Eucalbanins A, B and C, Monomeric and Dimeric Hydrolyzable Tannins from *Eucalyptus alba* REINW.

Takashi YOSHIDA,[a] Tetsuhiro MARUYAMA,[a] Aya NITTA[b] and Takuo OKUDA*,[a]

Faculty of Pharmaceutical Sciences, Okayama University,[a] Tsushima, Okayama 700, Japan and Faculty of Pharmaceutical Sciences, Kyoto University,[b] Sakyo-ku, Kyoto 606, Japan. *Received December 26, 1991*

Three new hydrolyzable tannins, eucalbanins A (5), B (8) and C (11), have been isolated from the fruit extract of *Eucalyptus alba* REINW., and their structures were elucidated on the basis of spectral data and chemical correlation with known tannins. Quercetin-3-*O*-α-L-arabinopyranoside, (+)-catechin, procyanidin B-7, and nine known hydrolyzable tannins [casuarinin, casuariin, pedunculagin, tellimagrandin I (1), gemin D (2), cornusiin B (3), 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose, penta-*O*-galloyl-β-D-glucose and oenothein B (4)] have also been isolated.

Keywords *Eucalyptus alba*; Myrtaceae; ellagitannin; tannin; ellagitannin dimer; eucalbanin A; eucalbanin B; eucalbanin C

The dried fruit of *Eucalyptus alba* REINW. (Myrtaceae), called Ceplik in Jamu medicine in Indonesia, has been used as a flavoring agent in traditional medicinal drugs.[1] In a chromatographic survey on the tannin constituents in medicinal plants, we found that Ceplik is rich in tannins, and have isolated thirteen polyphenolics including three new hydrolyzable tannins, named eucalbanins A, B and C.

The concentrated aqueous acetone homogenate of the dried fruits, purchased in Indonesia, was extracted with ethyl acetate and *n*-BuOH. These extracts were separately fractionated and purified by repeated column chromatography over Toyopearl HW-40 and/or MCI-gel CHP 20P to yield eucalbanins A (**5**), B (**8**) and C (**11**), along with quercetin-3-*O*-α-L-arabinopyranoside (guaijaverin),[2] (+)-catechin, procyanidin B-7,[3] and nine known hydrolyzable tannins, casuarinin,[4] casuariin,[4] pedunculagin,[4] tellimagrandin I (**1**),[4,5] gemin D (**2**),[6] cornusiin B (**3**),[7] 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose,[8] penta-*O*-galloyl-β-D-glucose[9] and oenothein B (**4**).[10]

Eucalbanin A, $[\alpha]_D$ +36° (MeOH), was assigned the structure **5** on the basis of the following findings. It showed the $(M+Na)^+$ ion peak at *m/z* 1109 in the fast-atom bombardment mass spectrum (FAB-MS). Upon methylation followed by methanolysis with sodium methoxide in MeOH, eucalbanin A (**5**) yielded methyl tri-*O*-methylgallate, dimethyl hexamethoxydiphenate (**6**) and methyl hexa-*O*-methyltergallate dilactone (**7**). The sugar compo-

