Glycosides of 14,15-Seco and 13,14:14,15-Disecopregnanes from the Roots of *Cynanchum sublanceolatum*

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Thirty-two new 13,14-seco and 13,14:14,15-disecopregnane glycosides were obtained from the roots of *Cynanchum sublanceolatum* (Asclepiadaceae). These glycosides were confirmed to be possessing five known compounds, cynajapogenin A, glaucogenin-A, 13-hydroxycynajapogenin A, 2α-hydroxyanhydrohirundigenin, atratogenin A and one new pregnane, 13-*epi*-cynajapogenin A, as their aglycones, using both spectroscopic and chemical methods.

Key words *Cynanchum sublanceolatum*; Asclepiadaceae; sublanceoside; 13,14-secopregnane glycoside; 13,14:14,15-disecopregnane glycoside; 2,6-dideoxyhexopyranose

Cynanchum sublanceolatum (MIQ.) MATUM. is an Asclepiadaceous plant and indigenous to the Honshu island of Japan. In the course of our research on phytochemicals, especially steroidal glycosides, in Asclepiadaceous plants, we have investigated the constituents of the roots of *C. sublanceolatum*. The paper describes the isolation and structural determination of thirty-two new pregnane glycosides along with three known compounds.

The MeOH extract from the dried roots of *C. sublamceolatum* was suspended in water. The suspension was extracted with diethyl ether and partitioned into an ether-soluble fraction and a water-soluble fraction. The residue of each fraction was chromatographed on a silica gel column, respectively, to give a fraction of 14,15-seco and 13,14:14,15-disecopregnane glycosides from which thirty-two new compounds were obtained.

In order to acquire the component aglycones and sugars, the fraction containing pregnane glycosides from a silica gel column chromatography was subjected to acid hydrolysis. The obtained known aglycones were identified as cynajapogenin A (**1a**),[1,2] glaucogenin-A (**2a**),[3,4] 2α-hydroxyanhydrohirundigenin (**12a**)[5] and atratogenin A (**18a**)[2] in view of the ^{1}H- and ^{13}C-NMR spectroscopic data.

The acquired sugar mixtures were fractionated to cymarose, digitoxose and a mixture of oleandrose and sarmentose using silica gel column chromatography. The absolute configurations of digitoxose and cymarose were believed to be the D-form and a mixture of the D- and L-forms on the basis of their optical rotation values, respectively. The absolute configuration of glucose was determined to be the D-form based on GC analysis following its reaction with D-cysteine methyl ester hydrochloride (see Experimental).

Compound **1** was suggested to have the molecular formula $C_{26}H_{36}O_7$ based on high resolution (HR)-FAB-MS [*m/z*: 483.2333 [M+Na]$^+$]. In the ^{1}H- and ^{13}C-NMR spectra of **1**, one anomeric proton and carbon signals were observed at δ 5.45 (1H, dd, J=9.5, 2.0 Hz)] and δ 98.3, in addition to signals due to the aglycone, which was identified as a 13,14-seco-type pregnane, cynajapogenin A (**1a**) by acid hydrolysis with 0.1 M H_2SO_4. The ^{13}C-NMR spectral comparison of **1** with **1a** showed that glycosylation shifts were presented at the C-2, -3 and -4 positions [C-2 (−2.2 ppm), C-3 (+9.1 ppm), C-4 (−2.8 ppm)].[6] Thus, **1** was glycosylated at the C-3 position, and was considered to be cynajapogenin A 3-*O*-monoglycoside. Acid hydrolysis of **1** showed that the sugar moiety consisted of digitoxose, and this sugar was identified as β-D-digitoxopyranose, as judged from the coupling constant of the anomeric proton signal (J=9.5, 2.0 Hz). Accordingly, **1** was established as cynajapogenin A 3-*O*-β-D-digitoxopyranoside that was named sublanceoside A_1.

The following compounds **2**—**34** and **35** were also glycosylated at the C-3 position of each aglycone based on observation of glycosylation shifts in the ^{13}C-NMR spectra.

HR-FAB-MS of compound **2** afforded a [M+Na]$^+$ peak at *m/z* 529.2413, suggesting the molecular formula, $C_{27}H_{38}O_9$. Compound **2** was considered to be a 13,14:14,15-diseco-type pregnane monoglycoside, according to appearance of signals due to one anomeric proton and carbon signals together with those of glaucogenin-A (**2a**) in the ^{1}H- and ^{13}C-NMR spectra. Being subjected to acid hydrolysis with 0.1 M H_2SO_4, **2** afforded **2a** and digitoxose. Thus, compound **2** was determined to be glaucogenin-A 3-*O*-β-D-digitoxopyranoside, and was named sublanceoside A_2.

The molecular formulae of compounds **3** and **4** were proposed to be $C_{33}H_{48}O_{10}$ and $C_{34}H_{50}O_{12}$ by HR-FAB-MS. The aglycone moieties of **3** and **4** were suggested to be **1a** and **2a**, respectively, on comparison of the ^{13}C-NMR spectroscopic data with those of **1** and **2**, which were confirmed by being subjected to acid hydrolysis. Because of the consistency of the ^{1}H- and ^{13}C-NMR spectroscopic data of the sugar moieties in **3** and **4**, both compounds were considered to possess the same sugar sequences. Furthermore, the presence of two anomeric proton and carbon signals at δ 5.19 (1H, dd, J=9.5, 2.0 Hz), 5.33 (1H, dd, J=9.5, 2.0 Hz) and δ 98.0 and 100.8 in the ^{1}H- and ^{13}C-NMR signals due to each sugar moiety suggested that **3** and **4** were cynajapogenin A 3-*O*-diglycoside and glaucogenin-A 3-*O*-diglycoside, respectively. On acid hydrolysis of **4**, cymarose and digitoxose were obtained, and their configurations were identified to be D-forms by their optical rotation values. The signals at δ 5.19 and 5.33 were assignable as the anomeric protons of β-D-cymaropyranose and β-D-digitoxopyranose based on the coupling constants and as a consequence of the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum. The sequence of the sugar moiety was determined by measurement of the difference nuclear Overhauser effect (NOE) spectra. Irradiation of

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the anomeric proton of each sugar in **4** showed NOEs as follows; δ 5.19 (H-1′ of β-D-cymaropyranose) and δ 3.56 (overlapping, H-3 of the aglycone), δ 5.33 (H-1″ of β-D-digitoxopyranose) and δ 3.54 (1H, dd, J=9.5, 3.0 Hz, H-4′ of β-D-cymaropyranose). Based on the above arguments, compounds **3** and **4** were determined to be cynajapogenin A 3-O-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside and glaucogenin-A 3-O-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside, and were named sublanceoside B_1 and B_2, respectively.

From the results of HR-FAB-MS, compounds **5** and **6** had the molecular formulae, $C_{39}H_{58}O_{13}$ and $C_{40}H_{60}O_{15}$, respectively. On the basis of the ^{1}H- and ^{13}C-NMR spectroscopic data, the aglycone moieties of **5** and **6** were identified to be **1a** and **2a**, and the sugar moieties of **5** and **6** were considered to have the same structures, owing to the coincidence of their NMR spectroscopic data. In the ^{1}H- and ^{13}C-NMR spectra of **6**, three anomeric proton and carbon signals and one set of methoxyl proton and carbon signals were observed [δ 5.38 (1H, dd, J=9.5, 2.0 Hz), 5.27 (1H, dd, J=9.5, 2.0 Hz), 5.07 (1H, dd, J=4.5, 2.0 Hz): δ 99.8, 98.5, 98.0 and δ 3.40 (3H, s): δ 56.8]. Thus, compound **6** was deduced to be glaucogenin-A 3-O-triglycoside. On acid hydrolysis of **6**, cymarose and digitoxose were afforded as the component sugars together with **2a**. In addition, the optical rotation value of each sugar suggested that the absolute configurations of digitoxose and cymarose were D- and L-forms, respectively. On the basis of the above evidence, the sugar moiety of **6** consisted of two D-digitoxopyranoses and one L-cymaropyranose. The measurements of the two dimensional (2D)-NMR (^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple quantum coherency (HMQC) and ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC)) of **6** revealed that the signals at δ 5.38, 98.0 and δ 5.27, 99.8 were assigned at the anomeric protons and carbons of two β-linked D-digitoxopyranoses, and the remaining signals at δ 5.07, 98.5 were assignable at the anomeric proton and carbon of one α-linked L-cymaropyranose. In addition, the HMBC experiment showed long-range correlations between δ 5.38 (H-1′ of β-D-digitoxopyranose) and δ 85.6 (C-3), δ 5.27 (H-1″ of β-D-digitoxopyranose) and δ 83.0 (C-4′ of β-D-digitoxopyranose), and δ 5.07 (H-1‴ of α-L-cymaropyranose) and δ 80.8 (C-4″ of β-D-digitoxopyranose). Hence, the structure of **6** was determined to be glaucogenin-A 3-O-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside, which was supported by observation of NOEs in the difference NOE experiment irradiating the anomeric proton signals. Furthermore, compound **5** was established to be cynajapogenin A 3-O-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside. Compounds **5** and **6** were named sublanceoside C_1 and C_2.

HR-FAB-MS showed that the molecular formulae of compounds **7** and **8** were $C_{45}H_{68}O_{18}$ and $C_{46}H_{70}O_{20}$, which suggested that they were larger than **5** and **6** for one hexose unit, respectively. Consistency of the NMR spectroscopic data of the sugar moieties of **7** and **8** showed that these compounds had the same sugar sequences. On acid hydrolysis, **7** and **8** afforded cymarose, digitoxose and glucose as the component sugars together with each aglycone, **1a** and **2a**. On enzymatic hydrolysis with cellulase, compounds **5** and **6** were detected from **7** and **8** on HPLC analyses, respectively. Comparison of the ^{13}C-NMR spectroscopic data of **7** with those of **5** revealed glycosylation shifts around the C-4 position of α-L-cymaropyranose [C-3‴ (−2.9 ppm), C-4‴ (+5.5 ppm), C-5‴ (−1.0 ppm)].[4] In addition, the HMBC experiment of **7** exhibited a long-range correlation between δ 5.01 (H-1⁗ of β-D-glucopyranose) and δ 78.2 (C-4‴ of α-L-cymaropyranose). Thus, the structure of **7** was elucidated to be cynajapogenin A 3-O-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside, and compound **8** was determined as shown in Chart 1. Compounds **7** and **8** were named sublanceoside D_1 and D_2, respectively.

