Anodendrosins J and K, Two Sucrose Bisglycosylnervogenates from the Seeds of *Anodendron affine* (Studies on *Anodendron*. IX)[1]

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Anodendrosins J and K, two diesters of 4-*O*-glycosyl-3,5-diprenyl-4-hydroxybenzoic acid with sucrose were obtained from the seeds of *Anodendron affine*, and their structures were determined by spectral and chemical means.

Keywords anodendrosin; sucrose bisglycosylnervogenate; nervogenic acid; 3,5-diprenyl-4-hydroxybenzoic acid; *Anodendron affine*; Apocynaceae

During our studies on the constituents of Apocynaceae plants, anodendrosins A—I, esters of 4-*O*-glycosyl-3,5-diprenyl-4-hydroxybenzoic acid (4-*O*-glycosylnervogenic acid)[2] (GNA) with sucrose or 1,3-di-*O*-methyl-*myo*-inositol (dambonitol), and ester glucosides of GNA, were isolated from the seeds of *Anodendron affine* DRUCE and their structures were established.[3] In order to study on the minor constituents of the seeds, reinvestigation was carried out and several homologous compounds (**1**—**9**) were obtained along with known anodendrosins and many cardenolide glycosides.[1,4] Among these, anodendrosins J (**1**) and K (**2**), diesters of GNA with sucrose, are described in this paper.

The fast atom bombardment mass spectrum (FAB-MS) of **1** afforded a $[M+Na]^+$ peak at *m/z* 1201.5026, suggesting the molecular formula to be $C_{58}H_{82}O_{25}$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the presence of two GNA residues in the molecule, based on a duplicate pair of phenyl proton signals at δ 8.07, 8.08 (2H each, s, H-2, 6, 2′, 6′), signals due to prenyl residues (δ 1.59, 1.63, 1.69, 1.70, 6H each, s, terminal methyl protons; δ 5.43, 5.45, 2H each, br t, H-β, β', β'', β'''; δ 3.81—4.01, 8H, H-α, α', α'', α'''), and two anomeric protons at δ 5.36, 5.38 (1H each, d, J=8 Hz). Two sets of signals corresponding to GNA residues were also observed in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum as shown in Table II. The carbohydrate esterified with the two GNA residues was identified as sucrose, and the ester linkages were assigned to the C-6-hydroxyl groups of glucose and fructose, based on acylation shifts of the corresponding carbinol carbon and proton signals, and a comparison of chemical shifts with those of anodendrosin E ($6_{glc.}$-*O*-(4-*O*-β-D-glucopyranosyl) nervogenoyl sucrose)[3] (**10**). The mild saponification of **1** with 0.2% $KHCO_3$ in aqueous MeOH afforded **1a**, **1b** and sucrose (Chart 1). While **1a** was identified as methyl 4-*O*-β-D-glucopyranosylnervogenate, **1b** showed different behavior from **10** on thin layer chromatography (TLC). One of the C-6 methylene protons in the glucose moiety was observed in the upper-field region, and there was an upfield shift of C-6 in comparison with the signals of **1**, so that the GNA residue in **1b** was located at the C-6 hydroxyl of fructose. Thus, **1** was confirmed to be sucrose $6_{glc.}$-*O*-, $6_{fruc.}$-*O*-bis-(4-*O*-β-D-glucopyranosyl) nervogenate

