Tannins of Rosaceous Medicinal Plants. V.¹⁾ Hydrolyzable Tannins with Dehydrodigalloyl Group from *Rosa laevigata* MICHX.

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Four new hydrolyzable tannins, laevigatins A—D, were isolated from *Rosa laevigata*, a Chinese medicinal plant. Among them, laevigatin B, laevigatin C and laevigatin D, were dimeric ellagitannins closely related to agrimoniin, which has a dehydrodigalloyl group in the molecule. Nine known tannins, including agrimoniin and agrimonic acids A and B, were also isolated. Structures of new tannins were assigned on the basis of proton and carbon-13 nuclear magnetic resonance spectral data, and chemical conversion of agrimonic acid A to laevigatin A, and of agrimoniin to laevigatins B and C.

Keywords *Rosa laevigata*; Rosaceae; tannin; ellagitannin; dimeric ellagitannin; laevigatin A; laevigatin B; laevigatin C; laevigatin D; agrimoniin

The fruits of *Rosa laevigata* MICHX., a Chinese medicinal plant, have been used as a diuretic, carminative and antitussive. They have also been used as a remedy for chronic diarrhea and intestinal inflammation.^{2,3)} The leaves of this plant have been used for the treatment of skin tumor, burn and ulcer in China. During a chromatographic survey of the distribution of oligomeric hydrolyzable tannins in the plants of Rosaceae, we found that *R. laevigata* contains a large quantity of agrimoniin, a dimeric ellagitannin which has been found only in certain species of the genera, *Agrimonia* and *Potentilla*.^{4,5)} We have now made a more detailed investigation on the polyphenolic constituents of this plant, and have isolated thirteen polyphenols including four new tannins, named laevigatins A (8), B (9), C (10), and D (11), as well as agrimoniin, the main component. We report here the structure elucidation of the new tannins which appear to be biogenetically correlated with each other.

An aqueous acetone homogenate of dried pericarps was extracted successively with ether, ethyl acetate and *n*-butanol. The ethyl acetate extract upon repeated chromatographic purification yielded eleven tannins, including laevigatins A (8)—D (11). Seven of them were identified as procyanidin B-3,⁶⁾ sanguiin H-4 (1),⁷⁾ pedunculagin (2),⁸⁾ potentillin (4),⁴⁾ agrimonic acids A (5) and B (6),⁴⁾ and a dimeric tannin, agrimoniin (7).⁴⁾ The ethyl acetate extract obtained from the aqueous acetone homogenate of the fresh leaves afforded laevigatin C (10), along with seven known compounds, (+)-catechin, procyanidin B-3, 2, casuarictin (3),⁸⁾ 4, 5, and 7.

All of the new tannins gave a characteristic colour (reddish brown→dark blue) of ellagitannins, upon spraying sodium nitrite reagent⁹⁾ on the thin-layer chromatography (TLC) plates.

Laevigatin A (8) was obtained as an off-white amorphous powder. Prominent peaks at m/z 825 ($M+Na$)⁺ and 801 ($M-H$)[−] were observed in its positive and negative fast atom bombardment mass spectra (FAB-MS), respectively, indicating the molecular formula, C₃₄H₂₆O₂₃. The proton nuclear magnetic resonance (¹H-NMR) spectrum exhibited typical signals for a dehydrodigalloyl (DHDG) group at δ 7.14 (s), 7.29 (d, $J=2$ Hz) and 6.88 (d, $J=2$ Hz), and a hexahydroxydiphenoyl (HHDP) group at δ 6.69 (s) and 6.41 (s). The chemical shifts and coupling pattern of the

