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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 → 3)-β-D-glucopyranoside, nagilactoside D as 1-deoxy-nagilactone A-2α-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside, and nagilactoside E as nagilactone A-1β-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyransoide.

INTRODUCTION

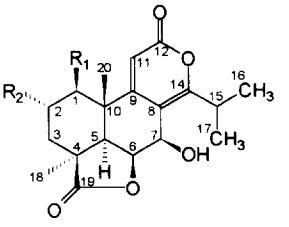
The diterpene dilactones of *Podocarpus nagi* (Thunb.) Zoll. et Mor. exZoll have been extensively studied because of their antitumour, plant inhibitory, insecticidal and other biological activities [1]. In the course of our studies on the antitumour constituents from the seed of *P. nagi*, we recently 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-7β in the aglycone was found at δ5.78 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 ^{13}C NMR 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' (δ87.4) indicated that the two sugars were linked through a 1 → 3 glycosidic bond. The 1 → 6 glycosidic linkage of **2** and **3** was established by the lowfield shift of the inner glucose C-6' (δ69.1 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 (δ > 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



	R_1	R_2
1.	H	β-D-glc-(1 → 3)-β-D-glc-
2.	H	β-D-glc-(1 → 6)-β-D-glc-
3.	β-D-glc-(1 → 6)-β-D-glc-	H
4.	H	β-D-glc-
5.	β-D-glc-	H
6.	H	OH
7.	OH	H

Table 1. ^{1}H NMR spectral data of compounds 1–3 (400 MHz, DMSO-d_6)*

H	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*
O-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*
CH_3-16, 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)
1"†	4.33, *d* (7.7)	4.25, *d* (7.4)	4.22, *d* (7.6)

*The signals were assigned by ^{1}H–^{1}H-COSY.

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

Table 2. ^{13}C NMR spectral data of compounds 1–3 (25.05 MHz, DMSO-d_6)*

C	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 → 3)-β-D-glucopyranoside, nagilactoside D (**2**) is 1-deoxy-nagilactone A-2α-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside (**2**) and nagilactoside E (**3**) is nagilactone A-1-β-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside.

EXPERIMENTAL

General. ^{1}H NMR: 400 MHz; ^{13}C 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 (30–60μ, Toso Co. Ltd.), MCI gel CHP 20P (25–150μ, Mitsubishi Chemical Industries Co. Ltd) and Cosmosil 75 C_{18}-OPN (42–105μ, Nacalai Tesque Inc.) columns. The crude cellulase (12 000γ 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 (× 3) with EtOH at room temp. After concn, the extract (700 g) was subjected to a silica gel CC with C_6H_6, CH_2Cl_2, Me_2CO and MeOH as eluent. The MeOH fraction (150 g) was applied to a MCI gel column (500 g), eluting with H_2O containing an increasing proportion of MeOH to afford 2 fractions. Fr. I (120 g), eluted with H_2O, 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 H_2O–MeOH (0–40% gradient) to afford frs. II-1–II-5. On Repeated chromatography over Cosmosil 75 C_{18}-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–H_2O (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° (H_2O; *c* 0.1). FAB-MS *m/z*: 695 $[M + Na]^+$, 673 $[M + H]^+$, 511 $[M + H - (Glc - H_2O)]^+$, 349 $[M + H - 2(Glc - H_2O)]^+$; UV $\lambda_{max}^{H_2O}$ nm: 300; IR ν_{max}^{KBr} cm^{-1}: 1765, 1705, 1625, 1545, 1080; CD: $\Delta\varepsilon_{208}$ + 9.98°, $\Delta\varepsilon_{235}$ − 1.10°, $\Delta\varepsilon_{295}$ + 0.92° (H_2O; *c* 0.3); ^1H and ^{13}C 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. $[\alpha]_D^{26}$ + 53.32° (H_2O; *c* 0.8) FAB-MS *m/z*: 695 $[M + Na]^+$, 673 $[M + H]^+$, 511 $[M + H - (Glc - H_2O)]^+$, 349 $[M + H - 2(Glc - H_2O)]^+$; UV $\lambda_{max}^{H_2O}$ 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° (H_2O; *c* 0.3); ^1H and ^{13}C 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° (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 $\lambda_{max}^{H_2O}$ nm: 300; IR ν_{max}^{KBr} cm^{-1}: 1760, 1700, 1620, 1545, 1080; CD: $\Delta\varepsilon_{208}$ + 7.30°, $\Delta\varepsilon_{235}$ + 2.00°, $\Delta\varepsilon_{295}$ + 0.40° (H_2O; *c* 0.3); ^1H and ^{13}C NMR: Tables 1 and 2.

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