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OENOTHEINS D, F AND G, HYDROLYSABLE TANNIN DIMERS FROM *OENOTHERA LACINIATA**

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Key Word Index—*Oenothera laciniata*; Onagraceae; roots; stems; tannins; oenotheins D, F and G.

Abstract—Three new dimeric hydrolysable tannins, oenotheins D, F and G, together with known tannins including the macrocyclic oligomers, oenotheins A and B, have been isolated from roots and stems of *Oenothera laciniata*. Their structures were elucidated on the basis of chemical and spectral evidence. Oenotheins D and F were macrocyclic dimers isomeric with oenothein B in relation to the orientation of the valoneoyl groups linking the monomers.

INTRODUCTION

Among more than 150 oligomeric hydrolysable tannins hitherto known [1], oenotheins A and B (**1**) which were isolated as major polyphenols from *Oenothera erythrosepala* and *O. biennis* constitute an important class of oligomers because of their unique macrocyclic structures [2, 3] and of interest biological activities such as host-mediated antitumour activity [4–7]. Oenothein B and related oligomers have also been found in several medicinal plants belonging to the Lythraceae [1, 2, 8, 9] and Myrtaceae [10]. We have now found that *O. laciniata* produces oenotheins A and B as major metabolites, as found in other *Oenothera* species. In addition, three new dimeric hydrolysable tannins with structures related to **1**, which were named oenotheins D (**5**), F (**8**) and G (**9**), were isolated from the roots and stems of this species. The present paper describes the structural elucidation of these new dimers. A minor trimar, oenothein E, was also isolated from plants collected in May; its structure will be reported elsewhere.

RESULTS AND DISCUSSION

The 70% aqueous acetone homogenate of the dried roots and stems of *O. laciniata* collected in September was subjected to a combination of column chromatography over polystyrene and/or polyvinyl gels using aqueous methanol, to yield the trimeric and dimeric ellagitannins, oenotheins A and B (**1**) as the major components. Five monomeric ellagitannins were also obtained and identified as 1,2,3- and 1,2,6-tri-*O*-galloyl-β-D-glucose [11], oenothein C (**2**) [3], cornusiin B (**3**) [12] and tellimagrandin I (**4**) [13]. A new dimer, oenothein D (**5**), was isolated as a minor constituent. On the other hand, fresh roots and stems of the same species collected in May produced two new dimers, oenotheins F (**8**) and G (**9**), different from oenothein D (**5**), and a known dimer (eucalbanin B [10]), as minor components, although the major tannins (oenotheins A and B) were the same in the plants collected in September and May.

The dimeric nature of the new tannins was suggested by their retention volumes on normal phase HPLC [14], which were almost the same as that of **1**.

Oenothein D (**5**), a light brown amorphous powder, yielded methyl tri-*O*-methylgallate (**6**) and trimethyl octa-*O*-methylvalonate (**7**) in a relative ratio of 1:1 upon methanolysis of the permethylated derivative (**5a**) with sodium methoxide in methanol. The ^{1}H NMR spectrum of **5** exhibited six 1H-singlets and two 2H-singlets in the aromatic region, indicating the presence of two galloyl and two valoneoyl groups in the molecule. Evidence of two $^4C_1$ glucopyranose residues in **5** was apparent from the characteristic coupling pattern of the well-resolved sugar proton signals which were assigned by a ^{1}H–^{1}H COSY spectrum (Table 1). Two anomeric proton signals appeared at δ4.85 (*d*, $J = 8$ Hz) and 5.89 (*d*, $J = 4$ Hz) indicating that the anomeric hydroxyl groups of both glucose cores are unacylated. Although this tannin forms an anomeric mixture, equilibration occurs to give a dominant anomer, as indicated by each proton signal accompanied by extremely small peaks due to anomerization. Namely, one glucose core (glucose-I) in **5** exists mostly in the α-form, the other (glucose-II) in the β-form. Such a formation might be caused by steric requirements probably due to the rigid macrocyclic structure. The low-field chemical shifts of the other glucose protons

*Part 3 in the series of Tannins from Onagraceous Plants. For Part 2, see ref. [1].

