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TWO MACROCYCLIC HYDROLYSABLE TANNIN DIMERS FROM *EUGENIA UNIFLORA*

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Key Word Index—*Eugenia uniflora*; Myrtaceae; leaves; tannin; hydrolysable tannin; macrocyclic tannin dimer; eugeniflorins D_1 and D_2; dehydrovaloneoyl group.

Abstract—Eugeniflorins D_1 and D_2, new hydrolysable tannin dimers, were isolated, together with four known polyphenols, from *Eugenia uniflora* leaves. Their macrocyclic structures were elucidated from spectral and chemical evidence. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The shrubby tree *Eugenia uniflora*, of Brazilian origin, is widely distributed in the tropics and subtropics, such as Brazil, India, Ceylon and southern China. Its cherry-like fruit, called 'pitangueira' in Brazil, is edible and its leaves are used in folk medicine [1–3]. Studies of *E. uniflora* have hitherto been focused on the essential oil in the leaf [4], which is used as a digestive, eupeptical, and carminative remedy [5], with no investigation of the polyphenolic constituents. In our study on the polyphenolics of the myrtaceous plants, leaves of this species were found to be rich in polyphenols and, after a chromatographic survey, six phenolic compounds, including two new macrocyclic hydrolysable tannin dimers, named eugeniflorins D_1 (**1**) and D_2 (**2**), were isolated.

RESULTS AND DISCUSSION

The aqueous acetone extract of dried leaves was subjected to a combination of column chromatography on Dia-ion HP-20, Fractogel TSK HW-40 and MCl-gel CHP-20P with aqueous methanol to afford six compounds. Four of them were identified as oenothein B (**3**) [6], 1,2,4,6-tetra-*O*-galloyl-β-D-glucose [7], gallocatechin and myricitrin. Oenothein B (**3**) is a macrocyclic hydrolysable tannin dimer, first isolated from *Oenothera* species [6] and was found to exhibit a remarkable host-mediated antitumour activity [8] and suppression of mouse mammary tumour gene expression [9]. Two were new compounds and were named eugeniflorin D_1 and eugeniflorin D_2.

Eugeniflorin D_1 (**1**), a light-brown amorphous powder, $[\alpha]_D+28°$ (methanol), showed an $[M+Na]^+$ ion peak at m/z 1743 in the FAB-mass spectrum, corresponding to the molecular formula $C_{75}H_{52}O_{48}$. Acid hydrolysis of **1** yielded gallic acid and valoneic acid dilactone (**4**). The sugar component was identified as glucose by GC of the trimethylsilyl derivative. The ^1H NMR spectrum of **1** showed marked broadening of several aromatic and sugar proton signals, which is a characteristic spectral feature of macrocyclic oligomers, such as woodfordin C [10] and oenotheins A [11] and B, attributable to a slow interconversion among metastable macro-ring conformations [6]. The presence of three galloyl groups and two valoneoyl groups was indicated by three 2H singlets (δ 6.96, 6.94, 6.91) and six 1H singlets (δ 6.98, 6.83, 6.69, 6.62, 6.53, 6.33) in the aromatic region. The coupling patterns of the sugar proton signals assigned by ^1H–^1H COSY were those of two $^4C_1$ glucopyranose residues. One of the anomeric hydroxyl groups of the two glucose moieties (glucose II) in **1** is free, as indicated by the chemical shift of an anomeric proton signal (δ 4.41, *d*, $J = 8.0$ Hz), but there is no duplication of the proton signal of this glucose. This glucose core therefore takes predominantly the β-form in a way analogous to **3** [6, 12]. The other anomeric centre is acylated, as evidenced by the signal at δ 6.38 (*d*, $J = 8.5$ Hz). There is a large chemical shift difference (δ 1.42 and 1.28) in the C-6 methylene proton signals of each glucose core, which indicates that a hexahydroxydiphenoyl part of the valoneoyl group is at *O*-4/*O*-6 of each glucose [13]. These spectral features,

