Revised Structure of Cercidinin A, a Novel Ellagitannin Having (*R*)-Hexahydroxydiphenoyl Esters at the 3,4-Positions of Glucopyranose

Takashi TANAKA,[a] Gen-ichiro NONAKA,[b,1)] Makoto ISHIMATSU,[b,2)] Itsuo NISHIOKA,[b] and Isao KOUNO*,[a]

School of Pharmaceutical Sciences, Nagasaki University,[a] 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,[b] 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.
Received October 30, 2000; accepted December 9, 2000

The structue of cercidinin A, an ellagitannin isolated from the bark of *Cercidiphyllum japonicum*, was revised to 1,2,6-tri-*O*-galloyl-3,4-(*R*)-hexahydroxydiphenoyl-β-D-glucose by two-dimensional NMR spectral analysis. Cercidinin A represents the first ellagitannin possessing a hexahydroxydiphenoyl group at the 3,4-positions of a modified $^4C_1$-glucopyranose core.

Key words *Cercidiphyllum japonicum*; Cercidiphyllaceae; cercidinin A; tannin; ellagitannin

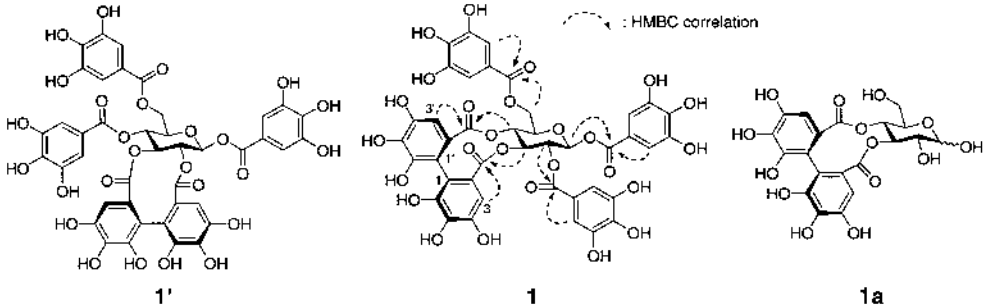
In the course of our chemical study on tannins, cercidinin A had been isolated from the bark of *Cercidiphyllum japonicum* SIEB. et ZUCC. (Cercidiphyllaceae) and characterized as 1,4,6-tri-*O*-galloyl-2,3-(*R*)-hexahydroxydiphenoyl (HHDP)-β-D-glucose (**1′**).[3)] Recently, Khanbabaee and Lötzerich synthesized the 1,4,6-tri-*O*-galloyl-2,3-(*R*)-HHDP-β-D-glucose and pointed out that the NMR data of the product were not identical to those reported for cercidinin A.[4)] This paper deals with reexamination and revision of the structure of cercidinin A and the significance of this compound as the first ellagitannin possessing a hexahydroxydiphenoyl group at the 3,4-position of glucose, from the viewpoint of biogenesis of ellagitannins.

Results and Discussion

In the previous work,[3)] cercidinin A was characterized by one-dimensional ^{1}H- and ^{13}C-NMR analysis and chemical examination. Methylation, followed by methanolysis, yielded (*R*)-dimethyl hexamethoxydiphenate ($[\alpha]_D$ +27.1°) and methyl trimethoxybenzoate, unequivocally indicating the presence of a (*R*)-HHDP and galloyl groups in the molecule. Presence of a glucose core was verified by acid hydrolysis. The location of the esters on the glucopyranose was deduced from the result of selective hydrolysis of galloyl groups with tannase. Because of low resolution of the ^{1}H-NMR spectra (100 MHz) in those days, complete assignment of the sugar proton signals of the hydrolysate, an anomer mixture of the HHDP-glucose, could not be achieved. However, complex signal patterns observed in the ^{1}H-NMR spectrum resembled that of 2,3-(*S*)-HHDP glucose.

