

CASUARICTIN AND CASUARININ, TWO NEW ELLAGITANNINS FROM CASUARINA STRICTA

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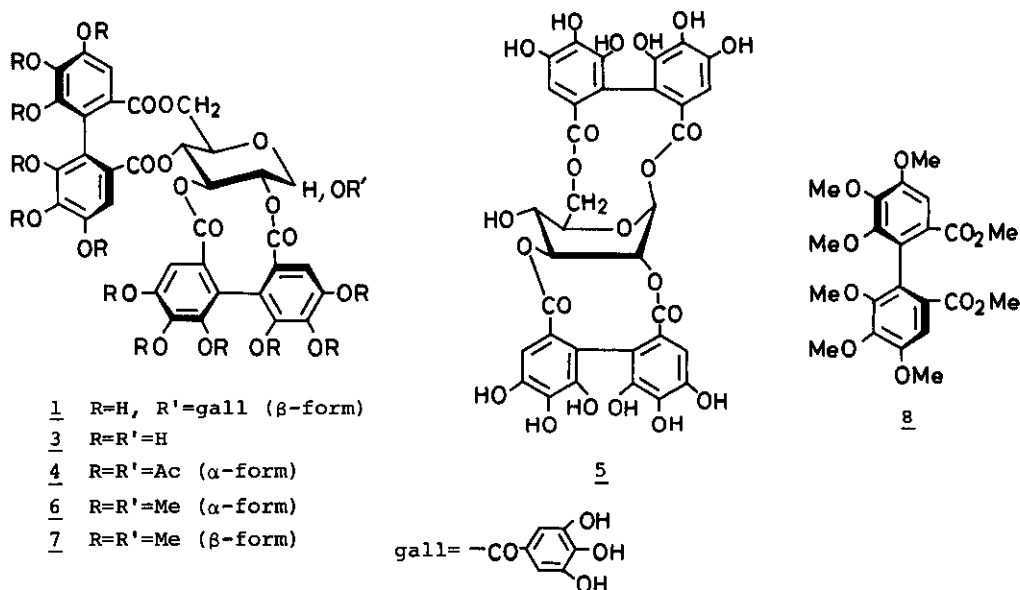
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Abstract — Two new ellagitannins, casuarictin (1) and casuarinin (2) were isolated from the leaves of *Casuarina stricta*, and their structures were elucidated. Pedunculagin (3) and tellimagrandin-I were also isolated, and the structure 3 including *S*-configurations of hexahydroxydiphenoyl groups was established.

The tannin of *Casuarina stricta* (Casuarinaceae), which is a tannin-rich tree, has been entirely unknown. We have isolated two new tannins, named casuarictin (1) and casuarinin (2), along with pedunculagin (3)^{1,2} and tellimagrandin-I³ (2,3-di-*O*-galloyl-4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-*D*-glucose). These tannins were obtained from the ethyl acetate soluble fraction of the leaf extract after droplet counter-current chromatography.

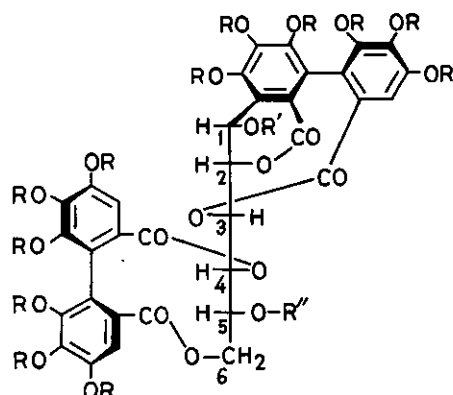
Pedunculagin (3) was obtained as an off-white amorphous powder, $C_{34}H_{24}O_{22} \cdot 5H_2O$, $[\alpha]_D +100^\circ$ ($c=1.0$, MeOH), which gave a tridecaacetate (α -anomer) (4), mp 240–241°, $C_{60}H_{50}O_{35} \cdot 2H_2O$, $[\alpha]_D -7.6^\circ$ ($c=1.4$, $CHCl_3$), and was identified by direct comparison with an authentic sample. Structure of pedunculagin was initially formulated by Schmidt *et al.*¹ as 3 without assignment of the configurations of hexahydroxydiphenoyl (HHDP) groups, and later structure 5 was postulated by Hillis *et al.*² based on the 1H nmr analysis. In the present investigation, the ^{13}C nmr spectrum (methanol- d_4) of 3 showed the signals of anomeric carbons at δ 92.2 and 95.6, and the double signals for the other carbons of glucose. The presence of free anomeric hydroxyl group in 3 was further confirmed by isolation of methylated anomers, (6) $C_{47}H_{50}O_{22}$, $[\alpha]_D +27.4^\circ$ ($c=1.0$, acetone), and (7), $[\alpha]_D -34.2^\circ$ ($c=1.0$, acetone), produced with diazomethane and also with dimethyl sulfate and potassium carbonate. The absolute configurations of the HHDP groups are assigned as *S*, since dimethyl hexamethoxydiphenate (8), $[\alpha]_D -38^\circ$ ($c=0.6$, EtOH), obtained by methanolysis of 6 or 7, was characterized as the enantiomer of (*R*)-dimethyl hexamethoxydiphenate