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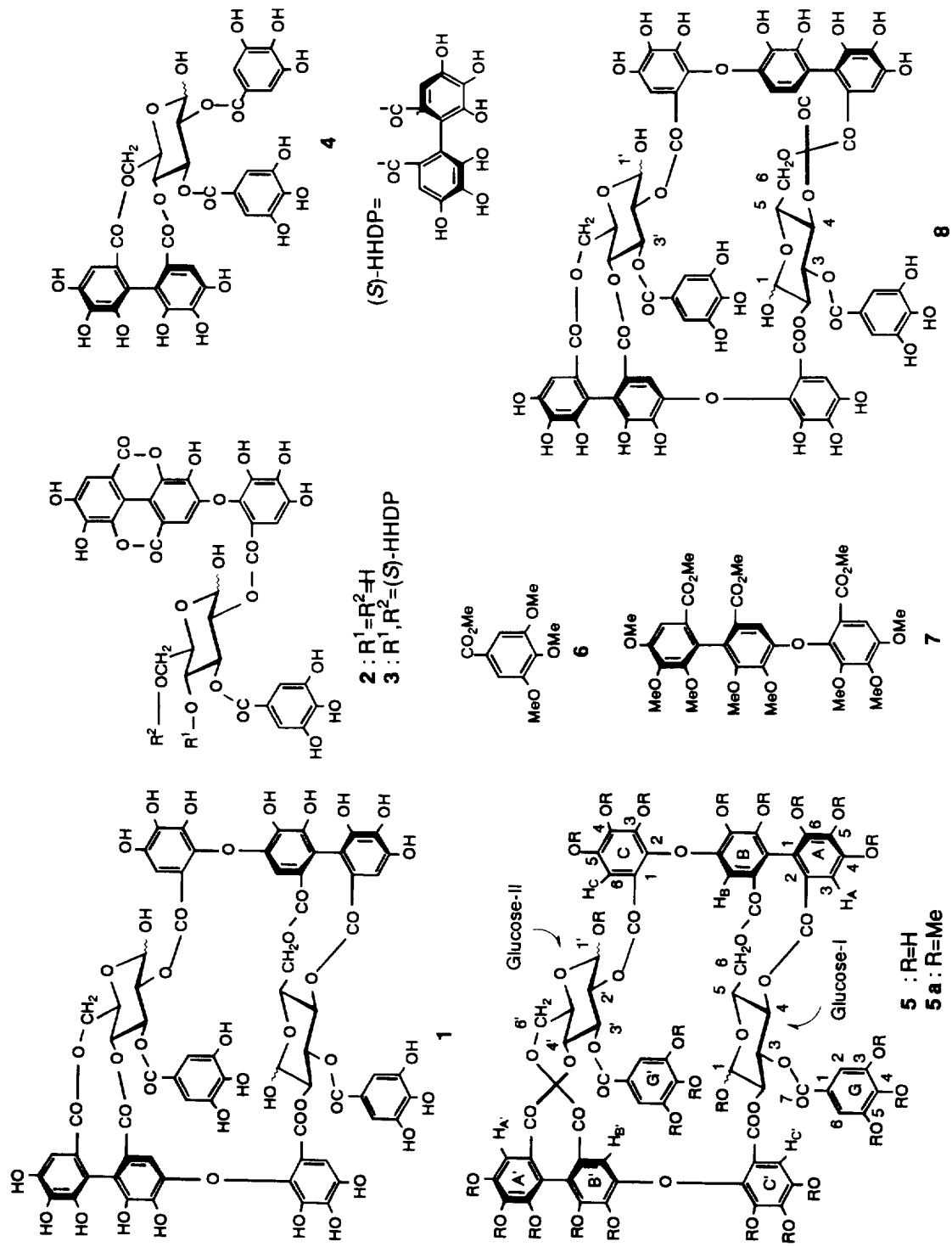

1
2 : R1=R2=H
3 : R1,R2=(S)-HHDP
(S)-HHDP=
4
5 : R=H
5a : R=Me
Glucose-II
Glucose-I
6
7
8

Table 1. ^1H and ^{13}C NMR data for glucose moieties of compounds **1*** and **5*** ($(CD_3)_2CO + D_2O$ (*J* in Hz))

	1		**5**	
Position	δ_H	δ_C	δ_H	δ_C
Glucose-I				
1	6.18 (*d*, *J* = 3)	91.5	5.89 (*d*, *J* = 4)	91.4
2	6.10 (*br*)	74.5	5.46 (*dd*, *J* = 4, 10)	74.6
3	6.08 (*br*)	70.3	5.93 (*t*, *J* = 10)	70.1
4	5.92 (*br*)	71.4	5.60 (*t*, *J* = 10)	71.6
5	4.56 (*dd*, *J* = 6, 10)	69.1	4.50 (*dd*, *J* = 6, 10)	69.2
6	5.24 (*dd*, *J* = 6, 13)	63.2	5.08 (*dd*, *J* = 6, 13)	63.8
	3.62 (*d*, *J* = 13)		3.76 (*d*, *J* = 13)	
Glucose-II				
1′	4.42 (*br*)	95.8	4.85 (*d*, *J* = 8)	94.4
2′	5.16 (*d*, *J* = 8, 10)	74.8	5.23 (*dd*, *J* = 8, 10)	76.3
3′	5.43 (*t*, *J* = 10)	73.5	5.86 (*t*, *J* = 10)	72.5
4′	4.88 (*t*, *J* = 10)	73.8	5.37 (*t*, *J* = 10)	74.8
5′	4.12 (*dd*, *J* = 6, 10)	71.9	4.45 (*dt*, *J* = 3, 5, 10)	69.9
6′	5.02 (*dd*, *J* = 6, 13)	65.3	4.70 (*t*, *J* = 10)	67.3
	3.85 (*d*, *J* = 13)		4.01 (*dd*, *J* = 3.5, 10)	

*Data for main anomeric form.

