

## Tannins and Related Compounds. CXVII.<sup>11</sup> Isolation and Characterization of Three New Ellagitannins, Lagerstannins A, B and C, Having a Gluconic Acid Core, from *Lagerstroemia speciosa* (L.) PERS

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Lagerstannins A (6), B (11) and C (8), ellagitannins having a gluconic acid core, have been isolated from the fruits and leaves of *Lagerstroemia speciosa* (L.) PERS. (= *L. flos-reginae* RETZ.) (Lythraceae). On the basis of chemical and spectroscopic evidence, their structures were established as 2,3,4,6-bis-*O*-(*S*)-hexahydroxydiphenoyl-*D*-gluconic acid (6), 2,3,5-*O*-(*S,R*)-flavogallonyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*D*-gluconic acid (11), and 5-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*D*-gluconic acid (8), respectively. Furthermore, the structure of an ellagitannin, punigluconin, previously isolated from the bark of *Punica granatum* L., was revised as 2,5-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*D*-gluconic acid (10), based on spectral re-examination.

**Keywords:** *Lagerstroemia speciosa*; Lythraceae; lagerstannin; punigluconin; *Punica granatum*; gluconic acid; flavogallonic acid; ellagitannin; tannin

*Lagerstroemia speciosa* (L.) PERS. (= *L. flos-reginae* RETZ.) (Lythraceae) is a deciduous tree native to regions from India and southern China to tropical Australia, and cultivated as an excellent avenue tree because of its beautiful flowers. It is known that the leaves and fruits have hypoglycemic properties in treating diabetes mellitus and contain large amounts of tannin.<sup>2)</sup> In continuing our chemical studies on tannins in *Lagerstroemia* spp.,<sup>3)</sup> we have isolated three new ellagitannins, lagerstannins A, B and C, having a gluconic acid core in the molecule. Furthermore, in the course of the structural studies on these tannins, we have noticed that the structure of a related ellagitannin, punigluconin,<sup>4)</sup> formerly isolated from the bark of *Punica granatum* as the first example of an ellagitannin having a gluconic acid core, should be revised. This paper deals with the isolation and structure elucidation of these compounds.

The aqueous acetone extract of the fresh fruit was subjected to a combination of Sephadex LH-20, MCI-gel CHP 20P, Cosmosil 75C<sub>18</sub>-OPN, Fuji-gel ODS-G3 and TSK-gel Toyopearl HW 40F column chromatographies to yield lagerstannins A (6) and B (11), together with five known tannins, gemin D (1),<sup>5)</sup> lagerstroemin (2),<sup>3,6)</sup> castalagin (3),<sup>6)</sup> vescalagin (4),<sup>7)</sup> grandinin (5)<sup>8)</sup> and hippophaenin A (7).<sup>9)</sup> On the other hand, similar chromatographic separation of the extract of the dried leaves<sup>3,10)</sup> yielded lagerstannin C (8), 4,6-*O*-(*S*)-hexahydroxydiphenoylgluconic acid (9)<sup>4)</sup> and 7.

Lagerstannin A (6) was characterized as an ellagitannin by color reactions with ferric chloride (dark blue) and sodium nitrite-acetic acid<sup>10)</sup> (reddish-brown) reagents. The proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectrum of

6 (Table I) showed four one-proton singlets ( $\delta$  6.62, 6.63, 6.77 and 6.79) suggestive of the presence of two hexahydroxydiphenoyl ester groups. The carbon-13 nuclear magnetic resonance (<sup>13</sup>C-NMR) spectrum exhibited, besides aromatic and ester carbon signals confirming the presence of two hexahydroxydiphenoyl groups, one carboxyl ( $\delta$  170.8) and five aliphatic carbon signals ( $\delta$  68.1, 69.0, 73.4, 76.1 and 76.6), the chemical shifts of which were similar to those of hippophaenin A (7), indicating the presence of an aldohexonic acid moiety.

Comparison of the <sup>1</sup>H-NMR spectra of 6 and 7 (Table I) showed a close relationship between these compounds. Namely, the chemical shifts and coupling patterns of the signals arising from the aldonic acid moiety in 6 were very similar to those of 7, except for the appearance of the signal due to H-5 at significantly upper field ( $\delta$  4.36). These findings suggested that 6 is 2,3,4,6-bis-*O*-hexahydroxydiphenoylgluconic acid, and this was also supported by the negative ion fast atom bombardment mass spectrum (FAB-MS) of 6, exhibiting the (M-H)<sup>-</sup> peak at *m/z* 799.

Selective hydrolysis of the galloyl group in 7 with tannase afforded 6 as expected. Furthermore, the absolute structure of 6, including the atropisomerism of the biphenyl linkages and the absolute configuration of the gluconic acid moiety, was confirmed as follows. Reduction of 6 with lithium aluminum hydride afforded many reduction products, among which a product (6a) could be isolated. The physical and spectroscopic data of 6a were found to be identical with those of 2,3,4,6-bis-*O*-(*S*)-hexahydroxydiphenoyl-*D*-glucitol obtained by reduction of 2,3,4,6-bis-*O*-(*S*)-hexahydroxydiphenoyl-*D*-glucopyranose (pedunculagin)<sup>11)</sup> with

