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C-Glucosidic ellagitannin oligomers from *Melaleuca squarrosa* Donn ex Sm., Myrtaceae

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Abstract

C-Glucosidic ellagitannin dimers were classified as types A–C according to a putative biogenetic oligomerization mode. They were characterized by different positions of the C–C bond between the phenolic acyl unit in one monomer and the benzylic C-1 of the open-chain glucose core in the other monomer. In recent years, four *C*-glucosidic tannins, melasquanins A–D (**18**–**21**), have been found in the leaves of *Melaleuca squarrosa* Donn ex Sm. (Myrtaceae). These are characterized as a dimer (melasquanin A) of a dimerization mode (type D), and trimers (melasquanins B–D) based on spectroscopic analysis including various two-dimensional nuclear magnetic resonance (2D NMR) experiments. Melasquanins B (**19**) and D (**21**) are *C*-glucosidic tannin trimers with a structure containing, non-repeating condensation modes, which was hitherto unknown.

Keywords: *Melaleuca squarrosa*; Myrtaceae; *C*-Glucosidic ellagitannin; Complex tannin; *C*-Glucosidic tannin trimer; Melasquanin B; Melasquanin C; Melasquanin D

1. Introduction

Vegetable tannins are divided into two large groups, hydrolyzable and condensed tannins. The former is further grouped into three sub-types; gallotannins, ellagitannins, and *C*-glucosidic tannins with an open-chain glucose core. Typical *C*-glucosidic tannins, castalagin (**1**) and vescalagin (**2**), having a flavogalloyl group were first characterized by Mayer et al. (1969,1971). Their analogs, casuarinin (**3**) and stachyurin (**4**), having a hexahydroxydiphenoyl (HHDP) group instead of the flavogalloyl unit, were later found in *Casuarina* and *Stachyurus* species (Okuda et al., 1980). This class of tannins was established to be widely distributed in many plant families such as in the Myrtaceae (Okuda et al., 1980), Combretaceae (Lin et al., 1991), Melastomataceae (Yoshida et al., 1992), Theaceae (Hatano et al., 1991), Lythraceae (Xu et al., 1991a,b), Betulaceae (Yoshida et al., 1991a), Elaeagnaceae (Yoshida et al., 1991b; Ito et al., 1999), Fagaceae (Mayer et al., 1969,1971; Nonaka et al., 1985), and Casuarinaceae (Okuda et al., 1983).

C-Glucosidic tannins show a unique reactivity at the anomeric center. For example, when castalagin (**1**) and casuarinin (**3**) with an α-hydroxyl group at C-1 are treated with boiling water, they are gradually isomerized to the respective anomers, vescalagin (**2**) (Mayer et al., 1967) and stachyurin (**4**) (Okuda et al., 1983). On the other hand, when **2** and **4** are treated with MeOH in the presence of trifluoroacetic acid at 37°C, they readily give corresponding 1-*O*-methylated derivatives with retention of configuration at C-1; **1** and **3** do not react under similar conditions (Yoshida et al., 1991c). The solvolysis of 1-*O*-methylated stachyurin in water is accompanied by retention of the

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configuration at C-1 to give **4** (Yoshida et al., 1991c). These results suggested that an α-site at this center suffers more severe steric hindrance compared to a β-site, and a β-site seems to be a convex site prone to nucleophilic attacks on a benzylic cation (C-1). Reflecting this reactivity, various condensates of the *C*-glucosidic tannin with other phenolic compounds have been found in nature. Such biogenetic condensates include "*C*-glucosidic tannin oligomers" and "complextannins," which are composed of *C*-glucosidic ellagitannin and catechin or epicatechin, e.g., stenophyllanins A (**5**) and B (**6**) (Nonaka et al., 1985).

Among more than 500 hydrolyzable tannins characterized to date, approximately 80 are *C*-glucosidic tannins or their condensates. Interestingly, all of these naturally occurring condensates have common structural features which include β-oriented C–C bonds between C-1 and the phenolic counterpart, and also all (*S*)-configurations at chiral HHDP and/or flavogalloyl units in the molecule.

2. Results and discussion

2.1. Structural diversity of C-glucosidic tannin dimers

Since the first discovery of *C*-glucosidic tannin dimers in 1991, about 30 dimers have been found in various plants. They can be classified into three types based on their biogenetic condensation modes. In type A, an anomeric carbon of a *C*-glucosidic monomeric unit binds to the benzene nucleus in an HHDP group at O-4/O-6 of the other *C*-glucosidic monomer [e.g., roburin A (**7**) (Herve du Penhoat et al., 1991) and alienanin B (**8**) (Nonaka et al., 1991; Yoshida et al., 1992)]. Dimers of this type have been found mainly in the Fagaceae (Herve du Penhoat et al., 1991; Nonaka et al., 1991; Peng et al., 1991; Tanaka et al., 1996) and in some species of the Elaeagnaceae (Yoshida et al., 1991b; Ito et al., 1999), Melastomataceae (Yoshida et al., 1992), and Combretaceae (Lin et al., 1991). In type B, the anomeric center of a monomer participates in the formation of a C–C bond with the O-5 galloyl group of the other monomer through apparent intermolecular dehydration as represented by casuglaunin A (**9**) (Shimokawa et al., 1991) and elaeagnatin B (**10**) (Ito et al., 1999). This type of dimer was found only in some elaeagnaceous plants and *Casuarina glauca*. The third type of condensation, C, leads to interesting dimers in which two moles of vescalagin type (or stachyurin type) monomer are connected through the A-ring of catechin. This type of tannin is quite rare in nature and only two such tannins, annogeissinin (**11**) (Lin et al., 1991) and casuglaunin B (**12**) (Shimokawa et al., 1991; Ito et al., 1999) were isolated from *Annogeissus acuminata* (Combretaceae) and *Casuarina glauca* (Casuarinaceae), respectively.

As for the *C*-glucosidic tannin oligomers reported to date, only castaneanins [dimer (**13**)–pentamer (**16**)] (Tanaka et al., 1996) from the heartwood of Japanese chestnut are known. They are characterized by repeating units derived from structures of castalagin (**1**) as a monomeric constituent in condensation mode A (Figs. 1–3).

In a recent phytochemical study, we found the third congener of type C dimer, cowaniin (**17**), in the rosaceous plant *Cowania mexicana* (Ito et al., 2007). Complex tannin dimer **17** was regarded as a biogenetical precursor of casuglaunin B (**12**) as substantiated by chemical transformation from **17** to **12**. We have also isolated **17** from *Melaleuca squarrosa* Donn ex Sm. (Myrtaceae), an evergreen shrub indigenous to southeastern Australia, which is rich in *C*-glucosidic tannins including four new *C*-glucosidic ellagitannin oligomers