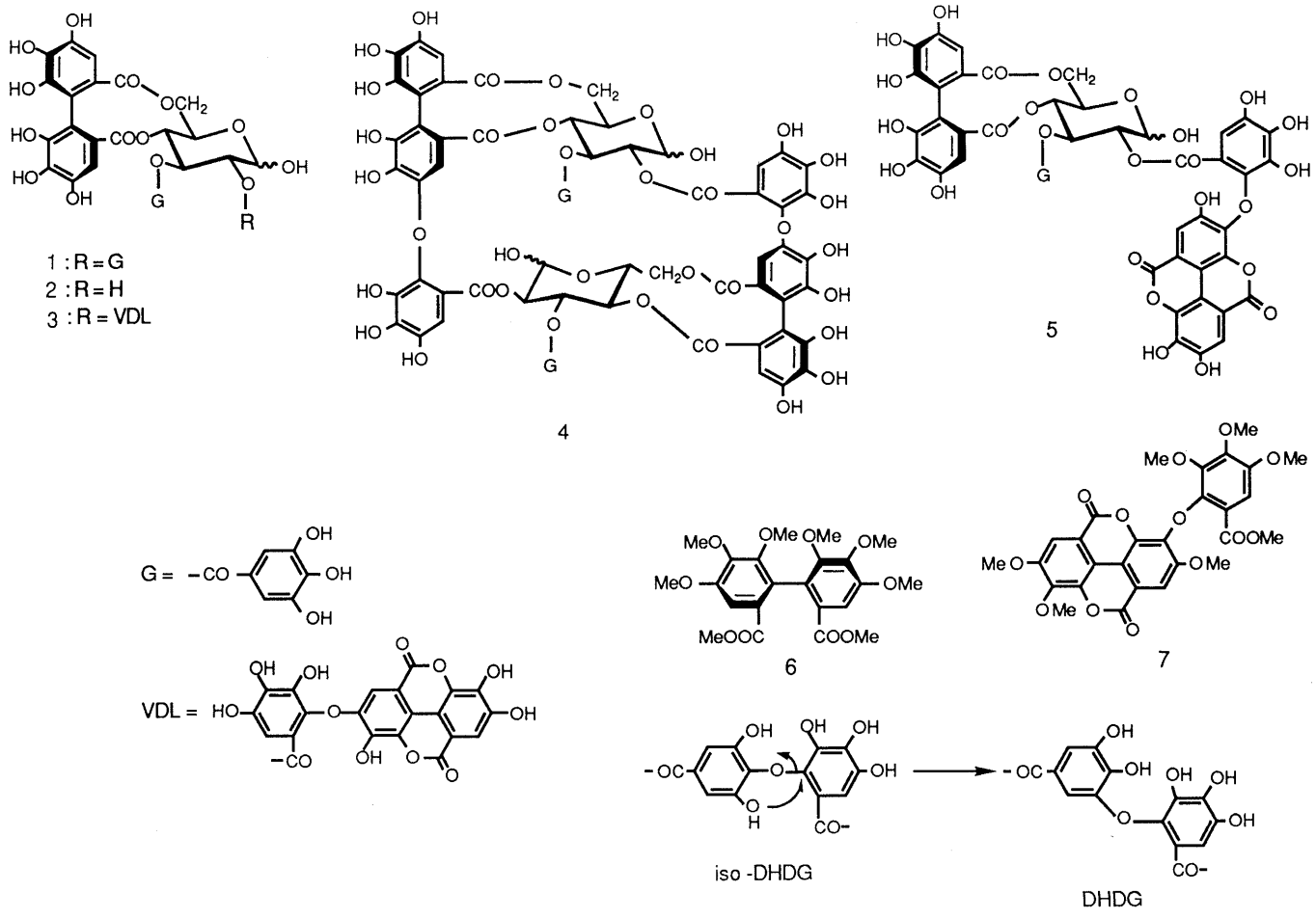

Chart 1

nent of **5** was identified as glucose by gas-liquid chromatography (GLC) of the trimethylsilyl derivative of the acid hydrolyzate. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **5** showed a dual peak for each proton, and the absence of an acylated anomeric proton signal in the region of δ 5.8—6.5, indicating the formation of an equilibrium mixture of two anomers. Paired signals of a 2H singlet [δ 6.78, 6.76 (2H in total)], and two 1H singlets [δ 6.40, 6.39 (1H in total) and 6.59, 6.58 (1H in total)] are ascribable to a galloyl group and a hexahydroxydiphenoyl (HHDP) group. The low field signals [δ 7.04, 7.00 (1H in total), 7.42, 7.41 (1H in total) and 7.52, 7.56 (1H in total)] are assigned to a dilactonized tergalloyl group. The glucose proton and ^{13}C signals, which were assigned on the basis of ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation (COSY) spectra, are closely similar to those of cornusiin B (**3**),[7] suggesting a gross structure **5** for eucalbanin A.

We recently found that an isodehydrodigalloyl (iso-DHDG) group in the molecule of hydrolyzable tannin is easily isomerized to a dehydrodigalloyl (DHDG) group, under weakly alkaline conditions.[11] This isomerization can be interpreted in terms of Smiles-type rearrangement as illustrated in Chart 1. This reaction has been successfully applied to chemical correlation between eucalbanin A (**5**) and cornusiin B (**3**): an aqueous solution, containing a small amount of 0.02 M phosphate buffer (pH 7.4), of eucalbanin A (**5**) was left standing at room temperature for 12 h, to give, almost quantitatively, an isomerized product identical with cornusiin B (**3**). Based on these data, eucalbanin A is represented by the structure **5**.

Eucalbanin B (**8**), $[\alpha]_D$ +78° (MeOH), was obtained as an off-white amorphous powder, and gave gallic acid, ellagic acid, valoneic acid dilactone[7] and glucose, upon acid hydrolysis. Its dimeric nature was indicated by the $(M+Na)^+$ ion peak at *m/z* 1593 in the FAB-MS and the fact that its retention time was analogous to that of a dimeric hydrolyzable tannin, *e.g.*, oenothein B (**4**), in high-performance liquid chromatography (HPLC) (normal phase).[12] The ^{1}H-NMR spectrum of **8** is complicated by the existence of four anomer combinations due to the presence of two glucose cores. These four tautomers were also revealed by eight ^{13}C signals at δ 90.9—91.4 and 96.5—97.1 (each four lines) attributable to α- and β-anomeric carbons in the ^{13}C-NMR spectrum of **8**. The presence of three galloyl, an HHDP and a valoneoyl groups was shown by three 2H singlets and five 1H singlets in the aromatic region of the ^{1}H-NMR spectrum, most of which are doubly duplicated (four line signals). Among the glucose proton signals, the C-6 and C-6′ methylene proton signals are observed at δ 3.66—3.92 and 5.15—5.26, which were shown by ^{1}H–^{1}H COSY to correlate through *geminal* couplings. A large difference of these chemical shifts between *geminal* protons at C-6 (C-6′) implies that the HHDP group and the HHDP part of the valoneoyl group are on O-4 (4′)/O-6 (6′) of the glucose cores.[5,13] These data indicate that eucalbanin B is a dimer of tellimagrandin I (**1**),[4,5] and this structure was substantiated by the ^{13}C-NMR spectrum, in which the glucose carbon resonances are in agreement with those of 2 mol of **1**[14] (Fig. 1).

