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Tannins of Rosaceous Medicinal Plants. I. Structures of Potentillin, Agrimonic Acids A and B, and Agrimoniin, a Dimeric Ellagitannin

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Four new ellagitannins, potentillin (**1**), agrimoniin (**2**), and agrimonic acids A (**3**) and B (**4**), have been isolated from the roots of *Agrimonia japonica* (MIQ.) KOIDZ., and their structures, with an α-glucosidic linkage, were elucidated on the basis of spectral and chemical evidence. Potentillin (**1**) and agrimoniin (**2**) have also been isolated from *Potentilla kleiniana* WIGHT *et* ARNOTT.

Keywords——*Agrimonia japonica*; *Potentilla kleiniana*; Rosaceae; tannin; hydrolyzable tannin; dimeric ellagitannin; potentillin; agrimoniin; agrimonic acid A; agrimonic acid B

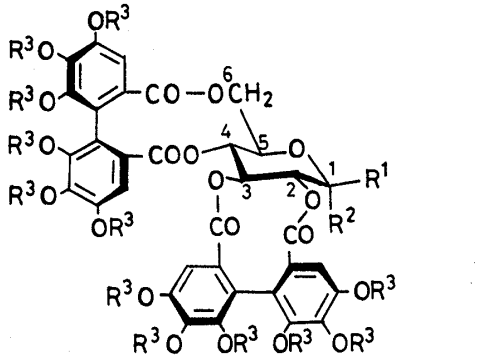
A number of plant species of Rosaceae are used as folk medicines for a variety of diseases in diverse areas of the world. Among them, *Agrimonia japonica* (MIQ.) KOIDZ. (Japanese name: kinmizuhiki), which is rich in tannin, has been used as an antidiarrheic and a hemostatic in Japan and China.[1)] With regard to chemical constituents of this plant, the occurrence of agrimonolide, vanillic acid, ellagic acid and *l*-taxifolin in the roots,[2)] and of apigenin-7-β-D-glucoside, luteolin-7-β-D-glucoside,[3)] and agrimols A-E[4)] in the leaves were previously reported, but no isolation and structural studies of tannins have yet been reported. We have examined the tannins of the roots of this plant, and have isolated four new ellagitannins named potentillin (**1**), agrimoniin (**2**), and agrimonic acid A (**3**) and B (**4**), along with pedunculagin (**5**)[5)] and casuarinin (**6**).[5)] Amongst them, **1**, **2** and **5** have also been isolated from *Potentilla kleiniana* WIGHT *et* ARNOTT (Japanese name: Ohebi-ichigo), which is also used similarly as a folk medicine in Japan. In a preliminary communication[6)] we reported the structures of potentillin (**1**) and agrimoniin (**2**); these compounds have an α-glucose core, which is rather unusual in nature, and the latter tannin was the first example of a dimeric ellagitannin to be fully characterized. This paper presents the detail of the isolation and structural elucidation of these new tannins.

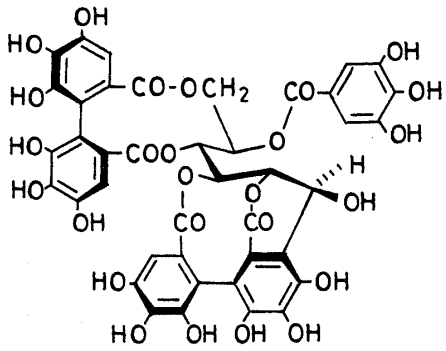
An aqueous acetone homogenate of the roots of *A. japonica* was extracted with ether and then with ethyl acetate. The ethyl acetate extract was separated by Sephadex LH-20 column chromatography into two fractions: one rich in condensed tannins and one rich in hydrolyzable tannins. The latter fraction was further subjected to droplet counter-current chromatography as described in the experimental section to yield six tannins, **1**—**6**. The tannins from *Potentilla kleiniana* was isolated in the same way as those from *A. japonica*.

Potentillin (**1**), an off-white amorphous powder, $C_{41}H_{28}O_{26}\cdot 5H_2O$, $[\alpha]_D$ +108°, showed the characteristic color of ellagitannin with the $NaNO_2$–AcOH reagent[7)] on a thin-layer chromatogram. Its ^1H nuclear magnetic resonance (NMR) spectrum (200 MHz, acetone-d_6) shows resonances at δ 6.68, 6.59, 6.48 and 6.38 (all 1H singlets), assignable to two hexahydroxydiphenoyl groups. The presence of a galloyl group was also indicated by a singlet

at δ 7.24 (2H). These peaks and the signals of the sugar protons are very similar to those of casuarictin (**7**) which was recently isolated from *Casuarina stricta*[5] and *Stachyurus praecox*,[8] except for the chemical shifts and coupling constants of the anomeric proton signals [δ 6.63 (d, J=4 Hz) for **1** and δ 6.22 (d, J=9 Hz) for **7**].

The presence of the structural units, gallic acid, hexahydroxydiphenic acid and glucose, in **1** was confirmed by the formation of methyl tri-*O*-methylgallate, dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (**8**), $[\alpha]_D$ −37° (EtOH), and glucose upon methanolysis of pentadeca-*O*-methylpotentillin (**9**), M^+ *m/z* 1146, which was prepared by methylation with dimethyl sulfate. These observations and a comparison of the ^{13}C-NMR spectrum of **1** with that of **7** (Table I) indicate that **1** is an α-isomer of **7**. This assumption was confirmed by selective hydrolysis of **1** with tannase, which yielded pedunculagin (**5**), 2,3-*O*-(4,4′,5,5′-6,6′-hexahydroxydiphenoyl)-D-glucose[9] and gallic acid. Thus, the structure of potentillin was determined as 1-*O*-galloyl-2,3-*O*,4,6-*O*-bis((*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl)-α-D-glucopyranose (**1**).