HR-FAB-MS suggested that the molecular formula of compound **9** was $C_{41}H_{62}O_{15}$. Although the optical rotation and the NMR spectroscopic data of **9** were super-imposable to those of the previously reported compounds, glaucoside-C[7,8] and cynanoside J,[9] these data could not illustrate which compounds were identical to **9**. On acid hydrolysis of **9**, digitoxose and cymarose were obtained in addition to **2a**. Digitoxose was confirmed to be the D-form by its optical rotation value. On the other hand, the rotation value of cymarose was almost zero (−0.71°), indicating that cymarose was a mixture of D- and L-forms in the ratio 1 : 1. The ^{1}H- and ^{13}C-NMR spectroscopic data of **9** showed the signals due to a terminal α-L-cymaropyranosyl group. Thus, compound **9** was identified to be cynanoside J.

The molecular formulae of **10**—**12** were considered to be $C_{40}H_{60}O_{13}$, $C_{40}H_{60}O_{14}$ and $C_{41}H_{62}O_{14}$ on the basis of HR-FAB-MS, respectively. The sugar moieties of **10**—**12** were deduced to have the same structures as that of **9**, because of the coincidence of their ^{1}H- and ^{13}C-NMR spectroscopic data with those of **9**. And the aglycones of **10**—**12** were considered to **1a**, 13-hydroxycynajapogenin A (**11a**)[10] and 2α-hydroxyanhydrohirundigenin (**12a**) based on the NMR spectroscopic data, respectively. Thus, the structures of **10**—**12** were determined as shown in Chart 1, and were named sublanceoside E_1, E_3 and E_4.

HR-FAB-MS suggested that the molecular formula of compound **13** was $C_{40}H_{60}O_{13}$, which was the same as that of **10**. The sugar moiety of **13** was also considered to be the same sequence as those of **9**—**12** due to its NMR spectroscopic data. However, the ^{13}C-NMR spectrum showed slight differences in chemical shifts due to the aglycone moiety of **13** from those of **10**. Acid hydrolysis of **13** afforded **13a** as the aglycone moiety. Comparing the ^{1}H-NMR spectroscopic data of **13a** with those of **1a**, the methin proton signal at δ 3.65 (1H, t, J=4.5 Hz) was observed instead of the signal at δ 3.62 (1H, dd, J=12.5, 5.0 Hz). The HMBC experiment exhibited long-range correlations from this methin signal (δ 3.65) to C-8 (δ 42.9), C-11 (δ 21.0), C-12 (δ 30.1), C-14 (δ 211.9), C-16 (δ 110.8), C-17 (δ 117.2) and C-20 (δ 148.9). Thus, this methin proton signal was assigned at the H-13 position. In addition, coupling constant of this signal showed that H-13 had α-orientation. Based on the above results, **13a** was believed to be 13-*epi*-cynajapogenin A, and the structure of **13** was established as shown in Chart 1. Compound **13** was named sublanceoside E_5.

HR-FAB-MS showed that the molecular formulae of compounds **14**—**18** were $C_{46}H_{70}O_{18}$, $C_{47}H_{72}O_{20}$, $C_{47}H_{72}O_{19}$, $C_{46}H_{70}O_{18}$ and $C_{47}H_{72}O_{18}$. Consistency of the NMR spectroscopic data of the sugar moiety of **14**—**18** and comparison of

their data with those of **7** and **9** suggested that the sugar sequences of **14**—**18** were 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside. This sugar sequence was confirmed by acid hydrolysis, enzymatic hydrolysis and the presence of glycosylation shifts in the ^{13}C-NMR spectrum and long-range correlations in the HMBC experiment. Namely, on acid hydrolysis, **14**—**18** afforded cymarose, digitoxose and glucose as the component sugars together with each aglycone, **1a**, **2a**, **12a**, **13a**, and atratogenin A (**18a**). On enzymatic hydrolysis with cellulase, compounds **14**, **15**, **16** and **17** yielded **10**, **9**, **12** and **13**, respectively. Comparing the ^{13}C-NMR spectroscopic data of **14** with those of **10**, the C-4 signal of α-L-cymaropyranose shifted downfield by 5.5 ppm. Moreover, in the HMBC spectrum of **14**, a long-range correlation appeared between the anomeric proton signal of β-D-glucopyranose [δ 5.00 (1H, d, J=8.0 Hz)] and the C-4 signal of α-L-cymaropyranose (δ 78.2). Therefore, the structures of **14**—**18** were determined as shown in Chart 1. Compounds **14** and **15** were reported to be the revisions[11] of cynascyroside C[1] and glaucoside-H.[4,8] Compounds **16**, **17** and **18** were named sublanceoside F_4, F_5 and F_6, respectively.

The molecular formulae of compounds **19**—**24** were proposed to be $C_{41}H_{62}O_{14}$, $C_{42}H_{64}O_{14}$, $C_{47}H_{72}O_{18}$, $C_{48}H_{74}O_{20}$, $C_{47}H_{72}O_{18}$ and $C_{48}H_{74}O_{18}$, respectively, by HR-FAB-MS. Because of the consistency of the ^{1}H- and ^{13}C-NMR spectroscopic data of the sugar moieties in **21**—**24**, these compounds were considered to have the same sugar sequences. The aglycone moieties of **21**—**24** were suggested to be **1a**, **2a**, **13a** and **18a** according to their ^{13}C-NMR spectroscopic data. Acid hydrolysis of compound **22** with 0.1 M H_2SO_4 afforded cymarose and disaccharide (**22b**) with **2a**. This cymarose was proved to have the D-form by its optical rotation value, and **22b** was proposed to be L-(4-*O*-β-D-glucopyranosyl)-cymarose,[4] according to TLC analysis and its optical rotation value. The NMR spectroscopic data of **22** suggested that the sugar moiety of **22** consisted of three cymaropyranoses and one terminal β-D-glucopyranose. In consideration of the above result of acid hydrolysis and the NMR spectroscopic data, it was presumed that, as to the sugar moiety of **22**, two cymaroses had β-linked D-forms and the remaining cymarose possessed an α-linked L-form, and the terminal β-D-glucopyranosyl group was attached at the C-4 position of this α-L-cymaropyranose. The sugar sequence of **22** was determined based on the results of the difference NOE and HMBC experiments. In the difference NOE spectra, irradiation of each anomeric proton signal revealed NOEs as follows; δ 5.18 (1H, dd, J=9.5, 2.0 Hz, H-1′ of β-D-cymaropyranose) and δ 3.58 (overlapping, H-3 of the aglycone), δ 5.06 (1H, dd, J=9.5, 2.0 Hz, H-1″ of β-D-cymaropyranose) and δ 3.44 (1H, dd, J=9.5, 3.0 Hz, H-4′ of β-D-cymaropyranose), δ 4.96 (1H, dd, J=4.5, 2.0 Hz, H-1‴ of α-L-cymaropyranose) and δ 3.42 (1H, dd, J=9.5, 3.0 Hz, H-4″ of β-D-cymaropyranose). The long-range correlations were presented between δ 85.5 (C-3 of the aglycone) and δ 5.18 (H-1′ of β-D-cymaropyranose), δ 100.4 (C-1″ of β-D-cymaropyranose) and δ 3.44 (H-4′ of β-D-cymaropyranose), δ 98.9 (C-1‴ of α-L-cymaropyranose) and δ 3.42 (H-4″ of β-D-cymaropyranose), and δ 79.0 (C-4‴ of α-L-cymaropyranose) and δ 5.00 (H-1⁗ of β-D-glucopyranose). Thus, the structure of **22** was established to be glaucogenin-A 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. The structures of **21**, **23** and **24** were also shown in Chart 1.

Compounds **19** and **20** were 13-hydroxycynajapogenin A 3-*O*-triglycoside and 2-hydroxyanhydrohirundigenin 3-*O*-triglycoside. Based on the NMR spectroscopic data and the results of acid hydrolysis, the sugar moieties of **19** and **20** consisted of three cymaropyranoses possessing two β-linkages and one α-linkage. Because the ^{1}H- and ^{13}C-NMR spectroscopic data of the sugar moieties of **19** and **20** were consistent with those of deglucosyl derivative **22a**, which was produced by the enzymatic hydrolysis of **22** (see Experimental), the sugar sequences of these compounds were determined to be 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Thus, the structures of **19** and **20** are shown in Chart 1. Compounds **19**—**24** were named sublanceoside G_3, G_4, H_1, H_2, H_5 and H_6, respectively.

The molecular formula of compound **25** was determined to be $C_{41}H_{62}O_{13}$ by HR-FAB-MS. Acid hydrolysis suggested that the aglycone of **25** was **1a** and the sugar moiety consisted of cymarose and diginose. The overall structure assignment was accomplished by a combination of 1D- and 2D-NMR spectra. From the ^{1}H- and ^{13}C-NMR spectra, it was apparent that **25** possessed the same sugar sequence as those of atrotoside-A,[2] cynatratoside-F,[8] tylophoside C,[5] cynanoside A and E.[9] This sugar sequence was confirmed by observation of the NOEs on irradiation of the anomeric proton signal of each sugar in the difference NOE experiment. Thus, the structure of **25** was established as cynajapogenin A 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside, and was named sublanceoside I_1.

