Ellagitannin Monomers and Oligomers from *Euphorbia prostrata* Ait. and Oligomers from *Loropetalum chinense* Oliv.[1)]

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Three new ellagitannins, prostratins A, B and C, along with eleven known polyphenols including three hydrolyzable tannin oligomers (rugosins D, E and G), have been isolated from the leaves of *Euphorbia prostrata* Ait. A hydrolyzable tannin trimer, prostratin B, and two dimers, rugosins D and E, were also obtained from the leaves of *Loropetalum chinense* Oliv.

Keywords *Euphorbia prostrata*; Euphorbiaceae; tannin; ellagitannin; prostratin A; prostratin B; prostratin C; *Loropetalum chinense*; Hamamelidaceae

Many euphorbiaceous plants are known to be rich in tannins. Geraniin, a dehydroellagitannin, is widely distributed in the species of Euphorbioideae, as found by a survey of tannins in the order Geraniales by high-performance liquid chromatography (HPLC).[2)] Recent investigations on the tannins of Euphorbia species have revealed the presence of a new class of dimeric dehydroellagitannins, such as euphorbins A—F, which contain the geraniin structure as a part of the molecule.[3)] In a continuing study on the polyphenolics of euphorbiaceous plants, we have isolated three new tannins, named prostratins A (**12**), B (**15**) and C (**18**),[4)] and eleven known polyphenols including ellagitannin oligomers, rugosins D (**10**), E (**11**) and G (**14**),[5)] from the leaves of *Euphorbia prostrata* Ait., which are a folk medicine used for treating diarrhoea, dysuria and rheumatism in China.[6a)] Prostratin B (**15**) and rugosins D—G are hydrolyzable tannin oligomers of a type different from euphorbins. These oligomers (**10**,

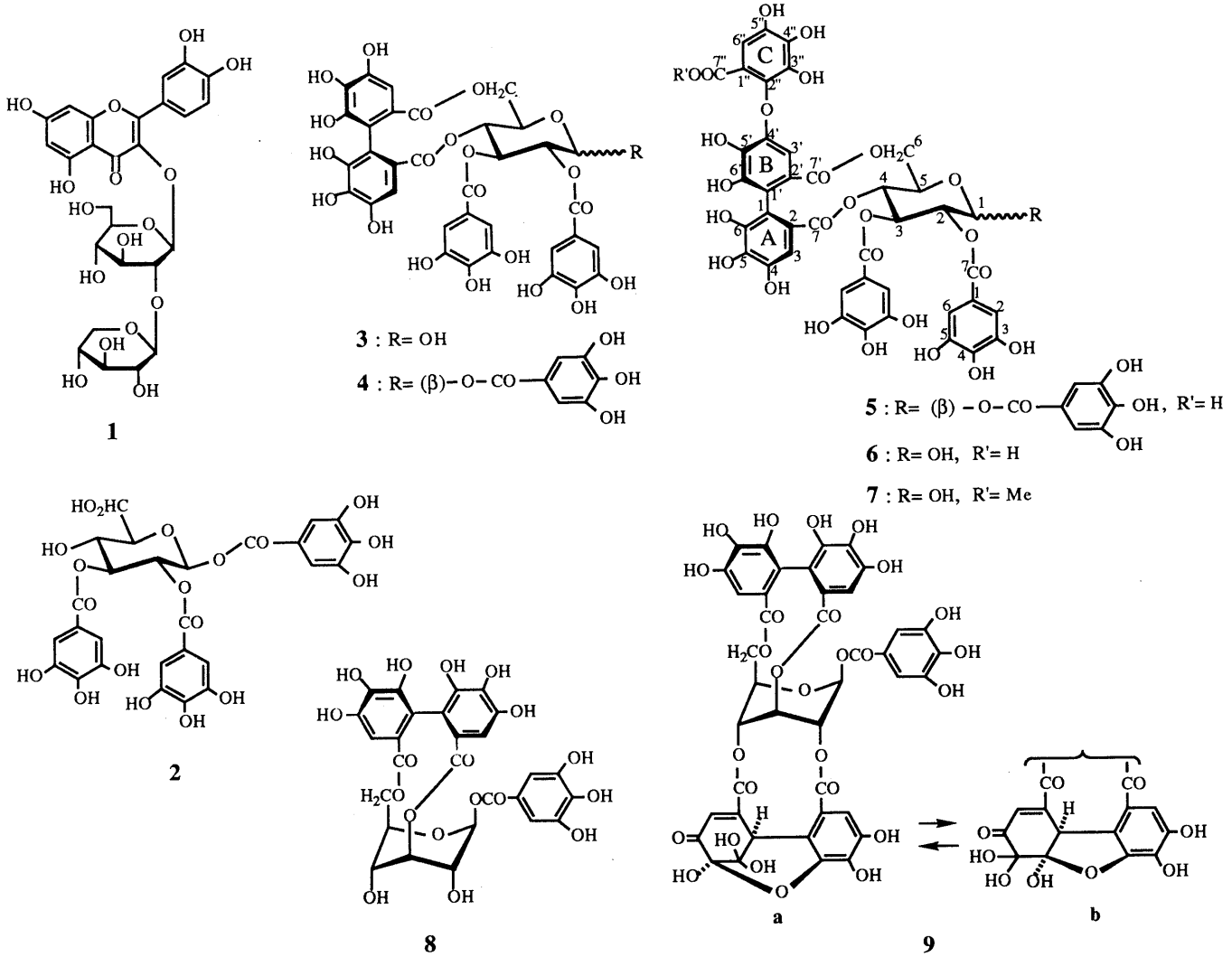


Chart 1

11, **15**) except rugosin G (**14**) were also isolated from the leaves of *Loropetalum chinense* OLIV. (Hamamelidaceae), a Chinese medicinal plant used to treat hemorrhage, dysuria and diarrhoea.[6b]

An aqueous acetone extract of dried leaves of *E. prostrata*, collected in China, was treated as described in the experimental section to yield prostratins A (**12**), B (**15**) and C (**18**), along with a flavonoid glycoside (**1**), seven hydrolyzable tannin monomers, 1,2,3-tri-*O*-galloyl-*β*-D-glucose (**2**),[7] tellimagrandin I (**3**), tellimagrandin II (**4**),[8,9] rugosin A (**5**), rugosin B (**6**),[10] corilagin (**8**) and geraniin (**9**),[11] and two dimers, rugosins D (**10**) and E (**11**),[5] and a trimer, rugosin G (**14**).[5]

Three oligomeric hydrolyzable tannins isolated from the leaf extract of *L. chinense* were characterized as rugosins D (**10**) and E (**11**), and prostratin B (**15**), respectively.

A flavonoid glycoside (**1**), $C_{26}H_{28}O_{16}\cdot 2H_2O$, gave glucose, xylose and quercetin upon acid hydrolysis. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** exhibited two anomeric proton signals [δ 5.49 (d, $J=7.5$ Hz) and 4.76 (diffused d, $J=7$ Hz)], whose coupling constants indicated the *β*-configuration of both glycosidic linkages. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed the aromatic carbon signals characteristic of 3-*O*-glycosylated quercetin[12] (see Experimental). The ^{13}C resonances of the sugar moiety of **1** were in accord with the data reported for a xylopyranosyl(1→2)-glucopyranose residue in a diterpene glycoside from *Salvia digitaloides* roots.[13] Therefore, **1** is clearly quercetin 3-*O*-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranoside, and hence appears to be identical with the flavone glycoside (Q-1, quercetin 3-*O*-*β*-sambubioside), which was previously isolated from *Hibiscus mutabilis* f. *versicolor*.[14] However, the structure of the disaccharide moiety of Q-1 in the previous report[14] was based only on the identification by paper partition chromatography. Therefore, to our knowledge, the present report appears to be the first to present a detailed spectroscopical characterization (see Experimental) of the structure **1**.