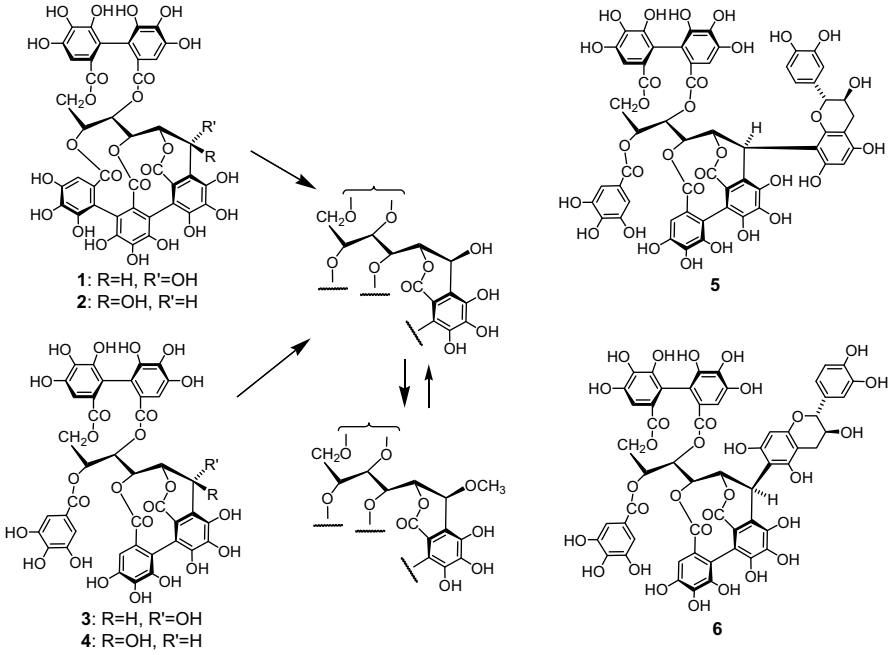


Fig. 1. Structures of compounds **1**–**6**.

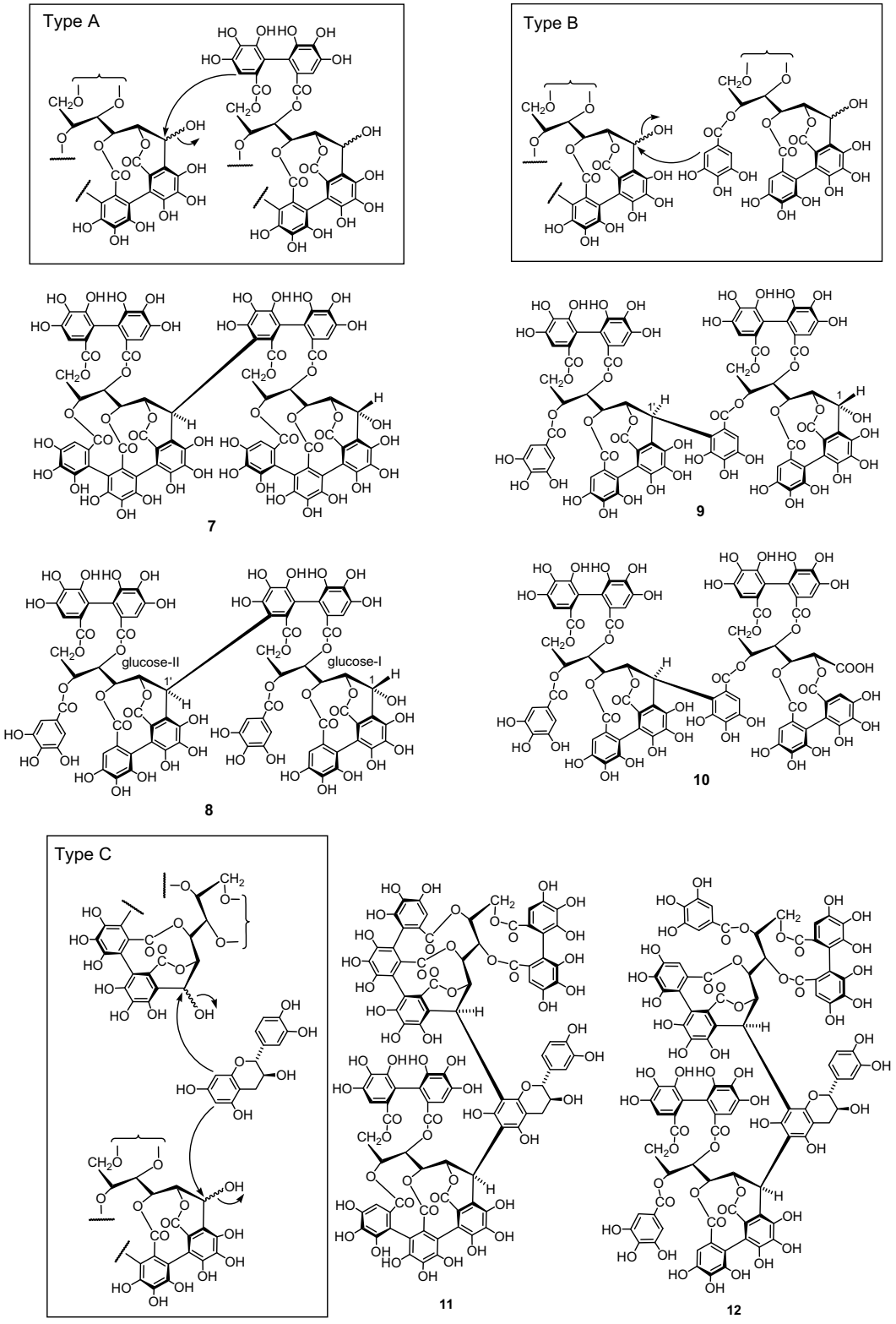
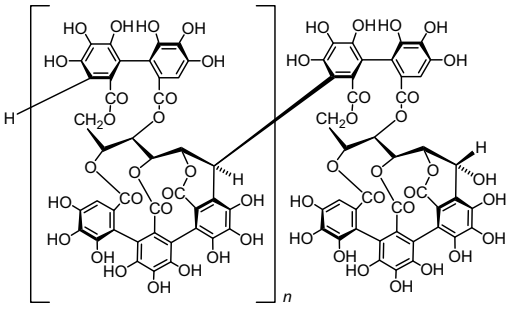


Fig. 2. Dimerization modes of *C*-glucosidic tannins (Types A–C) and their representative structures **7**–**12**.

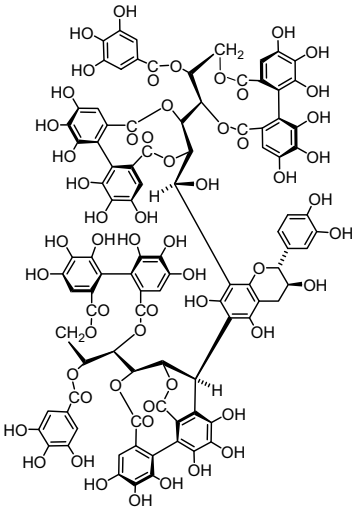
of different types. Here we describe in detail the isolation and characterization of the new tannins named melasquanins A–D (**18–21**) along with known tannins from *M. squarrosa*.

2.2. C-Glucosidic ellagitannin oligomers from M. squarrosa

A concentrated 70% aqueous acetone homogenate of the dried leaves of *M. squarrosa* was partitioned with



13: *n*=1
14: *n*=2
15: *n*=3
16: *n*=4



17

Fig. 3. Structures of compounds **13**–**17**.