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1 : $R^1 = R^3 = H$, R^2 = -OCO-(hexahydroxydiphenoyl)

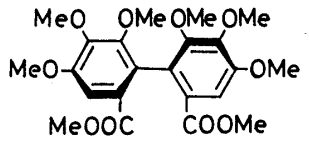
5 : R^1, R^2 = H, OH R^3 = H

7 : R^1 = -OCO-(hexahydroxydiphenoyl) , $R^2 = R^3 = H$

9 : R^1 = H, R^2 = -OCO-(hexamethoxydiphenoyl) R^3 = Me

12 : R^1 = H, R^2 = OMe , R^3 = Me

13 : R^1 = OMe, R^2 = H, R^3 = Me



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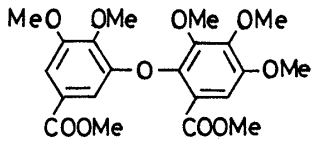

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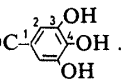
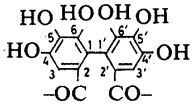
Chart 1

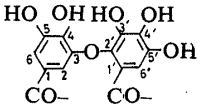
Agrimoniin (**2**), $C_{82}H_{54}O_{52} \cdot 13H_2O$, $[\alpha]_D$ +162°, was obtained as an off-white amorphous powder, and showed a positive $FeCl_3$ reaction and the characteristic blue color of ellagitannin with the $NaNO_2$–AcOH reagent. Its homogeneity was confirmed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (normal and reversed phases). Methylation of **2** with diazomethane afforded nonacosa-*O*-methylagrimoniin (**10**), which was subjected to methanolysis to give **8** and dimethyl penta-*O*-methyldehydrodigallate (**11**), mp 113—114 °C.[10] Gas chromatographic analysis revealed that the

TABLE I. ^{13}C Chemical Shifts of **1**—**3** and **7**

Carbon	7[d]	1[d]	2[e]	3[e]
Ester C=O	170.6	170.8	170.6, 170.4	171.7
	169.8	169.6 (2C)	169.8 (3C)	170.5 (2C)
	169.4		169.6	169.9, 169.5
	169.0	168.9	169.1, 169.0	166.8
	166.1	165.9	165.6, 165.4	
Gall[a]				
C-1	119.5	119.8		
2	110.2 (2C)	110.2 (2C)		
3	146.5 (2C)	146.5 (2C)		
4	140.6	140.6		
HHDP[b]				
C-1,1′	120.0, 116.3	116.1 (2C)	116.6, 116.5, 116.2, 115.0	117.2 (2C), 116.5
	115.3, 114.6	115.1, 114.7	115.6, 115.5, 115.2 (2C)	115.8
2,2′	126.6 (4C)	125.9 (4C)	126.8, 126.5 (2C), 126.3	127.1 (2C)
			126.2 (2C), 120.6, 125.7	126.9 (2C)
3,3′	110.6, 108.6	108.3	109.0 (2C), 108.8 (2C)	109.3 (2C)
	107.7 (2C)	107.4 (3C)	108.5 (2C), 108.8 (2C)	108.7, 108.6
4,4′, 6,6′	145.7 (5C)	145.7 (5C)	145.9 (7C), 145.8 (5C)	146.7 (6C)
	144.9 (3C)	144.6 (3C)	145.0, 144.8 (3C)	145.8 (2C)
5,5′	137.3 (4C)	137.3 (4C)	137.8 (2C), 137.7 (2C)	138.3 (4C)
			137.6 (2C), 137.5 (2C)	
DHDG[c]				
C-1 (1′)			120.2 (116.9)	120.6 (117.4)
2 (2′)			112.7 (137.3)	113.2 (138.3)
3 (3′)			148.4 (141.3)	149.8 (142.6)
4 (4′)			142.3 (140.4)	142.6 (141.6)
5 (5′)			147.4 (144.3)	147.9 (143.0)
6 (6′)			108.0 (110.5)	108.6 (110.9)
Glucose				
C-1	92.4	90.7	91.5, 91.9	91.7
2	76.0	74.1	75.1 (2C)	75.0
3	77.3	76.0	76.5, 76.7	76.9
4	69.3	69.1	69.7, 69.3	69.9
5	73.5	71.0	71.5 (2C)	71.8
6	63.1	63.2	63.8 (2C)	64.1

a) Gall= 

b) HHDP= 

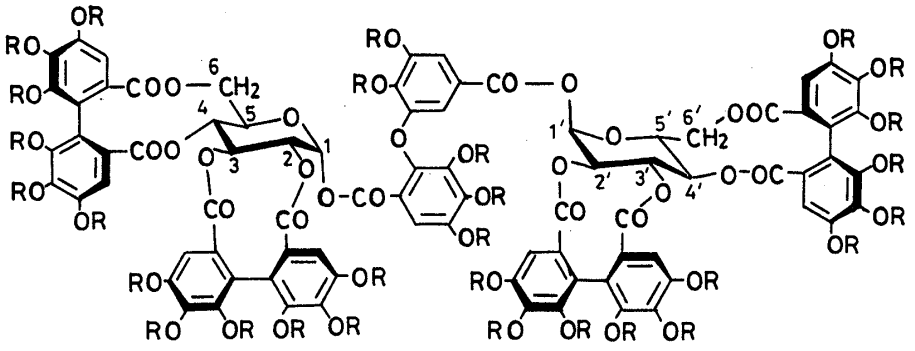
c) DHDG= 

d) Measured in acetone-d_6. *e*) Measured in MeOH-d_4.