The molecular formulae of compounds **26**—**28** were determined to be $C_{47}H_{72}O_{16}$, $C_{48}H_{74}O_{18}$ and $C_{48}H_{74}O_{17}$ based on

Table 1. ^{13}C-NMR Data of the Aglycone Moiety of Compounds **3**, **4**, **11**—**13** and **18**

	3	4	11	12	13	18
Carbon No.						
-1	45.5	44.8	45.4	44.9	45.4	45.4
-2	70.1	70.0	70.0	70.3	70.0	70.1
-3	85.5	85.5	85.2	84.8	85.2	85.3
-4	37.7	37.6	37.5	37.6	37.6	37.6
-5	139.0	139.9	138.9	140.4	139.2	139.2
-6	121.5	120.8	121.2	120.5	121.4	121.6
-7	25.9	28.5	25.8	25.9	26.0	27.0
-8	45.6	40.2	43.0	103.8	42.8	41.6
-9	52.1	53.1	52.5	45.1	50.5	50.7
-10	38.8	39.5	38.9	38.7	39.0	39.0
-11	25.5	23.9	22.4	20.3	20.9	20.4
-12	33.8	30.1	39.8	31.9	30.0	38.3
-13	47.9	118.6	76.3	53.8	46.4	47.7
-14	209.3	175.4	213.0	153.1	211.9	212.3
-15	140.2	67.8	140.8	72.4	140.9	139.3
-16	112.0	75.6	110.5	84.4	110.8	111.4
-17	118.0	56.2	121.4	63.7	117.2	a)
-18	—	143.9	—	76.7	—	23.9
-19	20.0	19.0	19.8	19.7	19.7	19.8
-20	148.3	114.4	a)	118.3	148.9	148.2
-21	11.9	24.8	12.8	22.7	12.0	14.6

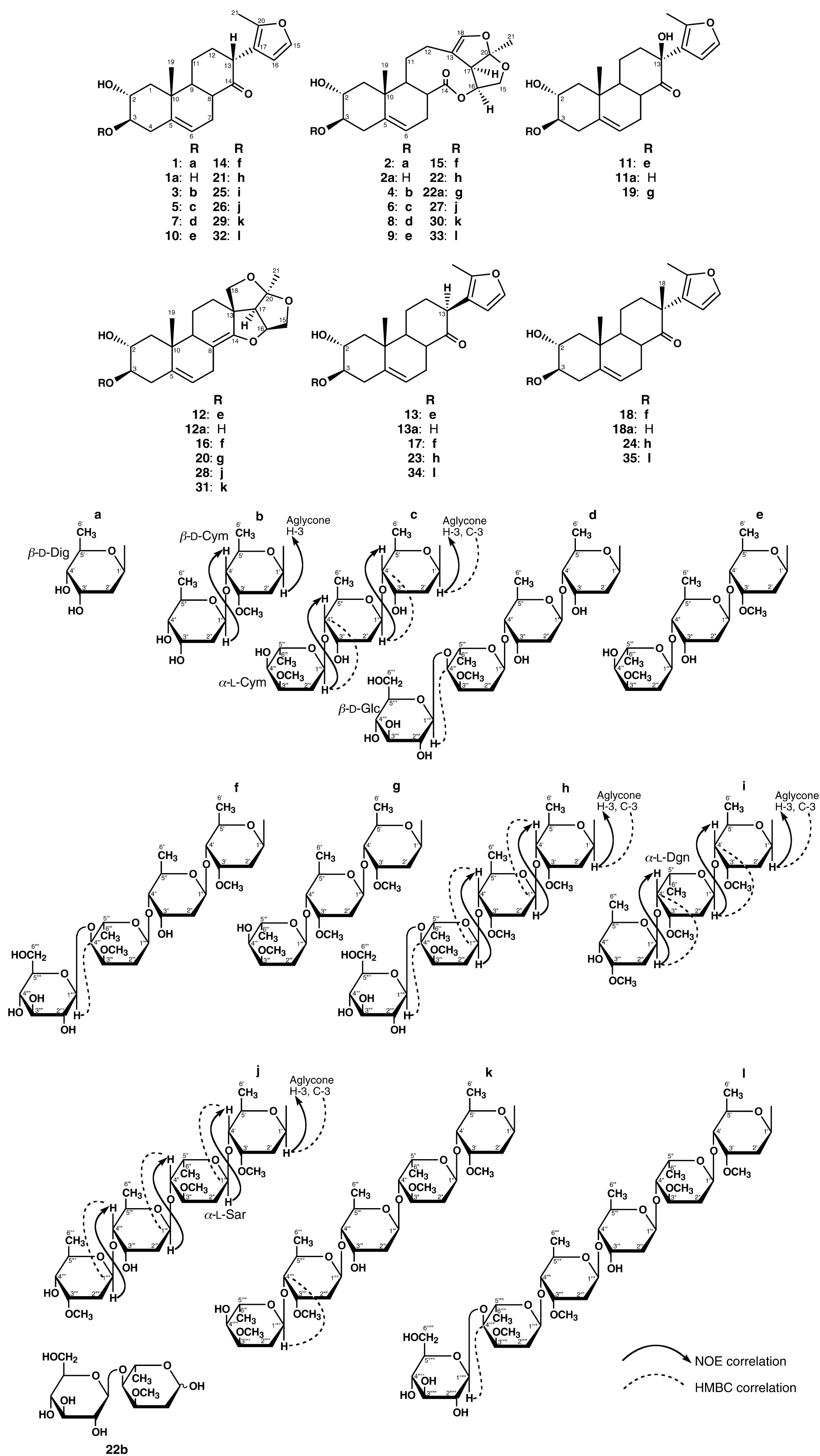
Measured in pyridine-d_5 at 35 °C. *a*) Overlapping with pyridine-d_5 signals.

HR-FAB-MS. The ^{1}H- and ^{13}C-NMR measurement suggested that **26**—**28** were cynajapogenin A 3-*O*-tetraglycoside, glaucogenin-A 3-*O*-tetraglycoside and 2α-hydroxyanhydrohirundigenin 3-*O*-tetraglycoside, respectively. And their NMR spectroscopic data were assigned as shown in Table 2 and Experimental, according to the results of 2D-NMR experiments. Consistency of the NMR spectroscopic data of the sugar moieties in **26**—**28** revealed that these compounds had the same sugar sequences. Acid hydrolysis of **26** and **27** afforded D-cymarose, D-digitoxose and L-sarmentose. In addition, observation of two characteristic H-3 signals of cymaropyranose and three methoxyl proton signals at δ 3.88 (1H, q, J=3.5 Hz), 3.72 (1H, q, J=3.5 Hz) and δ 3.63 (3H, s), 3.46 (3H, s), 3.37 (3H, s) in the ^{1}H-NMR spectrum of **26** showed that the component sugars of **26** were two β-D-cymaropyranoses, one α-L-sarmentopyranose and one β-D-digitoxopyranose. The sugar sequence of **26** was determined by the results of the difference NOE experiment irradiating at each anomeric proton signal and the HMBC measurement. NOEs were observed between δ 5.17 (H-1′ of β-D-cymaropyranose) and δ 3.54 (H-3 of the aglycone), δ 4.99 (H-1″ of α-L-sarmentopyranose) and δ 3.43 (H-4′ of β-D-cymaropyranose), δ 5.36 (H-1‴ of β-D-digitoxopyranose) and δ 3.74 (H-4″ of α-L-sarmentopyranose), and δ 5.13 (H-1⁗ of β-D-cymaropyranose) and δ 3.50 (H-4‴ of β-D-digitoxopyranose). And long-range correlations were exhibited between δ 85.4 (C-3 of the aglycone) and δ 5.17 (H-1′ of β-D-cymaropyranose), δ 99.5 (C-1″ of α-L-sarmentopyranose) and δ 3.43 (H-4′ of β-D-cymaropyranose), δ 100.7 (C-1‴ of β-D-digitoxopyranose) and δ 3.74 (H-4″ of α-L-sarmentopyra-

Table 2. ^{13}C-NMR Data of the Sugar Moiety of Compounds **1**, **4**, **6**, **7**, **9**, **14**, **19**, **22**, **22a**, **25**, **26**, **29** and **33**