The anomeric proton signal (H-1) of the β-anomer of a glucose core is shifted to higher field (δ 4.36, 4.37, each d, J=8 Hz) than that of **1** (δ 5.13 d, J=8 Hz).[5] This

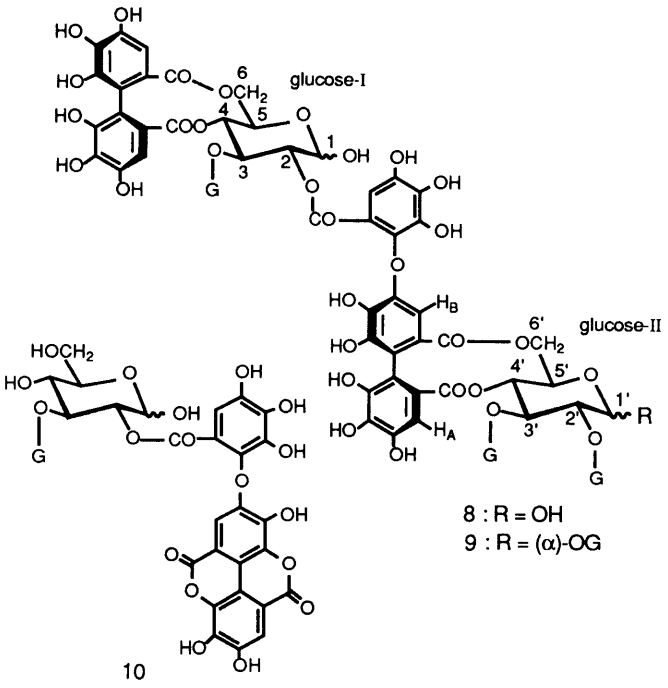

Chart 2

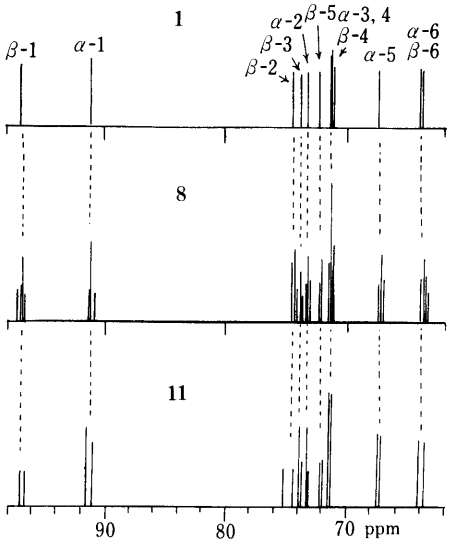

Fig. 1. ^{13}C-NMR Spectral Comparison of the Glucose Moieties of Tellimagrandin I (**1**), and Eucalbanins B (**8**) and C (**11**)

remarkable upfield shift is analogous to that observed in the ^{1}H-NMR spectra of cornusiin B (**3**),[7] oenothein B (**4**)[10] and many other tannins which have a galloyl part of the valoneoyl group at the adjacent O-2 in the glucose core.[10] The locations of the acyl groups on the glucose cores of **8** were confirmed by its partial hydrolysis in a weak acid, yielding cornusiin B (**3**), oenothein C (**10**)[7] and 2,3-di-*O*-galloyl-D-glucose.

These spectral and chemical findings indicate that eucalbanin B (**8**) is a degalloyl congener of woodfordin B (**9**), isolated from *Woodfordia fruticosa* (Lythraceae).[15] The (*S*)-configuration for both of the HHDP and valoneoyl groups in **8** was indicated by the circular dichroism (CD) spectrum, which was almost superimposable on that of **9**.[15] Finally, the structure of eucalbanin B was established as **8**,

by enzymatic hydrolysis of **9** with tannase, yielding **8**.

Eucalbanin C (**11**), $[\alpha]_D$ +57° (MeOH), showed the $(M+Na)^+$ ion peak at *m/z* 1593 in the FAB-MS, which is the same as that of **8**. Methylation of **11** with dimethyl sulfate and potassium carbonate in acetone, followed by methanolysis, gave methyl tri-*O*-methylgallate, dimethyl hexamethoxydiphenate (**6**) and trimethyl octa-*O*-methylter-gallate (**12**).[13,16] The ^{1}H-NMR spectrum of **11**, which is similar to that of eucalbanin B (**8**), indicated that this compound also exists as an equilibrium mixture of four tautomers. The spectrum showed the presence of three galloyl groups, an HHDP group and a tergalloyl group, by three 2H singlets and five 1H singlets appearing as doubly duplicated signals. A remarkable difference in the ^{1}H-NMR spectra between **8** and **11** was observed in the chemical shifts of the 4 line signal group arising from one of the five aromatic 1H protons: this signal group (valoneoyl H_B) observed around 6.1—6.2 ppm in **8** was greatly shifted to lower field (δ 6.80, 6.806, 6.819, 6.82, 1H in total) in **11**. This can be reasonably explained in terms of replacement of the valoneoyl group in **8** by the tergalloyl group, in which the nucleus H_B is free from steric compression by the polyphenol ether on the adjacent carbon. The glucose carbon signals in the ^{13}C-NMR spectrum of **11** were also almost superimposable on that of **8** (Fig. 1). Eucalbanin C was thus assumed to be an isomer of **8** concerning the position of an ether linkage on the linking unit of monomers.