obtained from geraniin⁴, by optical rotation and cd spectrum (Fig. 1). The structure of pedunculagin was thus established as 2,3-4,6-di-O-[(S)-hexahydroxydiphenoyl]-D-glucose (3).



Casuarictin (1) was obtained as an off-white amorphous powder, $\text{C}_{41}\text{H}_{28}\text{O}_{26} \cdot 6\text{H}_2\text{O}$, $[\alpha]_D +35^\circ$ ($c=0.2$, MeOH). The ^1H nmr spectrum (acetone- d_6) of 1 showed the presence of a galloyl (δ 7.18, 2H, s) and two HHDP groups (δ 6.68, 6.55, 6.47 and 6.38, 1H each, s). The doublet attributable to an anomeric proton was also exhibited at δ 6.21 ($J=8$ Hz). Upon the treatment with tannase, 1 gave pedunculagin (3). These data indicate that casuarictin is 1-O-galloyl-2,3-4,6-di-O-[(S)-hexahydroxydiphenoyl]- β -D-glucose.

Casuarinin (2) was isolated as a pale yellow amorphous powder, $\text{C}_{41}\text{H}_{28}\text{O}_{26} \cdot 7\text{H}_2\text{O}$, $[\alpha]_D +43.6^\circ$ ($c=1.0$, MeOH), $\text{uv}\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 221 (4.87), 267 (sh) (4.51). The ^1H nmr spectrum (acetone- d_6) of 2 exhibited the proton peak of a galloyl group (δ 7.12, 2H, s) and three other aromatic protons (δ 6.78, 6.56 and 6.49, 1H each, s), along with seven sugar protons (δ 4.06~5.64). Five ester carbonyl peaks (δ 170.3, 169.5, 161.2, 166.4 and 165.0) were shown in the ^{13}C nmr spectrum. Methylation of 2 with diazomethane afforded pentadeca-O-methylcasuarinin (9), $\text{C}_{56}\text{H}_{58}\text{O}_{26} \cdot \text{H}_2\text{O}$, $[\alpha]_D -41^\circ$ ($c=0.5$, CHCl_3), M^+ 1146, which was treated with sodium methoxide in methanol to give methyl tri-O-methylgallate, 8, $[\alpha]_D -38^\circ$ ($c=0.6$, EtOH), and a heptamethyl derivative (10), $\text{C}_{27}\text{H}_{34}\text{O}_{15}$, $[\alpha]_D -62^\circ$ ($c=0.8$, CHCl_3), M^+



- 2 R=R'=H, R''=gall
9 R=Me, R'=H, R''=gall(Me)₃
11 R=Me, R'=Ac, R''=gall(Me)₃
12 R=R'=R''=H
14 R=Me, R'=R''=H

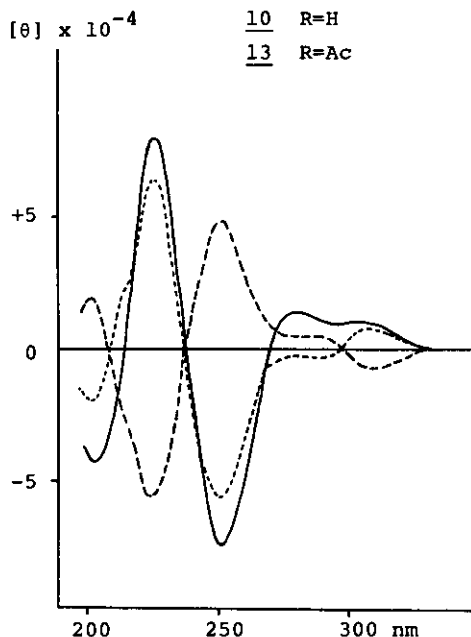
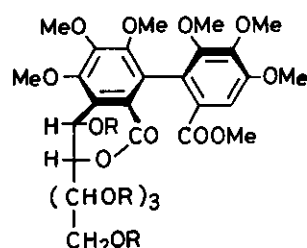
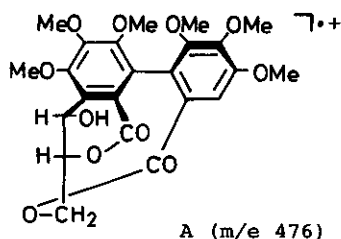
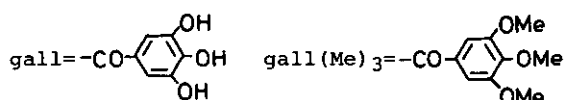