aliphatic proton signals were clearly indicative of the ⁴C₁ glucopyranose core in which the hydroxyl groups at O-1, O-2 and O-3 are acylated (Table I). The mode of the glucosidic linkage in 8 was deduced to be α -form, from the small coupling constant ($J=4$ Hz) of the anomeric proton signal (δ 6.43). These spectral characteristics are very similar to those of the co-existing sanguiin H-4 (1), except for replacement of the galloyl group by a DHDG group (Table I). The carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum of 8 is also consistent with the structure 1-*O*-dehydrodigalloyl-2,3-*O*-hexahydroxydiphenoyl- α -D-glucose. In order to confirm the structure of 8 including the linkage mode of the DHDG group at the anomeric center, partial hydrolyses of agrimonic acid A (5) and its isomer, agrimonic acid B (6), in boiling water were performed, and a hydrolysate obtained from the former was confirmed to be identical with laevigatin A (8). Evidence supporting structures 5 and 6 for agrimonic acids A and B, which were determined mainly based on the mass fragmentation of their methylated derivatives,⁴⁾ has now emerged from the ¹H-¹³C long-range shift correlation spectrum. Namely, the anomeric proton signal (δ 6.56, d, $J=3.5$ Hz) of 5 was shown to be coupled with the carbonyl carbon (C-7', δ 165.1) signal, which is also correlated with the *meta*-coupled aromatic proton signals (δ 6.91 and 7.35, each d, $J=2$ Hz, H-2' and H-6') through three-bond coupling. Based on these data, the structure of laevigatin A is represented by 8.

Laevigatin B (9), [α]_D +2.2° (MeOH) and laevigatin C (10), [α]_D +32° (MeOH), were obtained as pale brown

TABLE I. ¹H-NMR Data for 1 and 8 (500 MHz, Acetone-*d*₆-D₂O)

	1	8
HHDP	6.69 (1H, s), 6.43 (1H, s)	6.69 (1H, s), 6.41 (1H, s)
Galloyl	7.19 (2H, s)	
DHDG		7.14 (1H, s), 7.29 (1H, d, $J=2$), 6.88 (1H, d, $J=2$)
Glucose H-1	6.50 (d, $J=3.5$)	6.43 (d, $J=4$)
H-2	5.15 (dd, $J=3.5, 9$)	5.13 (dd, $J=4, 10$)
H-3	5.45 (t, $J=9$)	5.44 (t, $J=10$)
H-4	3.99 (t, $J=9$)	3.98 (t, $J=10$)
H-5	3.89 (dt, $J=5, 9$)	3.85 (ddd, $J=2, 4, 10$)
H-6	3.79 (2H, m)	3.77—3.81 (2H, m)

Chart 1

TABLE II. ¹H-NMR Spectral Data for 5—7, 9 and 10 (500 MHz, Acetone-*d*₆). Values in Parenthesis are Coupling Constants in Hz)

	5	6	7	9 ^{a)}	10 ^{a)}
Glucose (a)					
H-1	6.56 d (3.5)		6.58 d (3.5)	6.43 d (4)	6.54 d (4)
H-2	5.38 dd (3.5, 9)		5.38 dd (3.5, 9.5)	5.12 dd (4, 10)	5.39 dd (4, 9)
H-3	5.57 dd (9, 10)		5.56 t (9.5)	5.42 t (10)	5.60 dd (9, 10)
H-4	5.21 t (10)		5.21 t (9.5)	3.97 t (10)	5.21 t (10)
H-5	4.65 dd (6, 10)		4.67 dd (6, 9.5)	3.87 dt (3, 10)	4.65 dd (6, 10)
H-6	5.30 dd (6, 13) 3.80 d (13)		5.32 dd (6, 13) 3.80 d (13)	3.77 d (3.2, 2H)	5.32 dd (6, 13) 3.81 d (6, 13)
Glucose (b)					
H-1		6.52 d (4)	6.55 d (3.5)	6.55 d (4)	6.49 d (4)
H-2		5.33 dd (4, 9)	5.36 dd (3.5, 9.5)	5.33 dd (4, 10)	5.11 dd (4, 10)
H-3		5.48 dd (9, 10)	5.46 t (9.5)	5.46 t (10)	5.36 t (10)
H-4		5.14 t (10)	5.16 t (9.5)	5.12 t (10)	3.99 t (10)
H-5		4.41 dd (6, 10)	4.50 dd (6, 9.5)	4.44 dd (7, 10)	3.40 dt (2, 10)
H-6		5.19 dd (6, 13) 3.66 d (13)	5.24 dd (6, 13) 3.69 d (13)	5.18 dd (7, 13) 3.67 d (13)	3.68 dd (2, 13) 3.55 dd (2, 13)

a) In acetone-*d*₆-D₂O.