The isomerization of **11**, in a way similar to that from **5** to **3**, was then attempted, and the product obtained in high yield was identified as **8**. Consequently, the structure of eucalbanin C, including the orientation and the absolute configuration of the tergalloyl group at O-4′/O-6′, was determined as **11**.

Eucalbanins B (**8**) and C (**11**) and oenothein B (**4**) are the first examples of dimeric hydrolyzable tannins isolated from plant species of Myrtaceae, although many monomeric hydrolyzable tannins have been found in this family.[8,17–19] Oenothein B (**4**), a macrocyclic dimer, which exhibits a potent host-mediated antitumor activity,[20] was first isolated from *Oenothera erythrosepala*,[10] and later found in various Onagraceous plants and *Woodfordia fruticosa* (Lythraceae).[15] The present paper is also the first report of isolation of oenothein B from a plant of a family other than Onagraceae and Lythraceae.

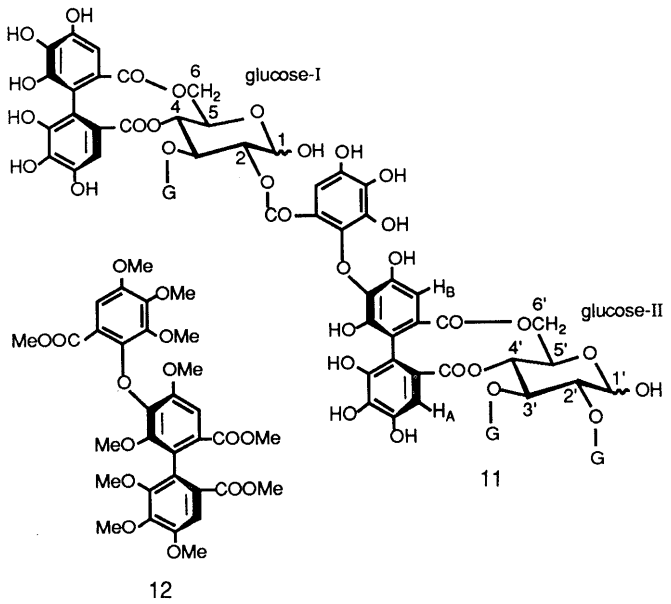

Chart 3

Experimental

General ^{1}H- (500 MHz) and ^{13}C-NMR (126 MHz) spectra were measured on a Varian VXR 500 instrument, in acetone-d_6+D_2O unless otherwise stated, and chemical shifts are given in δ (ppm) values relative to acetone-d_6 (2.04 ppm for ^{1}H and 29.8 ppm for ^{13}C). HPLC was conducted on Superspher Si 60 (4 mm × 119 mm) and LiChrospher RP-18 (4 mm × 250 mm) columns, using the following solvent systems: (A) hexane–MeOH–THF–HCOOH (60 : 45 : 15 : 1) and oxalic acid (500 mg/1.2 l) (B) hexane–MeOH–THF–HCOOH (55 : 33 : 11 : 1) and oxalic acid (450 mg/1.2 l) (C) 0.05 M phosphate buffer–CH_3CN (85 : 15), (D) 0.05 M phosphate buffer–CH_3CN (9 : 1), (E) 0.05 M phosphate buffer–CH_3CN (88 : 12). Column chromatography was performed on Toyopearl HW-40 (coarse and fine grades) (Tosoh) and MCI-gel CHP-20P (Mitsubishi Chemical Industry Co., Ltd.). The thin-layer chromatography (TLC) was carried out with Kieselgel PF_{254} using benzene–acetone (10 : 1). The solvent was evaporated off under reduced pressure below 40 °C.

Plant Materials The dried fruits (Ceplik) of *E. alba* Reinw. were purchased in a market at Sukabumi, Indonesia, in 1990, and a voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Okayama University.

Isolation of Tannins The dried fruits (2.7 kg) were homogenized in 70% aqueous acetone (9 l × 3) and filtered. The filtrate was concentrated to *ca.* 2 l, and extracted with ether, EtOAc and *n*-BuOH, successively. A part (3.7 g) of the EtOAc extract (39.9 g) was subjected to column chromatography over Toyopearl HW-40 (coarse) (2.2 × 30 cm) with aqueous MeOH (60% MeOH→70%) and MeOH–H_2O–acetone (7 : 2 : 1→6 : 2 : 2). The 60% MeOH eluate was further chromatographed over Toyopearl HW-40 (fine) with 50% MeOH to give quercetin 3-*O*-arabinopyranoside (23 mg) and (+)-catechin (3 mg). Rechromatography of the 70% MeOH eluate over MCI-gel CHP 20P (H_2O→10% MeOH→20%→25%→30%→40%)) yielded procyanidin B-7 (2.5 mg), pedunculagin (70 mg) and tellimagrandin I (**1**) (15 mg). The eluate from MeOH–H_2O–acetone (7 : 2 : 1) was similarly purified by a combination of rechromatography over MCI-gel CHP-20P (40% MeOH) and Toyopearl HW-40 (fine) (70% EtOH) to give penta-*O*-galloyl-*β*-D-glucose (20 mg) and eucalbanin C (**11**) (33 mg).

