Tannins and Related Compounds. CXXII.[1a] New Dimeric, Trimeric and Tetrameric Ellagitannins, Lambertianins A—D, from *Rubus lambertianus* SERINGE

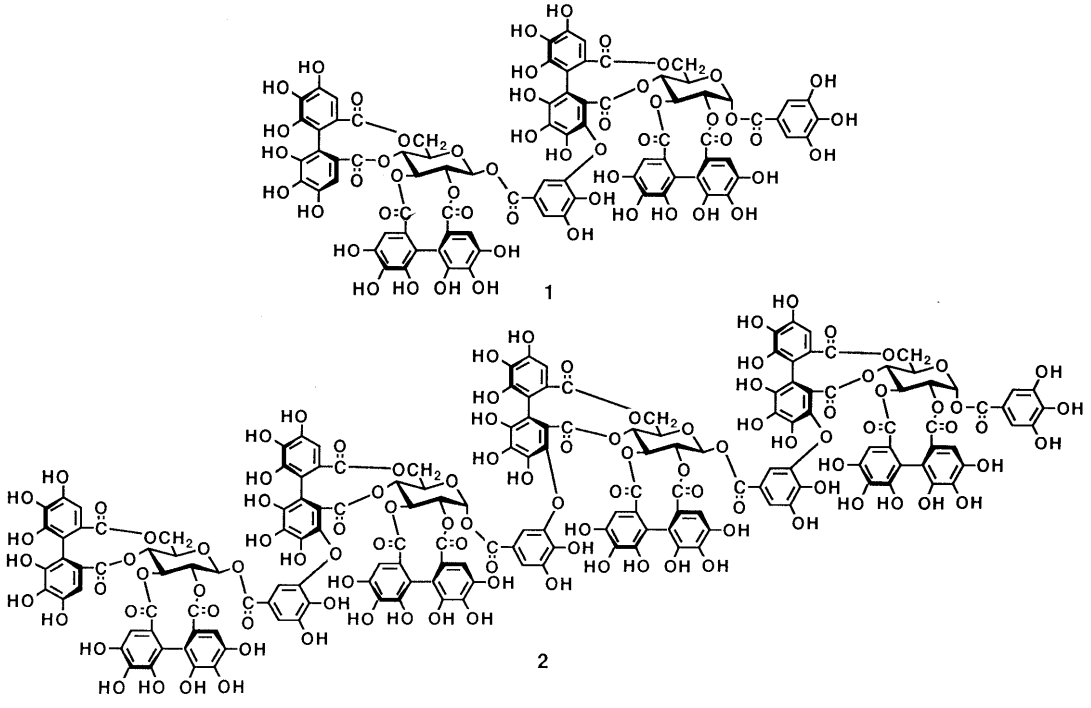
Takashi TANAKA,*[a,1b] Hideki TACHIBANA,[a] Gen-ichiro NONAKA,[a] Itsuo NISHIOKA,[a] Feng-Lin HSU,[b] Hiroshi KOHDA,[c] and Osamu TANAKA[c]

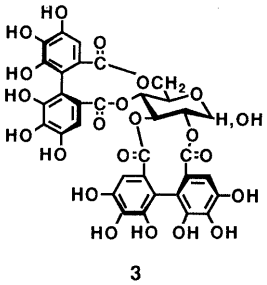
Faculty of Pharmaceutical Sciences, Kyushu University,[a] 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan, Taipei Medical College,[b] 250 Wu-Hsing Street, Taipei, Taiwan, and Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine,[c] 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan. Received December 10, 1992

Chemical examination of the leaves of *Rubus lambertianus* SERINGE (Rosaceae) has led to the isolation of four new ellagitannins, which were characterized on the basis of chemical and spectroscopic evidence to be dimers [lambertianins A (6) and B (7)], a trimer [lambertianin C (8)] and a tetramer [lambertianin D (10)], all having sanguisorbic acid ester group(s) as linking unit(s) between glucopyranose moieties. Furthermore, HPLC analyses of fifteen *Rubus* species collected in Japan and Taiwan revealed that the trimer (8) and the tetramer (10), together with sanguiin H-6 (1), occur widely in these species.

Keywords *Rubus lambertianus*; Rosaceae; tannin; ellagitannin; lambertianin; sanguiin

In previous papers,[2] we reported on the isolation, from the underground part of the Rosaceous plant *Sanguisorba officinalis* L. (Japanese name: jiyu), of dimeric [sanguiins H-3, H-6 (**1**) and H-8-H] and tetrameric [sanguiin H-11 (**2**)]ellagitannins having a sanguisorbic acid ester group as an intramolecularly "bridged" phenolcarboxyl group between the glucopyranose units. On the other hand, Haslam *et al.* at almost the same time reported the separation of sanguiin H-6 (**1**) (they denoted the compound as T_1) from some *Rubus* species.[3] It is now known that many members of Rosaceae, particularly of the genera *Rosa*,[4] *Potentilla*,[5] *Geum*[6] and *Agrimonia*,[5] predominantly produce a variety of oligomeric ellagitannins, which structurally differ from sanguiins, having a dehydrodigalloyl or a valoneoyl group as the linking unit. So far, only *Sanguisorba* and *Rubus* species have been found to metabolize oligomers in which each glucose unit is connected through a sanguisorboyl group.[7] In a continuation of our chemical studies on tannins in Rosaceous plants, we have investigated several species of the genus *Rubus*, which, in contrast to other genera, has not yet been examined in detail, except for Haslam's work[3] and a chemotaxonomical survey of sanguiins H-6 (1) and H-11 (2) by means of HPLC.[8] As a results, we have isolated, together with a large quantity of sanguiin H-6 (**1**), four new ellagitannins including two dimers [lambertianins A (**6**) and B(7)], a trimer [lambertianin C (**8**)] and a tetramer [lambertianin D (**10**)][9] from the leaves of *R. lambertianus* SERINGE collected in Taiwan. In addition, the presence of the trimer (**8**) in two *Rubus* species (*R. crataegifolius* BUNGE and *R. chingii* HU) was confirmed by isolation. Successive HPLC analyses revealed that most of the *Rubus* species collected in Japan and Taiwan contain the trimer (**8**) and the tetramer (**10**) instead of sanguiin H-11 (**2**), accompanied almost





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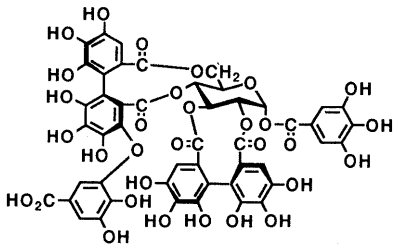

