Tannins and Related Compounds. XCVII.[1] Structure Revision of *C*-Glycosidic Ellagitannins, Castalagin, Vescalagin, Casuarinin and Stachyurin, and Related Hydrolyzable Tannins

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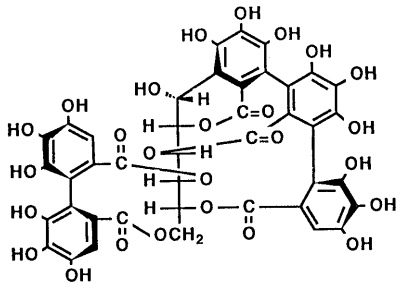
The structures of the *C*-glycosidic ellagitannins, castalagin, vescalagin, casuarinin and stachyurin, have been revised to 1—4 respectively, on the basis of two-dimensional nuclear Overhauser effect (NOE) spectroscopy and NOE difference spectroscopy. Furthermore, the structures of some related *C*-glycosidic tannins were re-examined by using similar techniques, and it was concluded that the configuration at the glucose C-1 position in all hitherto known *C*-glycosidic tannins must be revised.

Keywords structure revision; *C*-glycosidic ellagitannin; complex tannin; castalagin; vescalagin; casuarinin; stachyurin; nuclear Overhauser effect; tannin

Castalagin, vescalagin, casuarinin and stachyurin are representative *C*-glycosidic ellagitannins, which occur mostly as major phenolic metabolites in the plants of Fagaceae,[2] Myrtaceae,[3] Juglandaceae,[3b] Casuarinaceae,[4] Stachyuraceae,[4] Betulaceae,[5] Punicaceae,[6] Lythraceae,[3b] *etc.* The structures of castalagin and vescalagin were first elucidated by Mayer *et al.*[7] to have a novel triphenoyl (flavogallonyl) ester group attached through a carbon-to-carbon linkage to an open-chain form of the glucose moiety, and they were shown to be mutually epimeric at the glucose C-1 position (**1′** and **2′**, respectively). Later, casuarinin and stachyurin were characterized as structures **3′** and **4′**, respectively, having a similar relationship at the C-1 position, the configuration of the former corresponding to that of castalagin.[4] In the course of our studies on the conformation of *C*-glycosidic ellagitannins by employing ^{1}H-nuclear magnetic resonance (^{1}H-NMR) techniques, we have found that nuclear Overhauser enhancement and exchange spectroscopy (NOESY) of vescalagin and stachyurin showed, contrary to our expectation, a cross peak between the glycosyl H-1 and H-3 (Fig. 1), whereas in castalagin and casuarinin, this cross peak was not



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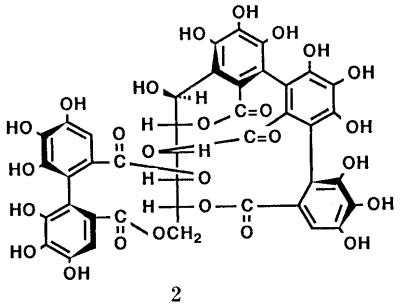

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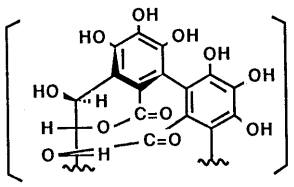
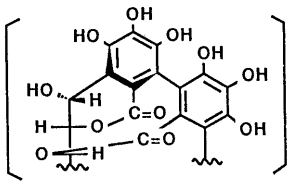
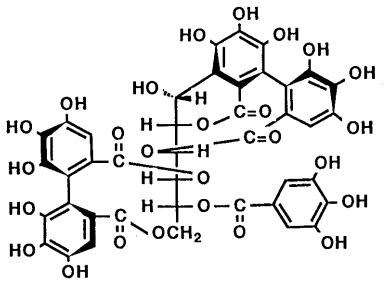


1′



2′



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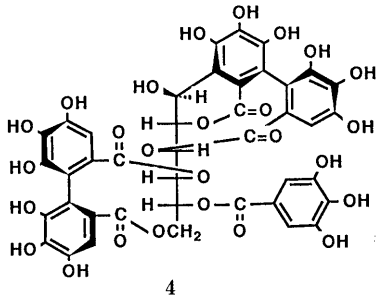

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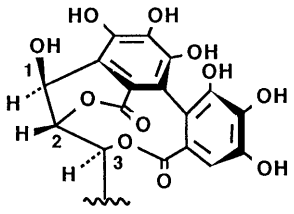
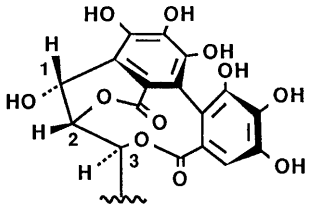