(Table 1) implied that all the hydroxyl groups except for the anomeric ones on glucose cores are acylated. These spectral features were closely similar to those of the macrocyclic dimer, oenothein B (**1**) [3]. The ^{13}C NMR spectrum of **5** was also very similar to that of **1**. Upon comparison of the sugar carbon resonances of **1** with those assigned by ^1H–^{13}C COSY of **5**, the signals of glucose-I showed close resemblance to each other, while those of glucose-II in **5** were significantly different from those of **1** (Table 1). Oenothein D (**5**) was thus assumed to be an isomer of **1** with regard to the orientation of the valoneoyl group at O-4′/O-6′ of the glucose-II. This was substantiated by a $[M + Na]^+$ ion peak at *m/z* 1591, the same as that of **1** in the FAB-mass spectrum.

One-bond and long-range ^1H–^{13}C COSY spectra of **5** allowed the assignment of aromatic proton and carbon signals as described in the Experimental. In the ^1H–^{13}C long-range COSY spectrum, the valoneoyl H_A and $H_{A'}$ signals (δ6.18 and 6.60) showed the cross-peaks by three-bond couplings with the ester carbonyl carbons at δ169.0 and 169.8, which in turn were correlated with the glucose H-4 and H-4′ signals. The valoneoyl H_B and $H_{B'}$ signals (δ6.39 and 7.28) disclosed similar long-range correlations with the carbonyl carbons (δ168.2 and 168.0, respectively) which were associated with the glucose H-6 (δ5.08) and H-6′ (δ4.70) signals, thus indicating that the HHDP part of each valoneoyl group was attached to O-4/O-6 on each glucose core. The connectivity between the galloyl part of the valoneoyl group and O-2 of each glucose core was shown by three-bond correlations in a combination of $H_{C(C')}$ (δ6.53 (6.68))-carbonyls (δ166.8 (167.0))-H-2′ (2) (δ5.23 (5.46)). Similarly, the positions of the galloyl groups were established to be at O-3 of each glucose core by long-range correlations.

Structures **5** was chemically substantiated by partial hydrolysis with hot water containing a small amount of trifluoroacetic acid, which afforded oenothein C (**2**) and 3-*O*-galloyl-D-glucose, along with gallic acid and valoneic acid dilactone. A small amount of ellagic acid, which was produced by cleavage of the ether bond of the valoneoyl group [15], was also detected by HPLC of the reaction mixture.

The absolute configuration at the chiral biphenyl moiety of each valoneoyl group was determined to be *S*, based on the strong positive Cotton effect at 220 nm and the negative one at 259 nm in the CD spectrum [16] of **5**. Based on these data, the structure of oenothein D has been established as shown in the formula (**5**), in which the orientations of the two valoneoyl groups are the same, unlike **1**.

Oenothein F (**8**), an off-white amorphous powder, was obtained as a minor constituent of plants collected in Spring. Methylation of **8** followed by methanolysis with sodium methoxide yielded **6** and **7**. The FAB-mass spectrum of **8** showed a $[M + Na]^+$ ion peak at *m/z* 1591, which is the same as those of **1** and **5**. These data, along with the production of **2** upon partial hydrolysis with hot 5% H_2SO_4, suggested that this tannin is also an isomer of **1**. Oenothein F was shown to exist as a mixture of four anomers by ^1H NMR, in which each aromatic 1H- and 2H-singlet formed four-lines in a ratio of *ca* 1:2:2:6. The relative peak intensity due to these anomers was changed to *ca* 1:4:4:23 after leaving the NMR sample in a solution of $(CD_3)_2CO$ containing two drops of D_2O for two days. The ^1H NMR spectrum of the most dominant anomer looked like that of a monomeric tannin. Namely, the four aromatic proton singlets appeared at δ6.21, 6.40, 7.30 (each 2H) and 7.04 (4H), which were assignable to

two valoneoyl and two galloyl groups. The proton signals due to two sugar residues were also observed as an apparently single set of sequentially coupled seven-spin systems (see Experimental). The chemical shifts and coupling patterns of these signals were closely similar to those of an α-anomer of tellimagrandin I (**4**) [3], indicating that oenothein F has two $^4C_1$ glucopyranose cores, in which the substitution mode of the acyl groups is similar to that of **4**. The *S*-configuration of both valoneoyl groups in **8** was evidenced from HPLC analysis using a chiral column for trimethyl octa-*O*-methylvalonate (**7**) prepared from **8** as mentioned earlier, which showed the same retention time as that of an authentic (*S*)-**7**. Taking a symmetrical structure suggested by the monomer-like NMR spectrum [17] into consideration, these data indicated that oenothein F has the macrocyclic structure (**8**), in which the two valoneoyl groups at the O-4/O-6 positions of each glucose core have the same orientation to one another. Oenothein F was thus regarded as an isomer of oenothein D (**5**), as represented by formula **8**.

