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^{13}C Nuclear Magnetic Resonance Spectra of Hydrolyzable Tannins. III.[1] Tannins Having $^1C_4$ Glucose and C-Glucosidic Linkage

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Two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy was utilized for the assignments of the glucose carbon signals in the ^{13}C-NMR spectra of hydrolyzable tannins in which the glucopyranose core takes a $^1C_4$ or related boat conformation, and of tannins possessing a C-glucosidic linkage. Remarkable changes in the sequences of glucose carbons were observed with change in the conformation of the glucose core, and with the formation of a C-glucosidic linkage. The chemical shifts of the C-2 signals of the glucose cores adopting the open-chain form in C-glucosidic tannins and in complex tannins can be utilized for the discrimination of the configurations at C-1 in these tannins.

Keywords——tannin; ^{13}C-NMR; 2D NMR; conformation; ellagitannin; C-glucosidic tannin; complex tannin; geraniin; casuarinin; guavin A

In the previous papers,[1,2] we reported the assignments of the glucose carbon signals in the ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrum of tannins possessing $^4C_1$ glucose. They include tannins having acylated anomeric centers[2] and those existing as anomer mixtures.[1] We also reported application of the spectral data of monomeric tannins to the structural analyses of dimeric tannins.[1,2] This paper deals with the ^{13}C signals of tannins in which the glucose core adopts a $^1C_4$ or related boat conformation, and of tannins having a C-glucosidic linkage.

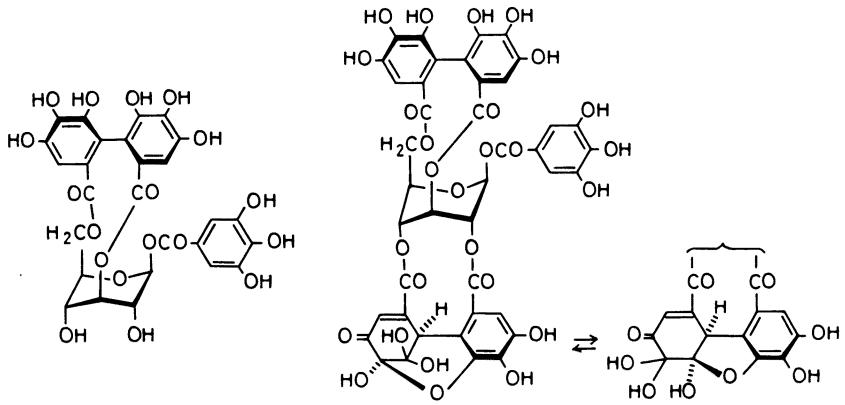
Results and Discussion

Hydrolyzable Tannins in Which the Glucose Core Adopts a $^1C_4$ or Boat Conformation

Among the tannins having a $^4C_1$ glucose core, galloylglucoses are metabolized into tannins having hexahydroxydiphenoyl (HHDP) groups (*viz.* ellagitannins) through oxidative coupling of the galloyl groups.[3,4] The oxidative coupling which occurs between the galloyl groups located at O-3 and O-6, and/or O-2 and O-4, causes the conformational change of the glucopyranose core from $^4C_1$ to $^1C_4$ or a related boat conformation.[4] This conformational change accompanied with the change in the orientation of the C–O bonding of hydroxyl (or ester) groups should affect the chemical shifts of the glucose carbon signals. However, no systematic study on the ^{13}C-NMR spectra of tannins having $^1C_4$ glucose has yet been presented except for the assignments of the ^{13}C signals of corilagin (**1**).[5]

The conformations of the glucose core in some of these tannins are different from each other, depending on the solvent.[6] Therefore, the conformation of the glucose cores of these tannins in each solvent should be determined in advance of assignment of the ^{13}C signals.

