August 1989 Chem. Pharm Bull. 37(8) 2071—2077 (1989)

Tannins and Related Compounds. LXXXV.[1] Structures of Novel *C*-Glycosidic Ellagitannins, Grandinin and Pterocarinins A and B

Gen-ichiro NONAKA, Kanji ISHIMARU, Ryutaro AZUMA, Makoto ISHIMATSU and Itsuo NISHIOKA*

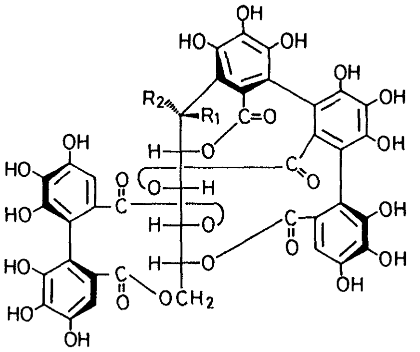
Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received February 8, 1989*

The structures of three hydrolyzable tannins, *i.e.*, grandinin (3) (isolated from various species of Myrtaceae, Fagaceae and Lythraceae), pterocarinin A (4) (from *Pterocarya steroptera* C.DC. and *Eucalyptus viminalis* LABILL.) and pterocarinin B (6) (from *P. stenoptera*), have been established on the basis of chemical and spectroscopic evidence as novel *C*-glycosidic ellagitannins in which a C_5-polyalcohol unit with lyxose-type configuration is linked through a carbon–carbon bond to the C-1 position of the *C*-glycosyl moiety. Successful biomimetic synthesis of 3 and 4 suggests that the C_5-polyalcohol unit is biosynthetically derived from L-ascorbic acid.

Keywords grandinin; pterocarinin A; pterocarinin B; *C*-glycosidic ellagitannin; lyxose-type polyalcohol; L-ascorbic acid; biomimetic synthesis; tannin

Previously, we reported on the isolation and characterization of several complex tannins, in which a *C*-glycosidic ellagitannin is connected through a carbon-to-carbon linkage (at the C-1 position) to the A-ring of a flavonoid glucoside,[2] flavan-3-ol[3] or procyanidin.[4] The isolation of these new classes of tannins suggests that the *C*-glycosidic C-1 position serves as a strong nucleophilic center during metabolism. In the course of our continuing chemical examination of tannins, we have isolated novel *C*-glycosidic ellagitannins named grandinin and pterocarinins A and B, which possess a unique C_5-polyalcohol moiety at the C-1 position. This paper deals with the isolation and structural elucidation of these compounds.

Grandinin (**3**) was obtained in relatively high yields in almost all cases from the aqueous acetone extracts of various species of the families Myrtaceae (*Eugenia grandis* WIGHT, *Syzygium samarangens* (BLUME) MERR. *et* PERRY, *Psidium guajava* L. and *Eucalyptus viminalis* LABILL.), Fagaceae (*Quercus stenophylla* MAKINO, *Q. acutissima* CARRUTHERS, *Q. mongolica* FISCHER *et* TURCZ. var. *grosseserrata* (BL.) REHDER *et* WILSON, *Q. philyraeoides* ASA GRAY, *Castanea crenata* SIEB. *et* ZUCC. and *C. mollissima* BLUME) and Lythraceae (*Lagerstroemia indica* L.), accompanied invariably with *C*-glycosidic ellagitannins, castalagin (**1**) and vescalagin (**2**).[5] The brown and dark blue colorations with the sodium nitrite–acetic acid[6] and ferric chloride reagents, respectively, were characteristic of ellagitannins. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum was extremely complicated owing to the existence of an equilibrium mixture. The integral curve in the aromatic field (δ 6.6—7.3), however, showed the presence a total of three aromatic protons, and this fact suggests that **3** possesses a *C*-glycosidic nature. The aliphatic signals consist of two parts. The lowfield signals between δ 4.30—6.10 were attributable to the protons of a polyalcohol core, while a block of upper field resonances between δ 3.6—4.2 suggested the presence of an extra polyalcohol moiety carrying no acyl group. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum exhibited, together with a complex polyalcohol signal pattern, signals (δ 102.5, 103.7, 1C in total) due to a hemiketal carbon, the chemical shifts being in good agreement with those of the C-2 carbon in the equilibrium forms of ketoses.



	R_1	R_2
1	OH	H
2	H	OH

When treated with methanolic hydrochloride at room temperature, **3** afforded many degradation products, among which the major compound **3a** could be isolated. The ^{1}H- and ^{13}C-NMR spectra of **3a** were, in contrast, simple and amenable to first-order analysis. The signal pattern in the aliphatic proton region was typical of *C*-glycosidic ellagitannins and the coupling patterns agreed well with those of **2**. In particular, a similar coupling constant ($J=1.5$ Hz) of the polyalcohol C-1 hydrogen signal clearly indicated the C-1 position to have the same configuration as that of **2**. Furthermore, in the aromatic field, three AMX-type signals newly appeared at δ 6.84 (1H, dd, $J=2$, 4 Hz), 7.67 (1H, d, $J=4$ Hz) and 8.24 (1H, d, $J=2$ Hz), in place of the above-mentioned high-field polyalcohol signals. Taking the chemical shifts and the coupling constants into account, these signals were presumed to arise from a mono-substituted furan ring. The ^{13}C-NMR spectrum of **3a** was consistent with the presence of the furan ring, showing four aromatic carbon signals at δ 152.6, 149.4, 120.3 and 113.9, the chemical shifts being analogous to those of furfural (δ 153.3, 148.5, 121.7, 112.9). In addition, the observation of a signal at δ 185.4 in **3a**, in conjunction with the good coincidence of the chemical shifts of the furan ring carbon signals, was indicative of the presence of a carbonyl group adjacent to the furan ring. These findings were further supported by fast atom bombardment mass spectral (FAB-MS) comparison of **3** and **3a**, which showed the loss of three moles of water from the molecule of **3** [**3**, positive mode *m/z*: 1067 $(M+H)^+$. **3a**, negative mode *m/z*: 1011 $(M-H)^-$]. The formation of the furan ring agreed well with the fact that on acid treatment, most ketoses readily liberate water to