3′



4′

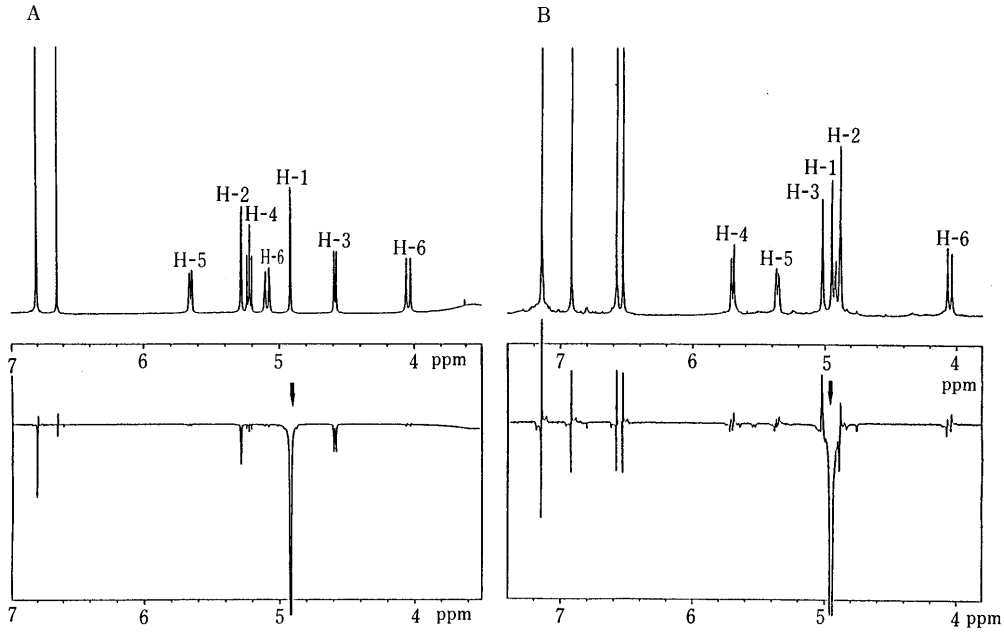


Fig. 1. NOE Difference Spectra (in Acetone-d_6 + D_2O, 400 MHz)
A, vescalagin (**2**); B, stachyurin (**4**).

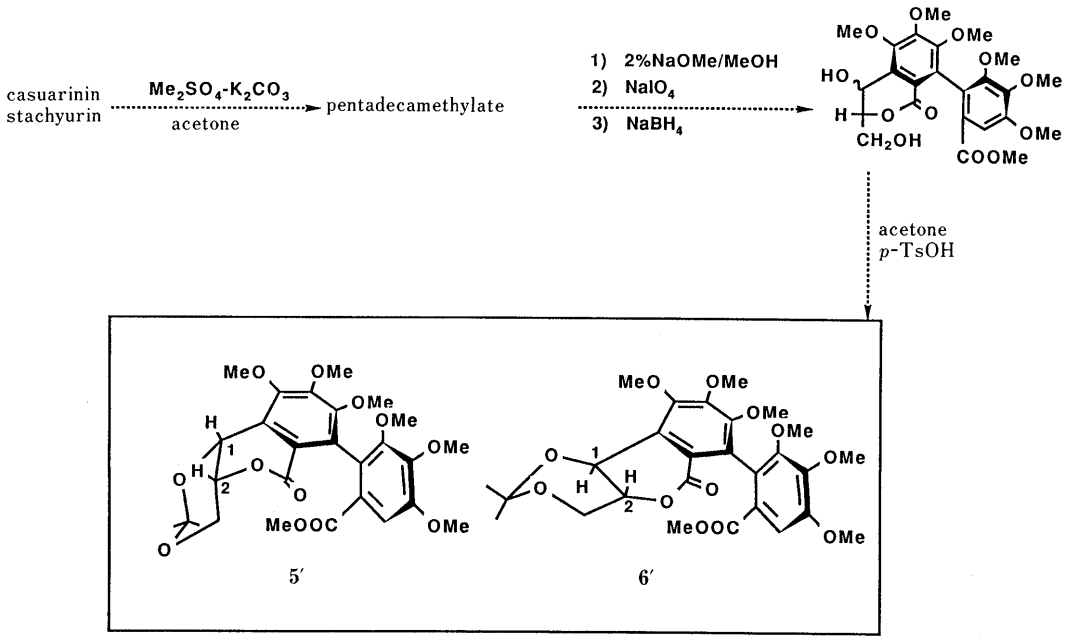


Chart 1

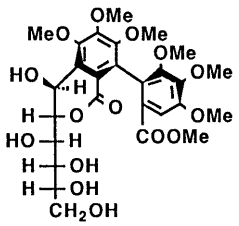
observed. These results were inconsistent with the proposed structures (**1′**—**4′**), thus prompting us to re-examine the structures.

