 mg). The 70% MeOH eluate (920 mg) was further purified by column chromatography over MCI gel CHP-20P to yield phyllanthusiin B (**12**) (50 mg). Fraction 3 was similarly chromatographed over Toyopearl HW-40 (coarse) developing with 50% MeOH → 60% → 70% to afford phyllanthusiin E (**16**) (255 mg), **4** (4.7 g), **3** (4.7 g), phyllanthusiin C (**14**) (63 mg), putranjivain A (**8**) (116 mg) and phyllanthusiin A (**9**) (73 mg). Rechromatography of fraction 4 over Toyopearl HW-40 (fine) gave phyllanthusiin D (**15**) (476 mg) and chebulagic acid (**5**) (70 mg).

**Brevifolincarboxylic Acid (2)** Yellow needles, mp 289—290 °C. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 278 (4.51), 350 (4.17). EI-MS *m/z*: 292 $(M^+)$, $^1$H-NMR (DMSO-$d_6$)$\delta$: 2.42 (1H, dd, $J=2.0$, 18.5 Hz, H-5a), 2.98 (1H, dd, $J=8.0$, 18.5 Hz, H-5b), 4.35 (1H, dd, $J=2.0$, 8.0 Hz, H-4), 7.29 (1H, s, H-3′). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 41.00 (C-4), 37.52 (C-5), 108.04 (C-3′), 113.13 (C-1′), 115.22 (C-2′), 139.16 (C-5′), 140.26 (C-4′), 143.90 (C-6′), 145.74 (C-3), 149.64 (C-2), 160.32 (C-7′), 173.60 (C-6), 193.42 (C-1). Methylation of **2** (10 mg) with an excess of ethereal $CH_2N_2$ gave methyl tri-*O*-methylbrevifolincarboxylate (**2a**) (6.6 mg), colorless fine needles, mp 162—164 °C. EI-MS *m/z*: 348 $(M^+)$. $^1$H-NMR (acetone-$d_6$) $\delta$: 2.54 (1H, dd, $J=2.0$, 18.5 Hz, H-5a), 3.01 (1H, dd, $J=8.0$, 18.5 Hz, H-5b), 3.71, 3.98, 4.01, 4.07 (each 3H, s, OMe), 4.46 (1H, dd, $J=2.0$, 8.0 Hz, H-1), 7.62 (1H, s, H-3′).

**Repandusinic Acid A (6)** A pale yellow amorphous powder. $^1$H-NMR (acetone-$d_6$) $\delta$: 4.21 [1H, br s, glucose (Glc) H-2], 4.24 (1H, dd, $J=8.0$, 11.5 Hz, Glc H-6), 4.57 (1H, br t, $J=8.0$ Hz, Glc H-6), 4.75 (1H, dd, $J=8.0$, 11.5 Hz, Glc H-5), 4.90 (1H, br s, Glc H-3), 5.40 (1H, d, $J=3.0$ Hz, H-2′), 5.54 (1H, d, $J=3.0$ Hz, H-3′), 5.61 (1H, br d, $J=3.5$ Hz, Glc H-4), 6.21 (1H, d, $J=3.5$ Hz, Glc H-1), 6.68, 6.85 (1H each, s, HHDP), 7.12 [1H, s, ring-D (D) H-3], 7.14 [2H, s, galloyl (Gal)].

**Putranjivain A (8)** A pale yellow amorphous powder. $^1$H-NMR (acetone-$d_6$–$D_2O$) $\delta$: 1.62 (1H, br d, $J=14.0$ Hz, H-3′), 2.71 (1H, dd, $J=1.5$, 14.0 Hz, H-3′), 3.85 (1H, dd, $J=2.5$, 9.5 Hz, H-5″), 4.05 (1H, br s, H-4″), 4.15 (1H, br s, H-3″), 4.16 (1H, dd, $J=6.0$, 9.5 Hz, H-5″), 4.38 (1H, dd, $J=8.0$, 11.0 Hz, Glc H-6), 4.68 (1H, d, $J=2.0$ Hz, H-1′), 4.76 (1H, t, $J=11.0$ Hz, Glc H-6), 4.83 (1H, br t, $J=13.0$ Hz, Glc H-5), 4.98 (1H, br s, H-1″), 5.29 (1H, br d, $J=3.0$ Hz, Glc H-4), 5.55 (1H, br s Glc H-2), 5.66 (1H, br s, Glc H-3), 6.48 (1H, br s, H-1), 6.12, 7.07 (1H each, s, HHDP), 7.16 (2H, s, Gal), 7.29 [1H, s, H-3 (D)].

