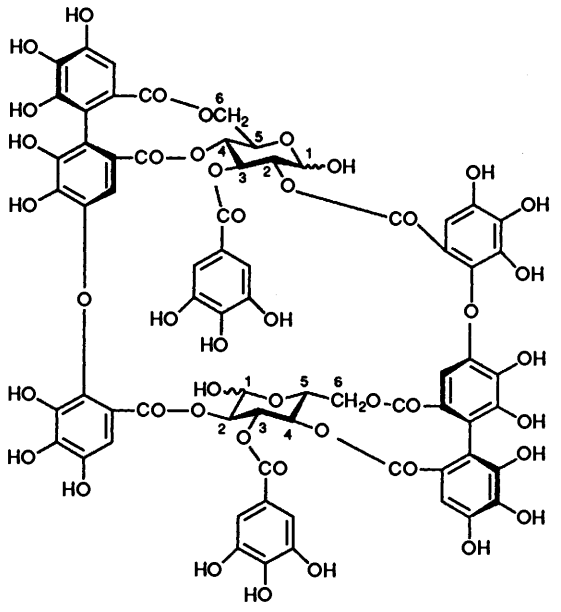
Oenothein B, a Dimeric, Hydrolysable Tannin with Macrocyclic Structure, and Accompanying Tannins from *Oenothera erythrosepala*

Tsutomu Hatano, Taeko Yasuhara, Muneto Matsuda, Kazufumi Yazaki, Takashi Yoshida, and Takuo Okuda *
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

A new dimeric, hydrolysable tannin which we have named oenothein B (**1**), and eight accompanying compounds, including oenothein C (**2**), were isolated from the leaves of *Oenothera erythrosepala*. The macrocyclic structure of compound (**1**) was established from chemical and spectroscopic data. The previously assigned structure of compound (**2**) was also confirmed by two-dimensional NMR spectroscopy.

Some oligomeric, hydrolysable tannins have been found to have noticeable biological activity,[1] such as antitumour[2] and antiviral[3,4] activity. Oenothein B (**1**), isolated from *Oenothera erythrosepala*, is one of these tannins.

Several *Oenothera* species grown in North and South America have been recorded as medicinal plants.[5–7] Detection of some phenolic acids, including gallic acid and ellagic acid, in extracts of *Oenothera* species has been reported without precise description of the accompanying tannins.[8] We have isolated nine polyphenolic compounds from *O. erythrosepala*, and have found that the main constituent, oenothein B, is a new dimeric, hydrolysable tannin of macrocyclic structure and the first example of this class.[9]

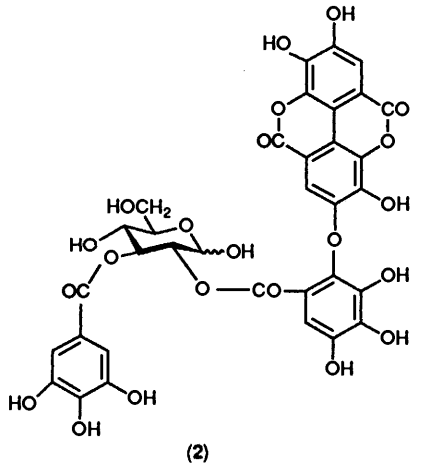


(**1**)

Results and Discussion

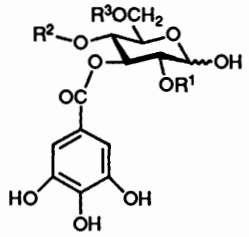
Isolation.—Leaves of *O. erythrosepala* were homogenized in aqueous acetone, and the concentrated filtrate from the homogenate was extracted successively with diethyl ether, ethyl acetate, and butan-1-ol. Oenothein B was obtained from the butan-1-ol extract, like many other oligomeric, hydrolysable tannins.[10–13] Oenothein C (**2**),[14] gemin D (**3**),[15] tellimagrandin I (**4**),[16,17] cornusiin B (**5**)[14] and 3-*O*-galloyl-D-glucose (**6**),[14,18] were also isolated from the butan-1-ol extract. The ethyl acetate extract afforded 1,2,6-tri-*O*-galloyl-β-D-glucose,[14,19] 1,2,3,6-tetra-*O*-galloyl-β-D-glucose,[14,19] and 1,2,4,6-tetra-*O*-galloyl-β-D-glucose,[19,20] as well as compounds (**3**), (**4**), and (**5**).

Constituent Sugar and Acyl Groups of Oenothein B.—The new tannin, oenothein B (**1**), was isolated as an off-white, amorphous powder. The FAB mass spectrum of this tannin showed the $(M + H)^+$ ion at *m/z* 1 569, indicating its molecular formula to be $C_{68}H_{48}O_{44}$. Treatment of compound (**1**) with diazomethane and then with sodium methoxide[14] afforded methyl tri-*O*-methylgallate (**7**) and trimethyl (*S*)-octa-*O*-methylvaloneate (**8**) in the molar ratio 1:1. The presence of a glucose moiety in compound (**1**) was shown by degradation of oenothein B with 5% sulphuric acid, followed by GC analysis. Therefore, oenothein B consists of two (*S*)-valoneoyl groups, two galloyl groups, and two glucose cores. The CD spectrum of compound (**1**) showed a positive Cotton effect in the short-wavelength region ($[\theta]_{218}$ $+3.8 \times 10^5$), also indicating the *S*-configuration[14,21] of the two valoneoyl groups in the molecule.

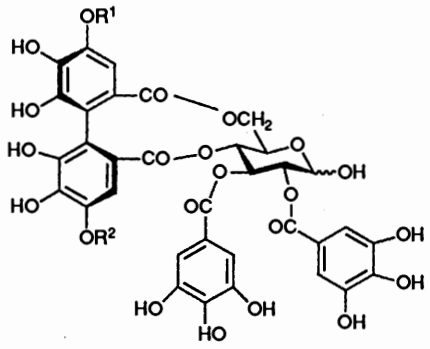


(**2**)

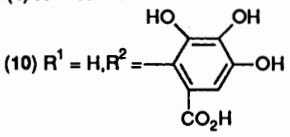
The ^{1}H and ^{13}C NMR Spectra of Oenothein B and Locations of Acyl Groups on its Glucose Cores.—The 200 MHz ^{1}H NMR



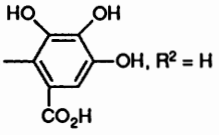
(3) R^1 = H, R^2R^3 = (S) – HHDP
(5) R^1 = LV, R^2R^3 = (S) – HHDP
(6) $R^1 = R^2 = R^3$ = H
(9) R^1 = galloyl, $R^2 = R^3$ = H



(4) $R^1 = R^2$ = H

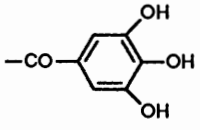


(10) R^1 = H, R^2 =

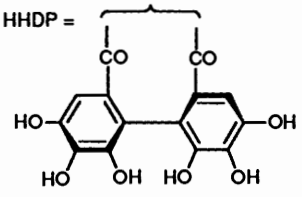


(11) R^1 =

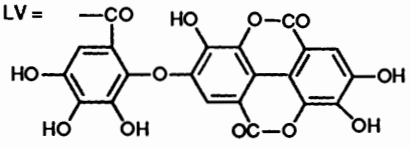
galloyl =

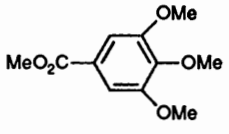


(S) – HHDP =

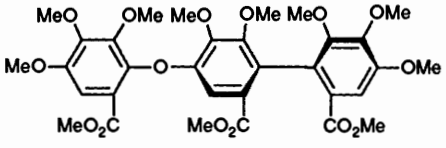


LV =





(7)



(8)

Table 1. ^{1}H NMR spectral data for the glucose cores in the main anomeric form of oenothein B (**1**): 200 MHz, at 50 °C; δ-values from $SiMe_4$ in [$^2H_6$]acetone, *J*-values in Hz.