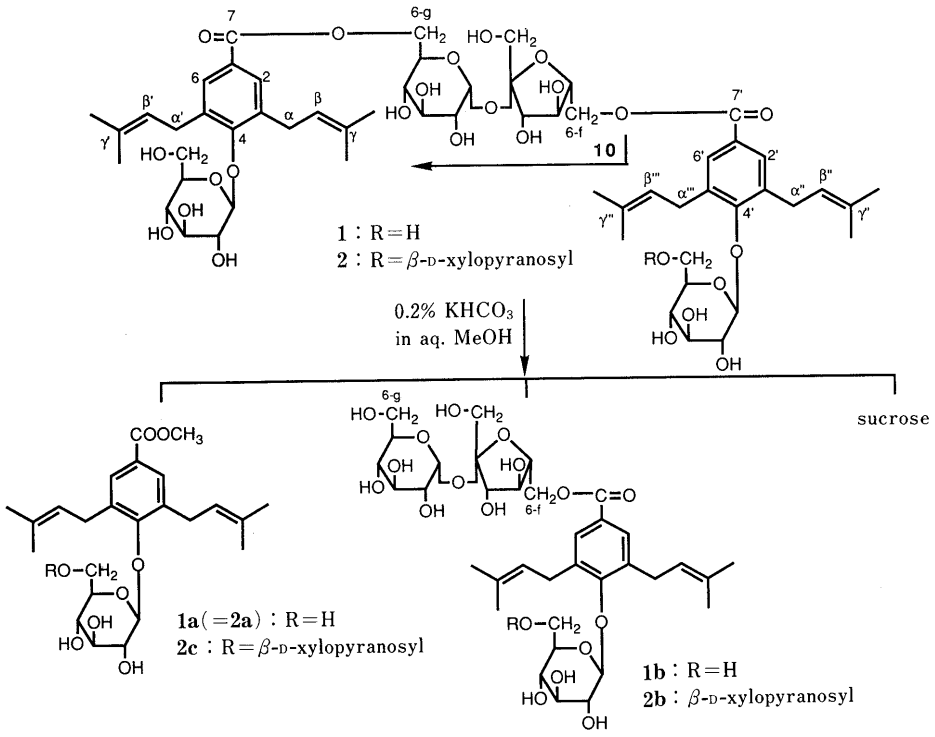

Chart 1

TABLE I. ^{1}H Chemical Shifts of **1**, **1b**, **2**, **2b** and **10**, δ (ppm) from Tetramethylsilane (TMS) in Pyridine-d_5 (J (Hz) in Parentheses)

H	**10**	**1**[a]	**1b**[a]	**2**[a]	**2b**[a]
2,6	8.08 (2H, s)	8.07 (2H, s)	8.08 (2H, s)	8.07 (2H, s)	8.08 (2H, s)
(2′,6′)		8.08 (2H, s)		8.08 (2H, s)	
α,α′	3.92	3.81—4.01 (4H)	3.86, 4.00	3.89—4.00 (4H)	3.92, 4.00
(α″,α‴)	(2H, br d, 7)		(dd, 16, 7)		(dd, 16, 7)
β,β′	5.45	5.43, 5.45	5.44	5.45, 5.46	5.48
(β″,β‴)	(2H, br t, 7)	(2H, br t, 7)	(2H, br t, 7)	(2H, br t, 7)	(2H, br t, 7)
γ,γ′-CH_3	1.63, 1.70	1.59, 1.63	1.59, 1.70	1.60, 1.63	1.60, 1.71
(γ″,γ‴-CH_3)	(6H, s)	1.69, 1.70 (6H, s)	(6H, s)	(6H, s)	(6H, s)
				1.71 (12H, s)	
[–COO–]					
Sucrose					
3 (Fruc.)	4.99 (d, 7)	5.03 (d, 7)	5.03 (d, 7)	5.01 (d, 7)	5.02 (d, 7)
4	4.98 (t, 7)	4.94 (t, 7)	5.00 (t, 7)	4.93 (t, 7)	4.99 (t, 7)
5	4.50 (m)	4.77 (td, 7, 4)	4.77 (m)	4.76 (m)	4.77 (m)
6a	4.22—4.35	5.09—5.14	5.15 (dd, 12, 3)	5.04—5.12	5.11 (dd, 12, 3)
6b	4.22—4.35	5.18 (dd, 11, 4)	5.25 (dd, 12, 8)	5.16 (dd, 12, 3)	5.22 (dd, 12, 8)
1 (Glc.)	6.19 (d, 3)	6.22 (d, 3)	6.19 (d, 4)	6.21 (d, 3)	6.19 (d, 4)
2		4.22 (dd, 9, 3)	4.16 (dd, 10, 4)	4.22 (dd, 9, 3)	4.18 (dd, 9, 4)
3	4.69 (t, 9)	4.65 (t, 9)	4.69 (t, 10)	4.69 (t, 9)	4.67 (t, 9)
4			4.19 (t, 10)		4.19 (t, 9)
6a	5.00—5.05	5.09—5.14	4.30—4.35	5.04—5.12	4.35 (dd, 12, 5)
6b	5.07 (dd, 12, 3)	5.09—5.14	4.53 (dd, 12, 4)	5.04—5.12	4.53 (dd, 12, 3)
[4-*O*-]					
1 (Glc.)	5.37 (d, 7)	5.36, 5.38 (d, 8)	5.39 (d, 7)	5.32, 5.36 (d, 7)	5.34 (d, 7)
1 (Xyl.)				4.77 (d, 7)	4.77 (d, 7)
2				3.87 (dd, 9, 7)	3.87 (dd, 9, 7)
3				4.01 (t, 9)	4.01 (t, 9)
4				4.21 (m)	4.11 (m)
5a				3.58 (dd, 12, 10)	3.58 (dd, 11, 10)
5b				4.20—4.26	4.20—4.26