Prostratin A (**12**), $[\alpha]_D$ +76° (MeOH), was obtained as a pale brown amorphous powder, and gave a dark-blue color with alcoholic ferric chloride, and also a positive coloration characteristic of ellagitannins with the $NaNO_2$–AcOH reagents.[15] In the negative fast atom bombardment mass spectrum (negative FAB-MS), it gave an $(M-H)^-$ ion peak at *m/z* 1237, indicating its molecular formula to be $C_{55}H_{34}O_{34}$. The ^{1}H-NMR spectrum of **12** exhibited the signals attributable to two galloyl groups

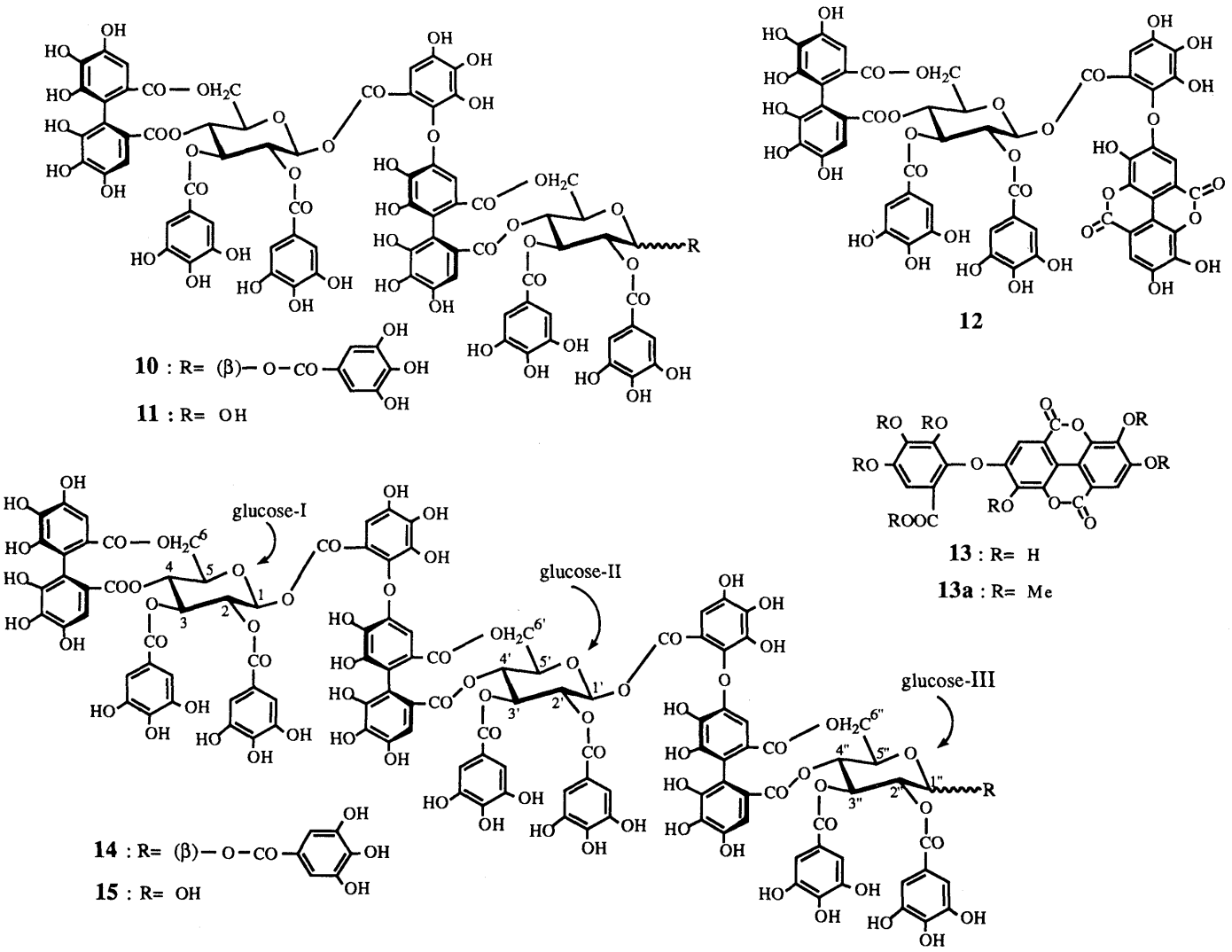


Chart 2

(δ 6.93 and 6.89, each 2H, s) and five one-proton singlets in the aromatic region. Among the latter signals, three at low field (δ 7.60, 7.22, 7.09) are attributable to a dilactonized valoneoyl group, which was substantiated by the ultraviolet (UV) absorptions at 218, 258 and 346 nm.[16] The other two one-proton singlets at δ 6.64 and 6.44 are assignable to the protons of a hexahydroxydiphenoyl (HHDP) group. The chemical shifts and coupling pattern of the aliphatic proton signals are characteristic of a fully acylated $^4C_1$ glucopyranose residue. The C-6 methylene protons of glucose at δ 5.23 (dd, J=6.5, 13.5 Hz) and 3.70 (dd, J=0.5, 13.5 Hz), which are similar to those of **4** (δ 5.20 and 3.89), indicate the presence of an HHDP group bridged at O-4—O-6.[8] Upon partial hydrolysis with hot water, prostratin A gave valoneic acid dilactone (**13**) and tellimagrandin I (**3**), leading to the structure **12** for prostratin A. This is the first report of **12** as a natural product, although it has been reported as a degradative product from isorugosin D.[17]