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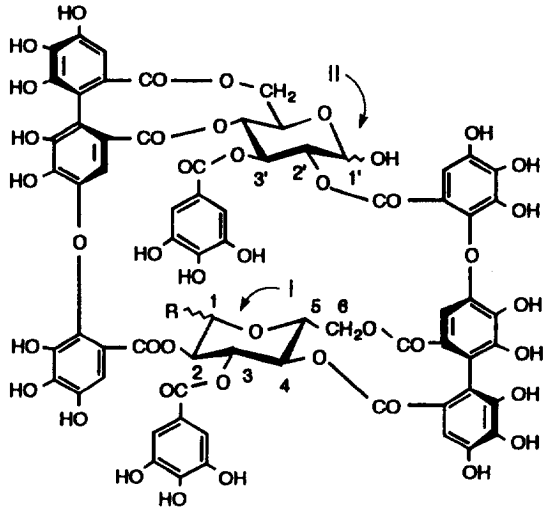
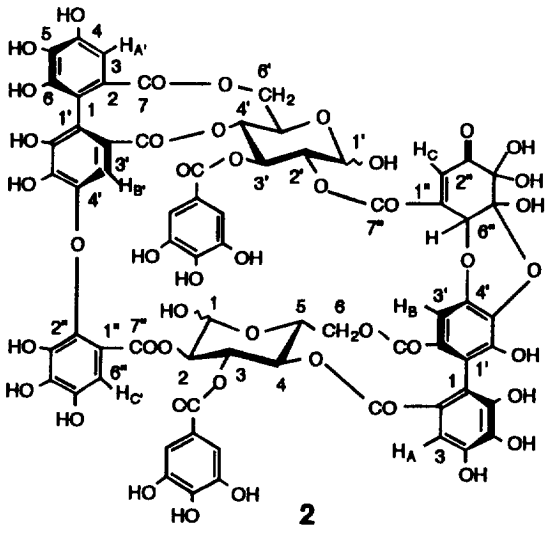