Carbon No.	**1**	**4**	**6**	**7**	**9**	**14**	**19**	**22**	**22a**	**25**	**26**	**29**	**33**
	D-Dig	D-Cym	D-Dig	D-Dig	D-Cym	D-Cym	D-Cym	D-Cym	D-Cym	D-Cym	D-Cym	D-Cym	D-Cym
-1′	98.3	98.0	98.0	97.9	97.9	97.9	97.8	97.9	97.9	97.6	97.8	97.8	97.8
-2′	39.8	37.0	38.8[a)]	38.9[a)]	37.1	37.1	37.1	37.0	37.1	35.4[a)]	37.0	37.0[a)]	37.0[a)]
-3′	68.7	77.9	67.5[b)]	67.5[b)]	77.9	77.9	77.9[a)]	77.9[a)]	77.9[a)]	77.5	77.7	77.7	77.7
-4′	73.9	83.0	83.0	83.0	82.9	82.9	82.9	82.9	82.9	82.1	82.0	82.0	82.0[b)]
-5′	70.8	69.5	69.0	69.0	69.4	69.4	69.4[b)]	69.4[b)]	69.4[b)]	69.6	69.7	69.7	69.7
-6′	18.7	18.3	18.5[c)]	18.4[c)]	18.5[a)]	18.5[a)]	18.6[c)]	18.5[c)]	18.6[c)]	18.4	18.3[a)]	18.3[b)]	18.3[c)]
		D-Dig	D-Dig	D-Dig	D-Dig	D-Dig	D-Cym	D-Cym	D-Cym	L-Dgn	L-Sar	L-Sar	L-Sar
-1″	—	100.8	99.8	99.8	100.4	100.4	100.4	100.4	100.4	101.1	99.5	99.5	99.5
-2″	—	39.6	38.2[a)]	38.3[a)]	38.4	38.5	37.1	37.0	37.1	32.5	28.6	28.6	28.6
-3″	—	68.6	67.7[b)]	67.7[b)]	67.7	67.7	77.7[a)]	77.7[a)]	77.7[a)]	74.6	75.9	75.9	75.9
-4″	—	74.0	80.8	81.0	80.8	80.9	82.1	82.2	82.1	73.9	77.3	77.3	77.3
-5″	—	70.4	69.0	69.0	68.9	68.8	69.3[b)]	69.5[b)]	69.3[b)]	67.7	63.2	63.2	63.1
-6″	—	19.0	18.4[c)]	18.3[c)]	18.4[a)]	18.2[a)]	18.5[c)]	18.5[c)]	18.5[c)]	17.9	16.9	16.9	16.9
			L-Cym	L-Cym	L-Cym	L-Cym	L-Cym	L-Cym	L-Cym	D-Cym	D-Dig	D-Dig	D-Dig
-1‴	—	—	98.5	98.4	98.4	98.3	99.0	98.9	99.0	99.5	100.7	100.6	100.6
-2‴	—	—	32.2	32.4	32.3	32.4	32.2	32.3	32.2	35.3[a)]	38.5	38.5	38.5
-3‴	—	—	76.5	73.6	76.6	73.6	76.5	73.4	76.5	79.0	67.4	67.5	67.5
-4‴	—	—	72.7	78.2	72.7	78.2	73.3	79.0	73.3	74.2	83.2	83.1	83.1
-5‴	—	—	67.3	66.3	67.3	66.3	66.5	65.2	66.5	71.1	68.7	68.7	68.7
-6‴	—	—	18.3[c)]	18.1[c)]	18.2[a)]	18.1[a)]	18.2[c)]	18.2[c)]	18.2[c)]	18.8	18.6[a)]	18.6[b)]	18.5[c)]
				D-Glc		D-Glc		D-Glc			D-Cym	D-Cym	D-Cym
-1⁗	—	—	—	102.5	—	102.5	—	102.4	—	—	99.7	99.7	99.7
-2⁗	—	—	—	75.4	—	75.4	—	75.3	—	—	35.7	36.9[a)]	36.8[a)]
-3⁗	—	—	—	78.6[d)]	—	78.6[b)]	—	78.6[d)]	—	—	78.8	77.7	77.7
-4⁗	—	—	—	71.9	—	71.9	—	71.9	—	—	74.1	82.0	82.2[b)]
-5⁗	—	—	—	78.5[d)]	—	78.5[b)]	—	78.5[d)]	—	—	71.0	69.4	69.3
-6⁗	—	—	—	63.0	—	63.0	—	63.1	—	—	18.9[a)]	18.5[b)]	18.5[c)]
												L-Cym	L-Cym
-1′′′′′	—	—	—	—	—	—	—	—	—	—	—	99.0	99.0
-2′′′′′	—	—	—	—	—	—	—	—	—	—	—	32.1	32.3
-3′′′′′	—	—	—	—	—	—	—	—	—	—	—	76.4	73.4
-4′′′′′	—	—	—	—	—	—	—	—	—	—	—	73.3	79.0
-5′′′′′	—	—	—	—	—	—	—	—	—	—	—	66.5	65.2
-6′′′′′	—	—	—	—	—	—	—	—	—	—	—	18.5[b)]	18.5[c)]
													D-Glc
-1′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	102.4
-2′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	75.4
-3′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	78.6[d)]
-4′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	71.9
-5′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	78.5[d)]
-6′′′′′′	—	—	—	—	—	—	—	—	—	—	—	—	63.1
-OMes	—	58.9	56.8	57.0	59.0	59.0	59.0	59.0	59.0	58.0	58.4	58.5	58.5
	—	—	—	—	56.8	57.1	58.5	58.6	58.5	57.4	58.1	58.4	58.4
	—	—	—	—	—	—	56.6	56.9	56.6	55.4	56.3	56.6	56.8
	—	—	—	—	—	—	—	—	—	—	—	56.3	56.3

Measured in pyridine-d_5 at 35 °C. *a*—*d*) Signal assignments may be interchangeable in each column. Dig=digitoxopyranose, Cym=cymaropyranose, Dgn=diginopyranose, Sar=sarmentopyranose, Glc=glucopyranose.



The important NOE and/or HMBC correlations were observed in compounds **4**, **6**, **7**, **14**, **22**, **25**, **26**, **29** and **33**.

Chart 1

nose), and δ 99.7 (C-1'''' of β-D-cymaropyranose) and 3.50 (H-4''' of β-D-digitoxopyranose). Thus, the structure of **26** was established to be cynajapogenin A 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-α-L-sarmentopyranosyl-(1→4)-β-D-cymaropyranoside, and the structures of **27** and **28** were also described in Chart 1. Compounds **26**—**28** were named sublanceoside J_1, J_2 and J_4.

HR-FAB-MS suggested that the molecular formulae of compounds **29**—**31** were $C_{54}H_{84}O_{19}$, $C_{55}H_{86}O_{21}$ and $C_{55}H_{86}O_{20}$. The NMR spectra showed that **29**—**31** were pentaglycosides, which possessed **1a**, **2a** and **12a** as each aglycone, respectively. Because the ^{1}H- and ^{13}C-NMR spectroscopic data of the sugar moieties of **29**—**31** were super-imposable, these compounds were considered to possess the same sugar sequences. The ^{1}H- and ^{13}C-NMR spectra of **29** were similar to those of **26**. However, compound **29** showed signals due to a terminal α-L-cymaropyranosyl group, the same as **19**. On comparison of the ^{13}C-NMR spectroscopic data of **29** with those of **26**, glycosylation shifts were observed around the C-4 position of the second β-D-cymaropyranose [C-3'''' (−1.2 ppm), C-4'''' (+7.9 ppm), C-5'''' (−1.6 ppm)]. Moreover, in the HMBC experiment, a long-range correlation was exhibited between the anomeric proton signal of the terminal α-L-cymaropyranose [δ 4.96 (1H, dd, J=4.5, 1.5 Hz)] and the C-4 signal of the second β-D-cymaropyranose (δ 82.0). Thus, the structure of **29** was established to be cynajapogenin A 3-*O*-α-L-cymaropyranose-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-α-L-sarmentopyranosyl-(1→4)-β-D-cymaropyranoside, and the structures of **30** and **31** are also shown in Chart 1. Compounds **29**—**31** were named sublanceoside K_1, K_2 and K_4.

The molecular formulae of compounds **32**—**35** were determined to be $C_{60}H_{94}O_{24}$, $C_{61}H_{96}O_{26}$, $C_{60}H_{94}O_{24}$ and $C_{61}H_{96}O_{24}$ based on HR-FAB-MS. The NMR spectroscopic data of the sugar moieties in **32**—**35** were consistent. Comparing the ^{1}H- and ^{13}C-NMR data of **33** with those of **30**, **33** had one more glucopyranosyl group with the anomeric proton signal resonating at δ 4.99 (1H, d, J=8.0 Hz). The enzymatic hydrolysis of **33** afforded **30**, and acid hydrolysis gave glucose in addition to cymarose, sarmentose, digitoxose and **2a**. The position of the glucopyranosyl group in **33** was suggested to be located at the C-4 position of α-L-cymaropyranose, according to observation of glycosylation shifts [C-3''''' (−3.0 ppm), C-4''''' (+5.7 ppm), C-5''''' (−1.3 ppm)]. This sugar sequence was also supported by a long-range correlation between the H-1 signal of β-D-glucopyranose [δ 4.99 (1H, d, J=8.0 Hz)] and the C-4 signal of α-L-cymaropyranose (δ 79.0) in the HMBC experiment. Thus, the structure of **33** was established to be glaucogenin-A 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranose-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-α-L-sarmentopyranosyl-(1→4)-β-D-cymaropyranoside, and the structures of **32**, **34** and **35** were also shown in Chart 1. Compounds **32**—**35** were named sublanceoside L_1, L_2, L_5 and L_6.

The previous papers reported the inhibition against acetylcholineesterase, moderate cytotoxic activity against HL-60 human promyelocytic leukemia cells and immunosuppressive activity of pregnane glycosides from *Cynanchum* spp.[9—12] We are interested in the biological activities of this plant, according to the similarity of the constituents of pregnane glycosides.

Experimental

General Procedure Instrumental analysis was carried out as described previously.[13]

Plant Material The roots of *Cynanchum sublanceolatum* were collected from the plants garden, University of Shizuoka in Japan.

Extraction and Isolation The dried roots of *C. sublanceolatum* (2.0 kg) were extracted three times with MeOH under reflux. The extract was concentrated under reduced pressure and the residue was suspended in H_2O. This suspension was extracted with Et_2O. The Et_2O layer was concentrated, and the residue (31.3 g) was then chromatographed on a silica gel column with a $CHCl_3$–MeOH (98 : 2—85 : 15) system and semi-preparative HPLC (Develosil-ODS, and YMC-ODS: 48—60% MeCN in water and 65—82.5% MeOH in water) to give compounds **1** (3 mg), **2** (7 mg), **3** (47 mg), **4** (42 mg), **5** (12 mg), **6** (44 mg), **9** (171 mg), **10** (29 mg), **11** (18 mg), **12** (11 mg), **13** (16 mg), **19** (4 mg), **20** (7 mg), **25** (4 mg), **26** (28 mg), **27** (16 mg), **28** (3 mg), **29** (29 mg), **30** (35 mg) and **31** (6 mg).

The H_2O layer of the MeOH extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column with absorbed material being eluted with MeOH–H_2O (1 : 1), MeOH–H_2O (7 : 3) and MeOH, respectively. The MeOH fraction from the porous polymer gel column was then evaporated to dryness, with the residue (32.4 g) was subjected to a silica gel CC with a $CHCl_3$–MeOH (95 : 5—8 : 2) system and semi-preparative HPLC (Develosil-ODS, and YMC-ODS: 40—47.5% MeCN in water and 72.5—82.5% MeOH in water) to give compounds **7** (11 mg), **8** (41 mg), **14** (49 mg), **15** (60 mg), **16** (7 mg), **17** (16 mg), **18** (6 mg), **21** (112 mg), **22** (118 mg), **23** (8 mg), **24** (4 mg), **32** (12 mg), **33** (18 mg), **34** (7 mg) and **35** (4 mg).