	α-Glucose	β-Glucose
1-H	6.20 (d, *J* 3.5)	4.48 (d, *J* 7.5)
2-H	5.87 (dd, *J* 3.5, 10)	5.19 (dd, *J* 7.5, 9.5)
3-H	6.11 (t, *J* 10)	5.45 (t, *J* 9.5)
4-H	5.60 (t, *J* 10)	4.92 (t, *J* 9.5)
5-H	4.59 (dd, *J* 6, 10)	4.16 (dd, *J* 5, 9.5)
6-H	5.25 (dd, *J* 6, 13),	5.00 (dd, *J* 5, 13),
	3.66 (d, *J* 13)	3.85 (d, *J* 13)

spectrum of oenothein B, recorded at 50 °C,* showed that this tannin forms an anomeric mixture. Table 1 summarizes the data of glucose protons of its main anomeric form, in which one glucose core takes the α-form and the other core takes the β-form. These assignments were confirmed by extensive decoupling experiments.

* The ^{1}H NMR analysis using a 500 MHz instrument at ambient temperature (21 °C) did not give a clear spectrum, not only because of multiplication of each signal due to anomerization in the two glucose cores (producing four anomeric forms), but also because of marked broadening of several signals, regarded as being due to a certain barrier against the conformational change such as restricted rotation around the ether linkages of two valoneoyl groups. The spectrum of compound (**1**) was therefore obtained at an elevated temperature on a spectrometer with a lower magnetic field (200 MHz).

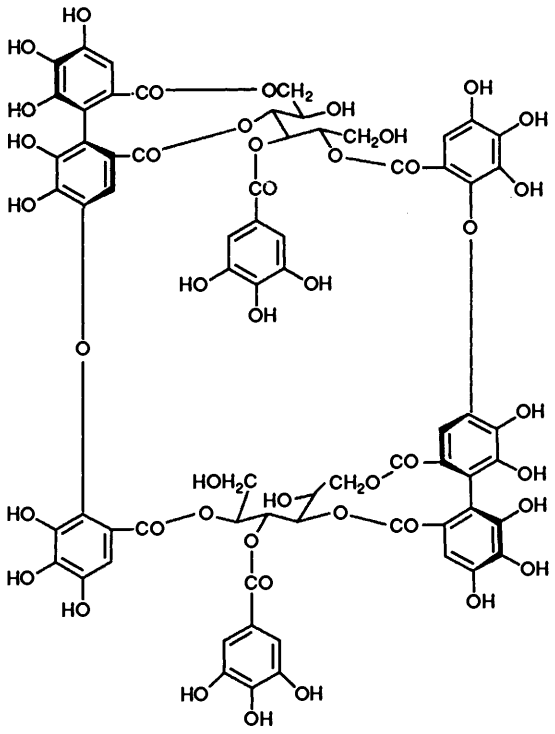
The coupling constants in Table 1 indicate that both of the two glucose cores adopt a $^4C_1$ conformation. The large difference in the chemical shifts between the two 6-H protons of each $^4C_1$ glucopyranose core (Δδ 1.59 and 1.15 ppm) indicates that the hexahydroxydiphenoyl (HHDP) moiety in each valoneoyl group is at O-4/O-6 of each glucose core.[16]

The chemical shift of 1-H of the β-glucose core (δ 4.48) indicates that the anomeric centre of this glucose core is not acylated. The anomeric centre of the α-glucose core is also not acylated as indicated by the chemical shift of 1-H (δ 6.20), at higher field than those of the other hydrolysable tannins having an α-oriented acyloxy grop at C-1, such as potentillin[22] (δ 6.63), and pentagalloyl -α-D-glucose[23] (δ 6.75).

The chemical shifts of the four anomeric carbon signals in the ^{13}C NMR spectrum (125.7 MHz; CD_3COCD_3 + D_2O; 40 °C) of oenothein B (**1**) [δ 95.9 (β-glucose, minor signal), 95.8 (β-glucose, major), 91.5 (α-glucose, major), and 90.8 (α-glucose, minor)] substantiate the absence of acyl groups at both anomeric centres in compound (**1**). These chemical shifts are almost in complete accord with the corresponding anomeric carbon signals of tellimagrandin I (**4**) [δ 96.7 (β-anomer) and 91.2 (α-anomer)], suggesting that compound (**4**) could be a constituent monomer of (**1**).[24,25]

The absence of acyl groups at these two anomeric centres was further confirmed by reduction of compound (**1**) with sodium borohydride,[26,27] which yielded (**12**).

Structure of the Reduction Product (**12**).—The ^{1}H NMR spectrum of compound (**12**) indicated the presence of two



(12)

Table 2. ^{1}H NMR spectral data for the glucitol cores in compound (**12**): 500 MHz; δ-values from $SiMe_4$ in [$^2H_6$]acetone + D_2O, *J*-values in Hz.

	Glucitol core I	Glucitol core II
1-H	3.47 (dd, *J* 2, 12), 3.35[a]	3.66 (dd, *J* 5, 11), 3.42 (dd, *J* 8, 11)
2-H	5.21 (ddd, *J* 2, 6, 9)	5.16 (dd, *J* 5, 8)
3-H	5.53 (d, *J* 6)	5.71 (d, *J* 9.5)
4-H	5.47 (d, *J* 7)	5.33 (dd, *J* 7, 9.5)
5-H	3.90 (br d, *J* 7)	4.01 (br d, *J* 7)
6-H	4.46 (dd, *J* 2, 12), 3.89 (br d, *J* 12)	4.57 (dd, *J* 2, 12), 3.64 (br d, *J* 12)

[a] Overlapped by the HDO signal.

glucitol cores in the molecule as shown in Table 2, in which assignments of glucitol proton signals were based on the ^{1}H–^{1}H shift-correlation spectrum.

The one-bond and long-range ^{1}H–^{13}C shift-correlation spectra of (**12**) showed cross-peaks summarized in Table 3. Among the cross-peaks in the long-range ^{1}H–^{13}C correlation spectrum, those which form four sets of the combination valoneoyl proton–ester carbonyl carbon–glucitol proton (δ_H 6.52 – δ_C 169.7 – δ_H 4.46, δ_H 6.41 – δ_C 167.4 – δ_H 5.47, δ_H 6.37 – δ_C 167.5 – δ_H 5.33, and δ_H 6.04 – δ_C 168.7 – δ_H 4.57) clearly indicate that the two carboxy groups of the HHDP moiety in each valoneoyl group are on O-4/O-6 of each glucitol core, and that the orientation[28] of the two valoneoyl groups are different from each other (Figure 1). Namely, the orientation of the valoneoyl group on O-4/O-6 of glucitol I [the upper glucitol core in formula (**12**)] is the same as that in isorugosin B (**10**),[28] and the orientation of the valoneoyl group on O-4/O-6 of glucitol II [the lower glucitol core in formula (**12**)] is the same as that in rugosin B (**11**).[28,29]

Figure 1 also exhibits the ^{1}H–^{13}C three-bond correlations of two galloyl groups (δ_H 7.08 – δ_C 166.5 – δ_H 5.53 and δ_H 7.14 – δ_C

Table 3. One-bond and long-range ^{1}H–^{13}C correlation data for compound (**12**).