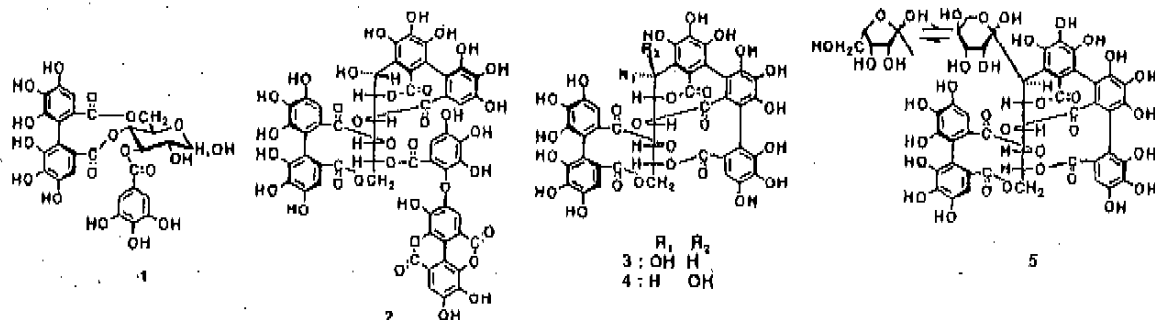
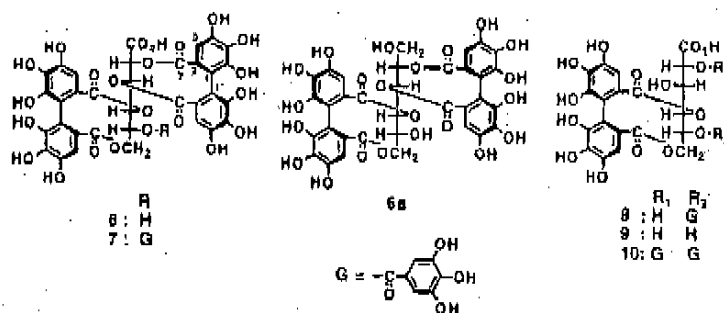


TABLE I.  $^1\text{H-NMR}$  Spectral Data for **6**–**11**, **11e**, **11f** and **11h** ( $\delta$  Values)

	6 <sup>a)</sup>	7 <sup>a)</sup>	8 <sup>a)</sup>	9 <sup>b)</sup>	10 <sup>c)</sup>	11 <sup>a)</sup>	11e <sup>d)</sup>	11f <sup>d)</sup>	11h <sup>d)</sup>
Gluconic acid									
H-2	5.36 (d, $J=10$ )	5.28 (d, $J=10$ )	4.25 (d, $J=3$ )	4.30 (d, $J=3$ )	5.36 (d, $J=4$ )	5.57 (d, $J=9$ )	5.65 (d, $J=10$ )	4.38 (d, $J=3$ )	4.08 <sup>e)</sup> (t, $J=6$ )
H-3	5.65 (dd, $J=2, 10$ )	5.59 (dd, $J=1, 10$ )	4.15 (t, $J=2$ )	4.27 (t, $J=2$ )	4.45 (dd, $J=2, 4$ )	5.39 (dd, $J=7, 9$ )	5.79 (dd, $J=4, 10$ )	4.09 (t, $J=3$ )	5.67 (dd, $J=6, 10$ )
H-4	5.55 (dd, $J=2, 8$ )	6.11 (dd, $J=1, 9$ )	5.47 (dd, $J=3, 8$ )	4.41 (dd, $J=3, 9$ )	5.82 (dd, $J=2, 8$ )	5.51 (dd, $J=3, 8$ )	5.65 (dd, $J=2, 7$ )	4.17 (t, $J=4$ )	5.42 (br s) <sup>d)</sup>
H-5	4.36 (dd, $J=3, 8$ )	5.41 (dd, $J=3, 9$ )	5.74 (dd, $J=3, 8$ )	5.13 (dd, $J=3, 9$ )	5.51 (dd, $J=3, 8$ )	5.65 (dd, $J=2, 7$ )	5.02 (ddd, $J=4, 5, 5$ )	5.42 (br s) <sup>d)</sup>	5.15 (dd, $J=5, 11$ )
H-6	4.79 (dd, $J=3, 12$ )	5.02 (dd, $J=3, 13$ )	4.95 (dd, $J=3, 14$ )	4.88 (dd, $J=3, 12$ )	4.82 (dd, $J=3, 13$ )	4.87 (dd, $J=2, 12$ )	3.86 (2H, br d, $J=5$ )	4.78 (dd, $J=3, 13$ )	4.50 (dd, $J=5, 11$ )
MHDP-H	6.62 (s)	6.59 (s)	6.58 (s)	6.53 (s)	6.54 (s)	6.53 (s)	6.54 (s)	6.54 (s)	6.64 (s)
	6.63 (s)	6.74 (s)	6.87 (s)	6.78 (s)	6.92 (s)	6.83 (s)	6.84 (s)	6.84 (s)	6.90 (s)
	6.77 (s)	6.76 (s)							
	6.79 (s)	6.79 (s)							
Flavogallonyl-H						6.61 (s)	6.52 (s)	7.39 (s)	7.29 (s)
Gallonyl-H						6.79 (s)	6.77 (s)	7.61 (s)	7.60 (s)
		7.27 (2H, s)	7.18 (2H, s)		7.12 (2H, s)				
					7.19 (2H, s)				

a) 270 MHz; in acetone- $d_6$  + D<sub>2</sub>O. b) 100 MHz; in acetone- $d_6$  + D<sub>2</sub>O. c) 100 MHz; in acetone- $d_6$ . d) Coupled with a hydroxyl signal ( $\delta$  5.29,  $J=6$  Hz), which was exchangeable with D<sub>2</sub>O. e) Due to the overlap of signals, the exact coupling constant could not be calculated.



sodium borohydride. On the basis of these findings, lagerstannin A was characterized as 2,3;4,6-bis-*O*-(*S*)-hexahydroxydiphenyl-*D*-gluconic acid (**6**).

Lagerstannin C (**8**) showed color reactions similar to those of **6**. In the  $^1\text{H-NMR}$  spectrum, the appearance of a two-proton singlet ( $\delta$  7.18) and two one-proton singlets ( $\delta$  6.58 and 6.87) suggested the presence of a galloyl and a hexahydroxydiphenyl group in the molecule. The presence of an aldohexonic acid moiety was revealed by the observation of six carbon signals at  $\delta$  64.9, 71.3, 71.7, 72.4, 73.1 and 174.9 in the  $^{13}\text{C-NMR}$  spectrum, and also of six aliphatic proton signals in the  $^1\text{H-NMR}$  spectrum (Table I). By examination of the two-dimensional  $^1\text{H-}^{11}\text{B}$  shift correlation ( $^1\text{H-}^1\text{H}$  COSY) spectrum of **8**, three aliphatic proton signals ( $\delta$  5.47, 5.74 and 4.96) appearing downfield were attributed to H-4, H-5 and H-6 in the aldonic acid moiety, indicating that these positions are acylated.