Prostratin B (**15**), $[\alpha]_D$ +125° (MeOH), was shown to be a trimeric hydrolyzable tannin by the retention time (close to that of **14**) on normal phase HPLC,[2] and by an $(M+Na)^+$ ion peak at m/z 2681 in FAB-MS. Acid hydrolysis of **15** yielded gallic acid, ellagic acid and **13**, which were characterized as their methyl derivatives. The sugar component was identified as glucose by gas-liquid chromatography (GLC) after trimethylsilylation. Although the ^{1}H-NMR spectrum of **15** was complicated by duplication of the signals due to equilibration of the α- and β-anomers of a glucose moiety, the presence of six galloyl groups, an HHDP group, and two valoneoyl groups in the molecule was suggested by the aromatic proton signals (see Experimental). These constituent units in **15** were indicated more clearly by six two-proton singlets (δ 6.95—7.13), and eight one-proton singlets [δ 6.08, 6.16, 6.45 (2H), 6.65, 6.83, 7.10, 7.13] in the ^{1}H-NMR spectrum of a dihydroprostratin B (**16**), which was prepared by reduction of **15** with $NaBH_4$. The ^{1}H-NMR spectrum of **16** also exhibited typical patterns of $^4C_1$ glucopyranose cores and a terminal glucitol core. The coupling constant (J=8.5 Hz) of two anomeric proton signals at δ 6.053 and 6.048 indicate β-orientation of the acyloxy groups at the anomeric centers of both glucopyranose cores (glucose-I and -II). Partial hydrolysis of **15** with hot water yielded rugosin E (**11**), and three monomers, among which two were identified as tellimagrandin I (**3**) and rugosin B (**6**). The ^{1}H-NMR spectrum of the third hydrolyzate (**17**), showed each signal as a dual peak owing to equilibration of the anomers. Close similarity of this spectrum to that of rugosin B (**6**) was observed except for the chemical shifts of three pairs of singlets attributable to the valoneoyl group [**17**, δ 7.16, 7.15 (1H in total), 6.93, 6.92 (1H in total), 6.50, 6.47 (1H in total); **6**, 7.13, 7.14 (1H in total), 6.44, 6.47 (1H in total), 6.30, 6.31 (1H in total)]. Marked differences in the ^{13}C-NMR spectra between **17** and **6** were also observed in the chemical shifts of the valoneoyl signals, whereas the other signals were essentially the same. These spectral features, coupled with an $(M+Na)^+$ peak at m/z 959 in FAB-MS which is 18 mass units lower than that of **6**, suggest that the valoneoyl group in **17** forms a depside linkage. This assumption was substantiated by facile formation of a methyl ester (**7**), m/z 991 $(M+Na)^+$ (FAB-MS), upon mild methanolysis, since the depsidic linkage is easily methanolyzed in this way.[18] The ^{1}H-NMR spectrum of **7** was closely similar to that of rugosin B (**6**) except for an extra signal due to a methoxyl group, and this indicates that **7** is a methyl ester of **6**. This correlation was proved by chemical conversion of **17** into **6**[10] upon further hydrolysis with hot water, to establish the structure of **17**, in which the valoneoyl group has the same orientation as that of **6**, and forms a depsidone ring. This structural assignment for **17** was confirmed by the ^{1}H–^{13}C long-range shifts correlation spectrum (J=7 Hz) which showed two- and three-bond long-range couplings as illustrated in the formula (Chart 3). The partial hydrolysis of **16** with hot water yielded a dihydro-congener (**19**) of **17** together with **3**, **6**, **17** and dihydrorugosin B (**20**).

The depsidic compounds such as **17** and **19**, which are easily hydrolyzed to yield the phenol-carboxylic acid congener, and thus regarded as the initial products of hydrolytic cleavage at the galloyl moiety in the valoneoyl

TABLE I. ^{13}C Resonances of **17**, **18** and **5** (126 MHz, Acetone-d_6–D_2O)

		17		**18**	**5**
		α-Anomer	β-Anomer		
Glucose	C-1	91.2	96.7	93.7	93.6
	C-2	73.1	74.1	71.7	71.7
	C-3	71.2	73.5	72.9	73.1
	C-4	71.2	71.2	70.8	70.7
	C-5	66.9	71.7	72.8	73.0
	C-6	64.3	64.3	63.9	63.0
Valoneoyl					
Ring-A	C-1	114.7	114.7	114.2	115.9
	C-2	124.9	124.9	125.1	125.5[a]
	C-3	107.4	107.4	107.5	107.7
	C-4	145.5	145.5	145.8	145.2
	C-5	136.6	136.5	136.6	136.7
	C-6	145.3	145.3	145.2	144.7
	C-7	167.9	167.8	167.6	167.6
Ring-B	C-1′	122.4	122.4	122.1	117.8
	C-2′	132.8	132.7	132.8	126.1[a]
	C-3′	110.5	110.5	111.2	106.1
	C-4′	151.6	151.6	151.7	146.8
	C-5′	135.4	135.4	135.7	137.4
	C-6′	149.0	149.0	148.6	144.7
	C-7′	167.4	167.4	167.0	167.8
Ring-C	C-1″	110.9	110.9	111.7	115.3
	C-2″	141.9	141.9	141.3	137.5
	C-3″	137.2	137.2	137.1	140.4
	C-4″	143.5	143.5	143.4	139.8
	C-5″	143.8	143.8	143.8	143.3
	C-6″	109.7	109.7	109.9	110.1
	C-7″	164.3	164.3	163.0	166.3
Galloyl	C-1	120.4	120.3	119.9	119.8
		120.4	120.8	120.5	120.5
				120.5	120.6
	C-2, C-6	109.0	110.0	110.1	110.1
		110.0	110.1	110.2	110.2
				110.3	110.3
	C-3, C-5	145.7	145.7	145.8	145.8
		145.9	145.9	145.9	145.9
				146.1	146.1
	C-4	139.1	139.0	139.2	139.1
		139.2	139.1	139.2	139.3
				139.8	139.8
	C-7	166.3	165.9	164.9	164.9
		166.9	166.6	165.3	164.9
				166.1	166.2

a) These values may be interchanged.

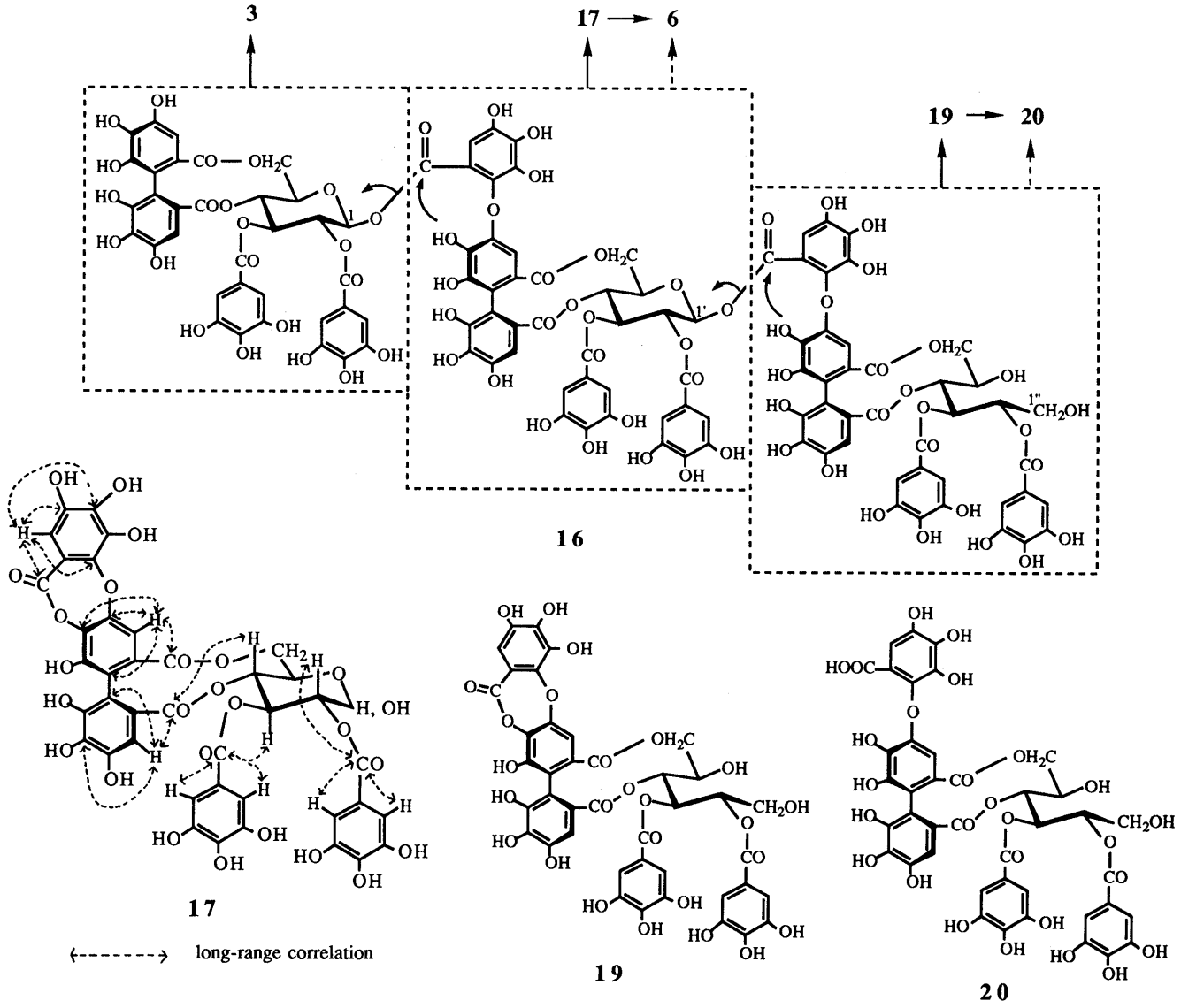
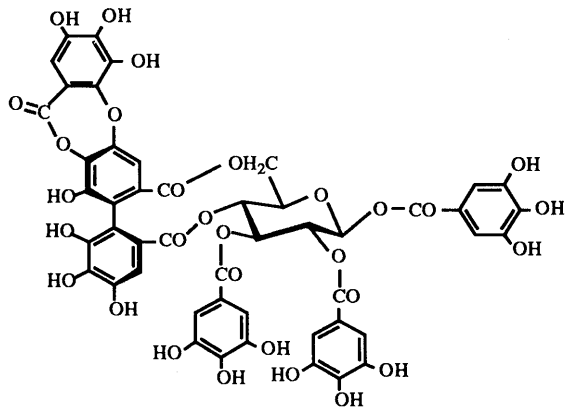