Geraniin (**2**),[7] widely distributed in plants,[8-11] possesses an HHDP group at C-3 and C-6 of the glucose core and a dehydrohexahydroxydiphenoyl (DHHDP) group at C-2 and C-4,



1 2a 2b

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Chart 1

TABLE I. ^{1}H-NMR Spectral Data for Glucose Residue Adopting $^1C_4$ Conformation in Hydrolyzable Tannins

	Corilagin (**1**)	Geraniin (**2**)		Chebulagic acid (**3**)
		2a form	**2b** form	
H-1	6.39 (br s)	6.60 (br s)	6.60 (br s)	6.56 (br s)
H-2	4.09 (br s)	5.60 (br s)	5.60 (br s)	5.54 (br s)
H-3	4.86 (br s)	5.50 (br s)	5.60 (br s)	5.96 (br s)
H-4	4.48 (br s)	5.56 (br s)	5.46 (br s)	5.26 (br s)
H-5	4.55 (dd, J=8, 11 Hz)	4.81 (m)	4.81 (m)	4.85 (dd, J=9, 10 Hz)
H-6	4.97 (t, J=11 Hz)	4.93 (t, J=11 Hz)	4.78 (m)	4.82 (t, J=10 Hz)
H-6	4.13 (dd, J=8, 11 Hz)	4.33 (dd, J=8, 11 Hz)	4.45 (dd, J=6, 9 Hz)	4.44 (dd, J=9, 10 Hz)

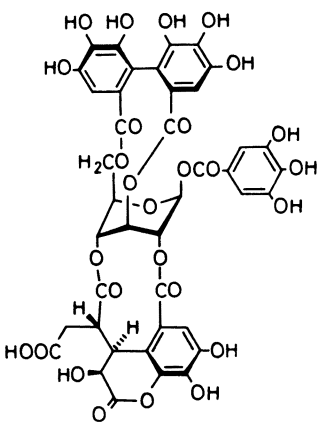
and exists as an equilibrium mixture of **2a** and **2b**, forming two hydrated hemiacetal structures of the DHHDP group. The ^{1}H–^{1}H correlation spectrum of geraniin exhibits cross peaks due to long-range couplings, along with those for vicinal and geminal couplings. Although the cross peaks due to the long-range couplings caused difficulty in the assignments of the proton signals, the nuclear Overhauser enhancement and exchange spectrosocpy (NOESY) allowed straightforward assignments of each proton, because of the absence of the cross peaks for the long-range couplings in the NOESY spectrum. The assignments of ^{1}H signals thus established are listed in Table I, which show some differences from the previously reported assignments.[12]

The glucopyranose core in geraniin (**2**) has been proposed to adopt the $^1C_4$ conformation, based on the small vicinal couplings of the glucose protons H-1—H-4, and the long-range couplings between H-1 and H-3, and between H-3 and H-5, due to the W-arrangements of these protons. The glucose carbon signals in the ^{13}C-NMR spectrum of geraniin were then assigned as shown in Table II on the basis of the ^{1}H–^{13}C correlation spectrum.

Corilagin (**1**),[5,6] which possesses an HHDP group at C-3 and C-6 of glucose, also shows broad singlets due to the glucose protons H-1—H-4 in the ^{1}H-NMR spectrum, and the cross peaks of the long-range couplings due to the W-arrangements (H-1 and H-3; H-2 and H-4; H-3 and H-5) in the ^{1}H–^{1}H correlation spectrum. Thus, the $^1C_4$ conformation of the glucopyranose core was also confirmed for corilagin. The reported assignments[5] of the ^{13}C signals of the glucose carbons were then verified by examination of the ^{1}H–^{13}C correlation

TABLE II. ^{13}C-NMR Spectral Data for Glucose Residue Adopting $^1C_4$ or a Related Boat Conformation in Hydrolyzable Tannins and Related Compounds

	C-1	C-2	C-3	C-4	C-5	C-6
Corilagin (**1**)	94.2	68.8	70.4	62.2	75.5	64.3
Geraniin (**2**)						
2a form	90.8	69.9	63.3	65.9	72.6	63.6
2b form	91.8	70.4	62.3	66.8	73.1	63.8
Chebulagic acid (**3**)	91.5	70.5	61.7	66.1	73.5	63.9
1-*O*-Galloyl-2,4;3,6-bis-*O*-hexahydroxydiphenoyl-*β*-D-glucose (**4**)	91.9	76.0	67.7	67.7	76.9	65.3
Geraniin-phenazine B (**5**)	91.6	76.6	68.7	67.6	76.8	65.2