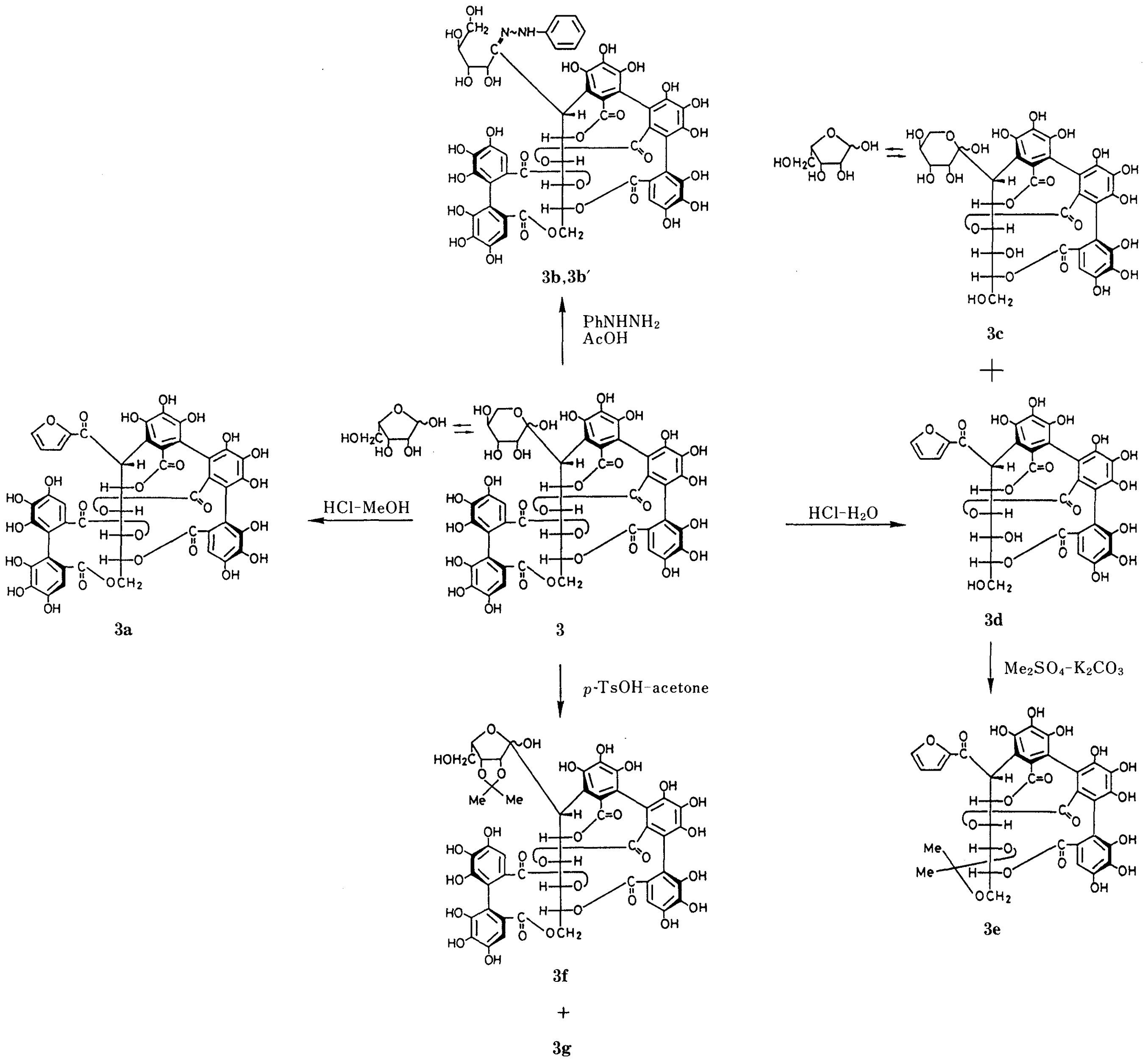


Chart 1

form furfural derivatives.

Further chemical work was done to confirm the presence of the ketose-type polyalcohol moiety in 3. On treatment with phenylhydrazine in the presence of acetic acid, followed by repeated reverse-phase chromatography, **3** yielded two hydrazones (**3b** and **3b**′), which were shown to be geometrical isomers about the nitrogen-nitrogen linkage by ^{1}H- and ^{13}C-NMR and negative FAB-MS [m/z 1155 $(M-H)^-$] comparisons. The ^{1}H- and ^{13}C- NMR spectra of **3b** and **3b**′ were straightforward. The absence of the hemiketal carbon signal and instead the appearance of a signal due to a nitrogen-bearing sp^2 carbon at δ 146.8 showed that the polyalcohol moiety possesses a linear chain structure like the phenylhydrazones of monosaccharides. Furthermore, the observation of four aliphatic carbon signals (δ 64.1—73.2) (in addition to six signals derived from the *C*-glycosyl core) indicated **3** to have an equilibrium ketopentose-type residue connected through a carbon-carbon bond to the C-1 position of the *C*-glycosyl core.

On heating in aqueous hydrochloric acid solution, **3** afforded, among others, two isolable products (**3c** and **3d**). The lack of two aromatic singlets in the ^{1}H-NMR spectrum of **3c**, as well as the appearance of a prominent $(M+H)^+$ peak at m/z 765 in the FAB-MS, established the structure of **3c** to be a deshexahydroxydiphenoyl derivative of **3**. On the other hand, the ^{1}H-NMR spectrum of **3d** showed the presence of a furan ring [δ 6.78 (1H, dd, $J=2$, 4 Hz), 7.57 (1H, d, $J=4$ Hz), 7.94 (1H, d, $J=2$ Hz)], together with only one aromatic singlet at δ 6.80. The negative FAB-MS data [m/z 709 $(M-H)^-$] were consistent with the deshexahydroxydiphenoyl furanoid structure (**3d**). The significant upfield shifts of the C-4 [δ 4.06 (dd, $J=7$, 8 Hz) and C-6 (δ 3.82—3.89) proton signals, in conjunction with the fact that **3d** readily produced an acetonide methylate (**3e**) when treated with dimethyl sulfate and potassium carbonate in dry acetone, indicated that the hexahydroxydiphenoyl ester group is located at the C-4 and C-6 positions of the *C*-glycosyl core.

The ^{13}C-NMR spectrum of **3**, although extremely complicated, exhibited two prominent (δ 69.0, 72.2) and two very small peaks (δ 80.0, 81.3), all assignable to C-4 of the polyalcohol moiety on the basis of ^{1}H–^{13}C two-dimensional shift correlation spectroscopy (2D-COSY), indicating that **3** consists of an equilibrium mixture of four structural isomers. Furthermore, considering that the C-4 signals in pentopyranoses are generally observed between δ 68—70, whereas those in pentofuranoses are between δ 81—85,[7] two pyranose-type forms predominate in **3**. Treatment of **3** with acetone in the presence of a trace amount of *p*-toluenesulfonic acid yielded two acetonides (**3f** and **3g**). The negative FAB-MS of **3f** and **3g** exhibited the same $(M-H)^-$ peak at *m/z* 1105, indicating that both are mono-isopropylidene derivatives. In the ^{13}C-NMR spectrum of **3f**, the observation of the C-4 signal at δ 80.6 implied the polyalcohol moiety to have a furanose-type form, while the lowfield shifts of the polyalcohol C-2 (δ 86.1) and C-3 (δ 82.4) signals indicated the formation of the acetonide at these positions. From these findings, the C-2 and C-3 hydroxyls are considered to have mutually *cis*-configuration. Although **3g** was regarded as a mono-acetonide having a pyranose-type form, assignment of the structure could not be made because of the complexities of the ^{1}H- and ^{13}C-NMR signal patterns. The stereostructural relationship between the polyalcohol C-3 and C-4 in grandinin was deduced to be as shown in **3** from the large coupling constant ($J=7$ Hz) between the H-3 and H-4 signals arising from one of the pyranose-type forms in the ^{1}H-NMR spectrum of **3**.