1 : R=(β)-OG
3 : R=OH



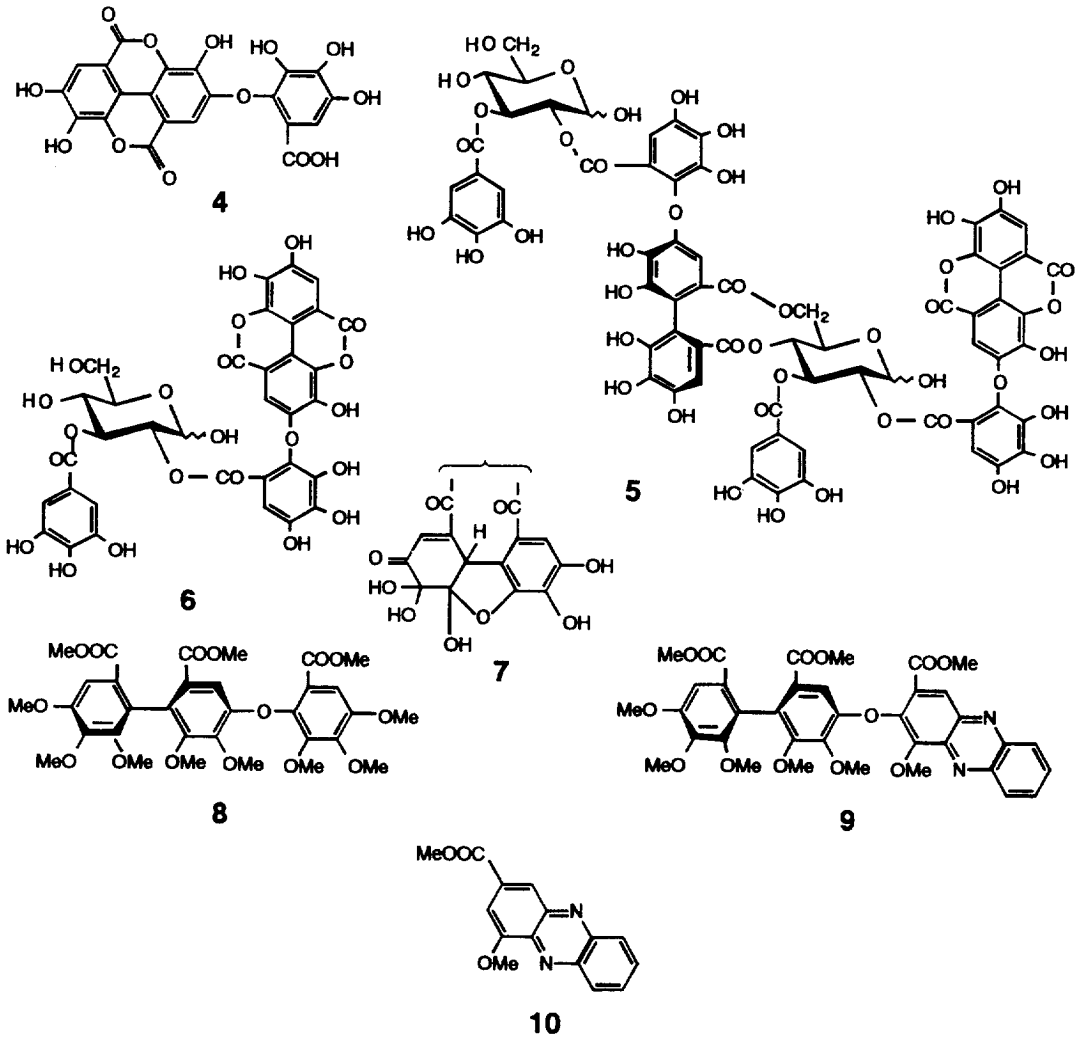
2

along with the other signals in the ^1H and ^{13}C NMR spectra of **1**, which were closely similar to those of **3** [6], implied that eugeniflorin D_1 is a monogallate of **3**. The additional galloyl group in **1** is at O-1 of glucose-I as shown by the difference in the H-1 signal and the similarity of the H-1′ signal between **1** and **3** (δ 6.20 (H-1, *d*, J = 3.0 Hz), 4.48 (H-1′, *d*, J = 7.5 Hz) in **3** and δ 6.38 (H-1, *d*, J = 8.5 Hz), 4.39 (H-1′, *d*, J = 8.0 Hz) in **1**). The structure **1** thus proposed for eugeniflorin D_1 was consistent with the following chemical evidence. Partial hydrolysis of **1** in dilute sulphuric acid furnished **4**, woodfordin G (**5**) [14] and oenothein C (**6**) [6]. Upon treatment with tannase, **1** gave degalloylated derivatives, one of which was identified as oenothein B (**3**). The CD spectrum of **1** established the *S*-configuration at the HHDP moiety in the two valoneoyl groups [15].

Eugeniflorin D_2 (**2**), $[\alpha]_D$ +37° (methanol), was obtained as a light-yellow amorphous powder. Its molecular formula was deduced as $C_{68}H_{48}O_{45}$ from the FAB-mass spectrum (*m/z* 1585 $[M+H]^+$) and the ^1H and ^{13}C NMR spectra. The ^1H–^1H COSY spectrum of **2** indicated the presence of two $^4C_1$ glucopyranose residues. The doublets at δ 5.98 (J = 3.0 Hz) and 5.20 (J = 8.0 Hz) of the anomeric protons resonate at higher field than those expected for the C-1 of acylated α- and β-anomers, showing that both anomeric centres are unacylated. However, each glucose core does not show anomerization. This is similar to **1** and oenothein B (**3**), which have glucose cores formed predominantly of one of the anomers, due to conformational restriction. Two 2H singlets (δ 7.30, 7.17) and three 1H singlets in the aromatic proton region are ascribable to those of two galloyl groups and a valoneoyl group. The other two 1H singlets at δ 6.80 and 6.57, and a vinyl proton signal at δ 7.11 (*d*, J = 2 Hz) coupled with a methine proton signal at δ 5.37 (*d*, J = 2 Hz), are attributable to a hitherto unknown oxidized valoneoyl group as follows. The ^{13}C NMR spectrum of **2** showed the presence of an α,β-unsaturated ketone system (δ 194.0, 135.4, 145.8) and two acetal carbon atoms (δ 93.5, 97.1), which are similar to those of the dehydrohexahydroxydiphenoyl (DHHDP) group (**7**) in geraniin [16]. A methine carbon signal shifted to lower field (δ 71.4) than that of the DHHDP group (δ 51.9), indicating that this carbon bears an oxygen atom. The other aromatic carbon signals and eight ester carbonyl resonances are similar to those of **3**.