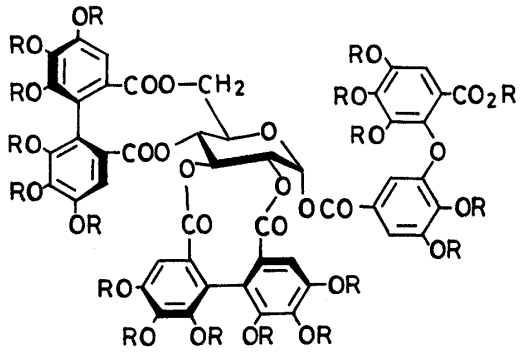
molar ratio of **8** and **11** is 3.9:1 (1% OV-1, column temp. 240 °C). Glucose was also liberated during the methanolysis, and was identified by gas liquid chromatography (GLC) after trimethylsilylation. Thus, nine aromatic 1H singlets at δ 6.36, 6.37, 6.38, 6.41, 6.44, 6.54, 6.60, 6.71 and 7.27, and two *meta*-coupled doublets (J=2 Hz) at δ 6.85 and 7.39 in the ^1H-NMR spectrum (200 MHz, MeOH-d_4) of **2** were accounted for by the presence of four hexahydroxydiphenoyl groups and a dehydrodigalloyl group. The presence of two glucose residues in **2** was shown by the two doublets (J=3.5 Hz) at δ 6.50 and 6.61 due to the anomeric protons, and also by the signals of twelve carbons, which partly overlap, in the ^{13}C-NMR spectrum (Table I).[11] These data, combined with the ten distinctive ester carbonyl resonances in the ^{13}C-NMR spectrum, indicate that agrimoniin is a dimeric ellagitannin in which hydroxyl groups on the two glucose cores are esterified by a dehydrodigalloyl group

and four hexahydroxydiphenoyl groups. The dimeric nature of **2** was also supported by the result of high-performance gel permeation chromatography, by which the molecular weight was estimated to be approximately twice that of **1**.

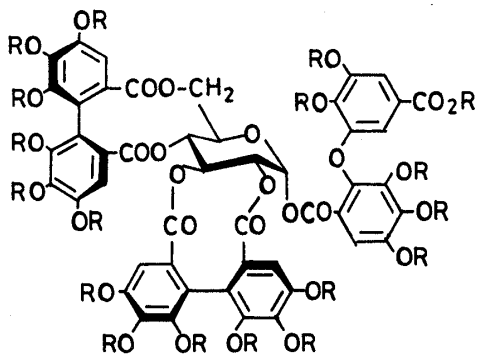
Methylation of agrimoniin with dimethyl sulfate and potassium carbonate in acetone provided useful information for determining the positions of the ester linkages. The nonacosamethyl ether (**10**) produced initially in the methylation was converted upon prolonged reaction (*ca.* 30 h) into six degradation products which were separated by preparative layer chromatography (PLC). Among them, four were identified as **8**, **11**, trideca-*O*-methyl-α-pedunculagin (**12**) and its β-anomer (**13**).[5] The remaining two products, **14** and **15**, showed the molecular ion peak at *m/z* 1356 ($C_{66}H_{68}O_{31}$) in the mass spectra. Their ^{1}H-NMR spectra exhibited the presence of a dehydrodigalloyl group in addition to two hexahydroxydiphenoyl groups, as revealed by five 1H singlets and two *meta*-coupled doublets in the aromatic region, and also showed signals due to eighteen methoxyl groups. A signal ascribable to an acylated anomeric proton was also observed as a doublet ($J=3.5$ Hz) at δ 6.52 in **14**, and at δ 6.62 ($J=3.5$ Hz) in **15**. Although first-order coupling between H-2 and H-3 of glucose in **15** was not detected because of complete overlapping, the pattern of the glucose proton signals of **14** was closely similar to that of **9**, suggesting the $^4C_1$ conformation of glucose. Based on these data, **14** and **15** are regarded as the methylated derivatives of 1-*O*-dehydrodigalloyl-2,3-*O*,4,6-*O*-bis(4,4′,5,5′,6,6′-hexahydroxydiphenoyl)-α-D-glucose, and thus they are isomeric to each other at the binding site of the dehydrodigalloyl group to the