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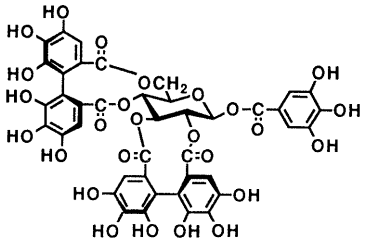

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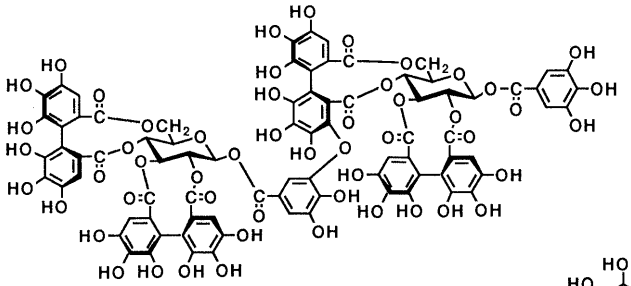
invariably with sanguiin H-6 (**1**). This paper deals with the isolation and structural elucidation of these new oligomeric ellagitannins from *R. lambertianus* and also describes the results of HPLC analysis.

The water-soluble portion, which was obtained by extraction of the dried leaves with aqueous acetone, was directly subjected to Sephadex LH-20 chromatography. Stepwise elution with water containing increasing amounts of methanol afforded phenolcarboxylic acids and monomeric ellagitannins, which were identified as gallic acid, ellagic acid, pedunculagin (**3**), sanguiin H-2 (**4**) and 1(*β*)-*O*-galloylpedunculagin (**5**) by comparisons of their physical and ^{1}H-NMR spectral data with those of authentic specimens. The oligomer fraction eluted with methanol alone and then with aqueous acetone was repeatedly chromatographed over Sephadex LH-20, MCI-gel CHP 20P, Fuji-gel ODS-G3 and Avicel cellulose to yield lambertianins A—D (**6**—**8**, **10**), together with a large amount of sanguiin H-6 (**1**).

As mentioned above, sanguiins were first isolated from *Sanguisorba* species and structurally elucidated. However, the orientation of the sanguisorboyl ester group invariably attached to the 4,6-positions of the glucopyranose moiety had remained unsolved. In order to elucidate this, the following NMR examinations were made. In the ^{1}H-NMR spectrum of **4**, signals due to glucose H-3 and H-5 appeared at δ 4.99 and 4.06, respectively, being shifted upfield as compared with those (δ 5.64 and 4.68) of 1(α)-*O*-galloylpedunculagin (the anomer of 5).[2] This fact clearly indicated that the aromatic ring of the "branched" gallic acid residue in the sanguisorboyl group affected magnetically the glucose 3,5-protons. Inspection of the Dreiding model showed that only in the case when the branched gallic acid moiety is bonded to the aromatic ring located at the glucopyranose C-4 position is the approach of this aromatic ring to the glucose 3,5-protons possible. Furthermore, the ^{13}C–^{1}H long-range shift correlation (COSY) spectrum of **4** exhibited a cross peak between one of the glucose H-6 signals at δ 3.80 and a carboxyl carbon signal at δ 167.7, the latter being further correlated with an aromatic proton singlet at δ 6.74. This fact clearly indicated that the aromatic ring located at the glucose C-6 position possesses an isolated aromatic proton, and thus the mode of the linkage of the sanguisorboyl group was determined to be as shown in the structural formula **4**. Since all of the other sanguiins having the sanguisorboyl group in the molecule were shown to liberate **4** and 1-desgalloyl **4** on treatment in hot water,[2] they could be concluded to possess the same orientation as that of **4**.

A new tannin, lambertianin A (**6**) showed a ^{1}H-NMR signal pattern closely related to that of the dimeric ellagitannin, sanguiin H-6 (**1**). Namely, in the aromatic field, a two-proton singlet (δ 7.15) and six one-proton singlets (δ 6.35, 6.36, 6.37, 6.56, 6.57, 6.67) corresponding to one galloyl and three 3,3′,4,4′,5,5′-hexahydroxydiphenoyl (HHDP) ester groups were observed. Furthermore, the observation of a pair of characteristic *meta*-coupled doublets (δ 7.23, 7.24, each J=2 Hz), together with a relatively lowfield one-proton singlet (δ 6.81), suggested the presence of a sanguisorboyl group. The aliphatic signals, including two sugar anomeric ones, were all found to be shifted fairly downfield and to have large coupling constants ($J_{1,2}=J_{2,3}=J_{3,4}=J_{4,5}$=9—10 Hz), indicating the occurrence of two *β*-glucopyranose moieties with $^4C_1$-conformation, whose hydroxyl groups are completely acylated. This was further supported by ^{13}C-NMR examination, which revealed twelve aliphatic signals, the chemical shifts being closely related to those of 1(*β*)-*O*-galloylpedunculagin (**5**).

The fact that the glucopyranose rings adopt the $^4C_1$-conformation implied that the HHDP and sanguisorboyl ester groups are located at the glucose C-2/3 and C-4/6 positions.[3] Furthermore, the appearance of two pairs of the fairly well separated glucose C-6 methylene signals (δ 4.01, 4.03 and 5.35, 5.78) supported the occurrence of the 4,6-bridge of the acyl group. The unusual lowfield shift (δ 5.78) of one of the glucose C-6 methylene signals was found to be analogous to those of sanguiins (*e.g.*, **1**: δ 5.58), and this fact indicated that the sanguisorboyl ester group is attached to the C-4 and C-6 positions as the linking unit



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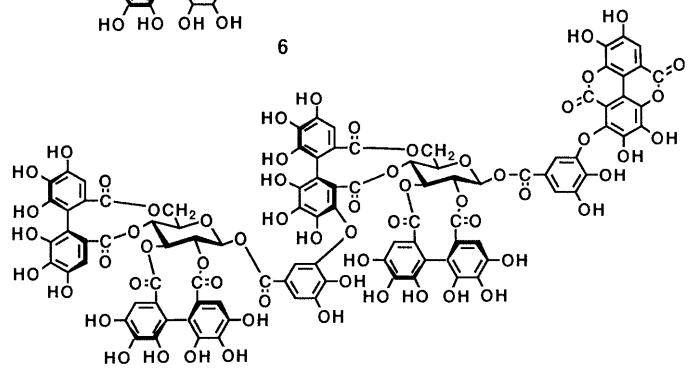

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of the two glucopyranose moieties. On the other hand, the observation of the above-mentioned upfield shifts (δ 5.25, 4.18) of H-3 and H-5 in one of the glucose moieties, as compared with those (δ 5.45, 4,49) in another glucose unit, established the orientation of the sanguisorboyl group to be the same as those of sanguiins.