The atropisomerism of the hexahydroxydiphenoyl and triphenoyl groups was established to be in the *S*- and *S,S*-series, respectively, by circular dichroism (CD) spectral comparison of **3** and **2** (Fig. 1).

From the above-mentioned chemical and spectroscopic evidence, the structure of grandinin is considered to be represented by the formula **3**, in which the polyalcohol hydroxyls possess lyxose-type configurations. From the biosynthetic point of view, however, the mode of the linkage between the polyalcohol and *C*-glycosyl ellagitannin moieties seems to be rather unusual. Taking into account the lyxose structure and the location of a hemiketal carbon atom at the C-1′ position, the polyalcohol unit is considered to be biosynthetically derived from ascorbic acid whose C-2 atom acts as a strong nucleophile.[8] Based on these speculations, an attempt was made to prepare **3** by coupling of ascorbic acid and **2**. Among various conditions tested, the reaction in aqueous acetic acid under reflux smoothly proceeded, as anticipated, to give a product, the $[\alpha]_D$ and the ^{1}H-NMR spectrum of which were identical with those of **3**, together with a structural isomer which is presumed to be a C-2′ epimer from a consideration of the reaction mechanism (Chart 2). On the basis of these results, the structure of grandinin, including the absolute stereostructure, was unequivocally established as shown in the formula **3**.

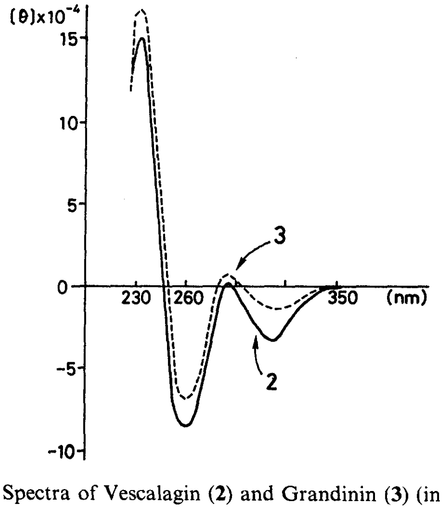


Fig. 1. CD Spectra of Vescalagin (**2**) and Grandinin (**3**) (in MeOH)

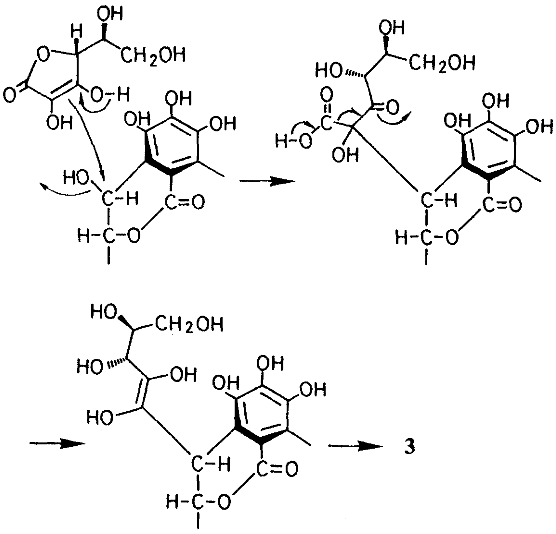


Chart 2. Possible Reaction Mechanism for the Formation of Grandinin (**3**)

Pterocarinin A (**4**) was isolated as a major tannin from the bark of *Pterocarya steroptera* (Juglandaceae) and also from the bark of *Eucalyptus viminalis* (Myrtaceae). The ^{1}H- and ^{13}C-NMR spectra showed that **4** exists as an equilibrium mixture, but the spectra were not as complicated as those of **1**. The appearance of a two-proton aromatic singlet at δ 7.11 and three one-proton singlets at δ 6.47, 6.57 and 6.81 suggested **4** to be a *C*-glycosidic ellagitannin having a galloyl group. The chemical shifts and coupling patterns of the aliphatic signals between δ 4.1—5.7 arising from the major conformer were similar to those of stachyurin (**5**),[9] except for the moderate upfield shift (δ 3.59) of the H-1 signal. The ^{13}C-NMR spectrum revealed the presence of a hemiketal carbon (δ 103.8), seven oxygen-bearing methines (δ 66.9—75.3) and two methylenes (δ 63.5, 64.5). The upfield shift (δ46.4) of the *C*-glycosyl C-1 signal, assigned on the basis of ^{1}H–^{13}C 2D-COSY analysis, indicated the location of a *C*-substituent at this position.

Enzymatic hydrolysis of **4** with tannase yielded a hydrolysate (**4a**), together with gallic acid and ellagic acid. On subsequent acid treatment, **4a** gave, among others, a product (**4b**), the negative FAB-MS of which exhibited the $(M-H)^-$ peak at *m/z* 559, in agreement with the elimination of three moles of water from **4a**. In the ^{1}H-NMR spectrum of **4b**, the observation of three additional aromatic signals [δ 6.81 (br d, $J=4$ Hz), 7.71 (d, $J=4$ Hz), 7.99 (br s)] similar to those found in **3a** indicated the pres-

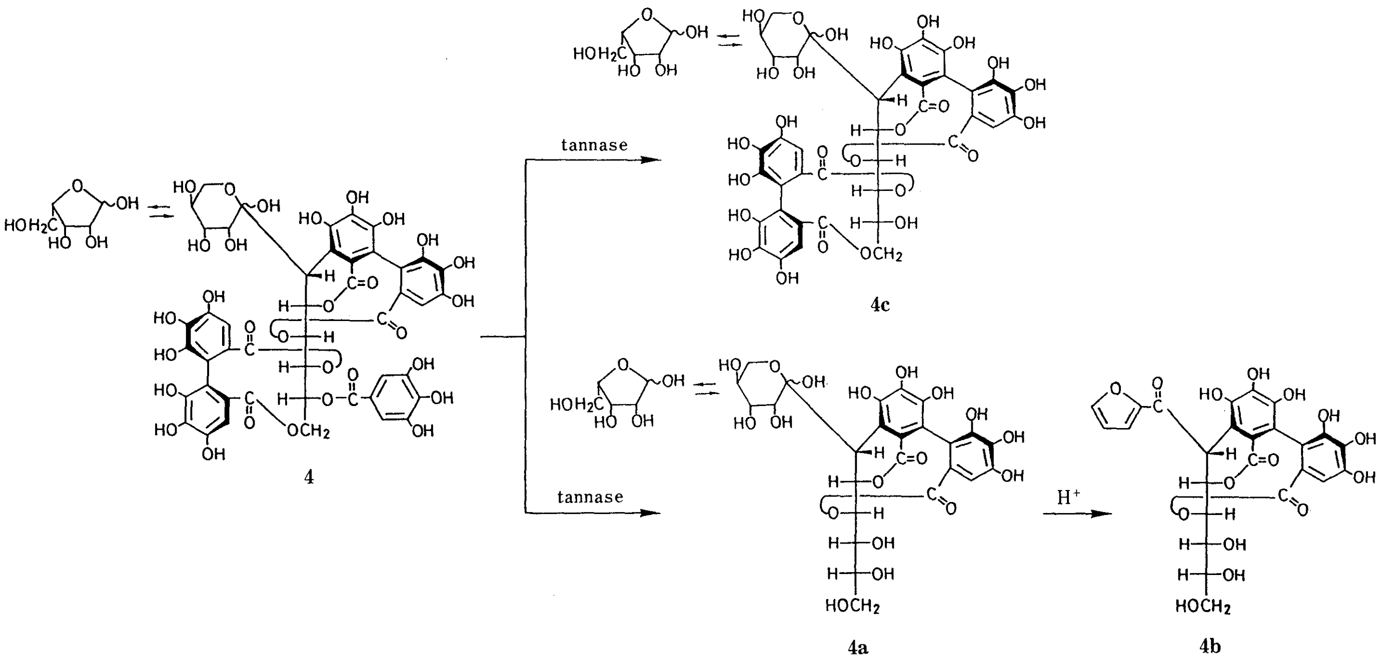
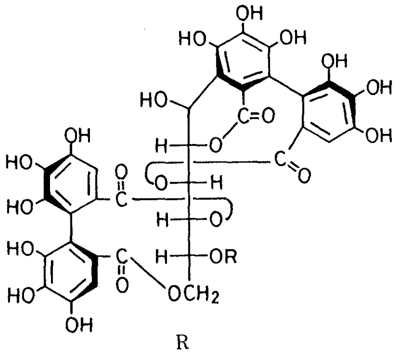