A remarkable difference in the NMR spectra between **5** and **8** of these apparently symmetrical structures may be due to a conformational difference of their macrorings, the latter of which might have much more flexible conformation.

Oenothein G (**9**) was also shown to have the same phenolic constituent units as those of **1** by methylation followed by methanolysis, which furnished **6** and (*S*)-**7**. However, its FAB-mass spectrum showed a $[M + Na]^+$ ion peak at *m/z* 1609, 18 larger than that of **1**. The ^1H NMR spectrum of **9** was complicated due to formation of four anomers. The aromatic proton signals were thus observed as a set of four lines with peak areas of *ca* 1:1:1:1 for each proton, although some of them partly overlapped to form two or three lines (see Experimental). These signals indicated the presence of two galloyl and two valoneoyl groups in the molecule. The sugar proton signals were assigned with the aid of a J-resolved 2-D spectrum and ^1H–^1H COSY (Table 2), indicating the presence of two moles of $^4C_1$ glucopyranose core in which both anomeric centres are unacylated. The chemical shifts of the other glucose proton signals suggested that a hydroxyl group at C-2 of one (glucose-I) of the glucose cores should also be unacylated (δ3.5–3.9 for H-2), while the other hydroxyl groups are all acylated. A remarkable upfield shift was observed for an anomeric proton (δ4.40) of the β-anomer of glucose-II. This phenomenon is characteristic of ellagitannins having a galloyl part of valoneoyl group at C-2 of $^4C_1$ glucoyranose, such as **2** and **3** [3, 11]. A large chemical shift difference (*ca* Δδ1.4 ppm) between the *geminal*-protons at C-6 of the glucose-I and II in **9** is also indicative of the presence of the biphenyl moiety of the valoneoyl group at O-4/O-6 of both glucose cores [12, 15]. Consequently, the galloyl groups should be attached to O-3 of each glucose. We previously reported that the orientation of the valoneoyl group at O-4/O-6 on a $^4C_1$ glucopyranose can be determined by the chemical shift of the valoneoyl H_A signal [15]; that is, the H_A resonates at δ6.42–6.53 in the orientation of type-A and at δ6.58–6.66 in that of type-B (Chart 2), although this empirical rule is not applicable to macrocyclic dimers. The relevant signals of **9** [δ6.61 and 6.60 (1H in total) and 6.47, 6.45, 6.42 and 6.38 (1H in total)] indicated that the two valoneoyl groups in the molecule are reversely oriented to each other. Partial hydrolysis of **9** in hot water containing a small amount of trifluoroacetic acid yielded 3-*O*-galloylglucose, **2**, valoneic acid dilactone and isoschimawalin A (**10**), which has a valoneoyl group of type-B at O-4/O-6 of the glucose core [18]. Based on these data, oenothein G was thus formulated as **9**.