Et_2O, EtOAc, and water-saturated *n*-BuOH to give the respective extracts and the water-soluble portion. The *n*-BuOH extract was fractionated by column chromatography over Diaion HP-20 followed by repeated column chromatography over polystylene and/or polyvinyl gels to yield cowaniin (**17**) (Ito et al., 2007) and melasquanin A (**18**), along with casuarinin (**3**) (Okuda et al., 1983), stachyurin (**4**) (Okuda et al., 1983), stenophyllanin A (**5**) (Nonaka et al., 1985), alienanin B (**8**) (Nonaka et al., 1991; Yoshida et al., 1992), casuglaunins A (**9**) (Shimokawa et al., 1991) and B (**12**) (Shimokawa et al., 1991; Ito et al., 1999), strictinin (Okuda et al., 1983), pedunculagin (Okuda et al., 1983), and pterocarinin A (Nonaka et al., 1989). The water-soluble portion was similarly separated by column chromatography to give melasquanins B (**19**), C (**20**), and D (**21**) as well as **8**, **9**, **12**, **17**, and pterocarinin A. Acid hydrolysis of the new tannins (**18**–**21**) yielded gallic acid and ellagic acid as the identifiable products, indicating that these tannins were ellagitannins possessing galloyl and HHDP units.

2.2.1. Structure of melasquanin A (*18*)

Melasquanin A (**18**) was obtained as a brownish powder and assigned a molecular formula of $C_{82}H_{54}O_{51}$, as derived from the ESIMS *m/z* 1872 $[M+NH_4]^+$, elemental analysis, and nuclear magnetic resonance (NMR) spectroscopic data. The dimeric nature of **18** was indicated by the ^1H NMR spectrum assigned by ^1H–^1H COSY, which exhibited two sets of sugar proton signals similar to those of *C*-glucosidic ellagitannins, **3** and **4**. Chemical shifts and coupling patterns of two anomeric proton signals [δ 4.65 (*br d*, $J = 1.0$ Hz, H-1′) and 5.55 (*d*, $J = 4.0$ Hz, H-1)] indicated the presence of a β-oriented C–C bond at the anomeric center (C-1′) and of the α-oriented hydroxyl group at the other anomeric center (C-1), as found in alienanin B (**8**). The ^1H NMR spectrum also displayed two 2H-singlets (δ 7.04 and 7.15) due to two galloyl groups and five 1H-singlets (δ 6.45, 6.51, 6.55, 6.75, and 6.99), while the ^{13}C NMR spectrum showed ten ester carbonyl carbon resonances at δ 164.9–169.9 along with eight signals characteristic of C-1 of the HHDP group (δ 104.8–117.0). These NMR spectroscopic data suggested that **18** was a *C*-glucosidic dimer isomeric to **8** and would be biogenetically derived from a new condensation mode of **3** and **4** different from type A. Upon comparing the ^{13}C NMR resonances of the glucose moieties between **18** and **8** (Table 1), the glucose-II core signals were similar, whereas the C-3–C-6 glucose-I core signals of **18** were considerably different from the corresponding signals of **8**. In the HMBC spectrum of **18**, all glucose proton signals except for H-3, H-1, and H-1′ showed three-bond correlations with the galloyl-H and HHDP-H through ester carbonyl carbons as illustrated in Fig. 4, indicating that the HHDP unit at O-2/O-3 of the glucose-I was fully substituted. The (*S*)-configurations at all chiral HHDP units in **18** were evidenced by a diagnostic Cotton effect at 237 nm (Okuda et al., 1982) in the circular dichroism (CD) spectrum,

Table 1
^{13}C NMR spectroscopic comparison for glucose moieties of **18**–**21** with those of **4**, **8**, and **9** in acetone-$d_6 + D_2O$

Position	4[a]	8[a]	9	18	19	20	21
C-1	64.4	67.4	67.0	68.8	68.9	67.3	67.1
C-2	80.8	77.0	76.7	76.9	77.0	77.1	76.4
C-3	71.6	69.2	70.9	72.2	72.0	69.2	68.7
C-4	72.7	74.59	75.1	75.3	75.7	74.6	74.2
C-5	70.6	69.4	73.0	72.3	71.7	69.5	68.9
C-6	64.0	65.6	64.1	64.7	65.4	65.66	65.2
C-1′		40.3	41.2	41.7	40.1	40.2	39.8
C-2′		80.8	81.7	81.8	80.8	81.1	79.6
C-3′		74.63	73.5	74.5	74.5	74.0	74.9
C-4′		73.3	74.0	73.4	73.29	73.5	72.4
C-5′		71.5	71.2	71.4	71.35	69.7	73.3
C-6′		64.3	63.8	64.3	64.4	65.66	63.1
C-1″					41.6	40.3	41.0
C-2″					81.8	80.9	81.2
C-3″					74.7	74.7	75.1
C-4″					73.29	73.3	73.4
C-5″					71.40	71.5	70.9
C-6″					64.2	64.3	63.8

[a] Assignments for these compounds were revised from previously reported data (Yoshida et al., 1992,1996) in this study.

