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DIMERIC ELLAGITANNINS, LAEVIGATINS E, F AND G, FROM *ROSA LAEVIGATA**

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Key Word Index—*Rosa laevigata*; Rosaceae; tannin; ellagitannin; dimeric ellagitannin; laevigatin E; laevigatin F; laevigatin G.

Abstract—Three new dimeric ellagitannins, laevigatins E, F and G, were isolated along with the known tannins, agrimonic acid B, sanguiin H-4, pedunculagin, agrimoniin, laevigatins B and D, from the polar fraction of the pericarp extract of *Rosa laevigata*. The structures of the new dimers, in which two glucose cores are linked through a dehydrodigalloyl group, were established by spectroscopic methods.

INTRODUCTION

In a previous paper, we reported on the isolation and chemical characterization of agrimoniin (**1**), the main tannin, laevigatin A, a new monomeric tannin, and laevigatins B (**2**), C (**3**) and D (**4**), dimeric ellagitannins [1], from dried pericarps and fresh leaves of *Rosa laevigata* Michx., a plant which has traditionally been used as a diuretic, an antitussive, and also for treatment of skin tumours and burns in China [2]. These dimers, each of which possesses a dehydrodigalloyl (DHDG) group connecting two glucose cores with each other, were of chemotaxonomical interest, since this type of dimer has not been found in any other species of the *Rosa* genus. Further investigation on the polyphenolics of the polar fraction of pericarp extract has led to the isolation of three additional new tannins which we have named laevigatins E (**5**), F (**6**) and G (**7**), together with previously reported agrimoniin (**1**), agrimonic acid B, sanguiin H-4, laevigatins B (**2**) and D (**4**) [1]. This paper deals with the structure elucidation of these new dimeric hydrolysable tannins.

* Part 7 in the series 'Tannins of Rosaceous Medicinal Plants'. For Part 6 see Yoshida, T., Jin, Z. X., and Okuda, T. (1989) *Phytochemistry* (in press).

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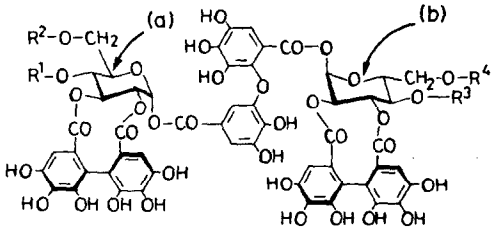
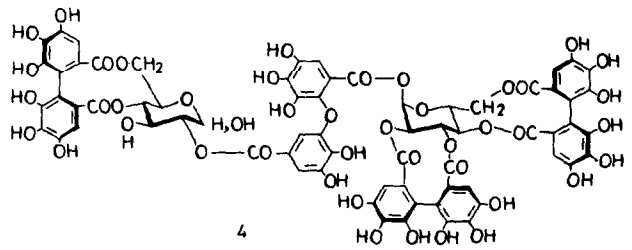
RESULTS AND DISCUSSION

Laevigatins E–G were positive to $FeCl_3$ and $NaNO_2$–HOAc reagent [3], suggesting that they were ellagitannins.

Laevigatin E (**5**), $[\alpha]_D$ +18.5° (MeOH), was shown to be an ellagitannin dimer by its ^1H NMR spectrum which established the presence of a DHDG group [δ7.24 (*s*), 7.32 (*d*, $J = 2$ Hz) and 6.88 (*d*, $J = 2$ Hz)], two hexahydroxydiphenoyl (HHDP) groups [δ6.70, 6.69, 6.52 and 6.44 (each, *s*)], and two $^4C_1$ glucopyranose residues. The



	R¹	R²	R³	R⁴
1	(S)-HHDP		(S)-HHDP	
2	H	H	(S)-HHDP	
3	(S)-HHDP		H	H
5	H	H	H	H



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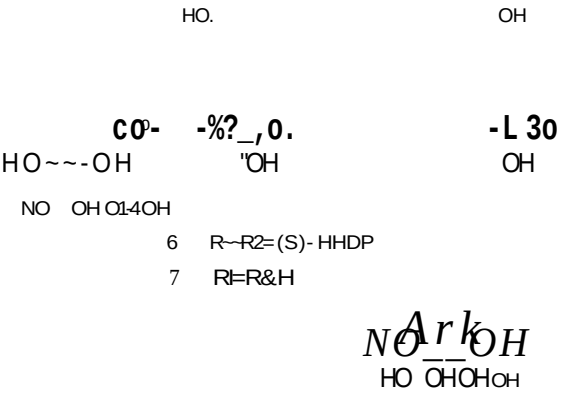