2 : R=H
10 : R=Me

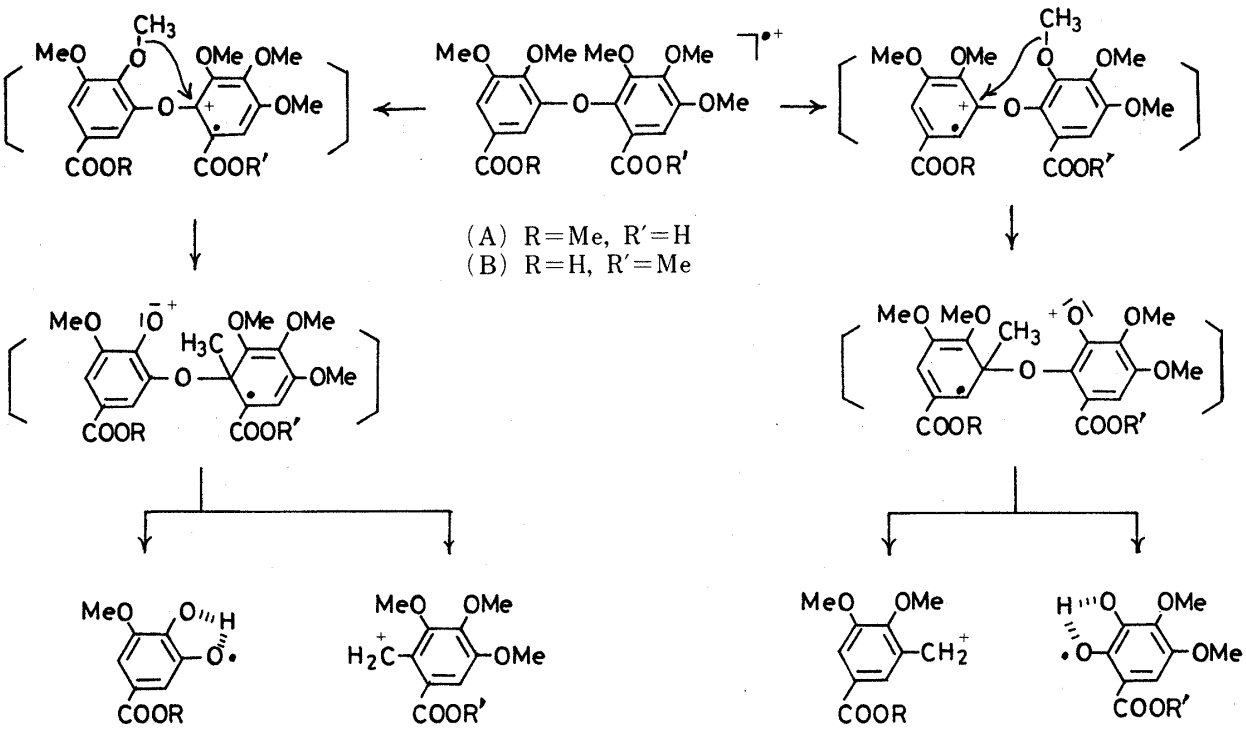


3 : R=H
14 : R=Me



4 : R=H
15 : R=Me

Chart 2



(A) R=Me, R′=H
(B) R=H, R′=Me

(C) R′=Me (*m/z* 239)
(E) R′=H (*m/z* 225)

(D) R=H (*m/z* 195)
(F) R=Me (*m/z* 209)

Chart 3

TABLE II. Rearrangement Ions Observed in Mass Spectra[a] of **14** and **15**

Compound	Fragment			
	C (*m/z* 239)	D (*m/z* 195)	E (*m/z* 225)	F (*m/z* 209)
14[b]	6.1%	4.6%	—	—
15[b]	—	—	5.0%	5.0%

a) Recorded at an ionizing potential of 20 eV.
b) Base peak: *m/z* 404.

anomeric center of glucose. The orientations of this group in **14** and **15** were determined from the fragmentation in the mass spectra (MS) as follows. The polymethoxy-biphenyl ether system is known to give an MS fragment ion which arises by cleavage of the biphenyl ether bond accompanied by the migration of a methoxyl group from one benzene ring to the other, followed by transfer of a hydrogen in the opposite direction.[12] The fragment ions of this type which originate from the monoester ions, A and B (*m/z* 422), were observed at *m/z* 239 (fragment C) and 195 (D) for **14**, and at *m/z* 225 (E) and 209 (F) for **15** (Chart 3 and Table II). These data are compatible with the structures **14** and **15**.

The (*S*)-configuration of the hexahydroxydiphenoyl groups in **2** was evident from the finding that the pedunculagin derivatives **12** and **13** of known absolute configurations[5] were obtained as products of the partial degradation of **10**. This assignment was also consistent with the circular dichroism (CD) spectrum of **2**, which displayed a positive Cotton effect at 235 nm of approximately twice the amplitude of that of **5**.[13] The data presented above led to

the unique dimeric structure **2** for agrimoniin.

Agrimonic acid A (**3**), $C_{48}H_{32}O_{31} \cdot 5H_2O$, $[\alpha]_D$ +66°, was obtained as a light brown amorphous powder. Its ^{1}H-NMR spectrum is very similar to that of **1** except that signals assignable to a dehydrodigalloyl group at δ 7.35, 6.92 (1H each, d, J=2 Hz) and 7.19 (1H, s) are present, in place of that attributable to the galloyl group. Thus, agrimonic acid A was presumed to be the 1-α-*O*-dehydrodigallate of pedunculagin. This structural assignment was confirmed by the identity of its methylated derivative with **14** (obtained upon methylation of **2**), as determined by direct comparison of the spectral and chromatographic data. Consequently, agrimonic acid A is represented by formula **3**.

Agrimonic acid B (**4**), $C_{48}H_{32}O_{31} \cdot 7.5H_2O$, $[\alpha]_D$ +72°, showed spectroscopic properties very similar to those of **3**, and its structure, which is isomeric to that of **3** at the binding site of the dehydrodigalloyl group to the anomeric center of glucose, was readily deduced from that of **3**. This structure (**4**) was confirmed by spectral comparison of its octadecamethyl derivative with **15**.

Although agrimoniin (**2**) and potentillin (**1**) coexist in both *A. japonica* and *P. kleiniana*, the presence of agrimonic acids A (**3**) and B (**4**), which was confirmed by HPLC, is limited to the former plant. Since the isolation procedures of tannins from these plants are practically the same, **3** and **4** are considered not to be artifacts derived from **2**. It is noteworthy that four tannins, **1**—**4**, described here possess the α-glucosidic linkage, which is rare in nature. Potentillin (**1**) is regarded as a key intermediate in the biosynthesis of these tannins. Agrimoniin (**2**) may have been formed by intermolecular C–O oxidative coupling between galloyl groups in two molecules of **1**. The hypothesis previously proposed for biogenesis of a dehydrodigallic acid by Schmidt and Mayer[14] is in accordance with this biogenetic pathway. Agrimonic acids A (**3**) and B (**4**) could have been similarly formed by the C–O oxidative coupling of a gallic acid molecule with the C-1 galloyl group of **1**, or could have been produced *via* the dimer in *A. japonica*.