The configuration at the C-1 position in these tannins had been determined on the basis of the coupling constant between H-1 and H-2.[4,7] Namely, the observation of a larger coupling constant (*ca.* 5 Hz) in castalagin and casuarinin than that (*ca.* 2 Hz) in vescalagin and stachyurin

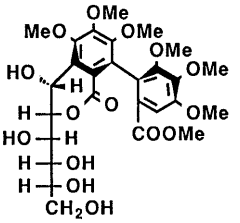
led to the conclusion that the former two possess a dihedral angle of less than 60°, while the latter two have a dihedral angle of more than 60°, in agreement with the proposed structures. Our re-examination of the Dreiding models, however, revealed that there is no marked difference in their dihedral angles and that the angle is, in all cases, *ca.* 60°.

In order to examine whether the cross peak in the above NOESY spectra are due to the actual nuclear Overhauser effect (NOE) or not, NOE difference spectra were measured. As shown in Fig. 2, when the H-1 signals (δ 4.92 in vescalagin and δ 4.96 in stachyurin) were irradiated, distinct NOE could be detected in the H-3 signals, although the sign of each NOE was opposite.

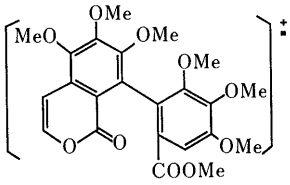
Next, to compare the coupling constants between the H-1 and H-2 signals in a more rigid conformation, we attempted to prepare the acetonides (**5′** and **6′**) from casuarinin and stachyurin (Chart 1). Alkaline methanolysis of the pentadecamethyl ether of casuarinin yielded the methanolysate (the structure **7′** was formerly proposed), whose physical



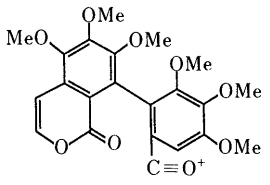
7′



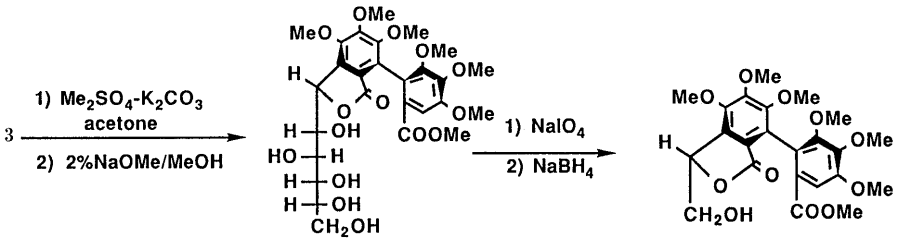
10′



m/z 460



m/z 429



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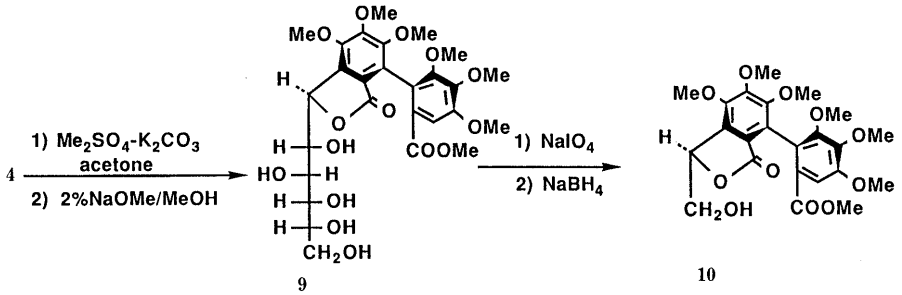
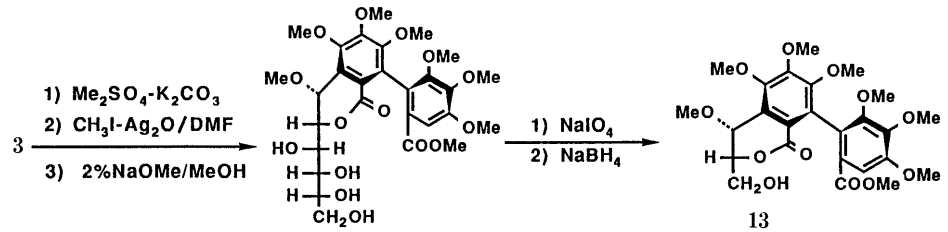


