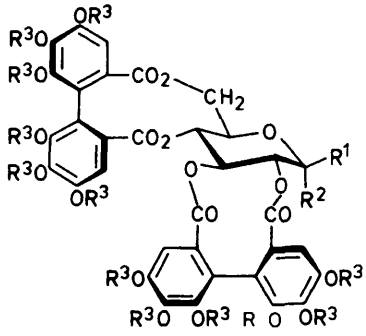
Tannins of *Casuarina* and *Stachyurus* Species. Part 1. Structures of Pedunculagin, Casuarictin, Strictinin, Casuarinin, Casuariin, and Stachyurin

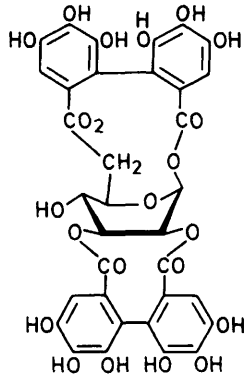
Takuo Okuda,* Takashi Yoshida, Mariko Ashida, and Kazufumi Yazaki
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

Three novel C-glucosidic ellagitannins, casuarinin, casauriin, and stachyurin, and two additional new ellagitannins, casuarictin and strictinin, were isolated from the leaves of *Casuarina stricta*, and their structures elucidated on the basis of chemical and spectral evidence. Pedunculagin and tellimagrandin I were also isolated, and the structure having (*S*)-4,4',5,5',6,6'-hexahydroxydiphenoyl groups was established for pedunculagin. These seven ellagitannins isolated from *C. stricta* have been found also in *Stachyurus praecox*.

Plants of the *Casuarina* species (Casuarinaceae), which are evergreen trees common in the Pacific islands, are regarded as rich tannin resources and some of them have been used as astringents and antidiarrhoeics in southeast asia.[1] Isolation of hinokiflavone from *C. stricta* Ait.,[2] ellagic acid from *C. equisetifolia* L., *C. junghuniana* Miq., *C. cunninghamiana* Miq., and *C. rigida* Miq.,[3] and of (+)-catechin and (+)-gallocatechin from *C. equisetifolia* L.[4,5] were reported. Kaempferol, quercetin, and their glycosides, in addition to the above four compounds, also occur in this genus as shown by chromatographic analysis, and this is of chemotaxonomic interest.[6,7] However, little is known about the tannins of these plants. We have isolated, in addition to the known compounds pedunculagin (1)[8,9] and tellimagrandin I (6),[10,11]† five new tannins from the leaves of *C. stricta* Ait., and have named them casuarictin (8), strictinin (9), casuarinin (10), casuariin (17), and stachyurin (20). We have also isolated these tannins, except for (17), from the leaves of *Stachyurus praecox* Sieb. et Zucc. (Stachyuraceae) whose fruits are known to be rich in tannins, and was formerly used in Japan as a substitute for Chinese gall. The existence of causariin (17) in the leaves of *S. praecox* was also shown by h.p.l.c. (normal and reversed phases). This paper deals with the structure elucidation of these new tannins,[12] and also with the establishment[12] of structure (1) for pedunculagin, for which two structures have previously been proposed.[8,9]



(1) R^1, R^2 = H, OH, R^3 = H
(2) R^1 = H, R^2 = OAc, R^3 = Ac
(3) R^1 = H, R^2 = OMe, R^3 = Me
(4) R^1 = OMe, R^2 = H, R^3 = Me



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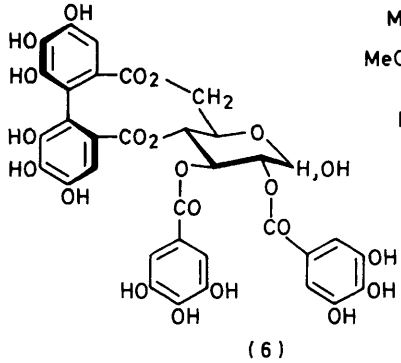

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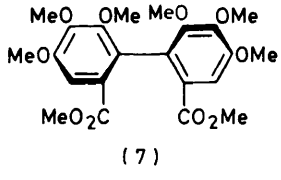


(7)

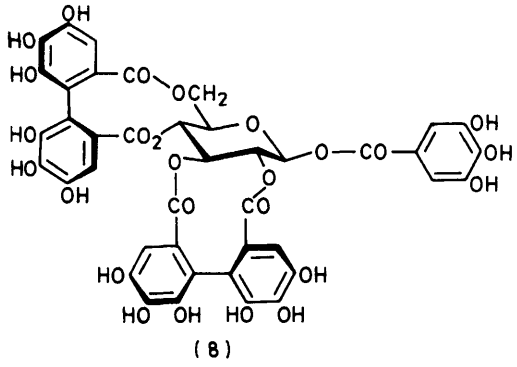
Results and Discussion

The ethyl acetate soluble portion of the leaf extract of *C. stricta* was fractionated by droplet counter-current chromatography followed by column chromatography on Sephadex LH-20 and cellulose, to give pedunculagin (1), tellimagrandin I (6), casuarictin (8), strictinin (9), and casuarinin (10). Casuariin (17) and stachyurin (20) were isolated from the aqueous layer after the extraction with ethyl acetate. Isolation of these tannins from *S. praecox* was achieved essentially in the same way as from *C. stricta*. All of these tannins gave the characteristic colour of ellagitannins with the $NaNO_2$–AcOH reagent.[13]

Pedunculagin (1) was isolated as an off-white, amorphous powder, $C_{34}H_{24}O_{22}\cdot 5H_2O$, $[\alpha]_D$ +100°, and was identified by direct comparison with an authentic sample. The identity was further confirmed by production of trideca-*O*-acetyl-α-pedunculagin (2),[8] m.p. 240—241 °C. This tannin was initially isolated from the gall of *Quercus pedunculata* Ehrh., and structure (1) was proposed by Schmidt *et al.*[8] without assigning the absolute configuration of the hexahydroxydiphenoyl (HHDP) groups. An alternative structure (5) was later postulated by Seikel and Hillis,[9] based on a ^{1}H n.m.r. analysis, and also on the poor reactivity of the aliphatic hydroxy group of pedunculagin upon attempted methylation with diazomethane.

The presence of a free anomeric hydroxy group in pedunculagin has now been demonstrated as follows. The 200-MHz ^{1}H n.m.r. spectrum exhibited two doublets, attributable to anomeric protons, at δ 5.22 (*J* 9 Hz) and 5.48 (*J* 3.5 Hz), although the other glucose-proton signals were complicated.

† The trivial names, tellimagrandin I and II for 2,3-di-*O*-galloyl-4,6-(*O*-4,4',5,5',6,6'-hexahydroxydiphenoyl)-D-glucose and 1,2,3-tri-*O*-galloyl-4,6-*O*-(4,4',5,5',6,6'-hexahydroxydiphenoyl)-β-D-glucose, respectively, have been conferred by Professor B. A. Bohm in a personal communication (1981).