Experimental

Optical rotations were measured on a JASCO DIP-4 polarimeter. Infrared (IR) spectra were recorded on a JASCO A-200 spectrometer and ultraviolet (UV) spectra on a Hitachi 200-10 spectrophotometer. MS were recorded on a Shimadzu LKB-9000 GC-MS spectrometer. The ionizing potential was 70 eV unless otherwise stated. NMR spectra were measured on a Hitachi R-22 FTS (90 MHz for ^{1}H-NMR and 22.6 MHz for ^{13}C-NMR) or a JEOL FX-200 (200 MHz for ^{1}H-NMR and 50.1 MHz for ^{13}C-NMR) spectrometer, with tetramethylsilane ($SiMe_4$) as an internal standard; chemical shifts are given in δ-values (ppm). Normal phase HPLC was performed on a column of Nomura Develosil 60-5, 4 × 150 mm, with hexane–MeOH–tetrahydrofuran–formic acid (55 : 33 : 11 : 1, v/v) containing oxalic acid (450 mg/l) as an eluant. Reversed phase HPLC was run on a column of Merck LiChrosorb RP-18 (10 μ), 4 × 300 mm, developing with 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (50 : 50 : 2 : 5, v/v). Detection was effected by UV absorption measurement at 280 nm. TLC was performed on cellulose (Funakoshi) plates (0.3 mm) in the following solvent systems: (A) 7% aqueous acetic acid, and (B) BuOH–AcOH–H_2O (4 : 1 : 5, upper layer). Kieselgel PF_{254} (Merck) was used for analytical and preparative TLC (0.25 and 0.5 mm, respectively) (solvent system C, benzene–acetone 6 : 1, v/v). The plates were visualized by UV irradiation (254 nm) or by spraying with $FeCl_3$ or $NaNO_2$–AcOH reagent. Sephadex LH-20 (100 μ) (Pharmacia Fine Chemicals) and Avicel microcrystalline cellulose (Funakoshi) were used for column chromatography. Solvents were removed by evaporation under reduced pressure below 40 °C. Light petroleum refers to that fraction boiling in the range of 85—120 °C.

Isolation of Tannins from *Agrimonia japonica* ——The fresh roots (1.93 kg) of *A. japonica*, collected in October, were homogenized in a mixture of acetone–H_2O (7 : 3) (3 × 10 l) and the homogenate was filtered. After removal of acetone, the aqueous solution was extracted with ether (9 × 1 l) and EtOAc (20 × 1 l) successively. The EtOAc extract (90 g) was chromatographed over Sephadex LH-20 (6.5 × 50 cm). Elution with EtOH removed most of the condensed tannins, and elution was continued with acetone–H_2O (7 : 3) to yield a dark brown residue (31 g) (hydrolyzable tannin-rich fraction). This residue was further subjected to column chromatography on Sephadex LH-20 (6.5 × 50 cm) with gradient elution (EtOH–H_2O–acetone, initially 90 : 10 : 0, then the concentration of H_2O and acetone were increased), and 12 g fractions were collected. Every fifth fraction was monitored by UV absorption measurement at 280 nm, TLC (cellulose) and HPLC. The following four fractions (fraction A, eluate with EtOH–

H_2O–acetone 60 : 30 : 10; fraction B, eluate with EtOH–H_2O–acetone 55 : 30 : 15; fraction C, eluate with EtOH–H_2O–acetone 50 : 30 : 20; fraction D, eluate with EtOH–H_2O–acetone 35 : 30 : 35) were further fractionated as described below.

Fraction A (9.7 g) was purified by droplet counter-current chromatography (3.2 mm i.d. × 120 cm, 100 glass tubes) with BuOH–PrOH–H_2O (2 : 1 : 3, v/v) (ascending method) to give pedunculagin (**5**) (215 mg), $[\alpha]_D$ +96° (c = 1.0, MeOH).

Fraction B (2.6 g) was chromatographed on a cellulose column (4.8 × 32 cm) with H_2O, and 10 ml portions of eluate were collected. Fractions 14—46 contained agrimonic acids A (**3**) and B (**4**), and fractions 65—85 contained casuarinin (**6**), all contaminated with condensed tannins. Removal of the condensed tannins from these fractions was effected by EtOAc extraction of the aqueous solutions at pH 6 and then pH 2 (adjusted with 0.1 N NaOH and 50% H_3PO_4). The EtOAc extract at pH 2 after further chromatography on Sephadex LH-20 and then on cellulose, gave pure casuarinin (**6**) (70 mg), and agrimonic acids A (**3**) (70 mg) and B (**4**) (25 mg). HPLC (reversed-phase) was used to monitor the separation of **3** and **4**.

Fraction C (3.7 g) was subjected to droplet counter-current chromatography in a similar way to that described above, and 12 ml portions of fractions were collected. Fractions 26—43 gave a dark brown residue (678 mg) which was rechromatographed over Sephadex LH-20 (1.9 × 24 cm) eluting initially with EtOH and then with EtOH containing increasing amounts of MeOH to afford pure potentillin (**1**) (254 nm). Fractions 44—67 from the above droplet counter-current chromatography provided a further crop of agrimonic acid A (**3**) (37 mg).

Fraction D (35 g) was purified on a column of Sephadex LH-20 (3.2 × 40 cm) with gradient elution (EtOH–H_2O–acetone), and on a column of cellulose (3 × 34 cm) eluting with H_2O, to give agrimoniin (**2**) (1.13 g).