Sublanceoside A_1 (**1**): Amorphous powder. $[\alpha]_D^{22}$ −57° (c=0.31, MeOH). FAB-MS m/z: 461 $[M+H]^+$, 483 $[M+Na]^+$. HR-FAB-MS m/z: 483.2333 (Calcd for $C_{26}H_{36}O_7Na$: 483.2359). ^{13}C-NMR: shown in Table 2. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.45 (1H, dd, 9.5, 2.0, H-1'), 4.44 (1H, q, 3.0, H-3'), 4.33 (1H, dq, 9.5, 6.0, H-5'), 1.54 (3H, d, 6.0, H-6'). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3** except for the C-3, H-2 and H-3 signals [δ 85.7 (C-3), 4.06 (1H, m, H-2), 3.64 (overlapping, H-3)].

Sublanceoside A_2 (**2**): Amorphous powder. $[\alpha]_D^{21}$ +58° (c=0.42, MeOH). FAB-MS m/z: 529 $[M+Na]^+$. HR-FAB-MS m/z: 529.2413 (Calcd for $C_{27}H_{38}O_9Na$: 529.2414). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **4** and **1**, respectively, except for the C-3 signal [δ 85.8 (C-3)]. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **4** and **1** except for the H-2 and H-3 signals [δ 4.03 (1H, m, H-2), 3.61 (overlapping, H-3)].

Sublanceoside B_1 (**3**): Amorphous powder. $[\alpha]_D^{22}$ −26.7° (c=1.09, MeOH). FAB-MS m/z: 605 $[M+H]^+$, 627 $[M+Na]^+$. HR-FAB-MS m/z: 605.3304, 627.3143 (Calcd for $C_{33}H_{49}O_{10}$: 605.3326 and $C_{33}H_{48}O_{10}Na$: 627.3145). ^{13}C-NMR: shown in Table 1. The ^{13}C-NMR spectroscopic data of the sugar moiety were in good agreement with those of **4**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 7.48 (1H, d, 2.0, H-15), 6.48 (1H, d, 2.0, H-16), 5.43 (1H, br d, 4.5, H-6), 4.02 (1H, m, H-2), 3.61 (overlapping, H-13), 3.57 (overlapping, H-3), 2.20 (3H, s, H-21), 1.07 (3H, s, H-19). The ^{1}H-NMR spectroscopic data of the sugar moiety were similar to those of **4**.

Sublanceoside B_2 (**4**): Amorphous powder. $[\alpha]_D^{21}$ +56° (c=0.71, MeOH). FAB-MS m/z: 673 $[M+Na]^+$. HR-FAB-MS m/z: 673.3193 (Calcd for $C_{34}H_{50}O_{12}Na$: 673.3200). ^{13}C-NMR: shown in Tables 1 and 2. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 6.46 (1H, br s, H-18), 5.44 (overlapping, H-16), 5.42 (1H, br s, H-6), 5.33 (1H, dd, 9.5, 2.0, H-1''), 5.19 (1H, dd, 9.5, 2.0, H-1'), 4.41 (1H, br s, H-3''), 4.26 (1H, dq, 9.5, 6.0, H-5''), 4.24 (1H, dq, 9.5, 6.0, H-5'), 4.23 (1H, dd, 8.5, 7.0, H-15), 4.11 (1H, q, 3.5, H-3'), 4.00 (1H, m, H-2), 3.95 (1H, dd, 9.5, 8.5, H-15), 3.56 (overlapping, H-3), 3.54 (1H, dd, 9.5, 3.0, H-4'), 3.54 (1H, br d, 8.0, H-17), 1.58 (3H, d, 6.0, H-6''), 1.54 (3H, s, H-21), 1.34 (3H, d, 6.0, H-6'), 0.91 (3H, s, H-19).

Sublanceoside C_1 (**5**): Amorphous powder. $[\alpha]_D^{22}$ −74.4° (c=1.30, MeOH). FAB-MS m/z: 757 $[M+Na]^+$. HR-FAB-MS m/z: 757.3781 (Calcd for $C_{39}H_{58}O_{13}Na$: 757.3775). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **3** and **6**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **3** and **6**.

Sublanceoside C_2 (**6**): Amorphous powder. $[\alpha]_D^{24}$ −7.99° (c=1.87, MeOH). FAB-MS m/z: 803 $[M+Na]^+$. HR-FAB-MS m/z: 803.3839 (Calcd for $C_{40}H_{60}O_{15}Na$: 803.3830). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **4**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.38 (1H, dd, 9.5, 2.0, H-1'), 5.27 (1H, dd, 9.5, 2.0, H-1''), 5.07 (1H, dd, 4.5, 2.0, H-1'''), 4.63 (1H, br s, H-3'), 4.44 (overlapping, H-3'', -5'''), 4.29 (1H, dq, 9.5, 6.5, H-5'), 4.12 (1H,

dq, 9.5, 6.5, H-5″) 3.71 (1H, q, 3.5, H-3‴) 3.47 (1H, dd, 9.5, 3.0, H-4′), 3.40 (3H, s, C-3‴-OMe), 3.39 (1H, dd, 9.5, 3.0, H-4″) 1.42 (3H, d, 6.5, H-6‴), 1.35 (3H, d, 6.5, H-6′), 1.32 (3H, d, 6.5, H-6″). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **4**.

Sublanceoside D_1 (**7**): Amorphous powder. $[\alpha]_D^{21}$ $-76.6°$ (c=1.04, MeOH). FAB-MS m/z: 919 $[M+Na]^+$. HR-FAB-MS m/z: 919.4308 (Calcd for $C_{45}H_{68}O_{18}Na$: 919.4303). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.39 (1H, dd, 9.5, 2.0, H-1′), 5.28 (1H, dd, 9.5, 2.0, H-1″), 5.03 (1H, t, 3.5, H-1‴), 5.01 (1H, d, 8.0, H-1⁗), 4.63 (1H, q, 3.0, H-3′), 4.60 (1H, dd, 8.0, 6.0, H-5⁗), 4.54 (1H, br d, 12.0, H-6⁗), 4.43 (1H, q, 3.0, H-3″), 4.36 (1H, dd, 12.0, 5.5, H-6⁗), 4.30 (1H, dq, 9.5, 6.0, H-5′), 4.13 (1H, dq, 9.5, 6.5, H-5″), 3.97 (1H, dd, 8.0, 3.0, H-4‴), 3.93 (overlapping, H-3‴), 3.48 (1H, dd, 9.5, 3.0, H-4′), 3.45 (3H, s, C-3‴-OMe), 3.38 (1H, dd, 9.5, 3.0, H-4″), 1.35 (6H, d, 6.0, H-6′, -6‴), 1.31 (3H, d, 6.5, H-6″). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **3**.

Sublanceoside D_2 (**8**): Amorphous powder. $[\alpha]_D^{21}$ $-13.6°$ (c=1.33, MeOH). FAB-MS m/z: 965 $[M+Na]^+$. HR-FAB-MS m/z: 965.4360 (Calcd for $C_{46}H_{70}O_{20}Na$: 965.4358). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **4** and **7**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **4** and **7**.

Compound **9**: ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **4**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.21 (1H, dd, 9.5, 2.0, H-1″), 5.18 (1H, dd, 9.5, 2.0, H-1′), 5.08 (1H, dd, 4.0, 2.0, H-1‴), 4.45 (overlapping, H-3″, -5‴), 4.21 (1H, dq, 9.5, 6.5, H-5′), 4.11 (1H, dq, 9.5, 6.5, H-5″), 4.07 (1H, q, 3.0, H-3′), 3.71 (1H, q, 3.5, H-3‴), 3.62 (3H, s, C-3′-OMe), 3.47 (1H, dd, 9.5, 3.0, H-4′), 3.42 (1H, dd, 9.5, 3.0, H-4″), 3.40 (3H, s, C-3‴-OMe), 1.43 (3H, d, 6.5, H-6‴), 1.38 (3H, d, 6.5, H-6″), 1.30 (3H, d, 6.5, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **4**.

Sublanceoside E_1 (**10**): Amorphous powder. $[\alpha]_D^{22}$ $-71°$ (c=0.71, MeOH). FAB-MS m/z: 771 $[M+Na]^+$. HR-FAB-MS m/z: 771.3937 (Calcd for $C_{40}H_{60}O_{13}Na$: 771.3932). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **3** and **9**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **3** and **9**.

Sublanceoside E_3 (**11**): Amorphous powder. $[\alpha]_D^{23}$ $-73°$ (c=0.91, MeOH). FAB-MS m/z: 787 $[M+Na]^+$. HR-FAB-MS m/z: 787.3873 (Calcd for $C_{40}H_{60}O_{14}Na$: 787.3881). ^{13}C-NMR: shown in Table 1. The ^{13}C-NMR spectroscopic data of the sugar moiety were in good agreement with those of **9**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 7.50 (1H, d, 2.0, H-15), 6.67 (1H, d, 2.0, H-16), 6.61 (1H, br s, C-13-OH), 5.43 (1H, br d, 5.0, H-6), 3.95 (1H, m, H-2), 3.55 (1H, m, H-3), 2.19 (3H, s, H-21), 0.87 (3H, s, H-19). The ^{1}H-NMR spectroscopic data of the sugar moiety were similar to those of **9**.

Sublanceoside E_4 (**12**): Amorphous powder. $[\alpha]_D^{24}$ $-44°$ (c=0.49, MeOH). FAB-MS m/z: 801 $[M+Na]^+$. HR-FAB-MS m/z: 801.4033 (Calcd for $C_{41}H_{62}O_{14}Na$: 801.4037). ^{13}C-NMR: shown in Table 1. The ^{13}C-NMR spectroscopic data of the sugar moiety were in good agreement with those of **9**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.38 (1H, br s, H-6), 4.77 (overlapping, H-16), 4.25 (1H, br d, 11.0, H-15), 4.08 (1H, d, 9.0, H-18), 3.95 (1H, m, H-2), 3.80 (1H, dd, 11.0, 4.5, H-15), 3.58 (1H, m, H-3), 2.80 (1H, d, 8.0, H-17), 1.58 (3H, s, H-21), 0.83 (3H, s, H-19). The ^{1}H-NMR spectroscopic data of the sugar moiety were similar to those of **9**.