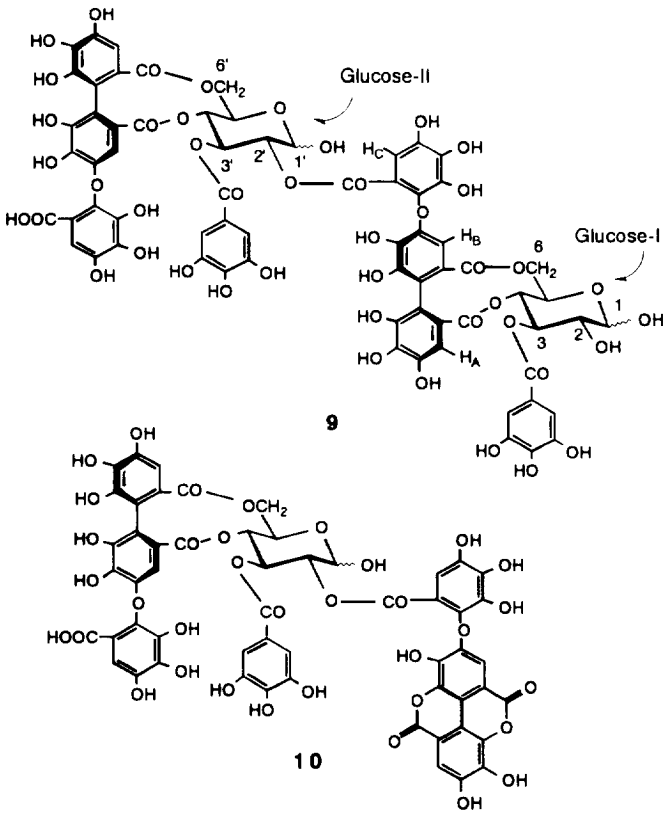
EXPERIMENTAL

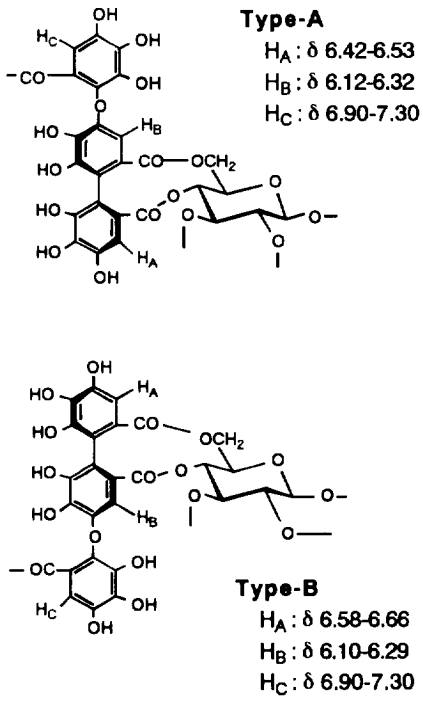
^1H and ^{13}C NMR spectra were measured at 500 and 126 MHz, respectively, in acetone-d_6 + D_2O unless

Table 2. ^1H NMR data for glucose moieties of compound **9** in $(CD_3)_2CO + D_2O$ (500 MHz, *J* in Hz)

	Glucose-I		Glucose-II	
	α-Anomer	β-Anomer	α-Anomer	β-Anomer
H-1	5.24, 5.19	4.69, 4.80	5.40, 5.39	4.40
	(*d*, *J* = 4)	(*d*, *J* = 8)	(*d*, *J* = 4)	(*d*, *J* = 8)
H-2	3.87, 3.79	3.55, 3.70	5.07, 5.05	5.11, 5.03
	(*dd*, *J* = 4, 10)	(*dd*, *J* = 8, 10)	(*dd*, *J* = 4, 10)	(*dd*, *J* = 8, 10)
H-3	5.47, 5.45	5.33, 5.30	5.69, 5.66	5.29, 5.27
	(*t*, *J* = 10)	(*t*, *J* = 10)	(*t*, *J* = 10)	(*t*, *J* = 10)
H-4	4.87, 4.86	4.93	5.00, 4.94	4.93
	(*t*, *J* = 10)	(*t*, *J* = 10)	(*t*, *J* = 10)	(*t*, *J* = 10)
H-5	4.56, 4.63	4.02*	4.51	4.02, 4.06*
	(*m*)	(*m*)	(*m*)	(*m*)
H-6	5.18	5.18†	5.07	5.12†
	(*dd*, *J* = 7, 14)	(*dd*, *J* = 7, 14)	(*dd*, *J* = 7, 14)	(*dd*, *J* = 7, 14)
	3.83	3.83‡	3.64	3.71‡
	(*d*, *J* = 14)	(*d*, *J* = 14)	(*d*, *J* = 14)	(*d*, *J* = 14)

*†‡ Interchangeable.





stated otherwise. Chemical shifts are given in δ values relative to that of solvent [acetone-d_6 (δ_H 2.04; δ_C 29.8)] on a TMS scale. Normal-phase HPLC was conducted on a Superspher Si60 (Merck) column (4 × 125 mm) developed with *n*-hexane–MeOH–THF–HCO_2H (55:33:11:1) containing oxalic acid (450 mg l^{-1}) (flow rate, 1.5 ml min^{-1}; detection 280 nm) at room temp. Reverse-phase HPLC was performed on a LiChrospher RP-18 column (4 × 250 mm) developed with 10 mM H_3PO_4–10 mM KH_2PO_4–MeCN (9:9:2) (flow rate, 1 ml min^{-1}, detection 280 nm) at 40°. Other chromatographic conditions are the same as those described in ref. [1].

Plant material. Stems and roots of *O. laciniata* were collected at the campus of the Okayama University in September and May. Voucher specimens are deposited at the Medicinal Herbal Garden of the Faculty of Pharmaceutical Sciences, Okayama University.