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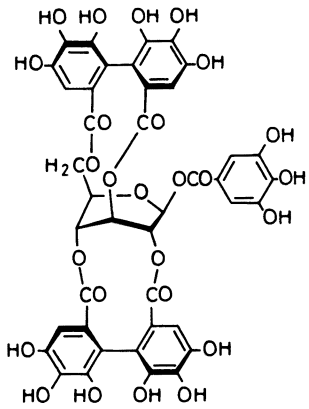

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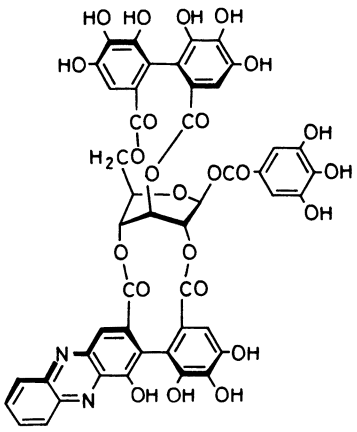

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Chart 2

spectrum (Table II).

Chebulagic acid (**3**)[13] possesses diester bridges involving a chebuloyl group at C-2 and C-4, and an HHDP group at C-3 and C-6. The $^1C_4$ conformation of the glucose core has been established in a way analogous to that for corilagin, and the ^{1}H and ^{13}C signals of chebulagic acid have been assigned as in Tables I and II.

The effects of substitution of the acyl groups at O-2 and O-4 on the $^1C_4$ glucose core (Table III) can be calculated from the data in Table II. These tables show significant downfield shifts of the C-4 signals of geraniin (**2**) and chebulagic acid (**3**), relative to the corresponding signal of corilagin (**1**), in contrast with rather small changes[2] in the chemical shifts of *α*-carbons occurring upon acylations on the $^4C_1$ glucose cores. The upfield shifts of the *β*-carbons (C-3) in **2** and **3** are also larger than the shifts of the *β*-carbons (C-5)[2] observed upon the substitutions at C-4 and C-6 of the $^4C_1$ glucose. These shifts affect the sequences of the glucose carbons (Table IV).

Although the glucose carbons of geraniin and chebulagic acid show analogous chemical shifts in spite of the difference in their diester bridges (DHHDP and chebuloyl) at C-2 and C-4, the compound having an HHDP bridge at C-2 and C-4 exhibits conspicuous differences in the chemical shifts from the above two compounds, due to the conformational change of the glucose core. Thus 1-*O*-galloyl-2,4;3,6-bis-*O*-(*R*)-hexahydroxydiphenoyl-*β*-D-glucose (**4**), which was prepared by catalytic reduction of geraniin, has a skew-boat conformation of glucose, as indicated by the coupling constants in the ^{1}H-NMR spectrum.[12] This spectrum

TABLE III. Effects of Acylation on the Chemical Shifts of Glucose Carbons of Hydrolyzable Tannins and Related Compounds in Which the Glucose Core Adopts $^1C_4$ Conformation

	C-1	C-2	C-3	C-4	C-5	C-6
1→2a	−3.4	+1.1	−7.1	+3.7	−2.9	−0.7
1→2b	−2.4	+1.6	−8.1	+4.6	−2.4	−0.5
1→3	−2.7	+1.7	−8.7	+3.9	−2.0	−0.4

TABLE IV. Sequence of the ^{13}C Signals of the Glucose Residue Adopting $^1C_4$ or a Related Boat Conformation in Hydrolyzable Tannins and Derivatives

	Sequences (lower field ↔ higher field)
1	C-1 ···· C-5 ···· C-3 ···· C-2 ···· C-6 ···· C-4
2a	C-1 ···· C-5 ···· C-2 ···· C-4 ···· C-6 ···· C-3
2b	C-1 ···· C-5 ···· C-2 ···· C-4 ···· C-6 ···· C-3
3	C-1 ···· C-5 ···· C-2 ···· C-4 ···· C-6 ···· C-3
4	C-1 ···· C-5 ···· C-2 ····· C-3, C-4 ····· C-6
5	C-1 ···· C-5 ···· C-2 ···· C-3 ···· C-4 ···· C-6

also shows a difference in the sequence of the ^{13}C signals of glucose (Table II) from that of geraniin (and that of chebulagic acid) (Table IV).

The ^1H-NMR data for the phenazine derivative (**5**) of geraniin[7] also suggest the skew-boat conformation[12] of glucose. The chemical shifts of the ^{13}C signals of glucose carbons in **5** are almost the same as those of **4** except for the small downfield shift of the C-3 signal (Table II).