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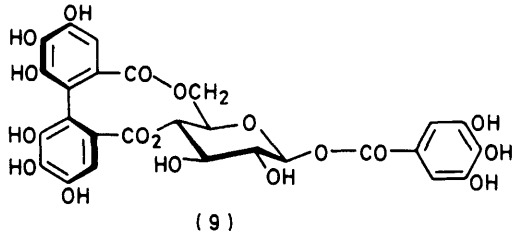


(9)

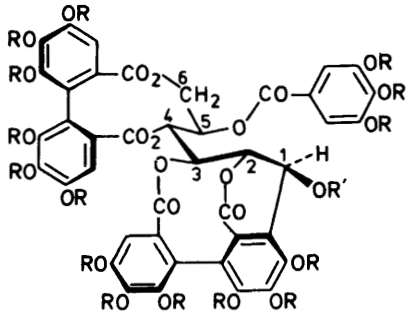
The ^{13}C n.m.r. spectrum similarly exhibited two signals for anomeric carbons at δ_C 92.2 and 95.6 p.p.m., and the double signals for each of the other glucose carbons. A deuterium-induced differential isotope shift (d.i.s.) measurement [14] showed both anomeric carbons as a couple of peaks (d.i.s. value 0.14 and 0.17 p.p.m., respectively), while the other carbon signals of the glucose were unaffected. The chemical shifts of the anomeric carbons were also comparable with those of tellimagrandin I (6) (δ_C 91.9 and 97.2 p.p.m.) and alnusiin [15] (δ_C 92.5 and 96.2 p.p.m.) which have a free hydroxy group at O-1 and form a mixture of α and β anomers. These observations unequivocally indicate that an ester group is lacking at the anomeric centre of pedunculagin, thus excluding the proposal that a mixture of the conformational isomers involving a 1,6-bridge is formed, as claimed by Seikel and Hillis.[9] Upon methylation with diazomethane or with dimethyl sulphate and potassium carbonate in acetone, pedunculagin afforded the α-anomer (3) and β-anomer (4) of trideca-*O*-methylpedunculagin which were separable by preparative layer chromatography (p.l.c.) on silica gel. Methanolysis of (3) and (4) gave the respective methyl glucoside, and the dimethyl hexamethoxydiphenate (7). The biphenyl configuration of compound (7) thus obtained was assigned as *S* by comparison of its specific rotation, $[\alpha]_D$ −37.7° (ethanol), and c.d. spectrum, $[\theta]_{225}$ $+6.7 \times 10^4$, $[\theta]_{250}$ -5.6×10^4, with those of the *R*-enantiomer obtained from geraniin [16,17] (Figure). Based on these results the structure, including the absolute configuration of the HHDP groups, of pedunculagin was established as 2,3:4,6-di-*O*-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-D-glucopyranose (1).

Casuarictin (8), $C_{41}H_{28}O_{26}\cdot 6H_2O$, $[\alpha]_D$ +35°, which forms an amorphous powder, is the most abundant tannin in *S. praecox*. Its i.r. absorption bands at 3 400 (OH) and 1 735 cm^{-1} (ester), and the u.v. maxima at 220 and 260sh nm, are similar to those of (1). The 200-MHz ^1H n.m.r. spectrum exhibited a 2-H singlet at δ 7.18, which is characteristic of a galloyl group, and four 1-H singlets assignable to two HHDP groups at δ 6.68, 6.55, 6.47, and 6.38. The sugar moiety was defined by the well separated signals of the seven-spin system which were assigned by the decoupling experiments. The axial orientation of these protons, as revealed by the coupling constants. indicates that the sugar residue of compound (8) is β-D-glucopyranose with the $^4C_1$ conformation. These data, and the ^{13}C n.m.r. spectrum (Table 1), indicate that a galloyl and two HHDP groups esterify β-D-glucose in the molecule of casuarictin. The location of the galloyl group at O-1 in (8) was confirmed by its hydrolysis with tannase to yield pedunculagin (1). Casuarictin was therefore characterized as 1-*O*-galloyl-2,3:4,6-di-*O*-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-β-D-glucopyranose (8).

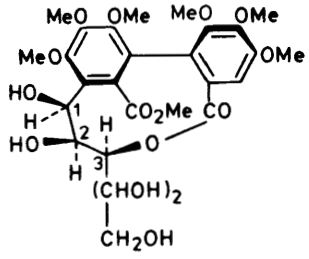
Strictinin (9), $C_{27}H_{22}O_{18}\cdot 2.5H_2O$, $[\alpha]_D$ −3°, was isolated as an off-white, amorphous powder. The ^1H n.m.r. spectrum of (9) showed the presence of a galloyl group and a HHDP group at δ 7.21 (2 H), 6.72 and 6.61 (1 H each). The signals assignable to 2-H and 3-H of the sugar are shifted upfield from those of (8) by *ca.* 1.5 p.p.m., while the other resonances are essentially the same as those of (8). The enzymatic hydrolysis of (9) with tannase afforded gallic acid and 4,6-*O*-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-D-glucose.[11,18] That compound (9) is the β-anomer is evident by the coupling constant (*J* 7 Hz) of 1-H of the glucopyranose ($^4C_1$ conformation) in the ^1H n.m.r. spectrum. Based on these data, the structure of strictinin can be represented by formula (9).

Casuarinin (10), $C_{41}H_{28}O_{26}\cdot 7H_2O$, $[\alpha]_D$ +43.6°, is the main tannin of *C. stricta*, and gives a characteristic reddish violet colour on t.l.c. with the $NaNO_2$–AcOH reagent; this colour rapidly changes to blue. This initial colouration was clearly distinguished from that of ordinary ellagitannins such as pedunculagin (1) and tellimagrandin I (6). Casuariin (17) and stachyurin (20) gave a similar colour with the same reagent, thus suggesting their structural analogy. The structures of these tannins were determined as follows. The ^1H n.m.r. spectrum of (10) exhibited three 1-H singlets at δ 6.78, 6.56, and 6.49 in the aromatic region, in addition to the signal at δ 7.12 due to a galloyl group, and the signals of the carbohydrate residue at δ 4.06—5.64. Methylation of (10) with diazomethane gave pentadeca-*O*-methylcasuarinin (11), $C_{56}H_{58}O_{26}\cdot H_2O$, M^+ 1 146, which upon methanolysis yielded methyl tri-*O*-methylgallate, dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (7), and a heptamethyl derivative (14), $C_{27}H_{34}O_{15}$, M^+ 598. However, the sugar was not liberated upon this methanolysis. Hydrolysis with boiling 0.5M-H_2SO_4 for 2 h, which in a parallel experiment liberated a significant amount of glucose from casuarictin (8), also did not liberate the sugar from (10). Acid hydrolysis of compound (10) for over 20 h was necessary for the liberation of the sugar which was identified as glucose by gas chromatography of the trimethylsilyl ether. This behaviour of (10) upon alkaline and acidic hydrolyses, which is analogous to that of some C-glycosides,[19,20] indicates that casuarinin should be a C-glucoside. Acetylation of (11) afforded a monoacetate (12), $C_{58}H_{60}O_{27}$, M^+ 1 188, the^1 H n.m.r. spectrum of which showed a doublet (*J* 5 Hz) at δ 6.73 which is shifted downfield from that at δ 5.50 for (11). The chemical shift and multiplicity of this signal are attributable to those of 1-H of glucose. However, the ^{13}C n.m.r. spectrum of (10) (Table 2), which showed six glucose carbons in the region δ_C 64.5—76.7 p.p.m., did not show the hemiacetal carbon which was expected at δ_C 90.0—100.0 p.p.m. These results, and the presence of five ester groups shown by the ^{13}C n.m.r. spectrum of (10) (Table 1), indicate that the glucose moiety in (10) is in the open-chain form. The β-configuration at O-1 in (10) was deduced by the analogy of the coupling constant ($J_{1,2}$ 5 Hz) in the ^1H n.m.r. spectrum to that of castalagin.[20]

The close resemblance in the sp^2-carbon region of the ^{13}C n.m.r. spectra between compounds (10) and (8) (Table 1) suggests that the two HHDP groups and a galloyl group are also present in (10). The hydrolysis of (10) with tannase afforded the degalloylated derivative, $C_{34}H_{24}O_{22}\cdot 5H_2O$, which was isolated by Sephadex LH-20 column chromatography,



(10) R = R′ = H
(11) R = Me, R′ = H
(12) R = Me, R′ = Ac
(13) R = Me, R′ = CONHCOCCl$_3$



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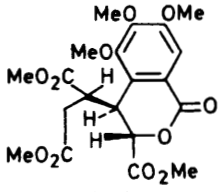