Tannase hydrolysis of **8** afforded gallic acid and a partial hydrolysate (**9**), whose  $^1\text{H-NMR}$  spectrum showed a fairly upfield shift of the signal due to H-5 ( $\delta$  1.33), indicating the location of the galloyl group to be at the C-5 hydroxyl group in **8**. Furthermore, the hydrolysate (**9**) was identified as 4,6-*O*-(*S*)-hexahydroxydiphenyl-*D*-gluconic acid<sup>4)</sup> by comparison of physical and spectral data. Hence, the structure of lagerstannin C was determined to be 5-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenyl-*D*-gluconic acid (**8**).

The locations of two galloyl groups in punigluconin (**10**)

were formerly reported to be at the C-2 and C-3 positions.<sup>4)</sup> However, in the  $^1\text{H-NMR}$  spectrum, which was closely related to that of **8** (Table I), the lowfield shift ( $\delta$  5.51) of the H-5 signal and the upfield shift ( $\delta$  4.45) of the H-3 signal were apparently inconsistent with the former structure.<sup>12)</sup> Thus, the structure of punigluconin was concluded to be revised as 2,5-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenyl-*D*-gluconic acid (**10**).

Lagerstannin B (**11**) was also positive to the ferric chloride (dark blue) and sodium nitrite-acetic acid (reddish-brown) tests. The  $^{13}\text{C-NMR}$  spectrum showed aromatic carbon signals due to five carboxy bearing pyrogallol rings whose chemical shifts were closely related to those of a hexahydroxydiphenyl group. In contrast to the numbers of aromatic rings, six carboxyl carbon signals appeared at  $\delta$  165.9, 166.4, 167.3, 168.0 (2C) and 168.8. This fact, combined with the observation of five aliphatic carbon signals ( $\delta$  65.7, 70.3, 71.3, 72.5 and 73.1), suggested the presence of an aldohexonic acid moiety. In the  $^1\text{H-NMR}$  spectrum, the observation of six aliphatic proton signals in the fairly lowfield (Table I) indicated that all the hydroxyl groups in the aldonic acid moiety were acylated, and the chemical shifts of four one-proton singlets in the aromatic region were similar to those of the hexahydroxydiphenyl groups in **7**. Furthermore, the negative ion FAB-MS showed the ( $M-1$ )<sup>-</sup> peak at  $m/z$  949, which was two mass units less than that found in the case of **7**. These spectroscopic findings suggested the presence of a triphenyl ester group

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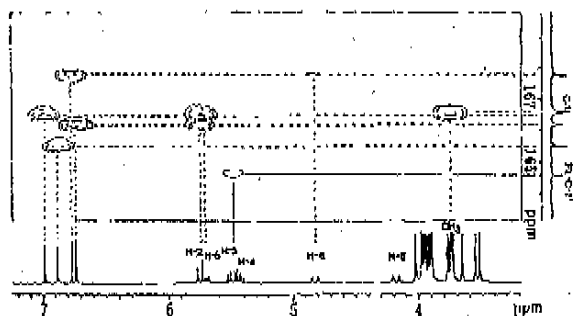
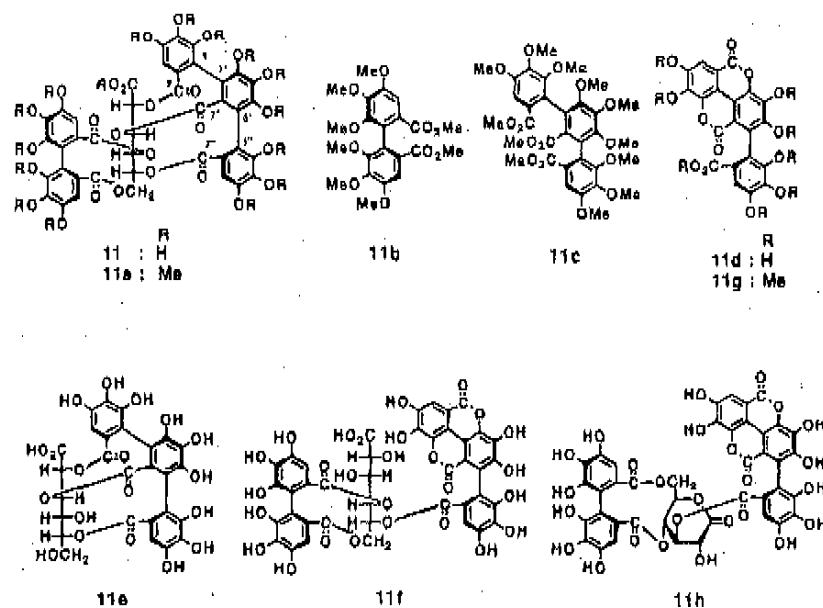


Fig. 1.  $^1\text{H}$ - $^{13}\text{C}$  Long-Range COSY Spectrum of **11a** in  $\text{CDCl}_3$ , ( $J_{\text{CH}}$  = 5 Hz). FL: Flavogallonyl group.

in the molecule, together with a hexahydroxydiphenoyl group.

Methylation of **11** with dimethyl sulfate and anhydrous potassium carbonate in dry acetone gave the hexadecamethylate (**11a**), which was subsequently subjected to alkaline methanolysis to give dimethyl (*S*)-hexamethoxydiphenate (**11h**) [ $[\alpha]_{\text{D}}^{25} = -23.3^\circ$  ( $\text{CHCl}_3$ )]<sup>13</sup> and trimethyl nonamethylflavogallonate (**11c**).<sup>14</sup> The latter was found to be optically inactive, indicating that this ester is a *meso*-type compound.