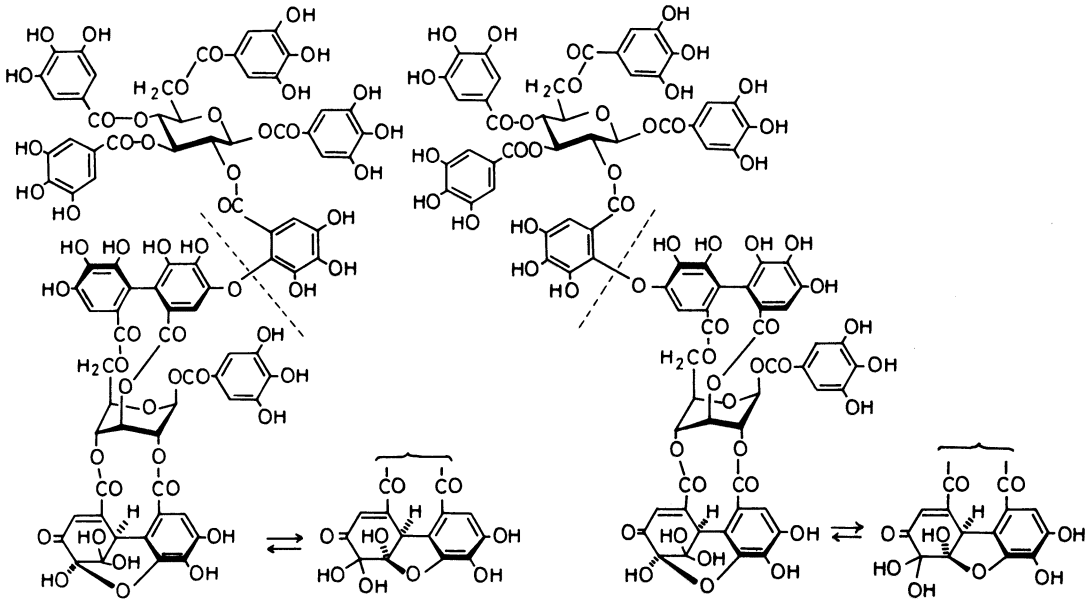
Two dimeric tannins composed of geraniin and a monomeric tannin having $^4C_1$ glucose [*i.e.*, euphorbin A (**6**) and euphorbin B (**7**)], have been isolated very recently.[14] Although the ^1H- and ^{13}C-NMR spectra of these tannins are complicated by the quilibration[7] between the six-membered hemiacetal form and the five-membered hemiacetal form of the geraniin moiety, the spectra of these tannins were simplified after the formation of the phenazine derivatives. Comparison of the simplified ^{13}C-NMR spectra of the phenazine derivatives of the dimeric tannins with the ^{13}C-NMR data of the glucose carbons of the phenazine derivative of geraniin helped in the structure elucidation of **6** and **7**.[14]

Tannins Having a C-Glucosidic Linkage

In some species of plants, hydrolyzable tannins having $^4C_1$ glucose cores are metabolized into C-glucosidic tannins [*e.g.*, casuarinin (**8**)[15] and stachyurin (**9**)[15]] *via* pedunculagin (**10**), which is a key intermediate,[3] and some C-glucosidic tannins are further metabolized into complex tannins [*e.g.*, guavin A (**11**)[16]] by condensation with catechins. The glucose carbon signals of tannins having a C-glucosidic linkage in the ^{13}C-NMR spectra have also been completely assigned as follows.

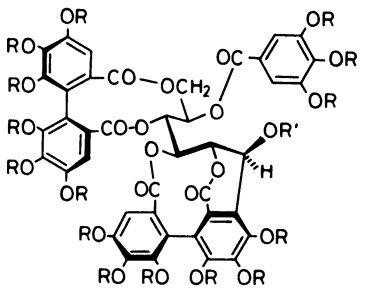
Previously reported assignments[15] of the ^{13}C signals of a C-glucosidic tannin, casuarinin (**8**), have been re-examined with the aid of two-dimensional (2D) NMR spectroscopy. Although the previous assignments for the glucose carbon signals of **8** were based on the acylation shifts observed upon addition of trichloroacetylisocyanate (TAI) reagent,[17] the measurement of the ^1H–^{13}C correlation spectrum led to revisions of the assignments as shown in Table V.