amorphous powders, and were found to be dimeric ellagitannins of molecular formula C₆₈H₄₈O₄₄, as indicated by their FAB-MS spectra [*m/z* 1567 (M-H)⁻ and *m/z* 1591 (M+Na)⁺]. Their ¹H-NMR spectra were quite similar to each other and disclosed aromatic proton signals attributable to a DHDG group and three HHDP groups. The anomeric proton signals with small coupling constant (*J* = 4 Hz) were observed at δ 6.55 and 6.43 for **9**, and at δ 6.54 and 6.49 for **10**. This fact and the coupling pattern of the other sugar proton signals of both **9** and **10** indicated that these tannins contain ⁴C₁ glucopyranose cores in which the anomeric centers have α-oriented acyloxy groups. These

TABLE III. ¹³C-NMR Spectral Data for 5—7 and 9 (126 MHz, Acetone-*d*₆)

Carbon	5	6	7	9 ^{a)}	
			Gluc. (a) Gluc. (b)	Gluc. (a) Gluc. (b)	
C-1	90.6	90.5	90.7	90.6	91.0 90.8
C-2	73.8	74.1	73.8	74.0	73.6 74.2
C-3	75.7	75.9	75.6	75.7	78.0 75.9
C-4	69.0	68.9	68.9	68.8	67.5 68.9
C-5	70.8	71.3	70.7	71.2	76.3 71.3
C-6	63.1	63.1	63.1	63.0	61.4 63.2

a) In acetone-*d*₆-D₂O.

Chart 2

spectral characteristics are comparable to those of agrimoniin (**7**) except for the lack of the signals due to an HHDP group and marked upfield shifts of H-4 and H-6 of one of the two glucose cores (Table II). Based on these data, **9** and **10** were presumed to be isomers differing from each other only in the presence of the hydroxyl groups at C-4 and C-6 of either glucose (a) or glucose (b) in place of the HHDP group in agrimoniin. Prior to locating the hydroxyl groups on one of the glucoses in **9** and **10**, the sugar proton and carbon signals of agrimoniin were fully assigned by analysis of the ¹H-¹H and ¹H-¹³C shift correlation 2D spectra, and by differentiation of each glucose core based on a comparison with the signals of agrimonic acids A (**5**) and B (**6**)⁴⁾ (Tables II and III).

Each proton signal of glucose core (a) in **7** appears at

somewhat lower field than the corresponding signal of glucose core (b). Although the differences of chemical shifts between each of the H-1—H-4 signals in glucose core (a) and that in glucose core (b) are not large, it is evident that the chemical shift of H-5 is diagnostic for discrimination of each glucose core [δ 4.67 for core (a), and δ 4.50 for core (b)]. The H-5 signal of the fully acylated glucose core in **9** appears at δ 4.44, while that in **10** is at δ 4.65, which are almost identical with those of the glucose cores (b) and (a) of **7**, respectively. Based on these observations, structures **9** and **10** were assigned for laevigatins B and C, respectively. The ^{13}C -NMR spectra were also consistent with the proposed structures (Table III). Final confirmation of the structures, including the absolute configurations of the HHDP groups, was obtained by chemical transformation of **7** to **9** and **10** by partial hydrolysis with tannase.