Isolation of Tannins from *Potentilla kleiniana*——Fresh whole plants (1.34 kg) of *P. kleiniana* were homogenized in acetone–H_2O (1 : 1) (3 × 4.2 l). The homogenate was filtered through Celite-545 and the filtrate was concentrated to *ca.* 500 ml, then extracted with ethyl ether (3 × 600 ml). The aqueous layer was further extracted with EtOAc (11 × 500 ml), and this extract was evaporated to give a dark brown residue (5.27 g). The aqueous layer was next extracted with BuOH saturated with H_2O (6 × 400 ml). The extract was evaporated to yield a hygroscopic brown residue (11 g). A portion (2 g) of the EtOAc extract was subjected to droplet counter-current chromatography (3.2 mm i.d. × 120 cm, 100 glass tubes) by the ascending method using BuOH–PrOH–H_2O (2 : 1 : 3). Every fifth fraction (8 g each) was monitored by UV absorption measurement at 280 nm, and by TLC (cellulose, solvent A). Fractions 85—155 were combined, and further chromatographed on a cellulose column (1.2 × 15 cm) with H_2O as the eluant to yield pure potentillin (**1**) (120 mg). A portion (2 g) of the BuOH extract was treated in a similar manner. Fractions 156—260 from the droplet counter-current chromatography were purified by column chromatography over cellulose (2.2 × 30 cm) eluting with H_2O, to give pedunculagin (**5**) (158 mg) and agrimoniin (**2**) (288 mg).

Potentillin (1)——An off-white amorphous powder, TLC (cellulose), *Rf* 0.42 (solvent A), *Rf* 0.35 (B), $[\alpha]_D$ +108° (c = 1.0, EtOH). *Anal.* Calcd for $C_{41}H_{28}O_{26} \cdot 5H_2O$: C, 47.96; H, 3.73. Found: C, 48.04; H, 3.47. UV λ_{max}^{MeOH} nm (log ε): 222 (4.81), 258 (4.55). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1735, 1610, 1505, 1445, 1360—1300, 1180, 1030. CD (MeOH) $[\theta]^{25}$ (nm): $+16 \times 10^4$ (236), -4.4×10^4 (260), $+3.7 \times 10^4$ (282). ^{1}H-NMR (200 MHz, acetone-d_6) δ: 7.24 (2H, s, galloyl), 6.68, 6.59, 6.48, 6.38 [1H each, s, 2 × hexahydroxydiphenoyl (HHDP)], 6.63 (1H, d, J = 4 Hz, H-1), 5.42 (1H, dd, J = 4, 10 Hz, H-2), 5.58 (1H, t, J = 10 Hz, H-3), 5.24 (1H, t, J = 10 Hz, H-4), 4.66 (1H, dd, J = 6, 10 Hz, H-5), 5.34 (1H, dd, J = 6, 13 Hz, H-6), 3.83 (1H, d, J = 13 Hz, H-6′).

Pentadeca-*O*-methylpotentillin (9)——Anhydrous potassium carbonate (100 mg) and dimethyl sulfate (60 μl) were added to a solution of potentillin (**1**) (20 mg) in dry acetone (1.5 ml). The reaction mixture was stirred for 17 h at room temperature, and then refluxed for 3 h. The mixture was filtered and the filtrate was evaporated to give a syrupy residue, which was purified by PLC (benzene–acetone 5 : 1, v/v), to give pentadeca-*O*-methylpotentillin (**9**) (14 mg) as a white amorphous solid, TLC (SiO_2, solvent C) *Rf* 0.71, $[\alpha]_D$ +60° (c = 1.1, acetone). *Anal.* Calcd for $C_{56}H_{58}O_{26} \cdot 2H_2O$: C, 56.75; H, 5.27. Found: C, 56.84; H, 5.26. MS m/z 1146 (M^+). UV λ_{max}^{MeOH} nm (log ε): 215 (5.13), 255 (sh) (4.70), 290 (sh) (4.26). IR ν_{max}^{KBr} cm^{-1}: 1755, 1590, 1483, 1460, 1390, 1240, 1200, 1160, 1130, 1100. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.31 (2H, s, galloyl), 6.83, 6.76, 6.61, 6.51 (1H each, s, 2 × HHDP), 6.53 (1H, d, J = 3.5 Hz, H-1), 5.39 (1H, dd, J = 3.5, 9 Hz, H-2), 5.70 (1H, dd, J = 9, 10 Hz, H-3), 5.26 (1H, t, J = 10 Hz, H-4), 4.51 (1H, dd, J = 6, 10 Hz, H-5), 5.28 (1H, dd, J = 6, 13 Hz, H-6), 3.98 (1H, d, J = 13 Hz, H-6′), 3.51—3.97 (15 × OMe).

Methanolysis of Pentadeca-*O*-methylpotentillin (9)——A mixture of **9** (10 mg) and 1% sodium methoxide (0.1 ml) in absolute MeOH (1 ml) was kept at room temperature for 4 h. The mixture was then neutralized with Amberlite IR-120 ion-exchange resin (H-form), and filtered. The filtrate was evaporated under a nitrogen gas stream at room temperature. The residue was treated with diazomethane, and then partitioned between dichloromethane and H_2O. The dichloromethane layer was evaporated and purified by PLC (light petroleum–dichloromethane–acetone 6 : 3 : 1, v/v) to afford dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (**8**) (4 mg), $[\alpha]_D$ −37° (c = 1.1, EtOH), MS m/z 450 (M^+), CD (MeOH) $[\theta]^{25}$ (nm): $+6.7 \times 10^4$ (225), -5.6×10^4 (250), and methyl tri-*O*-methylgallate (1.7 mg), mp 80—81 °C, MS m/z 226 (M^+). The aqueous layer gave glucose, which was identified by GLC (2.5% OV-1, column temperature, 170 °C) and GC-MS of the trimethylsilyl ether.