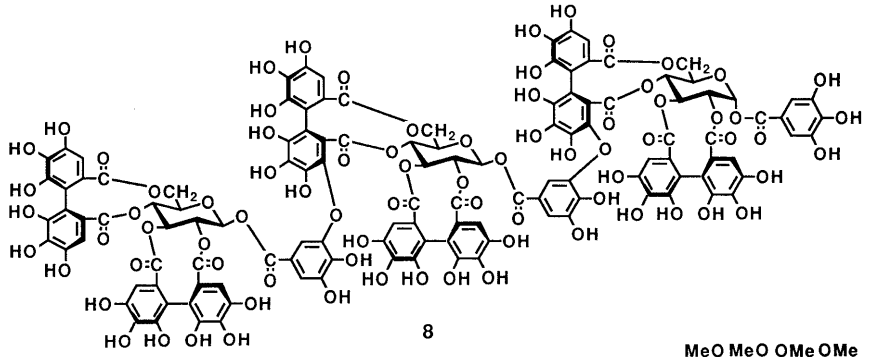
The negative FAB-MS, showing the same $[M-H]^-$ peak at m/z 1869 as that of **1**, was consistent with the above ^{1}H- and ^{13}C-NMR data, and lambertianin A was thus conlcuded to be the structural isomer (**6**) of **1**, having two β-glucopyranose cores.

The ^{1}H-NMR spectrum of lambertianin B (**7**) exhibited eight aromatic one-proton singlets (δ 6.15—7.57) and two pairs of *meta*-coupled doublets (δ 6.76, 7.15, 7.17, 7.28, each $J=2$ Hz), suggesting the presence of three HHDP and two sanguisorboyl ester groups. The aliphatic signal pattern was closely correlated with that of **6**, showing similar chemical shifts and coupling constants. In the ^{13}C-NMR spectrum, the chemical shifts of the sugar signals were found to be almost the same as those of **6**. The appearance of two carboxyl carbon signals at δ 157.0 and 160.5, which were shifted to relatively higher field than those in the ordinary phenolcarboxylic acids, suggested the presence of α,β; γ,δ-unsaturated lactone rings. Furthermore, the observation of a fairly lowfield shift (δ 7.57) of one of the aromatic singlets in the ^{1}H-NMR spectrum implied that one of the sanguisorboyl groups exists in a bis-lactone form. Taking into account the absence of the galloyl group and instead the appearance of the sanguisorbic acid bis-lactone signals, lambertianin B was concluded to have the structure **7**, which was consistent with the negative FAB-MS data, showing the $[M-H]^-$ peak at m/z 2169. Final structural confirmation was obtained by partial hydrolysis of **7** in hot water, which yielded 1-desgalloylsanguiin H-6.

The trimeric nature of lambertianin C (**8**) was readily deduced from the observation of three anomeric proton signals at δ 6.04 (d, $J=8$ Hz), 6.18 (d, $J=8$ Hz) and 6.54 (d, $J=4$ Hz) in the ^{1}H-NMR spectrum. In addition, from the coupling constants of these signals, combined with the appearance of sugar signals having large coupling constants ($J=9$—10 Hz), **8** was considered to be based on two β- and one α-glucopyranose cores each adopting $^4C_1$-conformation. In the aromatic field, the observation of a two-proton singlet at δ 7.15, eight one-proton singlets between δ 6.22—6.70 and two pairs of *meta*-coupled doublets at δ 7.07, 7.14, 7.16 and 7.24 (each $J=2$ Hz), accompanied by two relatively lowfield singlets at δ 6.78 and 6.87, suggested the presence of one galloyl, four HHDP and two sanguisorboyl ester groups, respectively.

Methylation of **8** with dimethyl sulfate and potassium carbonate in dry acetone yielded the tritetracontamethyl ether. Subsequent hydrolysis of this methylate, followd by diazomethane treatment, afforded methyl trimethoxybenzoate, dimethyl (*S*)-hexa-*O*-methoxydiphenoate (**8a**) and trimethyl (*S*)-octa-*O*-methylsanguisorboate (**8b**) in the molar ratio of 1 : 4 : 2. Thus, the existence of fifteen carboxyl groups in total from these phenolcarboxylic acid moieties was consistent with the lowfield shifts of all the aliphatic proton signals, which implied that the hydroxyl groups in the glucose moieties are completely esterified. Furthermore, the fact that the ^{13}C-NMR chemical shifts of the aliphatic signals including the anomeric signals are closely similat to those of **4** plus **5** indicated that the substitution systems in the glucopyranose rings are similar to those of **4** and **5**.

The locations of acyl groups were confirmed as follows. Treatment of **8** in hot water, followed by repeated chromatography over Sephadex LH-20 and MCI-gel CHP 20P, yielded, among others, three hydrolysis products. Of them, two were identical with the naturally occurring ellagitannins, 2,3-(*S*)-HHDP-D-glucose and sanguiin H-2 (**4**), and the remaining one was found to be identical with the product (**9**) obtained previously by similar partial hydrolysis of **2**. On the basis of these spectroscopic and chemical evidence, the structure of lambertianin C was established to be as shown by the formula **8**. This is the



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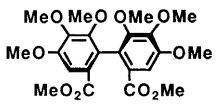

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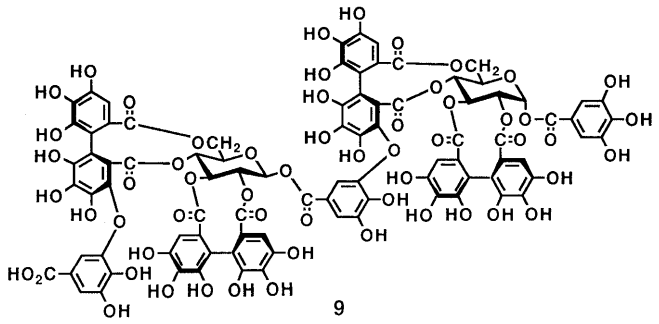

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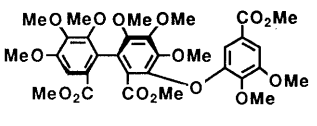