Laevigatin D (**11**), an off-white amorphous powder, $[\alpha]_{\text{D}} + 11.2^\circ$ (MeOH), exists as an equilibrium mixture of α - and β -anomers, as revealed by two peaks in the reversed-phase high performance liquid chromatography (HPLC) which were replaced by a single peak of different retention time upon reduction with NaBH_4 .¹⁰⁾ The ^1H -NMR spectrum of **11** exhibited the characteristic signals of a DHDG group and three HHDP groups, all of which form dual peaks. Although the sugar proton signals are also complicated because of their partial duplication, the ^1H - ^1H shift correlation (COSY) spectrum led to unequivocal assignments (Table IV). The proton signals on the fully acylated glucose core (b) were in accord with those of the glucose core (b) of agrimoniin (**7**). A pair of triplets ($J=10$ Hz) due to H-3 of the alternative glucose residue (a) was observed at δ 4.17 and 3.86, higher field compared with that of glucose core (b), indicating the presence of a free hydroxyl group at C-3. The anomeric proton signals of glucose (a) were at δ 5.33 (d, $J=4$ Hz) and 4.53 (d, $J=8$ Hz).

In the ^{13}C -NMR spectrum of **11**, each carbon signal attributed to the glucose core (b) appears as a single peak in contrast with the dual peaks exhibited by each carbon of the glucose core (a). The chemical shifts of the former were again in good agreement with those of signals of the glucose core (b) of agrimoniin (**7**). As for the glucose core (a) in **11**, the ^{13}C resonances of C-3 are shifted upfield by 0.7 ppm (α -anomer) and 0.6 ppm (β -anomer) with respect to tellimagrandin I (**12**), whereas those of C-2 and C-4 show downfield shifts of 2.4–2.7 ppm and 2.2–2.3 ppm, respectively, from the corresponding signals in **12** (Table IV). These data obviously indicate that laevigatin D possesses a monomeric unit which lacks a galloyl group at C-3 of **12**, when the

acylation shifts in the ellagitannins recently examined¹¹⁾ are taken into account. The (*S*)-configurations of both HHDP groups in **11** were established by a strong positive Cotton effect at 237 nm and a negative one at 260 nm in the circular dichroism (CD) spectrum.¹²⁾ The structure (**11**) was thus assigned to laevigatin D, and was confirmed by the result of partial hydrolysis in hot water, yielding agrimoniin acid B (**6**).

Although there has been a question as to whether laevigatins B, C, and D are artefacts of agrimoniin produced during drying of the plant materials and/or extraction, this possibility rather seems to be ruled out by the following considerations. The tannin components including dimers, agrimoniin and laevigatin C, in the leaves were shown by HPLC (normal and reversed phases) to be essentially the same before and after drying. Since pericarps

TABLE IV. NMR Spectral Data for Glucose Moieties of **11** and **12** (Acetone- d_6 - D_2O . Values in Parenthesis are Coupling Constants in Hz)

	¹ H-NMR			¹³ C-NMR			
	11			11	12		
Gluc. (a)	α -Ano- mer	β -Ano- mer		α -Ano- mer	β -Ano- mer	α -Ano- mer	β -Ano- mer
H-1	5.33 d (4)	4.53 d (8)	C-1	91.0	96.6	91.2	96.7
H-2	4.78 dd (4, 10)	4.92 dd (8, 10)	C-2	75.3	76.8	72.9	74.1
H-3	4.17 t (10)	3.86 t (10)	C-3	70.4	72.9	71.1	73.5
H-4	4.86 t (10)	4.88 t (10)	C-4	73.3	73.4	71.1	71.1
H-5	4.43 ddd (2, 6, 10)	3.86 ddd (1, 4, 10)	C-5	67.2	72.2	67.2	72.0
H-6	5.12 dd (6, 12)	5.14 dd (4, 12)	C-6	63.8	63.9	63.5	63.5
	3.92 dd (2, 12)	3.76 br d (12)					
Gluc. (b)							
H-1		6.51 d (3.5)	C-1		90.6		
H-2		5.35 dd (3.5, 10)	C-2		73.9		
H-3	5.55 t (10)	5.53 t	C-3		75.7		
H-4		5.17 t (10)	C-4		69.0		
H-5	4.58 ddd (1, 6, 10)	4.59 ddd	C-5		70.8		
H-6	5.24 dd (6, 13)	5.25 dd	C-6		63.2		
		3.77 brs (13)

Chart 3

and leaves were dried in a similar way, and the isolation procedure of the tannins is also similar, laevigatins B—D should be obtainable from the extract of fresh leaves, if laevigatins B—D from the pericarps were artefacts produced from agrimoniin. Laevigatins B (9) and C (10) are thus regarded as the dimers biogenetically formed by an intermolecular C—O oxidative coupling between galloyl groups at C-1 of sanguini H-4 (1) and potentillin (4), or through metabolic degradations of agrimoniin (7).