	δ_C	Proton coupled *via* one bond	Proton coupled *via* two or three bonds
Glucitol I			
C-1	60.7	3.35	
C-2	73.2	5.21	
C-3	72.7	5.53	
C-4	73.2	5.47	
C-5	69.7	3.90	
C-6	67.2[a]		
Glucitol II			
C-1	59.8	3.66	
C-2	72.0	5.16	
C-3	72.7	5.71	
C-4	73.4	5.33	
C-5	69.4	4.01	
C-6	67.4[a]		
Galloyl I			
C-1	119.9[b]		
C-2, C-6	110.6	7.08	
C-3, C-5	145.8		7.08 (galloyl I)
C-4	139.8		7.08 (galloyl I)
C-7	166.5		7.08 (galloyl I), 5.53 (3-H of glucitol I)
Galloyl II			
C-1	120.6[b]		
C-2, C-6	110.3	7.14	
C-3, C-5	145.8		7.14 (galloyl II)
C-4	139.2		7.14 (galloyl II)
C-7	167.1		7.14 (galloyl II), 5.71 (3-H of glucitol II)
Valoneoyl I			
C-1	114.4		6.52 (H_A)
C-2	124.6[c]		
C-3	107.0	6.52	
C-4	145.2		
C-5	135.8		6.52 (H_A)
C-6	144.3[d]		
C-7	169.7		6.52 (H_A), 4.46 (6-H of glucitol I)
C-1′	117.7		6.41 (H_B)
C-2′	127.1[c]		
C-3′	106.0	6.41	
C-4′	147.7		6.41 (H_B)
C-5′	137.3		6.41 (H_B)
C-6′	144.9[d]		
C-7′	167.4		6.41 (H_B), 5.47 (4-H of glucitol I)
C-1″	113.4[e]		
C-2″	137.5[f]		7.47 (H_C)[l]
C-3″	139.7[g]		
C-4″	140.6[h]		7.47 (H_C)[l]
C-5″	142.7[i]		7.47 (H_C)[l]
C-6″	110.9[j]	7.47	
C-7″	162.6[k]		7.47 (H_C)[l]
Valoneoyl II			
C-1	115.8		6.37 (H_A′)
C-2	125.2[c]		
C-3	108.5	6.37	
C-4	145.1		6.37 (H_A′)
C-5	136.6		6.37 (H_A′)
C-6	144.6[d]		
C-7	167.5		6.37 (H_A′), 5.33 (4-H of glucitol II)
C-1′	116.8		6.04 (H_B′)
C-2′	127.1[c]		

Table 3 (*continued*)

C-3′	104.8	6.04	
C-4′	146.8		6.04 (H_B')
C-5′	136.3		6.04 (H_B')
C-6′	145.0[d]		
C-7′	168.7		6.04 (H_B'), 4.57 (6-H of glucitol II)
C-1″	115.5[e]		
C-2″	137.2[f]		7.08 (H_C')[l]
C-3″	140.1[g]		
C-4″	140.1[h]		7.08 (H_C')[l]
C-5″	143.0[i]		7.08 (H_C')[l]
C-6″	109.1[j]	7.08	
C-7″	164.1[k]		7.08 (H_C')[l]

[a–k] Values with the same superscript are interchangeable. [l] Assignments of H_C and H_C' may be reversed.

167.1 – δ_H 5.71), which show that one galloyl group is at O-3 of glucitol I, and the other galloyl group is at O-3 of glucitol II.

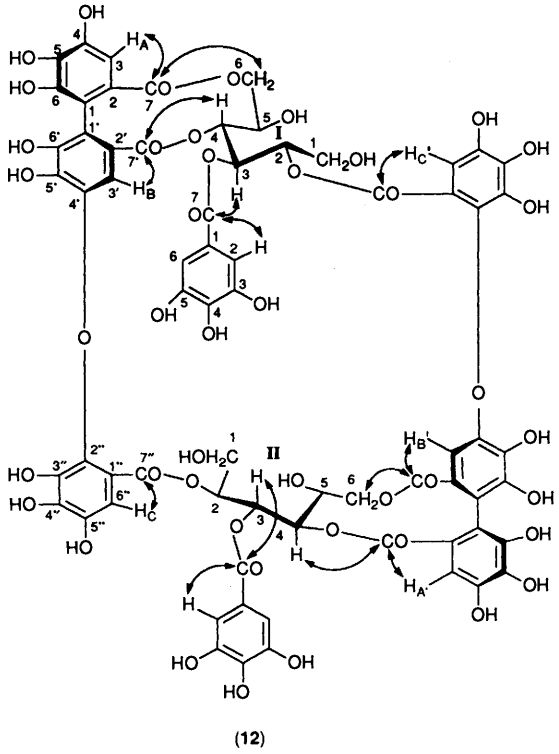
These findings, combined with the chemical shifts of the glucitol protons (Table 2) which show that all of the atoms O-2, -3, -4, and -6 of the two glucitol cores are acylated, indicate that the galloyl moiety in each valoneoyl group should be at O-2 of each glucitol core in structure (12). The macrocyclic structure (**12**) for this compound [and therefore structure (**1**) for oenothein B] was thus assigned.

Partial Hydrolysis of Oenothein B and the Structure of Products (**13**) *and* (**14**).—The macrocyclic structure (**1**) of oenothein B was further substantiated by partial degradation of compound (**1**) with 0.05M-sulphuric acid, which yielded compounds (**13**) and (**14**).

The FAB mass spectrum of compound (**13**) showed the $(M + Na)^+$ ion at m/z 1 591, indicating its molecular formula to be $C_{68}H_{48}O_{44}$. The ^1H NMR spectrum showed that this compound consists of a lactonized valoneoyl group, a valoneoyl group, two galloyl groups, and two glucose cores (see Experimental section), and that this compound, lacking the acyl groups at the two anomeric centres, forms a mixture of four anomers (α–α, α–β, β–α, and β–β).

The pattern of glucose carbon signals in the ^{13}C NMR spectrum of compound (**13**) is similar to that of the merged signals of glucose carbons of compounds (**2**) and (**5**) (Figure 2), to indicate similarity of locations of the acyl groups.[24,25] Namely, four galloyl groups (including two galloyl moieties in the lactonized valoneoyl group and the valoneoyl group) are at O-2 and O-3 on the two glucose cores in compound (**13**). The HHDP moiety of the valoneoyl group is on O-4/O-6 of one of the two glucose cores [the right-hand glucose core in formula (**13**)], and the hydroxy groups on C-4 and C-6 of the other glucose core [the left-hand glucose core in formula (**13**)] are not acylated.

Partial degradation of compound (**13**) in hot water afforded oenothein C (**2**),[14] together with compounds (**6**), (**5**), and (**9**). The latter two compounds should derive from cleavage of the ether linkage of the valoneoyl group in compound (**13**).[28,30] The locations of the lactonized valoneoyl group on O-2 and of galloyl group on O-3 of the glucose core of compound (**2**), which were assigned previously, have now been confirmed by measurement of the long-range ^1H–^{13}C shift-correlation



(12)