A part (25 g) of the BuOH extract (40 g) was chromatographed over Diaion HP-20 developing with H_2O→20% MeOH→30%→40%→50%→60%→70% in a stepwise gradient mode. The 20% MeOH eluate was further chromatographed over Toyopearl HW-40 (fine) (2.2 × 35 cm) with 70% MeOH to yield 2,3-hexahydroxydiphenoyl-D-glucose (35 mg), casuariin (17 mg), pedunculagin (52 mg), oenothein B (**4**) (48 mg), casuarinin (9 mg) and gemin D (**2**) (103 mg). The 40% MeOH eluate (3.1 g) was similarly chromatographed over Toyopearl HW-40 (coarse) (2.2 × 40 cm) developing with 70% MeOH and MeOH–H_2O–acetone (7 : 2 : 1). The MeOH–H_2O–acetone (7 : 2 : 1) eluate was divided into four fractions (I—IV), by analogy with the HPLC peaks. Each fraction was further purified by column chromatography over MCI-gel CHP-20P or Toyopearl HW-40 (fine) with aqueous MeOH to afford cornusiin B (**3**) (15 mg), eucalbanin C (**11**) (61 mg), eucalbanin B (**8**) (16 mg) and eucalbanin A (**5**) (19 mg), respectively.

The known compounds were identified by comparisons of their physico-chemical data with those of authentic samples, or with the reported data (see below).

Quercetin-3-*O*-α-L-arabinopyranoside (Guaijaverin) Yellow needles, mp 238—242 °C. $[\alpha]_D$ −56° (*c*=0.7, MeOH). FAB-MS *m/z*: 457 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 206 (5.58), 256 (5.30), 360 (5.23). ^{1}H-NMR (MeOH-d_4) δ: 6.23 (d, *J*=2 Hz, H-6), 6.42 (d, *J*=2 Hz, H-8), 6.91 (d, *J*=8.5 Hz, H-5′), 7.61 (dd, *J*=2, 8.5 Hz, H-6′), 7.79 (d, *J*=2 Hz, H-2′), 5.18 [d, *J*=6.5 Hz, arabinose (Arab) H-1], 3.94 (dd, *J*=6.5, 8.5 Hz, Arab H-2), 3.87 (dd, *J*=3.5, 9.5 Hz, Arab H-4), 3.85 (br s, Arab H-5), 3.69 (dd, *J*=3.5, 8.5 Hz, Arab H-3), 3.49 (dd, *J*=3, 13.5 Hz, Arab H-5). ^{13}C-NMR (DMSO-d_6) δ: 101.4 (Arab C-1), 70.7 (Arab C-2), 71.6 (Arab C-3), 66.0 (Arab C-4), 64.2 (Arab C-5), 93.5 (C-8), 98.6 (C-6), 103.9 (C-10), 115.3 (C-2′), 115.7 (C-5′), 120.9 (C-6′), 122.1 (C-1′), 133.7 (C-3), 144.9 (C-3′), 148.6 (C-4′), 156.2 (C-9), 161.2 (C-5), 164.1 (C-7), 177.5 (C-4). (MeOH-d_4, standard: MeOH = δ 49.0 ppm): 104.7 (Arab C-1), 72.9 (Arab C-2), 74.1 (Arab C-3), 69.1 (Arab C-4), 66.9 (Arab C-5), 94.8 (C-8), 100.0 (C-6),

105.5 (C-10), 116.2 (C-6'), 117.4 (C-2'), 122.8 (C-1'), 123.0 (C-5'), 135.6 (C-3), 145.9 (C-3'), 149.9 (C-4'), 158.4 (C-2), 158.6 (C-9), 162.9 (C-5), 166.4 (C-7), 179.4 (C-4).

Procyanidin B-7 A light brown amorphous powder. ^{1}H-NMR (acetone-d_6) δ: 6.97, 6.88 [each 1H, d, J=2 Hz, upper (U) and lower (L) units, H-2'], 6.77, 6.75 [each 1H, d, J=8 Hz, H-5' (U and L)], 6.73, 6.69 [each 1H, dd, J=2, 8 Hz, H-6' (U and L)], 6.08, 6.05 [each 1H, d, J=2 Hz, H-6, H-8 (U)], 6.00 [1H, br s, H-6 (L)], 4.93 [1H, br s, H-2 (U)], 4.60 [1H, br s, H-4 (U)], 4.47 [1H, d, J=8 Hz, H-2 (L)], 4.03 [1H, br s, H-3 (U)], 3.93 [1H, dt, J=5, 8 Hz, H-3 (L)], 2.78 [1H, dd, J=5, 11 Hz, H-4 (L)], 2.44 [1H, dd, J=5, 11 Hz, H-4 (L)].