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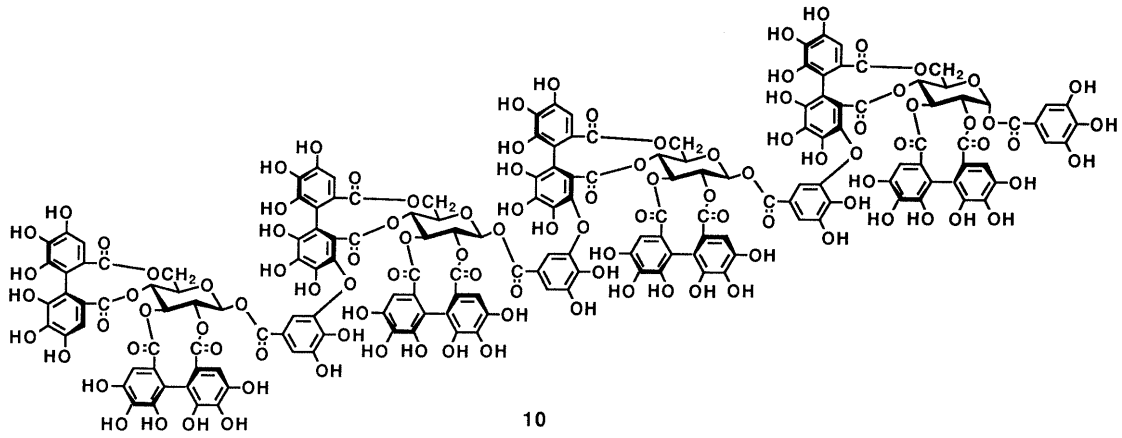

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first isolation of a trimeric ellagitannin from *Rubus* species.

The ^{1}H-NMR spectrum of lambertianin D (**10**) clearly showed four anomeric proton signals at δ 6.05 (2H, d, $J=8$ Hz), 6.20 (d, $J=9$ Hz) and 6.54 (d, $J=4$ Hz), and the chemical shifts and coupling patterns of aliphatic signals closely resembled those found in **8**. The aromatic signals consisted of a two-proton signlet (δ 7.16), thirteen one-proton singlets (δ 6.22—6.87) and three pairs of *meta*-coupled doublets (δ 7.05—7.23). These ^{1}H-NMR observations suggested **10** to be a tetrameric ellagitannin based on one α- and three β-glucopyranose cores to which one galloyl, five HHDP and three sanguisorboyl ester groups are attached. The ^{13}C-NMR spectrum of **10** exhibited twenty-four aliphatic resonances including one α- (δ 90.8) and three β-anomeric signals (δ 92.3, 92.4, 92.5). Among these signals, the chemical shifts of six were found to be in good agreement with those of **4**, while the remaining eighteen, appearing as six groups showed chemical shifts similar to those of **5**. Thus, **10** was considered to have a structure in which one additional 1(β)-*O*-galloylpedunculagin (**5**) unit is linked to **8**.

Treatment of **10** in hot water afforded many uncharacterized products, and their preparative-scale separation was extremely difficult. Analysis of the reaction mixture by means of normal-phase HPLC revealed that after 27 h, the peak corresponding to **10** disappeared and instead six major peaks (A—F in Fig. 1) were observed. Among these, the retention times of peaks E and F coincided with those of 2,3-(*S*)-HHDP-D-glucose and the partial hydrolysis product (**9**) obtained from **8**, respectively. Previously, we reported that there is a close correlation between the retention times in normal-phase HPLC and the molecular weights, and when the logarithmic scales of the retention times were plotted against the molecular weights, an almost straight line was obtained. In order to apply this method to the identification of the unknown peaks (A—D), we first attempted to obtain a calibration line by employing 2,3-(*S*)-HHDP-D-glucose and compounds **1**, **4**, **8** and **9** as reference tannins of known molecular weights. The results are shown in Fig. 2, actually giving a straight line. From this, the molecular weight of **10** was readily estimated to be *ca.* 3700, supporting the hypothesis that this compound is a tetrameric ellagitannin. On the other hand, peaks A and B, whose molecular weights were estimated to be *ca.* 3400 and 2950, were found to be derived from the successive losses of the HHDP and the 2,3-(*S*)-HHDP-glucose moiety from **10**, respectively, while peaks C and D were considered to correspond to trimeric hydrolysates formed by alternative losses of the HHDP and sanguisorboyl groups from compound B, respectively.

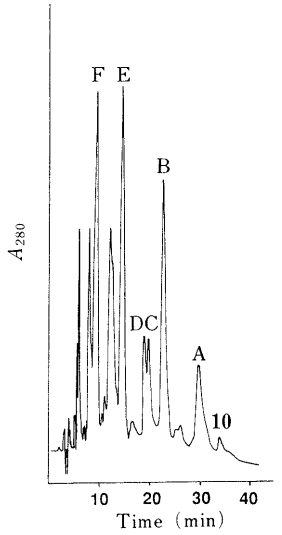

Fig. 1. HPLC Chromatogram of the Hydrolysis Products (after Reflux in Water for 27 h) from **10**

Conditions: column, Cosmosil 5 SL (4.6 mm i.d. × 250 mm); solvent, *n*-hexane–methanol–tetrahydrofuran–formic acid (45 : 40 : 13 : 1) containing oxalic acid (500 mg/l); flow rate, 0.7 ml/min; detection, 280 nm.

Based on the chemical and spectral evidence mentioned above, lambertianin D was concluded to have the structure (**10**), in which the 1(β)-*O*-galloylpedunculagin (**5**) moiety is oxidatively coupled with the lambertianin C (**8**) molecule. Compound **10** is a structural isomer of the tetrameric ellagitannin, sanguiin H-11 (**2**), differing only in the configuration of one of the anomeric centers.