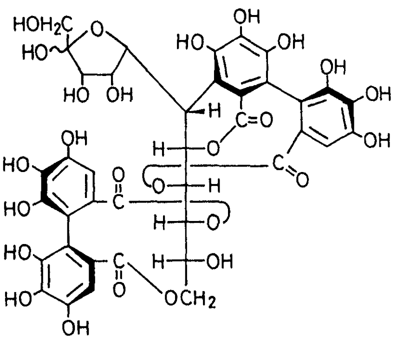
Chart 3

ence of a monosubstituted furan ring.

In the ^{13}C-NMR spectrum of **4**, the polyalcohol C-4 signal assigned by ^{1}H–^{13}C 2D-COSY appeared at δ66.9 and *ca.* 82, the former greatly predominating. This fact clearly indicated that **4** exists mostly as a pyranose-type form in solution. Furthermore, the ^{1}H-NMR spectrum of **4** showed signals attributable to the polyalcohol H-2, H-3 and H-4 and δ4.01 (d, $J=3$ Hz), 3.89 (dd, $J=3$, 8 Hz) and 4.00 (dd, $J=5$, 8 Hz), respectively. Consideration of the coupling constants of these signals led us to assume that the C-2 proton has equatorial configuration, while the C-3 and C-4 protons are axially oriented, consistent with lyxose-type configurations.



R
5 galloyl
7 H



6

The final structural confirmation was made by condensation of L-ascorbic acid and **5** in the same manner as described above to give **4**. Thus, pterocarinin A was assigned the structure **4**.

Pterocarinin B (**6**), isolated from *Pterocarya steroptera*, was readily deduced to be a *C*-glycosyl ellagitannin from three one-proton aromatic singlets at δ6.43, 6.56 and 6.77 in the ^{1}H-NMR spectrum. The appearance of the *C*-glycosyl H-5 signal at higher field (δ4.12) implied the absence of an acyl group at this position, and the lowfield aliphatic signal pattern was analogous to those of 5-desgalloylstachyurin (**7**) and 5-desgalloylpterocarinin A (**4c**). In addition, the presence of three methines [δ4.54 (dd, $J=5$, 10 Hz), 4.27 (dd, $J=2$, 5 Hz), 4.15 (d, $J=2$ Hz)] and one isolated methylene [δ3.64, 3.71 (each d, $J=13$ Hz)] was readily seen from the ^{1}H-NMR spectrum. Examination of the ^{1}H–^{1}H (Fig. 2) and ^{1}H–^{13}C 2D-COSY spectra permitted the assignment of the structure of **6**. The signal at δ4.54 was correlated with the signal at δ4.27, which further showed a correlation with the signal at δ4.15, thus indicating the partial structure $-\overset{\text{O}}{\mathrm{CH}}-\overset{\text{O}}{\mathrm{CH}}-\overset{\text{O}}{\mathrm{CH}}-\blacksquare$. Since the *C*-glycosyl H-1 signal at δ3.51, the assignment of which was based on the correlation with the carbon signal at δ40.7, was shown to be coupled with the signal at δ4.54, the terminal methine carbon should be linked to the *C*-glycosyl C-1 position. The observation of a hemiketal carbon signal at δ105.4, as well as the correlation of the signal at δ4.54 with the relatively lowfield carbon signal at δ81.4, led us to the structure of the polyalcohol moiety, although the relative configuration of each carbon atom is not definitive. The negative FAB-MS, showing an intense $(M-H)^-$ peak at *m*/*z* 915, was consistent with the proposed structure.

The configuration of the *C*-glycosyl C-1 atom was determined to be the same as those of **3** and **4** on the basis of

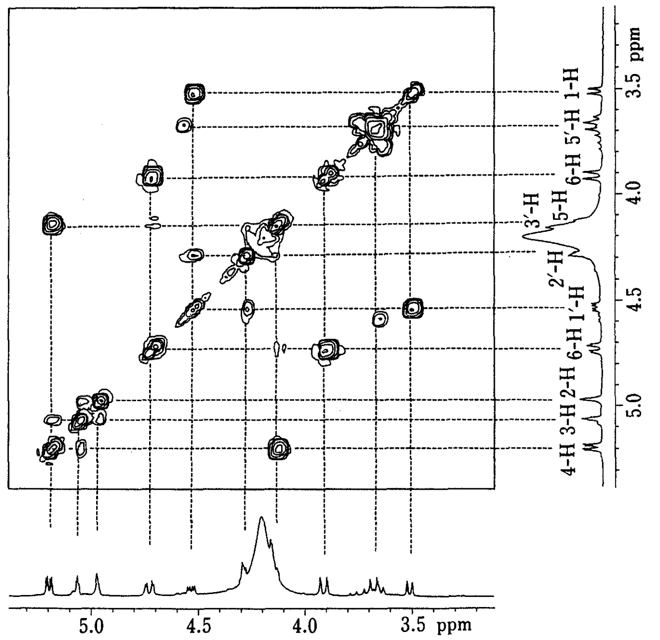


Fig. 2. ^1H–^1H 2D-COSY Spectrum of Pterocarinin B (6)

the samll coupling constant ($J=2$ Hz) between the H-1 and H-2 signals.

From these observations, the structure of pterocarinin B was concluded to be represented by the formula **6**.