Isolation of tanins. (*a*) *Tannins collected in September.* Dried roots and stems (1 kg) were homogenized in 70% aq. Me_2CO and filtered. The concd filtrate was subjected to CC over Diaion HP-20 with H_2O and aq. MeOH (10% → 20% → 30% → 40% → 60%). The 20% MeOH eluate (6.8 g) was found to be almost pure oenothein B (**1**) by normal and reverse-phase HPLC. A part (3 g) of the 30% MeOH eluate (7.97 g) was rechromatographed over Toyopearl HW-40 (fine) with aq. MeOH to yield **1** (1.22 g) and a trimer, oenothein A (175 mg). A combination of CC of the 40% MeOH eluate (9 g) over Toyopearl HW-40 and MCI-gel CHP 20P with aq. MeOH gave 1,2,6-trigalloyl-β-D-glucose (14 mg), 1,2,3-trigalloyl-β-D-glucose (5 mg), oenothein C (**2**) (30 mg), tellimagrandin I (**4**) (140 mg), oenothein D (**5**) (39 mg), cornusiin B (**3**) (40 mg), oenothein B (**1**) (27 mg) and oenothein A (405 mg). (*b*) *Tannins collected in May.* Fresh roots and stems (6 kg) were treated in a similar way to (a). The 20% MeOH (11 g) and 30% MeOH (12 g) eluates from CC of the concd extract over Diaion HP-20 contained **1** and a mixture of **1** and oenothein A, respectively, as in the case of (a). A part (6 g) of the 40% MeOH eluate (7.5 g) was chromatographed over Toyopearl HW-40 (fine grade) with aq. MeOH (50% → 60% → 70%MeOH) → MeOH–H_2O–Me_2CO (7:2:1) to give 1,2,6-tri-*O*-galloyl-β-D-glucose (76 mg), tellimagrandin I (**4**) (45 mg), oenotheins A (125 mg) and B (**1**) (161 mg), eucalbanin B (10 mg), and oenotheins F (**8**) (16.5 mg) and G (**9**) (17 mg).

Oenothein D (**5**). Light brown amorphous powder. $[\alpha]_D$ +81° (MeOH; *c* 1.0) (Found: C, 44.86; H, 4.07%. $C_{68}H_{48}O_{44} \cdot 14H_2O$ requires C, 44.84; H, 4.18%). FABMS: *m/z* 1591 $[M + Na]^+$. UV λ_{max}^{MeOH} nm: 213 (log ε 5.02), 265 (4.68). CD (MeOH): $[\theta]_{220}$ + 24.1 × 10^4, $[\theta]_{259}$ − 4.1 × 10^4, $[\theta]_{283}$ + 5.8 × 10^4, $[\theta]_{320}$ − 1.2 × 10^4. ^{1}H NMR of the predominant anomer: δ 7.34, 6.59 (each 2H, *s*, galloyl), 7.28 (valoneoyl (Val)-$H_{B'}$), 6.68 (Val-$H_{C'}$), 6.60 (Val-$H_{A'}$), 6.53 (Val-H_C), 6.39 (Val-H_B), 6.18 (Val-H_A) (each 1H, *s*), glucose protons, see Table 1. ^{13}C NMR: δ 110.1 (2C) [ring-G′ (G′) C-2, C-6], 111.4 (2C) (G C-2, C-6), 120.9 (G C-1), 121.7 (G C-1), 138.7 (G′ C-4), 137.7 (G C-4), 145.4 (2C) (G′ C-3,

C-5), 144.1 (2C) (G C-3, C-5), 105.9 (valoneoyl ring B (Val-B) C-3) 107.3 (Val-A C-3), 109.4 (Val-C C-6), 109.6 (Val-C' C-6), 109.6 (Val-C' C-6), 110.1 (Val-A' C-3), 114.9 (Val-B' C-1), 116.3 (Val-A C-1), 117.0 (Val-B' C-3), 117.5 (Val-B' C-2), 117.7 (Val-B C-1), 119.2 (Val-A' C-1), 115.9, 117.6, 126.5, 127.8, 120.3 (Val-A, A', B, C-2, Val-C, C' C-1), 134.9 (Val-B' C-5), 135.2 (Val-B C-5), 136.5 (Val-A C-5), 137.2 (Val-A' C-5), 134.2 (Val-C C-2), 138.8 (Val-C' C-2), 139.0, 139.9 (Val-C C', C-3), 139.1 (Val-C C-4), 140.7 (Val-C' C-4), 143.1 (Val-C' C-5), 143.7 (Val-C C-5), 144.9, 145.5, 147.1, 147.4 (Val-A, A', B, B' C-6), 147.8 (Val-B C-4), 145.1 (Val-A C-4), 144.7 (Val-A' C-4), 144.5 (Val-B' C-4), 169.8 (Val-A' C-7), 169.0 (Val-A C-7), 168.2 (Val-B C-7), 168.0 (Val-B' C-7), 167.0 (Val-C' C-7), 166.9 (ring G' C-7), 166.8 (Val-C C-7), 165.8 (ring G C-7) (ester carbonyl), glucose carbons, see Table 1.