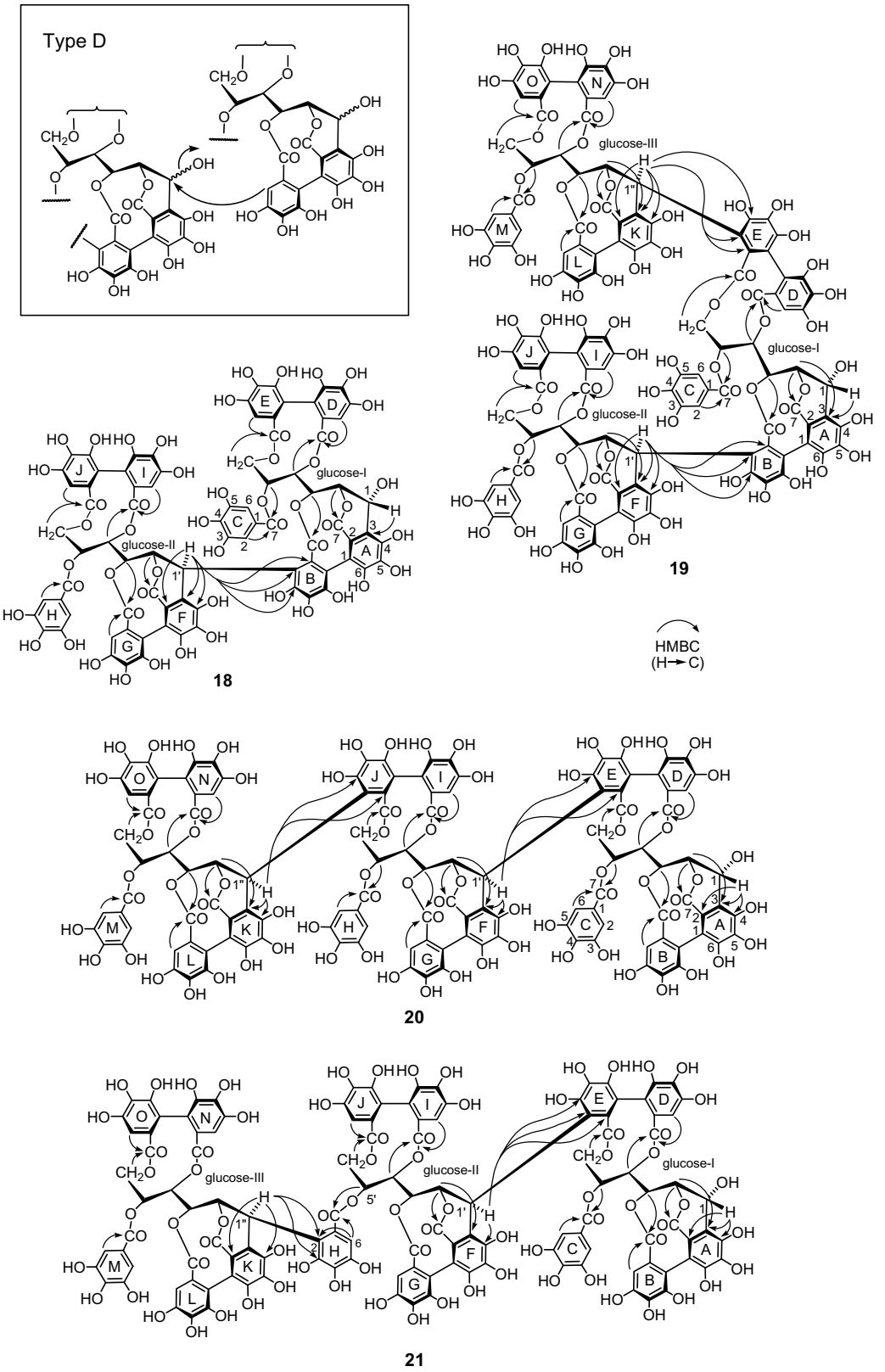


Fig. 4. Structures of compounds **18**–**21**.

although its amplitude ($[\theta]$ 21.8×10^4) was smaller than that of alienanin B (8) ($[\theta]_{232}$ 27.7×10^4). This discrepancy is discussed later.

Consequently, the structure of melasquanin A was represented by the formula **18**, and this new type of *C*-glucosidic tannin dimer was classified as type D.

It should be noted that the *M. squarrosa* leaves contain all types (A–D) (**8**, **9**, **12**, and **18**) of *C*-glucosidic ellagitannin dimers. These tannins, except for **12**, have common features that include the casuarinin part as a terminal unit as well as a β-oriented C–C bond at C-1 of the extension unit. Upon comparing the ^{1}H NMR spectroscopic data for the glucose moiety of the casuarinin part in **8**, **9**, and **18**, one of the C-6 methylene proton signals in type A (**8**) appeared at an unusual high field around δ 2.99, while the H-5 signal in the type-D dimer (**18**) showed a remarkable shift upfield by about 1.5 ppm (δ 3.79) compared to that of **3** (Table 2). On the other hand, a type-B dimer (**9**) had glucose proton signals comparable to those of **3**. Therefore, the H-6 or H-5 signal of the terminal monomer possessing the anomeric hydroxyl group in types-A, -B, and -D dimers was diagnostic to distinguish the condensation mode of *C*-glucosidic ellagitannins.

2.2.2. Structure of melasquanin B (19)

The trimeric nature of melasquanin B (**19**) was suggested by an $[M+NH_4]^+$ ion peak at *m/z* 2790 in its ESIMS spectrum, which is *m/z* 918 (corresponding to a monomeric unit) larger than that of **18**. In addition, a longer retention time than **18** was observed in normal-phase HPLC (Okuda et al., 1989). The ^{1}H NMR spectrum showed three 2H-singlets due to three galloyl groups and seven 1H-singlets attributable to HHDP groups in the aromatic proton region. The spectrum also exhibited three anomeric proton signals characteristic of *C*-glucosidic tannin at δ 5.57 (*d*, $J = 4.0$ Hz, H-1), 4.71 (*d*, $J = 1.0$ Hz, H-1′) and 4.61 (*br s*, H-1′′). The proton resonances of each glucose core were assigned starting from the anomeric proton resonances by analysis of COSY and TOCSY experiments. The H-5 and H-6 proton resonances of the glucose-I core in **19** were observed at δ 3.53 (*br dd*, $J = 3.5$, 10.5 Hz) and δ 3.11 (*d*, $J = 13.0$ Hz), which were comparable to the diagnostic shifts for the type-D and type-A dimer, respectively. The HMQC spectra of **19** also established glucose carbon resonances corresponding well to those of **18** and the glucose-II part of **8** (Table 1). The linking mode of the monomeric units was verified from the detailed HMBC experiment, which showed long-range correlations between glucose protons and acyl protons through ester carbonyl carbons in a similar fashion to those found in **8** and **18**, as shown in Fig. 4. The (*S*)-configuration of the HHDP units in **19** was indicated by a positive Cotton effect at 235 nm ($[\theta]$ 32.2×10^4) in the CD spectrum (Fig. 5), the intensity of which was consistent with the presence of two more (*S*)-HHDP groups than **18**. Based on these spectroscopic data, the structure **19** was deduced for melasquanin B.

2.2.3. Structure of melasquanins C (20) and D (21)

Melasquanins C (**20**) and D (**21**) showed pseudomolecular ion peaks $[M+NH_4]^+$ at *m/z* 2790 in the positive ESIMS, similarly to **19**. These trimers were condensates of the dimer, alienanin B (**8**), with an additional stachyurin by type-A or -B condensation modes as indicated by the following findings. The ^{1}H NMR spectra showed signals

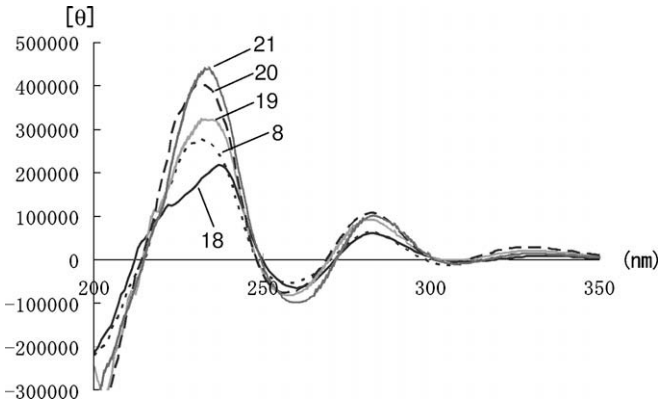


Fig. 5. CD spectra of compounds **18**–**21** and **8**.

Table 2
^{1}H NMR spectroscopic data for the glucose moieties of **3**, **8**, **9**, and **18** in acetone-d_6 + D_2O

Position	**3**[a]	**8**[b]	**9**[b]	**18**[a]
H-1	5.60 (*d*, 5.0)	5.62 (*d*, 4.8)[c]	5.51 (*d*, 4.8)	5.55 (*d*, 4.0)
H-2	4.63 (*dd*, 2.5, 5.0)	4.61 (*dd*, 1.8, 4.8)	4.72 (*dd*, 1.8, 4.8)	4.70 (*t*, 4.0)
H-3	5.42 (*t*, 2.5)	5.37 (*t*, 1.8)	5.46 (*br t*, 1.8)	4.82 (*dd*, 1.0, 4.0)
H-4	5.44 (*dd*, 2.5, 8.5)	5.28 (*dd*, 1.8, 9.0)	5.45 (*br dd*, 1.8, 7.2)	5.05 (*dd*, 1.0, 10.5)
H-5	5.31 (*dd*, 2.5, 8.5)	5.18 (*dd*, 1.8, 9.0)	5.39 (*br dd*, 2.4, 7.2)	3.79 (*dd*, 3.5, 10.5)
H-6	4.84 (*dd*, 2.5, 13.0)	4.03 (*dd*, 1.8, 12.6)	5.00 (*dd*, 2.4, 13.2)	4.60 (*dd*, 3.5, 13.5)
	4.04 (*d*, 13.0)	2.99 (*d*, 12.6)	4.30 (*d*, 13.2)	4.07 (*d*, 13.5)
H-1′		4.75 (*d*, 1.2)	4.81 (*br s*)	4.65 (*br d*, 1.0)
H-2′		4.92 (*br t*, 1.8)	4.91 (*br s*)	4.84 (*br t*, 1.5)
H-3′		4.98 (*br t*, 1.8)	5.22 (*t*, 1.8)	4.97 (*t*, 1.5)
H-4′		5.63 (*dd*, 1.8, 9.0)	5.80 (*dd*, 1.8, 7.8)	5.66 (*dd*, 1.5, 8.5)
H-5′		5.27 (*dd*, 3.0, 9.0)	5.35 (*dd*, 3.0, 7.8)	5.35 (*dd*, 3.5, 8.5)
H-6′		4.79 (*dd*, 3.0, 12.6)	4.95 (*dd*, 3.0, 13.2)	4.87 (*dd*, 3.5, 13.5)
		4.03 (*d*, 12.6)	4.03 (*d*, 13.2)	4.03 (*d*, 13.5)