Agrimoniin (7) and its analogues may be regarded as the active components that have led to the use of the leaves for the treatment of skin tumor and ulcer,²⁾ since 7 has recently been found to exhibit a potent antitumor activity against several kinds of tumors.¹³⁾

Experimental

The ¹H- and ¹³C-NMR spectra were measured on a Varian VXR 500 instrument (500 MHz for ¹H and 126 MHz for ¹³C) in the SC NMR Laboratory of Okayama University, and the chemical shifts are given in δ values downfield from tetramethylsilane. TLC was conducted on microcrystalline cellulose (Avicel SF, Funakoshi) plates developing with 7% HOAc and spots were visualized by spraying FeCl₃ or NaNO₂-HOAc reagents.⁹⁾ Column chromatography was performed using Toyopearl HW-40 (coarse and fine grades) (Tosoh), Diaion HP 20 and MCI gel CHP 20P (Mitsubishi Chemicals Industries). The solvent was evaporated off under reduced pressure at below 40 °C.

Isolation of Tannins Dried pericarps (800 g), collected in Chendgu, China, were homogenized in 70% aqueous acetone, and concentrated. The aqueous extract was extracted with ether, EtOAc and *n*-BuOH saturated with water, successively. A part (5 g) of the EtOAc extract (10 g) was chromatographed on a Toyopearl HW-40C column (2.2 cm i.d. $\times$ 40 cm) developing with a solvent system of EtOH-H₂O-acetone (7:3:0 $\rightarrow$ 6:3:1 $\rightarrow$ 5:3:2). The eluate with EtOH-H₂O (7:3) gave pedunculagin (2) and a mixture of sanguini H-4 (1), procyanidin B-3, and laevigatin A (8), which was separated into each component by column chromatography on MCI gel CHP 20P (1.1 cm i.d. $\times$ 15 cm) with H₂O containing increasing amount of MeOH. The eluate with EtOH-H₂O-acetone (6:3:1) was further subjected to column chromatography on Toyopearl HW-40F (2.2 cm i.d. $\times$ 28 cm, 70% aqueous EtOH) to give agrimonic acid A (5), agrimonic acid B (6), potentillin (4) and laevigatin B (9) eluted in that order. The eluate with EtOH-H₂O-acetone (5:3:2) gave agrimoniin (7). The fraction eluted before agrimoniin was chromatographed over Toyopearl HW-40F (1.1 cm i.d. $\times$ 30 cm) with a solvent system of EtOH-H₂O-acetone (7:3:0 $\rightarrow$ 6:3:1) to afford laevigatins C (10) and D (11).

The total yields of tannins from this chromatographic separation of EtOAc extract, carried out twice, were as follows: procyanidin B-3, 84 mg; sanguini H-4 (1), 103 mg; pedunculagin (2), 116 mg; potentillin (4), 11 mg; agrimonic acid A (5), 177 mg; agrimonic acid B (6), 18 mg; agrimoniin (7), 295 mg; laevigatin A (8), 34 mg; laevigatin B (9), 22 mg; laevigatin C (10), 42 mg; laevigatin D (11), 12 mg.

The fresh leaves (1 kg) of *R. laevigata* were extracted with 70% aqueous acetone (5 l). Concentration of the extract gave a dark brown residue (127 g), which was partitioned between H₂O (700 ml) and ether (300 ml). The aqueous layer was extracted with additional ether (300 ml $\times$ 3), EtOAc (600 ml $\times$ 5) and *n*-BuOH saturated with H₂O (400 ml $\times$ 5). The ether extract (4.5 g) was mostly composed of (+)-catechin. The EtOAc extract (16 g) was successively subjected to column chromatography over Toyopearl HW-40C, MCI gel CHP 20P, and Toyopearl HW-40F, with a solvent system of EtOH-H₂O-acetone, in a way similar to that described above for the EtOAc extract from the pericarps, to give (+)-catechin (1.44 g), procyanidin B-3 (269 mg), pedunculagin (2) (462 mg), casuarictin (3) (30 mg), potentillin (4) (201 mg), agrimonic acid A (5) (8 mg), agrimoniin (7) (1.56 g) and laevigatin C (10) (11 mg).