The  $^1\text{H}$   $^{13}\text{C}$  long-range COSY spectrum of the hexadecamethylate (**11a**) (Fig. 1) showed three-bond long-range correlations between four aromatic singlets ( $\delta$  6.74, 6.78, 6.89 and 6.99) and carboxyl carbon signals ( $\delta$  166.1, 167.7, 165.4 and 166.4, respectively), and between the methyl proton signal at  $\delta$  3.75 and the carboxyl carbon signal at  $\delta$  166.5, which is therefore assignable to C-1 of the aldonic acid moiety. The remaining ester carbon signal at  $\delta$  164.5, which was not correlated with any aromatic proton signal, was hence attributable to a carboxyl carbon (C-7') attached to the "central" aromatic ring of the flavogallonyl group. This carboxyl signal was found to be correlated with the

proton signal ( $\delta$  5.50, dd,  $J=6, 9$  Hz) due to H-3, clearly indicating that the "central" aromatic carboxyl group of the flavogallonyl group is located at the C-3 position of the aldonic acid moiety.

Partial hydrolysis of **11** in dilute sulfuric acid yielded ellagic acid, optically active flavogallonic acid (dilactone form, **11d**)<sup>15</sup> [ $[\alpha]_{\text{D}}^{25} +20.3^\circ$  (MeOH)] and two hydrolysates (**11e** and **11f**). The negative ion FAB-MS of **11e** showed the ( $M-H$ )<sup>-</sup> peak at  $m/z$  647, indicating that this hydrolysate is generated by hydrolysis of the hexahydroxydiphenoyl group. In the  $^1\text{H}$ -NMR spectrum (Table I) of **11e**, the lowfield shifts of the signals due to H-2 ( $\delta$  5.65), H-3 ( $\delta$  5.29) and H-5 ( $\delta$  5.02) indicated the locations of the flavogallonyl ester groups to be at these positions.

On the other hand, **11f** showed the same ( $M-H$ )<sup>-</sup> peak at  $m/z$  949 as that of **11**. The  $^1\text{H}$ -NMR spectrum (Table I) of **11f** exhibited four one-proton singlets, among which the chemical shifts of two signals ( $\delta$  7.38 and 7.61) in the lower region were similar to those of **11d** ( $\delta$  7.32 and 7.60), suggesting that the flavogallonyl group in **11f** forms dilactone rings. Furthermore, the chemical shifts and coupling patterns of the aliphatic signals were found to be in good agreement with those of lagerstannin C (**8**). Considering the co-generation of **11e** on hydrolysis, the structure of **11f** was regarded to be 5-*O*-flavogallonyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-D-gluconic acid.

The chirality of the biphenyl bond in the flavogallonyl moiety of **11e** was determined to be in the *R*-series by yielding methyl (*R*)-heptamethylflavogallonate (**11g**) [ $[\alpha]_{\text{D}}^{25} +17.7^\circ$  ( $\text{CHCl}_3$ )],<sup>15</sup> together with **11b**, on methylation, followed by alkaline methanolysis. Coupled with the above-mentioned results of  $^1\text{H}$ - $^{13}\text{C}$  long-range COSY spectral analysis of **11a**, this fact indicated that, in the molecule of **11**, the atropisomerism of the biphenyl bond between aromatic rings attached to the C-3 and C-5 positions is in the *R*-series. On the basis of these results, the structure of lagerstannin B was determined to be as

shown by the formula 11.

It is interesting to note that prolonged acid hydrolysis of 11 afforded another hydrolysate (11h), which was found to be generated by acyl migration of the flavogallonyl group and formation of a glucono-1,5-lactone ring. Characterization of 11h is as follows. The negative ion FAB-MS exhibited the  $(M-11)^-$  peak at  $m/z$  931 which is eighteen mass units less than that found in the case of 11f. On methylation, followed by alkaline methanolysis similar to the case of 11f, 11h afforded 11b and 11g. In the  $^1\text{H-NMR}$  spectrum (Table I), the coupling patterns of the signals due to the gluconic acid moiety were significantly different from those of 11f, and the lowfield shifts of the signals due to H-3 ( $\delta$  5.67), H-4 ( $\delta$  4.72), H-5 ( $\delta$  5.15) and H-6 ( $\delta$  4.50 and 4.39) indicated that these positions are acylated. Furthermore, the fairly upfield shift of the C-6 carbon signal ( $\delta$  58.4) as compared with that ( $\delta$  65.7) of 11 was consistent with a glucono-1,5-lactone structure [1,5-lactone,  $\delta$  60.8 (C-6); 1,4-lactone,  $\delta$  62.4; acid form,  $\delta$  63.6].<sup>16)</sup> These findings suggested that acyl migration of the flavogallonyl ester group from the C-5 to C-3 position occurred during hydrolysis. Thus, the structure of 11h was concluded to be 3-*O*-(*R*)-flavogallonyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-D-glucono-1,5-lactone.

Ellagitannins possessing a gluconic acid core have been rarely found in the plant kingdom, and so far isolated from *Punica granatum* (Punicaceae),<sup>4)</sup> *Hippophae rhamnoides* (Elaeagnaceae)<sup>21)</sup> and *Lagerstroemia subcostata*.<sup>17)</sup> In the case of *L. speciosa*, it is interesting from the viewpoint of the metabolism of hydrolyzable tannins that these tannins comprise a major group of the tannins in the fruit and co-exist with structurally related C-glycosidic ellagitannins such as 3 and 4.

#### Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FAB-MS were taken with a JEOL JMS-DX 300 instrument.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a JEOL FX-100 and JEOL GX-270 spectrometers, with tetramethylsilane as an internal standard, and chemical shifts are given in  $\delta$  (ppm). Column chromatography was performed with Sephadex LH-20 (25–100  $\mu$ , Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75–150  $\mu$ , Mitsubishi Chemical Industries, Ltd.), Cosmosil 75 C<sub>18</sub>-OPN (75  $\mu$ , Nacal Tesque Inc.), Fuji-gel ODS G-3 (43–65  $\mu$ , Fuji-gel Houbai Co., Ltd.), and Toyopearl HW-40F (Tosoh Corp.). Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F<sub>254</sub> plates (0.2 mm thick, Merck) with benzene-ethyl formate formic acid (1:7:1) and precoated cellulose F<sub>254</sub> plates (0.1 mm thick, Merck) with 2% acetic acid, and spots were detected by ultraviolet (UV) illumination and by spraying 1% ethanolic ferric chloride, sodium nitrite acetic acid or 5% sulfuric acid reagents. Optical resolution high performance liquid chromatography (HPLC) was performed on a Tosoh apparatus equipped with a CCPM solvent delivery system, a UV-8000 spectrometer and a Chiralcol OD (Daicel Chemical Industries, Ltd.) column (4.6 mm i.d.  $\times$  250 mm) [mobile phase, *n*-hexane-2-propanol (6:4); flow rate, 1.5 mL/min; column temperature, 24  $^\circ\text{C}$ ; detection, 280 nm].