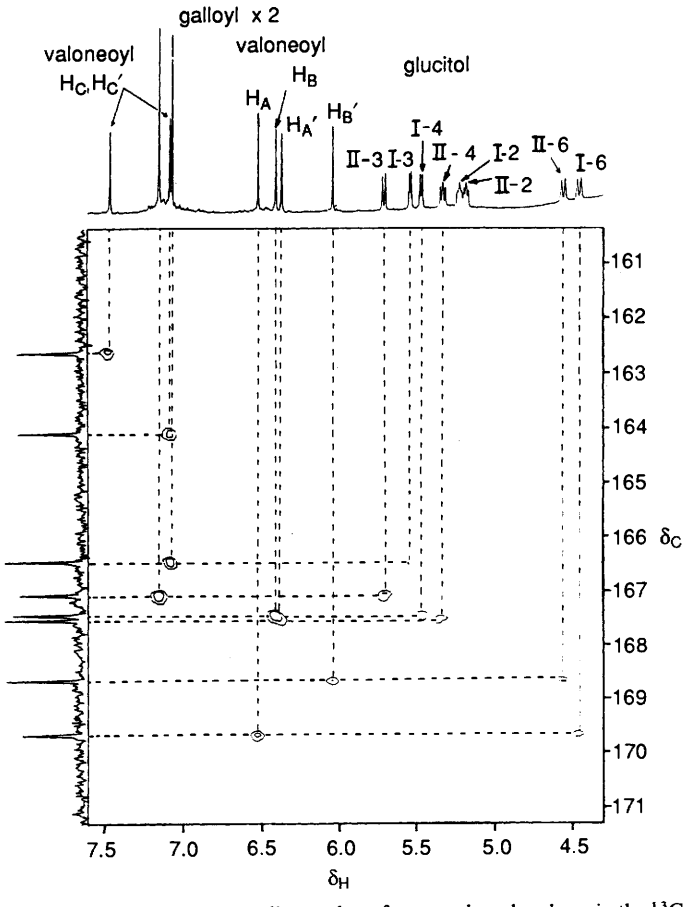
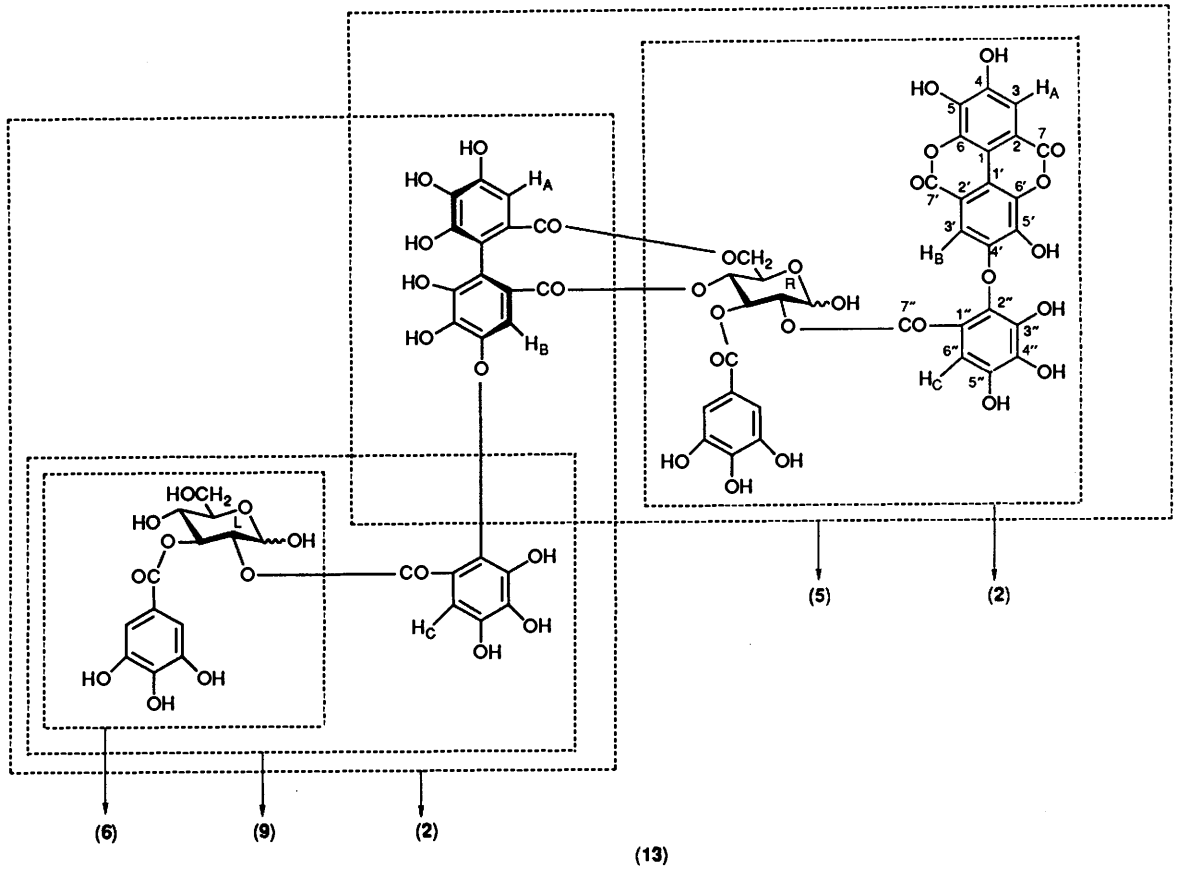
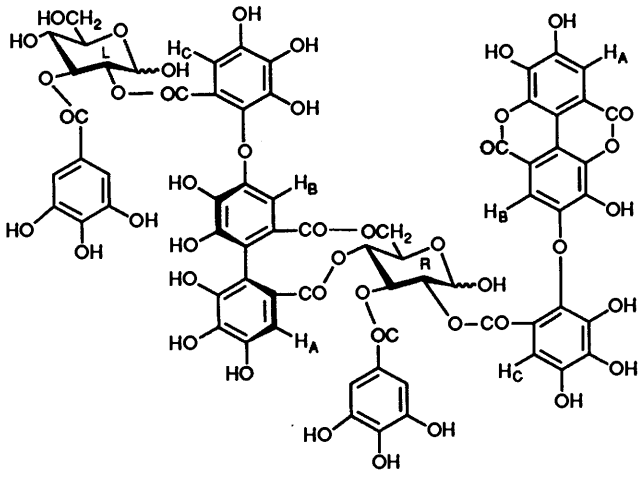


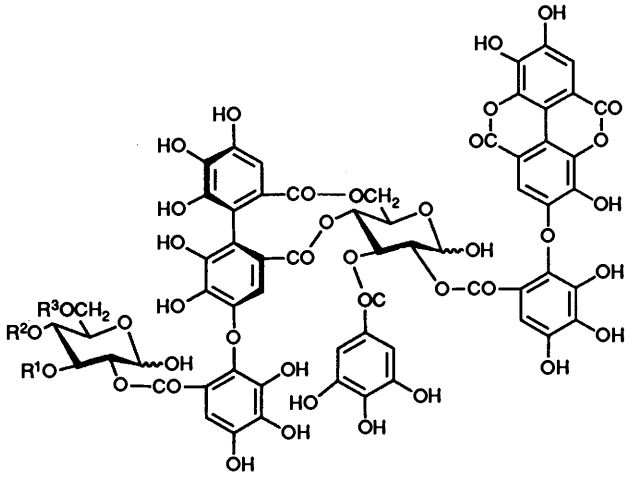
Figure 1. The long-range ^1H–^{13}C shift-correlation spectrum of compound (**12**). The region corresponding to that of ester carbonyl carbons in the ^{13}C NMR spectrum is shown. The average J_{CH}-value was set at 7 Hz. 'Glucitol II-3' means 3-H of glucitol core II, the lower glucitol core in formula (**12**).



(13)



(14)



(15) R^1 = galloyl, R^2R^3 = (*S*) - HHDP
(16) $R^1 = R^2 = R^3$ = H
(17) R^1 = H, R^2R^3 = (*S*) - HHDP

spectrum. The correlations of aromatic proton–ester carbonyl carbon–glucose proton, depicted in Figure 3, clearly indicate that the galloyl moiety of the lactonized valoneoyl group is on O-2 of the glucose core, and that the galloyl group is on O-3.

The four 1-H signals of β-glucose cores [δ_H 4.74, 4.71, 4.22, and 4.19 (each d, *J* 8 Hz)] in the three anomers (α–β, β–α, and β–β) of (**13**) showed remarkable upfield shifts, relative to the 1-H signal of the β-anomer of compound (**4**) (δ_H 5.13). These shifts are ascribable to the anisotropic effects of the lactonized valoneoyl group and the valoneoyl group in the molecule, and therefore the galloyl moiety in each of these two groups could be on O-2 of each glucose core, where these groups will be able to cause the upfield shifts of 1-H.[14,31]

The structure of compound (**13**) was finally established by tannase (E.c. 3.1.1.20) treatment of compound (**15**) (previously obtained from cornusiin C),[14] which yielded compounds (**13**), (**16**), and (**17**).