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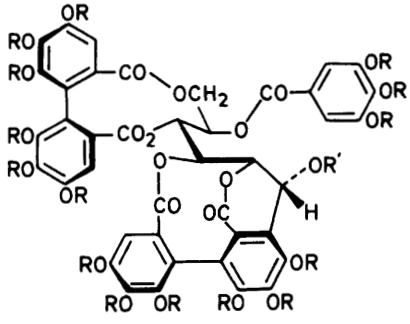
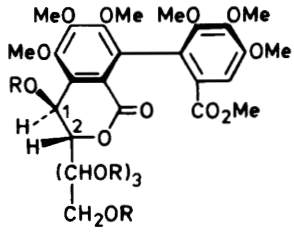
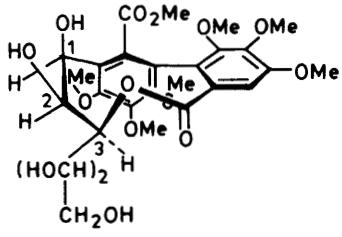


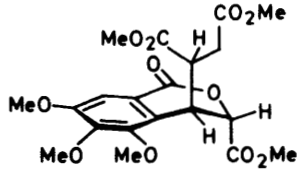
(20) R = R′ = H
(21) R = Me, R′ = H
(22) R = Me, R′ = Ac



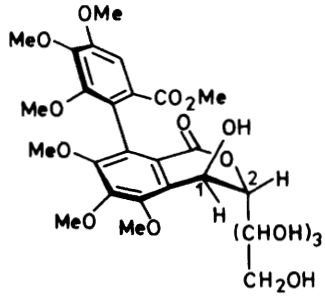
(14) R = H
(15) R = Ac



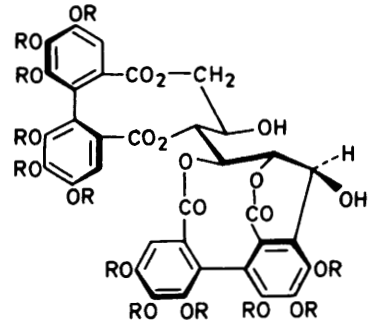
(16a)



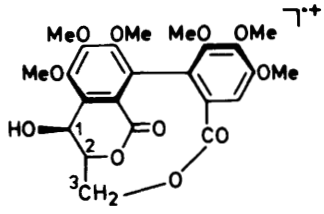
(19a)



(14a)



(17) R = H
(18) R = Me



fragment A

and was identified as casuariin (17). The ^{1}H n.m.r. spectrum of (17) exhibited the glucose protons as a fairly well resolved group of seven signals which were assigned by double-irradiation experiments (*cf.* Experimental section). A marked upfield shift (δ 5.38 ⟶ 4.18) of the 5-H signal occurred upon the degalloylation, thus indicating that the galloyl group in (10) was at O-5 of the glucose.

One of the two HHDP groups in (10) is regarded as participating in the C-glucoside formation since only three proton signals of these groups are found in the ^{1}H n.m.r. spectrum of (10), and also by the fact that the heptamethyl derivative (14), which possesses one aromatic proton as revealed by a singlet at δ 7.46, gave a penta-acetate (15), $C_{34}H_{44}O_{20}$, M^+ 808. The biphenyl configuration at the C-glucosidic HHDP group in (14) was established as *S* by its c.d. spectrum which exhibited Cotton effects at 204, 227, and 252 nm, similar to those positions for (7),[17] although small differences in amplitude are observed as shown in the Figure. Of the two possible structures (14) and (16) for the heptamethyl derivative, the former is compatible with the ^{1}H n.m.r. spectrum which showed the broad singlet due to 1-H of glucose at δ 5.74, since the dihedral angle between 1-H and 2-H in (14) is *ca.* 90°, whereas that in (16) should be 0—30° in the possible conformation (16a).

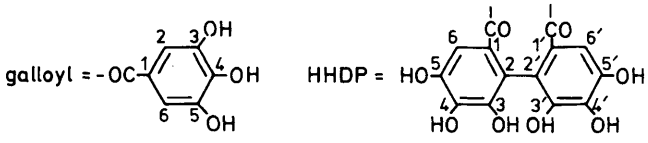
Because of the structural analogy of (14) with trimethyl tri-*O*-methylchebulate (19), whose absolute configuration was recently determined,[21] compound (14) may take the conformation (14a) which is similar to the conformation (19a). The chiral dihydroisocoumarin ring in compound (19) exhibits four c.d. bands at 205 ([θ] -4.4×10^4), 225 ([θ] $+3.4 \times 10^4$), 243 ([θ] -0.6×10^4), and 265 nm ([θ] $+2.4 \times 10^4$).[21] Thus, the c.d. spectrum of (14) should give Cotton effects similar to those of (19) which happen to overlap those (202, 225, and 250 nm) due to the biphenyl conjugation. A possible interpretation for the difference in the amplitude of the c.d. spectra between compounds (14) and (7) is provided on this basis.

The mode of the ester linkages at O-2 and -3 between the C-glucosidic HHDP group and glucose as shown in formula (10) was supported by a prominent peak at *m/z* 476, attribu-

Table 1. sp^2-Carbon resonances of (8), (10), (17), and (20) in CD_3COCD_3 [a]

	(8)	(10)	(17) [b]	(20) [c]
Ester C=O	171.1	170.3	171.3	168.9
	169.2	169.5	170.9	168.8
	168.8	169.2	169.7	168.4
	168.4	166.4	166.9	165.7
	165.4	165.0		165.2
Galloyl C-1	126.3	125.4		125.2
C-2, -6	110.6(2)	110.5(2)		110.1(2)
C-4	140.5	139.6		139.1
HHDP C-1, -1′ [e]	126.6(4)	128.0	127.8	127.9
		127.5	127.5	127.0
		121.4	125.5	122.8
		121.0	120.1	120.9
HHDP C-2, -2′	120.0	116.5	116.9	116.0(2)
	116.3	116.3	116.5(2)	115.7
	115.3	116.1	115.5	115.2
	114.6	115.4		
HHDP C-3, -3′ C-5, -5′ and galloyl C-3, -5	146.7(2)	146.8	146.9	146.5
	145.7(5)	146.4(3)	146.5	145.9(2)
	144.9(3)	145.7(2)	145.9(2)	145.7
		145.2	145.2(2)	145.2(2)
		145.0	144.6	144.5
		144.4	144.4	144.4
		144.1		144.1
				143.4
HHDP C-4, -4′	137.0(4)	138.8	139.9	137.8
		137.3	137.6	136.7
		136.3	136.5	135.9
		135.3	135.7	135.0
HHDP C-6, -6′	110.6	108.7	109.2	108.6
	108.6	107.5	107.7	107.3
	107.7(2)	105.7	105.8	105.7
		117.4 [d]	117.8 [d]	119.1 [d]

[a] Chemical shifts are given in δ-values (p.p.m.) with 1,4-dioxan (δ 67.4) as internal standard. Number in parentheses is the number of carbon atoms resonating at that δ-value shown. [b] Measured in CD_3COCD_3–D_2O. [c] Recorded on 50-MHz instrument. [d] Signal due to the carbon linked to C-1 of glucose. [e] The numbering of the HHDP group in this table corresponds to that of the galloyl group.