As mentioned in the introduction, an HPLC survey of tannins in Rosaceous plants including *Rubus* species has recently been made,[8] and it was reported that almost all the *Rubus* and *Sanguisorba* plants examined characteristically contained sanguiins H-6 (**1**) and H-11 (**2**). Our HPLC re-examination of fifteen *Rubus* and two *Sanguisorba* species collected in Japan and Taiwan (Table I) revealed that the presence of sanguiin H-11 (**2**) in the genus *Rubus* is questionable, and instead lambertianin D (**10**) occurs widely in these species. This discrepancy was considered to have arisen from the lack of the reference compound (**2**)

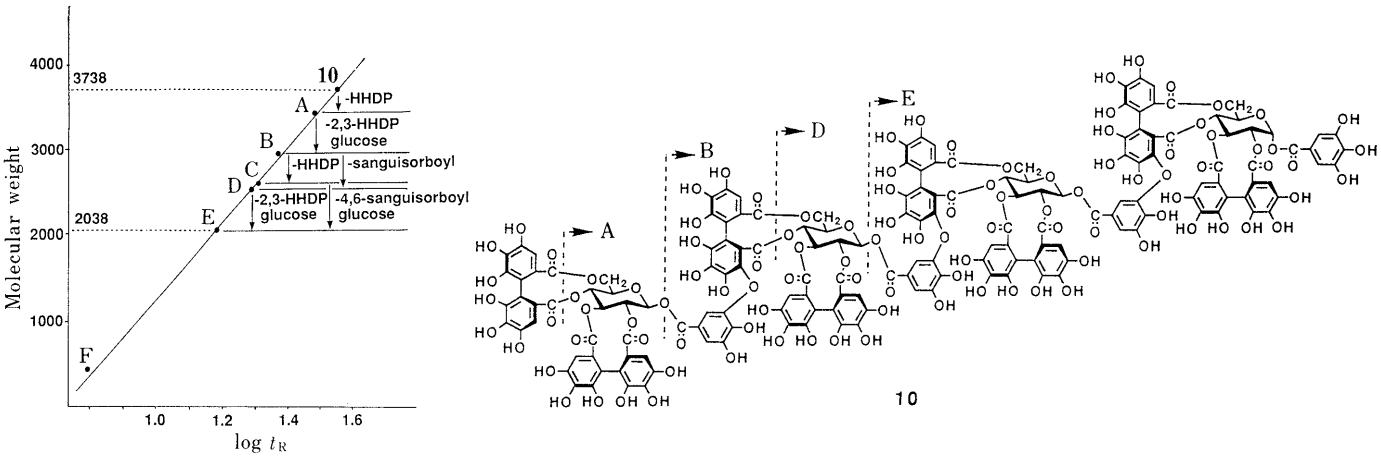

Fig. 2. Correlation between the HPLC Retention Times of Hydrolysis Products and Their Molecular Weights

TABLE I. Occurrence of Sanguiins H-6 (**1**) and H-11 (**2**) and Lambertianins C (**8**) and D (**10**) in *Rubus* and *Sanguisorba* Species

Species	Collected at	Compounds			
		1	**8**	**2**	**10**
R. hirsutus THUNB.	Sasaguri, Fukuoka	−	−	−	−
R. buergeri MIQUEL	Sasaguri, Fukuoka	−	−	−	−
R. crataegifolius BUNGE	Sasaguri, Fukuoka	+	+	−	+
	Mt. Kujyu, Ooita	+	+	−	+
R. parvifolius L.	Sasaguri, Fukuoka	+	+	−	+
R. palmatus THUNB.	Sasaguri, Fukuoka	+	+	−	+
	Mt. Abura, Fukuoka	−	−	−	−
	Mt. Wakasugi, Fukuoka	+	+	−	+
R. chingii HU	Kasumi, Hiroshima	+	+	−	+
R. sieboldii BLUMÉ	Hagi, Yamaguchi	+	+	−	+
R. corchorifolius L.	Asahison, Yamaguchi	+	+	−	+
R. palmatus THUNB. var *coptophyllus* KUNZE	Bibai, Hokkaido	+	+	−	+
R. idaeus L.	Bibai, Hokkaido	+	+	−	+
R. mesogeanus FOCKE	Bibai, Hokkaido	+	+	−	+
R. phoenicolasius MAXIM.	Bibai, Hokkaido	+	+	−	+
R. calycinoides HAYATO	Taiwan	+	+	−	+
R. swinhoei HANCE	Taiwan	−	−	−	−
R. lambertianus SERINGE	Taiwan	+	+	−	+
S. tenuifolia var *parviflora* MAXIM.	Sasaguri, Fukuoka	+	+	+	−
S. officinalis L.	Mt. Kujyu, Ooita	+	+	+	−

and also from the similarities of the retention times (**2**, 50.5 min; **10**, 51.6 min) in normal-phase HPLC. Sanguiin H-6 (**1**) was indeed found to occur, accompanied by lambertianin C (**8**), almost invariably as a major metabolite in all the species, except for *R. hirsutus*, *R. buergeri* and *R. swinhoei*. It is interesting to note here that even in the same season, almost all the species showed, depending on the collection places, remarkable variations in the contents of compounds **1**, **8** and **10**. In particular, the variation of tannin contents in *R. palmatus* was significant (**1**, 0—0.2%; **5**, 0—0.9%); some samples completely lacked tannins. It remains to be clarified whether these variations are due to genetic or environmental factors.

Experimental

Optical rotations were measured with a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL FX-100 and JEOL GX-270 instruments, and chemical shifts are given in the δ-scale. FAB-MS were recorded on JEOL DX-300/JMA 3500 and JEOL HX-100/JMA 3500 machines with dimethyl sulfoxide (DMSO) (or methanol)/glycerol as the matrix. Column chromatography was performed with Sephadex LH-20 (25 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (Mitsubishi Chemical Industries Co., Ltd.), Fuji-gel ODS-G3 (43—65 μ, Fujigel Hanbai Co., Ltd.), Avicel cellulose (Funakoshi) and Kieselgel 60 (70—230 mesh, Merck). TLC was carried out on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1:7:1 or 1:5:2, v/v) and dioxane–ethyl formate–formic acid (2:10:3, v/v), and on precoated cellulose plates (0.1 mm thick, Merck) with 2% acetic acid. Spots were detected first under a UV lamp (Manaslu light, 2536 Å) and then by the use of a ferric chloride reagent spray. HPLC was performed with a Toyo Soda CCPM machine equipped with a Toyo Soda UV-8000 detector (at 280 nm).

