Oxygenated Anodendrosins from the Seeds of *Anodendron affine* (Studies on *Anodendron.* XI)[1]

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The structures of several newly isolated anodendrosins, oxygenated derivatives of 4-*O*-glucosyl-3,5-diprenyl-4-hydroxybenzoic acid ester with sucrose, dambonitol or glucose, from the seeds of *Anodendron affine* were elucidated by spectral methods.

Keywords anodendrosin; oxygenated anodendrosin; nervogenic acid; 3,5-diprenyl-4-hydroxybenzoic acid; 3,5-bis-(γ-hydroxy-α,β-didehydroisopentyl)-4-hydroxybenzoic acid; prenyl benzofuran; prenyl benzopyran; sucrose nervogenic acid ester; *Anodendron affine*; Apocynaceae

Esters of 4-*O*-glycosyl-3,5-diprenyl-4-hydroxybenzoic acid (4-*O*-glucosylnervogenic acid) with carbohydrates such as sucrose, 1,3-di-*O*-methyl-*myo*-inositol (dambonitol) and glucose were obtained from the seeds of *Anodendron affine* DRUCE, and named anodendrosins A—I.[2] In the course of a reinvestigation, nine homologous compounds were obtained along with the known anodendrosins, and two of them (**1** and **2**) were determined to be sucrose bis-4-*O*-glycopyranosyl-3,5-diprenyl-4-hydroxybenzoates (anodendrosins J and K).[3] This paper deals with the remaining anodendrosins (**3**—**9**), most of which contain one or two hydroxyls in the prenyl side chains.