Chart 3

group, accompanied by transesterification with the nearby phenolic hydroxyl group. In conclusion, prostratin B is a trimer consisting of **3** and 2 mol of **6**, and is formulated as $C_{116}H_{82}O_{74}$, which is consistent with the FAB-MS data. The structure of prostratin B is therefore assigned as **15**. An attempt to correlate **15** with rugosin G (**14**) by selective degalloylation with tannase was unsuccessful, because of the formation of a complex mixture, probably due to facile hydrolysis of galloyl groups other than that of C-1″.

Prostratin C (**18**), $[\alpha]_D$ +79° (MeOH), was isolated as a light brown amorphous powder and showed the $[M-H]^-$ peak at *m/z* 1087, corresponding to the molecular formula $C_{48}H_{32}O_{30}$, in the negative FAB-MS. The ^{1}H-NMR spectrum of **18** exhibited three two-proton singlets at δ 7.12, 6.99 and 6.95, attributable to galloyl groups, and three one-proton singlets at δ 7.20, 6.93 and 6.49, which are closely similar to those of the depsidone-forming valoneoyl group in **17**. The presence of the depsidone-forming valoneoyl group in **18** was further supported by the ^{13}C-NMR spectral comparison with **17** (Table I). The glucose signals in the ^{1}H-NMR and ^{13}C-NMR (Table I) spectra of **18** were almost superimposable on those of rugosin A (**5**).[10] This suggests



18

Chart 4

that prostratin C is a depside-forming derivative of rugosin A (**5**). The structure (**18**) was finally proved by its partial hydrolysis with hot water to afford rugosin A (**5**), and also by production of prostratin C upon the treatment of rugosin

D (**10**) with hot water.

Although many hydrolyzable tannins having a valoneoyl group as a constituent unit, as exemplified by rugosins A—G, have been isolated from various plants,[2,19] prostratins A (**12**) and C (**18**) are rare examples of tannins having a depside-forming valoneoyl group in the molecule. Although rugosins D, E and/or F have been found in the plants of Rosaceae, Coriariaceae, Trapaceae and Stachyuraceae,[19] the isolation of rugosin-type oligomers from the plants of Euphorbiaceae and Hamamelidaceae suggests a rather wide distribution of these oligomers in plants.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument at 500 MHz for ^{1}H and 126 MHz for ^{13}C, and chemical shifts are given in δ (ppm) values from tetramethylsilane. FAB-MS were measured on a VG 70-SE mass spectrometer. Optical rotations were obtained on a JASCO DIP-4 digital polarimeter and circular dichroism (CD) spectra on a JASCO J500A. HPLC was carried out with a Shimadzu LC-6A and a Shimadzu SPD-6A using a Superspher Si60 column (4 mm i.d. × 125 mm) (Merck), with a solvent system consisting of hexane–MeOH–tetrahydrofuran–formic acid (55 : 33 : 11 : 1) containing oxalic acid (450 mg/l) and a flow rate of 1.5 ml/min for normal phase analysis, and using a LiChrospher RP-18 column (4 mm i.d. × 250 mm) (Merck) in an oven at 40°C, with a solvent system consisting of 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH–EtOAc (41 : 41 : 13 : 5) and a flow rate of 1.2 ml/min for reversed-phase analysis. Kieselgel PF_{254} (Merck) was used for preparative thin layer chromatography (TLC), and the spots were visualized under UV irradiation (254 nm). Sephadex LH-20 (100 μm) (Pharmacia Fine Chemicals), Toyopearl HW-40 (coarse, fine and superfine grades) (Tosoh), Diaion HP-20 and MCI gel CHP 20P (75—150 μm) (Mitsubishi Kasei Industry) were used for column chromatography. Solvents were removed by evaporation under reduced pressure below 40°C.