Laevigatin A (8) An off-white amorphous powder, $[\alpha]_D^{+61}$ ($c=0.5$, MeOH), TLC, *R*_f 0.54. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 223 (4.65), 263 (4.35). FAB-MS (+NaI) *m/z*: 825 (M+Na)⁺ (MW=802 for C₃₄H₂₆O₂₃). FAB-MS (+KI) *m/z*: 841 (M+K)⁺. FAB-MS (negative) *m/z*: 801 (M-H)⁻. ¹H-NMR (acetone-*d*₆-D₂O): see Table I. ¹³C-NMR (acetone-*d*₆-D₂O) δ : 165.1, 168.1, 168.9, 169.9 (ester carbonyl), 90.7 (glucose C-1), 73.6 (glucose C-2), 77.8 (glucose C-3), 67.0 (glucose C-4), 76.0 (glucose C-5), 60.8 (glucose C-6).

Preparation of Tannase Tannase was prepared according to a modification of Knudson's method¹⁴⁾ as follows. The mycelia of *Aspergillus niger* (strain 3010), maintained as a stock culture at 4 °C on agar medium adjusted pH 6.0 with 1 N NaOH [KNO₃ 1 g, KH₂PO₄ 0.5 g, MgSO₄·7H₂O 0.5 g, sucrose 10 g, and agar 2 g in 100 ml of H₂O], were firstly transferred to growth medium, and cultured at 25 °C in the dark for 1 week. The growth medium contained glucose 2 g, sodium glutamate 30 mg, DL-alanine 30 mg, L-aspartic acid 30 mg, NaNO₃ 0.2 g, KH₂PO₄ 0.1 g, KCl 50 mg, FeCl₃·6H₂O 1 mg, Arnon A₂ solution 0.1 ml, MgSO₄·7H₂O 25 mg, and agar 1.5 g in 100 ml of H₂O. For tannase induction, the mycelia were then inoculated into liquid medium (150 ml), which contained NaNO₃ (2 g), KH₂PO₄ (1 g), KCl (0.5 g), MgSO₄·7H₂O (0.5 g) and tannic acid (20 g) (sterilized separately by filtration) as a sole carbon source in H₂O (1000 ml), in 300 ml Erlenmeyer flasks, and cultured at 25 °C in the dark under agitation with a shaker for 3 d. Before spore production, the fungus mats were harvested by suction, washed with water, and homogenized in ice water. The homogenate was centrifuged at 15000 *g* for 10 min, and the supernatant thus obtained was used directly as crude tannase. It was stored at below -20 °C in a freezer. The enzymatic activity was confirmed by degalloylation of pentagalloylglucose, prior to use for degradation of tannins.

Conversion of Agrimonic Acid A (5) to Laevigatin A (8) A solution of 5 (50 mg) in H₂O (5 ml) was treated with tannase at 37 °C for 49 h. After addition of EtOH, the reaction mixture was evaporated to dryness. The residue was redissolved in H₂O containing a few drops of 1% HCl, and the insoluble material was removed by centrifugation. The supernatant was subjected to chromatography on a Diaion HP-20 column eluting with H₂O and MeOH. The MeOH eluate was further purified by column chromatography over MCI gel CHP 20P, with H₂O containing increasing amount of MeOH, to afford the partial hydrolysate, which was identical with laevigatin A (HPLC, TLC, ¹H-NMR).