The abnormal mode of the linkage between the *C*-glycosyl ellagitannin and C_5-polyalcohol moieties in compounds **3**, **4** and **6** suggests that biosynthetically the polyalcohol unit may not originate from common sugars. In the cases of **3** and **4**, the unit is probably derived from L-ascorbic acid by condensation, followed by decarboxylation, but the origin of the unit in **6** is not clear.

Experimental

Details of the instruments and chromatographic conditions used throughout this work are the same as described in the previous paper.[2)]

Isolation of Grandinin (3) As mentioned in the text, grandinin was isolated from various Myrtaceous, Fagaceous and Lythraceous plants. As a typical example of the isolation procedures, the case of *Eugenia grandis* (Myrtaceae) is described herein.

The dried leaves (2.3 kg) of *Eugenia grandis*, collected in Singapore, were extracted at room temperature with 60% aqueous acetone. The extract was concentrated under reduced pressure, and the resulting precipitates (consisting mainly of chlorophylls and waxes) were removed by filtration. The filtrate was directly subjected to Sephadex LH-20 chromatography. Elution with water gave non-phenolic compounds such as sugars, amino acids, *etc*. Further elution with water containing increasing proportions (30—80%) of methanol yielded a fraction containing gallic acid, ellagic acid, grandinin (**3**), castalagin (**1**) and vescalagin (**2**). Rechromatography of this fraction on a Sephadex LH-20 column with ethanol afforded gallic acid, ellagic acid, **3** (5.37 g) and a mixture of **1** and **2**. The separation of the mixture was achieved by chromatography over MCI-gel CHP 20P with water containing increasing amounts of methanol to give **1** (9.10 g) and **2** (21 mg).

Isolation of Pterocarinins A (4) and B (6) The fresh bark (20.8 kg) of *Pterocarya steroptera*, collected at the medicinal plant garden in Kyushu University, Fukuoka, Japan, was extracted with 80% aqueous acetone at room temperature. The acetone was removed by concentration under reduced pressure, and precipitates appearing in the aquous solution were filtered off. The filtrate was mixed with Celite 545, dried and packed in a column. Elution with acetone afforded an acetone-soluble fraction, which was chromatographed on a Sephadex LH-20 column with a solvent system of ethanol–water–acetone to give two fractions. The first fraction (715 g) was found to contain flavonoid glycosides and their gallates, gallic acid, ellagic acid and flavan-3-ol derivatives. The second fraction (375 g) was rechromatographed on Sephadex LH-20 with water containing increasing amounts of methanol to yield a further four fractions, among which the first and the third fractions gave pterocarinins B (**6**) (43 mg) and A (**4**) (2.34 g), respectively, on repeated chromatography over MCI-gel CHP 20P with a mixture of water and methanol.

Grandinin (3) A pale brown amorphous powder, $[\alpha]_D^{24}$ $-33.0°$ [$c=1.2$, H_2O–acetone (1 : 1)]. *Anal.* Calcd for $C_{46}H_{34}O_{30}\cdot 6H_2O$: C, 47.02; H, 3.94. Found: C, 46.79; H, 3.92. FAB-MS *m/z*: 1067 $(M+H)^+$. ^1H-NMR (400 MHz, acetone-d_6+D_2O): 3.80, 4.10 (1H in total, each br s, H-1), 3.6—4.2 (m, H-3′, 4′, 5′), 4.36 (1H, d, $J=13$ Hz, H-6), 4.52 (br s, H-2′), 5.14 (1H, t-like, $J=7$ Hz, H-3), 5.35 (1H, t, $J=7$ Hz, H-4), 5.5—6.1 (2H in total, H-2, 5), 6.6—7.3 (aromatic H). ^{13}C-NMR (100 MHz, acetone-d_6+D_2O): 48.3 (C-1), 65.2 (C-5′), 68.2 (C-6), 68.5 (C-6), 69.0 (C-4′), 72.1 (C-4), 72.2 (C-4′), 72.8 (C-4), 74.8 (C-2), 75.3 (C-2), 76.4 (C-3′), 77.1 (C-2′), 102.5 (C-1′), 110.3, 110.4, 112.4, 112.5, 112.6 (aromatic C), 168.9, 169.8, 170.1, 170.5, 170.6, 173.1 (–COO–). CD ($c=1.18\times10^{-5}$, MeOH) $[\theta]^{23}$ (nm): $+1.7\times10^5$ (232), -6.8×10^4 (260), $+9.0\times10^3$ (285), 1.4×10^4 (315).

Treatment of 3 with Methanolic Hydrochloride A solution of **3** (290 mg) in 2% methanolic hydrochloride (10 ml) was left standing at room temperature for 12 h. The reaction products were separated by Sephadex LH-20 and Fuji-gel ODS G3 chromatographies with water–methanol to afford, among others, the furan derivative (**3a**) (60 mg) as colorless needles (H_2O), mp 210 °C (dec.), $[\alpha]_D^{26}$ $-23.7°$ ($c=1.4$, MeOH). *Anal.* Calcd for $C_{46}H_{28}O_{27}\cdot 7H_2O$: C, 48.51; H, 3.71. Found: C, 48.35; H, 3.41. Negative FAB-MS *m/z*: 1011 $(M-H)^-$. ^1H-NMR (100 MHz, acetone-d_6): 3.98 (1H, d, $J=12$ Hz, H-6), 4.90 (1H, d, $J=6$ Hz, H-3), 4.84—5.02 (1H, dd, $J=6$, 12 Hz, H-6), 5.03 (1H, d, $J=1.5$ Hz, H-1), 5.33 (1H, t, $J=6$ Hz, H-4), 5.60 (1H, d, $J=1.5$ Hz, H-2), 5.42 (1H, d, $J=6$ Hz, H-5), 6.60, 6.75, 7.00 (each 1H, s, aromatic H), 6.84 (1H, dd, $J=2$, 4 Hz, furan H-4), 7.67 (1H, d, $J=4$ Hz, furan H-3), 8.24 (1H, d, $J=2$ Hz, furan H-5). ^{13}C-NMR (25.05 MHz, acetone-d_6): 49.1 (C-1), 65.6 (C-6), 70.3, 71.1 (2×C), 74.1 (C-2,3,4,5), 107.4, 109.0, 109.3 (aromatic C), 113.9 (furan C-4), 120.3 (furan C-3), 149.4 (furan C-5), 152.6 (furan C-2), 163.9, 165.3, 166.6 (2×C), 169.0 (–COO–), 185.4 (CO).