Eucalbanin A (5) A light brown amorphous powder, $[\alpha]_D$ +36° (c=1.0, MeOH). FAB-MS m/z: 1109 $(M+Na)^+$. *Anal.* Calcd for $C_{48}H_{30}O_{30}\cdot H_2O$: C, 45.50; H, 3.98. Found: C, 45.33; H, 3.94%. UV λ_{max}^{MeOH} nm (log ε): 216 (4.79), 255 (4.67), 370 (3.76). CD (MeOH) [θ] (nm): $+7.3\times10^4$ (236), -4.7×10^4 (259), $+4.2\times10^4$ (282), -1.2×10^4 (316). ^{1}H-NMR (a ratio of α- and β-anomer is *ca.* 2:1) δ: 6.78, 6.76 [each s, 2H in total, galloyl (Gal)], 6.40, 6.39 (each s, 1H in total, HHDP), 6.59, 6.58 (each s, 1H in total, HHDP), 7.04, 7.00 (each 1H, s), 7.42, 7.41 (each s, 1H in total), 7.52, 7.56 (each s, 1H in total) (dilactonized tergalloyl); glucose (α-anomer) 5.50 [d, J=4 Hz, glucose (Glc) H-1], 5.10 (dd, J=4, 10 Hz, Glc H-2), 5.78 (t, J=10 Hz, Glc H-3), 5.01 (t, J=10 Hz, Glc H-4), 4.58 (ddd, J=1.5, 5.5, 10 Hz, Glc H-5), 5.22 (dd, J=5.5, 13 Hz, Glc H-6), 3.70 (dd, J=1.5, 13 Hz, Glc H-6) (β-anomer); 4.78 (d, J=8 Hz, Glc H-1), 5.18 (dd, J=8, 10 Hz, Glc H-2), 5.32 (t, J=10 Hz, Glc H-3), 4.98 (t, J=10 Hz, Glc H-4), 4.07 (dd, J=5.5, 10 Hz, Glc H-5), 5.21 (dd, J=5.5, 13 Hz, Glc H-6), 3.75 (d, J=13 Hz, Glc H-6). ^{13}C-NMR α-anomer δ: 91.0 (Glc C-1), 72.9 (Glc C-2), 71.3 (C-3), 71.3 (Glc C-4), 67.0 (Glc C-5), 63.5 (Glc C-6), 107.8, 108.0 (HHDP C-3, C-3'), 109.7 (Gal C-2, C-6), 109.0, 112.3 [dilactonized tergalloyl (DLT) C-3, C-3', 6''], 159.2, 160.0, 165.6, 166.5, 167.5, 168.3 (carbonyl). β-anomer δ: 96.4 (Glc C-1), 74.0 (Glc C-2), 73.8 (Glc C-3), 71.2 (Glc C-4), 71.9 (Glc C-5), 63.5 (Glc C-6), 107.8, 107.9 (HHDP C-3, C-3'), 109.8 (Gal C-2, C-6), 108.8, 111.1, 112.1 (DLT C-3, C-3', C-6''), 159.0, 160.2, 165.4, 166.3, 167.6, 168.2 (carbonyl).

Methanolysis of Methylate of 5 A mixture of **5** (5 mg), K_2CO_3 (50 mg) and Me_2SO_4 (2 μl) in dry acetone (2 ml) was stirred at room temperature for 4 h, and then refluxed for 6 h. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness. The reaction mixture was directly methanolyzed in 1% NaOMe solution, and subjected to preparative TLC to give methyl tri-*O*-methylgallate, dimethyl hexamethoxydiphenate (**6**) and methyl hexa-*O*-methyltergallate dilactone (**7**) [^{1}H-NMR (acetone-d_6) δ: 7.14, 7.39, 7.71 (each 1H, s), 3.71, 3.77, 3.95, 3.97, 4.09, 4.27, 4.33 (each 3H, s)], which were identified by direct comparison with authentic samples (TLC, MS and ^{1}H-NMR).

Isomerization of Eucalbanin A (8) to Cornusiin B (3) A solution of **8** (2 mg) in H_2O (1 ml) and 2 drops of 0.02 M phosphate buffer (KH_2PO_4–Na_2PO_4, pH 7.4) was left standing at room temperature under monitoring of the reaction process by HPLC. After confirming the disappearance of the starting material, the reaction mixture was acidified with diluted HCl, applied to a BondElut C18 cartridge (Analytichem) and washed with water. The MeOH eluate gave an isomerized product (1.3 mg), which was identified as authentic cornusiin B (**3**) (HPLC and ^{1}H-NMR).