Fig. 1. CD spectra of 8 (----), (9)-dimethyl hexamethoxydiphenate(-.-.-) and 10 (—) in MeOH

598, ¹H nmr (CDCl₃): δ 7.43 (1H, s), 5.66 (1H, br.s), 4.03-3.54 (7 x OMe). The sugar was not liberated by the methanolysis described above. Upon the hydrolysis in refluxing 1N-H₂SO₄ for 2 hr, the sugar was not detected in the hydrolysates mixture from 2 by the gas chromatography after trimethylsilylation, while liberation of the sugar to a large extent was exhibited by the other ellagitannins having O-glycosidic linkage. Liberation of a small amount of glucose from 2 was observed only after refluxing for 20 hr. These behaviors of 2 are analogous to those of some C-glycosides^{5,6}, and indicate that 2 is a C-glucoside. Acetylation of 9 gave

a monoacetate (11), $C_{58}H_{60}O_{27}$, M^+ 1188, whose 1H nmr spectrum ($CDCl_3$) showed downfield shift of H-1 signal (d, $J=5$ Hz) (δ 5.50→6.73), to indicate the presence of a free hydroxyl group at C_1 of glucose in 2. The ^{13}C nmr spectrum (acetone- d_6) of 2, however, showed six glucose carbons at δ 64.5~76.7, excluding presence of the hemiacetal carbon whose peak is expected to be at δ 90.0~100.0. These results indicate that glucose in 2 is in the open-chain form. The orientation of the hydroxyl group at C_1 in 2 is regarded as in the structural formula by analogy of the coupling constant ($J_{1,2}=5$ Hz) in the 1H nmr spectrum, to that of castalagin⁷.

The treatment of 2 with tannase yielded degalloyl derivative (12), $C_{34}H_{24}O_{22} \cdot 5H_2O$. The marked upfield shift of the H-5 resonance in the 1H nmr spectrum of 12⁸ from that of 2 (δ 5.38→4.18), indicates that the galloyl group in 2 was at O-5 of glucose.

One of the two HHDP groups in 2 should be participating in the C-glucoside formation, since only three aromatic protons besides those of galloyl group are exhibited in the region of aromatic protons in the 1H nmr spectrum of 2, and the heptamethyl derivative (10) obtained by the methanolysis of 9 gave a pentaacetate (13), $C_{37}H_{44}O_{20}$, M^+ 808, 1H nmr ($CDCl_3$): δ 3.63~4.11 (7 x OMe), 1.75~2.29 (5 x OAc). The biphenyl configuration of 10 was determined to be S by comparison of the cd spectrum with that of 8 (Fig. 1). The ester linkage between the C-glucosidic HHDP group and glucose should be at O-2 and O-3 as indicated in 2 by the following findings. The mass spectrum of 14 which was obtained by methylation of 12 showed a prominent peak at m/e 476 due to the fragment ion A. Inspection of the molecular model of 2 with consideration of the coupling constants⁸ of glucose protons, excludes the reversed arrangement of two ester linkages at O-2 and O-3. The second HHDP group in 2 is, therefore, linked to O-4 and O-6 of glucose.

Based on these data, structure 2 is proposed for casuarinin.

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References and Notes

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8. Coupling constants in the ^1H nmr spectrum of 12; $J_{1,2}=5$, $J_{2,3}=3$, $J_{3,4}=3$, $J_{4,5}=9$, $J_{5,6}=3$, $J_{5,6'}=0$, $J_{6,6'}=12$ Hz. The assignments of these protons and the protons of 2 were confirmed by the decoupling experiments.

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