Formation of Phenylhydrazones (3b, 3b′) from 3 A mixture of **3** (100 mg), acetic acid (0.5 ml) and phenylhydrazine (0.5 ml) in methanol (10 ml) was kept at room temperature for 10 min. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was subjected to MCI-gel CHP 20P and Fuji-gel ODS G3 chromatographies with water–methanol to furnish two phenylhydrazones **3b** (30 mg) and **3b′** (10 mg). **3b**: A pale yellow amorphous powder, $[\alpha]_D^{26}$ $+41.2°$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{52}H_{40}N_2O_{29}\cdot 7H_2O$: C, 48.67; H, 4.24; N, 2.18. Found: C, 48.69; H, 4.09; N, 2.16. Negative FAB-MS *m/z*: 1155 $(M-H)^-$. ^1H-NMR (100 MHz, acetone-d_6): 4.34 (1H, br s, H-1), 4.74 (1H, d, $J=6$ Hz, H-3), 4.84 (1H, dd, $J=12$, 2Hz, H-6), 5.26 (1H, t, $J=6$ Hz, H-4), 5.64 (2H, m, H-2,5), 6.64, 6.78, 7.18 (each 1H, s, aromatic H), 6.60—7.20 (5H, m, phenylhydrazone-H). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 42.6 (C-1), 64.1 (C-5′), 65.7 (C-6), 107.1, 108.8, 109.3, 113.8, 119.9, 129.5 (aromatic C), 141.7 (HN–C=), 146.8 (C-1′), 165.9, 166.8, 167.3, 169.5 (–COO–). **3b′**: A pale brown amorphous powder, $[\alpha]_D^{26}$ $-35.6°$ ($c=0.4$, MeOH). *Anal.* Calcd for $C_{52}H_{40}N_2O_{29}\cdot 7H_2O$: C, 48.67; H, 4.24; N, 2.18. Found: C, 48.65; H, 4.08; N, 2.26. Negative FAB-MS *m/z*: 1155 $(M-H)^-$. ^1H-NMR (100 MHz, acetone-d_6+D_2O): 4.38 (1H, br s, H-1), 4.64 (1H, d, $J=6$ Hz, H-3), 5.02 (1H, dd, $J=12$, 2Hz, H-6), 5.24 (1H, t, $J=6$ Hz, H-4), 5.48 (1H, br s, H-2), 5.64 (1H, br d, $J=6$ Hz, H-5), 6.60, 6.78, 7.26 (each 1H, s, aromatic H), 6.60—7.20 (5H, m, phenylhydrazone-H).

Treatment of 3 with Hydrochloric Acid A solution of **3** (320 mg) in 3% aqueous hydrochloric acid was heated on a water bath for 3 h. The reaction mixture was applied to a column of Sephadex LH-20, and elution with water containing increasing proportions of methanol gave **3c** (10 mg) and **3d** (80 mg). **3c**: A pale brown amorphous powder $[\alpha]_D^{26}$ $+54.8°$($c=0.9$, MeOH). *Anal.* Calcd for $C_{32}H_{28}O_{22}\cdot 9/2H_2O$: C, 45.45; H, 4.41. Found: C, 45.49; H, 4.20. FAB-MS *m/z*: 765 $(M+H)^+$. ^1H-NMR (100 MHz, acetone-d_6+D_2O): 4.50 (1H, H-3), 5.15 (1H, H-5), 5.70 (1H, H-2), 6.81 (1H, s, aromatic H). **3d**: Colorless needles (H_2O), mp 220°C (decomp.), $[\alpha]_D^{26}$ 0° ($c=0.5$, MeOH). *Anal.* Calcd for $C_{32}H_{22}O_{19}\cdot 5H_2O$: C, 48.01; H, 4.02. Found: C, 48.20; H, 3.77. Negative FAB-MS *m/z*: 709 $(M-H)^-$. ^1H-NMR (100 MHz, acetone-d_6+D_2O): 3.8—3.9 (2H, m, H-6), 4.06 (1H, dd, $J=7$, 8 Hz, H-4), 4.68 (1H, d, $J=1.5$ Hz H-1), 4.77 (1H, dd, $J=1.5$, 7 Hz, H-3), 5.10 (1H, m, H-5), 5.80 (1H, t, $J=1.5$ Hz, H-2), 6.78 (1H, dd, $J=2$, 4 Hz, furan H-4), 6.80 (1H, s, aromatic H), 7.58 (1H, d, $J=4$ Hz, furan H-3), 7.94 (1H, d, $J=2$ Hz, furan H-5). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 49.4 (C-1), 61.8 (C-6), 68.7, 73.2, 73.7, 74.3 (C-2,3,4,5), 108.8 (aromatic C), 113.9 (furan C-4), 121.2 (furan C-3), 149.6 (furan C-5), 151.3 (furan C-2), 165.9, 166.6, 168.4 (–COO–), 186.0 (CO).

Formation of 3e A mixture of **3d** (30 mg), dimethyl sulfate (0.3 ml) and anhydrous potassium carbonate (0.7 g) in dry acetone was heated under reflux for 3 h. The inorganic salts were removed by filtration, and the filtrate, after concentration, was subjected to silica gel chromatography with benzene–acetone to furnish the acetonide methyl ether (**3e**) (8 mg) as colorless needles (MeOH), mp 178 °C, $[\alpha]_D^{26}$ +251.9° (c=0.3, acetone). *Anal.* Calcd for $C_{45}H_{46}O_{19}$: C, 60.66; H, 5.20. Found: C, 60.21; 5.17. FD-MS m/z: 890 $(M)^+$. ^{1}H-NMR (100 MHz, $CDCl_3$): 1.38, 1.48 (each 3H, s, Me), 3.47—4.13 (OMe), 4.86 (1H, d, J=7 Hz, H-3), 5.40 (1H, m, H-5), 5.88 (1H, br s, H-2), 6.46 (1H, dd, J=2, 4 Hz, furan H-4), 6.69 (1H, d, J=4 Hz, furan H-3), 6.91 (1H, s, aromatic H), 7.58 (1H, d, J=2 Hz, furan H-5).

Formation of Isopropylidenes (3f, 3g) from 3 A solution of **3** (100 mg) in dry acetone (20 ml) containing p-toluenesulfonic acid (1 mg) was left standing at room temperature for 5 h. After evaporation of the solvent under reduced pressure, the residue was chromatographed over Sephadex LH-20 with ethanol to give the isopropylidenes **3f** (29 mg) and **3g** (20 mg). **3f**: A pale brown amorphous powder. *Anal.* Calcd for $C_{49}H_{38}O_{30}\cdot 6H_2O$: C, 48.44; H, 4.15. Found: C, 48.55; H, 3.91. Negative FAB-MS m/z: 1105 $(M-H)^-$. ^{1}H-NMR (400 MHz, acetone-d_6+D_2O): 1.28, 1.34 (each 3H, s, Me), 3.89 (1H, dd, J=12, 7 Hz, H-5′), 3.98 (1H, dd, J=12, 5 Hz, H-5′), 4.42 (1H, ddd, J=3, 5, 7 Hz, H-4′), 4.61 (1H, dd, J=12, 2 Hz, H-6), 4.77 (1H, d, J=8 Hz, H-3), 5.14 (1H, dd, J=3, 6 Hz, H-3′), 5.17 (1H, d, J=6 Hz, H-2′), 5.19 (1H, t, J=8 Hz, H-4), 5.56 (1H, s, H-2), 5.58 (1H, br d, J=8 Hz, H-5), 6.60, 6.70, 7.08 (each 1H, s, aromatic H). ^{13}C-NMR (100 MHz, acetone-d_6+D_2O): 25.2, 26.8 (Me), 46.2 (C-1), 60.6 (C-5′), 66.7 (C-6), 70.3 (C-4), 71.0 (C-5), 72.5 (2 × C) (C-2,3), 80.6 (C-4′), 82.4 (C-3′), 86.1 (C-2′), 107.1, 108.7, 110.3 (aromatic C), 165.9, 166.9, 167.6, 167.8, 170.0 (–COO–). **3g**: A pale brown amorphous powder. *Anal.* Calcd for $C_{49}H_{38}O_{30}\cdot 11/2H_2O$: C, 48.80; H, 4.10. Found: C, 48.82; H, 3.86. Negative FAB-MS m/z: 1105 $(M-H)^-$. The ^{1}H-NMR (400 MHz) spectrum was extremely complicated.