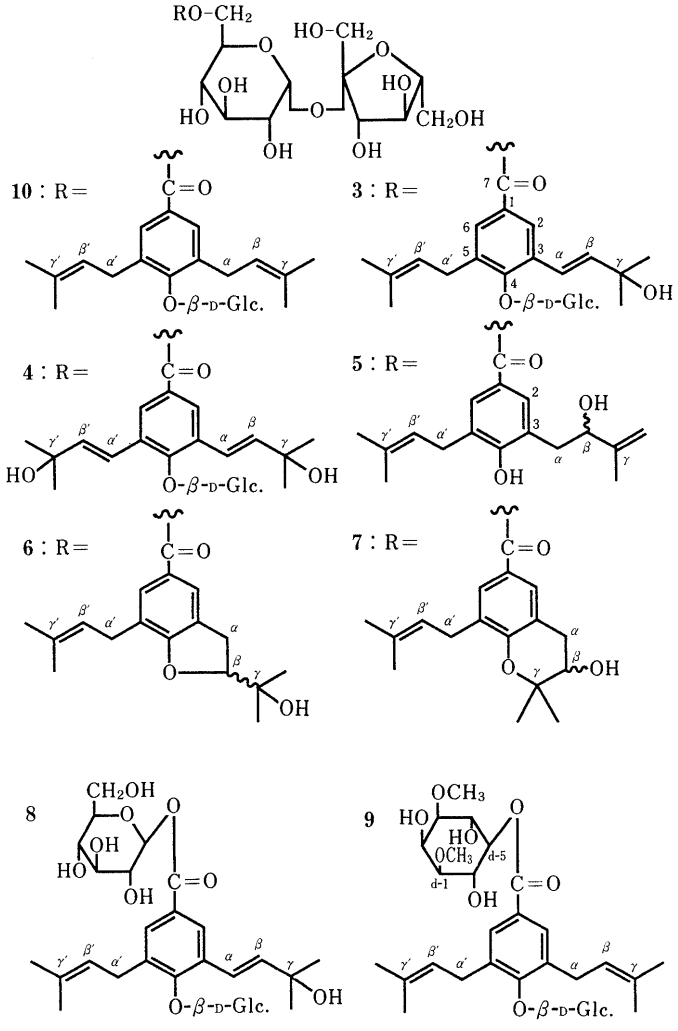

Chart 1

On the basis of carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, **3**—**7** seemed to be esters of 3,5-disubstituted 4-hydroxybenzoic acid (DHB) with sucrose, linking at the C-6 hydroxyl group of the glucose side, as in anodenrosin E (**10**) (Table I). The fast atom bombardment mass spectrum (FABMS) of **3** suggested the molecular formula to be $C_{35}H_{52}O_{19}$, having one additional oxygen as compared with **10**. The signals due to 4-*O*-glucoside were observed with almost the same chemical shifts as those in **10**. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, two phenyl protons of DHB (H-2, 6) were observed at δ 8.10 and 8.48 (1H each, d, J=2 Hz), separately. While only one set of signals due to a prenyl side chain was present, two disubstituted *trans* olefinic protons (δ 7.93, 6.80 (1H each, d, J=16 Hz)) and two tertiary methyl groups were observed. Therefore, **3** was characterized as a derivative of **10**, in which one of the prenyl side chains was transformed into a γ-hydroxy-α,β-didehydroisopentyl structure.

FABMS of **4** afforded an $[M+Na]^+$ peak at m/z 815.2944 and the molecular formula was suggested to be $C_{35}H_{52}O_{20}$, with one oxygen more than **3**. Unlike **3**, two phenyl protons were observed as a duplicated singlet signal at δ 8.55, indicating the symmetric structure of the DHB moiety. The presence of two γ-hydroxy-α,β-didehydroisopentyl side chains was confirmed based on the proton signals due to two disubstituted olefinic linkages at δ 8.01 and 6.82 (2H each, d, J=16 Hz) and four tertiary methyl groups at δ 1.61 (6H, s) and 1.62 (6H, s). Compound **4** was thus characterized as a dihydroxyderivative of **10**.

Compound **5** showed no anomeric proton signal due to the glucose residue which was usually linked to the phenyl hydroxyl group as observed in **3** and **4**. The $[M+Na]^+$ peak in FABMS at m/z: 637.2477 suggested the molecular formula to be $C_{29}H_{42}O_{14}$. Besides the signals due to one prenyl group, a carbinyl methine proton signal was observed at δ 4.65 (br d, J=9 Hz), showing cross peaks with methylene proton signals at δ 3.06 (1H in total with a signal at δ 3.09) and δ 3.20 (1H) in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum. The signals at δ 4.86, 4.87 (1H in total, d, J=2 Hz) and δ 5.21, 5.22 (1H in total, br s) seemed to be due to an exomethylene group formed by dehydrogenation of one of the terminal methyl groups in the side chain.

In order to assign the location of the hydroxyl group in the side chain, the long range ^{1}H–^{13}C COSY spectrum was measured. Cross peaks were observed between H-2/C-α and H-α/C-2, C-3. The hydroxyl group was thus

TABLE I. ^{13}C Chemical Shifts of **3**—**9** and **10**, δ (ppm) from Tetramethylsilane (TMS) in Pyridine-d_5