**Isolation of Tannins. 1) From Fruits** The fresh fruits (6.0 kg), collected in Taiwan in September, were crushed into small pieces and extracted five times with 80% aqueous acetone at room temperature. The combined extracts were concentrated under reduced pressure, and the resulting precipitates were removed by filtration. The filtrate was applied to a Sephadex LH-20 column (10.5 cm i.d.  $\times$  35 cm) with water containing increasing proportions of methanol and finally with 50% aqueous acetone to give four fractions; Ia (3.7 g), Ib (10.0 g), Ic (9.5 g) and Id (53.7 g). The first fraction was rechromatographed over MCI-gel CHP 20P (4.0 cm i.d.  $\times$  30 cm), Cosmosil 75 C<sub>18</sub>-OPN (3.0 cm i.d.  $\times$  30 cm) and Sephadex

LH-20 (3.0 cm i.d.  $\times$  50 cm) with a mixture of water and methanol to afford lagerstannins A (6) (66 mg) and B (11) (1.28 g). Repeated chromatography of the second fraction on MCI-gel CHP 20P, Fuji-gel ODS G-3 and Sephadex LH-20 with water containing increasing amounts of methanol furnished vesicagin (4) (98 mg) and grandinin (5) (338 mg). The third fraction was rechromatographed on MCI-gel CHP 20P (4.0 cm i.d.  $\times$  30 cm) and then Toyopearl HW-40F (4.0 cm i.d.  $\times$  25 cm) with water containing increasing proportions of methanol to yield gemin D (7) (67 mg) and castalagin (3) (1.74 g). On similar chromatographic separation, final fraction afforded lagerstannin (2) (404 mg) and hippophaenin A (7) (343 mg).

**2) From Leaves** Fraction 1, which was previously obtained from the 70% aqueous acetone extract of the dried leaves by Sephadex LH-20 chromatography,<sup>14)</sup> was rechromatographed on Sephadex LH-20 with ethanol and then MCI-gel CHP 20P with water containing an increasing amount of methanol to yield 4,6-*O*-(*S*)-hexahydroxydiphenoyl gluconic acid (9) (20 mg). Repeated chromatography of fraction 2<sup>14)</sup> on MCI-gel CHP 20P, Cosmosil 75 C<sub>18</sub>-OPN, Toyopearl HW-40F and Sephadex LH-20 with water and methanol furnished lagerstannin C (8) (40 mg) and 7 (100 mg).

**Lagerstannin A (6)** A tan amorphous powder,  $[\alpha]_D^{25}$  +76.3 $^\circ$  ( $c=0.5$ , MeOH-H<sub>2</sub>O (5:3)). *Anal.* Calcd for C<sub>32</sub>H<sub>34</sub>O<sub>12</sub>·1/2H<sub>2</sub>O: C, 50.44; H, 3.11. Found: C, 50.18; H, 3.06. Negative ion FAB-MS  $m/z$ : 799 ( $M-11$ ).  $^1\text{H-NMR}$ : Table I.  $^{13}\text{C-NMR}$  (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 67.8 MHz): 68.1 [Gluconic acid (GlcA)-6], 69.0, 73.4, 76.1, 76.6 (GlcA-2, 3, 4, 5), 107.3, 107.5, 107.7, 108.6 [hexahydroxydiphenoyl (HHDp)-3, 3'], 113.7, 114.2, 115.2, 116.1 (HHDp-1, 1'), 125.5, 126.7, 126.9, 127.1 (HHDp-2, 2'), 135.9 (2C), 136.2, 136.7 (HHDp-5, 5'), 144.2, 144.4, 144.5, 144.6, 145.2, 145.3 (3C) (HHDp-4, 4', 6, 6'), 168.6, 169.4, 169.7, 170.0 (HHDp-7, 7'), 170.8 (GlcA-1).

**LIAH<sub>4</sub> Reduction of 6** A solution of 6 (25 mg) in tetrahydrofuran (2 ml) was stirred with LiAlH<sub>4</sub> (50 mg) at room temperature for 2 h. To the reaction mixture, water (1 ml) and 0.5 N sulfuric acid (5 ml) was slowly added at 0  $^\circ\text{C}$ , and the solution was chromatographed on Sephadex LH-20 (1.0 cm i.d.  $\times$  15 cm) with water containing increasing proportions of methanol. The fractions obtained by elution with 50–60% methanol were collected and concentrated to dryness. The residue was applied to a column of MCI-gel CHP 20P (1.0 cm i.d.  $\times$  5 cm) with 10% aqueous methanol to yield 6a (3 mg) as a white amorphous powder,  $[\alpha]_D^{25}$  +177.5 $^\circ$  ( $c=0.3$ , MeOH). *Anal.* Calcd for C<sub>32</sub>H<sub>34</sub>O<sub>12</sub>·1/2H<sub>2</sub>O: C, 51.33; H, 3.42. Found: C, 51.03; H, 3.42.  $^1\text{H-NMR}$  (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 100 MHz): 3.89–4.02 (3H, m, H-1, 6), 4.29 (1H, dd,  $J=3, 9$  Hz, H-5), 4.71 (1H, dd,  $J=3, 12$  Hz, H-6), 5.08 (1H, m, H-2), 5.22 (1H, dd,  $J=1, 9$  Hz, H-4), 5.60 (1H, dd,  $J=1, 10$  Hz, H-3), 6.55, 6.67, 6.88, 6.69 (each 1H, s, HHDp-H).  $^{13}\text{C-NMR}$  (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 25.05 MHz): 60.4 (C-1), 68.2 (C-6), 68.8, 72.7, 74.9, 77.1 (C-2, 3, 4, 5), 106.9, 107.5, 107.8, 108.3 (HHDp-3, 3'), 113.8, 114.1, 115.0, 116.5 (HHDp-1, 1'), 125.1, 127.1, 127.4 (2C) (HHDp-2, 2'), 135.7, 135.9, 136.1, 136.9 (HHDp-5, 5'), 144.1, 144.3, 144.4, 144.7, 145.1, 145.3 (3C) (HHDp-4, 4', 6, 6'), 168.3, 169.3, 169.4, 169.7 (HHDp-7, 7').