9 10

Chart 2



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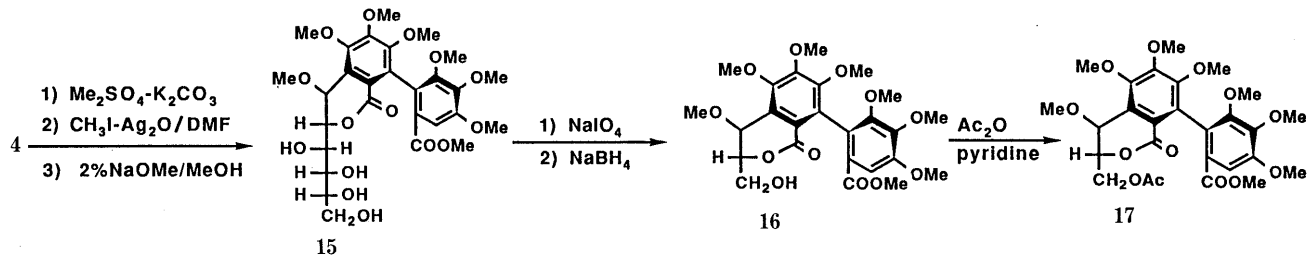


15 16 17

Chart 3

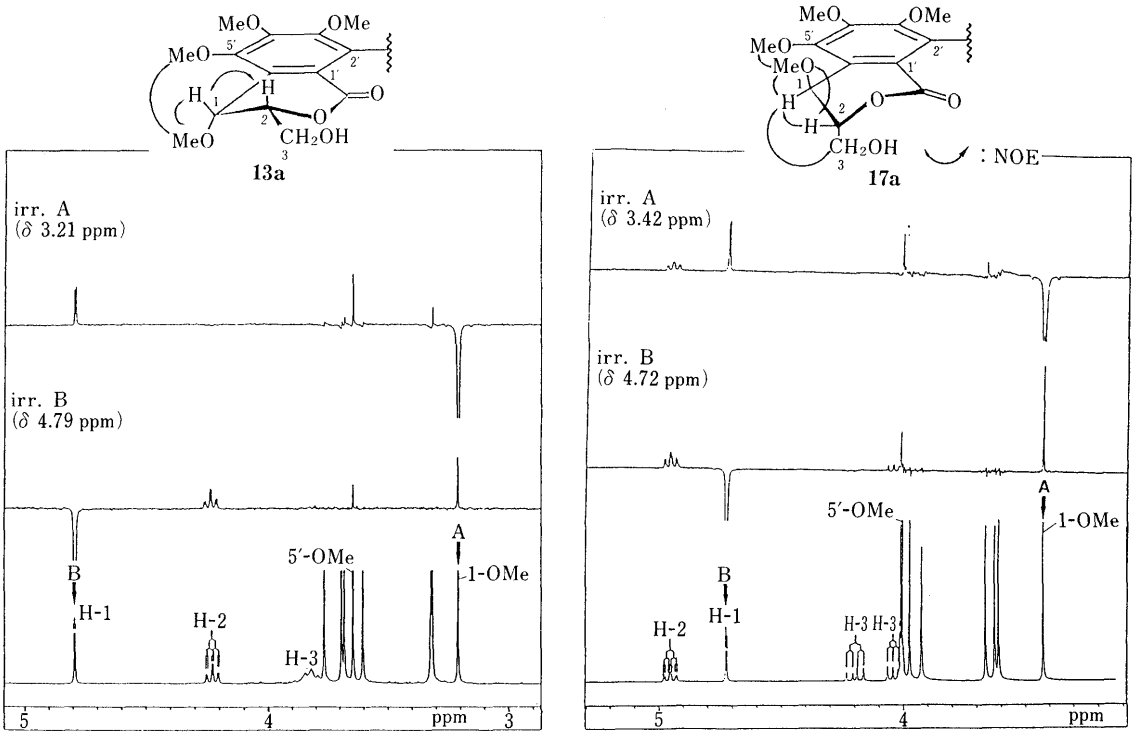


Fig. 2. NOE Difference Spectra of **13** and **17** (**13** in Benzene and **17** in $CDCl_3$, 270 MHz)