C	**10**	**3**[a]	**4**	**5**[a]	**6**	**7**[b]	**8**	**9**
1	127.1	127.2	127.3	121.8	122.8[c]	120.7	126.7	128.0
2	129.7	126.5	126.6	131.8	124.9	129.5[c]	126.5	130.0
3	136.4	132.9	133.1	126.7	123.0[c]	122.2	132.5	136.3
4	157.5	157.1	156.2	159.6	162.7	155.7	157.5	157.3
5	136.4	137.3	133.1	129.7	128.0	129.7	137.5	136.3
6	129.7	130.3	126.6	130.7	130.9	130.4[c]	130.6	130.0
7	166.7	166.8	166.7	167.1	167.0	167.0	165.7	166.8
α	29.2	122.1	122.0	39.3, 39.4	30.5	32.2	122.0	29.4
β	123.7	141.4	141.0	76.5, 76.6	90.9	68.9	141.3	123.9
γ	132.7	70.8	70.6	147.9	70.9	78.9	70.5	132.2
γ-CH_3	18.0	30.4	30.3	18.3, 18.4	25.3	21.0	30.40	18.0
	25.6	30.5	30.4		26.4	26.2	30.43	25.6
γ=CH_2				110.7				
α'	29.2	29.4	122.0	29.2	28.4	28.9	29.5	29.4
β'	123.7	123.4	141.0	123.2	122.3	123.0	123.5	123.9
γ'	132.7	132.1	70.6	132.4	132.8	132.2	132.1	132.2
γ'-CH_3	18.0	18.1	30.3	17.8	17.7	17.9	18.0	18.0
	25.6	25.8	30.4	25.7	25.7	25.7	25.6	25.6
[–COO–]								
Sucrose						Glucose	Dambonitol	
1 (Fruc.)	64.8	64.9	65.6	64.7	64.7	64.7	96.6	83.4
2	105.7	106.0	106.2	105.8	105.7	105.8	74.2	65.7
3	80.2	79.9	79.8	79.9	79.8	79.9	79.6	83.4
4	75.9	75.8	75.9	75.8	75.8	75.8	71.1[d]	71.3
5	84.4	84.3	84.3	84.4	84.4	84.4	78.6[c]	78.7
6	63.0	63.2	63.2	63.3	63.3	63.3	62.3	71.3
1 (Glc.)	93.0	93.8	93.7	93.6	93.5	93.5		(OCH_3 × 2)
2	73.4	73.4	73.3	73.3	73.3	73.3		57.8
3	74.8	74.8	74.7	74.8	74.9	74.8		
4	71.2	71.5[c]	71.7	71.5	71.4	71.5		
5	71.9	72.1	72.1	72.1	72.1	72.1		
6	64.2	64.6	64.4	64.5	64.4, 64.5	64.5, 64.6		
[4-*O*-]								
1 (Glc.)	106.2	106.4	106.5				106.4	106.3
2	75.7	75.8	75.6				75.4	75.7
3	78.4	78.5	78.1[c]				78.4	78.4
4	71.9	71.4[c]	70.5				71.3[d]	71.8
5	78.6	78.6	78.3[c]				78.5[c]	78.7
6	63.0	62.6	61.9				62.6	62.9

a) Signal assignments were made based on 2D ^{13}C–^1H COSY and long-range ^{13}C–^1H COSY spectra. *b*) Signal assignment was done based on the 2D ^{13}C–^1H COSY spectrum. *c*, *d*) Signals may be interchangeable in each column.

located at the β-position in the isopentyl side chain. Since some of the protons of the hydroxylated side chain appeared as twin signals, **5** is considered to be an epimeric mixture as regards the orientation of the hydroxyl group.

Based on FABMS, **6** and **7** both have the same molecular formula, $C_{29}H_{42}O_{14}$, as **5**. The presence of one prenyl group was confirmed by the NMR spectra. In **6** and **7**, a methine proton on carbon bearing oxygen (H-β) showed cross peaks with neighboring methylene protons in the ^1H–^1H COSY spectra. The signals of carbons bearing oxygen appeared at δ 90.9 (d) and 70.9 (s) in **6**, and δ 68.9 (d) and 78.9 (s) in **7**. Since the unsaturation index was 9, cyclization seemed to have occurred between one of the prenyl side chains and the phenyl hydroxyl group to afford a benzopyran or a benzofuran ring, as in anodendrosins H and I.[3]

Acetylation of **6** and **7** with Ac_2O and pyridine afforded a heptaacetate and an octaacetate, respectively. Consequently, **6** and **7** were determined to have benzofuran and benzopyran structures, respectively. Compounds **6** and **7** were also mixtures concerning the orientation of the hydroxyisopropyl residue (**6**) or the secondary hydroxyl (**7**) as in **5**, since some of the protons of the furan or the pyran ring appeared as twin signals.