Sublanceoside E_5 (**13**): Amorphous powder. $[\alpha]_D^{24}$ $-76.3°$ (c=1.58, MeOH). FAB-MS m/z: 771 $[M+Na]^+$. HR-FAB-MS m/z: 771.3929 (Calcd for $C_{40}H_{60}O_{13}Na$: 771.3932). ^{13}C-NMR: shown in Table 1. The ^{13}C-NMR spectroscopic data of the sugar moiety were in good agreement with those of **9**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 7.49 (1H, d, 2.0, H-15), 6.52 (1H, d, 2.0, H-16), 5.42 (1H, br s, H-6), 3.99 (1H, m, H-2), 3.57 (overlapping, H-3), 2.20 (3H, s, H-21), 1.01 (3H, s, H-19). The ^{1}H-NMR spectroscopic data of the sugar moiety were similar to those of **9**.

Compound **14**: ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.23 (1H, dd, 9.5, 2.0, H-1″), 5.18 (1H, dd, 9.5, 2.0, H-1′), 5.05 (1H, t, 3.5, H-1‴), 5.00 (1H, d, 8.0, H-1⁗), 4.60 (1H, dq, 8.0, 6.5, H-5⁗), 4.54 (1H, dd, 12.0, 2.0, H-6⁗), 4.43 (1H, br s, H-3″), 4.36 (1H, dd, 12.0, 5.5, H-6⁗), 4.21 (overlapping, H-5′), 4.12 (1H, dq, 9.5, 6.0, H-5″), 4.07 (1H, q, 3.0, H-3′), 3.93 (overlapping, H-3‴), 3.62 (3H, s, C-3′-OMe), 3.47 (1H, dd, 9.5, 3.0, H-4′), 3.46 (3H, s, C-3‴-OMe), 3.41 (1H, dd, 9.5, 3.0, H-4″), 1.37 (3H, d, 6.0, H-6″), 1.35 (3H, d, 6.5, H-6‴), 1.29 (3H, d, 6.5, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **3**.

Sublanceoside F_4 (**16**): Amorphous powder. $[\alpha]_D^{23}$ $-49°$ (c=0.69, MeOH). FAB-MS m/z: 963 $[M+Na]^+$. HR-FAB-MS m/z: 963.4569 (Calcd for $C_{47}H_{72}O_{19}Na$: 963.4566). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **12** and **14**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **12** and **14**.

Sublanceoside F_5 (**17**): Amorphous powder. $[\alpha]_D^{22}$ $-65°$ (c=0.51, MeOH). FAB-MS m/z: 933 $[M+Na]^+$. HR-FAB-MS m/z: 933.4453 (Calcd for $C_{46}H_{70}O_{18}Na$: 933.4460). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **13** and **14**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **13** and **14**.

Sublanceoside F_6 (**18**): Amorphous powder. $[\alpha]_D^{24}$ $-89°$ (c=0.60, MeOH). FAB-MS m/z: 947 $[M+Na]^+$. HR-FAB-MS m/z: 947.4617 (Calcd for $C_{47}H_{72}O_{18}Na$: 947.4616). ^{13}C-NMR: shown in Table 1. The ^{13}C-NMR spectroscopic data of the sugar moiety were in good agreement with those of **14**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 7.42 (1H, d, 2.0, H-15), 6.40 (1H, d, 2.0, H-16), 5.44 (1H, br t, 3.5, H-6), 4.03 (1H, m, H-2), 3.59 (overlapping, H-3), 2.22 (3H, s, H-21), 1.48 (3H, s, H-18), 1.10 (3H, s, H-19). The ^{1}H-NMR spectroscopic data of the sugar moiety were similar to those of **14**.

Sublanceoside G_3 (**19**): Amorphous powder. $[\alpha]_D^{22}$ $-69°$ (c=0.35, MeOH). FAB-MS m/z: 801 $[M+Na]^+$. HR-FAB-MS m/z: 801.4035 (Calcd for $C_{41}H_{62}O_{14}Na$: 801.4037). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **11**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.17 (1H, dd, 9.5, 2.0, H-1′), 5.06 (1H, dd, 9.5, 2.0, H-1″), 4.98 (1H, dd, 4.0, 2.0, H-1‴), 4.53 (1H, dq, 9.0, 6.5, H-5‴), 4.20 (1H, dq, 9.5, 6.0, H-5′), 4.17 (1H, dq, 9.5, 6.0, H-5″), 4.05 (1H, q, 3.0, H-3′), 3.87 (1H, q, 3.0, H-3″), 3.71 (1H, q, 3.5, H-3‴), 3.61 (3H, s, C-3′-OMe), 3.56 (3H, s, C-3″-OMe), 3.44 (1H, dd, 9.5, 3.0, H-4″), 3.43 (1H, dd, 9.5, 3.0, H-4′), 3.38 (3H, s, C-3‴-OMe), 1.52 (3H, d, 6.5, H-6‴), 1.36 (3H, d, 6.0, H-6″), 1.30 (3H, d, 6.0, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **11**.

Sublanceoside G_4 (**20**): Amorphous powder. $[\alpha]_D^{22}$ $-51°$ (c=0.20, MeOH). FAB-MS m/z: 815 $[M+Na]^+$. HR-FAB-MS m/z: 815.4187 (Calcd for $C_{42}H_{64}O_{14}Na$: 815.4194). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **12** and **19**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **12** and **19**.

Sublanceoside H_1 (**21**): Amorphous powder. $[\alpha]_D^{22}$ $-66.5°$ (c=1.78, MeOH). FAB-MS m/z: 947 $[M+Na]^+$. HR-FAB-MS m/z: 947.4639 (Calcd for $C_{47}H_{72}O_{18}Na$: 947.4616). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **3** and **22**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **3** and **22**.

Sublanceoside H_2 (**22**): Amorphous powder. $[\alpha]_D^{21}$ $-8.02°$ (c=1.44, MeOH). FAB-MS m/z: 993 $[M+Na]^+$. HR-FAB-MS m/z: 993.4680 (Calcd for $C_{48}H_{74}O_{20}Na$: 993.4671). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **4**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.18 (1H, dd, 9.5, 2.0, H-1′), 5.06 (1H, dd, 9.5, 2.0, H-1″), 5.00 (1H, d, 8.0, H-1⁗), 4.96 (1H, dd, 4.5, 2.0, H-1‴), 4.69 (1H, dq, 8.0, 6.5, H-5‴), 4.56 (1H, br d, 12.0, H-6⁗), 4.38 (1H, m, H-6⁗), 4.21 (overlapping, H-5′), 4.16 (1H, dq, 9.5, 6.0, H-5″), 4.05 (1H, q, 3.0, H-3′), 3.94 (overlapping, H-3‴), 3.87 (1H, q, 3.0, H-3″), 3.61 (3H, s, C-3″-OMe), 3.58 (3H, s, C-3′-OMe), 3.45 (3H, s, C-3‴-OMe), 3.44 (1H, dd, 9.5, 3.0, H-4′), 3.42 (1H, dd, 9.5, 3.0, H-4″), 1.48 (3H, d, 6.5, H-6‴), 1.34 (3H, d, 6.0, H-6″), 1.32 (3H, d, 6.0, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **4**.

Sublanceoside H_5 (**23**): Amorphous powder. $[\alpha]_D^{21}$ $-63°$ (c=0.49, MeOH). FAB-MS m/z: 947 $[M+Na]^+$. HR-FAB-MS m/z: 947.4621 (Calcd for $C_{47}H_{72}O_{18}Na$: 947.4616). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **13** and **22**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **13** and **22**.

Sublanceoside H_6 (**24**): Amorphous powder. $[\alpha]_D^{24}$ $-83°$ (c=0.43, MeOH). FAB-MS m/z: 961 $[M+Na]^+$. HR-FAB-MS m/z: 961.4775 (Calcd for $C_{48}H_{74}O_{18}Na$: 961.4773). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **18** and **22**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **18** and **22**.

Sublanceoside I_1 (**25**): Amorphous powder. $[\alpha]_D^{22}$ $-69°$ (c=0.38, MeOH). FAB-MS m/z: 785 $[M+Na]^+$. HR-FAB-MS m/z: 785.4080 (Calcd for $C_{41}H_{62}O_{13}Na$: 785.4088). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.19 (1H, dd, 9.5, 2.0, H-1′), 5.16 (1H,

br d, 3.5, H-1″), 5.13 (1H, dd, 9.5, 2.0, H-1‴), 4.25 (1H, dq, 9.5, 6.0, H-5′), 4.24 (overlapping, H-5″), 4.11 (1H, dq, 9.5, 6.0, H-5‴), 4.09 (overlapping, H-4″), 3.93 (1H, q, 3.0, H-3′), 3.82 (1H, ddd, 12.0, 4.0, 3.5, H-3″), 3.73 (1H, q, 3.0, H-3‴), 3.56 (3H, s, C-3′-OMe), 3.46 (overlapping, H-4′), 3.46 (3H, s, C-3‴-OMe), 3.44 (3H, s, C-3″-OMe) 1.53 (3H, d, 6.0, H-6‴), 1.46 (3H, d, 6.0, H-6″), 1.32 (3H, d, 6.0, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **3**.