Isolation of Tannins from *E. prostrata* The dried leaves (1.2 kg) of *E. prostrata*, collected in Fuzhou, Fujian, China in September, were homogenized three times with acetone–H_2O (7 : 3) and the combined homogenates were filtered. After removal of acetone, the aqueous concentrate was extracted with ether, EtOAc and *n*-BuOH saturated with H_2O, successively. A part (5 g) of the EtOAc extract (22 g) was chromatographed over Toyopearl HW-40 (coarse) (2.2 cm i.d. × 65 cm) developing with EtOH–H_2O (3 : 2)→EtOH–H_2O (7 : 3)→EtOH–H_2O–acetone (7 : 2 : 1) in a stepwise gradient mode. The following eight fractions were obtained: fractions I and II eluted with EtOH–H_2O (3 : 2); fractions III—V eluted with EtOH–H_2O (7 : 3); factions VI—VIII eluted with EtOH–H_2O–acetone (7 : 2 : 1). Fraction I (200 mg) was rechromatographed over Toyopearl HW-40 (fine) with EtOH–H_2O (1 : 1) to yield gallic acid (11 mg). Fraction II (257 mg) was rechromatographed over Sephadex LH-20 with EtOH to yield corilagin (**8**) (49 mg) and 1,2,3-tri-*O*-galloyl-β-D-glucose (**2**) (11 mg). Fraction III gave tellimagrandin I (**3**) (580 mg). Fraction IV (270 mg) was further purified by a combination of chromatography over Sephadex LH-20 [EtOH–H_2O (7 : 3)] and Toyopearl HW-40 (fine) [EtOH–H_2O (4 : 1)] to yield geraniin (**9**) (46 mg) and rugosin A (**5**) (29 mg). Fraction V gave tellimagrandin II (**4**) (165 mg). Fraction VI (47 mg) was rechromatographed over Sephadex LH-20 with EtOH–H_2O (8 : 2) to yield prostratin A (**12**) (19 mg). Fractions VII and VIII gave rugosin E (**11**) (100 mg) and rugosin D (**10**) (100 mg), respectively. The remaining EtOAc extract (17 g) was similarly chromatographed over Toyopearl HW-40 (coarse) (5 cm i.d. × 26 cm) developing with EtOH–H_2O (3 : 2)→EtOH–H_2O (7 : 3)→EtOH–H_2O–acetone (7 : 2 : 1)→EtOH–H_2O–acetone (6 : 2 : 2). The fraction eluted with EtOH–H_2O (3 : 2) gave tellimagrandin I (**3**) (330 mg). The EtOH–H_2O (7 : 3) eluate was divided into two fractions; one of them gave tellimagrandin II (**4**) (416 mg), and the other fraction, obtained after elution of **4**, was further chromatographed over Toyopearl HW-40 (fine) with EtOH–H_2O (7 : 3) to yield prostratin A (**12**) (68 mg) and prostratin C (**18**) (26 mg). Rugosin E (**11**) (720 mg) and rugosin D (**10**) (825 mg) were obtained from the fractions eluted with EtOH–H_2O–acetone (7 : 2 : 1). The fraction (256 mg) eluted with EtOH–H_2O–acetone (6 : 2 : 2) was further chromatographed over Toyopearl HW-40 (fine) with the same eluant to yield prostratin B (**15**) (21 mg) and rugosin G (**14**) (24 mg). A part (13 g) of the *n*-BuOH extract (28 g) was fractionated by column chromatography over Diaion HP-20 (2.8 cm i.d. × 45 cm) with H_2O→H_2O–MeOH (4 : 1)→H_2O–MeOH (7 : 3)→H_2O–MeOH (1 : 1)→MeOH in a stepwise gradient mode. The fraction (2.3 g) eluted with H_2O–MeOH (7 : 3) was chromatographed over Toyopearl HW-40 (fine) using the solvent system, EtOH–H_2O (3 : 2)→EtOH–H_2O (7 : 3)→EtOH–H_2O–acetone (7 : 2 : 1)→EtOH–H_2O– acetone (6 : 2 : 2). Two fractions were obtained with EtOH–H_2O (3 : 2) and one of them gave quercetin 3-*O*-β-D-xylopyranosyl (1→2)-β-D-glucopyranoside (**1**) (101 mg). The other fraction was rechromatographed over Sephadex LH-20 with EtOH–H_2O (6 : 4) to yield rugosin B (**6**) (67 mg). The eluate with EtOH–H_2O–acetone (7 : 2 : 1) gave rugosin E (**11**) (386 mg) and rugosin D (**10**) (96 mg). The fraction obtained with EtOH–H_2O–acetone (6 : 2 : 2) gave prostratin B (**15**) (195 mg). The yield of each compound per dried leaves was as follows: **1**, 0.018%; **2**, 0.004%; **3**, 0.21%; **4**, 0.061%; **5**, 0.011%; **6**, 0.012%; **8**, 0.018%; **9**, 0.017%; **10**, 0.11%; **11**, 0.15%; **12**, 0.007%; **14**, 0.003%; **15**, 0.037%; **18**, 0.003%.

Isolation of Tannins from *L. chinense* The dried leaves (700 g) of *L. chinense*, collected in Henan, China, in September, were homogenized in acetone–H_2O (7 : 3). After filtration and removal of acetone by evaporation, the concentrated solution was directly subjected to chromatography on a Diaion HP-20 column (9.5 cm i.d. × 100 cm) developing with H_2O→H_2O–MeOH (9 : 1)→H_2O–MeOH (4 : 1)→H_2O–MeOH (3 : 2)→H_2O–MeOH (2 : 3)→MeOH in a stepwise gradient mode. A part (12 g) of the H_2O–MeOH (3 : 2) eluate (36 g) was further chromatographed over Toyopearl HW-40 (coarse), developing with MeOH–H_2O (3 : 2)→MeOH–H_2O (7 : 3)→MeOH–H_2O–acetone (8 : 1 : 1)→MeOH–H_2O–acetone (6 : 2 : 2), to give rugosin E (**11**) (1.16 g) [eluate from MeOH–H_2O–acetone (8 : 1 : 1)]. A part (24 g) of the H_2O–MeOH (2 : 3) eluate (39 g) from the Diaion HP-20 column was similarly rechromatographed over Toyopearl HW-40 (coarse), to afford rugosin D (**10**) (497 mg) and prostratin B (**15**) (1.37 g), from the eluates with MeOH–H_2O–acetone (8 : 1 : 1) and (4 : 3 : 3), respectively. The yield of each compound per dried leaves was as follows: **10**, 0.12%; **11**, 0.5%; **15**, 0.32%.

Quercetin 3-*O*-β-D-Xylopyranosyl(1→2)-β-D-glucopyranoside (Quercetin 3-*O*-β-Sambubioside) (1) A pale yellow solid, $[\alpha]_D$ −64° (c = 2.0, MeOH). *Anal.* Calcd for $C_{26}H_{28}O_{16}\cdot 2H_2O$: C, 49.37; H, 5.10. Found: C, 49.68; H, 4.97. UV λ_{max}^{MeOH} nm (log ε): 210 (4.54), 256 (4.31), 357 (4.22). FAB-MS m/z: 619 $(M+Na)^+$, 597 $(M+H)^+$. ^{1}H-NMR (MeOH-d_4) δ: 4.76 (diffused d, J = 7 Hz, xylose H-1), 5.49 (d, J = 7.5 Hz, glucose H-1), 3.21—3.99 (sugar), 6.16 (d, J = 2Hz, H-6), 6.35 (d, J = 2 Hz, H-8), 6.86 (d, J = 8.5 Hz, H-5′), 7.62 (dd, J = 2.5, 8.5 Hz, H-6′), 7.64 (d, J = 2.5 Hz, H-2′) (aglycone). ^{13}C-NMR (DMSO-d_6) δ: 60.6 (C-6), 69.4 (C-4), 76.8 (C-3), 77.6 (C-5), 81.8 (C-2), 97.9 (C-1) (glucose), 65.6 (C-5), 69.6 (C-4), 73.9 (C-2), 76.2 (C-3), 104.5 (C-1) (xylose), 93.4 (C-8), 98.6 (C-6), 103.9 (C-10), 115.2 (C-2′), 116.0 (C-5′), 121.2 (C-1′), 121.9 (C-6′), 133.0 (C-3), 144.9 (C-3′), 148.5 (C-4′), 155.3 (C-2), 156.2 (C-9), 161.2 (C-5), 164.0 (C-7), 177.4 (C-4) (aglycone).