**NaBH<sub>4</sub> Reduction of Pedunculagin** A solution of pedunculagin (400 mg) in water (20 ml) was stirred with NaBH<sub>4</sub> (400 mg) at room temperature for 24 h. The reaction mixture was acidified with 2 N HCl and directly subjected to Sephadex LH-20 column chromatography (3.0 cm i.d.  $\times$  20 cm) with water containing increasing proportions of methanol. Elution of 50% methanol afforded 6a (204 mg).

**Hippophaenin A (7)** A white amorphous powder,  $[\alpha]_D^{25}$  +62.2 $^\circ$  ( $c=0.4$ , MeOH-H<sub>2</sub>O (1:1)). Negative ion FAB-MS  $m/z$ : 931 ( $M-11$ ).  $^1\text{H-NMR}$ : Table I.  $^{13}\text{C-NMR}$  (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 67.8 MHz): 64.6 (GlcA-6), 70.5, 71.9, 75.4, 75.7 (GlcA-2, 3, 4, 5), 110.1 (galloyl-2, 6), 113.5, 114.2, 115.5, 116.1 (HHDp-1, 1'), 120.4 (galloyl-1), 125.4, 126.5, 126.8, 127.0 (HHDp-2, 2'), 135.7, 136.2 (2C), 136.8 (HHDp-5, 5'), 139.5 (galloyl-4), 144.2, 144.5, 144.8, 145.2 (3C), 145.3, 145.4, 146.1 (2C) (HHDp-4, 4', 6, 6', galloyl-3, 5), 167.1, 168.8, 169.4, 169.5, 169.7 (HHDp-7, 7', galloyl-7), 171.1 (GlcA-1).

**Enzymatic Hydrolysis of 7 with Tannase** A solution of 7 (50 mg) in water (3 ml) was incubated with tannase (20 mg) at room temperature for 7 h. The reaction mixture was chromatographed on MCI-gel CHP 20P (2.0 cm i.d.  $\times$  25 cm) with water containing increasing proportions of methanol to give gallic acid (4 mg) and 6 (20 mg).

**Lagerstannin C (8)** A white amorphous powder,  $[\alpha]_D^{25}$  +125.0 $^\circ$  ( $c=0.4$ , MeOH). *Anal.* Calcd for C<sub>31</sub>H<sub>32</sub>O<sub>12</sub>: C, 49.86; H, 3.41. Found: C, 49.36; H, 3.40. Negative ion FAB-MS  $m/z$ : 649 ( $M-11$ ).  $^1\text{H-NMR}$ : Table I.  $^{13}\text{C-NMR}$  (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 25.15 MHz): 64.9 (GlcA-6), 71.1, 71.7, 72.4, 73.1 (GlcA-2, 3, 4, 5), 107.5, 109.0 (HHDp-3, 3'), 110.1 (galloyl-3, 6), 115.4, 116.0 (HHDp-1, 1'), 120.9 (galloyl-1), 126.3, 127.1 (HHDp-2, 2'), 136.0, 136.5 (HHDp-5, 5'), 139.4 (galloyl-4), 143.3, 144.3, 145.3 (2C)

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(HHDP-4, 4', 6, 6'), 146.1 (galloyl-3, 5), 166.4, 167.9, 169.1 (COO), 174.9 (GlcA-1).

**Enzymatic Hydrolysis of 8 with Tannase** A solution of 8 (20 mg) in water (2 ml) was incubated with tannase (10 mg) at room temperature for 3 h. The reaction mixture was chromatographed as described for 7 to give gallic acid (2 mg) and 9 (10 mg), as a white amorphous powder,  $[\alpha]_D^{25} +150.8^\circ$  ( $c=0.6$ , MeOH). FAB-MS  $m/z$ : 521 (M+Na)<sup>+</sup>, 499 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR: Table I.

**Punigluconin (10)** A tan amorphous powder,  $[\alpha]_D^{25} +45.5^\circ$  ( $c=0.7$ , MeOH). Anal. Calcd for C<sub>24</sub>H<sub>32</sub>O<sub>13</sub>·2H<sub>2</sub>O: C, 48.70; H, 3.61. Found: C, 48.32; H, 3.60. <sup>1</sup>H-NMR: Table I. <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 25.05 MHz): 64.9 (GlcA-6), 70.7, 71.5, 73.0, 73.8 (GlcA-2, 3, 4, 5), 107.4, 109.3 (HHDP-3, 3'), 110.2, 110.7 (galloyl-2, 6), 115.0, 116.0 (HHDP-1, 1'), 120.9 (2C) (galloyl-1), 125.8, 127.2 (HHDP-2, 2'), 135.8, 136.7 (HHDP-5, 5'), 139.2 (2C) (galloyl-4), 144.3, 145.2, 145.8, 146.0 (HHDP-4, 4', 6, 6', galloyl-3, 5), 165.9, 166.2, 167.6, 169.0, 169.9 (COO, GlcA-1).