[a] 500 MHz.
[b] 600 MHz.
[c] *J* values (Hz) are in parentheses.

due to three galloyl groups at δ 7.12 (4H) and 7.11 (2H) and seven 1H-singlets for **20**, and two 2H-singlets due to two galloyl groups and nine 1H-singlets for **21**. Complete ^{1}H and ^{13}C NMR assignments for three glucose units in each tannin were achieved by 2D NMR analysis (Table 1). Among the glucose carbon signals of both compounds in the ^{13}C NMR spectra, those due to two glucose residues showed a close similarity to those of alienanin B (**8**), implying that **20** and **21** were trimers composed of **8** and a monomeric unit. The terminal monomeric unit of **20** and **21** had an α-hydroxyl group as indicated by a doublet ($J = 4.8$ Hz) of H-1 at δ 5.60 and 5.61, respectively. Melasquanin C (**20**) displayed signals due to the C-6 methylene protons of each glucose core at δ 2.93 (*d*, $J = 12.6$ Hz)/4.03 (dd, $J = 3.6$, 12.6 Hz), 2.96 (*d*, $J = 13.2$ Hz)/4.01 (*dd*, $J = 3.6$, 13.2 Hz), and 4.05 (*d*, $J = 13.2$ Hz)/4.78 (*dd*, $J = 2.4$, 13.2 Hz) in the ^{1}H NMR spectrum. Two signals at around δ 2.9 were indicative of two type-A linking modes in the molecule, suggesting that the third monomeric unit was linked via the type-A mode to the alienanin B part in **20**. The assumed structure **20** was verified by HMBC, which showed long-range correlations as illustrated in Fig. 4.

In the ^{1}H NMR spectrum of melasquanin D (**21**), the H-6 proton signal characteristic of the type-A alienanin B part was observed at δ 3.04 (*d*, $J = 13.2$ Hz), while the other C-6 methylene protons appeared in the normal region. Taking one less galloyl and two more 1H-singlets compared to those of **20** into consideration, one of the galloyl groups in **8** was thought to participate in a C–C bond formation with the anomeric carbon of the third monomer. This suggested it was a trimer produced by a combination of type-A and type-B condensation of **3** and/or **4**. In the HMBC spectrum of **21**, the H-1'' signal (δ 4.81, *br s*) showed long-range correlations with two galloyl carbons at δ 146.1 and 120.7, the latter of which also correlated with the galloyl proton (ring-H, H-6) signal at δ 6.88. This galloyl group was located on the C-5′ of glucose-II by the correlation between the galloyl proton and H-5′ signals through an ester carbonyl carbon (δ 167.5). Other long-range couplings, as observed in Fig. 4, were consistent with the structure **21** for melasquanin D. The *S*-configurations at the biphenyl moieties in both **20** and **21** were determined by strong positive Cotton effects at 231 and 234 nm, respectively, in their CD spectra (Fig. 5). The intensity of the Cotton effect at ca. 230 nm in ellagitannins was demonstrated to be additive for the number of HHDP unit irrespective of its binding position (Okuda et al., 1982). When the amplitude ([θ] 6.9×10^4) for one (*S*)-HHDP unit estimated from the CD of the known dimer **8** is adopted, the Cotton effects of **20** and **21** well correspond to those due to six (*S*)-HHDP groups in the molecules. Smaller amplitudes of the corresponding Cotton effects in **18** and **19** can be explained by a smaller twisted angle between the biphenyl rings at O-2/O-3 forsed by intermolecular linkage, than that of **8**, **20** and **21**. A slight bathochromic shift (ca. 5 nm) of the Cotton effect in **18** compared with **8** (232 nm) may reflect such reduced twisted angle (Fig. 5).

2.2.4. Concluding remarks

To the best of our knowledge, melasquanins B (**19**) and D (**21**) are the first examples of the *C*-glucosidic ellagitannin trimers possessing a structure consisting of non-repeating units.

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. Elemental analyses were recorded on a Yanaco CHN recorder MT-5. UV spectra were obtained on a HITACHI U-2000-10 spectrophotometer and CD spectra on a JASCO J-820W spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian VXR-500 (500-4 for ^{1}H and 126 MHz for ^{13}C) or Varian INOVA 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometers with chemical shifts given in δ (ppm) values relative to those of the solvent [acetone-d_6 (δ_H 2.04; δ_C 29.8)] on a tetramethylsilane scale. The standard pulse sequences that were programmed into the instrument (VXR-500) were used for each two-dimensional measurement. The J_{CH} value was set at 6 Hz in the HMBC spectra. ESIMS spectra were obtained on a Micromass Auto Spec OA-Tof mass spectrometer (solvent: MeOH–H_2O (1:1, v/v) containing 0.1% NH_4OAc). Normal phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC Co., Ltd.) column (4.6 i.d. × 250 mm) eluted with *n*-hexane/MeOH/tetrahydrofuran/HCO_2H (55:33:11:1) containing oxalic acid (450 mg/1 L; flow rate: 1.5 mL/min; 280 nm UV detection) at room temperature. Reversed-phase HPLC was performed on a YMC-Pack ODS-A A-302 (YMC Co., Ltd.) column (4.6 i.d. × 150 mm) eluted with 10 mM H_3PO_4/10 mM KH_2PO_4/CH_3CN (44:44:12) [flow rate: 1.0 mL/min; detection, 280 nm UV or diode array detector (DAD; HITACHI L-7445), 200–600 nm] at 40 °C. Column chromatography was carried out on Diaion HP-20, MCI GEL CHP-20P (Mitsubishi Kasei Co.), Toyopearl HW-40 (coarse grade; Tosoh Co.), and Sephadex LH-20 (Pharmacia).

3.2. Plant material

Leaves of *M. squarrosa* were supplied from the Herbal Garden of POLA Chemical Industries, Inc. A voucher specimen OKP-MY98005 is deposited at the Medicinal Herbal Garden of the Faculty of Pharmaceutical Sciences, Okayama University.