Prostratin A (12) A pale brown amorphous powder, $[\alpha]_D$ +76° (c = 0.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 218 (5.01), 258 (4.81), 346 (3.90). FAB-MS (negative) m/z: 1237 $(M-H)^-$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 6.93, 6.89 (each 2H, s, galloyl), 7.60, 7.22, 7.09 (each 1H, s, dilactonized valoneoyl), 6.64, 6.44 (each 1H, s, HHDP), 6.08 (d, J = 8 Hz, H-1), 5.71 (t, J = 10 Hz, H-3), 5.49 (dd, J = 8, 10 Hz, H-2), 5.23 (dd, J = 6.5, 13.5 Hz, H-6), 5.14 (t, J = 10 Hz, H-4), 4.41 (ddd, J = 0.5, 6.5, 10 Hz, H-5), 3.70 (dd, J = 0.5, 13.5 Hz, H-6) (glucose). This compound was identical with that obtained from isorugosin D by partial hydrolysis.[17]

Partial Hydrolysis of Prostratin A (12) An aqueous solution (30 ml) of **12** (30 mg) was heated on a boiling-water bath for 8 h. The reaction mixture was evaporated, and the residue was chromatographed over Toyopearl HW-40 (fine) developing with H_2O–MeOH (1 : 1) to yield gallic acid (6.8 mg), valoneic acid dilactone (**14**) (1.1 mg), and tellimagrandin I (**3**) (7 mg), which were identical with authentic specimens as judged from HPLC and ^{1}H-NMR spectral comparisons.

Prostratin B (15) A pale brown amorphous powder, $[\alpha]_D$ +125°C (c = 1.0, MeOH). *Anal.* Calcd for $C_{116}H_{82}O_{74}\cdot 21H_2O$: C, 45.86; H, 4.11. Found: C, 45.86; H, 3.91. UV λ_{max}^{MeOH} nm (log ε): 220 (5.27), 276 (4.96). FAB-MS m/z: 2681 $(M+Na)^+$. CD (MeOH): $[\theta]_{223}$ $+24\times10^4$, $[\theta]_{258}$ -8.4×10^4, $[\theta]_{281}$ $+12\times10^4$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.06, 7.05 (each 2H, s, galloyl), 7.60, 7.22, 7.09 (each 1H, s, dilactonized valoneoyl), 2H in total), 6.99 (2H, s), 6.98 (2H, s), 6.952, 6.949 (each s, 2H in total) (galloyl), 7.16, 7.14 (each s, 1H in total), 7.122, 7.119 (each s, 1H in total), 6.65, 6.64 (each s, 1H in total), 6.47, 6.460, 6.457, 6.449 (each s, 3H in total), 6.180, 6.175 (each s, 1H in total), 6.164, 6.156 (each s, 1H in total) (valoneoyl and HHDP); glucose (α-anomer), 6.05 (d, J = 8.5 Hz, H-1), 5.522 (dd, J = 8.5, 10 Hz, H-2), 5.75 (t, J = 10 Hz, H-3), 5.14 (t, J = 10 Hz, H-4), 4.43 (dd, J = 6, 10 Hz, H-5), 5.26 (dd, J = 6, 13 Hz, H-6), 3.78 (d, J = 13 Hz,

H-6), 6.08 (d, J=8.5 Hz, H-1'), 5.54 (dd, J=8.5, 10 Hz, H-2'), 5.77 (t, J=10 Hz, H-3'), 5.099 (t, J=10 Hz, H-4'), 4.44 (dd, J=6, 10 Hz, H-5'), 5.23 (dd, J=6, 13 Hz, H-6'), 3.73 (d, J=13 Hz, H-6'), 5.524 (d, J=4 Hz, H-1''), 5.105 (dd, J=4, 10 Hz, H-2''), 5.84 (t, J=10 Hz, H-3''), 5.041 (t, J=10 Hz, H-4''), 4.62 (dd, J=6, 10 Hz, H-5''), 5.15 (dd, J=6, 13 Hz, H-6''), 3.65 (d, J=13 Hz, H-6''); glucose (β-anomer), 6.05 (d, J=8.5 Hz, H-1), 5.522 (dd, J=8.5, 10 Hz, H-2), 5.75 (t, J=10 Hz, H-3), 5.14 (t, J=10 Hz, H-4), 4.43 (dd, J=6, 10 Hz, H-5), 5.26 (dd, J=6, 13 Hz, H-6), 3.78 (d, J=13 Hz, H-6), 6.06 (d, J=8.5 Hz, H-1'), 5.53 (dd, J=8.5, 10 Hz, H-2'), 5.76 (t, J=10 Hz, H-3'), 5.108 (t, J=10 Hz, H-4'), 4.42 (dd, J=6, 10 Hz, H-5'), 5.21 (dd, J=6, 13 Hz, H-6'), 3.72 (d, J=13 Hz, H-6'), 5.06 (d, J=8 Hz, H-1''), 5.24 (dd, J=8, 10 Hz, H-2''), 5.58 (t, J=10 Hz, H-3''), 5.044 (t, J=10 Hz, H-4''), 4.22 (dd, J=6, 10 Hz, H-5''), 5.19 (dd, J=6,13 Hz, H-6''), 3.74 (d, J=13 Hz, H-6''). ^{13}C-NMR (acetone-d_6–D_2O) δ: 62.9, 63.0 (α and β-anomers, C-6, C-6'), 63.5 (α and β, C-6''), 66.9 (α, C-5''), 70.5, 70.6 (α and β, C-4, C-4'), 71.0 (α, C-4''), 71.1 (β, C-3''), 71.3 (α, C-3''), 71.6 (α and β, C-2, C-2'), 71.8 (β, C-5''), 72.7, 72.9 (α and β, C-5, C-5'), 73.0 (α, C-2''), 73.2 (α and β, C-3, C-3'), 73.6 (α, C-3''), 74.0 (β, C-2''), 91.0 (α, C-1''), 92.9, 93.1 (α and β, C-1, C-1'), 96.5 (β, C-1'') (glucose), 162.10, 162.14, 162.3, 165.82, 165.86, 165.95, 166.3, 166.4, 166.5, 166.8, 167.6, 167.7, 167.8, 167.9, 168.0 (ester carbonyl).

Acid Hydrolysis of Prostratin B (15) A solution of **15** (20 mg) in 5% H_2SO_4 (1 ml) was heated on a boiling-water bath for 5 h. After cooling, deposited precipitates were filtered off and washed with H_2O. The filtrate was extracted with EtOAc. The precipitates and EtOAc-soluble portion were methylated with an excess of ethereal CH_2N_2, and the products were purified by preparative TLC [benzene–acetone (15:1)] to afford methyl tri-*O*-methylgallate (7.0 mg), tetra-*O*-methylellagic acid (1.8 mg) and methyl hexa-*O*-methylvalonate dilactone (**13a**) (5.1 mg). The sugar component in the aqueous layer that remained after EtOAc extraction was identified as glucose by GLC (G-250; column temperature, 180°C) of the trimethylsilyl derivative.