a) Signal assignments were done based on two-dimensional (2D) ^{1}H–^{1}H correlation spectroscopy (COSY) spectra.

and is named anodendrosin J.

FAB-MS of **2** afforded a $[M+Na]^+$ peak at m/z 1333.5479 ($C_{63}H_{90}NaO_{29}$), suggesting a structure with one additional pentose as compared with **1**. In the ^{13}C-NMR spectrum, signals due to the sucrose moiety were identical with those of **1**, indicating that the two GNA residues were linked to sucrose at the C-6 hydroxyls of glucose and fructose. Signals due to the pentose suggested it to be D-xylose. The xylose unit was linked to the C-6 hydroxyl of the glucose residue in one of the GNA groups, forming primverose as in anodendrosins C, D and G,[3] since one of the C-6 signals showed a glycosylation shift (+7.0 ppm).

In order to determine whether the primverosylnervogenic acid was linked to the glucose side or the fructose side, **2** was treated with 0.2% $KHCO_3$ in aqueous MeOH to yield three products (**2a**, **2b** and **2c**) and sucrose (Chart 1). By comparison with **1a**, **2a** was identified as methyl 4-*O*-glucopyranosylnervogenate. Compound **2b** showed the molecular formula, $C_{40}H_{60}O_{22}$, based on FAB-MS, and the presence of sucrose and primverose moieties was confirmed by the ^{1}H- and ^{13}C-NMR spectra. The C-6 methylene proton signals of fructose were assignable in the lower field (δ 5.11, 5.22), as in **2**, by tracing from $H_{fruc.}$-4 in the homonuclear Hartmann Hahn spectrum (HOHAHA), while the C-6 methylene proton signals of the glucose unit in **2b** were observed in the upper field (δ 4.35, 4.53) as in **1b**. Compound **2c** was characterized as methyl 4-*O*-β-primverosylnervogenate based on ^{1}H-NMR and FAB-MS considerations. Compound **2** was thus determined to be sucrose $6_{glc.}$-*O*-(4-*O*-β-D-glucopyranosyl)-nervogenoyl, $6_{fruc.}$-*O*-(4-*O*-β-primverosyl)nervogenate, and is named anodendrosin K.

Since the anodendrosins previously obtained were monoesters of nervogenic acid with carbohydrates,[3] this is the first time that sucrose bisnervogenates have been isolated from this plant. Anodendrosins having primverosylnervogenic acid so far obtained are an ester glucoside (anodendrosin D) or an ester with dambonitol (C), and **2** is the first diester in which primverosylnervogenic acid is linked to the fructose side of sucrose. The biological significance of anodendrosins in the seeds is to be investigated. The structures of **3**—**9** will be reported elsewhere.