6 R~-R2=(S)-HHDP

7 RI=R&H

dimeric nature was further demonstrated by the peak at *m/z* 1267 [M+H] + in FABMS which is consistent with the molecular formula, C~4H42036. The ~HNMR spectrum, confirmed by 1H-all COSY measurement, exhibited H-4 and H-6 signals of both glucose cores at higher field than the corresponding signals of agrimoniin (1), suggesting the presence of free hydroxyl groups at these positions (Table l). These data along with the or-glycosidic linkage, as revealed by the small coupling constants (d = 3.5 Hz) for the anomeric proton signals (36.43 and 6.45), led to the structure 5 for laevigatin E. This structure was finally confirmed by the partial hydrolysis of agrimoniin (1) with tannase to give laevigatin E.

Laevigatin F (6), [~]D + 108° (MeOH), was assigned the molecular formula C6sH4.gO4¢by FABMS *[m/z* 1569 [M +HI + and 1567 (M-H)-]. The ~HNMR spectrum exhibited two *meta-coupled* doublets (J = 2 Hz) and seven one-proton singlets ascribable to a DHDG group and three HHDP groups. The sugar proton signals of 6 were fully assigned by ~H-~HCOSY measurements to reveal that 6 is composed of 2 tool of the 4C~ glucopyranose, each of which possesses an ~-oriented acyloxy group at the anomeric centre. These spectral features suggested that laevigatin F is a dimeric ellagitannin closely related to agrimoniin (1), and is an isomer of 2 and 3. The ~HNMR spectrum also indicated that one of the glucose residues is fully acylated, while the other has two free hydroxyl groups at C-2 and C-3, as evidenced by the appearances of H-2 (63.78) and H-3 (63.77) at high field (Table 1). The chemical shifts and multiplicity of the proton signals of the former were virtually identical with those of the glucose *core* (a) of agrimoniin (1) El]. The CD spectrum of 6 showed close similarity to that of laevigatin D [1], which allows assignment of S-configuration *to* the HHDP groups in 6 [4]. These spectroscopic data, in *conjunction* with the ~3CNMR spectrum (Table 2), led to the *structure* 6 for laevigatin F.

Laevigatin G (7), [~-I, + 63° (MeOH), was indicated by the ~H and ~3CNMR spectra to consist of a dehydrodi-

Table 1. 1HNMR spectral data of agrimoniin (1), laevigatins B (2), E (5), F (6) and G (7) (500 MHz, MezCO-ds-D20, values in parenthesis are coupling constants in Hz)

H	1	2	5	6	7
Glucose (a)					
1	6.58	6.43	6.43	6.53	6.41
	d (3.5)	d (4)	d (3.5)	d (4)	d (3.5)
2	5.38	5.12 *dd*	5.14 *dd*	5.35	5.11
	dd (3.5/9.5)	*dd* (4/10)	*dd* (3.5/9.5)	*dd* (4/10)	*dd* (3.5/9.5)
3	5.56	5.42	5.46	5.54	5.41
	t (9.5)	t (10)	t (9.5)	t (10)	t (9.5)
4	5.21	3.97	3.97	5.16	3.94
	t (9.5)	t (10)	t (9.5)	t (10)	t (9.5)
5	4.67	3.87	3.86	4.62	3.87
	dd (6/9.5)	*dt* (3/10)	*ddd* (2/4/13)	*dd* (7/10)	*ddd (2/4.5/9.5)*
6	5.32	3.77 (2H)	3.75	5.26	3.77
	dd (6/13)	d (3)	*dd* (4/13)	*dd* (7/13)	*dd* (2/12)
	3.80		3.82	3.76	3.72
	d (13)		*dd* (2/13)	d (13}	*dd* (4.5/I2)
Glucose (b)					
1	6.55	6.55	6.45	6.27	6.25
	d (3.5)	d (4)	d (3.5)	d (3.5)	d (4)
2	5.36	5.33	5.10	3.78	3.78
	dd (3.5/9.5)	*dd* (4/10)	*dd* (3.5/10)	*dd* (3.5/10)	*dd* (4/10)
3	5.46	5.46	5,35	3.77	3.67
	t (9,5)	t (I0)	t (I0)	t (10)	t (10)
4	5.16	5,46	3,96	4,79	4.76
	t (9.5)	t (10)	t (10)	t (10)	t (10)
5	4.50	4.44	3.45	4.25	4,18
	dd (6/9.5)	*dd* (7/10)	*dt* (3/10)	*dd* (6/10)	*dd* (6/10)
6	5.24	5.18	3.68	5.08	5.0d
	dd (6/13)	*dd* (7/13)	*dd* (3/13)	*dd* (6/13)	*ad* (6/I 3)
	3.69	3.67	3.56	3,62	3.61
	d (13)	d (13)	dd (3/13)	d (13)	d (I3)