Methylation of **5**. A mixt. of **5** (5 mg), Me_2SO_4 (0.05 ml), anhyd. K_2CO_3 (500 mg) and Me_2CO (2 ml) was stirred overnight at room temp. and refluxed for 3 hr. After removal of inorganic materials by centrifugation, the reaction mixt. was concd and subjected to prep. TLC (Kieselgel PF_{254}, benzene–Me_2CO, 4:1) to yield the tetracosamethyl derivative (**5a**) (1.4 mg). FAB-MS: *m/z* 1927 $[M + Na]^+$, 1905 $[M + H]^+$. ^1H NMR (main anomer): δ7.55, 6.89 (each 2H, *s*, galloyl), 7.25, 6.99, 6.73, 6.66, 6.47, 6.26 (each 1H, *s*, valoneoyl), 4.61 (*d*, $J = 8$ Hz, H-1), 5.66 (*dd*, $J = 8$, 10 Hz, H-2), 5.70 (*t*, $J = 10$ Hz, H-3), 5.48 (*t*, $J = 10$ Hz, H-4), 4.10 (*m*, H-5), 4.78 (*t*, $J = 11$ Hz, H-6), 5.22 (*d*, $J = 4$ Hz, H-1'), 5.19 (*dd*, $J = 4$, 10 Hz, H-2'), 5.75 (*t*, $J = 10$ Hz, H-3'), 5.53 (*t*, $J = 10$ Hz, H-4'), 4.31 (*dd*, $J = 6$, 10 Hz, H-5'), 5.01 (*dd*, $J = 6$, 13 Hz, H-6'), 3.50–4.10 (24 × OMe).

Methanolysis of **5a**. A soln of **5a** (1 mg) in MeOH (2 ml) and 1% NaOMe (0.1 ml) was left standing at room temp. for 8 hr. After acidification with a few drops of HOAc, the solvent was removed *in vacuo*. The residue was re-dissolved in MeOH and an aliquot analysed by reverse-phase HPLC which indicated the production of Me tri-*O*-methylgallate (**6**) and triMe octa-*O*-methylvalonate (**7**) in a molar ratio of 2:2. The molar ratio was determined from HPLC peak areas, which were the same as those observed upon similar treatment of permethylated **1**. Prep. TLC (Kieselgel PF_{254}, benzene–Me_2CO, 6:1) of the residue afforded **6** (0.5 mg), (EIMS: *m/z* 226 $[M]^+$) and **7** (0.4 mg) (EIMS: *m/z* 669 $[M]^+$).

Partial hydrolysis of **5**. An aq. soln of **5** (5 mg) containing one drop of CF_3CO_2H was heated at 100° for 8 hr. The reaction mixt. was passed through Bond Elut C-18, and eluted with water and MeOH. Reverse-phase HPLC of the water eluate showed peaks due to 3-*O*-galloylglucose and gallic acid. A mixt. of valoneic acid dilactone and ellagic acid precipitated out upon concn of the MeOH eluate which was removed by centrifugation. The supernatant gave oenothein C (**2**) (1 mg) which was identified by HPLC (normal- and reverse-phase) and ^1H NMR spectral comparisons.

Oenothein F (**8**). Off-white amorphous powder. $[\alpha]_D$ + 45° (acetone, *c* 1.0). FAB-MS: *m/z* 1591 $[M + Na]^+$, CD (MeOH): $[\theta]_{222} + 13.0 \times 10^4$, $[\theta]_{248} + 6.7 \times 10^4$, $[\theta]_{260} - 4.9 \times 10^4$, $[\theta]_{283} + 4.6 \times 10^4$. ^1H NMR of predominant anomer: δ7.04 (4H, *s*, galloyl), 7.29, 6.39, 6.21 (each 2H, *s*, valoneoyl), 5.56 (2H, *d*, $J = 4$ Hz, H-1, 1'), 5.42 (2H, *dd*, $J = 4$, 10 Hz, H-2, 2'), 5.78 (2H, *t*, $J = 10$ Hz, H-3, 3'), 5.04 (2H, *t*, $J = 10$ Hz, H-4, 4'), 4.61 (2H, *dd*, $J = 5$, 10 Hz, H-5, 5'), 5.03 (2H, *dd*, $J = 5$, 13 Hz, H-6, 6'), 3.80 (2H, *d*, $J = 13$ Hz, H-6, 6').

Acid hydrolysis of oenothein F (**8**). A soln of **8** (1 mg ml^{-1}) in 5% H_2SO_4 was heated at 100° and the reaction monitored by reverse-phase HPLC. The formation of oenothein C (**2**) and gallic acid accompanied by the disappearance of **8** was observed after 2 hr. The reaction mixt. after 11 hr was passed through Bond Elut C-18 and washed with water. The MeOH eluate was methylated with CH_2N_2, followed by purification by prep. TLC (benzene–Me_2CO, 15:1) to give tetra-*O*-methylellagic acid (0.1 mg) (EIMS: *m/z* 358 $[M]^+$) and Me hexa-*O*-methylvaloneic acid dilactone (0.3 mg) (EIMS: *m/z* 568 $[M]^+$).

Methylation of **8** *followed by methanolysis*. Oenothein F (**8**) (1 mg) was methylated with CH_2N_2 and the products without separation were methanolized with 1% NaOMe soln. After acidification with HOAc, followed by evaporation, the reaction mixt. was partitioned between water and EtOAc. HPLC analysis of the EtOAc-soln. portion using a chiral column (Sumichiral OA-3300, 4 × 250 mm, solvent: *n*-hexane–CH_2Cl_2–EtOH, 200:25:1) gave peaks due to **6** and **7**, the latter of which was identified as triMe (*S*)-octa-*O*-methylvalonate (R_t 18.8 min); ref. (*R*)-**7**, R_t 16.3 min.