Isolation of Tannins The air-dried leaves (2.9 kg) of *R. lambertianus*, collected in Nantou, Taiwan, were extracted with 70% aqueous acetone at room temperature. After concentration of the extract under reduced pressure, the resulting precipitates were removed by filtration, and the filtrate was subjected to Sephadex LH-20 chromatography. Elution first with water gave a mixture of sugars, non-aromatic glycosides, *etc.*, and stepwise elution with water containing increasing amounts of methanol and then with aqueous acetone afforded four fractions consisting of tannins and related compounds. The first fraction, after concentration, yielded pale brown precipitates (45 mg), mp > 300 °C, which were identified as ellagic acid. The mother liquor was passed through a Sephadex LH-20 column with ethanol to afford gallic acid (70 mg) as colorless needles (water), mp 253—257 °C. The second and the third fractions, which contained monomeric ellagitannins, were separately subjected to MCI-gel CHP 20P chromatography with water containing increasing proportions of methanol to furnish pedunculagin (**3**) (93 mg), sanguiin H-2 (**4**) (13 mg) and 1(β)-*O*-galloylpedunculagin (**5**) (3.0 g). The oligomeric ellagitannin fraction was rechromatographed over Sephadex LH-20 with the solvent system of water–methanol–acetone to give further three fractions. Rechromatography of the first fraction over Fuji-gel ODS-G3 with water–methanol (7:3) gave large amounts of sanguiin H-6 (**1**) (8.9 g) and lambertianin C (**8**) (2.7 g), while the last two fractions were separately chromatographed over Sephadex LH-20 with the same solvent system to afford lambertianin D (**10**) (340 mg) and a mixture of lambertianins A (**6**) and B (**7**). Separation of **3** and **4** was achieved by chromatography over Avicel cellulose with 2% acetic acid, followed by removal of acetic acid with MCI-gel CHP 20P, and the yields were 30 and 140 mg, respectively.

Pedunculagin (3) A pale brown amorphous powder, $[\alpha]_D^{20}$ +55.3° (c=1.5, acetone). ^{1}H-NMR (100 MHz, acetone-d_6): 3.78 (1/2H, d, J=13 Hz, β-H-6), 3.85 (1/2H, d, J=13 Hz, α-H-6), 4.26 (1/2H, m, α-H-5), 4.69 (1/2H, m, β-H-5), 6.33, 6.51, 6.56, 6.60, 6.67, 6.68 (each s, HHDP-H).

Sanguiin H-2 (4) A pale brown amorphous powder, $[\alpha]_D^{20}$ +12.6° (c=1.8, acetone). ^{1}H-NMR (100 MHz, acetone-d_6): 3.80 (1H, d, J=13 Hz, H-6), 4.06 (1H, dd, J=6, 8 Hz, H-5), 4.93 (1H, t, J=8 Hz, H-4), 4.99 (1H, t, J=8 Hz, H-3), 5.28 (1H, dd, J=3, 8 Hz, H-2), 5.45 (1H, dd, J=6, 13 Hz, H-6), 6.35, 6.40 (each 1H, s, HHDP-H), 6.54 (1H, d, J=3 Hz, H-1), 6.74 [1H, s, sanguisorboyl (SS)-H], 7.13, 7.28 (each 1H, d, J=2 Hz, SS-H), 7.09 (2H, s, galloyl H).

1(β)-*O*-Galloylpedunculagin (5) A pale brown amorphous powder, $[\alpha]_D^{20}$ +25.5° (c=1.3, methanol). ^{1}H-NMR (100 MHz, acetone-d_6): 3.88 (1H, d, J=14 Hz, H-6), 4.51 (1H, dd, J=7, 9 Hz, H-5), 5.18 (1H, t, J=

9 Hz, H-4), 5.22 (1H, t, J=9 Hz, H-2), 5.37 (1H, dd, J=7, 14 Hz, H-6), 5.47 (1H, t, J=9 Hz, H-3), 6.22 (1H, d, J=8 Hz, H-1), 6.37, 6.47, 6.55, 6.68 (each 1H, s, HHDP-H), 7.18 (2H, s, galloyl H).

Sanguiin H-6 (1) A pale brown amorphous powder, $[\alpha]_D^{22}$ +52.5° (c=0.5, acetone). ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 3.86 (1H, d, J=13 Hz, H-6), 3.94 (1H, d, J=13 Hz, H-6′), 4.34 (1H, dd, J=6, 8 Hz, H-5), 4.36 (1H, dd, J=6, 8 Hz, H-5′), 5.04 (1H, t, J=8 Hz, H-4), 5.11 (2H, t, J=8 Hz, H-2′, H-4′), 5.20 (1H, t, J=8 Hz, H-3), 5.29 (1H, dd, J=6, 13 Hz, H-6′), 5.32 (1H, dd, J=4, 8 Hz, H-2), 5.38 (1H, t, J=8 Hz, H-3′), 5.58 (1H, dd, J=6, 13 Hz, H-6), 6.18 (1H, d, J=8 Hz, H-1′), 6.26, 6.32, 6.40, 6.48, 6.53 (each 1H, s, HHDP- and SS-H), 6.54 (1H, d, J=4 Hz, H-1), 7.12 (2H, s, galloyl H), 7.14, 7.27 (each 1H, d, J=2 Hz, SS-H).

Lambertianin A (6) A pale brown amorphous powder, $[\alpha]_D^{20}$ −6.76° (c=3, methanol). *Anal.* Calcd for $C_{82}H_{54}O_{52}\cdot 8H_2O$: C, 49.08; H, 3.25. Found: C, 48.98; H, 3.49. Negative FAB-MS m/z: 1869 $[M-H]^-$. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 4.01 (1H, d, J=13 Hz, H-6), 4.03 (1H, d, J=13 Hz, H-6′), 4.18 (1H, dd, J=6, 9 Hz, H-5), 4.49 (1H, dd, J=6, 10 Hz, H-5′), 4.94 (1H, t, J=9 Hz, H-4), 5.04 (1H, t, J=10 Hz, H-4′), 5.14 (1H, t, J=10 Hz, H-2′). 5.17 (1H, t, J=9 Hz, H-2), 5.25 (1H, t, J=9 Hz, H-3), 5.35 (1H, dd, J=6, 13 Hz, H-6′), 5.45 (1H, t, J=10 Hz, H-3′), 5.78 (1H, dd, J=6, 13 Hz, H-6), 6.04 (1H, d, J=9 Hz, H-1), 6.30 (1H, d, J=10 Hz, H-1′), 6.35, 6.36, 6.37, 6.56, 6.57, 6.67 (each 1H, s, HHDP-H), 6.81 (1H, s, SS-H), 7.15 (2H, br s, galloyl H), 7.23, 7.24 (each 1H, d, J=2 Hz, SS-H). ^{13}C-NMR (65.05 MHz, acetone-d_6+D_2O): 63.0, 63.1, 69.3 (×2), 73.5, 73.8, 75.7, 76.0, 76.8, 77.5, 92.2, 92.4 (glucose-C), 107.5, 108.3 (HHDP-C-3, C-3′), 110.3 (galloyl C-2, C-6), 165.4, 166.4, 168.1, 168.3 (×2), 168.6, 168.9, 169.7 (–COO–).