3.3. Isolation and purification of tannins

Dried leaves of *M. squarrosa* (1.4 kg) were homogenized in H_2O-acetone (3:7, v/v, 11 L). The filtered homogenate was concentrated to 1 L and extracted with Et_2O (1 L × 3), EtOAc (1 L × 3), and *n*-BuOH (1 L × 3), successively, to furnish the Et_2O (53.7 g), EtOAc (35.4 g), *n*-BuOH (82.9 g) extracts, and a water-soluble portion

(135.8 g). Fractionations were achieved by monitoring normal- and/or reversed-phase HPLC. The *n*-BuOH extract (82.9 g) was subjected to Diaion HP-20 (6.5 i.d. × 70 cm) chromatographed with H_2O → aqueous MeOH (10% → 20% → 30% → 50% → 100% MeOH) → H_2O–acetone (3:7, v/v). The H_2O–MeOH (1:4, v/v) eluate was further applied to Toyopearl HW-40 (2.2 i.d. × 60 cm) with aqueous MeOH (50% → 60% → 70% MeOH → MeOH–H_2O–acetone (7:2:1) → H_2O–acetone (3:7)) and MCI GEL CHP-20P (1.1 i.d. × 40 cm) columns, eluted with aqueous MeOH (10% → 20% → 30% → 50% → 100% MeOH) to give strictinin (28.9 mg), pedunculagin (23.0 mg), pterocarinin A (120.4 mg), casuarinin (**3**) (482.6 mg), stachyurin (**4**) (35.3 mg), stenophyllanin A (**5**) (47.2 mg), and alienanin B (**8**) (96.8 mg). The H_2O–MeOH (7:3, v/v) eluate was purified by column chromatography over Toyopearl HW-40 (2.2 i.d. × 60 cm) with aqueous MeOH (50% → 60% → 70% MeOH → MeOH–H_2O–acetone (7:2:1) → MeOH–H_2O–acetone (7:1:2) → H_2O–acetone (3:7)) and MCI GEL CHP-20P (1.1 i.d. × 50 cm) columns eluted with aqueous MeOH (10% → 20% → 30% → 50% → 100% MeOH) to yield strictinin (64.3 mg), **3** (394.7 mg), **5** (66.6 mg), **8** (121.7 mg), casuglaunins A (**9**) (18.8 mg) and B (**12**) (14.8 mg), cowaniin (**17**) (7.0 mg), and melasquanin A (**18**) (13.9 mg). The water-soluble portion (37.58 g) was subjected to column chromatography over Diaion HP-20 CC (6.5 i.d. × 45 cm) with H_2O → aqueous MeOH (10% → 20% → 30% → 50% → 100% MeOH) → H_2O–acetone (3:7, v/v). The H_2O–MeOH (7:3, v/v) eluate was further applied to Toyopearl HW-40 (2.2 i.d. × 65 cm) with aqueous MeOH (70% MeOH → MeOH → MeOH–H_2O–acetone (7:2:1) → MeOH–H_2O–acetone (7:1:2) → H_2O–acetone (3:7)), MCI GEL CHP-20P (1.1 i.d. × 40 cm) with aqueous MeOH (20% → 30% → 40% → 50% → 100% MeOH) and Sephadex LH-20 (1.1 i.d. × 40 cm) with EtOH and MeOH to give pterocarinin A (701.6 mg), **3** (193.5 mg), **8** (747.3 mg), **9** (7.6 mg), and melasquanins C (**20**) (33.6 mg) and D (**21**) (27.0 mg). The eluate of the separation via MeOH–H_2O (1:1, v/v) (3.57 g) over Diaion HP-20 was similarly subjected to CC over Toyopearl HW-40 (2.2 i.d. × 45 cm) with aqueous MeOH (70% MeOH → MeOH → MeOH–H_2O–acetone (7:2:1) → MeOH–H_2O–acetone (7:1:2) → H_2O–acetone (3:7)) and MCI GEL CHP-20P (1.1 i.d. × 40 cm) with aqueous MeOH (20% → 30% → 40% → 50% → 100% MeOH) to yield **8** (7.6 mg), **9** (11.4 mg), casuglaunin B (**12**) (68.2 mg), **17** (17.2 mg), and melasquanin B (**19**) (9.4 mg). The remainder (78.21 g) of the portion was similarly separated and purified by a combination of column chromatographic steps over Diaion HP-20, Toyopearl HW-40, and MCI GEL CHP-20P with aqueous MeOH, to give **8** (265.8 mg), **12** (236.1 mg), and **19** (35.3 mg).

3.4. Melasquanin A (*18*)

A brownish amorphous powder; $[\alpha]_D^{23}$ + 39.1 (*c* 1.0, MeOH); CD (MeOH) [θ] (nm): $+21.8 \times 10^4$ (237), -6.4×10^4 (260), $+6.1 \times 10^4$ (283); ^{1}H NMR spectroscopic data (500 MHz, acetone-d_6 + D_2O): δ 6.45 (1H, *s*, H_E-3), 6.51, 6.55 (each 1H, *s*, $H_{J,G}$-3), 6.75 (1H, *s*, H_I-3), 6.99 (1H, *s*, H_D-3), 7.04 (2H, *s*, H_C-2,6), 7.15 (2H, *s*, H_H-2,6); for sugar protons, see Table 2; ^{13}C NMR spectroscopic data (126 MHz, acetone-d_6 + D_2O): δ 110.0 (2C, C_H-2,6), 110.5 (2C, C_C-2,6), 106.0 (C_G-3), 106.6 (C_E-3), 107.4 (C_J-3), 108.9 (C_I-3), 109.2 (C_D-3), 114.8, 115.1, 115.4, 115.48, 115.50, 115.8, 115.6 (2C), 116.3, 116.6, 117.0 ($C_{A,B,D–G,I,J}$-1, $C_{A,B,F}$-3), 125.0, 125.9 ($C_{C,H}$-1), 120.4, 120.4, 120.7, 123.9, 126.2, 126.76, 126.84, 127.6 ($C_{A,B,D—G,I,J}$-2), 139.37, 139.39 ($C_{C,H}$-4), 134.2, 135.8, 136.0, 136.2, 136.4, 136.6, 137.3, 139.7 ($C_{A,B,D–G,I,J}$-5), 145.8, 146.1 (each 2C, $C_{C,H}$-3,5), 143.5, 143.8, 143.9, 144.0, 144.36, 144.37, 144.6, 144.8, 145.0, 145.15, 145.19, 145.22, 145.7, 146.1, 146.2, 146.9 ($C_{A,B,D–G,I,J}$-4,6), 164.9 (C_A-7), 165.3 (C_F-7), 166.0 (C_H-7), 166.5 (C_C-7), 168.3 (C_I-7), 169.0 (C_J-7), 169.1 (C_G-7), 169.4 (C_D-7), 169.6 (C_E-7), 169.9 (C_B-7); for sugar carbons, see Table 1; ESIMS *m/z*: 1872 $[M+NH_4]^+$; *Anal.* Found: C, 45.4; H, 4.23. $C_{82}H_{54}O_{51} \cdot 17H_2O$ requires: C, 45.6; H, 4.07%.