The value of the downfield shift of the α-carbon and the upfield shift of the β-carbon resulting from trichloroacetylcarbamoylation at C-1 of the pentadeca-*O*-methyl derivative of



6 7

Chart 3



8: R = R' = H

12: R = Me, R' = H

13: R = Me, R' = -CONHCOCCl$_3$

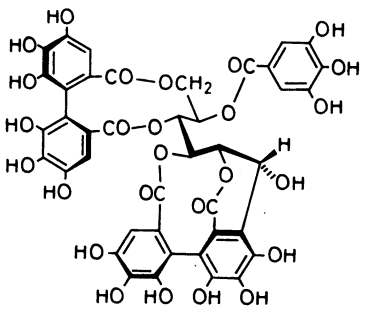


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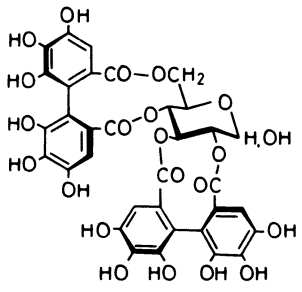
Chart 4



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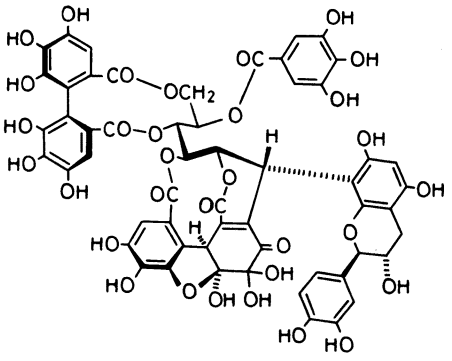

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Chart 5

TABLE V. ^{13}C-NMR Spectral Data for Glucose Residue in C-Glucosidic Tannins and Complex Tannins

	C-1	C-2	C-3	C-4	C-5	C-6
Casuarinin (**8**)[a]	67.6	76.7	69.8	74.2	71.2	64.6
Stachyurin (**9**)	65.5	81.0	70.9	73.3	72.0	64.5
Casuariin (**14**)	68.5	77.1	70.8	77.1	68.5	67.2
Stenophyllanin A (**15**)	38.3	81.4	69.8	74.2	73.1	64.8
Guavin A (**11**)[a]	46.5	81.2	75.8	71.2	71.7	64.4

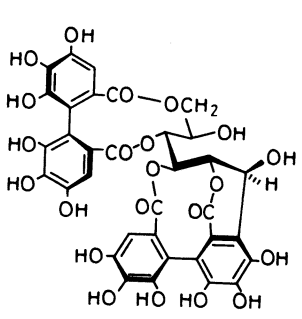
a) Assignments are based on the ^{1}H–^{13}C correlation spectra.

TABLE VI. Effects of Trichloroacetylcarbamoylation on the Chemical Shifts of the Glucose Carbons of Pentadeca-*O*-methylcasuarinin

	C-1	C-2	C-3	C-4	C-5	C-6
Pentadeca-*O*-methylcasuarinin (**12**)	67.0	76.3	69.9	73.6	71.4	64.7
Pentadeca-*O*-methyl-(1-*O*-trichloroacetylcarbamoyl)casuarinin (**13**)	68.5	74.7	70.5	73.7	71.0	64.7
Difference (**12**→**13**)	+1.5	−1.6	+0.6	+0.1	−0.4	0

TABLE VII. Sequence of the ^{13}C Signals of the Glucose Residue in Hydrolyzable Tannins Having a C-Glucosidic Linkage

	Sequence (lower field ↔ higher field)
8	C-2 ···· C-4 ···· C-5 ···· C-3 ···· C-1 ···· C-6
9	C-2 ···· C-4 ···· C-5 ···· C-3 ···· C-1 ···· C-6
14	C-2, C-4 ····· C-3 ···· C-5, C-1 ····· C-6
15	C-2 ···· C-4 ···· C-5 ···· C-3 ···· C-6 ···· C-1
11	C-2 ···· C-3 ···· C-5 ···· C-4 ···· C-6 ···· C-1