Reduction of Prostratin B (15) A 0.16 M aqueous solution (5 ml) of $NaBH_4$ was added dropwise to a solution of **15** (100 mg) in H_2O (10 ml), over 30 min at room temperature, under vigorous shaking. The course of the reaction was monitored by reversed-phase HPLC until the starting material disappeared, and then the reaction was terminated by adding a few drops of dilute HCl. The reaction mixture was passed through a column of Diaion HP-20 and eluted with H_2O→H_2O–MeOH (4:1)→H_2O–MeOH (3:2)→MeOH in a stepwise gradient mode. The eluate with H_2O–MeOH (3:2) gave dihydroprostratin B (**16**) (93 mg) as an off-white amorphous powder, $[\alpha]_D$ +123° (c=1.0, MeOH). *Anal.* Calcd for $C_{116}H_{84}O_{74}\cdot 20H_2O$: C, 45.83; H, 4.18. Found: C, 46.06; H, 4.14. UV λ_{max}^{MeOH} nm (log ε): 221 (5.31), 275 (5.01). ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.13 (3H, s), 7.06, 7.00, 6.99, 6.97, 6.95 (each 2H, s), 7.10, 6.83, 6.65, 6.16, 6.08 (each 1H, s), 6.45 (2H, s) (galloyl, HHDP and valoneoyl), 6.048 (d, J=8.5 Hz, H-1), 5.53 (dd, J=8.5, 10 Hz, H-2), 5.75 (t, J=10 Hz, H-3), 5.13 (t, J=10 Hz, H-4), 4.43 (dd, J=7, 10 Hz, H-5), 3.78 (d, J=13.5 Hz, H-6), 6.053 (d, J=8.5 Hz, H-1'), 5.51 (dd, J=8.5, 10 Hz, H-2'), 5.77 (t, J=10 Hz, H-3'), 5.10 (t, J=10 Hz, H-4'), 4.43 (dd, J=7, 10 Hz, H-5'), 3.74 (d, J=13.5 Hz, H-6'), 3.97 (dd, J=4.5, 12.5 Hz, H-1''), 3.82 (dd, J=4.5, 12.5 Hz, H-1''), 5.97 (dd, J=3, 7.5 Hz, H-3''), 4.09 (dd, J=3, 10 Hz, H-5''), 4.55 (dd, J=3, 12.5 Hz, H-6''), 3.79 (d, J=12.5 Hz, H-6''), 5.33—5.19 (complicated peaks; H-6, H-6', H-2'', H-4'').

Partial Hydrolysis of Prostratin B (15) An aqueous solution (10 ml) of **15** (100 mg) was heated on a boiling-water bath for 45 min. After cooling, the solvent was evaporated off and the residue was chromatographed over Toyopearl HW-40 (superfine), developing with EtOH–H_2O (3:2)→EtOH–H_2O (7:3)→EtOH–H_2O–acetone (7:2:1). The fractions eluted with EtOH–H_2O (3:2) gave rugosin B (**6**) (6.8 mg) and tellimagrandin I (**3**) (9.0 mg). The eluates from EtOH–H_2O (7:3) and from EtOH–H_2O–acetone (7:2:1) gave a partial hydrolyzate (**17**) (10.1 mg) and rugosin E (**11**) (4.0 mg), respectively.

The Partial Hydrolyzate (**17**): A pale yellow amorphous powder, $[\alpha]_D$ +144° (c=1.0, MeOH). *Anal.* Calcd for $C_{41}H_{28}O_{26}\cdot 5H_2O$: C, 47.96; H, 3.73. Found: C, 48.12; H, 4.01. UV λ_{max}^{MeOH} nm (log ε): 217 (4.92), 280 (4.54). FAB-MS m/z: 959 (M+Na)$^+$, 937 (M+H)$^+$. CD (MeOH): $[\theta]_{215}$ $+9.6\times10^4$, $[\theta]_{259}$ -2.9×10^4, $[\theta]_{286}$ $+4.9\times10^4$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.04, 7.03 (each s, 2H in total), 6.97, 6.92 (each s, 2H in total) (galloyl), 7.16, 7.15 (each s, 1H in total), 6.93, 6.92 (each s, 1H in total), 6.50, 6.47 (each s, 1H in total) (valoneoyl); glucose (α-anomer), 5.55 (d, J=4 Hz, H-1), 5.103 (dd, J=4, 10 Hz, H-2), 5.86 (t, J=10 Hz, H-3), 5.096 (t, J=10 Hz, H-4), 4.67 (ddd, J=1, 6, 10 Hz, H-5), 5.20 (dd, J=6, 13 Hz, H-6), 3.84 (dd, J=1, 13 Hz, H-6); glucose (β-anomer), 5.06 (d, J=8 Hz, H-1), 5.24 (dd, J=8, 10 Hz, H-2), 5.60 (t, J=10 Hz, H-3), 5.090 (t, J=10 Hz, H-4), 4.28 (br dd, J=6, 10 Hz, H-5), 5.21 (dd, J=6, 13 Hz, H-6), 3.92 (br d, J=13 Hz, H-6). ^{13}C-NMR: see Table I.

Partial Methanolysis of 17 A solution of **17** (10 mg) in a mixture of MeOH (10 ml) and 0.2 M acetate buffer (pH 6.0, 1 ml) was kept at 37°C for 24 h. After acidification with dilute HCl and removal of MeOH by evaporation, the reaction mixture was extracted with EtOAc. The EtOAc extract was chromatographed over MCI gel CHP 20P using MeOH–H_2O (2:3) as an eluant to give a methanolyzate (**7**) (3.4 mg) as an off-white amorphous powder, $[\alpha]_D$ +118° (c=1.0, MeOH). *Anal.* Calcd for $C_{42}H_{32}O_{27}\cdot 7H_2O$: C, 46.08; H, 4.24. Found: C, 45.96; H, 4.17. UV λ_{max}^{MeOH} nm (log ε): 221 (4.80), 272 (4.48). FAB-MS m/z: 991 (M+Na)$^+$, 1007 (M+K)$^+$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.045, 7.038 (each s, 2H, in total), 6.98, 6.94 (each s, 2H in total) (galloyl), 7.060, 7.057 (each s, 1H in total), 6.47, 6.44 (each s, 1H in total), 6.26, 6.25 (each s, 1H in total) (valoneoyl); glucose (α-anomer), 5.50 (d, J=4 Hz, H-1), 5.07 (dd, J=4, 10 Hz, H-2), 5.84 (t, J=10 Hz, H-3), 5.06 (t, J=10 Hz, H-4), 4.61 (dd, J=6.5, 10 Hz, H-5), 5.19 (dd, J=6.5, 13 Hz, H-6), 3.66 (d, J=13 Hz, H-6); glucose (β-anomer), 5.03 (d, J=8 Hz, H-1), 5.22 (dd, J=8, 10 Hz, H-2), 5.58 (t, J=10 Hz, H-3), 5.05 (t, J=10 Hz, H-4), 4.21 (dd, J=6.5, 10 Hz, H-5), 5.21 (dd, J=6.5, 13 Hz, H-6), 3.74 (d, J=13 Hz, H-6), 3.67 (3H, s, OMe).

Partial Hydrolysis of Dihydroprostratin B (16) An aqueous solution (10 ml) of **16** (100 mg) was heated on a boiling-water bath for 50 min. After removal of the solvent, the residue was chromatographed over Toyopearl HW-40 (superfine) with MeOH–H_2O (1:1)→MeOH–H_2O (3:2)→MeOH–H_2O (7:3). The MeOH–H_2O (1:1) eluate gave dihydrorugosin B (**20**) (5.1 mg). The MeOH–H_2O (3:2) eluate was further chromatographed over Toyopearl HW-40 (superfine) with EtOH–H_2O (3:2) to give rugosin B (**6**) (7.2 mg). The fraction eluted after **6** was rechromatographed over MCI gel CHP 20P with aqueous MeOH to give tellimagrandin I (**3**) (19.4 mg) and the dihydro derivative (**19**) (5.9 mg) of **17**. The fraction eluted with EtOH–H_2O (7:3) gave **17** (9.4 mg). The dihydro derivatives (**20** and **19**) were identified by direct comparisons with those prepared by $NaBH_4$ reduction of rugosin B (**6**) and **17**.