Oenothein G (**9**). light brown amorphous powder, $[\alpha]_D$ + 36° (acetone, *c* 1.0). FAB-MS: *m/z* 1609 $[M + Na]^+$. UV λ_{max}^{MeOH} nm: 217 (log ε 5.09), 265 (4.80). ^1H NMR: δ7.06, 7.05, 7.03, 7.02 (each *s*, 2H in total, galloyl), 6.96, 6.94, 6.93, 6.88 (each *s*, 2H in total, galloyl), 7.24, 7.23, 7.22, 7.21 (each *s*, 1H in total), 7.03, 7.02, 6.99, 6.98 (each *s*, 1H in total) (valoneoyl-$H_{C,C'}$), 6.62, 6.61 (each *s*, 1H in total, valoneoyl-$H_{A'}$), 6.47, 6.45, 6.42, 6.39 (each *s*, 1H in total, valoneoyl-H_A), 6.28, 6.26 (each *s*, 1H in total), 6.12, 6.09, 6.04 (each *s*, 1H in total) valoneoyl-$H_{B,B'}$), glucose protons, see Table 2.

Methylation of **9** *followed by methanolysis*. Oenothein G (**9**) (2 mg) was methylated with CH_2N_2 and the products without separation were methanolized with 1% NaOMe soln. Usual work-up and prep. TLC in a way similar to that described for **5** gave **6** (0.7 mg) and **7** (0.5 mg). $[\alpha]_D$ − 15° (Me_2CO, *c* 0.25).

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REFERENCES

1. Okuda, T., Yoshida, T. and Hatano, T. (1993) *Phytochemistry* **32**, 507.

2. Yoshida, T., Chou, T., Matsuda, M. Yasuhara, T., Yazaki, K., Hatano, T., Nitta, A. and Okuda, T. (1991) *Chem. Pharm. Bull.* **39**, 1157.
3. Hatano, T., Yasuhara, T., Matsuda, M., Yazaki, K., Yoshida, T. and Okuda, T. (1990) *J. Chem. Soc. Perkin Trans. I* 2735.
4. Nakashima, H., Murakami, T., Yamamoto, N., Sakagami, H. Tanuma, S., Hatano, T., Yoshida, T. and Okuda, T. (1992) *Antiviral Res.* **18**, 91.
5. Miyamoto, K., Nomura, M., Murayama, T., Furukawa, T., Hatano, T., Yoshida, T., Koshiura, R. and Okuda, T. (1993) *Biol. Pharm. Bull.* **16**, 379.
6. Miyamoto, K., Nomura, M., Sasakura, M., Matsui, E., Koshiura, R., Murayama, T., Furukawa, T., Hatano, T., Yoshida, T. and Okuda, T. (1993) *Jpn J. Cancer Res.* **84**, 99.
7. Miyamoto, K., Murayama, T., Nomura, M., Hatano, T., Yoshida, T., Fukuyama, T., Koshiura, R. and Ouda, T. (1993) *Anticancer Res.* **13**, 37.
8. Yoshida, T., Chou, T., Nitta, A., Miyamoto, K., Koshiura, R. and Okuda, T. (1990) *Chem. Pharm. Bull.* **38**, 1211.
9. Yoshida, T., Chou, T., Nitta, A. and Okuda, T. (1992) *Chem. Pharm. Bull.* **40**, 2023.
10. Yoshida, T., Maruyama, T., Nitta, A. and Okuda, T. (1992) *Chem. Pharm. Bull.* **40**, 1750.
11. Haddock, E. A., Gupta, R. J., Al-Shafi, S. M. K., Haslam, E. and Magnolato, D. (1982) *J. Chem. Soc. Perkin Trans. I* 2515.
12. Hatano, T., Ogawa, N., Kira, R., Yasuhara, T. and Okuda, T. (1989) *Chem. Pharm. Bull.* **37**, 2083.
13. Okuda, T., Yoshida, T., Ashida, M. and Yazaki, K. (1983) *J. Chem. Soc. Perkin Trans. I* 1765.
14. Okuda, T., Yoshida, T. and Hatano, T. (1989) *J. Nat. Prod.* **52**, 1.
15. Yoshida, T., Hatano, T., Kuwajima, T. and Okuda, T. (1992) *Heterocycles* **33**, 463.
16. Okuda, T., Yoshida, T., Hatano, T., Koga, T., Toh, N. and Kuriyama, K. (1982) *Tetrahedron Letters* **23**, 3937.
17. Yoshida, T., Ahmed, A. F., Memon, M. U. and Okuda, T. (1993) *Phytochemistry* **39**, 197.
18. Yoshida, T., Chou, T., Nitta, A. and Okuda, T. (1991) *Chem. Pharm. Bull.* **39**, 2247.