CASUARICTIN AND CASUARININ, TWO NEW ELLAGITANNINS FROM CASUARINA STRICTA

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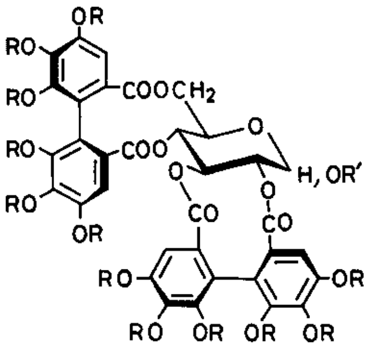
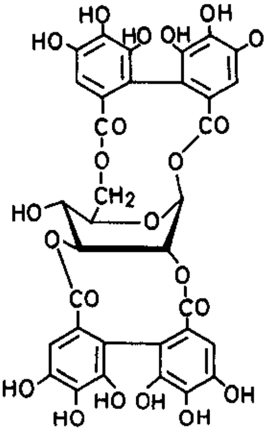
Abstract — Two new ellagitannins, casuarictin (1) and casuarinin (2) were isolated from the leaves of Casuarina stricta, and their structures were elucidated. Pedunculagin (3) and tellimagrandin-I were also isolated, and the structure 3 including S-configurations of hexahydroxydiphenoyl groups was established.

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-I[3] (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° (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° (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° (c=1.0, acetone), and (7), $[\alpha]_D$ -34.2° (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° (c=0.6, EtOH), obtained by methanolysis of 6 or 7, was characterized as the enantiomer of (R)-dimethyl hexamethoxydiphenate obtained from geraniin[4], 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).



1 R=H, R'=gall (β-form)
3 R=R'=H
4 R=R'=Ac (α-form)
6 R=R'=Me (α-form)
7 R=R'=Me (β-form)



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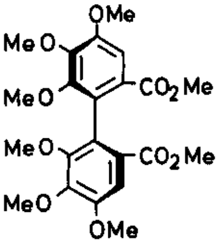
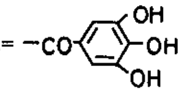


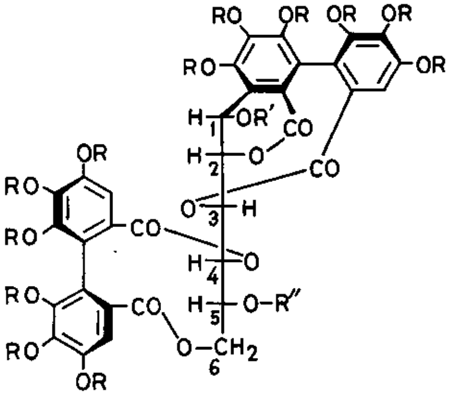
8

gall=

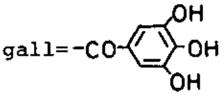


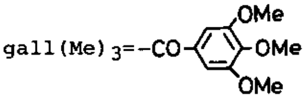
Casuarictin (1) was obtained as an off-white amorphous powder, $C_{41}H_{28}O_{26} \cdot 6H_2O$, $[\alpha]_D$ +35° (c=0.2, MeOH). The ^1H nmr spectrum (acetone-d_6) of 1 showed the presence of a galloyl (δ 7.18, 2H, s) and two HHDP groups (δ 6.68, 6.55, 6.47 and 6.38, 1H each, s). The doublet attributable 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.

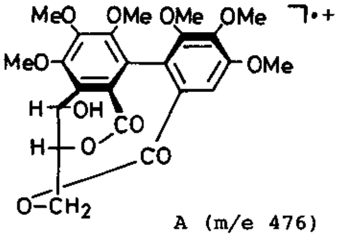
Casuarinin (2) was isolated as a pale yellow amorphous powder, $C_{41}H_{28}O_{26} \cdot 7H_2O$, $[\alpha]_D$ +43.6° (c=1.0, MeOH), uvλ_{max}^{MeOH} nm (log ε): 221 (4.87), 267 (sh)(4.51). The ^1H nmr spectrum (acetone-d_6) of 2 exhibited the proton peak of a galloyl group (δ 7.12, 2H, s) and three other 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), $C_{56}H_{58}O_{26} \cdot H_2O$, $[\alpha]_D$ -41° (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° (c=0.6, EtOH), and a heptamethyl derivative (10), $C_{27}H_{34}O_{15}$, $[\alpha]_D$ -62° (c=0.8, $CHCl_3$), M^+



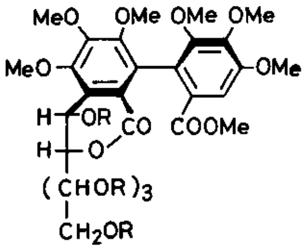
2 R=R'=H, R"=gall
9 R=Me, R'=H, R"=gall(Me)$_3$
11 R=Me, R'=Ac, R"=gall(Me)$_3$
12 R=R'=R"=H
14 R=Me, R'=R"=H







A (m/e 476)



10 R=H
13 R=Ac

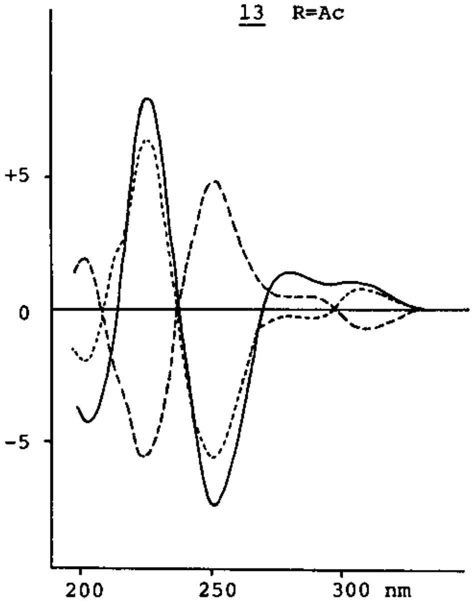


Fig. 1. Cd spectra of 8 (-----), (R)-dimethyl hexamethoxydiphenate(---) and 10 (—) in MeOH

598, ^{1}H nmr (CDCl$_3$): 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-H_2SO_4 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 castalagin[7].

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 12[8] 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 constants[8] 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 ^{1}H 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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