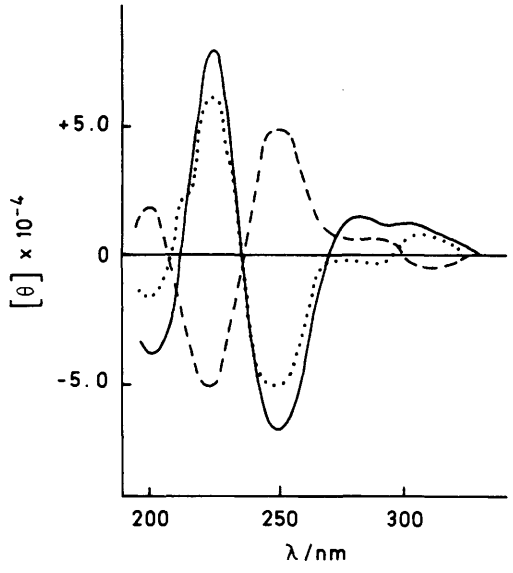


Figure. C.d. spectra of (7) (· · · ·), its *R*-enantiomer (— — —), and the heptamethyl derivative (14) (——) in methanol

Table 2. ^{13}C N.m.r. chemical shifts (p.p.m.) of the glucose moiety of (10), (17), and (20) in CD_3COCD_3, and their derivatives in $CDCl_3$

	(10)	(17) [a]	(20)	(11)	(13)	(21)	(22)
C-1	71.1	70.8	72.0	71.4	73.7	71.6	72.8
C-2	76.7	77.1	81.0	76.3	71.0	80.4	77.4
C-3	67.8	68.5	65.0	67.0	68.5	65.5	65.5
C-4	74.3	77.1	73.3	73.6	74.7	73.4	71.2
C-5	69.8	68.5	70.9	69.9	70.5	71.0	70.9
C-6	64.5	67.2	64.5	64.7	64.7	64.6	64.8

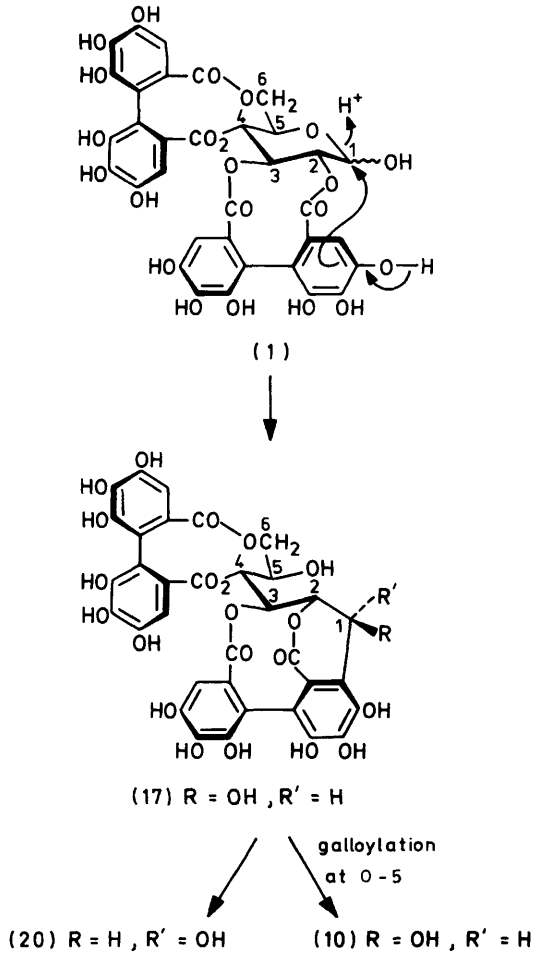
[a] In CD_3COCD_3–D_2O solution.

table to the fragment A in the mass spectrum of compound (18), which was prepared by the methylation of casuariin (17) with diazomethane. Consequently, the second HHDP group in (10), and also in (17), is linked to both O-4 and O-6 of the glucose.

The change of coupling constant of 1-H in (10) (*J* 5 Hz) and (14) (*J ca.* 0) is attributable to the conformational change upon cleavage of the ester linkages at O-3 -4, -5, and -6 in (10). The structures (10) for casuarinin and (17) for casuariin are thus satisfactory for all these data.

Stachyurin (20), $C_{41}H_{28}O_{26}\cdot 5H_2O$, $[\alpha]_D$ +39°, was isolated with casuariin (17) from the aqueous layer after the extraction with ethyl acetate. These two tannins, (17) and (20), were indistinguishable on p.p.c. (paper partition chromatography) and cellulose t.l.c. developed with butan-1-ol–acetic acid–water [4:1:5, (v/v)] and 7% acetic acid, but showed a distinct difference in retention time on h.p.l.c. (normal phase). The ^1H n.m.r. spectrum of (20) is rather similar to that of casuarinin (10), except for a remarkable difference of the chemical shift and coupling constant of 1-H of the sugar [(20); δ 4.93, d, *J* 2 Hz. (10); δ 5.64, d, *J* 5 Hz].

Methylation of compound (20) with diazomethane yielded a pentadeca-*O*-methylstachyurin (21), $C_{56}H_{58}O_{26}$, M^+ 1 146, which is isomeric with (11). Acetylation of (21) gave a monoacetate (22), $C_{58}H_{60}O_{27}$, M^+ 1 188, whose ^1H n.m.r. spectrum exhibited the 1-H signal as a broad singlet at δ 6.18, which is lower than that of 1-H in compound (21) by *ca.* 1 p.p.m. As in (10), the hemiacetal carbon signal of compound (20) was not observed in the region δ 90.0—100.0 p.p.m. in the ^{13}C n.m.r. spectrum. The carbon resonances of the glucose moiety in (10) and (20) were assigned as in Table 2, based on the off-resonance decoupling spectra and on the acylation shift [22,23] observed upon the transformations of (10) ⟶ (17), (11) to its trichloroacetylcarbamate derivative (13), and (21) ⟶ (22). Two marked differences in the ^{13}C n.m.r. spectra were observed between (10) and (20). (i) The resonance due to the aromatic carbon directly bound to C-1 of the sugar in (20) is shifted downfield by 1.7 p.p.m. with respect to that in (10) (Table 1). (ii) The signal assigned to C-2 of glucose in (10) i



shifted downfield by 4.3 p.p.m. in (20) (Table 2). These data indicate that stachyurin could be a C-1 epimer of casuarinin (10), and this assumption was confirmed by the transformation of casuarinin (10) to (20) in boiling water, which occurred in a way analogous to the epimerization of castalagin.[20]

In the proposed structure (10) the dihedral angle between 1-H and 2-H is 30—40°, whereas this angle approaches 90° in (20). These angles are consistent with the coupling constants in their ^{1}H n.m.r. spectra.

It is conceivable that the biogenetic precursor of compounds (10), (17), and (20) is pedunculagin (1), as (10), (17), and (20) can be formed from (1) through the transformations illustrated in the Scheme. It is notable that all seven tannins discussed in this paper, which have mutually correlated structures should coexist in two plant species which are taxonomically far apart from each other.

Since C-glucosidic ellagitannins [20,24,25] have been hitherto detected only in plants of the family Fagaceae, the present findings are the first example of the isolation of C-glucosidic tannins from the plants of other families.

Experimental

I.r. spectra were recorded on a JASCO A-102 spectrophotometer and u.v. spectra on a Hitachi 200-10 spectrophotometer. Optical rotations were measured on a JASCO DIP-4 Digital polarimeter. Mass spectra were recorded on a Shimadzu LKB-9 000 GC-MS spectrometer. ^{1}H N.m.r. (90 MHz) and ^{13}C n.m.r. (22.6 MHz) spectra were measured on a Hitachi R-22FTS instrument using $SiMe_4$ as internal standard, and the chemical shifts were given in δ-values (p.p.m.). G.l.c. was performed on a Hitachi Gas Chromatograph 163 equipped with a glass column (3 mm i.d. × 2 m) packed with 1% OV-1 on Chromosorb W. P.p.c. was done on Toyo No. 50 paper in the solvent systems (A) butan-1-ol–acetic acid–water (4 : 1 : 5, v/v; upper layer) and (B) 7% acetic acid in water (v/v), and spots were detected either by spraying with iron(III) chloride solution, or with the $NaNO_2$–AcOH reagent. Normal-phase h.p.l.c. was run on a column of Nomura Develosil 60–5, 4 × 150 mm, with n-hexane–methanol–tetrahydrofuran–formic acid (55 : 33 : 11 : 1, v/v) as eluant, containing oxalic acid (450 mg l^{-1}). Reversed-phase h.p.l.c. was carried out on a column of Merck LiChrosorb RP-18 (10 μm), 4 × 300 mm, with 0.1M-H_3PO_4–0.1M-KH_2PO_4–ethanol–ethyl acetate (50 : 50 : 2 : 5, v/v) as eluant. Detection was effected by u.v. absorption at 280 nm. Avicel microcrystalline cellulose (Funakoshi) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography, and Merck silica gel PF_{254} for analytical and preparative t.l.c. Solvents were evaporated under reduced pressure below 40 °C. Light petroleum refers to that fraction boiling in the range 75—125 °C.