Hydrolysis of Potentillin (1) with Tannase——A solution of potentillin (**1**) (30 mg) in H_2O (2 ml) was treated with tannase[15] (0.1 ml) at 37 °C for 4 d. The reaction mixture was directly applied to a Sephadex LH-20 column

(1.1 × 15 cm). Elution with H_2O yielded 2,3-*O*-hexahydroxydiphenoyl-D-glucose (6.5 mg), and the fraction eluted with 40% aq. acetone gave pedunculagin (**5**) (5 mg). The products were identified by direct comparisons (HPLC, TLC, $[\alpha]_D$ and ^{1}H-NMR spectra) with authentic samples.

Agrimoniin (2)——An off-white amorphous powder, TLC (cellulose) *Rf* 0.41 (solvent A), *Rf* 0.21 (B), $[\alpha]_D$ +162° (*c* = 1.0, EtOH). *Anal.* Calcd for $C_{82}H_{54}O_{52} \cdot 13H_2O$: C, 46.76; H, 3.83. Found: C, 46.59; H, 3.61. UV λ_{max}^{MeOH} nm (log ε): 232 (5.20), 270 (sh) (5.05). CD (MeOH) $[\theta]^{25}$ (nm): +36.5 × 10^4 (235), −10.6 × 10^4 (260), +9.7 × 10^4 (282). IR ν_{max}^{KBr} cm^{-1}: 3425 (br), 1735, 1615, 1510, 1420, 1350, 1180, 1015. ^{1}H-NMR (200 MHz, acetone-d_6) δ: 6.67, 6.66, 6.57, 6.44, 6.35, 6.36 (1H each, s), 6.62 (2H, s) (4 × HHDP), 7.31 (1H, s), 7.40 (1H, d, *J* = 2 Hz), 6.93 (1H, d, *J* = 2 Hz) [dehydrodigalloyl (DHDG)], 6.55, 6.58 (1H each, d, *J* = 3.5 Hz, H-1, 1′), 5.36, 5.38 (1H each, dd, *J* = 3.5, 9.5 Hz, H-2, 2′), 5.46, 5.56 (1H each, t, *J* = 9.5 Hz, H-3, 3′), 5.16, 5.21 (1H each, t, *J* = 9.5 Hz, H-4, 4′), 4.67, 4.50 (1H each, br dd, *J* = 6, 9.5 Hz, H-5, 5′), 5.24, 5.32 (1H each, dd, *J* = 6, 13 Hz, H-6, 6′), 3.69, 3.80 (1H, each, d, *J* = 13 Hz, H-6, 6′).

Methylation of Agrimoniin (2) with Diazomethane——A solution of **2** (100 mg) in dry acetone (2 ml) was treated with excess ethereal diazomethane for 40 h at room temperature. Purification of the products by PLC (solvent C, double development) gave nonacosa-*O*-methylagrimoniin (**10**) (11 mg) as a white amorphous solid, TLC (SiO_2, solvent C) *Rf* 0.48, $[\alpha]_D$ +53.7° (*c* = 1.3, $CHCl_3$). *Anal.* Calcd for $C_{111}H_{112}O_{52} \cdot 5H_2O$: C, 56.29; H, 5.19. Found: C, 56.35; H, 4.86. UV λ_{max}^{MeOH} nm (log ε): 218 (5.39), 255 (sh) (4.97), 295 (sh) (4.41). IR ν_{max}^{KBr} cm^{-1}: 1750, 1590, 1570, 1275, 1240, 1200, 1160, 1100, 1060. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.29 (1H, s), 7.24 (1H, d, *J* = 2 Hz), 6.76 (1H, d, *J* = 2 Hz) (DHDG), 6.77, 6.74, 6.70, 6.64, 6.53, 6.52, 6.51, 6.38 (1H each, s, 4 × HHDP), 6.39, 6.52 (1H each, d, *J* = 4.6 Hz, H-1, 1′), 3.50—4.08 (29 × OMe).

Methanolysis of Nonacosa-*O*-methylagrimoniin (10)——A mixture of **10** (40 mg) and 1% sodium methoxide (0.2 ml) in absolute MeOH (3 ml) was left for 12 h at room temperature. The mixture was then neutralized with Amberlite IR-120 resin (H-form), and filtered. The filtrate was evaporated under a stream of nitrogen at room temperature. The residue was treated with diazomethane and partitioned between dichloromethane and H_2O. The dichloromethane layer was evaporated and the residue was purified by PLC (solvent C), affording dimethyl penta-*O*-methyldehydrodigallate (**11**) (9 mg), mp 113—114 °C, MS *m/z* 436 (M$^+$), ^{1}H-NMR (acetone-d_6) δ: 7.31 (1H, s), 7.30 (1H, d, *J* = 2 Hz), 6.78 (1H, d, *J* = 2 Hz), 3.69—3.94 (7 × OMe), and **8** (13 mg), $[\alpha]_D$ −37° (*c* = 1.0, EtOH), MS *m/z* 450 (M$^+$). The aqueous layer gave glucose, which was identified by GLC and GC-MS of the trimethylsilyl ether.

Methylation of Agrimoniin (2) with Dimethyl Sulfate——Anhydrous potassium carbonate (500 mg) and dimethyl sulfate (0.35 ml) were added to a solution of agrimoniin (**2**) (100 mg) in dry acetone (6 ml). The reaction mixture was stirred for 13 h at room temperature, and then refluxed for 28 h. The mixture was filtered and the filtrate was concentrated to a syrupy residue, which was purified by PLC (benzene–acetone 14 : 1, v/v, developed four times) to give **11** (4 mg), **8** (5 mg), trideca-*O*-methyl-α-pedunculagin (**12**) (10.2 mg), trideca-*O*-methyl-β-pedunculagin (**13**) (5.8 mg), the octadecamethyl derivative (**14**) (5.8 mg) and its isomer (**15**) (6 mg), and **10** (19 mg).