In the present work, we first measured the ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) and the heteronuclear single quantum coherence (HSQC) spectrum of cercidinin A and made complete assignment for the proton and carbon signals arising from the glucose moiety. In the heteronuclear multiple bond correlation (HMBC) spectrum, signals attributable to glucose H-3 (δ 5.63, dd, J=8.8, 10.1 Hz) and H-4 (δ 5.36, dd, J=8.8, 10.1 Hz) were correlated with the ester carboxyl signals at δ 169.1 and 168.7, respectively. The ester carbons, in turn, coupled with the proton signals at δ 6.47 and δ 6.71 (each 1H, s), which were attributable to the aromatic protons of the HHDP group. Furthermore, the remaining carboxyl carbons of three galloyl groups, resonated at δ 165.0, 165.9, and 166.5, were coupled with the glucose H-1 (δ 6.28, d, J=8.1 Hz), H-2 (δ 5.58, dd, J=8.1, 10.1 Hz) and H-6 (δ 4.69, br d, J=10.8 Hz, δ 4.54, dd, J=4.2, 10.8 Hz), respectively. These HMBC correlations unambiguously clarified the location of the (*R*)-HHDP esters at the C-3 and C-4 positions of the glucose core. The 500 MHz ^{1}H-NMR signals of the tannase hydrolysate, 3,4-(*R*)-HHDP-glucose (**1a**), were also completely assigned, and a large low field shift of the signals, attributable to the glucose H-3 and H-4, confirmed the above conclusion (see Experimental). On the basis of these results, the structure of cercidinin A was revised as shown in formula **1**. Cercidinin B,[3)] which had been characterized as 1-desgalloylcercidinin A, was also revised to 2,6-di-*O*-galloyl-3,4-(*R*)-HHDP-D-glucopyranose.

The ^{1}H-NMR coupling constant between H-3 and H-4 (8.8 Hz) of **1** was slightly smaller than that observed for normal $^4C_1$ glucopyranose ($J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9—10 Hz), suggesting that this glucose possessed a modified $^4C_1$ conformation. In addition, the coupling patterns of the pyranose ring protons of **1a** indicated that the pyranose conformation of the α-form ($J_{2,3}$=10.3 Hz, $J_{3,4}$=9.4 Hz, $J_{4,5}$=10.5 Hz) was slightly different from that of the β-form, taking $^4C_1$ conformation ($J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.7 Hz).



1′ **1** **1a**

* To whom correspondence should be addressed. e-mail: ikouno@net.nagasaki-u.ac.jp

Most monomeric ellagitannins are structurally and biogenetically classified into two groups.[5] Tannins, belonging to the first group (group A), have the $^4C_1$ glucopyranose core, and usually the (*S*)-HHDP esters are located at the 2,3-positions and/or 4,6-positions of the pyranose ring. C-Glycosidic ellagitannins, having an open-chain glucose core, are also biogenetically related to this group.[6] Tannins, belonging to the other group (group B), possess the glucose core with $^1C_4$ or related boat conformation. In these ellagitannins, (*R*)- or (*S*)-HHDP esters (or its oxidized form, dehydrohexahydroxydiphenoyl esters) are attached to the 1,6-; 1,3-; 2,4-; or 3,6-positions of the pyranose ring.[7] Cercidinin A belongs to the group A because it possesses a modified $^4C_1$-glucopyranose core and represents the first ellagitannin having an HHDP group at the C-3 and C-4 positions. The biphenyl bond of the HHDP group is formed by oxidative coupling between two galloyl groups attached to the glucopyranose core,[5] and its atrop isomerism depends on the position where the galloyl groups are located, though there are a few exceptions.[3,6,8] In this respect, cercidinin A is the first and sole example indicating that oxidative coupling between two galloyl groups, located at the 3,4-positions of glucose, generates an (*R*)-HHDP group.

Experimental

General ^{1}H- and ^{13}C-NMR spectra were obtained with Varian Unity plus 500 spectrometer operating at 500 MHz for ^{1}H, and 125 MHz for ^{13}C, respectively. the HMBC experiment ($^nJ_{CH}$ optimized for 8 Hz) was performed using standard Varian pulse sequences.