Isolation of Tannins from Casuarina stricta.—(i) The dried leaves (1 kg) of *Casuarina stricta* were homogenized in acetone–water (1 : 1; 20 l), and the extract was filtered through Celite 545. The homogenate was concentrated to *ca.* 900 ml and then extracted with diethyl ether (500 ml × 4). The aqueous layer was subsequently extracted with ethyl acetate (500 ml × 30). The ethyl acetate layer was evaporated to give a dark brown residue (22 g) which was partitioned between the organic phase and aqueous phase of chloroform–methanol–propan-1-ol–water (9 : 12 : 2 : 8, v/v). Evaporation of the aqueous layer afforded the crude tannin fraction (14 g) (extract I). A portion (1.5 g) of extract I was subjected to droplet counter-current chromatography (100 glass tubes; 3.2 mm × 120 cm) with ascending elution using butan-1-ol–propan-1-ol–water (2 : 1 : 3, v/v) as eluant. Every fifth fraction (10 ml each) was monitored by u.v. absorption at 280 nm, and by t.l.c. (cellulose; 7% acetic acid). Fractions 26—50 (400 mg) were combined, and further chromatographed on Sephadex LH-20 with ethanol as eluant to yield casuarictin (8) (94 mg) and tellimagrandin I (6) (20 mg). Fractions 62—90 (550 mg) contained strictinin (9) (27 mg) and casuarinin (10) (320 mg) which were separated by Sephadex LH-20 column chromatography with ethanol as eluant. Fractions 120—165 (640 mg) gave pedunculagin (1) (480 mg) after purification by column chromatography on Sephadex LH-20 and then on cellulose.

(ii) The dried leaves (200 g) were treated in a way similar to (i) and the remaining aqueous solution (300 ml) after ethereal extraction was concentrated to half volume and was added to methanol (400 ml). After removal of insoluble materials by centrifugation, the supernatant was evaporated to afford the crude tannin fraction (32 g) (extract II). A portion (15 g) of extract II was roughly divided into the fraction containing pedunculagin and that containing the other tannins by column chromatography on cellulose (water as eluant). The latter fraction (3 g) was further fractionated by droplet counter-current chromatography in a similar manner to extract I. Fractions 140—200 (580 mg) gave casuariin (17) (90 mg) and stachyurin (20) (115 mg) which were separated by successive column chromatography over cellulose and Sephadex LH-20. Total yield of each tannin from dried leaves: pedunculagin (1), 0.22%; tellimagrandin I (6), 0.01%; casuarictin (8), 0.04%; strictinin (9), 0.025%; casuarinin (10), 0.30%; casuariin (17), 0.10%; stachyurin (20), 0.12%.

Isolation of Tannins from Stachyurus praecox.—Fresh leaves (125 g) of *Stachyurus praecox* collected in April were homogenized in acetone–water (7 : 3). The homogenate was filtered and the acetone was evaporated. The resulting aqueous solution (60 ml) was extracted with diethyl ether (60 ml × 10) and then with ethyl acetate (40 ml × 10). The ethyl acetate layer was evaporated to give a pale yellow residue (1.83 g) (ethyl acetate extract). The remaining aqueous layer was concentrated and triturated with methanol (500 ml). The methanol-soluble fraction was evaporated to give a brownish residue (8.8 g) (aqueous extract). A portion (1.8 g) of the ethyl acetate extract was fractionated by droplet counter-current chromatography (100 glass tubes; 3.2 mm × 120 cm) in an analogous way to that described above. Fractions 31—60 (896 mg) were chromatographed over Sephadex LH-20 (1.1 × 30 cm) to give tellimagrandin I (6) (108 mg) from the ethanol eluate and casuarictin (8) (717 mg) from the methanol eluate. Fractions 65—100 (218 mg) gave, in a similar way, strictinin (9) (17 mg) and casuarinin (10) (180 mg). Fractions 110—150 yielded pedunculagin (1) (199 mg). The aqueous extract (4.4 g) was chromatographed on a cellulose column (3.2 × 28 cm) with water as eluant; 25-ml fractions were collected. This column chromatography was performed twice, and the combined crude tannin fraction (fractions 4—10) was further fractionated by droplet counter-current chromatography and also by Sephadex LH-20 chromatography to give strictinin (9) (38 mg), casuarinin (10) (381 mg), pedunculagin (1) (110 mg), and stachyurin (20) (207 mg).

Total yield of each tanin from fresh leaves: pedunculagin (1), 0.25%; tellimagrandin I (6), 0.09%; casuarictin (8), 0.57%; strictinin (9), 0.04%; casuarinin (10), 0.45%; stachyurin (20), 0.17%.

Casuariin (17) in the leaf extract was detected by h.p.l.c. (normal and reversed phases).

Pedunculagin (1).—An off-white, amorphous powder, R_F (A) 0.24, R_F (B) 0.50; $[\alpha]_D$ +100°, $[\alpha]_{578}$ +102° (*c* 1.0, MeOH) {lit.,[8] $[\alpha]_{578}$ +106° (MeOH)} (Found: C, 46.45; H, 3.65. Calc. for $C_{34}H_{24}O_{22}\cdot 5H_2O$: C, 46.7; H, 3.9%); $\nu_{max.}$ (KBr) 3 400, 1 735, 1 615, 1 510, 1 440, 1 350—1 300, 1 170, 1 030, and 1 005 cm^{-1}; $\lambda_{max.}$ (MeOH) 232 (log ε 4.34) and 256sh nm (4.57); c.d. (MeOH) $[\theta]_{233}$ +16.8 × 10^4, $[\theta]_{259}$ −5.4 × 10^4, and $[\theta]_{282}$ +2.1 × 10^4; δ_H (200 MHz; CD_3COCD_3) 6.68 and 6.67 (1 H, in total), 6.60 and 6.59 (1 H, in total), 6.56 and 6.51 (1 H, in total), 6.33 and 6.32 (1 H in total), 5.48 (d, *J* 3.5 Hz), and 5.22 (d, *J* 9 Hz) (α : β anomer 11 : 9).

The deuterium-induced differential isotope shift spectrum of (1) was obtained with a dual sample tube consisting of a 5-mm tube (0.4M in H_2O–CD_3OD, 4 : 1) and a 8 mm tube 0.4M in D_2O–CD_3OD, (4 : 1).

Trideca-O-*acetyl*-α-*pedunculagin* (2) was obtained by acetylation of (1) with acetic anhydride and pyridine and had m.p. 240—241 °C (lit.,[8] 231—236 °C); $[\alpha]_D$ −7.6° (*c* 1.4, $CHCl_3$) (Found: C, 52.85; H, 3.8. Calc. for $C_{60}H_{50}O_{35}\cdot 2H_2O$: C, 52.7; H, 4.0%); δ_H ($CDCl_3$) 7.50, 7.30, 7.28, and 7.14 (each 1 H, s, 2 ArH), 6.38 (1 H, d, *J* 4 Hz, 1-H), 5.61 (1 H, t, *J* 9 Hz, 3-H), 5.37 (1 H, dd, *J* 4 and 9 Hz, 2-H), 5.34 (1 H, dd, *J* 8 and 13 Hz, 6-H), 5.31 (1 H, dd, *J* 8 and 9 Hz, 4-H), 4.50 (1 H, m, 5-H), and 3.92 (1 H, dd, *J* 3 and 13 Hz, 6-H).