Treatment of **2** with *o*-phenylenediamine gave a phenazine derivative, which after methylation and subsequent methanolysis afforded methyl tri-*O*-methylgallate, trimethyl octa-*O*-methylvaloneate (**8**) and also a nonamethyl derivative (**9**) and a dimethyl derivative (**10**) of partial methanolysis. The tentative structure (**9**) was deduced from its EI-mass spectrum (*m/z* 702 $[M]^+$), corresponding to the molecular formula $C_{36}H_{34}O_{13}N_2$, and the ^1H NMR spectrum exhibiting the signals of the phenazine moiety (δ 8.23 (2H, *dd*, J = 2, 8 Hz), 7.94 and 7.98 (each 1H, *dt*, J = 2, 8 Hz)) besides three 1H singlets at δ 7.36, 7.42 and 8.39. The dimethyl derivative (**10**; EI-mass spectrum *m/z* 268 $[M]^+$) showed the phenazine signals (δ 8.01 (2H, *dd*, J = 3, 7.5 Hz), 8.26 and 8.31 (each 1H, *dt*, J = 3, 7.5 Hz), and 7.64 and 8.46 (each 1H, *d*, J = 2 Hz)), in addition to two methoxyl signals at δ 4.02 and 4.20. The dimethyl derivative (**10**) could be produced from **9** by cleavage of the ether linkage in a manner similar to that of the valoneoyl group in several hydrolysable tannin oligomers [17].

Eugeniflorin D_2 (**2**) was transformed to oenothein B (**3**) when kept in aqueous solution at 80° for 1 day. This transformation of the dehydrovaloneoyl group in **2** into the valoneoyl group is regarded as reduction of a cyclohexenetrione moiety through an intermolecular disproportionation reaction. These findings clearly indicate that eugeniflorin D_2 has the structure in which one of two valoneoyl groups in **3** is replaced by the dehydrovaloneoyl group. The locations of the acyl groups on the glucose cores in **2** were determined



by ^{1}H–^{13}C long-range COSY. The valoneoyl proton signals ($H_{C'}$) at δ 7.18 was correlated with the H-2 of glucose-I, through three-bond coupling with the ester carbonyl carbon resonance at δ 164.5. The singlet at δ 5.88 was correlated with the signal at δ 168.3, which was also correlated with glucose H-6′ through three-bond coupling, and assigned to the valoneoyl $H_{A'}$. Similarly, the valoneoyl $H_{B'}$ was correlated with glucose H-4′. The location of the valoneoyl group was thus established at O-2, O-4′ and O-6′, and that of the dehydrovaloneoyl group at O-4, O-6 and O-2′, as shown in formula **2** (Table 1).

The absolute configurations at the biphenyl moieties in **2** were both determined to be (*S*), based on the formation of **3** from **2** and the circular dichroism (CD) spectrum of **2** which showed strong Cotton effects at 215 and 236 nm [18]. Eugeniflorin D_2 was thus represented by the formula **2**, although the stereochemistry at the methine carbon atom of the dehydrovaloneoyl group remains to be determined. Eugeniflorin D_2 is the first natural product possessing a dehydrovaloneoyl group in the molecule.