3.5. Melasquanin B (*19*)

A brownish amorphous powder; $[\alpha]_D^{23}$ + 41.2 (*c* 1.0, MeOH); CD (MeOH) [θ] (nm): $+32.2 \times 10^4$ (232), -8.2×10^4 (258), $+9.3 \times 10^4$ (282); ^{1}H NMR spectral data (500 MHz, acetone-d_6 + D_2O): δ 3.11 (1H, *d*, *J* = 13.0 Hz, H-6), 3.53 (1H, *br dd*, *J* = 3.5, 10.5 Hz, H-5), 3.83 (1H, *dd*, *J* = 3.5. 13.0 Hz, H-6), 4.02 (1H, *br d*, *J* = 13.0 Hz, H-6''), 4.03 (1H, *br d*, *J* = 13.0 Hz, H-6′), 4.61 (1H, *br s*, H-1''), 4.65 (1H, *t*, *J* = 4.0 Hz, H-2), 4.71 (1H, *d*, *J* = 1.0 Hz, H-1′), 4.75 (1H, *dd*, *J* = 1.0, 4.0 Hz, H-3), 4.77 (1H, *br s*, H-2''), 4.77 (1H, *br dd*, *J* = 3.0, 13.0 Hz, H-6′), 4.85 (1H, *br dd*, *J* = 3.0, 13.0 Hz, H-6''), 4.89 (1H, *br d*, *J* = 10.5 Hz, H-4), 4.90 (1H, *br t*, *J* = 2.0 Hz, H-3''), 4.91 (1H, *br t*, *J* = 1.0 Hz, H-2′), 4.97 (1H, *t*, *J* = 1.0 Hz, H-3′), 5.25 (1H, *br dd*, *J* = 3.0, 8.5 Hz, H-5''), 5.27 (1H, *br dd*, *J* = 3.0, 8.5 Hz, H-5′), 5.57 (1H, *d*, *J* = 4.0 Hz, H-1), 5.60 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-4), 5.63 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-4''), 6.47 (1H, *s*, H_L-3), 6.53 (1H, *s*, H_O-3), 6.538, 6.541 (each 1H, *s*, $H_{G,J}$-3), 6.73, 6.75 (each 1H, *s*, $H_{I,N}$-3), 6.89 (1H, *s*, H_D-3), 7.06 (2H, *s*, H_C-2, 6), 7.08, 7.14 (each 2H, *s*, $H_{H,M}$-2,6); ^{13}C NMR spectroscopic data (126 MHz, acetone-d_6 + D_2O): δ 110.0 (4C, $C_{H,M}$-2,6), 111.00 (2C, C_C-2,6), 106.1 (C_L-3), 106.3 (C_D-3), 107.2, 107.3 ($C_{G,J}$-3), 108.89 (3C, $C_{D,I,N}$-3), 114.0, 114.3, 114.8, 115.0, 115.3, 115.4 (2C), 115.6 (2C), 115.8 (2C), 116.1, 116.3, 116.9 (3C), 117.0 ($C_{A,B,D–G,I–L,N,O}$-1, $C_{A,B,E,F,K}$-3), 120.7 (2C), 121.6 ($C_{C,H,M}$-1), 120.4, 123.3, 124.8, 124.9, 125.0, 125.8, 125.9, 126.1, 126.2, 126.8, 126.8, 126.9 ($C_{A,B,D–G,I–L,N,O}$-2), 134.1, 134.3, 135.9, 136.0, 136.07, 136.13, 136.6, 136.7, 137.07, 137.13 (2C), 138.3 ($C_{A,B,D–G,I—L,N,O}$-5), 139.2, 139.27, 139.33 ($C_{C,H,M}$-4), 143.0, 143.3, 143.7 (2C), 143.8, 143.9, 144.27, 144.34 (3C), 144.5 (2C), 145.08 (2C), 145.17 (3C), 145.28, 145.34, 145.7, 145.9 (3C), 146.2 ($C_{A,B,D–G,I–L,N,O}$-4,6), 145.3, 145.8, 146.0 (each 2C, $C_{C,H,N}$-3,5), 164.8

(C_A-7), 165.2 (C_K-7), 165.9 (2C, $C_{H,M}$-7), 166.9 (C_F-7), 167.1 (C_C-7), 168.09, 168.12 ($C_{I,N}$-7), 168.8 (C_E-7), 168.96 (C_O-7), 169.02 (C_J-7), 169.1 (C_G-7), 169.3 (C_L-7), 169.4 (C_D-7), 169.6 (C_B-7); for sugar carbons, see Table 1; ESIMS *m/z*: 2790 $[M+NH_4]^+$; *Anal.* Found: C, 48.2; H, 3.97. $C_{123}H_{80}O_{76} \cdot 17H_2O$ requires: C, 48.0; H, 3.70%.

3.6. Melasquanin C (**20**)

A brownish amorphous powder; $[\alpha]_D^{23}$ + 54.6 (*c* 1.0, MeOH); CD (MeOH) [θ] (nm): $+40.3 \times 10^4$ (231), -7.5×10^4 (257), $+10.8 \times 10^4$ (282); ^{1}H NMR spectroscopic data (600 MHz, acetone-$d_6 + D_2O$): δ 2.93 (1H, *d*, $J = 12.6$ Hz, H-6′), 2.96 (1H, *d*, $J = 13.2$ Hz, H-6), 4.01 (1H, *dd*, $J = 3.6$, 13.2 Hz, H-6), 4.03 (1H, *dd*, $J = 3.6$, 12.6 Hz, H-6′), 4.05 (1H, *d*, $J = 13.2$ Hz, H-6″), 4.60 (1H, *dd*, $J = 1.8$, 4.8 Hz, H-2), 4.70 (1H, *d*, $J = 1.2$ Hz, H-1′), 4.75 (1H, *d*, $J = 1.2$ Hz, H-1″), 4.78 (1H, *dd*, $J = 2.4$, 13.2 Hz, H-6″), 4.83 (1H, *t*, $J = 1.2$ Hz, H-2′), 4.89 (1H, *dd*, $J = 1.2$, 1.8 Hz, H-3′), 4.91 (1H, *dd*, $J = 1.2$, 1.8 Hz, H-2″), 4.99 (1H, *t*, $J = 1.8$ Hz, H-3″), 5.11 (1H, *dd*, $J = 3.6$, 9.0 Hz, H-5′), 5.15 (1H, *dd*, $J = 3.6$, 9.0 Hz, H-5), 5.25 (1H, *dd*, $J = 1.8$, 9.0 Hz, H-4), 5.28 (1H, *dd*, $J = 2.4$, 8.4 Hz, H-5″), 5.35 (1H, *t*, $J = 1.8$ Hz, H-3), 5.50 (1H, *dd*, $J = 1.8$, 9.0 Hz, H-4′), 5.60 (1H, *d*, $J = 4.8$ Hz, H-1), 5.63 (1H, *dd*, $J = 1.8$, 8.4 Hz, H-4″), 6.38 (1H, *s*, H_G-3), 6.42 (1H, *s*, H_B-3), 6.46 (1H, *s*, H_L-3), 6.56 (1H, *s*, H_O-3), 6.65 (1H, *s*, H_I-3), 6.71 (1H, *s*, H_D-3), 6.78 (1H, *s*, H_N-3), 7.11 (2H, *s*, H_H-2,6), 7.12 (4H, *s*, $H_{C,M}$-2,6); ^{13}C NMR spectroscopic data (150 MHz, acetone-$d_6 + D_2O$): δ 110.0 (2C, C_H-2,6), 110.3, 110.4 (each 2C, $C_{C,M}$-2,6), 105.2 (C_B-3), 105.6 (C_G-3), 105.8 (C_L-3), 107.3 (C_O-3), 107.7 (C_D-3), 108.1 (C_I-3), 108.9 (C_N-3), 113.7, 113.8, 114.1, 114.4, 115.4, 115.45 (2C), 115.54, 115.8 (2C), 116.2, 116.3, 116.5, 116.6 ($C_{A,B,D—G,I—L,N,O}$-1, $C_{E,J}$-3), 116.4 (2C, $C_{F,K}$-3), 117.2 (C_A-3), 120.2, 121.66, 121.71 ($C_{C,H,M}$-1), 120.7, 123.9, 124.1, 124.9, 125.0, 125.1, 126.1, 126.2, 126.78, 126.82, 126.9, 127.5 ($C_{A,B,D–G,I–L,N,O}$-3), 132.0, 134.3, 134.5, 134.9, 135.67, 135.73, 136.1, 136.7, 136.87, 136.90, 137.0, 138.6 ($C_{A,B,D–G,I–L,N,O}$-5), 138.9, 139.0, 139.3 ($C_{C,H,M}$-4), 143.3 (C_F-4), 143.4 (C_K-4), 143.67 (C_A-4), 144.3 (2C, $C_{E,J}$-4), 143.6, 143.7 (2C), 143.9, 144.0, 144.4, 144.5, 145.15, 145.22, 145.3, 145.35, 145.38, 145.46, 145.50, 145.58, 145.61, 145.7, 145.8, 146.2 ($C_{B,D,G,I,L,N,O}$-4, $C_{A,B,D–G,I–L,N,O}$-6), 145.70, 145.71, 146.0 (each 2C, $C_{C,H.M}$-3,5), 164.8 (C_A-7), 166.00 (C_M-7), 166.02 (C_F-7), 166.17 (C_H-7), 166.22 (C_K-7), 166.3 (C_C-7), 168.1 (C_I-7), 168.2 (C_N-7), 168.3 (C_J-7), 168.4 (C_E-7), 168.6 (C_D-7), 168.9 (C_O-7), 169.1 (C_G-7), 169.3 (C_B-7), 169.4 (C_L-7); for sugar carbons, see Table 1; ESIMS *m/z*: 2790 $[M+NH_4]^+$; *Anal.* Found: C, 46.5; H, 3.99. $C_{123}H_{80}O_{76} \cdot 23H_2O$ requires: C, 46.3; H, 3.95%.