Methylation of Pedunculagin (1).—To a solution of compound (1) (50 mg) in dry acetone (3 ml) was added anhydrous potassium carbonate (250 mg) and dimethyl sulphate (0.25 ml). The reaction mixture was stirred for 12 h at room temperature, and then refluxed for 2 h. Removal of the solvent after filtration gave a syrupy residue which was purified by p.l.c. [light petroleum–dichloromethane–acetone (5 : 2 : 3 v/v); double development] to give the α-anomer (3) (15 mg) and the β-anomer (4) (17 mg) of trideca-*O*-methylpedunculagin. *Trideca*-O-*methyl*-α-*pedunculagin* (3): amorphous powder, $[\alpha]_D$ +27.4° (*c* 1.0, acetone) (Found: C, 58.45; H, 5.4. $C_{47}H_{50}O_{22}$ requires C, 58.4; H, 5.2%); $\nu_{max.}$ (KBr) 3 450, 1 755, 1 595, 1 490, 1 460, 1 430, 1 395, 1 275, 1 245, 1 200, 1 100, and 1 050 cm^{-1}; $\lambda_{max.}$ (MeOH) 222 (log ε 4.84), 247infl (4.63), and 288sh nm (4.01); δ_H (CCl_4) 6.70, 6.67, 6.65, and 6.48 (each 1 H, s, ArH).

Trideca-O-*methyl*-β-*pedunculagin* (4): amorphous powder, $[\alpha]_D$ −34.2° (*c* 1.0, acetone) (Found: C, 58.1; H, 5.1. $C_{47}H_{50}O_{22}$ requires C, 58.4; H, 5.2%); $\nu_{max.}$ (KBr) 3 450, 1 775, 1 595, 1 575, 1 490, 1 460, 1 395, 1 275, 1 245, 1 200, 1 160, 1 100, and 1 050 cm^{-1}; $\lambda_{max.}$ (MeOH) 222 (log ε 4.86), 247infl (4.66), and 288sh nm (4.01); δ_H (CCl_4) 6.72, 6.65, 6.61, and 6.50 (each 1 H, s, ArH); *m*/*z* 966 (M^+).

Methanolysis of (3) *and* (4).—A mixture of compound (3) (15 mg) and 1% sodium methoxide (0.1 ml) in absolute methanol (1 ml) was left overnight at room temperature. The mixture was neutralized with acetic acid and the solvent was evaporated in a stream of nitrogen at room temperature. The residue, after treatment with diazomethane, was partitioned between chloroform and water. The chloroform phase was evaporated and purified by p.l.c. [light petroleum–dichloromethane–acetone (4 : 2 : 1, v/v)] to afford dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (7) (11 mg), $[\alpha]_D$ −37.7° (*c* 0.6, EtOH); $\lambda_{max.}$ (EtOH) 221 (log ε 4.65), 253 (4.24), and 300 nm (3.74); c.d. (MeOH) $[\theta]_{225}$ +6.7 × 10^4 and $[\theta]_{250}$ −5.6 × 10^4; *m*/*z* 450 (M^+). The aqueous phase gave methyl α-D-glucoside which was identified by g.l.c. and g.c.-m.s. of the trimethylsilyl ether.

In an analogous way compound (4) afforded methyl β-D-glucoside and (7).

Casuarictin (8).—An off-white, amorphous powder, R_F (A) 0.28, R_F (B) 0.32; $[\alpha]_D$ +35.2° (*c* 0.2, MeOH) (Found: C, 46.9; H, 3.55. $C_{41}H_{28}O_{26}\cdot 6H_2O$ requires C, 47.15; H, 3.85%); $\nu_{max.}$ (KBr) 3 400, 1 735, 1 615, 1 510, 1 445, 1 345—1 310, 1 180, 1 030, and 1 005 cm^{-1}; $\lambda_{max.}$ (MeOH) 220 (log ε 4.90) and 260sh nm (4.59); c.d. (MeOH) $[\theta]_{235}$ +20.2 × 10^4, $[\theta]_{261}$ −5.3 × 10^4, $[\theta]_{281}$ +0.7 × 10^4, and $[\theta]_{306}$ −1.8 × 10^4; δ_H (CD_3COCD_3) 7.18 (2 H, s, galloyl ArH), 6.68, 6.55, 6.47, and 6.38 (each 1 H, s, ArH), 6.22 (1 H, d, *J* 9 Hz, 1-H), 5.45 (1 H, dd, *J* 9 and 10 Hz, 3-H), 5.37 (1 H, dd, *J* 7 and 13 Hz, 6-H), 5.18 (1 H, t, *J* 9 Hz, 2-H), 5.17 (1 H, t, *J* 10 Hz, 4-H), 4.50 (1 H, dd, *J* 7 and 10 Hz, 5-H), and 3.88 (1 H, d, *J* 13 Hz, 6-H).

Hydrolysis of (8) *with Tannase*.—A solution of compound (8) (29 mg) in water (2 ml) was treated with tannase at 37 °C for 15 h. The reaction mixture was directly chromatographed over Sephadex LH-20 to yield gallic acid (5 mg) from the water eluate, and pedunculagin (1) (20 mg) from the methanol–water (1 : 1) eluate. They were identified by direct comparison with authentic samples ($[\alpha]_D$, ^1H n.m.r., and h.p.l.c.).

Strictinin (9).—An off-white, amorphous powder, R_F (A) 0.26, R_F (B) 0.37; $[\alpha]_D$ −3.1° (*c* 0.4, MeOH) (Found: C, 47.75; H, 3.9. $C_{27}H_{22}O_{18}\cdot 2.5H_2O$ requires C, 47.7; H, 4.0%); $\nu_{max.}$ (KBr) 3 400, 1 725, 1 615, 1 515, 1 445, 1 350—1 315, 1 230—1 195, and 1 040—1 010 cm^{-1}; $\lambda_{max.}$ (MeOH) 218 (log ε 4.78) and 267 nm (4.44); c.d. (MeOH) $[\theta]_{235}$ +13.1 × 10^4, $[\theta]_{265}$ −3.2 × 10^4, $[\theta]_{288}$ −0.2 × 10^4, and $[\theta]_{312}$ −1.1 × 10^4; δ_H (CD_3COCD_3) 7.21 (2 H, s, galloyl ArH), 6.72 and 6.61 (each 1 H, s, ArH), 5.76 (1 H, d, *J* 7 Hz, 1-H), 5.22 (1 H, dd, *J* 6 and 14 Hz, 6-H), 4.91 (1 H, t, *J* 9 Hz, 4-H), 4.11 (1 H, dd, *J* 6 and 9 Hz, 5-H), 3.84 (1 H, t, *J* 9 Hz, 3-H), 3.79 (1 H, d, *J* 14 Hz, 6-H), and 3.66 (1 H, dd, *J* 7 and 9 Hz, 2-H).

Hydrolysis of (9) *with Tannase*.—Strictinin (9) (23 mg) was hydrolysed with tannase in an analogous way as described above. Separation of the mixture by Sephadex LH-20 column chromatography with water as eluant yielded gallic acid and 4,6-*O*-[(*S*)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl]-D-glucose (6 mg), $[\alpha]_D$ +44° (*c* 0.5, EtOH), which was identical with an authentic sample prepared from tellimagrandin I (6).