Cercidinin A (1) An off-white amorphous powder, $[\alpha]_D$ −71.6° (*c*=1.0, acetone), FAB-MS *m/z*: 961 $(M+Na)^+$, ^{1}H-NMR (500 Hz, acetone-d_6+D_2O, 9 : 1, v/v) δ: 7.15 (2H, s, C-6 galloyl-H), 7.11 (4H, s, C-1 and C-2 galloyl-H), 6.71 (1H, s, HHDP-3′), 6.47 (1H, s, HHDP-3), 6.28 (1H, d, *J*=8.1 Hz, H-1), 5.63 (1H, dd, *J*=8.8, 10.1 Hz, H-3), 5.58 (1H, dd, *J*=8.1, 10.1 Hz, H-2), 5.36 (1H, dd, *J*=8.8, 10.1 Hz, H-4), 4.69 (1H, br d, *J*=10.8 Hz, H-6), 4.54 (1H, dd, *J*=4.2, 10.8 Hz, H-6), 4.51 (1H, br dd, *J*=4.2, 10.1 Hz, H-5). ^{13}C-NMR (125 MHz, acetone-d_6+D_2O) δ: 169.1 [COO (connected to glucose C-3)], 168.7 [COO (C-4)], 166.5 [COO (C-6)], 165.9 [COO (C-2)], 165.0 [COO (C-1)], 146.1, 146.0 [galloyl-3, 5 (C-1, 2)], 145.9 [galloyl-3, 5 (C-6)], 145.2, 145.1 (HHDP-4, 4′), 144.4, 144.3 (HHDP-6, 6′), 139.8, 139.5 [galloyl-4 (C-1, 2)], 139.0 [galloyl-4 (C-6)], 136.4, 136.4 (HHDP-5, 5′), 126.1, 126.0 (HHDP-2, 2′), 120.9 [galloyl-1 (C-6)], 120.16. 119.5 [galloyl-1 (C-1, 2)], 114.6, 114.5 (HHDP-1, 1′), 110.2, 110.0 [galloyl-2, 6 (C-1, 2)], 109.9 [galloyl-2, 6 (C-6)], 107.5 (HHDP-3′), 107.3 (HHDP-3), 93.5 (C-1), 77.0 (C-3), 72.7 (C-5), 72.6 (C-4), 70.5 (C-2), 62.5 (C-6).

Tannase Hydrolysis Compound **1** (45 mg) in water (3 ml) was stirred with tannase at room temperature for 4 h. The mixture was directly subjected to Sephadex LH-20 column chromatography (1.5 cm i.d.×25 cm) with water containing increasing proportions (0→40%) of MeOH to afford gallic acid (23.5 mg) and **1a** (20.0 mg) as a white amorphous powder, $[\alpha]_D$ +12.9° (*c*=1.0, MeOH), ^{1}H-NMR (500 Hz, acetone-d_6+D_2O, 9 : 1, v/v) α form δ: 6.72, 6.61 (each s, HHDP-H), 5.34 (dd, *J*=9.4, 10.3 Hz, H-3), 5.31 (d, *J*=3.7 Hz, H-1), 4.91 (dd, *J*=9.4, 10.5 Hz, H-4), 4.16 (ddd, *J*=2.3, 5.5, 10.5 Hz, H-5), 3.80 (dd, *J*=3.7, 10.3 Hz, H-2), 3.77 (dd, *J*=2.3, 12.1 Hz, H-6a), 3.68 (dd, *J*=5.5, 12.1 Hz, H-6b); β form δ: 6.70, 6.61 (each s, HHDP-H), 5.05 (t, *J*=9.7 Hz, H-3), 4.91 (t, *J*=9.7 Hz, H-4), 4.75 (d, *J*=7.6 Hz, H-1), 3.81 (dd, *J*=2.3, 12.1 Hz, H-6a), 3.76 (ddd, *J*=2.3, 5.5, 9.7 Hz, H-5), 3.66 (dd, *J*=5.5, 12.1 Hz, H-6b), 3.58 (dd, *J*=7.6, 9.7 Hz, H-2); (α : β molar ratio=3 : 2).

References and Notes

1) Present address: *Saga Prefectural Institute for Pharmaceutical Research, Shuku-machi, Tosu, Saga 841–0052, Japan.*
2) Present address: *Tsumura Central Research Laboratories, 3586 Yoshihara, Ami-machi, Inashiki- gun, Ibaragi 300–1192, Japan.*
3) Nonaka G., Ishimatsu M., Ageta M., Nishioka I., *Chem. Pharm. Bull.*, **37**, 50—53 (1989).
4) Khanbabaee K., Lötzerich K., *J. Org. Chem.*, **63**, 8723—8728 (1998).
5) Haslam E., Cai Y., *Nat. Prod. Rep.*, **1994**, 41—66.
6) Tanaka T., Kirihara S., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **41**, 1708—1716 (1993).
7) Lee S., Tanaka T., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **39**, 630—638 (1991).
8) Ishimatsu M., Tanaka T., Nonaka G., Nishioka I., Nishizawa M., Yamagishi T., *Chem. Pharm. Bull.*, **37**, 129—134 (1989).