From a comparison of the ^1H- and ^{13}C-NMR spectra with those of **3**, **8** was considered to have the same asymmetric glucosyl-phenolcarboxylic acid moiety as **3**, along with the ester glucosyl residue. One of the anomeric proton signals was observed at lower field, at δ 6.64 (d, J=7 Hz). All signals in the ^{13}C-NMR spectrum due to two glucose units attached to the phenyl hydroxyl and the carboxyl functions were in good agreement with those of anodendrosin B. Compound **8** was therefore determined to be a monohydroxyderivative of anodendrosin B.

Compound **9** afforded an $[M+Na]^+$ peak at m/z 649.2839 in the FABMS, suggesting the molecular formula to be $C_{31}H_{46}O_{13}$. While the presence of a 4-*O*-β-D-glucopyranosyl-nervogenic acid moiety was confirmed in the NMR spectra, the carbohydrate moiety was assigned as dambonitol[2] based on the molecular formula and spectral considerations. Two methoxyl protons were observed as a duplicated singlet signal at δ 3.62, suggesting the esterified dambonitol to retain a symmetric form, and an acylated carbinyl methine proton was observed at δ 6.00 (1H, t,

TABLE II. ^{1}H Chemical Shifts of **3**—**9** and **10**, δ (ppm) from TMS in Pyridine-d_5 (J (Hz) in Parentheses)[a]

H	10	3	4	5	6	7	8	9
2, 6	8.08 (2H, s)	8.10 (d, 2) 8.48 (d, 2)	8.55 (2H, s)	8.13 (d, 2) 8.17 (d, 2)	8.00 (d, 2) 8.05 (d, 2)	7.97 (d, 2) 8.05 (d, 2)	8.15 (d, 2) 8.48 (d, 2)	8.12 (2H, s)
α	3.92 (2H, br d, 7)	7.93 (d, 16)	8.01 (d, 16)	3.06, 3.09[b] (dd, 15, 3) 3.20 (dd, 15, 9)	3.14, 3.15[b] (dd, 16, 10) 3.44 (dd, 16, 8)	2.97 (dd, 17, 8) 3.12, 3.13[b] (dd, 17, 2)	7.93 (d, 16)	3.78, 3.95 (dd, 16, 7)
α'	3.92 (2H, br d, 7)	3.88, 3.95 (dd, 16, 8)	8.01 (d, 16)	3.59 (br d, 7)	3.32 (br d, 7)	3.42 (br d, 7)	3.81, 4.03 (dd, 16, 7)	3.78, 3.95 (dd, 16, 7)
β	5.45 (br t, 7)	6.80 (d, 16)	6.82 (d, 16)	4.65 (br d, 9)	4.80 (dd, 10, 8)	3.97, 3.98[b] (dd, 8, 2)	6.68 (d, 16)	5.34 (br t, 7)
β'	5.45 (br t, 7)	5.49 (br t, 7)	6.81 (d, 16)	5.50 (br t, 7)	5.36 (br t, 7)	5.39 (br t, 7)	5.46 (br t, 7)	5.34 (br t, 7)
γ,γ'-CH_3	1.63 (6H, s) 1.70 (6H, s)	1.63, 1.64 1.66, 1.70 (3H, s)	1.61 (6H, s) 1.62 (6H, s)	1.66, 1.67 1.84 (3H, s)	1.40, 1.44 1.64, 1.65 (3H, s)	1.46, 1.50 1.68, 1.69 (3H, s)	1.60 (6H, s) 1.61, 1.67 (3H, s)	1.53 (6H, s) 1.64 (6H, s)
$\gamma=CH_2$				4.86, 4.87[b] (d, 2) 5.21, 5.22[b] (br s)				
[–COO–]								
Sucrose							Glucose	Dambonitol
1 (Glc.)	6.19 (d, 3)	6.20 (d, 3)	6.21 (d, 4)	6.16, 6.17[b] (d, 3)	6.18, 6.19[b] (d, 3)	6.18, 6.19[b] (d, 3)	6.64 (d, 7)	
2				4.17 (dd, 9, 3)	4.20 (dd, 9, 3)	4.18 (dd, 9, 3)		4.84 (t, 3)
3	4.69 (t, 9)	4.68 (t, 9)	4.68 (t, 9)	4.68 (t, 9)	4.69 (t, 9)	4.69 (t, 9)		
4				4.20 (t, 9)		4.20 (t, 9)		4.89 (t, 10)
5		4.91 (m)		4.92 (m)	4.93 (m)	4.92 (m)	4.12 (m)	6.00 (t, 10)
6	5.07 (dd, 12, 3)	5.12 (dd, 12, 2)		5.14, 5.17 (dd, 12, 2)	5.14, 5.15[b] (dd, 12, 2)	5.03 (dd, 12, 7) 5.14, 5.17[b] (dd, 12, 2)	3.38 (dd, 12, 5) 4.49 (dd, 12, 2)	4.89 (t, 10) (–OCH_3) 3.62 (6H, s)
1 (Fruc.)				4.31, 4.37 (d, 12)	4.32, 4.38 (d, 12)	4.32, 4.37 (d, 12)		
5	4.50 (m)	4.52 (ddd, 9, 5, 3)	4.52 (ddd, 8, 5, 3)	4.52 (m)	4.54 (m)	4.52 (m)		
6	4.46 (dd, 12, 3)			4.39 (dd, 12, 3)		4.31 (dd, 12, 4) 4.39 (dd, 12, 2)		
[4-*O*-]								
1 (Glc.)	5.37 (d, 7)	5.34 (d, 8)	5.26 (d, 8)				5.37 (d, 7)	5.34 (d, 7)