Casuarinin (10).—A pale yellow, amorphous powder, R_F (A) 0.18, R_F (B) 0.20; $[\alpha]_D$ +43.6° (*c* 1.0, MeOH) (Found: C, 46.15; H, 3.7. $C_{41}H_{28}O_{26}\cdot 7H_2O$ requires C, 46.35; H, 4.0%); $\nu_{max.}$ (KBr) 3 400, 1 720, 1 610, 1 500sh, 1 445, 1 355—1 300, 1 175, and 1 085 cm^{-1}; $\lambda_{max.}$ (MeOH) 221 (log ε 4.87) and 267sh nm (4.51); c.d. (MeOH) $[\theta]_{231}$ +16.7 × 10^4, $[\theta]_{262}$ −3.3 × 10^4, and $[\theta]_{283}$ +1.2 × 10^4; δ_H (CD_3COCD_3) 7.12 (2 H, s, galloyl ArH), 6.78, 6.56, and 6.50 (each 1 H, s, ArH), 5.64 (1 H, d, *J* 5 Hz, 1-H), 5.39 (3 H, br s, 3-, 4-, and 5-H), 4.67 (1 H, dd, *J* 2 and 5 Hz, 2-H), 4.18 (1 H, dd, *J* 3 and 13 Hz, 6-H), and 4.06 (1 H, d, *J* 13 Hz, 6-H).

Casuariin (17).—A pale yellow, amorphous powder, R_F (A) 0.14, R_F (B) 0.29; $[\alpha]_D$ +162° (*c* 0.5, MeOH) (Found: C, 47.9; H, 3.95. $C_{34}H_{24}O_{22}\cdot 4H_2O$ requires C, 47.65; H, 3.75%); $\nu_{max.}$ (KBr) 3 400, 1 720, 1 610, 1 450, and 1 360—1 295 cm^{-1}; $\lambda_{max.}$ (MeOH) 213 (log ε 4.70), 233 (4.74), and 257sh nm (4.57); δ_H (CD_3COCD_3) 6.67, 6.53, and 6.43 (each 1 H, s, ArH), 5.66 (1 H, d, *J* 5 Hz, 1-H), 5.44 (1 H, t, *J* 3 Hz, 3-H), 5.02 (1 H, dd, *J* 3 and 9 Hz, 4-H), 4.75 (1 H, dd, *J* 3 and 5 Hz, 2-H), 4.65 (1 H, dd, *J* 3 and 12 Hz, 6-H), 4.11 (1 H, dd, *J* 3 and 9 Hz, 5-H), and 3.84 (1 H, d, *J* 12 Hz, 6-H).

Pentadeca-O-methylcasuarinin (11).—A solution of compound (10) (50 mg) in methanol (2 ml) was treated with an excess of ethereal diazomethane for 1.5 h at room temperature. Purification of the product by p.l.c. using light petroleum–dichloromethane–acetone (2 : 1 : 1, v/v) as eluant (double development) gave *pentadeca-O-methylcasuarinin* (11) (28 mg) as an amorphous powder, $[\alpha]_D$ −42.3° (*c* 0.6, $CHCl_3$) (Found: C, 57.8; H, 5.15. $C_{56}H_{58}O_{26}\cdot H_2O$ requires C, 57.75; H, 5.2%); *m/z* 1 146 (M^+), 422, and 212; $\nu_{max.}$ (KBr) 3 480, 1 745, 1 590, 1 455, 1 400, 1 335, 1 200, 1 160, and 1 100 cm^{-1}; $\lambda_{max.}$ (MeOH) 219 (log ε 4.94) and 258sh nm (4.54); δ_H (200 MHz; $CDCl_3$) 7.35 (2 H, s, galloyl ArH), 7.13, 6.76, and 6.56 (each 1 H, s, ArH), 5.74 (1 H, dd, *J* 3 and 9 Hz, 5-H), 5.50 (1 H, d, *J* 5 Hz, 1-H), 5.41 (1 H, dd, *J* 2 and 9 Hz, 4-H), 5.35 (1 H, t, *J* 2 Hz, 3-H), 5.03 (1 H, dd, *J* 3 and 13 Hz, 6-H), 4.95 (1 H, dd, *J* 2 and 5 Hz, 2-H), 4.26 (1 H, d, *J* 13 Hz, 6-H), and 4.04—3.46 (15 × OMe).

The ^{13}C n.m.r. spectrum of the trichloroacetyl carbamate derivative (13) was obtained by *in situ* derivatization according to the literature method.[23]

Pentadeca-O-methylcasuarinin Monoacetate (12).—The pentadecamethyl derivative (11) (10 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. The solvent was removed by repeated co-evaporation with toluene. The product was purified by p.l.c. with benzene–acetone (4 : 1, v/v) as eluant to afford the *monoacetate* (12) (9.6 mg) as an amorphous powder, $[\alpha]_D$ −53° (*c* 1.0, $CHCl_3$) (Found: C, 57.7; H, 4.75. $C_{58}H_{60}O_{27}\cdot H_2O$ requires C, 57.7; H, 5.0%); *m/z* 1 188 (M^+); $\nu_{max.}$ (KBr) 3 450, 1 755, 1 590, 1 460, 1 405, 1 335, 1 200, 1 160, and 1 100 cm^{-1}; $\lambda_{max.}$ (MeOH) 216 (log ε 5.04) and 256sh nm (4.64); δ_H ($CDCl_3$) 7.33 (2 H, s, galloyl ArH), 7.13, 6.78, and 6.63 (1 H each, s, ArH), 6.73 (1 H, d, *J* 5 Hz, 1-H), 5.67 (1 H, dd, *J* 3 and 14 Hz, 5-H), 5.46 (1 H, dd, *J* 2 and 9 Hz, 4-H), 5.38 (1 H, t, *J* 2 Hz, 3-H), 5.01 (1 H, dd, *J* 14 and 3 Hz, 6-H), 4.83 (1 H, dd, *J* 2 and 5 Hz, 2-H), 4.22 (1 H, d, *J* 14 Hz, 6-H), 4.06—3.71 (15 × OMe), and 2.12 (3 H, s, OAc).

Methanolysis of Pentadeca-O-methylcasuarinin (11).—To a solution of compound (11) (50 mg) in absolute methanol (2 ml) was added 1% sodium methoxide (0.2 ml). After 16 h at room temperature, the mixture was acidified with acetic acid and evaporated to dryness. The residue was treated with ethereal diazomethane for 20 min, and purified by p.l.c. [chloroform–methanol (8 : 1, v/v)] to yield the *methanolysate* (14) (18 mg) (R_F 0.30) as an amorphous powder, $[\alpha]_D$ −62° (*c* 0.9, $CHCl_3$) (Found: C, 52.05; H, 5.7. $C_{27}H_{34}O_{15}\cdot 1.5H_2O$ requires C, 51.85; H, 5.95%); *m/z* 598 (M^+); $\nu_{max.}$ (KBr) 3 400, 1 750, 1 590, 1 480, 1 450, 1 420, 1 395, 1 345, 1 220, 1 095, and 1 020 cm^{-1}; $\lambda_{max.}$ (MeOH) 221 (log ε 4.67), 253 (4.20), and 301 nm (3.75); c.d. (MeOH) $[\theta]_{204}$ −4.4 × 10^4, $[\theta]_{227}$ +8.2 × 10^4, $[\theta]_{252}$ −7.6 × 10^4, and $[\theta]_{278}$ +1.3 × 10^4; δ_H (CD_3COCD_3) 7.46 (1 H, s, ArH), 5.74 (1 H, br s, 1-H), and 4.08—3.60 (7 × OMe).

The material eluted from the band of R_F 0.8—1.0 was further subjected to p.l.c. with light petroleum—dichloromethane–acetone (6 : 3 : 1, v/v) as eluant to give methyl tri-*O*-methylgallate (4 mg), m.p. 82—83 °C and dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (7) (6 mg), $[\alpha]_D$ −38° (*c* 1.0, EtOH), which were identical with authentic samples by m.s., i.r., and ^1H n.m.r. spectra.