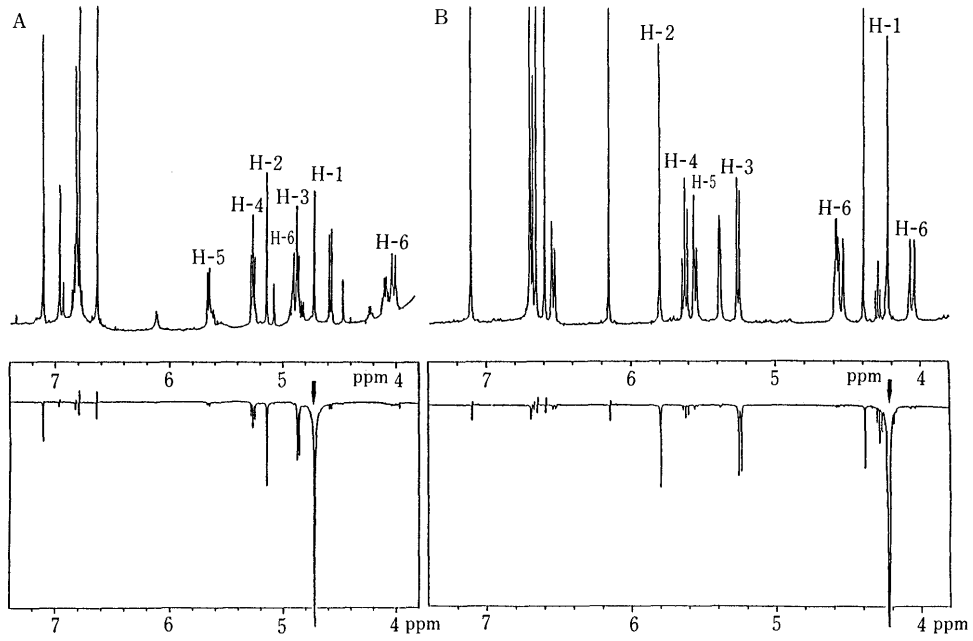
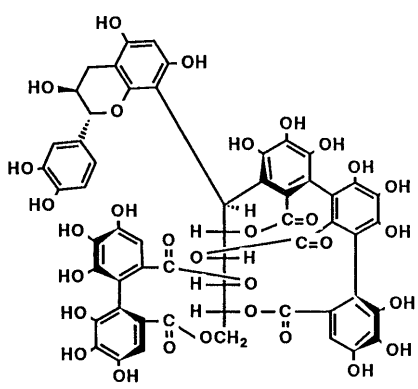


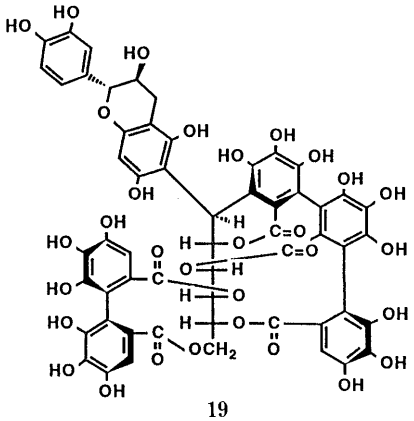
Fig. 3. NOE Difference Spectra (in Acetone-d_6 + D_2O, 400 MHz)
A, acutissimin B (**19**); B, mongolicain A (**20**).

and spectral data coincided well with those reported in the literature.[4] In the ^{1}H-NMR spectrum of the methanolysate, the change in the coupling constant (*ca.* 0 Hz) of the H-1 signal was notable, and this was reported to be attributable to the conformational change upon the cleavage of the ester bonds at the C-3, C-4, C-5 and C-6 positions.[4] However, the facts that on acetylation of the methanolysate, there was no change in the chemical shift (δ 5.74) of the H-1 signal and that periodate oxidation of the methanolysate, followed by sodium borohydride reduction, gave the degradation product (**8**)[8] clearly indicated that the structure **7'** for the methanolysate should be revised to **7**.

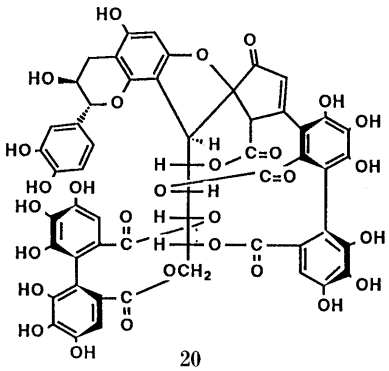
Such acyl migration also occurred in the case of stachyurin to give compound **9**, upon methanolysis in an alkaline medium. Several attempts to obtain the unmigrated product were unsuccessful. Thus, the C-1 hydroxyl group in the respective pentadecamethyl ethers was methylated by Kuhn's method, and the permethyl ethers (**11** and **14**) were subjected to periodate oxidation, followed by sodium borohydride reduction. The electron-impact mass spectra



18



19



20

(EI-MS) [*m/z*: 522 $(M)^+$, 460 (base peak), 429] of the degradation products thus obtained were consistent with the six-membered lactone structures (**13** and **16**). The NOE difference spectrum of the monoacetate (**17**) obtained from **16** clearly showed the NOE between the aliphatic methoxyl group (δ 3.42) and H-2 (δ 4.95), as well as the NOE between H-1 (δ 4.72) and H-3 (δ 4.04). On the other hand, such NOE signals were not observed in the degradation product (**13**) derived from casuarinin (Fig. 3). These findings were consistent with the stereostructures (**13a** and **17a**), and therefore the structures of casuarinin and stachyurin should be revised to **3** and **4**, respectively.

