

Pergamon

THREE DITERPENE DILACTONE GLYCOSIDES FROM PODOCARPUS NAGI

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Key Word Index—*Podocarpus nagi*; Podocarpaceae; diterpene dilactone; glycoside; nagilactosides C-E.

Abstract—Three water-soluble constituents, nagilactosides C–E, were isolated from *Podocarpus nagi*. Their structures were determined by chemical and spectroscopic methods, respectively. Nagilactoside C was identified as 1-deoxy-nagilactone A- 2α -O- β -D-glucopyranosyl- $(1 \rightarrow 3)$ - β -D-glucopyranoside, nagilactoside D as 1-deoxy-nagilactone A- 2α -O- β -D-glucopyranosyl- $(1 \rightarrow 6)$ - β -D-glucopyranoside, and nagilactoside E as nagilactone A- 1β -O- β -D-glucopyranoside, or spectral constraints and the set of the

INTRODUCTION

The diterpene dilactones of *Podocarpus nagi* (Thunb.) Zoll. et Mor. exZoll have been extensively studied because of their antitumour, plant inhibitory, insecticidal and other biological activities [1]. In the course of our studies on the antitumour constituents from the seed of *P. nagi*, we recently discovered two diterpene dilactone glycosides each of which contained one sugar moiety, nagilactosides A (4) [2] and B (5) [3]. Further investigation of the polar fractions has now led to the isolation of another three new glycosides each with a disaccharide moiety, nagilactosides C (1), D (2) and E (3).

RESULTS AND DISCUSSION

Compounds 1-3 were all obtained as amorphous powders. The characteristic absorptions of A-type diterpene dilactone were observed at 1760–1765 cm⁻¹ (γ -lactone), 1700–1705, 1620–1625, 1540–1545 cm⁻¹ (α -pyranone) in their IR spectra and at 300 nm in their UV spectra [1]. In their positive FAB-mass spectra, 1-3 showed the same molecular ion peak at m/z 673 [M + H]⁺ and two fragment ion peaks at m/z 511 [MH - 162]⁺ and 349 $[MH - 2 \times 162]^+$ indicative of the successive loss of two sugar residues. After hydrolysis with cellulase HPTLC of the hydrolysates from 1 and 2 afforded nagilactoside A (4), 1-deoxy- 2α -hydroxy-nagilactone A (6) and glucose, while that from 3 gave nagilactoside B (5), nagilactone A and glucose. These findings suggested that 1 and 2 were composed of 4 and a second glucose unit, while 3 was composed of 5 and a second glucose unit.

In the ¹H NMR spectra (Table 1), the proton signals of the OH-7 β in the aglycone was found at δ 5.78 for 1, 5.77

for 2 and 5.83 for 3. This indicated that the sugars in 1–3 existed as a disaccharide moiety. Analysis of the ¹³CNMR spectral data (Table 2) led to the determination of the linkage position and the configuration of the bond between the two sugars. For 1, the diagnostic lowfield shift of the inner glucose C-3' (δ 87.4) indicated that the two sugars were linked through a 1 \rightarrow 3 glycosidic bond. The 1 \rightarrow 6 glycosidic linkage of 2 and 3 was established by the lowfield shift of the inner glucose C-6' (δ 69.1 for 2 and 68.7 for 3). The glycosidic configurations of 1–3 were assigned as β from the chemical shifts of the anomeric carbons ($\delta > 100$) and the coupling constant of the anomeric protons ($J \approx 7.5$ Hz).