Table 2. ^{13}C NMR spectral data of agrimoniin (1), laevigatins B (2), F (6) and G (7) (126 MHz, Me_2CO-d_6–D_2O)

C	1*	2	6	7
Glucose (a)				
1	90.7	91.0	90.6	90.0
2	73.8	73.6	74.0	73.6
3	75.6	78.0	75.8	77.8
4	68.9	67.5	69.1	67.1
5	70.7	76.3	70.9	75.9
6	63.1	61.4	63.2	61.0
Glucose (b)				
1	90.6	90.8	93.0	93.0
2	74.0	74.2	72.6	72.3
3	75.7	75.9	73.2	72.8
4	68.8	68.9	72.6	72.3
5	71.2	71.3	71.0	70.8
6	63.0	63.2	63.8	63.7

* Measured in Me_2CO-d_6.

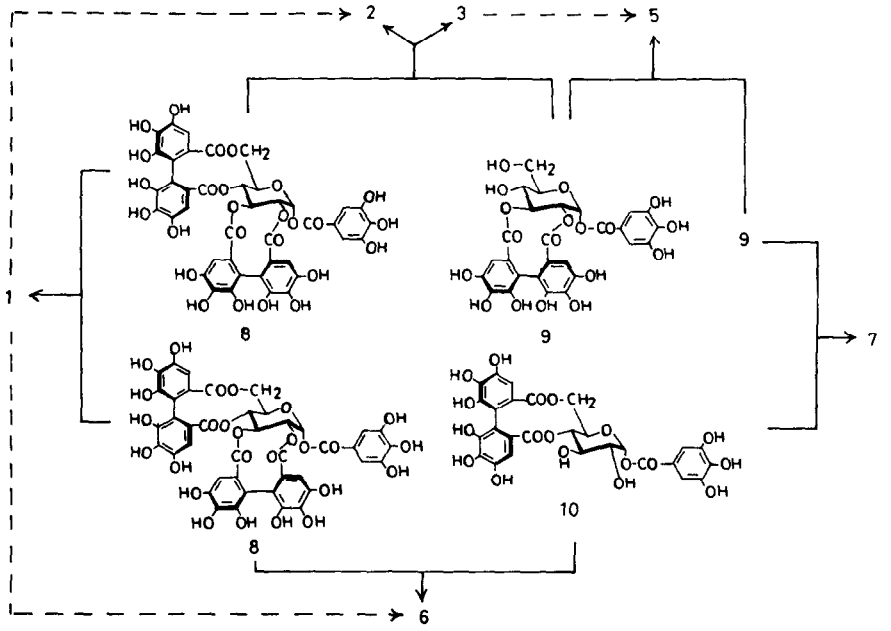
galloyl group, two HHDP groups and two $^4C_1$ glucopyranose residues possessing α-glycosidic linkages. Therefore, 7 was regarded as an ellagitannin dimer isomeric to 5 with regard to the positions of the HHDP groups. The locations of two HHDP groups in 7 were deduced to be at C-2 ~ C-3 in one of the glucose cores, and at C-4 ~ C-6 in the other, since the relevant proton signals resonated at low field as expected for those on ester-bearing carbons. Upon ^1H NMR spectral comparison of 7 with laevigatins B (2) and F (6), it was apparent that the individual proton signals of the glucose core of 7, in which C-1, C-2 and C-3 are acylated, were in good agreement with those of the glucose core (a) of 2, while those of the other glucose core in which C-1, C-4 and C-6 are acylated, coincided well with those of the glucose core (b) of 6 as shown in Table 1. Similar correlations were also demonstrated in the ^{13}C NMR spectra (Table 2). Based on these spectroscopic findings, laevigatin G was assigned the formula 7.