a) Signal assignments of **3**—**9** were made based on two dimensional (2D) ^{1}H–^{1}H COSY spectra. b) Two signals were observed as 1H in total.

$J=10$ Hz) in an axial position, as in anodendrosin G.[3] The ester linkage of nervogenic acid to dambonitol was located at the C-5″-hydroxyl group, and the structure was determined to be dexylosyl-anodendrosin G.

Since some of the oxygenated anodendrosins were obtained as mixtures of diastereomers, the prenyl side chain of nervogenic acid seems to be oxidized non-enzymatically during the drying process of the seeds. In fact, the extract from the fresh, ripe seeds contained normal anodendrosins such as B, C, D, E and J, but no oxygenated ones, and facile conversion of **10** into **4** was observed upon introduction of air into the solution. The possibility that anodendrosins have a role in preventing from the oxidation of other components in the seeds can not be excluded.

Experimental

^{1}H- and ^{13}C-NMR 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, dd=doublet of doublets, br s=broad singlet, br d=broad doublet. FABMS were recorded on a JEOL JMS-DX300-FD and a JEOL JMS-HX110. Optical rotations were measured on a JASCO DIP 360 polarimeter. UV spectra were recorded on a Shimadzu UV 200S spectrophotometer in MeOH. HPLC was conducted with a Waters ALC-200 machine equipped with a Radial-pack C_{18} column (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); 2, $CHCl_3$–MeOH–H_2O (7 : 2 : 1—7 : 3 : 1, bottom layer); 3, EtOAc–MeOH–H_2O (4 : 1 : 4—4 : 1 : 1, top layer); 4, $CHCl_3$–MeOH for a regular phase column, 60—80% MeOH or 40—45% MeCN for a reversed phase column, YMC-gel, MCI-gel (CHP-20, Mitsubishi Chem. Co.), Fuji-gel RQ-1 or ODS G3.

Extraction and Isolation The procedures were described in Part IX of this series, and 9 anodendrosins (**1**—**9**) as well as the known anodendrosins were obtained, each as a solid. Among these, **1** and **2** were described in the preceding paper.[3]

Compound 3 Yield, 360 mg, $[\alpha]_D^{30}$ +41.8° (c=0.5, MeOH). FABMS m/z: 799.2999 (Calcd for $C_{35}H_{52}NaO_{19}$: 799.3001). UV λ_{max} nm (log ε): 238 (4.50), 287 (sh, 3.79).