Sublanceoside J$_1$ (**26**): Amorphous powder. $[\alpha]_D^{22}$ −47° (c=0.84, MeOH). FAB-MS m/z: 915 [M+Na]$^+$. HR-FAB-MS m/z: 915.4710 (Calcd for $C_{47}H_{72}O_{16}$Na: 915.4718). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.36 (1H, dd, 9.5, 2.0, H-1‴), 5.17 (1H, dd, 9.5, 2.0, H-1′), 5.13 (1H, dd, 9.5, 2.0, H-1⁗), 4.99 (1H, br d, 4.5, H-1″), 4.63 (overlapping, H-3‴), 4.61 (overlapping, H-5″), 4.27 (1H, dq, 9.5, 6.0, H-5‴), 4.21 (1H, dq, 9.5, 6.0, H-5′), 4.09 (1H, dq, 9.5, 6.0, H-5⁗), 4.01 (overlapping, H-3″), 3.88 (1H, q, 3.5, H-3′), 3.74 (1H, br s, H-4″), 3.72 (1H, q, 3.5, H-3⁗), 3.63 (3H, s, C-3′-OMe), 3.50 (1H, dd, 9.5, 3.0, H-4⁗), 3.46 (3H, s, C-3⁗-OMe), 3.43 (1H, dd, 9.5, 3.0, H-4′), 3.37 (3H, s, C-3″-OMe), 1.44 (3H, d, 6.0, H-6⁗), 1.43 (3H, d, 6.0, H-6‴), 1.35 (3H, d, 6.5, H-6″), 1.29 (3H, d, 6.0, H-6′). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **3**.

Sublanceoside J$_2$ (**27**): Amorphous powder. $[\alpha]_D^{23}$ +15.0° (c=1.29, MeOH). FAB-MS m/z: 961 [M+Na]$^+$. HR-FAB-MS m/z: 961.4790 (Calcd for $C_{48}H_{74}O_{18}$Na: 961.4773). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **4** and **26**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **4** and **26**.

Sublanceoside J$_4$ (**28**): Amorphous powder. $[\alpha]_D^{24}$ −26° (c=0.31, MeOH). FAB-MS m/z: 945 [M+Na]$^+$. HR-FAB-MS m/z: 945.4838 (Calcd for $C_{48}H_{74}O_{17}$Na: 945.4824). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **12** and **26**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **12** and **26**.

Sublanceoside K$_1$ (**29**): Amorphous powder. $[\alpha]_D^{22}$ −75.2° (c=1.33, MeOH). FAB-MS m/z: 1059 [M+Na]$^+$. HR-FAB-MS m/z: 1059.5508 (Calcd for $C_{54}H_{84}O_{19}$Na: 1059.5505). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **3**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.35 (1H, dd, 9.5, 2.0, H-1‴), 5.17 (1H, dd, 9.5, 2.0, H-1′), 5.15 (1H, dd, 9.5, 2.0, H-1⁗), 4.99 (1H, br d, 4.0, H-1″), 4.96 (1H, dd, 4.5, 1.5, H-1⁗′), 4.61 (overlapping, H-5″, -3‴), 4.51 (1H, dq, 9.0, 6.5, H-5⁗′), 4.24 (1H, dq, 9.5, 6.0, H-5‴), 4.21 (1H, dq, 9.5, 6.0, H-5′), 4.15 (1H, dq, 9.5, 6.0, H-5⁗), 4.00 (overlapping, H-3″), 3.89 (1H, q, 3.0, H-3′), 3.85 (1H, q, 3.0, H-3⁗), 3.74 (1H, br s, H-4″), 3.70 (1H, q, 3.5, H-3⁗′), 3.62 (3H, s, C-3′ or -3⁗-OMe), 3.55 (3H, s, C-3⁗ or -3′-OMe), 3.46 (1H, dd, 9.5, 3.0, H-4‴), 3.43 (1H, dd, 9.5, 3.0, H-4′), 3.38 (overlapping, H-4⁗), 3.37 (6H, s, C-3″ and -3⁗′-OMes), 1.52 (3H, d, 6.5, H-6⁗′), 1.39 (3H, d, 6.0, H-6‴), 1.34 (3H, d, 6.5, H-6″), 1.30 (3H, d, 6.0, H-6′), 1.28 (3H, d, 6.0, H-6⁗). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **3**.

Sublanceoside K$_2$ (**30**): Amorphous powder. $[\alpha]_D^{22}$ −20.5° (c=1.25, MeOH). FAB-MS m/z: 1105 [M+Na]$^+$. HR-FAB-MS m/z: 1105.5560 (Calcd for $C_{55}H_{86}O_{21}$Na: 1105.5559). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **4** and **29**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **4** and **29**.

Sublanceoside K$_4$ (**31**): Amorphous powder. $[\alpha]_D^{22}$ −55° (c=0.63, MeOH). FAB-MS m/z: 1089 [M+Na]$^+$. HR-FAB-MS m/z: 1089.5614 (Calcd for $C_{55}H_{86}O_{20}$Na: 1089.5610). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **12** and **29**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **12** and **29**.

Sublanceoside L$_1$ (**32**): Amorphous powder. $[\alpha]_D^{21}$ −78.5° (c=1.49, MeOH). FAB-MS m/z: 1221 [M+Na]$^+$. HR-FAB-MS m/z: 1221.6036 (Calcd for $C_{60}H_{94}O_{24}$Na: 1221.6033). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **3** and **33**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **3** and **33**.

Sublanceoside L$_2$ (**33**): Amorphous powder. $[\alpha]_D^{21}$ −30.4° (c=1.55, MeOH). FAB-MS m/z: 1267 [M+Na]$^+$. HR-FAB-MS m/z: 1267.6113 (Calcd for $C_{61}H_{96}O_{26}$Na: 1267.6088). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **4**. ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 5.35 (1H, dd, 9.5, 2.0, H-1‴), 5.17 (1H, dd, 9.5, 2.0, H-1′), 5.13 (1H, dd, 9.5, 2.0, H-1⁗), 5.00 (overlapping, H-1″), 4.99 (1H, d, 8.0, H-1⁗″), 4.93 (1H, dd, 4.5, 2.0, H-1⁗′), 4.68 (1H, dq, 8.0, 6.5, H-5⁗′), 4.61 (overlapping, H-5″, -3‴), 4.55 (1H, br d, 12.0, H-6⁗″), 4.37 (1H, dd, 12.0, 5.5, H-6⁗″), 4.24 (1H, dq, 9.5, 6.5, H-5‴), 4.22 (overlapping, H-5′), 4.15 (1H, dq, 9.5, 6.5, H-5⁗), 4.01 (overlapping, H-3″), 3.94 (overlapping, H-3⁗′), 3.88 (1H, q, 3.0, H-3′), 3.86 (1H, q, 3.0, H-3⁗), 3.74 (1H, br s, H-4″), 3.63 (3H, s, C-3′ or -3⁗-OMe), 3.57 (3H, s, C-3⁗ or -3′-OMe), 3.46 (1H, dd, 9.5, 3.0, H-4‴), 3.44 (1H, dd, 9.5, 3.0, H-4′), 3.44 (3H, s, C-3⁗′-OMe), 3.37 (1H, dd, 9.5, 3.0, H-4⁗), 3.37 (3H, s, C-3″-OMe), 1.48 (3H, d, 6.5, H-6⁗′), 1.40 (3H, d, 6.5, H-6‴), 1.34 (3H, d, 6.5, H-6″), 1.30 (3H, d, 6.5, H-6′), 1.26 (3H, d, 6.5, H-6⁗). The ^{1}H-NMR spectroscopic data of the aglycone moiety were similar to those of **4**.

Sublanceoside L$_5$ (**34**): Amorphous powder. $[\alpha]_D^{22}$ −61° (c=0.68, MeOH). FAB-MS m/z: 1221 [M+Na]$^+$. HR-FAB-MS m/z: 1221.6035 (Calcd for $C_{60}H_{94}O_{24}$Na: 1221.6033). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **13** and **33**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **13** and **33**.

Sublanceoside L$_6$ (**35**): Amorphous powder. $[\alpha]_D^{21}$ −75° (c=0.35, MeOH). FAB-MS m/z: 1235 [M+Na]$^+$. HR-FAB-MS m/z: 1235.6195 (Calcd for $C_{61}H_{96}O_{24}$Na: 1235.6189). The ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **18** and **33**, respectively. The ^{1}H-NMR spectroscopic data of the aglycone and sugar moieties were similar to those of **18** and **33**.

Acid Hydrolysis of a Mixture of Pregnane Glycosides The fraction of pregnane glycosides eluted with the $CHCl_3$–MeOH (98 : 2) system on a silica gel column (590 mg) was heated at 60 °C for 4 h with 0.1 M H_2SO_4 (2 ml) and dioxane (8 ml) to obtain the aglycones and sugars. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was concentrated to dryness. Cynajapogenin A (**1a**), glaucogenin-A (**2a**), 2α-hydroxyanhydrohirundigenin (**12a**) and atratogenin A (**18a**) were afforded from the residue by purification with HPLC (column, YMC-ODS 20 mm×25 cm; solvent, 50—57.5% MeOH in water).

The H_2O layer was passed though an Amberlite IRA-60E column and the eluate was concentrated to dryness. The residue was chromatographed on silica gel with a $CHCl_3$–MeOH–H_2O (7 : 1 : 1.2 bottom layer) system to obtain component sugars. Cymarose and digitoxose were afforded, and as to the absolute configuration, digitoxose was believed to have a D-form and cymarose was a mixture of D- and L-forms based on their optical rotation values.

Cymarose: $[\alpha]_D^{22}$ +12.0° (c=1.25, 24 h after dissolution in H_2O). (lit: D-cymarose: $[\alpha]_D^{15}$ +51.6° (c=1.02, H_2O)[14]).

D-Digitoxose: $[\alpha]_D^{22}$ +44.6° (c=1.32, 24 h after dissolution in H_2O). (lit: $[\alpha]_D^{21}$ +48° (c=1.8, H_2O)[7]).

Oleandrose and sarmentose could not be purified completely.

Acid Hydrolysis of Compounds 11 and 13 Compounds **11** (12 mg) and **13** (9 mg) were dissolved in dioxane (0.6 ml) and 0.1 M H_2SO_4 (0.15 ml), respectively. The solution was heated at 60 °C for 1 h, and the reaction mixture was diluted H_2O and extracted with EtOAc. The EtOAc layer was dried with Na_2SO_4 anhydride overnight, and evaporated off *in vacuo* after removing Na_2SO_4 anhydride by filtration. When the residue of the EtOAc layer was chromatographed by HPLC (column, YMC-ODS 10 mm×25 cm; solvent, 47.5% MeOH in water and 60% MeOH in water), **11a** (3 mg) and **13a** (2 mg) were afforded. Compound **11a** was identified to be 13-hydroxycynajapogenin A on the basis of the ^{1}H- and ^{13}C-NMR spectroscopic data.