Seven dimeric ellagitannins including previously reported dimers [agrimoniin (1), laevigatins B (2), C (3), and D (4)] were isolated from *R. laevigata*. Laevigatins B (2), C (3) and F (6) are three of the four possible isomers of 1 which lack an HHDP group. Based on the concept that dimeric hydrolysable tannins are biogenetically formed by an intermolecular C–O oxidative coupling between polyphenolic ester groups of two glucose cores [5, 6], the biogenesis of the dimers of *R. laevigata* can be depicted as in Scheme 1. Although the biogenetic precursors, potentillin (8) and sanguiin H-4 (9) co-exist with the dimers in the same plant, another precursor, 1-*O*-galloyl-4,6-(*S*)-HHDP-α-D-glucose (10) has not yet been found. Therefore, it is also possible that laevigatins F (6) and G (7) are metabolites of agrimoniin (1).

EXPERIMENTAL

^1H and ^{13}C NMR: 500 MHz and 126 MHz respectively. The chemical shifts are given in δ(ppm) values relative to Me_2CO-d_6 and converted into the TMS scale by adding 2.04 ppm (^1H) and 29.8 ppm (^{13}C). CC: Toyopearl HW-40 (coarse and fine grades) (TOSOH, Japan), Diaion HP-20 and MCI gel CHP-20P (Mitsubishi Chemical Industries Co. Ltd.). TLC: cellulose (Avicel SF, microcrystalline cellulose, Funakoshi, Japan) developed with 7% HOAc, and visualized by spraying $FeCl_3$ reagent or $NaNO_2$–HOAc reagent [3]. Solvents were evapd under red. pres. below 40°.

Isolation of tannins. Dried pericarps of *R. laevigata*, collected at Chengdu, China, were extracted with a mixture of Me_2CO–H_2O (7:3), and concd. The aq. soln was extracted successively with Et_2O, EtOAc and *n*-BuOH. The BuOH extract (10 g) was subjected to CC over Diaion HP-20 eluted with H_2O containing increasing amounts of MeOH ($H_2O \rightarrow$ 10% aq. MeOH → 30% aq. MeOH → 50% aq. MeOH → MeOH → 70%



Scheme 1.

aq. MezCO). The 30% aq. MeOH eluate (2.66 g) was chromatographed on Toyopearl HW-40C (2.2cm i.d. × 30cm) using as solvent system, EtOH-H20-Me2CO (5:5:0--*6:4:0 --7:3:046:3:1--.5:3:2) to give Fr. I-III (EtOH-H20 1:1), Fr. IV (EtOH-H20 3:2), Fr. V (EtOH-H20 7:3), and Fr. VI (EtOH-H20-Me2CO 6:3:1). Fraction I was further purified by CC on Toyopearl HW-40F (70% aq. MeOH) to afford sanguiin H-4 (32 mg). Fractions II and III were subjected to CC over MCI gel CHP-20P to give laevigatin E (35 mg) and pedunculagin (32 mg). Fractions IV-VI afforded agrimonic acid B (13 mg), laevigatin B (37 mg) and agrimoniin (34 mg), respectively. The 50% aq. MeOH eluate in the above mentioned CC on Diaion HP-20P was similarly chromatographed over Toyopearl HW-40C (2.2 cm i.d. × 40cm) eluting with EtOH-H20-MezCO (7: 3:0 ~ 6: 3: 1~ 5:3:2) to give laevigatin F (39 mg), laevigatin D (31 mg) and agrimoniin (499 mgl.