Lambertianin B (7) A pale brown amorphous powder, $[\alpha]_D^{20}$ −4.1° (c=1.2, MeOH). Negative FAB-MS m/z: 2169 $[M-H]^-$, 1402, 1084, 469. *Anal.* Calcd for $C_{96}H_{56}O_{58}\cdot H_2O$: C, 53.49; H, 2.62. Found: C, 53.22; H, 2.43. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 3.95 (1H, d, J=13 Hz, H-6), 3.96 (1H, d, J=14 Hz, H-6′), 4.08 (1H, dd, J=6, 10 Hz, H-5), 4.45 (1H, dd, J=6, 10 Hz, H-5′), 4.87—5.00 (3H in total, m, H-2-4), 5.12 (each 1H, t, J=10 Hz, H-2′, 4′), 5.31 (1H, dd, J=6, 14 Hz, H-6′), 5.42 (1H, t, J=10 Hz, H-3′), 5.67 (1H, dd, J=6, 10 Hz, H-6), 5.87 (1H, d, J=8 Hz, H-1), 6.22 (1H, d, J=8 Hz, H-1′), 6.15, 6.30, 6.35, 6.52, 6.57, 6.67 (each 1H, s, HHDP-H), 6.77 (1H, s, SS-H), 6.76, 7.15, 7.17, 7.28 (each 1H, d, J=2 Hz, SS-H), 7.57 (1H, s, SS-H). ^{13}C-NMR 65.05 MHz, acetone-d_6+D_2O): 63.0, 63.1, 69.3 (×2), 73.5, 73.8, 75.5, 76.1, 76.6, 77.5, 92.2. 92.4 (glucose-C), 157.0, 160.5 (sanguisorbic acid dilactone-C), 165.0, 165.3, 166.2, 168.0, 168.2, 168.3, 168.4, 168.6, 169.6 (–COO–).

Partial Hydrolysis of 7 A solution of **7** (10 mg) in water (2 ml) was heated under reflux for 27 h. Analysis of the hydrolysis products by HPLC [column, Cosmosil 5 SL (4.6 mm i.d. × 250 mm); solvent, n-hexane–methanol–tetrahydrofuran–formic acid (45 : 40 : 13 : 1, v/v) containing oxalic acid (500 mg/l); flow rate, 0.7 ml/min] showed, among others, a peak (t_R, 13.5 min) corresponding to 1-desgalloylsanguiin H-6.

Lambertianin C (8) A pale brown amorphous powder, $[\alpha]_D^{21}$ −6.7° (c=0.9, acetone). *Anal.* Calcd for $C_{123}H_{80}O_{78}\cdot 8H_2O$: C, 50.08; H, 3.28. Found: C, 50.22; H, 3.57. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 3.90, 3.93 (1H and 2H, respectively, each d, J=13 Hz, H-6, 6′, 6″), 4.12 (1H, dd, J=6, 10 Hz, H-5′), 4.33 (1H, dd, J=6, 10 Hz, H-5), 4.46 (1H, dd, J=6, 10 Hz, H-5′), 4.96 (1H, t, J=10 Hz, H-4′), 5.02, 5.04 5.12, 5.13 (each 1H, t, J=10, 10, 9, 10 Hz, respectively, H-4, 2′, 3′, 4′), 5.18 (1H, t, J=9 Hz, H-2″), 5.22 (1H, t, J=10 Hz, H-3), 5.32 (1H, dd, J=3, 10 Hz, H-2), 5.34 (1H, dd, J=6, 13 Hz, H-6″), 5.44 (1H, t, J=9 Hz, H-3″), 5.51, 5.58 (each 1H, dd, J=6, 13 Hz, H-6, 6′), 6.04 (1H, d, J=8 Hz, H-1′), 6.18 (1H, d, J=8 Hz, H-1″), 6.22, 6.23, 6.36, 6.37, 6.40 (each 1H, s, HHDP-H), 6.54 (1H, d, J=4 Hz, H-1), 6.55, 6.57, 6.70, 6.78, 6.87 (each 1H, s, HHDP-H and SS-H), 7.07, 7.14 (each 1H, d, J=2 Hz, SS-H), 7.15 (2H, s, galloyl H), 7.16, 7.24 (each 1H, d, J=2 Hz, SS-H). ^{13}C-NMR (65.05 MHz, acetone-d_6+D_2O): 63.2 (×3), 69.4 (×3), 71.3, 73.2, 74.0 (×2), 75.2, 75.8, 75.9, 76.7, 77.4, 90.8, 92.4 (×2) (glucose-C), 165.4, 165.5, 166.3, 166.4, 168.2, 168.4, 169.7 (–COO–).

Methylation of 8 A mixture of **8** (100 mg), dimethyl sulfate (3 ml) and anhydrous K_2CO_3 (3 g) in dry acetone (30 ml) was refluxed with stirring for 7 h. After removal of inorganic salts by filtration, the filtrate was concentrated *in vacuo*, and the residue was purified by chromatography over silica gel. Stepwise elution with benzene containing increasing amounts of acetone yielded the tritetracontamethyl ether (36 mg) as a white amorphous powder, $[\alpha]_D^{20}$ −49.5° (c=0.9, acetone). ^{1}H-NMR (270 MHz, $CHCl_3$): 3.43—4.00 (OMe), 4.95—5.63 (sugar-H), 5.88, 6.05 (each 1H, br s, H-1′, 1″), 6.40 (1H, s, aromatic H), 6.41 (1H, br s, H-1), 6.51 (2H), 6.62 (2H), 6.75, 6.81, 6.95 (2H), 7.01, 7.13 (2H), 7.14 (3H) (aromatic H).

Alkaline Hydrolysis of the Methylate A solution of the methylate (26 mg) in 10% NaOH (2 ml) and methanol (2 ml) was heated at 90 °C for 2 h. After cooling, the solution was acidified with 12 N HCl, and extracted twice with ether (10 ml). The ether layer was washed with water, dried over Na_2SO_4 and concentrated to give a residue, which was treated with ethereal CH_2N_2 for 30 min. The reaction mixture was separated by silica gel chromatography with benzene–acetone (47 : 3) to give methyl trimethoxybenzoate (0.8 mg), dimethyl (*S*)-hexa-*O*-methoxydiphenoate (**8a**) (16 mg), colorless syrup, $[\alpha]_D^{27}$ −28.4° (c=0.3, $CHCl_3$), and trimethyl (*S*)-octa-*O*-methylsanguisorboate (**8b**) (4.3 mg), colorless syrup, $[\alpha]_D^{27}$ −32.9° (c=0.2, $CHCl_3$).