Consequently, the structure of nagiloctoside C (1) is 1-deoxy-nagilactone A- 2α -O- β -D-glucopyranosyl



н	1	2	3
1α	1.77, dd (6.3, 13.8)	1.72, dd (6.1, 13.6)	
1β	2.28, dd (9.5, 13.5)	2.28, dd (9.8, 13.2)	3.44, m
2α			1.68, m
2β	4.06, m	4.06, m	2.25, m
3α	1.88, dd (5.3, 13.5)	1.87, dd (4.7, 13.2)	1.49, m
3β	2.00, t (13.1)	2.00, dd (12.9, 13.1)	1.82, m
5α	1.92, d (5.0)	1.94, d (6.3)	1.87, d (4.5)
6α	5.01, brs	5.04, brs	4.98, m
7α	5.19, dd (9.2, 4.5)	5.20, dd (4.2, 8.2)	5.18, m
Ο-7β	5.78, d (4.3)	5.77, d (3.9)	5.83, d (4.3
11	5.94, s	5.94, s	5.87, s
15	3.23, m	3.25, m	3.23, m
CH₃-16, 17	1.19, d (6.8)	1.19, d (6.7)	1.19, d (6.7)
	1.15, d (6.8)	1.16, d (6.7)	1.16, d (6.7)
CH ₃ -18, 20	1.36, s	1.36, s	1.44, s
	1.29, s	1.31, s	1.31, s
1'†	4.37, d (7.7)	4.25, d (7.4)	4.28, d (7.8)
1Ӡ	4.33, d (7.7)	4.25, d (7.4)	4.22, d (7.6)

Table 1. ¹H NMR spectral data of compounds 1–3 (400 MHz, DMSO-d₆)*

*The signals were assigned by ¹H-¹H-COSY.

†Other sugar proton signals showed a very complicated pattern and could not be distinguished from each other.

Table 2. ¹³C NMR spectral data of compounds 1-3 (25.05 MHz, DMSO-d₆)*

С	1	2	3
1	40.4	40.4	82.4
2	71.9	72.1	26.4
3	33.5	33.6	30.9
4	42.3	42.6	46.5
5	47.7	47.9	50.9
6	74.1	74.9	74.1
7	59.1	59.3	59.5
8	111.6	111.8	111.5
9	169.9	170.4	169.2
10	36.8	37.0	35.3
11	105.6	105.9	105.5
12	162.5	163.2	161.4
14	167.1	167.4	166.3
15	28.9	29.1	28.3
16, 17	20.6	20.8	19.8
	20.6	20.8	20.1
18, 20	26.7	27.1	21.9
	23.8	24.0	21.4
19	182.2	182.7	176.2
1′	101.6	102.1	103.3
2'	72.8	73.9	72.7
3'	87.4	77.0	76.8
4′	68.7	70.5	70.2
5'	77.1	76.0	75.7
6'	61.4	69.1	68.7
1″	104.1	103.7	103.3
2″	74.6	73.9	73.5
3″	76.4	77.0	76.8
4″	70.5	70.5	70.2
5″	76.4	77.0	76.0
6″	61.4	61.6	61.6

*Signals were assigned by DEPT and comparison with known compounds.

 $(1 \rightarrow 3)$ - β -D-glucopyranoside, nagilactoside D (2) is 1-deoxy-nagilactone A-2 α -O- β -D-glucopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (2) and nagilactoside E (3) is nagilactone A-1- β -O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside.

EXPERIMENTAL

General. ¹H NMR: 400 MHz; ¹³C NMR: 25MHz; FAB-MS: Hp-5989 spectrometer; TLC: precoated silica gel 60 F_{254} plates (Merck, 0.2 mm thickness); CC: TSK gel Toyopearl HW-40F (30–60 μ , Toso Co. Ltd.), MCI gel CHP 20P (25–150 μ , Mitsubishi Chemical Industries Co. Ltd) and Cosmosil 75 C₁₈-OPN (42–105 μ , Nacalai Tesque Inc.) columns. The crude cellulase (12 000 γ mg⁻¹) preparation was produced in our laboratory.