**Lagerstanin B (11)** A tan amorphous powder,  $[\alpha]_D^{25} +19.3^\circ$  ( $c=0.6$ , MeOH-1), 0 (2:1). Anal. Calcd for C<sub>41</sub>H<sub>54</sub>O<sub>22</sub>·11.5H<sub>2</sub>O: C, 50.84; H, 2.92. Found: C, 50.60; H, 2.93. Negative ion FAB-MS  $m/z$ : 949 (M-H)<sup>-</sup>, 451, 425, 301. <sup>1</sup>H-NMR: Table I. <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 25.05 MHz): 65.7 (GlcA-6), 70.3, 71.3, 72.5, 73.1 (GlcA-2, 3, 4, 5), 106.0, 107.1, 107.9, 108.6 (HHDP-3, 3', flavogalloyl (FLV)-3, 3'), 112.0, 112.1, 112.4, 112.7, 114.5, 116.2 (HHDP-1, 1', FLV-1, 1', 1', 3'), 124.7, 125.3, 126.0, 127.2, 127.5 (HHDP-2, 2', FLV-2, 2', 2'), 135.4, 135.6, 135.7, 136.3, 136.9 (HHDP-5, 5', FLV-5, 5', 5'), 144.6, 145.0, 145.1, 145.4, 146.0 (HHDP-4, 4', 6, 6', FLV-4, 4', 4', 6, 6, 6'), 165.9, 166.4, 167.3, 168.0 (2C), 168.8 (COO, GlcA-1).

**Methylation of 11** A mixture of 11 (56 mg), dimethyl sulfate (2 ml) was anhydrous potassium carbonate (2.0 g) in dry acetone (20 ml) was heated under reflux for 3 h. The inorganic compounds were removed by filtration, and the filtrate, after concentration, was applied to a silica gel column. Elution with benzene-acetone (23:2) yielded the hexadecamethyl ether (11a) (38.3 mg) as colorless needles from methanol, mp 289–290 °C.  $[\alpha]_D^{25} -16.3^\circ$  ( $c=0.6$ , CHCl<sub>3</sub>). Anal. Calcd for C<sub>57</sub>H<sub>72</sub>O<sub>22</sub>: C, 58.26; H, 4.97. Found: C, 57.73; H, 4.97. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 270 MHz): 3.52, 3.55, 3.66, 3.73, 3.74, 3.75, 3.77, 3.89, 3.90, 3.92 (×2), 3.94, 3.95, 3.97, 3.98, 4.02 (48H in total, each s, OCH<sub>3</sub>), 4.18 (1H, d, *J*=13 Hz, H-6), 4.82 (1H, dd, *J*=3, 13 Hz, H-6), 5.43 (1H, dd, *J*=6, 8 Hz, H-4), 5.50 (1H, dd, *J*=6, 9 Hz, H-3), 5.70 (1H, dd, *J*=3, 8 Hz, H-5), 5.76 (1H, d, *J*=9 Hz, H-2), 6.74, 6.78, 6.89, 6.99 (each 1H, s, aromatic-H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 67.8 MHz): 53.3, 56.0, 56.1, 56.2, 56.4, 60.7, 60.8, 60.9, 61.0, 61.1, 61.2 (OCH<sub>3</sub>), 64.8 (GlcA-6), 69.7, 70.5, 72.6, 73.0 (GlcA-2, 3, 4, 5), 104.4, 105.0, 106.4, 106.8 [hexamethoxydiphenoyl (HMDP)-3, 3', nonamethylflavogalloyl (MFL)-3, 3'], 120.6, 121.5, 121.8, 122.2, 122.7, 123.1 (HMDP-1, 1', MFL-1, 1', 1', 3'), 126.4, 127.1, 127.6, 128.1, 128.7 (HMDP-2, 2', MFL-2, 2', 2'), 144.0, 144.3, 144.9, 145.0 (HMDP-5, 5', MFL-5, 5'), 147.1 (MFL-5), 152.5, 152.8, 152.9, 153.2, 153.3, 153.4 (HMDP-4, 4', 6, 6', MFL-4, 4', 4', 6, 6, 6'), 164.5 (MFL-7), 165.5 (HMDP-7), 166.1 (MFL-7), 166.4 (MFL-7'), 166.5 (GlcA-1), 167.8 (HMDP-7).

**Alkaline Methanolysis of 11a** A solution of 11a (20 mg) in methanol (0.5 ml) and 5% aqueous NaBH<sub>4</sub> (0.5 ml) was heated at 80 °C for 1 h. The reaction mixture was acidified with 10% HCl (5 ml) and extracted with ether. The organic layer was washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was treated with ethereal diazomethane for 1 h. After concentration, the products were separated by silica gel chromatography. Elution with benzene-acetone (24:1) yielded dimethyl hexamethoxydiphenate (11b) (9.1 mg) as a colorless syrup,  $[\alpha]_D^{25} -23.3^\circ$  ( $c=0.9$ , CHCl<sub>3</sub>) and trimethyl nonamethylflavogalloylate (11c) (5.3 mg) as a colorless syrup,  $[\alpha]_D^{25} 0^\circ$  ( $c=0.5$ , CHCl<sub>3</sub>). EI-MS  $m/z$ : 674 (M<sup>+</sup>), <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 100 MHz): 3.62, 3.66, 3.78, 3.90, 3.92, 3.96 (36H in total, OCH<sub>3</sub>), 7.24 (2H, s, aromatic-H).

**Partial Hydrolysis of 11** 1) A solution of 11 (150 mg) in 0.5 N sulfuric acid (6 ml) was heated under reflux for 1 h. The resulting yellow needles (18 mg), mp >300 °C, which were identified as ellagic acid by co-TLC, were collected by filtration. The filtrate was chromatographed on a Sephadex LH-20 column (3.0 cm i.d. × 30 cm) with water containing increasing proportions of methanol to afford 11d (7.5 mg), 11e (5.7 mg), 11f (23.4 mg) and the starting material (11) (12 mg). 11d: Flavogallic acid. A yellow powder,  $[\alpha]_D^{25} +20.3^\circ$  ( $c=0.7$ , MeOH). <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 100 MHz): 7.32, 7.60 (each 1H, s). 11e: A yellow amorphous powder,  $[\alpha]_D^{25} +7.1^\circ$  ( $c=0.4$ , MeOH) (H<sub>2</sub>O (7:3)). Anal. Calcd for C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>·3.5H<sub>2</sub>O: C, 48.01; H, 3.43. Found: C, 48.01; H, 3.33. Negative ion FAB-MS  $m/z$ : 602 (M-H)<sup>-</sup>. <sup>1</sup>H-NMR: Table I. 11f: A yellow amorphous powder,  $[\alpha]_D^{25} +15.7^\circ$  ( $c=0.7$ , MeOH). Anal. Calcd

for C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>·2H<sub>2</sub>O: C, 49.91; H, 3.06. Found: C, 49.85; H, 2.94. Negative ion FAB-MS  $m/z$ : 949 (M-H)<sup>-</sup>. <sup>1</sup>H-NMR: Table I.