**Phyllanthusiin A (9)** A pale yellow amorphous powder, $[\alpha]_D$ −94.5° ($c=1.0$, acetone). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 222 (4.85), 278 (4.47). CD (MeOH) $[\theta]$ (nm): $-8.5\times10^4$ (241), $-1.8\times10^4$ (265), $-4.1\times10^4$ (282). Negative FAB-MS *m/z*: 951 $(M-H)^-$. *Anal.* Calcd for $C_{41}H_{28}O_{27}\cdot6H_2O$: C, 46.22; H, 3.77. Found: C, 46.33; H, 3.57. $^1$H-NMR: see Table I. $^{13}$C-NMR: see Table II.

**Partial Hydrolysis of Phyllanthusiin A (9)** An aqueous solution (1 ml) of phyllanthusiin A (1 mg) was heated in a boiling-water bath for 4 h, and the reaction mixture, after evaporation of the solvent, was analyzed by HPLC (normal and reversed phases) to detect corilagin (**4**).

**Methylation of Phyllanthusiin A (9)** A solution of **9** (10 mg) in EtOH (1 ml) was treated with ethereal $CH_2N_2$ for 3 h at room temperature. After removal of the solvent, the residue was purified by preparative TLC (benzene–acetone, 7 : 1) to yield a tridecamethylate (**9a**) (3.2 mg) as a white amorphous powder $[\alpha]_D$ −118° ($c=0.6$, acetone). EI-MS *m/z*: 1134 $(M^+)$. $^1$H-NMR (acetone-$d_6$) $\delta$: 3.13 (1H, br d, $J=16.5$ Hz, H-5′), 3.28 (1H, dd, $J=7.0$, 16.5 Hz, H-5′), 3.40 (1H, br d, $J=7.0$ Hz, H-4′), 3.39, 3.65, 3.83, 3.87, 3.89, 3.90, 3.94 (each 3H, s, OMe), 3.66 (9H, s, OMe), 3.93 (6H, s, OMe), 4.43 (1H, dd, $J=8.0$, 11.0 Hz, Glc H-6), 4.63 (1H, br t, $J=8.0$ Hz, Glc H-5), 5.06 (1H, t, $J=11.0$ Hz, Glc H-6), 5.27 (1H, br s, Glc H-4), 5.48 (1H, br s, Glc H-2), 5.72 (1H, br s, Glc H-3), 6.46 (1H, br s, Glc H-1), 6.95, 7.13 (1H, s, HHDP), 7.25 (2H, s, Gal), 7.39 [1H, s, H-3 (D)].

**Methanolysis of Tridecamethylate (9a)** A mixture of **9a** (0.5 mg) and 1% NaOMe (0.05 ml) in MeOH (1 ml) was left standing overnight at room temperature. The reaction mixture was acidified with a few drops of AcOH and evaporated *in vacuo*. TLC analysis (benzene–acetone, 4 : 1) of the residue showed the formation of methyl tri-*O*-methylgallate (*Rf* 0.75) and dimethyl hexamethoxydiphenate (*Rf* 0.36).

**Phyllanthusiin B (12)** A pale yellow amorphous powder, $[\alpha]_D$ −43°

($c=1.0$, acetone). *Anal.* Calcd for $C_{41}H_{30}O_{28}\cdot 4H_2O$: C, 47.22; H, 3.65. Found: C, 46.98; H, 3.71. Negative FAB-MS $m/z$: 969 $(M-H)^-$. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 222 (4.89), 280 (4.50). CD (MeOH) $[\theta]$ (nm): $-2.8\times 10^4$ (240), $+1.1\times 10^4$ (256), $-4.3\times 10^4$ (283). $^1$H-NMR: see Table I. $^{13}$C-NMR: see Table II.