Partial Hydrolysis of 8 A solution of **8** (500 mg) in water (10 ml) was heated under reflux for 60 h. The reaction mixture was separated by Sephadex LH-20 and MCI-gel CHP 20P chromatographies with water containing increasing proportions of methanol to give 2,3-(*S*)-HHDP-D-glucose (28 mg), sanguiin H-2 (**4**) (18 mg) and the hydrolysate (**9**) (76 mg) as a pale brown amorphous powder, $[\alpha]_D^{22}$ +19.5° (c=1.4, acetone). Negative FAB-MS m/z: 2037 $[M-H]^-$. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 3.88 (1H, d, J=14 Hz, H-6 or 6′), 3.89 (1H, dd, J=7, 10 Hz, H-5′), 3.93 (1H, d, J=14 Hz, H-6 or 6′), 4.40 (1H, dd, J=7, 10 Hz, H-5), 4.83 (1H, t, J=10 Hz, H-4′), 4.90 (1H, t, J=10 Hz, H-3′), 5.01 (1H, t, J=10 Hz, H-4), 5.06 (1H, dd, J=8, 10 Hz, H-2′), 5.22 (1H, t, J=10 Hz, H-3), 5.31 (1H, dd, J=4, 10 Hz, H-2), 5.57 (2H, dd, J=7, 14 Hz, H-6, 6′), 5.87 (1H, d, J=8 Hz, H-1′), 6.19, 6.30, 6.36, 6.40 (each 1H, s, HHDP-H), 6.53 (1H, d, J=4 Hz, H-1), 6.80, 6.83 (1H, s, SS-H), 7.06, 7.12 (each 1H, d, J=2 Hz, SS-H), 7.13 (2H, s, galloyl H), 7.17, 7.21 (each 1H, d, J=2 Hz, SS-H). This compound was identified as the product obtained previously by hydrolysis of sanguiin H-11 (**2**).

Lambertianin D (10) A pale brown amorphous powder, $[\alpha]_D^{21}$ −5.1° (c=0.9, acetone). *Anal.* Calcd for $C_{164}H_{106}O_{104}\cdot 13H_2O$: C, 49.56; H, 3.35. Found: C, 49.45; H, 3.14. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 3.92, 3.94, 3.96, 3.99 (each 1H, d, J=14, 14, 12 and 14 Hz, respectively, H-6, 6′, 6″, 6‴), 4.14, 4.21—4.37 (1H and 2H, respectively, m, H-5, 5′, 5″), 4.46 (1H, dd, J=7, 9 Hz, H-5‴), 4.96, 4.98, 5.04 (each 1H, t, J=10 Hz, H-4, 4′, 4″), 5.09 (2H, t, J=10 Hz, H-3′, 3″), 5.10 (1H, t, J=9 Hz, H-4‴), 5.13 (2H, t, J=10 Hz, H-2′, 2″), 5.18 (1H, t, J=9 Hz, H-2‴), 5.26 (1H, t, J=10 Hz, H-3), 5.32 (1H, dd, J=4, 10 Hz, H-2), 5.34 (1H, dd, J=7, 12 Hz, H-6‴), 5.43 (1H, t, J=9 Hz, H-3‴), 5.49, 5.54, 5.61 (each 1H, dd, J=6, 14 Hz, H-6, 6′, 6″), 6.05 (2H, d, J=8 Hz, H-1′, 1″), 6.19 (2H, s, HHDP-H), 6.20 (1H, d, J=9 Hz, H-1‴), 6.22 (1H, s, HHDP-H), 6.37 (×2), 6.40, 6.43, 6.53 (each s, HHDP-H), 6.54 (1H, d, J=4 Hz, H-1), 6.57, 6.71, 6.78, 6.81, 6.87 (each 1H, s, HHDP- and SS-H), 7.05—7.07, 7.11—7.14 (each 2H, SS-H), 7.16 (3H, br s, galloyl and SS-H), 7.23 (1H, d, J=2 Hz, SS-H). ^{13}C-NMR (270 MHz, acetone-d_6+D_2O): 63.1 (×4), 69.4 (×4), 71.3, 73.3, 74.0 (×3), 75.2, 75.9 (×2), 76.0, 76.7 (×2), 77.4, 90.8, 92.3, 92.4, 92.5 (glucose-C), 165.3, 165.4, 165.5, 166.3, 166.4, 168.2, 168.4, 168.5, 168.7, 168.8, 169.6 (–COO–).

Partial Hydrolysis of 10 A solution of **10** (20 mg) in water was heated under reflux for 27 h. The products were analyzed by HPLC in the same manner as described above, and the results are shown in Fig. 1.

HPLC Analysis of *Rubus* and *Sanguisorba* Species The fresh samples (5 g each) (0.5 g, when dried) were extracted three times with 80% aqueous acetone (50 ml) at room temperature. The combined extracts were concentrated *in vacuo*, and the resulting precipitates, consisting of chlorophylls, waxes, *etc.*, were removed by filtration. The filtrate was adjusted to 50 ml with 80% aqueous acetone, and an aliquot (5 ml) was pippetted out. After evaporation of the solvent *in vacuo*, the residue was redissolved in water and applied to a Sep Pak C_{18} cartridge. After washing with water, elution with 40% aqueous methanol gave the tannin fraction, which, after concentration, was redissolved in methanol (10 ml) and subjected to HPLC analysis: column, Cosmoisl 5 SL (4.6 mm i.d. × 250 mm); solvent, n-hexane–methanol–tetrahydrofuran–formic acid (100 : 30 : 10 : 1) containing oxalic acid (500 mg/l); flow rate, 0.7 ml/min; t_R, sanguiin H-6 (**1**) (22.3 min), lambertianin C (**8**) (36.3 min), sanguiin H-11 (**2**) (50.5 min), lambertianin C (**10**) (51.6 min).

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1) *a*) Part CXXI: H.-F. Chen, T. Tanaka, G. Nonaka, T. Fujioka, and K. Mihashi, *Phytochemistry*, "accepted"; *b*) Present address: *Faculty*

of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-cho, Nagasaki 852, Japan.

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