Another product, (**14**), produced by partial degradation of oenothein B (**1**), was found to be the isomer of (**13**) with regard to the orientation of valoneoyl group as follows. The FAB mass spectrum of compound (**14**) exhibited the $(M + Na)^+$ ion at *m/z* 1 591, which is consistent with its molecular formula of

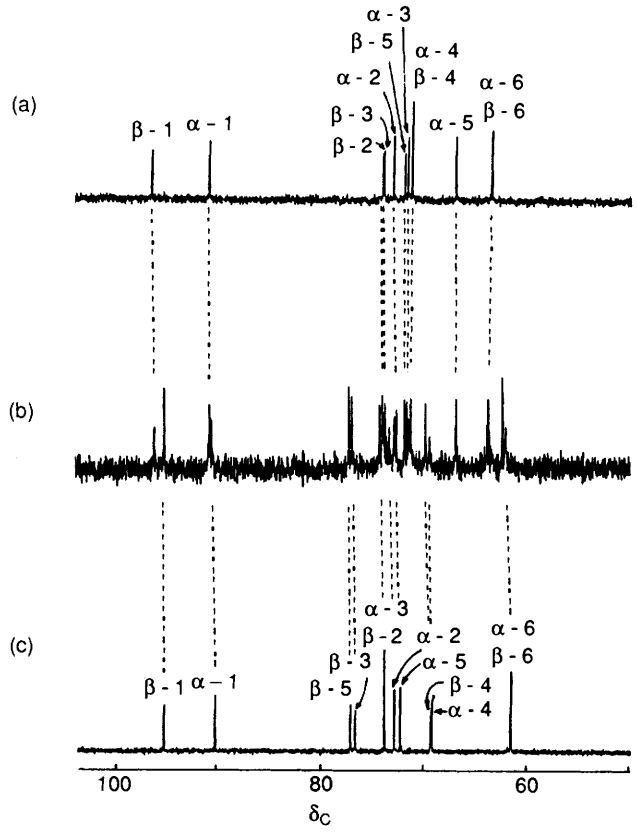


Figure 2. Comparison of the glucose carbon signals in the ^{13}C NMR spectra of (a) cornusiin B (**5**), (b) compound (**13**), and (c) oenothein C (**2**). 'β-1' Means C-1 of the glucose core of the β-anomer.

$C_{68}H_{48}O_{44}$. The ^1H NMR spectrum showed that compound (**14**) forms a mixture of four anomers, and consists of a lactonized valoneoyl group, a valoneoyl group, two galloyl groups, and two glucose cores (see Experimental section). The four 1-H signals of the β-glucose cores in the three anomeric forms of this compound [δ_H 4.73, 4.68, 4.16, and 4.16 (each d, J 8 Hz)] showed upfield shifts analogous to those of compound (**13**). These upfield shifts, ascribable to the lactonized valoneoyl group and the valoneoyl group, indicate that the galloyl moieties in these two acyl groups are on O-2 of the two glucose cores. The chemical shifts of the HHDP moiety of the valoneoyl group [δ_H 6.49–6.42 (H_A), 6.08–6.01 (H_B)] are distinctively different from those of the corresponding protons in compound (**13**) [δ_H 6.58, 6.57 (H_A), 6.20–6.13 (H_B)], reflecting the difference in orientation of the valoneoyl group as found in compounds (**10**) and (**11**).[28] Partial degradation of compounds (**14**) afforded products (**2**), (**5**), (**6**), and (**9**). Based on these findings, structure (**14**) was assigned for the product from oenothein B (**1**).

The structures of products (**13**) and (**14**) indicate that they were produced respectively through cleavage of the ester linkages at C-4 and C-6 of each glucose core in compound (**1**). The macrocyclic structure (**1**) of oenothein B, in which the orientations of the two valoneoyl groups are different from each other, have been thus assigned. Biogenetically, compound (**1**) is regarded as the product from two molecules of compound (**4**) which is present in the same plant.

Experimental

FAB mass spectra were recorded on a JEOL GMS-HX100 spectrometer. CD spectra were recorded on a JASCO J-500 machine equipped with a DP-501 data processor. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^1H, and 125.7 MHz for ^{13}C). A JEOL JMN-

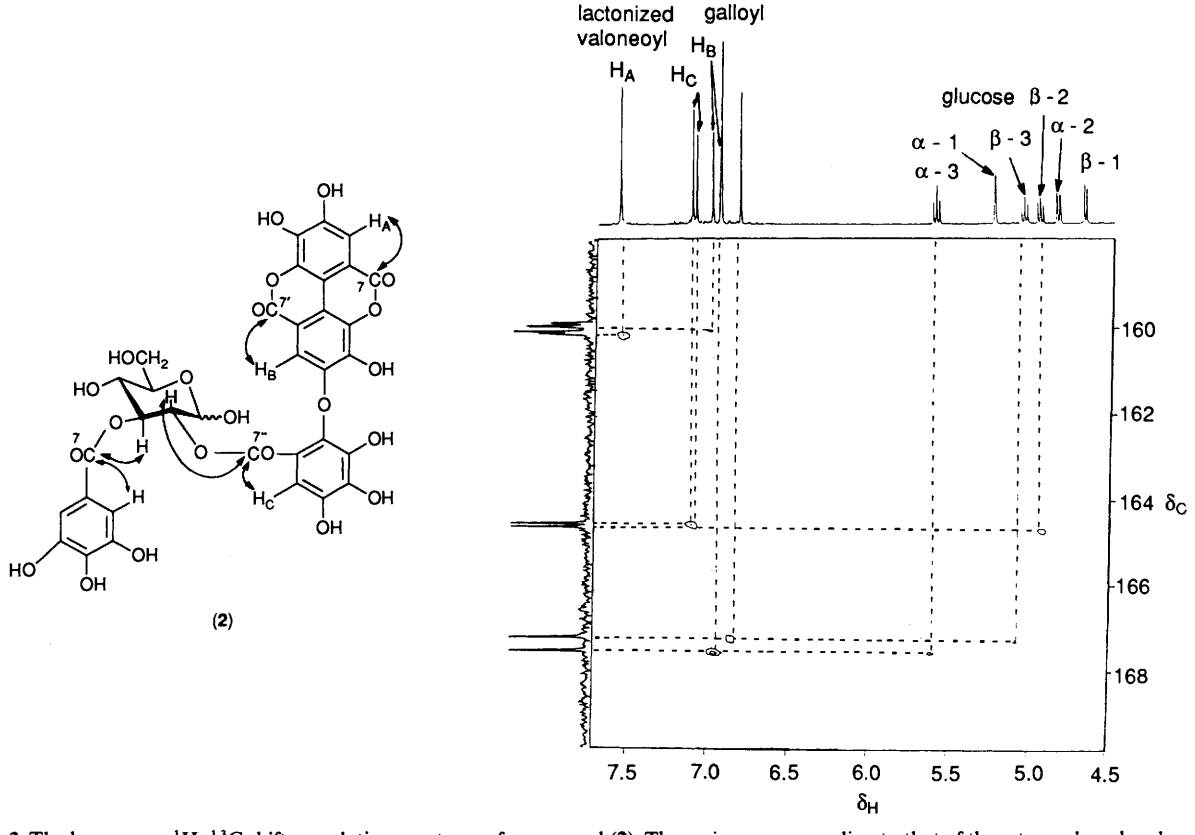


Figure 3. The long-range ^1H–^{13}C shift-correlation spectrum of compound (**2**). The region corresponding to that of the ester carbonyl carbons in the ^{13}C NMR spectrum is shown. The average J_{CH}-value was set at 7 Hz. 'α-3' Means 3-H of the α-anomer.