EXPERIMENTAL

General. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded and chemical shifts are given in δ (ppm) values from TMS. Normal phase HPLC was conducted on a YMC-pack SIL-06 (250 mm × 4.6 mm i.d.) column with the following solvent systems: (N1) *n*-hexane–MeOH–THF–HCO_2H (55:33:11:1) and oxalic acid 450 mg l^{-1}, at a flow rate 1 ml min^{-1}, (N2) *n*-hexane–MeOH–THF–HCO_2H (60:45:15:1) and oxalic acid 500 mg 1.2 l^{-1}, at a flow rate 1.5 ml min^{-1}. Reverse-phase HPLC at 40° using the following solvent systems: (R1) 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (50:50:4:5) (YMC-pack ODS-A, 250 mm × 4.6 mm i.d.), (R2) 0.1 M H_3PO_4–0.1 M KH_2PO_4–MeCN (42.5:42.5:15) (YMC-pack ODS-A, 250 mm × 4.6 mm i.d.), (R3) 0.01 M H_3PO_4–0.01 M KH_2PO_4–MeCN (9:9:2) (YMC pack ODS-AQ 302, 150 mm × 4.6 mm i.d.), (R4) 0.01 M H_3PO_4–0.01 M KH_2PO_4–MeCN (9:9:2) (YMC pack ODS-AQ 312, 150 mm × 6.0 mm i.d.), at a flow rate 1 ml min^{-1}. Detection for the HPLC analysis was at 280 nm. CC was carried out on Fractogel TSK HW-40

Table 1. One-bond and long-range $^{1}H-^{13}C$ correlation data for compound **2**

C	δ_C	Proton coupled via one bond	Proton coupled via two or three bonds
Glucose-I			
1	91.8	5.98	
2	75.4	5.70	
3	70.3	5.91	
4	71.1	4.92	
5	68.6	4.50	
6	64.4	3.17, 4.70	
Glucose-II			
1	96.3	5.20	
2	74.9	5.24	
3	72.4	5.56	
4	72.5	4.92	
5	71.5	4.05	
6	63.5	3.67, 5.10	
Galloyl-I			
1	121.2[a]		
2, 6	110.7	7.30	
3, 5	145.7		7.30 (galloyl-I)
4	138.6		7.30 (galloyl-I)
7	168.1		7.30 (galloyl-I)
			5.91 (3-H of glucose-I)
Galloyl-II			
1	121.5[a]		
2, 6	110.9	7.17	
3, 5	145.0		7.17 (galloyl-II)
4	138.5		7.17 (galloyl-II)
7	166.1		7.17 (galloyl-II)
Valoneoyl			
1	116.1	5.88 ($H_{A'}$)	
2	125.5[b]		
3	107.4	5.88	
4	145.2[c]		
5	131.1		5.88 ($H_{A'}$)
6	144.4[c]		
7	168.3		5.88 ($H_{A'}$)
			3.67 (6-H of glucose-II)
1′	115.8		6.37 ($H_{B'}$)
2′	129.1		6.37 ($H_{B'}$)
3′	106.9	6.37	
4′	145.0		6.37 ($H_{B'}$)
5′	136.2		6.37 ($H_{B'}$)
6′	144.5[c]		
7′	166.2		6.37 ($H_{B'}$)
			4.92 (4-H of glucose-II)
1″	113.7		
2″	140.2		7.18 ($H_{C'}$)
3″	140.3		
4″	141.9		7.18 ($H_{C'}$)
5″	143.2		7.18 ($H_{C'}$)
6″	109.3	7.18	
7″	164.5		7.18 ($H_{C'}$)
			5.70 (2-H of glucose-I)

Table 1.—*Continued.*

C	δ_C	Proton coupled via one bond	Proton coupled via two or three bonds
Dehydrovaloneoyl			
1	119.4		6.80 (H_A)
2	126.1[b]		
3	113.0	6.80	
4	145.1		6.80 (H_A)
5	139.6		6.80 (H_A)
6	145.3[c]		
7	167.4		6.80 (H_A)
			4.92 (4-H of glucose-I)
1′	115.0		6.57 (H_B)
2′	126.3		
3′	107.6	6.57	
4′	145.2[c]		
5′	136.2		6.57 (H_B)
6′	144.5[c]		
7′	168.2		6.57 (H_B)
1″	154.8		
2″	135.4	7.11	
3″	194.0		
4″	93.5		
5″	97.1		
6″	71.4		
7″	163.8		7.11 (H_C)

[a-c] Values with the same superscript are interchangeable.