Compound 4 Yield, 25 mg, $[\alpha]_D^{30}$ +50.2° (c=0.5, MeOH). FABMS m/z: 815.2944 (Calcd for $C_{35}H_{52}NaO_{20}$: 815.2949). UV λ_{max} nm (log ε): 248 (4.62).

Compound 5 Yield, 20 mg. FABMS m/z: 637.2477 (Calcd for $C_{29}H_{42}NaO_{14}$: 637.2472).

Compound 6 Yield, 14 mg. FABMS m/z: 637.2472 (Calcd for $C_{29}H_{42}NaO_{14}$: 637.2472). Compound **6** (10 mg) was acetylated with Ac_2O and pyridine at room temperature for 20 h. The acetate was purified on a silica gel column with solvent 1 (8 : 1) to give a heptaacetate (**6a**) (4.4 mg), FABMS m/z: 931.3213 (Calcd for $C_{43}H_{56}NaO_{21}$: 931.3212). ^{1}H-NMR δ: 8.09, 8.11 (1H each, br s, H-2, 6), 1.33, 1.40, 1.46, 1.69 (3H, each, s, γ,γ'-CH_3), 6.18, 6.19 (1H in total, d, J=3 Hz, Glc. -1 of Suc.), 2.03, 2.05, 2.06, 2.08, 2.11, 2.14, 2.24 (3H each, s, $OCOCH_3$).

Compound 7 Yield, 39 mg. FABMS m/z: 637.2472 (Calcd for $C_{29}H_{42}NaO_{14}$: 637.2472). Compound **7** (10 mg) was acetylated in the

same manner as in **6** to give an octaacetate (4.7 mg), a solid. FABMS *m/z*: 973.3317 (Calcd for $C_{45}H_{58}NaO_{22}$: 973.3318). ^{1}H-NMR δ: 8.01, 8.11 (1H each, br s, H-2, 6), 5.22 (1H, t, $J=5$ Hz, H-β), 1.29 1.38, 1.72, 1.73 (3H each, s, γ,γ'-CH_3), 6.18 (d, $J=3$ Hz, Glc. -1 of Suc.), 1.98, 2.04, 2.05, 2.06, 2.08, 2.10, 2.14, 2.24 (3H each, s, $OCOCH_3$).

Compound 8 Yield, 20 mg, $[\alpha]_D^{30}$ +8.8° ($c=1.0$, MeOH). FABMS *m/z*: 637.2472 (Calcd for $C_{29}H_{42}NaO_{14}$: 637.2472). UV λ_{max} nm (log ε): 238 (4.44), 287 (sh, 3.91).

Compound 9 (Dexylosyl-anodendrosin G) Yield, 70 mg, $[\alpha]_D^{25}$ +1.7° ($c=0.92$, MeOH). FABMS *m/z*: 649.2839 (Calcd for $C_{31}H_{46}NaO_{13}$: 649.2836).

Isolation of Anodendrosins from the Fresh, Ripe Seeds The seeds harvested in March, 1991 (950 g) were immediately soaked in MeOH, then homogenized and percolated with MeOH. The MeOH extract was worked up in the same manner as described above. The 80% and 100% MeOH eluates from the MCI-gel column were combined and chromatographed on silica gel and reversed-phase columns to afford anodendrosins B (180 mg), C (60 mg), D (60 mg), E (**10**) (460 mg) and J (**1**) (60 mg).

Oxidation of 10 Air was bubbled into a solution of **10** (25 mg) in dioxane–H_2O (1 : 1) (20 ml) for 70 h. The solvent was then evaporated *in vacuo* and the residue was chromatographed on a silica gel column with solvent 2 to afford **4** (7 mg) and **10** (6.4 mg).

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References and Notes

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