The Dihydro Derivative (**19**): An off-white amorphous powder, $[\alpha]_D$ +136° (c=1.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 224 (4.78), 277 (4.47). FAB-MS m/z: 961 (M+Na)$^+$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.16, 7.06 (each 2H, s, galloyl), 6.99, 6.93, 6.92 (each 1H, s, valoneoyl), 5.97 (dd, J=3, 7 Hz, H-3), 5.28 (H-2, overlapped with H-4 signals), 5.27 (dd, J=3, 8.5 Hz, H-4), 4.50 (dd, J=3, 12.5 Hz, H-6), 4.09 (dd, J=3, 8.5 Hz, H-5), 3.93 (d, J=12.5 Hz, H-6), 3.88 (dd, J=4.5, 12 Hz, H-1), 3.72 (dd, J=4, 12 Hz, H-1).

Dihydrorugosin B (**20**): An off-white amorphous powder, $[\alpha]_D$ +147° (c=1.1, MeOH). *Anal.* Calcd for $C_{41}H_{32}O_{27}\cdot 8H_2O$: C, 44.74; H, 4.40. Found: C, 44.57; H, 3.99. UV λ_{max}^{MeOH} nm (log ε): 223 (4.78), 268 (4.49). FAB-MS m/z: 979 (M+Na)$^+$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.13, 7.05 (each 2H, s, galloyl), 7.06, 6.85, 6.25 (each 1H, s, valoneoyl), 5.94 (dd, J=3, 7 Hz, H-3), 5.291 (H-2, overlapped with H-4 signals), 5.287 (dd, J=3, 8.5 Hz, H-4), 4.58 (dd, J=3, 12.5 Hz, H-6), 4.04 (dd, J=3, 8.5 Hz, H-5), 3.94 (dd, J=5, 12 Hz, H-1), 3.79 (d, J=12.5 Hz, H-6), 3.77 (dd, J=4, 12 Hz, H-1).

Prostratin C (18) A light brown amorphous powder, $[\alpha]_D$ +79° (c=1.0, MeOH). *Anal.* Calcd for $C_{48}H_{32}O_{30}\cdot 11H_2O$: C, 44.80; H, 4.23. Found: C, 44.53; H, 3.82. UV λ_{max}^{MeOH} nm (log ε): 218 (5.05), 280 (4.70). FAB-MS (negative) m/z: 1087 (M−H)$^-$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.12, 6.99, 6.95 (each 2H, s, galloyl), 7.20, 6.93, 6.49 (each 1H, s, valoneoyl), 6.20 (d, J=8 Hz, H-1), 5.85 (t, J=10 Hz, H-3), 5.61 (dd, J=8, 10 Hz, H-2), 5.30 (dd, J=6.5, 13.5 Hz, H-6), 5.20 (t, J=10 Hz, H-4), 4.57 (dd, J=6.5, 10 Hz, H-5), 3.97 (d, J=13.5 Hz, H-6). ^{13}C-NMR; see Table I.

Conversion of Prostratin C (18) to Rugosin A (5) An aqueous solution (0.5 ml) of **18** (0.5 mg) was heated on a boiling-water bath for 45 min. HPLC of the reaction mixture showed peaks identical with those of rugosin A (**5**) [t_R 5.98 min (reversed-phase), t_R 6.00 min (normal phase)] and **18** [t_R 13.9 min (reversed-phase), t_R 5.19 (normal phase)].

Partial Hydrolysis of Rugosin D (10) An aqueous solution (10 ml) of rugosin D (**10**) (100 mg) was heated on a boiling-water bath for 1 h. The reaction mixture was evaporated and chromatographed over MCI gel CHP 20P using water containing increasing amounts of EtOH in a stepwise elution to give tellimagrandin I (**3**) (13.7 mg), tellimagrandin II (**4**) (1.7 mg), rugosin A (**5**) (18.9 mg) and prostratin C (**18**) (4.9 mg) together with the starting material (7.6 mg).

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References and Notes

1) Part VI in the series on "Tannins and Related Polyphenols of Euphorbiaceous Plants." For Part V, see ref. 3*b*. This also constitutes part IV in the series on "Tannins of Hamamelidaceous Plants." Part III, see ref. 17.
2) T. Okuda, K. Mori, and T. Hatano, *Phytochemistry*, **19**, 547 (1980).
3) *a*) T. Yoshida, L. Chen, T. Shingu and T. Okuda, *Chem. Pharm. Bull.*, **36**, 2940 (1988); *b*) T. Yoshida, O. Namba, L. Chen and T. Okuda, *ibid.*, **38**, 86 (1990); *c*) T. Yoshida, O. Namba, K. Yokoyama, L. Chen and T. Okuda, 31st Symposium on the Chemistry of Natural Products, Nagoya, Symposium Papers, p. 601 (1989).
4) The structure of prostratin C was reported in a preliminary communication: T. Hatano, O. Namba, T. Yasuhara, K. Yazaki, T. Yoshida and T. Okuda, *Heterocycles*, **31**, 1221 (1990).
5) T. Okuda, T. Hatano and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4234 (1982).
6) *a*) Fujian medical Institute ed., "Dictionary of Fujian Herbs (Fukken Yakubutsushi)," Vol. 1, Fujian, People's Publishing House, Fuzhou, 1977, p. 269; *b*) Chiang Su New Medicinal College ed., "Dictionary of Traditional Chinese Crude Drugs," Shanghai Scientific Technologic Publishers, Shanghai, 1977, p. 4093.
7) T. Okuda, T. Hatano, K. Yazaki and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4230 (1982).
8) C. K. Wilkins and B. A. Bohm, *Phytochemistry*, **15**, 211 (1976).
9) T. Okuda, T. Yoshida, M. Ashida and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765.
10) T. Okuda, T. Hatano, K. Yazaki and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4230 (1982).
11) T. Okuda, T. Yoshida and T. Hatano, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 9.
12) T. Brasseur and L. Angenot, *Phytochemistry*, **27**, 1487 (1988).
13) T. Tanaka, O. Tanaka, Z.-W. Lin, J. Zhou and H. Ageta, *Chem. Pharm. Bull.*, **31**, 780 (1983).
14) N. Ishikura, *Agric. Biol. Chem.*, **49**, 1705 (1982).
15) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
16) O. T. Schmidt and E. Komarek, *Justus Liebigs Ann. Chem.*, **591**, 156 (1954).
17) T. Hatano, R. Kira, T. Yasuhara and T. Okuda, *Chem. Pharm. Bull.*, **36**, 3920 (1988).
18) R. Armitage, G. S. Bayliss, J. W. Gramshaw, E. Haslam, R. D. Haworth, K. Jones, H. J. Rogers and T. Searle, *J. Chem. Soc.*, (*C*), **1961**, 1842.
19) T. Okuda, T. Yoshida and T. Hatono, *Heterocycles*, **30**, 1195 (1990).