As mentioned above, since castalagin and vescalagin were shown to have the same relationship at the C-1 position as **3** and **4**, respectively, their structures are also revised to **1** and **2**.

So far, more than thirty structurally related *C*-glycosidic tannins including complex tannins have been isolated,[2b,d,e,3b] and their configuration at the C-1 position was exclusively determined by comparison of the H-1 coupling constant with those of the above tannins (**1**—**4**). Thus, we have selected several complex tannins, acutissimins A (**18**) and B (**19**)[9a] and mongolicain A (**20**)[9b] as appropriate model compounds, and measured their NOESY and NOE difference spectra. Some of the results are shown in Fig. 4, and in all cases, the NOE was clearly observed between the glycosyl H-1 and H-3 signals. Accordingly, the configuration at the C-1 position in acutissimins A and B and mongolicain A should be assigned as shown in the formulae **18**, **19** and **20**, respectively.

From the accumulated data mentioned above, we conclude that in the *C*-glycosidic hydrolyzable tannins, a small coupling constant (0—2 Hz) of the H-1 signal corresponds to the β-linkage of the substituent, whereas a larger coupling constant (*ca.* 5 Hz) indicates the α-linkage, and that all the proposed structures so far reported must therefore be revised.

Experimental

The following instruments were used to obtain physical and spectral data. Optical rotation, a JASCO DIP-4 digital polarimeter; ^{1}H-NMR, JEOL FX-100, JEOL GX-270 and JEOL GX-400 spectrometers; EI-MS, a JEOL DX-300/ JMS 3100 mass spectrometer; fast-atom bombardment mass spectrum (FAB-MS), JEOL DX-300/JMA 3500 and JEOL JMS-HX 100/JMA 3500 mass spectrometers.

Materials Castalagin (**1**) and vescalagin (**2**) used in this work were isolated from various Fagaceous and Myrtaceous species, *i.e.*, *Quercus stenophylla*,[2b] *Q. mongolica* var. *grosseserrata*[2d,e] *Castanea mollissima*,[2c] and *Eugenia grandis*,[3a] and their identities were confirmed by comparison of their physical and ^{1}H-NMR spectral data with those reported in the literature. Casuarinin (**3**) and stachyurin (**4**) were obtained from *Q. stenophylla*,[2b] *C. mollissima*[2c] and *Stachyurus praecox*,[4] and identified by physical and spectral comparisons.

Methylation of 3 A mixture of **3** (500 mg), dimethyl sulfate (3 ml) and anhydrous potassium carbonate (3 g) in dry acetone (40 ml) was heated under reflux for 2 h. After removal of the inorganic salts by filtration, the filtrate was subjected to silica gel chromatography. Elution with benzene–acetone (9 : 1) yielded the pentadecamethyl ether (275 mg) as a white amorphous powder, $[\alpha]_D^{22}$ −42.3° (c=0.6, $CHCl_3$). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.46—4.04 (OCH_3), 4.26 (1H, d, J=13 Hz, H-6), 4.95 (1H, dd, J=5, 2 Hz, H-2), 5.03 (1H, dd, J=13, 3 Hz, H-6), 5.35 (1H, t, J=2 Hz, H-3), 5.41 (1H, dd, J=9, 2 Hz, H-4), 5.50 (1H, d, J=5 Hz, H-1), 5.74 (1H, dd, J=9, 3 Hz, H-5), 6.56, 6.76, 7.13 (each 1H, s, aromatic H), 7.35 (2H, s, trimethoxybenzoyl H). A mixture of the pentadecamethyl ether (500 mg), methyl iodide (2 ml) and freshly prepared silver oxide (1 g) in dimethyl formamide (DMF) (2 ml) was stirred with ice-cooling for 1 h. After removal of the inorganics by filtration, the filtrate was concentrated under reduced pressure to a syrup, which was partitioned between ether and H_2O. The ether layer was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue was subjected to silica gel chromatography with benzene–acetone (8 : 1) to give the permethyl ether as a white amorphous powder (380 mg), $[\alpha]_D^{13}$ −35.8° (c=0.6, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{60}O_{26}\cdot H_2O$: C, 58.11; H, 5.31. Found: C, 58.23; H, 5.17. FAB-MS *m/z*: 1061 $(M+H)^+$. ^{1}H-NMR (100 MHz, $CDCl_3$): 3.41—4.02 (OCH_3), 4.24 (1H, d, J=13 Hz, H-6), 4.9—5.1 (3H, m, H-1, 2, 6), 5.40 (2H, m, H-3, 5), 5.68 (1H, dd, J=8, 2 Hz, H-4), 6.63, 6.74, 7.13 (each 1H, s, aromatic H), 7.36 (2H, s, trimethoxybenzoyl H).