Preparation of 3 A mixture of **2** (320 mg) and L-ascorbic acid (70 mg) in 0.1 M acetic acid was heated under reflux for 8 h. The reaction mixture was directly applied to a column of Sephadex LH-20. Elution with water containing increasing amounts of methanol yielded two crude products, which were separately purified by MCI-gel CHP 20P with water–methanol to furnish **3** (60 mg) and a structural isomer (121 mg) [negative FAB-MS m/z: 1065 $(M-H)^-$], which is probably an epimer at the C-2 position of the polyalcohol moiety.

Pterocarinins A (4) A pale brown amorphous powder, $[\alpha]_D^{21}$ +68.4° (c=1.0, acetone): *Anal.* Calcd for $C_{46}H_{36}O_{30}\cdot 4H_2O$: C, 48.43; H, 3.89. Found: C, 48.42; H, 3.83. FAB-MS m/z: 1069 $(M+H)^+$. ^{1}H-NMR (400 MHz, acetone-d_6) 3.59 (1H, d, J=1 Hz, H-1), 3.69 (1H, d, J=12 Hz, H-5′), 3.85 (1H, dd, J=5, 12 Hz, H-5′), 3.89 (1H, dd, J=3, 8 Hz, H-3′), 4.00 (1H, dd, J=5, 8 Hz, H-4′), 4.01 (1H, d, J=3 Hz, H-2′), 4.09 (1H, d, J=13 Hz, H-6), 4.92 (1H, dd, J=3, 13 Hz, H-6), 4.95 (1H, d, J=2 Hz, H-3), 5.18 (1H, br s, H-2), 5.33 (1H, dd, J=3, 9 Hz, H-5), 5.63 (1H, dd, J=3, 9 Hz, H-4), 6.47, 6.57, 6.81 (each 1H, s, aromatic H), 7.11 (2H, s, galloyl-H). ^{13}C-NMR (100 MHz, acetone-d_6): 46.1 (C-1), 63.2 (C-5′), 64.5 (C-6), 66.9 (C-4′), 71.1 (C-5), 72.2 (C-2′,3′), 73.5 (C-4), 74.2 (C-3), 75.3 (C-2), 102.0 (C-1′), 105.2, 107.4, 108.8 (HHDP[10] C-6), 110.1 (galloyl C-2,6), 115.0 (HHDP C-6), 115.2, 115.8, 116.4 (HHDP C-2), 120.3 (galloyl C-1), 124.3, 124.7, 126.7, 126.8 (HHDP C-1), 135.1, 136.1, 136.9, 138.4 (HHDP C-4), 139.5 (galloyl C-4), 143.3, 144.0, 144.6, 145.3, 145.7, 146.0, 146.6 (galloyl C-3, 5 and HHDP C-3, 5), 166.3, 167.0, 169.0, 169.2, 169.4 (–COO–).

Enzymatic Hydrolysis of 4 with Tannase A solution of **4** (502 mg) in water (20 ml) was incubated with tannase at room temperature for 12 h. After concentration of the reaction mixture under reduced pressure, the product was separated by chromatography over Sephadex LH-20 with water–methanol to afford the hydrolysate (**4a**) (235 mg), ellagic acid (111 mg) and gallic acid (38 mg). **4a**: A pale brown amorphous powder, $[\alpha]_D^{27}$ +71.2° [c=0.4, acetone–H_2O (1 : 1)]. *Anal.* Calcd for $C_{25}H_{26}O_{18}\cdot 2H_2O$: C, 46.16; H, 4.65. Found: C, 46.28; H, 4.65. ^{1}H-NMR (100 MHz, acetone-d_6+D_2O): 3.4—4.3 (10H in total, H-1,4,5,6,2′,3′,4′,5′), 4.88 (1H, br s, H-3), 5.22 (1H, br s, H-2), 6.44 (1H, aromatic H). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 47.0 (C-1), 63.3 (C-5′), 63.8 (C-6), 67.0 (C-4′), 71.9 (C-5), 72.1, 72.4 (C-2′, 3′), 74.4 (C-4), 75.6 (C-3), 76.7 (C-2), 102.1 (C-1′), 105.1, 105.2 (HHDP C-6), 116.4 (HHDP C-2), 127.5 (HHDP C-1), 135.0, 138.3 (HHDP C-4), 143.6, 144.0, 145.7, 146.5 (HHDP C-3, 5), 167.0, 171.4 (–COO–).

Treatment of 4a with Hydrochloric Acid A solution of **4a** (207 mg) in 1 N aqueous hydrochloric acid (12 ml) was heated under reflux for 50 min. The reaction mixture was directly separated by Sephadex LH-20 chromatography with water–methanol to yield a furan derivative (**4b**) (5 mg) as a pale brown amorphous powder, $[\alpha]_D^{30}$ +109.5° (c=0.5, acetone–H_2O): *Anal.* Calcd for $C_{25}H_{20}O_{15}\cdot 9/2H_2O$: C, 46.81; H, 4.56. Found: C, 46.8; H, 4.09. Negative FAB-MS m/z: 559 $(M-H)^-$. ^{1}H-NMR (100 MHz, acetone-d_6): 3.32—3.92 (4H, in total, H-4, 5, 6), 4.94 (1H, d, J=1 Hz, H-1), 5.02 (1H, m, H-3), 5.30 (1H, br s, H-2), 6.51 (1H, s, aromatic H), 6.81 (1H, d, J=4 Hz, furan H-4), 7.71 (1H, d, J=4 Hz, furan H-3), 7.99 (1H, s, furan H-5).

Methylation of 4, Followed by Alkaline Methanolysis A mixture of **4** (100 mg), dimethyl sulfate (1 ml) and anhydrous potassium carbonate (1.2 g) in dry acetone (15 ml) was refluxed with stirring for 4 h. After removal of the inorganic precipitates by filtration, the filtrate was concentrated to dryness. The residue was treated with 2% methanolic sodium methoxide (25 ml) at room temperature for 8 h. The reaction mixture was neutralized with Amberlite IRA-120B (H^+ form), and the solution was concentrated to afford a residue, which was chromatographed on silica gel with benzene–acetone, giving methyl tri-O-methyl gallate (5 mg), an uncharacterized hydrolysate (6 mg) and dimethyl hexamethoxydiphenoate (6.5 mg) as colorless needles (MeOH), mp 80 °C, $[\alpha]_D^{22}$ −33.2° (c=0.7, MeOH).