3.7. Melasquanin D (**21**)

Brownish amorphous powder; $[\alpha]_D^{23}$ + 58.4 (*c* 1.0, MeOH); CD (MeOH) [θ] (nm): $+43.9 \times 10^4$ (234), -9.8×0^4 (259), $+10.1 \times 10^4$ (283); ^{1}H NMR spectroscopic data (600 MHz, acetone-$d_6 + D_2O$): δ 3.04 (1H, *d*, $J = 13.2$ Hz, H-6), 4.02 (1H, *dd*, $J = 4.2$, 12.6 Hz, H-6), 4.07 (1H, *br d*, $J = 12.6$ Hz, H-6″), 4.44 (1H, *br s*, H-6′), 4.62 (1H, *dd*, $J = 1.8$, 4.8 Hz, H-2), 4.81 (1H, *br s*, H-1″), 4.87 (1H, *br s*, H-1′), 4.90 (1H, *dd*, $J = 3.0$, 13.2 Hz, H-6″), 4.95 (1H, *br d*, $J = 11.4$ Hz, H-6′), 4.98 (1H, *br s*, H-2′), 5.06 (1H, *br s*, H-2″), 5.14 (1H, *br s*, H-3′), 5.20 (1H, *dd*, $J = 4.2$, 9.0 Hz, H-5), 5.29 (1H, *br s*, H-3″), 5.33 (1H, *br s*, H-4), 5.35 (1H, *m*, H-5″), 5.37 (1H, *t*, $J = 1.8$ Hz, H-3), 5.41 (1H, *br d*, $J = 7.2$ Hz, H-5′), 5.58 (1H, *br s*, H-4′), 5.61 (1H, *d*, $J = 4.8$ Hz, H-1), 5.86 (1H, *br s*, H-4″), 6.34, 6.54, 6.59, 6.71 (each 1H, *br s*, $H_{G,I,L,N}$-3), 6.42 (1H, *s*, H_B-3), 6.55 (1H, *s*, H_J-3), 6.69 (1H, *s*, H_O-3), 6.94 (1H, *br s*, H_D-3), 6.88 (1H, *br s*, H_H-6), 7.13 (2H, *s*, H_C-2,6), 7.19 (2H, *s*, H_M-2,6); ^{13}C NMR spectroscopic data (150 MHz, acetone-$d_6 + D_2O$): δ 104.6 (C_B-3), 107.2 (C_O-3), 107.4 (C_J-3), 108.4 (C_D-3), 105.0, 107.2, 108.2 (2C), 109.7 ($C_{G,I,K,L,N}$-3), 109.6 (2C, C_C-2,6), 110.0 (2C, C_M-2,6), 110.3 (C_H-6), 112.8, 113.8, 114.7, 114.85, 114.93 (2C), 115.3 (2C), 115.7 (2C), 115.9, 116.5 ($C_{A,B,D–G,I–L,N,O}$-1), 115.5 (C_E-3), 115.7 (C_F-3), 116.3 (C_A-3), 120.7 (C_H-2), 120.3, 123.4 ($C_{C,M}$-1), 120.0 (C_A-2), 122.4 (C_K-2), 125.9 (C_E-2), 121.4, 124.8 (2C), 124.9, 125.9, 126.3, 126.4, 126.5, 127.1, 127.7 ($C_{B,D,F,G,I,J,L,N,O}$-2, C_H-1), 133.9, 134.3, 134.5, 135.0, 135.5 (2C), 135.9, 136.2, 136.5 (2C), 137.0, 137.3, 137.8, 138.3, 138.7 ($C_{C,H,M}$-4, $C_{A,B,D–G,I–L,N,O}$-5), 142.1 (C_F-4), 142.4 (C_K-4), 143.0 (C_A-4), 143.7 (C_E-4), 146.1 (C_H-3), 142.9 (2C), 143.0, 143.2 (2C), 143.7 (3C), 143.9 (2C), 144.5 (2C), 144.6 (2C), 144.9 (2C), 145.1 (4C), 145.7, 146.1, 146.4 (3C) (C_H-5, $C_{C,M}$-3,5, $C_{A,E,F,K}$-3, $C_{B,D,G,I,J,L,N,O}$-4,6), 163.8 (C_A-7), 165.1 (C_F-7), 165.4 (C_C-7), 165.5 (C_M-7), 167.0 (C_I-7), 167.5 (C_H-7), 167.7 (C_E-7), 168.1 (C_D-7), 168.3 (C_O-7), 168.5 (C_J-7), 168.7 (C_B-7), 167.1, 168.2, 168.8, 169.0 ($C_{G,K,L,N}$-7); for sugar carbons, see Table 1; ESIMS *m/z*: 2790 $[M+NH_4]^+$; *Anal.* Found: C, 46.5; H, 3.99. $C_{123}H_{80}O_{76} \cdot 23H_2O$ requires: C, 46.3; H, 3.95%.

3.8. Acid hydrolysis of melasquains A–D (**18–21**)

A solution of each compound (1 mg) in 1 M HCl (1 mL) was heated in boiling H_2O for 10 h. The reversed phase HPLC analysis of the reaction mixtures showed peaks identical to those of gallic acid (2.54 min) and ellagic acid (23.84 min).

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