Laevigatin B (9) A pale brown amorphous powder. $[\alpha]_D^{+2.2}$ ($c=0.2$, MeOH). TLC, *R*_f 0.50. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 225 (5.00), 255 (sh), (4.75). FAB-MS (+NaI) *m/z*: 1591 (M+Na)⁺ (MW=1568 for C₆₈H₄₈O₄₄). FAB-MS (negative) *m/z*: 1567 (M-H)⁻. ¹H-NMR (acetone-*d*₆-D₂O) δ : 6.68, 6.62, 6.59, 6.55, 6.40, 6.33 (1H each, s) (HHDP), 7.22 (1H, s), 7.33, 6.95 (1H each, d, *J*=2 Hz) (DHDG), glucose protons, see Table II. ¹³C-NMR (acetone-*d*₆-D₂O) δ : 163.2, 165.1, 167.9, 168.3 ($\times$ 2C), 168.7, 169.4, 169.7 (ester carbonyl), glucose carbon, see Table III.

Laevigatin C (10) A pale brown amorphous powder. $[\alpha]_D^{+32}$ ($c=0.5$, MeOH). TLC, *R*_f 0.42. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 205 (5.06), 216 (sh), (5.02), 257 (sh), (4.64). FAB-MS (+NaI) *m/z*: 1591 (M+Na)⁺ (MW=1568 for C₆₈H₄₈O₄₄). FAB-MS (negative) *m/z*: 1567 (M-H)⁻. ¹H-NMR (acetone-*d*₆-D₂O) δ : 6.70, 6.66, 6.62, 6.53, 6.47, 6.36 (1H each, s) (HHDP), 7.26 (1H, s), 7.38, 6.91 (1H each, d, *J*=2 Hz) (DHDG), glucose protons, see Table II.

Partial Hydrolysis of Agrimoniin (7) A solution of 7 (150 mg) in H₂O (10 ml) was treated with tannase at 37 °C for 59 h. The reaction mixture was evaporated to dryness, and the residue was treated with EtOH. The EtOH-soluble portion was chromatographed over Toyopearl HW-40C (1.1 cm i.d. $\times$ 12 cm, 50% EtOH) to give laevigatin B (9) (25 mg) and laevigatin C (10) (5 mg).

Laevigatin D (11) A pale brown amorphous powder. $[\alpha]_D^{+11.2}$ ($c=0.5$, MeOH). TLC, *R*_f 0.40. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 206 (5.06), 220 (sh) (5.0), 260 (4.06). FAB-MS *m/z*: 1569 (M+H)⁺ (MW=1568 for C₆₈H₄₈O₄₄). FAB-MS (negative) *m/z*: 1567 (M-H)⁻. CD ($c=0.01$, MeOH), $[\theta] \times 10^{-4}$ (nm): +16.46 (237), -7.45 (260), +4.04 (281), -2.17 (313). ¹H-NMR (acetone-*d*₆-D₂O) δ : 6.35 (1H, s), 6.42, 6.43 (1H in total), 6.59, 6.58 (1H in total), 6.62, 6.61 (1H in total), 6.64 (1H, s), 6.71, 6.68 (1H in total) (3 $\times$ HHDP), 7.22, 7.20 (1H in total, s), 7.35, 7.34 (1H in total, d, *J*=2 Hz), 6.85, 6.83 (1H in total, d, *J*=2 Hz) (DHDG), glucose protons, see Table IV. ¹³C-NMR (acetone-*d*₆-D₂O) δ : 169.4 (1C), 168.6, 168.65 (1C in total), 168.4 (1C), 168.3 (1C), 168.2 (1C), 167.9 (1C), 164.9 (1C), 164.7 (1C), glucose carbons, see Table IV.

Partial Hydrolysis of 11 An aqueous solution (1 ml) of 11 (1 mg) was partially hydrolyzed in a boiling water bath, and the reaction process was monitored by reversed-phase HPLC. After 40 min, two peaks of 11 (retention time, 5.7 and 8 min) were almost completely replaced by several newly formed peaks, among which the main peak (retention time, 6.5 min) was shown to be identical with that of agrimonic acid B (6) by co-chromatography with an authentic specimen. Agrimonic acid A (5) (retention time, 8.2 min) was not detected in the reaction mixture.

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