Acetylation of (14).—The methanolysate (14) (9.2 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. Work-up as described above, followed by p.l.c. [benzene–ethanol (14 : 1, v/v)], gave the *penta-acetate* (15) (7.5 mg) as plates, m.p. 157—158 °C, $[\alpha]_D$ −5° (*c* 0.4, $CHCl_3$) (Found: C, 54.15; H, 5.5. $C_{37}H_{44}O_{20}\cdot 1.5H_2O$ requires C, 54.35; H, 5.55%); *m/z* 808 (M^+); $\nu_{max.}$ (KBr) 3 450, 1 780, 1 750, 1 725, 1 590, 1 480, 1 455, 1 420, 1 400, 1 365, 1 350, 1 215, and 1 040 cm^{-1}; $\lambda_{max.}$ (MeOH) 271 (log ε 4.71) 252sh (4.22), and 303 nm (3.77); δ_H 7.47 (1 H, s, ArH), 5.74 (4 H, br s,), 5.73 (1 H, d, *J* 10 Hz), 5.15 (1 H, dd, *J* 4 and 10 Hz), 4.22 (1 H, t, *J* 4 Hz), 4.11—3.63 (7 × OMe), 2.29 (3 H, s, OAc), 2.10 (6 H, s, 2 × OAc), 2.04 (3 H, s, OAc), and 1.75 (3 H, s, OAc).

Hydrolysis of (10) *with Tannase*.—A solution of casuasinin (10) (100 mg) in 0.1M acetate buffer (pH 5.4) (3 ml) was incubated for 48 h at 37 °C. The mixture was chromatographed over Sephadex LH-20 (1.3 mm i.d. × 6 cm). Elution with water yielded gallic acid (4 mg), m.p. 236—239 °C. Elution with methanol–water (1 : 3) then gave degalloylcasuarinin (39 mg) as a pale yellow, amorphous powder, identical with *casuariin* (17) (see above).

Dodeca-O-methylcasuariin (18).—A solution of casuariin (17) (20 mg) in methanol (1 ml) was treated with ethereal diazomethane for 2 h at room temperature. The crude methyl ether was purified by p.l.c. with benzene–acetone (1 : 1, v/v) as eluant to give the *title compound* (18) (3 mg) as an amorphous solid (Found: C, 57.1; H, 5.1. $C_{46}H_{48}O_{22}$ requires C, 56.9; H, 5.2%); *m/z* 952 (M^+), 476, 361, and 360; δ_H ($CDCl_3$) 6.99, 6.73, and 6.58 (each 1 H, s, ArH), 5.47 (1 H, d, *J* 5 Hz, 1-H), and 4.04—3.55 (12 × OMe).

Stachyurin (20).—An off-white, amorphous powder, R_F (A) 0.14, R_F (B) 0.26; $[\alpha]_D$ +39° (*c* 0.4, MeOH) (Found: C, 47.95; H, 3.95. $C_{41}H_{28}O_{26}\cdot 5H_2O$ requires C, 47.95; H, 3.75%); $\nu_{max.}$ (KBr) 3 400, 1 720, 1 610, 1 500sh, 1 445, and 1 340—1 300 cm^{-1}; $\lambda_{max.}$ (MeOH) 221 (log ε 4.88) and 267sh nm (4.53); c.d. (MeOH) $[\theta]_{230}$ +16 × 10^4, $[\theta]_{259}$ −4.4 × 10^4, $[\theta]_{282}$ +2.4 × 10^4, and $[\theta]_{308}$ −1.0 × 10^4; δ_H (200 MHz; CD_3COCD_3) 7.14 (2 H, s, galloyl ArH), 6.82, 6.55, and 6.50 (each 1 H, s, ArH), 5.62 (1 H, dd, *J* 2 and 9 Hz, 4-H), 5.36 (1 H, dd, *J* 9 and 3 Hz, 5-H), 4.98 (1 H, t, *J* 2 Hz, 3-H), 4.93 (1 H, d, *J* 2 Hz,1-H), 4.86

(1 H, t, *J* 2 Hz, 2-H), 4.84 (1 H, dd, *J* 3 and 13 Hz, 6-H), and 4.02 (1 H, d, *J* 13 Hz, 6-H).

Pentadeca-O-methylstachyurin (21).—A solution of stachyurin (20) (30 mg) in methanol (1 ml) was methylated with ethereal diazomethane for 1.5 h at room temperature. After removal of the solvent, the residue was purified by p.l.c. [benzene–acetone (4 : 1, v/v)] to give the *pentadecamethyl derivative* (21) (7 mg) as an amorphous powder, $[\alpha]_D$ −24° (*c* 1.0, $CHCl_3$) (Found: C, 57.65; H, 4.9. $C_{56}H_{58}O_{26}\cdot H_2O$ requires C, 57.75; H, 5.2%); *m/z* 1 146 (M^+); $\nu_{max.}$ (KBr) 3 450, 1 745, 1 590, 1 460, 1 395, 1 330, 1 200, 1 160, 1 120, and 1 100 cm^{-1}; $\lambda_{max.}$ (MeOH) 216 (log ε 4.96) and 257sh nm (4.53); δ_H (CD_3COCD_3) 7.36 (2 H, s, galloyl ArH), 7.16, 6.87, and 6.84 (each 1 H, s, ArH), 5.72 (1 H, dd, *J* 2 and 7 Hz, 5-H), 5.32 (1 H, dd, *J* 7 and 9 Hz, 4-H), 5.17 (1 H, s, 1-H), 5.06 (1 H, dd, *J* 2 and 9 Hz, 3-H), 4.98 (1 H, d, *J* 2 Hz, 2-H), 4.81 (1 H, dd, *J* 3 and 13 Hz, 6-H), 4.23 (1 H, d, *J* 13 Hz, 6-H), and 4.01—3.49 (15 × OMe).

Pentadeca-O-methylstachyurin Monoacetate (22).—A mixture of the alcohol (21) (8 mg), acetic anhydride (0.2 ml), and pyridine (0.2 ml) was kept overnight at room temperature. Removal of the solvent by co-evaporation with toluene gave the chromatographically homogeneous *monoacetate* (22) (8 mg), $[\alpha]_D$ −19° (*c* 0.4, $CHCl_3$) (Found: C, 57.65; H, 4.8. $C_{58}H_{60}O_{27}\cdot H_2O$ requires C, 57.7; H, 5.0%); *m/z* 1 188 (M^+); $\nu_{max.}$ (KBr) 3 450, 1 750, 1 590, 1 460, 1 405, 1 330, 1 200, 1 160, and 1 100 cm^{-1}; $\lambda_{max.}$ (MeOH) 214 (log ε 5.01) and 256sh nm 4.58); δ_H (CD_3COCD_3) 7.35 (2 H, s, galloyl ArH), 7.24, 6.86, and 6.66 (each 1 H, s, ArH), 6.18 (1 H, br s, 1-H), 5.70 (1 H, dd, *J* 3 and 7 Hz, 5-H), 5.56 (1 H, dd, *J* 7 and 9 Hz, 4-H), 5.21—5.11 (2 H, m, 2- and 3-H), 4.76 (1 H, dd, *J* 3 and 13 Hz, 6-H), 4.25 (1 H, d, *J* 13 Hz, 6-H), 4.01—3.53 (15 × OMe), and 2.12 (3 H, s, OAc).

Isomerization of Casuarinin (10) *to Stachyurin* (20).—A solution of casuarinin (10) (100 mg) in water (100 ml) was refluxed for 17.5 h. The solvent was evaporated to yield a brown residue which was methylated with ethereal diazomethane for 2 h at room temperature. The resulting residue was separated by p.l.c. with benzene–acetone (5 : 1, v/v; double development) to afford the methylated derivative (11) (2.3 mg) (R_F 0.6) and a pale yellow, amorphous powder (7 mg) (R_F 0.4) which was identical with pentadeca-*O*-methylstachyurin (21) ($[\alpha]_D$, m.s., and ^1H n.m.r. spectrum).

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