Octadecamethyl Derivative (**14**): An off-white amorphous powder, TLC (SiO_2, solvent C) *Rf* 0.67, $[\alpha]_D$ +45° (*c* = 1.3, acetone), *Anal.* Calcd for $C_{66}H_{68}O_{31} \cdot H_2O$: C, 57.64; H, 5.13. Found: C, 57.50; H, 5.21. UV λ_{max}^{MeOH} nm (log ε): 219 (5.36), 249 (sh) (5.05), 287 (sh) (4.57). MS *m/z* 1356 (M$^+$), (20 eV) *m/z* 404 (100%), 239 (6.1), 195 (4.6). ^{1}H-NMR (acetone-d_6) δ: 7.33 (1H, s), 7.40, 6.84 (1H each, d, *J* = 2 Hz) (DHDG), 6.95 (2H, s), 6.67, 6.64 (1H each, s) (2 × HHDP), 6.52 (1H, d, *J* = 3.5 Hz, H-1), 5.41 (1H, dd, *J* = 3.5, 9 Hz, H-2), 5.58 (1H, dd, *J* = 9, 10 Hz, H-3), 5.14 (1H, t, *J* = 10 Hz, H-4), 4.55 (1H, dd, *J* = 6, 10 Hz, H-5), 5.19 (1H, dd, *J* = 6, 13 Hz, H-6; H-6′ was overlapped by the signals of OMe), 3.57—3.98 (18 × OMe).

Octadecamethyl Derivative (**15**): White amorphous solid, TLC (SiO_2, solvent C) *Rf* 0.61, $[\alpha]_D$ +46° (*c* = 1.3, acetone). *Anal.* Calcd for $C_{66}H_{68}O_{31} \cdot 2H_2O$: C, 56.90; H, 5.21. Found: C, 56.82; H, 5.46. UV λ_{max}^{MeOH} nm (log ε): 217 (5.35), 249 (sh) (5.03), 284 (sh), (4.55). IR ν_{max}^{KBr} cm^{-1}: 1755, 1590, 1485, 1390, 1335, 1200, 1160, 1100, 1020. MS *m/z* 1356 (M$^+$), (20 eV) *m/z* 404 (100%), 225 (5), 209 (5). ^{1}H-NMR (acetone-d_6) δ: 7.39 (1H, s), 7.34, 6.89 (1H each, d, *J* = 2 Hz) (DHDG), 6.97, 6.91, 6.79, 6.60 (1H each, s, 2 × HHDP), 6.62 (1H, d, *J* = 3.5 Hz, H-1), 4.02—3.60 (18 × OMe).

Agrimonic Acid A (3)——Isolated as a light brown amorphous powder, TLC (cellulose, solvent A) *Rf* 0.4, HPLC (reversed phase) t_R 5.97 min, $[\alpha]_D$ +66° (*c* = 1.0, EtOH). *Anal.* Calcd for $C_{48}H_{32}O_{31} \cdot 5H_2O$: C, 48.25; H, 3.53. Found: C, 48.56; H, 3.80. ^{1}H-NMR (200 MHz, acetone-d_6) δ: 7.19 (1H, s), 7.35, 6.92 (1H each, d, *J* = 2 Hz) (DHDG), 6.66, 6.63, 6.43, 6.36 (1H each, s, 2 × HHDP), 6.60 (1H, d, *J* = 3.5 Hz, H-1), 5.38 (1H, dd, *J* = 3.5, 9 Hz, H-2), 5.57 (1H, dd, *J* = 9, 10 Hz, H-3), 5.21 (1H, t, *J* = 10 Hz, H-4), 4.65 (1H, dd, *J* = 6, 10 Hz, H-5), 5.30 (1H, dd, *J* = 6, 13 Hz, H-6), 3.80 (1H, d, *J* = 13 Hz, H-6′).

Agrimonic Acid B (4)——Obtained as a light tan hygroscopic amorphous powder, TLC (cellulose, solvent A) *Rf* 0.4, HPLC (reversed phase) t_R 3.93 min, $[\alpha]_D$ +72° (*c* = 1.0, EtOH). *Anal.* Calcd for $C_{48}H_{32}O_{31} \cdot 7.5H_2O$: C, 46.50; H, 3.82. Found: C, 46.43; H, 3.90. UV λ_{max}^{MeOH} nm (log ε): 202 (5.29), 275 (4.41). ^{1}H-NMR (200 MHz, acetone-d_6) δ: 7.30 (1H, s), 7.28, 6.85 (1H each, d, *J* = 2 Hz) (DHDG), 6.64, 6.61, 6.51, 6.34 (1H each, s, 2 × HHDP), 6.52 (1H, d, *J* = 4 Hz, H-1), 5.33 (1H, dd, *J* = 4, 9 Hz, H-2), 5.48 (1H, dd, *J* = 9, 10 Hz, H-3), 5.14 (1H, t, *J* = 10 Hz, H-4), 4.41 (1H, dd, *J* = 6, 10 Hz, H-5), 5.19 (1H, dd, *J* = 6, 13 Hz, H-6), 3.66 (1H, d, *J* = 13 Hz, H-6′).

Methylation of Agrimonic Acids A (3) and B (4)——A mixture of **3** (70 mg), dimethyl sulfate (0.2 ml) and potassium carbonate (260 mg) in dry acetone (5 ml) was stirred for 12 h at room temperature, and then refluxed for 5 h. The reaction mixture was filtered and the filtrate was evaporated to give a syrupy residue (82 mg). PLC (benzene–

acetone 14 : 1, v/v) gave methyl heptadeca-*O*-methylagrimonate A (14 mg), which was identified by direct comparison with the methyl derivative (**14**) obtained from agrimoniin (**2**).

Agrimonic acid B (**4**) was also methylated in the same manner as described for **3**, and the product was identified by direct comparison with authentic **15**.

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