Experimental

The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, br d = broad doublet. FAB-MS were recorded on JEOL JMS-DX300-FD and JEOL JMS-HX110 spectrometers. Optical rotations were measured on a JASCO DIP 360 polarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi UV 200S spectrophotometer in MeOH. High performance liquid chromatography (HPLC) was conducted with a Waters ALC-200 machine equipped with a Radial-pack C_{18} (10 μm, 8 mm i.d. × 10 cm) and eluted with 15—40% MeCN. For column and thin layer chromatographies, the following solvent systems were applied; solvent 1, benzene–acetone (1 : 1); solvent 2, $CHCl_3$–MeOH–H_2O (7 : 2 : 1—7 : 3 : 1, bottom layer); solvent 3, EtOAc–MeOH–

TABLE II. ^{13}C Chemical Shifts of **1**, **1b**, **2**, **2b** and **10**, δ (ppm) from TMS in Pyridine-d_5

C	10	1[a]	1b	2	2b
1(1′)	127.1	127.1	127.0	127.1	127.1
2(2′)	129.7	129.9, 130.0	130.0	129.8, 130.0	130.0
3(3′)	136.4	136.4, 136.5	136.5	136.3, 136.5	136.6
4(4′)	157.5	157.5, 157.6	157.6	157.5	157.5
5(5′)	136.4	136.4, 136.5	136.5	136.3, 136.5	136.6
6(6′)	129.7	129.9, 130.0	130.0	129.8, 130.0	130.0
7(7′)	166.7	166.6, 166.7	166.7	166.5, 166.7	166.7
α(α″)	29.2	29.3, 29,4	29.4	29.2, 29.3	29.3
β(β″)	123.7	123.8	123.8	123.9	123.9
γ(γ″)	132.7	132.6, 132.7	132.5	132.5, 132.6	132.5
γ(γ″)-CH_3	18.0	18.1	18.0	18.0, 18.1	18.1
	25.6	25.7, 25.8	25.6	25.6, 25.7	25.6
α′(α‴)	29.2	29.3, 29.4	29.4	29.2, 29.3	29.3
β′(β‴)	123.7	123.8	123.8	123.9	123.9
γ′(γ‴)	132.7	132.6, 132.7	132.5	132.5, 132.6	132.5
γ′(γ‴)-CH_3	18.0	18.1	18.0	18.0, 18.1	18.1
	25.6	25.7, 25.8	25.6	25.6, 25.7	25.6
[–COO–]					
Sucrose					
1 (Fruc.)	64.8	64.3	64.5	64.2	64.5
2	105.7	106.4	106.0	106.4	106.0
3	80.2	79.5	79.5	79.4	79.5
4	75.9	77.5	77.0	77.6[b]	77.1[b]
5	84.4	81.1	81.0	81.0	80.9
6	63.0	67.6	67.4	67.4	67.4
1 (Glc.)	93.0	93.7	93.3	93.6	93.3
2	73.4	73.4	73.4	73.4	73.4
3	74.8	74.9	75.1	74.9	75.1
4	71.2	71.4	71.7	71.3	72.1
5	71.9	72.0	74.6	71.9	74.6
6	64.2	64.6	62.7[b]	64.5	62.7
[4-*O*-]					
1 (Glc.)	106.2	106.4 (×2)	106.4	106.3, 106.4	106.2
2	75.7	75.7 (×2)	75.7	75.4, 75.7	75.4
3	78.4	78.4 (×2)	78.4	78.2, 78.3	78.2
4	71.9	71.8 (×2)	71.7	71.4, 71.8	71.4
5	78.6	78.7 (×2)	78.7	77.9, 78.6	77.9
6	63.0	62.8 (×2)	62.8[b]	69.8, 62.8	69.8
1 (Xyl.)				105.6	105.5
2				74.5	74.5
3				77.1[b]	77.2[b]
4				70.9	70.9
5				66.9	66.9

a) Signal assignment was done based on 2D ^{13}C–^1H COSY spectra. *b*) Signals may be interchangeable in each column.

H_2O (4:1:4—4:1:1, top layer); solvent 4, $CHCl_3$–MeOH for a normal phase column, 60—80% MeOH or 40—50% MeCN for a reversed phase column. (YMC-gel, MCI-gel HP-20, Fuji gel RQ-1, octadecyl silica (ODS) G3).

