CASUARICTIN AND CASUARININ, TWO NEW ELLAGITANNINS FROM CASUARINA STRICTA

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

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 of including 5-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) and tellimagrandin-I (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}.5H_{2}O, [α]D+100° (c=1.0, MeOH), which gave a tridecaacetate (α-anomer) (4), mp 240-241°, C_{60}H_{50}O_{35}.2H_{2}O, [α]D-7.6° (c=1.4, CHCl₃), 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 ¹³C nmr spectrum (methanol-d₄) 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}, [α]D+27.4° (c=1.0, acetone), and (7), [α]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 5, since dimethyl hexamethoxydiphenate (6), [α]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\textsuperscript{4}, by optical rotation and cd spectrum (Fig. 1). The structure of pedunculagin was thus established as 2,3-4,6-di-β-O-[(S)-hexahydroxydiphenoyl]-β-D-glucose (3).

![Chemical structures]

1. \(R=H, R'=\text{gall (β-form)}\)
2. \(R=R'=\text{H}\)
3. \(R=R'=\text{Ac (α-form)}\)
4. \(R=R'=\text{Me (α-form)}\)
5. \(R=R'=\text{Me (β-form)}\)

Casuarictin (1) was obtained as an off-white amorphous powder, \(C_{41}H_{28}O_{26}\cdot6H_2O, [\alpha]_D+35^\circ (c=0.2, \text{MeOH})\). The \(^1H\) nmr spectrum (acetone-d\textsuperscript{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, \(C_{41}H_{28}O_{26}\cdot7H_2O, [\alpha]_D+43.6^\circ (c=1.0, \text{MeOH}), \text{uvλ}_{\text{max}}^\text{MeOH} \text{nm (log ε)}: 221 (4.87), 267 (sh)(4.51).\) The \(^1H\) nmr spectrum (acetone-d\textsuperscript{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 \(^13C\) nmr spectrum.

Methylation of 2 with diazomethane afforded pentadeca-O-methylcasuarinin (9), \(C_{56}H_{58}O_{26}\cdotH_2O, [\alpha]_D-41^\circ (c=0.5, \text{CHCl}_3), M^+1146,\) which was treated with sodium methoxide in methanol to give methyl tri-O-methylgallate, 8, \([\alpha]_D-38^\circ (c=0.6, \text{EtOH})\), and a heptamethyl derivative (10), \(C_{27}H_{34}O_{15}, [\alpha]_D-62^\circ (c=0.8, \text{CHCl}_3), M^+\)

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1682---
2: R=R'=H, R''=gall
3: 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

gall=\text{-CO-}\text{OH} \quad \text{gall(Me)₃=}\text{-CO-}\text{OMe} \quad \text{OMe}

Gallenate

\text{MeO} \quad \text{OMe} \quad \text{OMe} \quad \text{OMe} \quad \text{OMe}

A (m/e 476)

Fig. 1. \text{C}δ \text{ spectra of 8 (-----), (R)\text{-dimethyl hexamethoxydiphenate(----)}} \quad \text{and 10 (-----) in MeOH}

598, \text{H 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-glycosides⁵,⁶, 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) (\( \delta 5.50 \rightarrow 6.73 \)), 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 \( \delta 64.5-76.7 \), excluding presence of the hemiacetal carbon whose peak is expected to be at \( \delta 90.0-100.0 \). 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 from that of 2 (\( \delta 5.38 \rightarrow 4.18 \)), 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 methanolation of 9 gave a pentaacetate (13), \( C_{37}H_{44}O_{20} \), \( M^+ 808 \), \( ^1H \) nmr (CDCl\(_3\)): \( \delta 3.63-4.11 \) (7 x OMe), 1.75-2.29 (5 x OAc). The biphenyl configuration of 10 was determined to be \( \alpha \) 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 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
3. C. K. Wilkins and B. A. Bohm, Phytochemistry, 15, 211 (1976). The trivial name, in addition to tellimagrandin-II for 1,2,3-tri-O-galloyl-4,6-O-NNDP-β-D-glucose, has been informed by these authors in a private communication. The absolute configurations at the biphenyl moiety in these tannins were determined recently: T. Okuda, T. Hatano, and T. Yasui, Heterocycles, in press.


8. Coupling constants in the $^1$H nmr spectrum of 12: $J_{1,2}=5$, $J_{2,3}=3$, $J_{3,4}=3$, $J_{4,5}=9$, $J_{5,6}=3$, $J_{6,7}=0$, $J_{6,7}=12$ Hz. The assignments of these protons and the protons of 2 were confirmed by the decoupling experiments.

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