2) A solution of 11 (200 mg) in 0.5 N sulfuric acid (10 ml) was refluxed for 3 h. The reaction mixture was separated by Sephadex LH-20 and MCI-gel CHP-20 column chromatographies (H<sub>2</sub>O-MeOH) to afford ellagic acid, 11e (10.9 mg), 11d (14.9 mg) and 11h (10.4 mg). 11h: A yellow amorphous powder,  $[\alpha]_D^{25} +60.8^\circ$  ( $c=0.3$ , MeOH) (H<sub>2</sub>O (7:3)). Anal. Calcd for C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>: C, 51.80; H, 2.76. Found: C, 51.60; H, 2.86. Negative ion FAB-MS  $m/z$ : 931 (M-H)<sup>-</sup>, 451, 301. <sup>1</sup>H-NMR: Table I. <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>+D<sub>2</sub>O, 67.8 MHz): 58.4 (GlcA-6), 66.9, 70.9, 75.7, 79.3 (GlcA-2, 3, 4, 5), 107.1, 108.8, 109.5, 111.0, 111.2, 111.3, 113.8, 114.1, 116.4, 116.9, 118.5, 120.1, 120.9, 125.2, 125.8, 136.8, 136.9, 137.5, 137.9, 138.7, 139.2, 139.8, 144.3, 144.9, 145.0, 145.1, 145.2, 145.4, 146.7, 148.6 (aromatic C), 158.8, 160.9 (FLV-7, 7'), 165.8, 167.2, 167.6 (HHDP-7, 7', FLV-7'), 174.3 (GlcA-1). CD ( $c=0.013$ , methanol) ( $\theta$ ) (nm): -5115 (325), +20751 (294), -30546 (268), +83965 (232), -84550 (207).

**Methylation of 11f, Followed by Alkaline Methanolysis** A solution of 11f (20 mg) in methanol (5 ml) was treated with ethereal diazomethane for 3 h. The reaction mixture was concentrated to dryness under reduced pressure, and the crude methylate thus obtained was kept with 0.5% methanolic sodium methoxide (5 ml) at room temperature for 21 h. The reaction mixture was neutralized with Amberlite IR 120B (H<sup>+</sup> form), and subjected to silica gel column chromatography. Elution with hexane-acetone (4:1 and then 3:1) furnished 11h (2.3 mg) and 11g (4.8 mg). 11g: a white powder,  $[\alpha]_D^{25} +17.7^\circ$  ( $c=0.6$ , CHCl<sub>3</sub>). Chiral resolution-HPLC analysis of 11g showed a peak at *t*<sub>R</sub> 8.5 min which corresponded to the R form. [S form]<sup>12</sup>: *t*<sub>R</sub> 30.5 min. The racemic form used as a standard sample was prepared by methylation of racemic flavogallic acid isolated from the leaves of *Mallotus philippinensis*<sup>10</sup>).

**Methylation of 11h, Followed by Alkaline Methanolysis** A solution of 11h (5 mg) in methanol (0.5 ml) was methylated with ethereal diazomethane for 24 h. The reaction mixture was concentrated to dryness by blowing nitrogen gas to give the methyl derivative, which was treated with 0.5% methanolic sodium methoxide (0.5 ml) at room temperature for 20 h. Work up as described for 11f yielded 11b (0.5 mg) and 11g (1.0 mg).

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#### References and Notes

- 1) T. Tanaka, N. Ishida, M. Ishimatsu, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **40**, 2092 (1992).
- 2) L. M. Perry, "Medicinal Plants of East and Southeast Asia," The MIT Press, Cambridge, 1980, p. 248; E. Quisumbing, "Medicinal Plants of the Philippines," Katha Publishing Co., Inc., Quezone, 1978, pp. 640–642.
- 3) a) Y.-M. Xu, T. Sakai, T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **39**, 639 (1991); b) Y.-M. Xu, T. Tanaka, G. Nonaka and I. Nishioka, *ibid.*, **39**, 647 (1991); c) T. Tanaka, M. Ageta, G. Nonaka and I. Nishioka, Abstracts of Papers, The 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, April 1988, p. 278.
- 4) T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 656 (1986).
- 5) T. Yoshida, Y. Maruyama, M. U. Memon, T. Shingu and T. Okuda, *Phytochemistry*, **24**, 1041 (1985).
- 6) W. Mayer, H. Seitz and J. C. Joehims, *Justus Liebig's Ann. Chem.*, **721**, 186 (1969).
- 7) W. Mayer, H. Seitz, J. C. Joehims, K. Schaefer and G. Schilling, *Justus Liebig's Ann. Chem.*, **751**, 60 (1971).
- 8) G. Nonaka, K. Ishimaru, R. Aozuma, M. Ishimatsu and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 2071 (1989).
- 9) T. Yoshida, K. Tanaka, X.-M. Chen and T. Okuda, *Phytochemistry*, **30**, 663 (1991).
- 10) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
- 11) G. T. Schmidt, L. Würtele and A. Harzous, *Justus Liebig's Ann. Chem.*, **690**, 150 (1965).
- 12) Formerly, the signals observed at  $\delta$  5.51 and 4.45 were erroneously assigned to 11-1 and 11-5, respectively.
- 13) Y. Ikeda, H. Taguechi, I. Yoshioka and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1283 (1979).

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14) G. Nonaka, K. Ishimaru, M. Watanabe, I. Nishioka, T. Yamauchi and A. S. C. Wu, *Chem. Pharm. Bull.*, **35**, 217 (1987).  
 15) T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 1039 (1986).  
 16) K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, **41**, 27

(1983).  
 17) Hippophenonin A (7) was isolated from the leaves. K. Ishimaru, unpublished data.  
 18) R. Saijo, G. Nonaka, I. Nishioka, I.-S. Chen and T.-H. Hwang, *Chem. Pharm. Bull.*, **37**, 2940 (1989).