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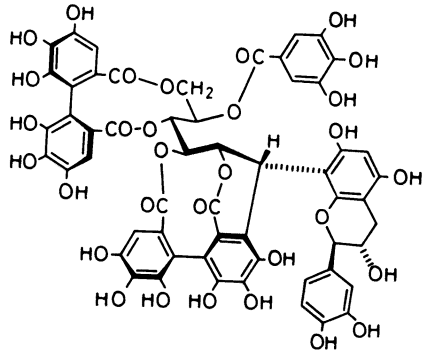

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Chart 6

8 (**12**→**13**),[15] were revealed to be smaller (Table VI) than the generally accepted values.[17] Based on the revised assignments for **8**, the ^{13}C signals of related C-glucosidic tannins, stachyurin (**9**, C-1 epimer of casuarinin) and casuariin (**14**), were also assigned as shown in Table V. The sequences of the glucose carbons in these tannins, which are different from those

of the other types of hydrolyzable tannins[1,2] (including tannins which possess a $^1C_4$ glucose core), and illustrated in Table VII.

The ^{13}C-NMR spectrum of stachyurin (**9**) thus assigned shows a significant downfield shift of the C-2 signal (+4.3 ppm) and upfield shift of the C-1 signal (−2.1 ppm), relative to the signals of casuarinin (**8**), while changes in the chemical shifts of the other glucose carbon signals are within ± 1.1 ppm. The C-2 signals of C-glucosidic tannins appear at the lowest field among the glucose carbon signals, and therefore the characteristic difference of the chemical shifts of C-2 signals described above can be utilized in differentiating the configuration at C-1 of the glucose core in the C-glucosidic tannins.

The ^{13}C signals of glucose carbons of stenophyllanin A (**15**),[18] which is composed of stachyurin (**9**) and (+)-catechin, have not been assigned except for the signals of C-1 and C-6. These signals have now been readily assigned as shown in Table V, by comparisons of their chemical shifts with those of corresponding signals of stachyurin (**9**).

The ^{13}C chemical shift of the C-2 signal of the glucose core in **15** (δ 81.4) is almost the same as that of the corresponding signal in **9** (δ 81.0), and is distinctively lower from those in the case of casuarinin (δ 76.7) and casuariin (δ 77.1). The small coupling constant between H-1 and H-2 of the glucose core in **15**[18] indicates that the orientation of the bond between C-1 of the glucose core and C-8 of the (+)-catechin moiety is the same as that of the C–O bond at C-1 of glucose in **9**. Therefore the chemical shifts of C-2 signals should be also useful for differentiation of the configuration at C-1 in these complex tannins, and has been applied to guavin A (**11**) as follows.

Guavin A (**11**)[16] showed the C-2 signal at δ 81.2, which is similar to that of stachyurin (**9**) (Table V), and therefore the configuration at C-1 of guavin A has been assigned as in structure **11**. This assignment coincides with that deduced from the coupling constant between H-1 and H-2 in the ^{1}H-NMR spectrum.[16]

Experimental

Instrument——NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in acetone-d_6 or acetone-d_6 + D_2O at ambient temperature, using tetramethylsilane as an internal standard. Chemical shifts are given in δ values (ppm).

Materials——1-*O*-Galloyl-2,4;3,6-bis-*O*-(*R*)-hexahydroxydiphenoyl-β-D-glucose (**4**) was obtained by catalytic reduction of **2**,[12] and casuarinin (**8**) was isolated from the leaves of *Lagerstroemia indica* L. (see below). All the other tannins and derivatives measured in the present study were obtained by the methods described in the previous papers.[7,13,15,16,19]

Casuarinin (8) from *Lagerstroemia indida*——Fresh leaves of *L. indica* (33 g) were homogenized in a mixture of acetone and water (7 : 3, v/v), and the homogenate was filtered. The filtrate was concentrated and extracted with ether, ethyl acetate and *n*-butanol, successively. The *n*-butanol extract afforded castalagin.[20] The ethyl acetate extract (0.3 g) was subjected to column chromatography over Sephadex LH-20 (1.1 i.d. × 44 cm) and developed with 70% EtOH. The eluate was collected 400-drop portions. Casuarinin (10 mg) was isolated from the combined fractions 60—84 by rechromatography over Sephadex LH-20, and identified by comparison with an authentic sample.

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