GX400 spectrometer (400 MHz), a Varian XL-200 instrument (200 MHz), and a Hitachi R22-FTS spectrometer (90 MHz) were also used for measurements of the ^{1}H NMR spectra. Chemical shifts are given in δ-values from tetramethylsilane. Normal-phase HPLC was conducted on a Merck Superspher Si60 cartridge column (4 mm i.d. × 125 mm) with solvent system N1 which consisted of hexane–EtOAc (2:1, v/v), or solvent system N2 which consisted of hexane–MeOH–tetrahydrofuran–HCO_2H (55:33:11:1) containing oxalic acid (450 mg l^{-1}), at a flow rate of 1.5 ml min^{-1}. Reversed-phase HPLC was performed on a Merck LiChrospher RP-18 cartridge column (4 mm i.d. × 250 mm) in an oven set at 40 °C, with solvent system R1 (0.01M-H_3PO_4–0.01M-KH_2PO_4–EtOH–EtOAc, 15:15:2:1) at a flow rate 1.0 ml min^{-1}, solvent system R2 (0.01M-H_3PO_4–0.01M-KH_2PO_4–MeCN, 9:9:2) at a flow rate 1.3 ml min^{-1}, solvent system R3 (0.01M-H_3PO_4–0.01M-KH_2PO_4–EtOH, 10:10:1) at a flow rate 1.2 ml min^{-1}, or solvent system R4 (0.01M-H_3PO_4–0.01M-KH_2PO_4–MeCN, 11:11:3) at a flow rate 1.3 ml min^{-1}. The spectrophotometric detector was set at 280 nm. GLC was performed on a Hitachi 163 gas chromatograph equipped with a glass column (3 mm i.d. × 2 m) packed with 2.5% OV-17 on Chromosorb W. Known compounds were identified by comparison of their ^{1}H NMR spectral data with those of authentic samples, and by HPLC.

Isolation of Tannins and Related Phenolics from Leaves of O. erythrosepala.—Fresh leaves (6.5 kg) of *O. erythrosepala* were homogenized in 70% aqueous acetone. The concentrated filtrate (940 ml) from the homogenate was extracted successively with diethyl ether (940 ml × 10), ethyl acetate (940 ml × 20), and butan-1-ol (400 ml × 5). A portion (2.7 g) of the ethyl acetate extract (17 g) was chromatographed on a column of Sephadex LH-20, to afford 1,2,6-tri-*O*-galloyl-β-D-glucose[14,19] (27 mg), gemin D (**3**)[15] (68 mg), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose[14,19] (5 mg), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose[19,20] (6 mg), tellimagrandin I (**4**)[16,17] (17 mg), and cornusiin B (**5**)[14] (47 mg).

The ethanol-soluble portion (5 g) of a portion (7 g) of the butan-1-ol extract (32 g) was chromatographed over Sephadex LH-20, to give 3-*O*-galloyl-D-glucose (**6**)[14,18] (15 mg), gemin D (**3**) (46 mg), oenothein C (**2**)[14] (5 mg), tellimagrandin I (**4**) (5 mg), cornusiin B (**5**) (30 mg), and oenothein B (**1**) (530 mg).

Oenothein B (**1**).—This was obtained as an off-white, amorphous *powder*, $[\alpha]_D$ +179° (*c* 1 in MeOH) (Found: C, 47.5; H, 3.6. $C_{68}H_{48}O_{44}\cdot 8H_2O$ requires C, 47.67; H, 3.77%); *m/z* (FAB) 1 569 $[(M + H)^+]$; λ_{max}(MeOH) 218 (log ε 5.09) and 265 nm (4.77); ν_{max}(KBr) 1 730–1 710 (ester carbonyl) and 1 610 cm^{-1}; $[\theta]_{218}$ +3.8 × 10^5, $[\theta]_{236}$ + 1.5 × 10^5, $[\theta]_{258}$ −4.3 × 10^4, and $[\theta]_{280}$ +1.1 × 10^5.

Methanolysis after Methylation of Oenothein B (**1**).—Ethereal diazomethane (2 ml) was added to an ethanolic solution (2 ml) of oenothein B (**1**) (40 mg), and the mixture was kept for 3 h at room temperature. After removal of the solvent under a nitrogen stream, 0.6% sodium methoxide in methanol (2 ml) was added to the residue, and the mixture was left overnight, then was acidified with acetic acid, and the solvent was evaporated off. Preparative TLC of this residue over Merck Kieselgel 60 PF_{254} (0.5 mm thick) with hexane–chloroform–acetone (4:6:3 and then 6:3:1) gave methyl tri-*O*-methylgallate (**7**) (2.8 mg) and trimethyl (*S*)-octa-*O*-methylvaloneate (**8**) (7.4 mg), $[\alpha]_D$ −22° (*c* 1 in acetone).

Determination of the Phenolic Acids of Oenothein B (**1**).[14]—Ethereal diazomethane (0.5 ml) was added to an ethanolic solution (0.2 ml) of oenothein B (**1**) (1 mg). After 30 min, the solvent was removed under a nitrogen stream, and the residue was treated with 0.5% sodium methoxide in methanol (0.2 ml) overnight. The solution was acidified with acetic acid and the solvent was evaporated off. The residue was further treated with ethereal diazomethane (0.5 ml) for 30 min, and the solvent was removed under a nitrogen stream. HPLC analysis (normal phase; solvent system N1) of the residue showed the presence of compounds (**7**) and (**8**) in a 1:1 molar ratio.

Treatment of Oenothein B (**1**) *with 5% Sulphuric Acid.*—A solution (1 ml) of compound (**1**) (8 mg) in 5% sulphuric acid in a sealed tube was heated in a boiling-water-bath for 20 h. The solution was neutralized using Amberlite IRA-410 resin, and was then evaporated. After trimethylsilylation of the residue, the reaction mixture was analysed by GLC, which showed the presence of glucose in the residue.

Reduction of Oenothein B (**1**).—Treatment of compound (**1**) (103 mg) with sodium borohydride (0.43 g), in a way similar to that reported previously,[27] afforded the main product (**12**) accompanied by unchanged compound (**1**). The mixture was separated by column chromatography over MCI gel CHP-20P with aq. methanol (0% ⟶ 20% ⟶ 25%). The 20% methanol eluate afforded compound (**1**) (25 mg), and the 25% methanol eluate gave compound (**12**) (8 mg).

Compound (**12**). This was obtained as an off-white, amorphous *powder*, $[\alpha]_D$ +25° (*c* 1 in MeOH) (Found: C, 48.45; H, 4.8. $C_{68}H_{52}O_{44}\cdot 7H_2O$ requires C, 48.07; H, 3.92%); *m/z* (FAB) 1 595 $[(M + Na)^+]$; δ_H(500 MHz; CD_3COCD_3 + D_2O) 7.47, 7.08, 6.52, 6.41, 6.37, and 6.04 [each 1 H, s, valoneoyl (Val) × 2] and 7.14 and 7.08 (each 2 H, s, galloyl × 2). Glucitol protons: see Table 2.

Partial Degradation of Oenothein B (**1**).—A solution (50 ml) of compound (**1**) (500 mg) in 0.05M-sulphuric acid in a sealed tube was heated in a boiling-water-bath for 13 h. The reaction mixture was chromatographed over MCI gel CHP-20P with aq. methanol (0% ⟶ 20% ⟶ 30%). The 20% methanol eluate afforded compound (**2**) (100 mg) and unchanged compound (**1**) (84 mg). The 30% methanol eluate afforded compound (**13**) (20 mg) and a mixture (54 mg) of compounds (**14**) and valoneic acid dilactone. The mixture was further separated by column chromatography on Toyopearl HW-40 (superfine grade) with 40% aq. ethanol, to give valoneic acid dilactone (9 mg) and compound (**14**) (25 mg).