(fine and superfine grades, Merck), ODS-AQ (YMC), Dia-ion HP-20 and MCl-gel CHP-20P (Mitsubishi Chemical Industry Co. Ltd). Prep. TLC was performed on silica gel (PSC-Fertigplatten Kieselgel 60 F_{254}, Merck) with toluene–Me_2CO (4:1) and *n*-hexane–$CHCl_3$–Me_2CO (6:3:1).

Plant material. Leaves of *E. uniflora* L. were collected in October at Heng-Chun Tropical Botanic Garden, Taiwan.

Isolation of tannins. Dried leaves (1 kg) were homogenized × 3 in Me_2CO–H_2O (7:3) and the combined homogenates filtered and the solvent evapd *in vacuo*. A part of the extract (50 g) was subjected to CC over Dia-ion HP-20 (30 cm × 7.2 cm i.d.) with aq. MeOH increasing the concn of MeOH. A part (3 g) of the 25% MeOH eluate was further chromatographed over Fractogel TSK HW-40 (fine grade) (25 cm × 3.0 cm i.d.) with MeOH–H_2O (3:2) → MeOH–H_2O–Me_2CO (7:2:1) → MeOH–H_2O–Me_2CO (3:1:1) → Me_2CO–H_2O (7:3) in a stepwise gradient. The eluate with 60% MeOH gave eugeniflorin D_2 (**2**) (20 mg) and the MeOH–H_2O–Me_2CO (7:2:1) eluate gave oenothein B (**3**) (15 mg). The 30% MeOH eluate from the Dia-ion HP-20 column was similarly rechromatographed on Fractogel TSK HW-40 gel and the eluate with MeOH–H_2O–Me_2CO (7:2:1) gave eugeniflorin D_1 (**1**) (3 mg). The 50% MeOH eluate (2 g) from the Dia-ion HP-20 column gave 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (1 mg), gallocatechin (15 mg) and myricitrin (12 mg).

Eugeniflorin D_1 (**1**). Light-brown amorphous powder. $[\alpha]_D$ +28° (MeOH; *c* 1.0). UV λ_{max}^{MeOH} nm (log ε): 218 (5.16), 268 (4.80). (Found: C, 49.73; H, 3.68. $C_{75}H_{52}O_{48}\cdot 6H_2O$ requires: C, 49.73; H, 3.17%). FABMS: *m/z* 1743 $[M+Na]^+$. CD (MeOH): $[\theta]_{222}+2.5\times10^5$, $[\theta]_{260}-6.9\times10^4$, $[\theta]_{285}+5.9\times10^4$. ^{1}H NMR [$(CD_3)_2CO+D_2O$)]: δ 6.96, 6.94, 6.91 (2H each, *s*, galloyl), 6.98, 6.83, 6.69, 6.62, 6.53, 6.33 (1H each, Val), 6.38 (*d*, *J* = 8.5 Hz, H-1), 5.75 (*t*, *J* = 10 Hz, H-3), 5.42 (*dd*, *J* = 7.5, 13 Hz, H-6′), 5.38 (*dd*, *J* = 8.5, 10 Hz, H-3′), 5.21 (*dd*, *J* = 8.5, 10 Hz, H-2), 5.21 (*d*, *J* = 13 Hz, H-6), 5.20 (*t*, *J* = 10 Hz, H-4), 5.17 (*t*, *J* = 10 Hz, H-4′), 5.03 (*t*, *J* = 8.5 Hz, H-2′), 4.58 (1H in total, H-5), 4.37 (*dd*, *J* = 5.5, 10 Hz, H-5′), 4.41 (*d*, *J* = 8 Hz, H-1′), 4.00 (*dd*, *J* = 3.5, 13 Hz, H-6′), 3.93 (*d*, *J* = 13 Hz, H-6). ^{13}C NMR [$(CD_3)_2CO+D_2O$)]: δ 170.4, 168.9, 168.8, 168.1, 168.1, 166.9, 166.9, 165.9, 164.8 (ester carbonyl), 95.9 (Glc C-1′), 93.1 (Glc C-1), 75.6 (Glc C-2), 74.6 (Glc C-2′), 74.0 (Glc C-4′), 72.2 (Glc C-3′), 72.1 (Glc C-5), 71.9 (Glc C-5′), 71.8 (Glc C-3), 71.0 (Glc C-4), 66.1 (Glc C-6′), 63.2 (Glc C-6).