**Methylation of Phyllanthusiin B (12)** A mixture of **12** (47 mg), dimethyl sulfate (0.2 ml) and potassium carbonate (200 mg) in dry acetone (5 ml) was stirred overnight at room temperature and then refluxed for 1 h. After removal of the inorganic material by centrifugation, the reaction mixture was subjected to preparative TLC (benzene–acetone, 7 : 1), which gave a tridecamethylate (**12a**) (29 mg) as a white solid mass, $[\alpha]_D$ $-39°$ ($c=1.0$, acetone), FAB-MS $m/z$: 1153 $(M+H)^+$, 1175 $(M+Na)^+$. $^1$H-NMR (acetone-$d_6$) $\delta$: 3.20 (1H, d, $J=17.0$ Hz, H-5′), 3.29 (1H, d, $J=17.0$ Hz, H-5′), 3.23, 3.64, 3.65, 3.70, 3.77, 3.85, 3.86, 3.87, 3.88, 3.92, 3.93 (each 3H, s, OMe), 3.66 (6H, s, OMe), 4.43 (1H, br s, 4′-OH), 4.48 (1H, d, $J=2.0$ Hz, H-3′), 4.53 (1H, dd, $J=8.0$, 10.5 Hz, Glc H-6), 4.91 (1H, br t, $J=8.0$ Hz, Glc H-5), 5.06 (1H, t, $J=10.5$ Hz, Glc H-6), 5.25 (1H, br s, Glc H-4), 5.44 (1H, br s, Glc H-2), 5.66 (1H, d, $J=2.0$ Hz, H-2′), 6.21 (1H, br s, Glc H-3), 6.57 (1H, br s, Glc H-1), 6.88, 7.05 (each 1H, s, HHDP), 7.16 [1H, s, H-3 (D)], 7.24 (2H, s, G). $^{13}$C-NMR (acetone-$d_6$) $\delta$: 43.25 (C-5′), 55.66 (C-3′), 52.13, 53.94, 55.96, 56.42, 56.61, 56.76 (3C), 60.63, 60.74, 60.85, 60.95 (2C) (OMe × 13), 61.29 (Glc C-3), 64.39 (Glc C-6), 67.35 (Glc C-4), 69.37 (Glc C-2), 73.13 (Glc C-5), 77.71 (C-4′), 82.00 (C-2′), 93.28 (Glc C-1), 105.53 [ring-C (C) C-3], 107.66 (2C) [ring-A (A) C-2, C-6], 109.01 [ring-B (B) C-3], 109.21 [C-3 (D)], 119.16 [C-1 (D)], 121.41 [C-2 (D)], 122.08 [C-1 (C)], 124.39 [C-1 (A)], 124.83 [C-1 (B)], 127.01, 129.83 [C-2 (B, C)], 139.01 [C-5 (D)], 144.12 [C-4 (A)], 144.58 [C-5 (C)], 146.14 [C-5 (B)], 153.37, 153.42 [C-6 (B, C)], 153.77 [C-4 (B)], 154.02 [C-4 (D)], 154.36 [C-4 (C)], 154.41 (2C) [C-2, C-6 (A)], 154.80 [C-6 (D)], 164.62 [C-7 (A)], 165.05 [C-7 (D)], 166.00 [C-7 (B)], 168.33 [C-7 (C)], 171.00 (C-6′), 171.71 (C-1′), 172.64 (C-7′).

**Methanolysis of the Tridecamethylate (12a)** A mixture of **12a** (10 mg) and 1% NaOMe (1 ml) in absolute MeOH (1 ml) was kept standing overnight at room temperature and worked up as usual. Preparative TLC (light petroleum–$CHCl_3$–acetone, 2 : 1 : 1) furnished methyl tri-*O*-methylgallate (**10a**) [1 mg; EI-MS $m/z$ 226 $(M^+)$], dimethyl hexamethoxydiphenate (**11**) [0.5 mg; EI-MS $m/z$ 450 $(M^+)$] and the hexamethyl derivative (**13**) (1.5 mg), $[\alpha]_D$ $-17°$ ($c=0.5$, acetone), FAB-MS $m/z$: 457 $(M+H)^+$, 479 $(M+Na)^+$. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 221 (4.38), 264 (3.84), 300 (3.48). CD (MeOH) $[\theta]$ (nm): $+1.1\times 10^4$ (228), $-1.9\times 10^4$ (264), $+0.7\times 10^4$ (300). $^1$H-NMR (acetone-$d_6+D_2O$) $\delta$: 2.55 (1H, d, $J=16.5$ Hz, H-5′), 2.89 (1H, d, $J=16.5$ Hz, H-5′), 3.56, 3.64, 3.70, 3.85, 3.86, 3.93 (each 3H, s, OMe), 4.31 (1H, d, $J=1.5$ Hz, H-3′), 5.50 (1H, d, $J=1.5$ Hz, H-2′), 7.08 (1H, s, H-3).

**Phyllanthusiin C (14)** A pale yellow amorphous powder, $[\alpha]_D$ $-49°$ ($c=1.0$, acetone). *Anal.* Calcd for $C_{40}H_{30}O_{26}\cdot 5H_2O$: C, 47.24; H, 3.94. Found: C, 47.38; H, 3.70. Negative FAB-MS $m/z$: 925 $(M-H)^-$. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 223 (4.85), 278 (4.49). CD $[\theta]$ (nm): $-3.3\times 10^4$ (241), $+0.4\times 10^4$ (258), $-4.1\times 10^4$ (284). $^1$H-NMR: see Table I, $^{13}$C-NMR: see Table II.