Plant material. The seed of Podocarpus nagi was collected in the Renghua district, Guangdong, China. A voucher specimen is deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation of the compounds. Defatted seed powder (15 kg) was extracted (\times 3) with EtOH at room temp. After concn, the extract (700 g) was subjected to a silica gel CC with C₆H₆, CH₂Cl₂, Me₂CO and MeOH as eluent. The MeOH fraction (150 g) was applied to a MCI gel column (500 g), eluting with H₂O containing an increasing proportion of MeOH to afford 2 fractions. Fr. I (120 g), eluted with H₂O, gave a large amount of sucrose (120 g), and fr. II (25 g), eluted with aq. 40% MeOH, was rechromatographed on a MCI gel column (300 g) with H₂O-MeOH (0-40% gradient) to afford frs. II-1-II-5. On Repeated chromatography over Cosmosil 75 C₁₈-OPN, TSK gel Toyopearl HW-40F and MCI gel CHP 20P with 30-40% MeOH, frs. II-3 and II-4 gave 1 (2.5 g), 2 (1.8 g), and 3 (0.5 g). Cellulase hydrolysis of compounds 1–3. A soln of 1, 2 or 3 (2 mg) in 2 ml 0.1 M HOAc–NaOAc buffer (pH 4.5) was incubated at 40° with cellulase (1 mg) for 0.5 hr. The partially hydrolysed products were identified by comparison with authentic samples on silica gel HPTLC with EtOH–EtOAc–H₂O (200:100:1) as developing solvent. After 4 hr the glycosides were completely hydrolysed and only glucose and aglycone were detected.

Compound 1. Amorphous powder. Found: C, 52.21; H, 6.44. $C_{31}H_{44}O_{16} \cdot 9/4 H_2O$ requires: C, 52.21; H, 6.85. $[\alpha]_D^{26} + 41.09^{\circ}$ (H₂O; c0.1). FAB-MS m/z: 695 $[M + Na]^+$, 673 $[M + H]^+$, 511 $[M + H - (Glc - H_2O)]^+$; UV $\lambda_{max}^{H_2O}$ nm: 300; IR ν_{max}^{Har} cm⁻¹: 1765, 1705, 1625, 1545, 1080; CD: $\Delta \varepsilon_{208} + 9.98^{\circ}$, $\Delta \varepsilon_{235} - 1.10^{\circ}$, $\Delta \varepsilon_{295} + 0.92^{\circ}$ (H₂O; c0.3); ¹H and ¹³C NMR: Tables 1 and 2.

Compound 2. Amorphous powder. Found: C, 52.65; H, 6.61. $C_{31}H_{44}O_{16} \cdot 2H_2O$ requires: C, 52.54; H, 6.83. $[\alpha]_{2}^{D^6} + 53.32^{\circ}$ (H₂O; c0.8) FAB-MS m/z: 695 [M + Na]⁺, 673 [M + H]⁺, 511 [M + H - (Glc - H₂O)]⁺, 349 [M + H - 2(Glc - H₂O)]⁺; UV $\lambda_{max}^{H_{2}O}$ nm: 300; IR ν_{max}^{KBr} cm⁻¹: 1760, 1700, 1620, 1540, 1070; CD: $\Delta \varepsilon_{208}$ + 6.78°, $\Delta \varepsilon_{235}$ - 1.05°, $\Delta \varepsilon_{295}$ + 0.98° (H₂O; *c* 0.3); ¹H and ¹³C NMR: Tables 1 and 2.

Compound 3. Amorphous powder. Found: C, 53.23;H, 6.73. $C_{31}H_{44}O_{16} \cdot 3/2 H_2O$ requires: C, 53.21; H, 6.77. $[\alpha]_D^{26} - 12.73^{\circ} (H_2O; c 0.5)$. FAB-MS m/z: 695 $[M + Na]^+$, 673 $[M + H]^+$, 511 $[M + H - (Glc - H_2O)]^+$; 349 $[M + H - 2(glc - H_2O)]^+$; UV $\lambda_{max}^{H_{20}}$ nm: 300; IR ν_{max}^{KBr} cm⁻¹: 1760, 1700, 1620, 1545, 1080; CD: $\Delta \varepsilon_{208} + 7.30^{\circ}$, $\Delta \varepsilon_{235} + 2.00^{\circ}$, $\Delta \varepsilon_{295} + 0.40^{\circ}$ (H₂O; c 0.3); ¹H and ¹³C NMR: Tables 1 and 2.

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