Alkaline Methanolysis of Casuarinin Pentadecamethyl Ether A solution of the pentadecamethyl ether (200 mg) in 2% NaOMe–MeOH (4 ml) was heated at 70 °C for 4 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form), and chromatographed over silica gel. Elution with benzene containing increasing amounts of acetone yielded the methanolysate (**7**) (90 mg), together with trimethoxybenzoate (10 mg) and dimethyl (*S*)-hexamethoxydiphenoate (42 mg). **7**: a white amorphous powder, $[\alpha]_D^{22}$ −62.0° (c=0.9, $CHCl_3$). EI-MS *m/z* (%): 598 (M^+, 4), 447 (87), 416 (100). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.50—3.96 (7 × OCH_3), 5.65 (1H, br s, H-1), 7.39 (1H, s, aromatic H).

$NaIO_4$ Oxidation of 7, Followed by $NaBH_4$ Reduction A solution of **7** (50 mg) in MeOH–H_2O (5 : 1) (1.2 ml) was treated with $NaIO_4$ (25 mg) at room temperature for 30 min. The reaction mixture was diluted with H_2O (3 ml) and extracted with ethyl acetate. The ethyl acetate layer was washed with H_2O, dried over Na_2SO_4 and concentrated to a syrup, which was treated with $NaBH_4$ (10 mg) at room temperature for 30 min. The excess reagent was decomposed by addition of acetic acid, and the solvent was evaporated off. The residue was chromatographed over silica gel with benzene–acetone (3 : 1) to furnish the degradation product (**8**) as a white amorphous powder (15 mg), $[\alpha]_D^{13}$ −4.52° (c=0.6, $CHCl_3$). EI-MS *m/z* (%): 478 $(M)^+$, 447 (base peak). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.61—3.99 (7 × OCH_3), 3.87 (1H, dd, J=12, 5 Hz, H-2), 4.20 (1H, dd, J=12, 3 Hz, H-2), 5.51 (1H, dd, J=5, 3 Hz, H-1), 7.44 (1H, s, aromatic H).

Alkaline Methanolysis of Casuarinin Permethyl Ether A solution of the permethyl ether (200 mg) in 2% NaOMe–MeOH (5 ml) was heated at 70 °C for 6 h. Work-up as described above yielded methyl trimethoxybenzoate (11 mg), dimethyl (*S*)-hexamethoxydiphenoate (32 mg) and the methanoly-

sate (**12**) (63 mg) as a white amorphous powder, $[\alpha]_D^{13}$ −28.8° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{36}O_{15}\cdot 2H_2O$: C, 51.90; H, 6.22. Found: C, 52.05; H, 5.77. EI-MS m/z (%): 612 (M^+, 22), 460 (100), 429 (37). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.27—4.02 (OCH_3), 4.50 (2H, m, H-2, 3), 4.95 (1H, br s, H-1), 7.44 (1H, s, aromatic H).

$NaIO_4$ Oxidation of 12, Followed by $NaBH_4$ Reduction A mixture of **12** (50 mg) and $NaIO_4$ (25 mg) in MeOH–H_2O (5 : 1, 1.2 ml) was kept at room temperature for 30 min. The reaction mixture was diluted with H_2O (3 ml) and partitioned with ethyl acetate. The ethyl acetate layer was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue was treated with $NaBH_4$ (10 mg) in MeOH (2 ml) at room temperature for 30 min. The excess reagent was decomposed with acetic acid, and the solvent was evaporated off. The residue was chromatographed over silica gel with benzene–acetone (3 : 1) to afford the degradation product (**13**) as a white amorphous powder (15 mg), $[\alpha]_D^{13}$ −39.4° (c=0.7, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{30}O_{12}\cdot H_2O$: C, 55.60; H, 5.97. Found: C, 55.85; H, 5.95. EI-MS m/z (%): 522 (M^+, 84), 460 (100), 429 (22). ^{1}H-NMR (270 MHz, C_6D_6): 3.21—3.77 (8 × OCH_3), 4.23 (1H, dt, J=7, 2 Hz, H-2), 4.79 (1H, d, J=2 Hz, H-1), 7.65 (1H, s, aromatic H).