**Methylation of Phyllanthusiin C (14)** a) A solution of **14** (50 mg) in EtOH (1 ml) was treated with ethereal $CH_2N_2$ at room temperature for 5 h, and the reaction mixture, after removal of the solvent, was purified by preparative TLC (light petroleum–$CHCl_3$–acetone, 2 : 1 : 1) to give a dodecamethylate (**14a**) (9 mg), as a white amorphous powder. FAB-MS $m/z$: 1095 $(M+H)^+$, 1117 $(M+Na)^+$. $^1$H-NMR (acetone-$d_6$) $\delta$: 2.29 [(1H, br d, $J=10.5$ Hz, H-3′ (E)], 2.39 [1H, dd, $J=6.5$, 10.5 Hz, H-3′ (E)], 3.20, 3.41, 3.66, 3.70, 3.85, 3.86, 3.88, 3.90, 3.92, 3.95 (each 3H, s, OMe), 3.67 (6H, s, OMe), 4.18 [1H, d, $J=8.5$ Hz, 4′-OH (E)], 4.45 [1H, s, 2′-OH (E)], 4.55 (1H, dd, $J=7.5$, 10.5 Hz, Glc H-6), 4.65 [1H, ddd, $J=6.5$, 8.5, 10.5 Hz, H-4′ (E)], 4.74 [1H, s, H-1′ (E)], 5.00 (1H, br t, $J=7.5$ Hz, Glc H-5), 5.12 (1H, t, $J=10.5$ Hz, Glc H-6), 5.44 (1H, br s, Glc H-4), 5.66 (1H, br s, Glc H-2), 5.69 (1H, br s, Glc H-3), 6.53 (1H, br s, Glu H-1), 6.90, 7.06, 7.05 [each 1H, s, HHDP and ring-D H-3′), 7.25 (2H, s, Gal).

b) A mixture of **14** (30 mg), dimethyl sulfate (0.1 ml) and potassium carbonate (100 mg) in dry acetone (5 ml) was stirred overnight at room temperature and refluxed from 2 h. The reaction mixture was worked up as described for the methylation of **12** to yield deca-*O*-methylcorilagin (**4a**) (6 mg) and methyl tri-*O*-methylbrevifolincarboxylate (**2a**) (2.3 mg).

**Methanolysis of Dodecamethylphyllanthusiin C (14a)** A solution of **14a** (2 mg) in absolute MeOH (1 ml) containing a few drops of 1% NaOMe was left standing overnight at room temperature. After addition of AcOH followed by evaporation of the solvent, the residue was subjected to preparative TLC ($CHCl_3$–acetone 20 : 1) to give **10a**, **11** and methyl tri-*O*-methylbrevifolincarboxylate (**2a**), which were identified by co-chromatography on TLC with authentic samples.

**Phyllanthusiin D (15)** Colorless needles, mp 245—247 °C ($H_2O$–MeOH). $[\alpha]_D$ $-93°$ ($c=1.0$, acetone). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 223 (4.82), 280 (4.46). CD (MeOH) $[\theta]$ (nm): $-2.9\times 10^4$ (240), $+4.9\times 10^4$ (260), $-3.0\times 10^4$ (287). *Anal.* Calcd for $C_{44}H_{32}O_{27}\cdot 5H_2O$: C, 48.80; H, 3.88. Found: C, 48.86; H, 3.56. Negative FAB-MS $m/z$: 991 $(M-H)^-$. $^1$H-NMR: see Table I. $^{13}$C-NMR: see Table II.

**Methylation of Phyllanthusiin D (15)** A mixture of **15** (20 mg), dimethyl sulfate (0.1 ml) and potassium carbonate (100 mg) in dry acetone (5 ml), was stirred overnight at room temperature and then refluxed for 17 h. Preparative TLC (light petroleum–$CHCl_3$–acetone, 6 : 3 : 2) after removal of the inorganic material furnished deca-*O*-methylcorilagin (**4a**) (2.3 mg).

**Synthesis of Phyllanthusiin D (15) from Geraniin (3)** A solution of **3** (50 mg) in absolute acetone (50 ml) containing $CF_3COOH$ (2 ml) was refluxed for 1 week. The concentrated reaction mixture was submitted to column chromatography over MCI-gel CHP-20P, developing with $H_2O$ containing increasing amounts of MeOH. The eluate with 40% MeOH gave phyllanthusiin D (**15**) (10 mg).

**Phyllanthusiin E (16)** A brown amorphous powder. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 223 (4.36), 280 (4.21), 340 (3.97). *Anal.* Calcd for $C_{13}H_8O_8\cdot H_2O$: C, 51.87; H, 3.57. Found: C, 50.32; H, 3.23. Negative FAB-MS $m/z$: 291 $(M-H)^-$. $^1$H-NMR: see text. $^{13}$C-NMR (MeOH-$d_4$) $\delta$: 32.27 (C-13), 68.04 (C-11), 112.18 (C-2), 112.71 (C-3), 114.16 (C-7), 114.84 (C-9), 139.53 (C-5), 140.88 (C-6), 143.56 (C-10), 150.91 (C-4), 162.35 (C-8), 163.80 (C-1), 173.05 (C-12).

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