Eucalbanin B (8) An off-white amorphous powder, $[\alpha]_D$ +78° (c=1.0, MeOH). FAB-MS m/z: 1593 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 218 (5.10), 273 (4.75). CD (MeOH) [θ] (nm): $+13.9\times10^4$ (223), $+11.3\times10^4$ (235), -7.5×10^4 (263), $+7.8\times10^4$ (287), -3.2×10^3 (320). ^{1}H-NMR δ: 6.96, 6.98 (each s, 2H in total), 7.00, 7.01, 7.03 (each s, 2H in total), 7.02, 7.04, 7.05 (each s, 2H in total) (Gal), 6.47, 6.50, 6.51, 6.52 (each s, 1H in total), 6.62, 6.63, 6.64, 6.67 (each s, 1H in total) (HHDP), 6.11, 6.19, 6.21 (each s, 1H in total), 6.43, 6.46, 6.47 (each s, 1H in total), 7.05, 7.06, 7.07, 7.08 (each s, 1H in total) (valoneoyl), 5.48, 5.50, 5.51, 5.61 (each d, J=4 Hz, Glc α-anomer, H-1, H-1'), 4.36, 4.37 (each d, J=8 Hz, Glc β-anomer H-1), 5.82, 5.84, 5.86, 5.87, 5.88, 5.54, 5.57, 5.62 (each t, J=10 Hz, Glc H-3, H-3'), 5.04—5.15 (Glc H-2, H-2', H-4, H-4', H-6, H-6', β-anomer H-1'), 5.19, 5.24, 5.26 (each dd, J=6, 13 Hz, Glc H-6, H-6'), 4.65 (m, Glc β-anomer H-5, H-5'), 4.22, 4.27, 4.07 (each dd, J=6, 10 Hz, Glc α-anomer H-5, H-5'), 3.92, 3.85, 3.84, 3.78, 3.77, 3.76, 3.71, 3.66 (each d, J=13 Hz, Glc H-6, H-6'). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 90.9, 91.2, 91.3, 91.4 (Glc α-anomer, C-1, C-1'), 96.6, 96.6, 96.7, 97.1 (Glc β-anomer, C-1, C-1'), 72.7, 72.8, 72.9, 73.0 (Glc α-anomer C-2, C-2'), 74.2, 74.1, 74.0, 73.9 (Glc, β-anomer C-2, C-2'), 73.3, 73.4, 73.5 (Glc β-anomer, C-3, C-3'), 70.8, 70.9, 71.0, 71.1, 71.2, 71.5 (Glc α-anomer C-3, C-4, β-anomer C-4), 71.9, 72.0, 72.2 (Glc β-anomer C-5), 67.1, 67.2, 67.3, 67.4 (Glc α-anomer C-5), 63.4, 63.5, 63.6, 63.8 (Glc α- and β-anomer C-6, C-6'), 164.3, 166.0, 166.4, 166.5, 166.7, 167.6, 167.8, 168.1 (ester carbonyl).

Acid Hydrolysis of Eucalbanin B (8) a) A solution of **8** (1 mg) in 5% H_2SO_4 (1 ml) was heated in a boiling-water bath for 18 h. After cooling, the reaction mixture was applied to a BondElut C18 cartridge and washed with water. The aqueous washing was neutralized with Amberlite IRC-400 (OH form), evaporated and analyzed by GLC after trimethylsilylation to detect glucose. The HPLC analysis (reversed-phase; solvent D) of the MeOH eluate from BondElut C18 indicated the formation of gallic acid (t_R 2.7 min), ellagic acid (t_R 12.3 min) and valoneic acid dilactone (t_R 5.5 min).

b) Eucalbanin B (**8**) (1 mg) was dissolved in H_2O (0.5 ml) containing a few drops of 5% H_2SO_4, and heated in a boiling-water bath for 16 h. The HPLC (normal phase; solvent A) of the reaction mixture showed peaks identical with those of authentic cornusiin B (**3**) (t_R 6.20 min), oenothein C (**10**) (t_R 4.25 min) and 2,3-di-*O*-galloylglucose (t_R 3.58, 3.72 min). The identities of these products were further confirmed by reversed-phase HPLC using the solvent systems, C and D.

Enzymatic Hydrolysis of Woodfordin B (9) A solution of **9** (1 mg) in H_2O (1 ml) was incubated with tannase at 37 °C for 3 h. The product was identified as eucalbanin B (**8**) by normal phase HPLC with solvent B (t_R 6.95 min) and reversed-phase HPLC with solvent C (t_R 4.8, 5.2 min), solvent D (t_R 8.8, 9.3, 9.8, 10.7 min) and solvent E (t_R 6.7, 7.0 min).

Eucalbanin C (11) An off-white amorphous powder, $[\alpha]_D$ +57° (c=1.0, MeOH). FAB-MS m/z: 1593 $(M+Na)^+$. *Anal.* Calcd for $C_{68}H_{50}O_{44}\cdot 10H_2O$: C, 46.63; H, 4.01. Found: C, 46.89; H, 4.37%. UV λ_{max}^{MeOH} nm (log ε): 217 (5.15), 270 (4.80). CD (MeOH) [θ] (nm): $+11.0\times10^4$ (220), $+13.3\times10^4$ (235), -6.7×10^4 (262), $+5.9\times10^4$ (285), -7.4×10^4 (320). ^{1}H-NMR δ: 6.982, 6.985, 6.986 (each s, 2H in total), 6.989, 7.023, 7.025 (each s, 2H in total), 7.047, 7.058 (each s, 2H in total) (Gal), 6.475, 6.481, 6.483, 6.486 (each s, 1H in total), 6.486, 6.500, 6.513, 6.526 (each s, 1H in total), 6.635, 6.638, 6.644, 6.646 (each s, 1H in total), 6.800, 6.806, 6.820, 6.821 (each s, 1H in total), 6.937, 6.921 (each s, 1H in total) (HHDP and tergalloyl), 5.56, 5.62 (each d, J=4 Hz, Glc α-anomer H-1, H-1'), 5.018, 5.020, 5.26 (each d, J=8 Hz, Glc β-anomer H-1, H-1'), 5.88, 5.87, 5.86, 5.676, 5.672, 5.61 (each t, J=10 Hz, Glc H-3, H-3'), 5.07—5.20 (Glc H-2, H-2', H-4, H-4'), 5.27—5.33 (Glc H-6, H-6'), 4.65, 4.68 (each dd, J=5.5, 10 Hz, Glc β-anomer H-5, H-5'), 4.27, 2.28 (each d, J=6, 10 Hz, Glc α-anomer H-5, H-5'), 3.78, 3.80, 3.86, 3.87 (each d, J=13 Hz, Glc H-6, H-6'). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 90.8, 91.2 (Glc α-anomer C-1, C-1'), 96.4, 96.6 (Glc β-anomer C-1, C-1'), 73.1, 73.7 (Glc α-anomer C-2, C-2'), 74.1, 75.0 (Glc β-anomer C-2, C-2'), 73.5, 73.6 (Glc β-anomer C-3, C-3'), 71.7, 72.0 (Glc β-anomer C-5, C-5'), 71.0, 71.2 (Glc α-anomer C-3, C-3', C-4, C-4', β-anomer C-4), 66.9, 67.0 (Glc α-anomer C-5, C-5'), 63.4, 63.8 (Glc α- and β-anomer C-6, C-6'), 165.8, 166.2 (1C in total), 166.2, 166.5 (1C in total), 166.8, 166.9, 167.7, 168.1 (each 1C), 167.9, 168.1 (1C in total), 168.2, 168.3 (1C in total) (ester carbonyl).