Methylation of 4 A mixture of **4** (500 mg), dimethyl sulfate (3 ml) and anhydrous potassium carbonate (3 g) in dry acetone (40 ml) was refluxed with stirring for 1.5 h. Work-up as described for **3** gave the pentadecamethyl ether as a white amorphous powder (275 mg), $[\alpha]_D^{21}$ −24.0° (c=1.0, $CHCl_3$). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.45—4.04 (OCH_3), 4.18 (1H, br d, J=13 Hz, H-6), 4.70—4.87 (2H, m, H-2, 6), 5.04 (2H, m, H-1, 3), 5.42 (1H, br d, J=9 Hz, H-5), 5.68 (1H, t, J=9 Hz, H-4), 6.60, 6.73, 7.14 (each 1H, s, aromatic H), 7.27 (2H, s, trimethoxybenzoyl H). Further methylation of the pentadecamethyl ether (500 mg) with methyl iodide (2 ml) and silver oxide (1 g) in DMF (2 ml) as described above afforded the permethyl ether as a white amorphous powder (380 mg), $[\alpha]_D^{13}$ −34.5° (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{60}O_{26}\cdot 2H_2O$: C, 57.24; H, 5.39. Found: C, 57.31; H, 5.25. ^{1}H-NMR (100 MHz, $CDCl_3$): 3.47—4.06 (OCH_3), 4.17 (1H, d, J=12 Hz, H-6), 4.30 (1H, d, J=2 Hz, H-1), 4.64 (1H, s, H-2), 4.95 (1H, dd, J=12, 2 Hz, H-6), 5.13 (1H, t-like, J=3 Hz, H-3), 5.36 (1H, dd, J=7, 3 Hz, H-5), 5.68 (1H, dd, J=7, 3 Hz, H-4), 6.60, 6.74, 7.13 (each 1H, s, aromatic H), 7.27 (2H, s, trimethoxybenzoyl).

Alkaline Methanolysis of Stachyurin Pentadecamethyl Ether A solution of the pentadecamethyl ether (150 mg) was methanolyzed with 2% NaOMe–MeOH (3 ml) at 70 °C for 4 h. Work-up as described above furnished the product (**15**) (80 mg), together with methyl trimethoxybenzoate (15 mg) and dimethyl (*S*)-hexamethoxydiphenoate (38 mg). **15**: a white amorphous powder, $[\alpha]_D^{13}$ +44.1° (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{36}O_{15}$: C, 54.95; H, 5.93. Found: C, 55.67; H, 6.08. EI-MS m/z (%): 612 (M^+, 34), 586 (8), 520 (11), 488 (22), 460 (100), 429 (52), 249 (18). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.41—4.00 (8 × OCH_3), 4.90 (2H, m, H-1, 2), 7.34 (1H, s, aromatic H).

$NaIO_4$ Oxidation of 15, Followed by $NaBH_4$ Reduction A solution of **15** (50 mg) was treated with $NaIO_4$ (25 mg) in MeOH–H_2O (5 : 1) (1.2 ml) at room temperature for 30 min. Work-up as described above afforded the oxidation product, which was reduced with $NaBH_4$ (10 mg) in MeOH (2 ml) at room temperature for 30 min. The reaction product was separated as above to afford **16** as a white amorphous powder (10 mg), $[\alpha]_D^{13}$ +56.2° (c=0.2, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{30}O_{12}$: C, 57.52; H, 5.79. Found: C, 58.05; H, 5.90. EI-MS m/z (%): 522 (M^+, 70), 460 (100), 429 (32). ^{1}H-NMR (100 MHz, $CDCl_3$): 3.42—4.00 (8 × OCH_3), 4.80 (1H, d, J=1.5 Hz, H-1), 4.83 (1H, m, H-2), 7.34 (1H, s, aromatic H).

Acetylation of 16 A solution of **16** (10 mg) in acetic anhydride (0.5 ml) and pyridine (0.5 ml) was kept standing at room temperature for 3 h. Work-up as usual gave the monoacetate (**17**) as a white amorphous powder (7 mg), $[\alpha]_D^{13}$ +46.3° (c=0.6, $CHCl_3$). EI-MS m/z (%): 564 (M^+, 100), 447 (21), 429 (6). ^{1}H-NMR (270 MHz, $CDCl_3$): 2.07 (3H, s, $OCOCH_3$), 3.42—4.01 (8 × OCH_3), 4.04 (1H, t, J=6 Hz, H-3), 4.19 (1H, dd, J=12, 7 Hz, H-3), 4.72 (1H, d, J=1 Hz, H-1), 4.95 (1H, ddd, J=12, 6 Hz, H-2), 7.37 (1H, s, aromatic H).

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