Extraction and Isolation The seeds were collected during 1988—1990 from *Anodendron affine* DRUCE cultivated in the medicinal plant garden of Fukuoka University. The seeds (1.1 kg) were homogenized in MeOH and then percolated with MeOH (10 l) in April 1991. The MeOH solution was then concentrated and defatted with hexane. The defatted MeOH extract (112.8 g) was fractionated through an MCI-gel HP-20 column to 25%, 50%, 80%, and 100% MeOH eluates. The 80% and 100% MeOH eluates were combined and chromatographed on a silica gel column (solvents 2 and 3) and an ODS column, and finally 9 new and 7 known anodendrosins were isolated by HPLC.

Known Anodendrosins Anodendrosins A (430 mg), B (110 mg), C (248 mg), D (110 mg), E (610 mg), F (130 mg), H (10 mg).

Anodendrosin J (1) A solid, 200 mg, $[\alpha]_D^{24}$ +7.9° (c=3.35, MeOH), FAB-MS m/z: 1201.5026 (Calcd for $C_{58}H_{82}NaO_{25}$: 1201.5043). Compound **1** (50 mg) was dissolved in 0.2% $KHCO_3$ in 80% MeOH (5 ml) and the mixture was allowed to stand at room temperature for 30 h, then neutralized with Amberlite IRB-120 and concentrated *in vacuo* to dryness. The residue was fractionated on a silica gel column (solvent 3) to give **1a** (9 mg), **1b** (7 mg), **1** (14 mg) and sucrose (8 mg). **1a**: a solid. FAB-MS m/z: 473.2146 (Calcd for $C_{24}H_{34}NaO_8$: 473.2151). ^1H-NMR δ: 8.06 (2H, s, H-2, 6), 5.50 (2H, br t, J=7 Hz, H-β, β′), 5.39 (1H, d, J=7 Hz, H-1′), 4.02, 3.96 (2H each, dd, J=16, 7 Hz, H-α, α′), 3.79 (3H, s, –COOC$\underline{H}_3$), 1.72, 1.62 (6H each, s, γ, γ′-CH_3). **1b**: a solid, $[\alpha]_D^{27}$ +24.0° (c=0.13, MeOH). Sucrose was identified on TLC by comparison with an authentic sample (solvents 2 and 3).

Anodendrosin K (2) A solid, 30 mg, $[\alpha]_D^{28}$ −6.8° (c=0.5, MeOH). FAB-MS m/z: 1333.5479 (Calcd for $C_{63}H_{90}NaO_{29}$: 1333.5466). **2** (30 mg) was dissolved in 0.2% $KHCO_3$ in 80% MeOH (5 ml) and allowed to stand at room temperature for 30 h. The mixture was then worked up as described for **1**, and three compounds, **2a** (6 mg), **2b** (3 mg), **2c** (3 mg) and sucrose (7 mg) were obtained. **2a**: a solid. In a TLC comparison, (solvents 1, 2, 3), **2a** showed the same *Rf* value as **1a**. **2b**: a solid, $[\alpha]_D^{28}$ +14.0° (c=0.15, MeOH). FAB-MS m/z: 915.3470 (Calcd for $C_{40}H_{60}NaO_{22}$: 915.3473). **2c**: a solid, FAB-MS m/z: 605.2572 (Calcd for $C_{29}H_{42}NaO_{12}$: 605.2574). ^1H-NMR δ: 8.06 (2H, s, H-2, 6), 5.54 (2H, br t, J=6 Hz, H-β, β′), 5.34 (1H, d, J=7 Hz, H-1′), 4.79 (1H, d, J=7 Hz, H-1″), 3.89 (1H, dd, J=9, 7 Hz, H-2″), 3.76 (3H, s, –COOC$\underline{H}_3$), 3.59 (1H, dd, J=11, 10 Hz, H-5″a), 1.73, 1.62 (6H, each s, γ, γ′-CH_3).

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References and Notes

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