Acid hydrolysis of **1**. A soln of **1** (1 mg) in 5% H_2SO_4 (0.3 ml) was heated at 100° for 5 hr. After cooling, the reaction mixt. was extracted with EtOAc. The EtOAc extract was analysed by HPLC (reverse-phase: system R3) to show two peaks identical with those of authentic gallic acid (R_t 3.2 min) and valoneic acid dilactone (R_t 7.6 min). The sugar component in the aq. layer remaining after EtOAc extraction was identified as glucose by GC (capillary column G-250; column temp. 170°) after trimethylsilylation.

Partial hydrolysis of **1**. A soln of **1** (0.5 mg) in 0.5% H_2SO_4 (0.5 ml) was heated at 100° for 4 hr. After cooling, the reaction mixt. was analysed by HPLC

(reverse-phase: system R3) which showed peaks identical with those of authentic woodfordin G (**5**) (R_t 10.3, 11.2 min), oenothein C (**6**) (R_t 5.9 min) and dilactonized valoneic acid (**4**) (R_t 12.5 min).

Degalloylation of **1** *with tannase*. An aq. soln of **1** (1 mg ml^{-1}) was incubated at 37° for 36 hr with tannase (10 drops), which was prepd from *Aspergillus niger* [17]. The reaction mixt. was analysed by HPLC (reverse-phase: system R4), and a peak identical with that of authentic oenothein B (**3**) (R_t 9.0 min, normal phase, system N2; R_t 11.3 min, reverse-phase, system R4) was detected.

Eugeniflorin D_2 (**2**). Light-yellow amorphous powder. $[\alpha]_D + 37°$ (MeOH, *c* 1.0). UV λ_{max}^{MeOH} nm (log ε): 216 (4.89), 272 (4.48). (Found: C, 46.59; H, 3.29. $C_{68}H_{48}O_{45} \cdot 9H_2O$ requires: C, 46.73; H, 3.78%). FABMS: *m/z* 1585 $[M+H]^+$. CD(MeOH): $[\theta]_{215} + 1.2 \times 10^5$, $[\theta]_{236} + 5.2 \times 10^4$, $[\theta]_{257}$ 6.5×10^4, $[\theta]_{283} + 4.2 \times 10^4$. ^{1}H NMR [$(CD_3)_2CO + D_2O$)]: δ 7.30, 7.17 (each *s*, 2H in total, galloy-I), 7.18, 6.37, 5.88 (each 1H, *s*, Val), 7.11 (1H, *d*, $J = 2$ Hz), 6.80, 6.57 (each 1H, *s*), 5.37 (1H, *d*, $J = 2$ Hz) (dehydrovaloneoyl), 5.98 (*d*, $J = 3$ Hz, H-1), 5.91 (*t*, $J = 10$ Hz, H-3), 5.70 (*dd*, $J = 3$, 10 Hz, H-2), 5.56 (*dd*, $J = 9$, 10 Hz, H-3′), 5.24 (*dd*, $J = 8$, 9 Hz, H-2′), 5.20 (*d*, $J = 8$ Hz, H-1′), 5.10 (*dd*, $J = 6.5$, 13 Hz, H-6′), 4.92 (*t*, $J = 10.5$ Hz, H-4), 4.92 (*t*, $J = 10$ Hz, H-4′), 4.70 (*dd*, $J = 5.5$, 13 Hz, H-6), 4.50 (*dd*, $J = 5.5$, 10.5 Hz, H-5), 4.05 (*dd*, $J = 6.5$, 10 Hz, H-5′), 3.71 (*d*, $J = 13$ Hz, H-6), 3.67 (*d*, $J = 13$ Hz, H-6′). ^{13}C NMR: see Table 1.