Partial Hydrolysis of 4 with Tannase A stirred solution of **4** (60 mg) in water (20 ml) was treated with tannase at room temperature for 3 min. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was treated with ethanol. The ethanol-soluble portion was subjected to Sephadex LH-20 chromatography with ethanol to give gallic acid (5 mg) amd 5-desgalloyl pterocarinin A (**4c**) (42 mg) as a pale brown amorphous powder, $[\alpha]_D^{27}$ −90.7° (c=1.0, acetone). *Anal.* Calcd for $C_{39}H_{32}O_{26}.\ 5H_2O$: C, 46.52; H, 4.21. Found: C, 46.48; H, 3.94. ^{1}H-NMR (100 MHz, acetone-d_6+D_2O): 3.2—4.2 (7H in tatal, H-5,6,2′,3′,4′,5′), 3.57 (1H, s, H-1), 4.75 (1H, dd, J=3, 13 Hz, H-6), 4.94 (1H, br s, H-3), 5.24 (2H, m, H-2,4), 6.41, 6.59, 6.78 (each 1H, s, aromatic H). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 46.5 (C-1), 63.1 (C-5′), 66.8 (C-6), 68.3 (C-4′), 68.4 (C-5), 72.1 (C-2′, 3), 75.0 (C-4), 75.2 (C-3), 76.2 (C-2), 102.0 (C-1′), 105.1, 107.2, 108.8, 115.1 (HHDP C-6), 116.0, 116.4 (HHDP C-2), 124.5, 127.0 (HHDP C-1), 135.0, 135.8, 136.8, 138.5 (HHDP C-4), 143.4, 144.0, 144.4, 145.2, 146.7 (HHDP C-3, 5), 167.3, 169.1, 170.0, 170.2 (–COO–).

Preparation of 4 A mixture of **5** (100 mg) and L-ascorbic acid (30 mg) in 0.1 M acetic acid was heated on a water bath for 14 h. The reaction mixture was subjected to Sephadex LH-20 chromatography, and elution with water, and then with water–methanol (1 : 4) gave a crude product, which was further chromatographed on Bondapak C_{18} Porasil B with water–methanol to yield **4** (28 mg).

Pterocarinin B (6) A pale brown amorphous powder, $[\alpha]_D^{27}$ +115.7° (c=1.0, acetone+H_2O). *Anal.* Calcd for $C_{39}H_{32}O_{26}\cdot 5H_2O$: C, 46.53; H, 4.21. Found: C, 46.39; H, 3.93. Negative FAB-MS m/z: 915 $(M-H)^-$. ^{1}H-NMR (400 MHz, acetone-d_6+D_2O): 3.51 (1H, dd, J=2, 10 Hz, H-1), 3.64 (1H, d, J=13 Hz, H-5′), 3.71 (1H, d, J=13 Hz, H-5′), 3.91 (1H, d, J=13 Hz, H-6), 4.12 (1H, m, H-5), 5.14 (1H, br s, H-3′), 4.27 (1H, dd, J=2, 5 Hz, H-2′), 4.54 (1H, dd, J=5, 10 Hz, H-1′), 4.73 (1H, dd, J=3, 13 Hz, H-6), 4.98 (1H, br s, H-2), 5.06 (1H, t, J=3 Hz, H-3), 5.19 (1H, dd, J=3, 9 Hz, H-4), 6.43, 6.56, 6.77 (each 1H, s, aromatic H). ^{13}C-NMR (100 MHz, acetone-d_6+D_2O): 40.7 (C-1), 65.1 (C-5′), 68.3 (C-6), 68.7 (C-5), 75.1 (C-3), 76.8 (C-2,4), 77.1 (C-2′), 78.1 (C-3′), 81.4 (C-1′), 105.4 (C-4′), 105.6, 107.6, 109.1, 118.7 (HHDP C-6), 115.5, 116.4, 116.8, 117.2 (HHDP C-2), 122.7, 125.6, 127.5 (HHDP C-1), 135.4, 136.2, 137.1, 139.4 (HHDP C-4), 143.4, 144.3, 144.8, 145.5, 145.6, 146.1, 146.5 (HHDP C-3, 5), 166.9, 169.4, 170.1, 170.6 (–COO–).

Acknowledgements The authors would like to thank Associate Professors Y. Shoyama (Medicinal Plant Garden, Kyushu University), A.S.C. Wan (Department of Pharmacy, National University of Singapore, Singapore), T.-H.Hwang (Tajen Pharmaceutical College, Taiwan) and H. Feng (Nanjing Forestry University, China), and Professor T. Nohara (Faculty of Pharmaceutical Sciences, Kumamoto University) for providing plant materials. Thanks are also due to Associate Professor S. Saeki (Faculty of Pharmaceutical Sciences, Kyushu University) for valuable discussions, Dr. T. Tanaka (Sankyo Co., Ltd.) for the gift of tannase, Dr. K. Mihashi (Faculty of Pharmaceutical Sciences, Fukuoka University) for high-resolution ^{1}H- and ^{13}C-NMR measurements, Mr. Y. Tanaka and Miss Y. Soeda for ^{1}H- and ^{13}C-NMR measurements and Mr. R. Isobe for MS measurements.

References and Notes

1) Part LXXXIV: R. Saijo, G. Nonaka and I. Nishioka, *Chem. Pharm.*

Bull., **37**, 2063 (1989).

2) K. Ishimaru, M. Ishimatsu, G. Nonaka, K. Mihashi, Y. Iwase and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 3312 (1988).

3) G. Nonaka, H. Nishimura and I. Nishioka, *J. Chem. Soc. Perkin Trans. 1*, **1985**, 163; G. Nonaka, K. Ishimru, K. Mihashi, Y. Iwase, M. Ageta and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 857 (1988).

4) K. Ishimaru, M. Ishimatsu, G. Nonaka, K. Mihashi, Y. Iwase and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 3319 (1988).

5) G. Nonaka, K. Ishimaru, M. Watanabe, I. Nishioka, T. Yamauchi and A. S. C. Wan, *Chem. Pharm. Bull.*, **35**, 217 (1987).

6) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).

7) E. Breitmaier and W. Voelter, "^{13}C-NMR Spectroscopy, Monographs in Modern Chemistry," Verlag Chemie GmbH, D-6940, Weinheim, 1978.

8) As a typical example, we have recently isolated and structurally elucidated elaeocarpusin, which possesses an ascorbic acid moiety connected at the C-2 position through a carbon-to-carbon bond to a dehydrohexahydroxydiphenoyl ester group. T. Tanaka, G. Nonaka, I. Nishioka, K. Miyahara and T. Kawasaki, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 369.

9) T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 656 (1986).

10) HHDP = hexahydroxydiphenoyl.