Compound (**13**). This was obtained as an off-white, amorphous powder, $[\alpha]_D$ +23° (*c* 1 in MeOH); *m/z* (FAB) 1 591 $[(M + Na)^+]$; δ_H(500 MHz; CD_3COCD_3 + D_2O) 7.60 and 7.59 [1 H in total, H_A of lactonized valoneoyl (LV) group], 7.12–7.02 [5 H in total, LV H_B and H_C, Val H_A, galloyl (2 H)] 6.83, 6.81, 6.72, and 6.68 (2 H in total, galloyl), 6.58 and 6.57 (1 H, in total, Val H_A), 6.20, 6.16, 6.14, and 6.13 (1 H, in total, Val H_B), 5.73, 5.72, 5.63, and 5.58 (each t, *J* 10 Hz, α-glucose, 3-H), and 4.74, 4.71, 4.22, and 4.19 (each d, *J* 8 Hz, β-glucose, 1-H); δ_C (125.7 MHz; CD_3COCD_3 + D_2O) 62.0, 62.1, and 62.3 (glu_L C-6, α- and β-form), 63.6, 63.7, and 63.8 (glu_R C-6, α- and β-form), 66.8 and 66.9 (glu_R C-5, α-form), 69.2–69.9 (glu_L C-4, α- and β-form), 71.2–71.9 (glu_R C-3 and -4, α-form; glu_R C-4 and -5, β-form), 72.6–72.9 (glu_R C-2, α-form; glu_L C-5, α-form), 73.3–74.3 (glu_R C-2 and -3, β-form; glu_L C-2 and -3, α-form; glu_L C-2, β-form), 76.9 and 77.0 (glu_L C-3, β-form), 77.3 (glu_L C-5, β-form), 90.6–90.9 (glu_L C-1, α-form; glu_R C-1, α-form), 95.2 (glu_L C-1, β-form), 96.2 (glu_R C-1, β-form) [glu_L = the left-hand glucose core in formula (**13**), glu_R = the right-hand glucose core], 104.1, 104.3, and 105.8 (Val C-3′), 107.2–107.8 (Val C-3), 108.8–110.8 (LV C-2, -2′, -3′, and -6′; Val C-6″; galloyl C-2 and -6), 111.3–111.4 (LV C-3), 113.3–117.3 (LV C-1, -1′, and -1″; Val C-1, -1′, and -1″), 120.3–121.0 (galloyl C-1), 125.5–125.9 (Val C-2 and -2′), 135.8–137.7 (LV C-2″, -6, and -6′; Val C-2′, -5, and -5′),

138.8–139.0 (galloyl C-4), 139.9–141.2 (LV C-3″, -4″, -5, and -5′; Val C-3″ and -4″), 143.0–143.7 (LV C-6 and -6′; Val C-5″), 144.4–145.9 (Val C-4, -6, and -6′; galloyl C-3 and -5), 146.6 and 147.7 (Val C-4′), 149.0 (LV C-4), 149.8 (LV C-4′), 159.8 and 159.9 (LV C-7′), 160.1, 160.2, and 160.3 (LV C-7), and 164.1–168.6 (LV C-7″; Val C-7, -7′, and -7″; galloyl C-7).

Compound (**14**). This was obtained as an off-white, amorphous powder, $[\alpha]_D$ +61° (*c* 1 in MeOH); *m/z* (FAB) 1 591 [$(M + Na)^+$]; δ_H(500 MHz; $CD_3COCD_3 + D_2O$) 7.61–7.60 (1 H in total, LV H_A), 7.15–7.00 [5 H in total, LV H_B and H_C, Val H_C, galloyl (2 H)], 6.87, 6.86, 6.78, and 6.77 (2 H in total, galloyl), 6.49, 6.47, 6.45, and 6.42 (1 H in total, Val H_A), 6.08, 6.06, 6.03, and 6.01 (1 H in total, Val H_B), 5.75–5.68 (α-glucose, 3-H), and 4.73, 4.68, 4.16, and 4.16 (each d, *J* 8 Hz, β-glucose, 1-H); δ_C(125.7 MHz; $CD_3COCD_3 + D_2O$) 61.8 and 62.4 (glu_L C-6, α- and β-form), 63.3, 63.4, and 64.0 (glu_R C-6, α- and β-form), 66.7 and 66.9 (glu_R C-5, α-form), 69.1, 69.2, and 69.7 (glu_L C-4, α- and β-form), 70.8–72.0 (glu_R C-3 and -4, α-form; glu_R C-4 and -5, β-form), 72.6–72.9 (glu_R C-2, α-form; glu_L C-2 and -5, α-form), 73.7–74.1 (glu_R C-2 and -3, β-form; glu_L C-3, α-form; glu_L C-2, β-form), 76.8 (glu_L C-3, β-form), 77.5 and 77.6 (glu_L C-5, β-form), 90.8–90.9 (glu_L C-1, α-form; glu_R C-1, α-form), 95.3 (glu_L C-1, β-form), 96.3 (glu_R C-1, β-form) [glu_L = the left-hand glucose core in formula (**14**), glu_R = the right-hand glucose core], 103.9–104.7 (Val C-3′), 107.2 and 107.6 (Val C-3), 108.8–110.4 (LV C-2, -2′, -3′, and -6′; Val C-6″; galloyl C-2 and -6), 111.4 (LV C-3), 113.3–117.6 (LV C-1, 1′, and -1″; Val C-1, -1′, and -1″), 120.1–121.2 (galloyl C-1), 125.3–125.9 (Val C-2 and -2′), 136.1–137.6 (LV C-2″, -6, and -6′; Val C-2″, -5, and -5′), 138.9, 139.0, and 139.1 (galloyl C-4), 139.9–141.3 (LV C-3″, -4″, -5, and -5′; Val C-3″ and -4″), 143.1–143.7 (LV C-6 and -6′; Val C-5″), 144.5–145.8 (Val C-4, -6, and -6′; galloyl C-3 and -5), 146.5, 146.6, and 147.8 (Val C-4′), 149.0 (LV C-4), 149.8 (LV C-4′), 159.7 and 159.9 (LV C-7′), 160.3 and 160.4 (LV C-7), and 164.1–169.3 (LV C-7″; Val C-7, -7′, and -7″; galloyl C-7).

Partial Degradation of Compound (**13**).—An aq. solution (0.5 ml) of compound (**13**) (1 mg) in a sealed tube was heated in a boiling-water-bath for 9 h. Normal-phase and reversed-phase HPLC analyses of the reaction mixture showed the presence of compounds (**6**) (solvent systems N2, R1, R2, and R3), (**9**) (N2, R2, and R3), (**2**) (N2, R1, and R2), and (**5**) (N2, R1, and R2), along with gallic acid (N2, R1, and R2), ellagic acid (N2, R1, and R4), and valoneic acid dilactone (N2, R1, and R2).

Partial Degradation of Compound (**14**).—An aq. solution (0.4 ml) of compound (**14**) (1 mg) in a sealed tube was heated in a boiling-water-bath for 4 h. HPLC analyses (normal-phase and reversed-phase modes) showed the presence of compounds (**2**), (**5**), (**6**), and (**9**), gallic acid, ellagic acid, and valoneic acid dilactone in the reaction mixture.