Phenazine derivative from **2**. A soln of *o*-phenylenediamine (2.5 mg) in 50% HOAc (7.5 ml) was added to a soln of **2** (5 mg) in MeOH (0.5 ml) and the reaction mixt. left standing overnight at room temp. The residue was obtained after evapn of solvent, and dried *in vacuo*. After adding Me_2CO to the residue, insoluble material was removed by centrifugation. Evapn of solvent, followed by reprecipitation from Me_2CO–$CHCl_3$, gave the phenazine derivative (7.5 mg).

Methylation of phenazine derivative followed by methanolysis. CH_2N_2–Et_2O (2 ml) was added to an EtOH soln (0.2 ml) of the phenazine derivative (7.5 mg) and the mixt. kept for 2 hr at room temp. After removal of solvent under N_2, the residue was methanolized with 0.5% NaOMe in MeOH (1 ml) overnight at room temp. The reaction mixt. was acidified with HOAc and the solvent evapd. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was evapd and the residue further treated with CH_2N_2–Et_2O (1 ml) for 30 min and the solvent evapd. Prep. TLC of the residue with toluene–Me_2CO (4:1) and *n*-hexane–$CHCl_3$–Me_2CO (6:3:1) gave methyl tri-*O*-methylgallate (0.6 mg), trimethyl octa-*O*-methylvaloneate (**8**) (0.5 mg), the nonamethyl derivative (**9**) (0.8 mg) and the dimethyl derivative (**10**) (1.2 mg).

Nonamethyl derivative (**9**). Orange amorphous powder. EI-MS *m/z*: 702 $[M]^-$. ^{1}H NMR [$(CD_3)_2CO + D_2O$)]: δ 8.23 (2H, *dd*, $J = 2$, 8 Hz), 7.94, 7.98 (each 1H, *dt*, $J = 2$, 8 Hz), 7.36, 7.42, 8.39 (each 1H, *s*, phenazine signals), 3.54, 3.57, 3.61, 3.62, 3.73, 3.87, 3.93, 3.95, 3.96 (each 3H, *s*, methoxyl).

Dimethyl derivative (**10**). Yellow amorphous powder. EI-MS *m/z*: 268 $[M]^+$. ^{1}H NMR [$(CD_3)_2CO + D_2O$)]: δ 8.01 (2H, *dd*, $J = 3$, 7.5 Hz), 8.26, 8.31 (each 1H, *dt*, $J = 3$, 7.5 Hz), 7.64, 8.46 (each 1H, *d*, $J = 2$ Hz, phenazine signals), 4.02, 4.20 (each 3H, *s*, methoxyl).

Transformation of **2** *to* **3**. An aq. soln (10 ml) of **2** (50 mg) was kept at 80° for 24 hr. The mixt. was concd and then subjected to a combination of CC over Fractogel HW-40 (fine grade) (38 cm × 1.1 cm i.d.) with 60% MeOH → MeOH–H_2O–Me_2CO (7:2:1) → Me_2CO–H_2O (7:3), ODS column (25 cm × 1.1 cm i.d.) with 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc and MCl-gel CHP-20P with H_2O → MeOH to afford **3** (3.6 mg), which was identified by comparison with an authentic specimen by HPLC and ^{1}H NMR.

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