Methylation of Eucalbanin C (11) Followed by Methanolysis A mixture of **11** (3 mg), potassium carbonate (20 mg), and dimethyl sulfate (2 drops) in dry acetone (2 ml) was stirred overnight at room temperature, and then refluxed for 5 h. After removal of inorganic material by centrifugation, the supernatant was concentrated and purified by preparative TLC to give methyl tri-*O*-methylgallate (1.1 mg), dimethyl hexamethoxydiphenate (**6**) (0.5 mg) and trimethyl octa-*O*-methyltergallate (**12**) (0.4 mg), which were identified by comparisons of their electron impact-MS and ^{1}H-NMR spectra with those of authentic samples.

Isomerization of Eucalbanin C (11) to Eucalbanin B (8) A solution of **11** (5 mg) in H_2O (0.5 ml) and 0.02 M phosphate buffer (pH 7.4) (2 drops) was left standing at room temperature for 23 h. The reaction mixture was acidified with dilute HCl, passed through a cartridge of BondElut C18 and washed with water. Elution with 30% MeOH yielded an isomerized product (2.7 mg) identical with eucalbanin B (**8**) (HPLC and ^{1}H-NMR).

Acknowledgements The authors are grateful to Dr. N. Toh, Kyushu Kyoritsu University, for CD measurements, and to Mr. Iwadow of our Faculty for FAB-MS measurements. The NMR experiments were carried out using a VXR 500 instrument at the SC-NMR Laboratory of Okayama University.

References and Notes

1) A. Nitta, *Iyakuhin Kenkyu*, **20**, 1092 (1989).
2) H. El Khadem and Y. S. Mohamed, *J. Chem. Soc.*, **1958**, 3320; P. Pachaly and M. Klein, *Planta Medica*, **1987**, 442.
3) G. Nonaka, F.-L. Su and I. Nishioka, *J. Chem. Soc. Chem. Comm.*, **1981**, 781.
4) T. Okuda, T. Yoshida, M. Ashida and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765.

5) C. K. Wilkins and B. A. Bohm, *Phytochemistry*, **15**, 211 (1976).
6) T. Yoshida, Y. Maruyama, M. U. Memon, T. Shingu and T. Okuda, *Phytochemistry*, **24**, 1041 (1985).
7) T. Hatano, N. Ogawa, R. Kira, T. Yasuhara and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2083 (1989).
8) M. K. Seikel and W. E. Hillis, *Phytochemistry*, **9**, 1115 (1970).
9) O. Th. Schmidt, J. Schluz and H. Fiesser, *Justus Liebigs Ann. Chem.*, **706**, 187 (1967).
10) T. Hatano, T. Yasuhara, M. Matsuda, K. Yazaki, T. Yoshida and T. Okuda, *J. Chem. Soc., Perkin Trans. 1*, **1990**, 2735.
11) T. Yoshida, T. Hatano, T. Kuwajima and T. Okuda, *Heterocycles*, **33**, 463 (1992).
12) T. Okuda, T. Yoshida and T. Hatano, *J. Nat. Prod.*, **52**, 1 (1989).
13) T. Yoshida, T. Hatano and T. Okuda, *Heterocycles*, **30**, 1195 (1990).
14) T. Hatano, T. Yoshida, T. Shingu and T. Okuda, *Chem. Pharm. Bull.*, **36**, 2925 (1988).
15) T. Yoshida, T. Chou, A. Nitta, K. Miyamoto, R. Koshiura and T. Okuda, *Chem. Pharm. Bull.*, **38**, 1211 (1990).
16) T. Yoshida, K. Yazaki, M. U. Memon, I. Maruyama, K. Kurokawa and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2655 (1989).
17) T. Okuda, T. Yoshida, T. Hatano, K. Yazaki and M. Ashida, *Phytochemistry*, **21**, 2871 (1982).
18) G. Nonaka, M. Harada and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 685 (1980).
19) E. A. Haddock, R. K. Gupta, S. M. K. Al-Shafi, K. Layden, E. Haslam and D. Magnolato, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1049.
20) K. Miyamoto, N. Kishi, R. Koshiura, T. Hatano, T. Yoshida and T. Okuda, *Chem. Pharm. Bull.*, **35**, 814 (1987).