Partial Degradation of Compound (**15**) *with Tannase*.—An aq. solution (10 ml) of compound (**15**)[14] (20 mg) was treated with tannase[32] at 37 °C for 24 h. The solution was then acidified with 1M-HCl, and then passed through a Bond Elute C_{18} cartridge. The adsorbed compounds were eluted with water, and then with methanol. The methanol eluate was further separated by column chromatography on Toyopearl HW-40 (superfine grade) with 40% aq. ethanol, to give compounds (**16**) (1 mg), (**13**) (1 mg), and (**17**) (3 mg), and unchanged substrate (**15**) (4 mg).

Compound (**16**). This was obtained as an off-white, amorphous powder, δ_H(500 MHz; $CD_3COCD_3 + D_2O$) 7.58, 7.57, 7.55, and 7.54 (each s, 1 H, in total, H_A of LV group), 7.25 and 7.21 (each s, 1 H in total) and 7.11, 7.10, 7.05, 7.04, and 7.03 (each s, 2 H in total) (LV H_B and H_C, Val H_C), 6.80, 6.75, 6.72, and 6.67 (each s, 2 H in total, galloyl), 6.58 and 6.57 (each s, 1 H in total, Val H_A), 6.19, 6.17, 6.14, and 6.13 (each s, 1 H in total, Val H_B), 5.58 and 5.54 (each t, *J* 10 Hz, α-glucose 3-H), 5.38 and 5.36 (each d, *J* 4 Hz, α-glucose 1-H), and 4.69, 4.67, 4.26, and 4.26 (each d, *J* 8 Hz, β-glucose, 1-H).

Compound (**17**). This was obtained as an off-white, amorphous powder; δ_H(500 MHz; $CD_3COCD_3 + D_2O$) 7.57, 7.57, 7.54, and 7.53 (each s, 1 H in total, LV H_A), 7.24, 7.22, and 7.21 (each s, 1 H in total) and 7.10–7.01 (2 H in total) (LV H_B and H_C, Val H_C), 6.78, 6.76, 6.74, and 6.72 (each s, 2 H in total, galloyl), 6.65–6.55 [3 H in total, Val H_A, HHDP (2 H)], 6.16, 6.13, 6.11, and 6.09 (each s, 1 H in total, Val H_B), 5.56 and 5.54 (each t, *J* 10 Hz, α-glucose, 3-H), 5.37, 5.36, 5.33, and 5.33 (each d, *J* 4 Hz, α-glucose, 1-H), and 4.53 and 4.52 (each d, *J* 8 Hz, β-glucose, 1-H).

Acknowledgements

We thank Professor K. Takaishi and Dr. H. Kuwajima, Faculty of Pharmaceutical Sciences, Kinki University, for the FAB mass spectra. We also thank Professor Y. Asakawa and Dr. Y. Shiobara, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, for the decoupling experiments on the ^1H NMR spectral measurements on a JEOL JMN-GX400 spectrometer. The Varian VXR-500 instrument used was that in the SC-NMR Laboratory of Okayama University. This study was in part supported by a Grant-in-Aid for Scientific Research of the Ministry of Education, Science and Culture.

References

1 T. Okuda, T. Yoshida, and T. Hatano, *Heterocycles*, 1990, **30**, 1195.
2 K. Miyamoto, N. Kishi, R. Koshiura, T. Yoshida, T. Hatano, and T. Okuda, *Chem. Pharm. Bull.*, 1987, **35**, 814.
3 M. Asanaka, T. Kurimura, R. Koshiura, T. Okuda, M. Mori, and H. Yokoi, Abstracts, 4th International Conference on Immunopharmacology, May 1988, Osaka, p. 47.
4 K. Fukuchi, H. Sakagami, T. Okuda, T. Hatano, S. Tanuma, K. Kitajima, Y. Inoue, S. Inoue, S. Ichikawa, M. Nonoyama, and K. Konno, *Antiviral Res.*, 1989, **11**, 285.
5 C. F. Millspaugh, 'American Medicinal Plants,' Dover Publications, New York, 1974, pp. 233–236.
6 S. von Reis Altschul, 'Drugs and Foods from Little-Known Plants,' Harvard University Press, Cambridge, Massachusetts, 1973, p. 210.
7 S. von Reis and F. J. Lipp, Jr., 'New Plant Sources for Drugs and Foods from The New York Botanical Garden Herbarium,' Cambridge, Massachusetts, 1982, p. 219.
8 H. D. Zinsmeister and S. Bartl, *Phytochemistry*, 1971, **10**, 3129.
9 For a preliminary report, see T. Hatano, T. Yasuhara, M. Matsuda, K. Yazaki, T. Yoshida, and T. Okuda, *Chem. Pharm. Bull.*, 1989, **37**, 2269.
10 T. Yoshida, T. Hatano, and T. Okuda, *J. Chromatogr.*, 1989, **467**, 139.
11 T. Yoshida, K. Tanaka, X.-M. Chen, and T. Okuda, *Phytochemistry*, 1989, **28**, 2451.
12 T. Hatano, Y. Ikegami, T. Shingu, and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 2017.
13 T. Hatano, S. Hattori, and T. Okuda, *Chem. Pharm. Bull.*, 1986, **34**, 4533.
14 T. Hatano, N. Ogawa, R. Kira, T. Yasuhara, and T. Okuda, *Chem. Pharm. Bull.*, 1989, **37**, 2083.
15 T. Yoshida, Y. Maruyama, T. Okuda, M. U. Memon, and T. Shingu, *Phytochemistry*, 1985, **24**, 1041.
16 C. K. Wilkins and B. A. Bohm, *Phytochemistry*, 1976, **15**, 211.
17 T. Okuda, T. Yoshida, M. Ashida, and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1765.
18 O. T. Schmidt and A. Schach, *Justus Liebigs Ann. Chem.*, 1951, **571**, 29.
19 E. A. Haddock, R. K. Gupta, S. M. K. Al-Shafi, E. Haslam, and D. Magnolato, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2515.
20 T. Hatano, R. Kira, M. Yoshizaki, and T. Okuda, *Phytochemistry*, 1986, **25**, 2787.
21 T. Okuda, T. Yoshida, T. Hatano, T. Koga, N. Toh, and K. Kuriyama, *Tetrahedron Lett.*, 1982, **23**, 3937.
22 T. Okuda, T. Yoshida, M. Kuwahara, M. U. Memon, and T. Shingu, *Chem. Pharm. Bull.*, 1984, **32**, 2165.

23 G. Nonaka, M. Ishimatsu, T. Tanaka, I. Nishioka, M. Nishizawa, and T. Yamagishi, *Chem. Pharm. Bull.*, 1987, **35**, 3127.
24 T. Yoshida, T. Hatano, T. Okuda, M. U. Memon, T. Shingu, and K. Inoue, *Chem. Pharm. Bull.*, 1984, **32**, 1790.
25 T. Hatano, T. Yoshida, T. Shingu, and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 2925.
26 T. Hatano, T. Yoshida, and T. Okuda, *J. Chromatogr.*, 1988, **435**, 285.
27 T. Okuda, T. Hatano, T. Kaneda, M. Yoshizaki, and T. Shingu, *Phytochemistry*, 1987, **26**, 2053.
28 T. Hatano, R. Kira, T. Yasuhara, and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 3920.
29 T. Okuda, T. Hatano, K. Yazaki, and N. Ogawa, *Chem. Pharm. Bull.*, 1982, **30**, 4230.
30 T. Yoshida, L. Chen, T. Shingu, and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 2940.
31 T. Hatano, T. Yasuhara, and T. Okuda, *Chem. Pharm. Bull.*, 1989, **37**, 2665.
32 T. Yoshida, K. Tanaka, X.-M. Chen, and T. Okuda, *Chem. Pharm. Bull.*, 1989, **37**, 920.

Paper 0/01847C
Received 9*th April* 1990
Accepted 2*nd May* 1990