The aq. extract remaining after BuOH extraction was fractionated by CC over Diaion HP-20 (H20 containing increasing amount of MeOH). The 30% aq. MeOH eluate was further purified by a combination of CC over Toyopearl HW-40F and MCI gel CHP-20P, with the same solvent system, to give laevigatin G (15 mg).

Laevioatin E (5). A pale brown amorphous powder, "[~]o + 18.5° (MeOH; c 0.5). TLC, RI 0,46. UV).~,"°H nm Cloge): 206 (4.87), 260 (4.50); 1H NMR (Me2CO-dr-D20): 67.24 (IH, s), 7.32 (IH, d, J = 2 Hz), 6.88 (IH, d, J = 2 Hz) (DHDG), 6.70, 6.69, 6.52, 6.44 (1H each, s) (HHDP), glucose protons, see Table 1; FABMS *m/z:* 1267 [M+H]+; (negative) 1265 [M-H]- (C54H42036 , 1266).

Enzymatic hydrolysis ofl. A soln of I (150 mg) in HzO (10 ml) was incubated with tannase, which was prepared from *Aspergillus niger* [1], at 37° for 59 hr. After removal of the solvent, the residue was treated with EtOH. The EtOH soluble portion was subjected to CC over Toyopearl HW-40C developing with 50% aq. EtOH to afford 5 (25 mg), in addition to 2 and 3 [1].

Laevigatin F (6). An off-white amorphous powder, [~]D + 108° (MeOH; c 0.5), TLC, *Rf* 0.39. UV 2~ °n nm Clog e): 212 (5.03), 224 (5.05), 257 (4.75); CD (MeOH; c 0.01) [0] × 10-4 (nm): +26.03 (236), - 8.78 (260), + 6.59 (282), - 2.20 (314); 1H NMR (MezCO-dr-D20): ~57.26 (1H, s), 7.38. 6,88 (1H each, d, d = 2 Hz) (DHDG), 6.76, 6.63, 6.60, 6.57, 6.41, 6.34 (IH each, s) (HHDP), glucose protons, see Table 1; 13CNMR (Me2CO-dr): ?J169.4, 168.7, 168.4 (2C), 168.3, 167.9, 164.8, 163.8 (ester carbonyl), glucose carbons, see Table2; FAB-MS *m/z:* 1569 [M+H] +, 1567 [M - H] - (C68H4sO44, 1568).

Laevigatin G (7). A pale brown amorphous powder, [~]o + 63~' (MeOH; c 0.5), TLC Rf 0.49. UV 2~ °H nm (log e): 205 (4.56), 223 (sh) (4.51), 264(4.30); 1H NMR (Me2CO-d 6+ DzO): 67.23 (1H, s), 7.34, 6.85 (IH each, d, J = 2 Hz) (DHDG), 6.78, 6.68, 6.57, 6.40 (1H each, s) (HHDP), glucose protons, see Table 1; 13C NMR (Me2CO-d 6+ D20): 6164.4, 165.2, 168.6, 169.1 (2C), 169.9 (ester carbonyl), glucose carbons, see Table 2; FAB-MS (negative) *re~z:* 1265 [M--HI (C~,tH,~2Od~. 1266!.

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REFERENCES

I. Yoshida, T., Tanaka, K., Chen, X.-M. and Okuda, T. (1989) *Chem. Pharm. Bull.* (in press).
2. Chiang Su New Medicinal College (ed.) (1977) *Dictionary of Traditional Chinese Drugs*, p. 1406. Shanghai Scientific Technologic Publishers, Shanghai.
3. Okuda, T., Yoshida, T. and Mori, K. (1975) *Yakugaku Zasshi* 95, 1462.
4. Okuda, *T.*, Yoshida, T., Hatano, T., Koga, T., Toh, N. and Kuriyama, K. (1982) *Tetrahedron Letters* 23, 3937.
5. Haddock, E. A., Gupta, R. K., AI-Shafi, S. M. K. and Haslam, E. (1982) *J. Chem. Soc. Perkin Trans. 1*, 2525.
6. Okuda, T., Yoshida, T., Kuwafiara, M., Memon, M. U.. and Shingu, T. (1984) *Chem. Pharm. Bull.* 32, 2165.