13-*epi*-Cynajapogenin A (**13a**): Amorphous powder. $[\alpha]_D^{21}$ −68° (c=0.22, MeOH). FAB-MS m/z: 331 [M+H]$^+$. HR-FAB-MS m/z: 331.1908 (Calcd for $C_{20}H_{27}O_4$: 331.1909). ^{13}C-NMR (pyridine-d_5 at 35 °C) δ: 211.9 (C-14), 148.9 (C-20), 140.8 (C-15), 140.3 (C-5), 120.7 (C-6), 117.2 (C-17), 110.8 (C-16), 76.6 (C-3), 72.4 (C-2), 50.7 (C-9), 46.4 (C-13), 46.2 (C-1), 42.9 (C-8), 40.6 (C-4), 39.6 (C-10), 30.1 (C-12), 26.0 (C-7), 21.0 (C-11), 19.9 (C-19), 12.0 (C-21). ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 7.49 (1H, d, 2.0, H-15), 6.53 (1H, d, 2.0, H-16), 4.13 (1H, m, H-2), 3.79 (1H, br q, 8.5, H-3), 3.65 (1H, t, 4.5, H-13), 2.66 (1H, td, 10.5, 5.5, H-8), 2.64 (2H, overlapping, H-4), 2.40 (1H, dd, 12.5, 4.5, H-1), 2.20 (3H, s, H-21), 1.91 (1H, tdd, 12.0, 5.5, 4.0, H-12) 1.83 (1H, qd, 12.0, 3.5, H-11), 1.64 (1H, td, 12.0, 4.5, H-9), 1.08 (3H, s, H-19).

Subsequently, the H_2O layer was neutralized with an Amberlite IRA-60E column, and reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120B column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with MeOH, and the residue was acetylated overnight with acetic anhydride and pyridine (5 drops each) at room temperature. After evaporation of the reagents under a stream of air, cymaritol acetate and digitoxitol acetate were detected by GC analysis. GC conditions: column, Supelco SP-2380^{TM} capillary column 0.25 mm×30 m, carrier gas, N_2; column tempera-

ture 200 °C; t_R 7.4 min (cymaritol acetate), 10.5 min (digitoxitol acetate).

Acid Hydrolysis of Compounds 1—6, 9, 10, 12, 19, 20, 25—30 and 31 Solutions of compounds **1**—**6**, **9**, **10**, **12**, **19**, **20**, **25**—**30** and **31** (*ca.* 0.5 mg) in dioxane (80 μl) and 0.1 M H_2SO_4 (20 μl) were heated at 60 °C for 1 h. After hydrolysis, this solution was passed through an Amberlite IRA-60E column and concentrated to dryness. The residues were partitioned between H_2O and EtOAc, and the EtOAc extract was analyzed by HPLC to identify the aglycone *via* comparison with authentic samples. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; 60% MeOH in water; t_R, 10.8 min (cynajapogenin A (**1a**)), 12.4 min (glaucogenin-A (**2a**)); 52.5% MeOH in water; t_R, 12.2 min (2α-hydroxyanhydrohirundigenin (**12a**)), 10.8 min (13-hydroxycynajapogenin A (**11a**)). Cynajapogenin A was detected from **1**, **3**, **5**, **10**, **25**, **26** and **29**. Similarly, glaucogenin-A, 2α-hydroxyanhydrohirundigenin and 13-hydroxycynajapogenin A were detected from **2**, **4**, **6**, **9**, **27**, **30** and **12**, **20**, **28**, **31** and **19**, respectively.

Subsequently, the H_2O layer was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The following procedures for detection of the component sugars were described above. GC conditions: column, Supelco SP-2380™ capillary column 0.25 mm×30 m, carrier gas, N_2; column temperature 215 °C; t_R 5.6 min (cymaritol acetate), 5.8 min (diginitol acetate), 6.7 min (sarmentitol acetate), 7.2 min (digitoxitol acetate); 200 °C; t_R 7.4 min (cymaritol acetate), 8.2 min (diginitol acetate), 9.5 min (sarmentitol acetate), 10.5 min (digitoxitol acetate). Cymaritol acetate was detected from **3**—**6**, **9**, **10**, **12**, **19**, **20**, **25**—**30** and **31**. Diginitol acetate was found from **25**. Sarmentitol acetate was observed from **26**—**30** and **31**, and digitoxitol acetate was afforded from **1**—**6**, **9**, **10**, **12**, **26**—**30** and **31**.

Acid Hydrolysis of a Mixture of Pregnane Glycosides to Determine the Configuration of Glucose The fraction of pregnane glycosides eluted from the $CHCl_3$–MeOH (9 : 1) system formed a silica gel column (*ca.* 20 mg) was heated at 98 °C for 1 h with 0.05 M HCl and dioxane (0.2 ml each). After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was neutralized with an Amberlite IRA-60E column, and the eluate was concentrated to dryness. This residue was stirred with D-cysteine methyl ester hydrochloride hexamethyldisilazane and trimethylsilylchloride in pyridine, as described in the previous report.[15,16] After reactions, the supernatant was subjected to GC analysis. GC conditions: column, GL capillary column TC-1 0.25 mm×30 m (GL Science Co.), carrier gas, N_2; column temperature 230 °C; t_R 21.4 min (D-glucose), 20.6 min (L-glucose). D-Glucose was detected from the mixture of pregnane glycosides.

Acid Hydrolysis of Compounds 7, 8, 14—18, 21—24, 32—34 and 35 Solutions of compounds **7**, **8**, **14**—**18**, **21**—**24**, **32**—**34** and **35** (*ca.* 0.5 mg) in dioxane and 0.05 M HCl (50 μl each) were heated at 98 °C for 1 h. The following procedures, HPLC and GC conditions for the detection of the component aglycones and sugars were described above. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; 62.5% MeOH in water; t_R, 13.2 min (13-*epi*-cynajapogenin A (**13a**)); 60% MeOH in water; t_R, 15.8 min (atratogenin A (**18a**)). Cynajapogenin A was detected from **7**, **14**, **21** and **32**. Glaucogenin-A was found from **8**, **15**, **22** and **33**. 13-*epi*-Cynajapogenin A was observed from **17**, **23** and **34**. Atratogenin A was observed from **18**, **24** and **35**. 2α-Hydroxyanhydrohirundigenin was yielded from **16**. GC conditions: column, Supelco SP-2380™ capillary column 0.25 mm×30 m, carrier gas, N_2; column temperature 250 °C; t_R 11.6 min (glucitol acetate). Cymaritol acetate and glucitol acetate were detected from **7**, **8**, **14**—**18**, **21**—**24**, **32**—**34** and **35**. Digitoxitol acetate was found from **7**, **8**, **14**—**18**, **32**—**34** and **35**. Sarmentitol acetate was not observed from **32**—**34** and **35**. However, when compounds **32**—**34** and **35** (*ca.* 0.5 mg) were hydrolyzed with 0.1 M H_2SO_4 (20 μl) and dioxane (80 μl) at 60 °C for 1 h, sarmentitol acetate was detected together with cymaritol acetate and digitoxitol acetate from these compounds.

Acid Hydrolysis of Compounds 4, 6, 9, 22, 26, 27, 29 and 30 Compounds **4** (34 mg), **6** (35 mg), **9** (92 mg), **22** (55 mg), **26** (21 mg), **27** (13 mg), **29** (24 mg) and **30** (31 mg) were dissolved in 0.1 M H_2SO_4–dioxane (1 : 4) (0.8—1.5 ml) and heated at 60 °C for 1 h, respectively. The following procedures were described above. The H_2O layers were chromatographed on silica gel with the $CHCl_3$–MeOH–H_2O (7 : 1 : 1.2 bottom layer, 7 : 2 : 1.2 bottom layer) or benzene–acetone (5 : 1) systems to obtain component sugars.

From the H_2O layer of compound **4**, D-cymarose and D-digitoxose were afforded. D-Cymarose: $[\alpha]_D^{23}$ +49° (c=0.29, 24 h after dissolution in H_2O): D-digitoxose: $[\alpha]_D^{23}$ +42° (c=0.48, 24 h after dissolution in H_2O).

From the H_2O layer of compound **6**, L-cymarose and D-digitoxose were obtained. L-Cymarose: $[\alpha]_D^{23}$ −34° (c=0.21, 24 h after dissolution in H_2O) (lit: $[\alpha]_D^{13}$ −50.4° (c=1.00, H_2O)[14]): D-digitoxose: $[\alpha]_D^{23}$ +40° (c=0.61, 24 h after dissolution in H_2O).

From the H_2O layer of compound **9**, cymarose and D-digitoxose were yielded. Cymarose: $[\alpha]_D^{24}$ −0.71° (c=0.35, 24 h after dissolution in H_2O): D-digitoxose: $[\alpha]_D^{24}$ +43° (c=0.75, 24 h after dissolution in H_2O).

From the H_2O layer of compound **22**, D-cymarose and 4-*O*-(β-D-glucopyranosyl)-L-cymarose were afforded. D-Cymarose: $[\alpha]_D^{21}$ +54° (c=0.57, 24 h after dissolution in H_2O): 4-*O*-(β-D-glucopyranosyl)-L-cymarose (**22b**): $[\alpha]_D^{21}$ −65° (c=0.76, 24 h after dissolution in H_2O) (lit: $[\alpha]_D$ −72.6° (c=0.81, H_2O)[4]). An approximate *Rf* value for **22b** using a silica gel TLC was 0.24 with a $CHCl_3$–MeOH–EtOAc–H_2O (43 : 17 : 38 : 2) system.

From the mixture of the H_2O layer of compounds **26** and **27**, D-cymarose, L-sarmentose and D-digitoxose were obtained. D-Cymarose: $[\alpha]_D^{23}$ +45° (c=0.62, 24 h after dissolution in H_2O): L-sarmentose: $[\alpha]_D^{23}$ −9.4° (c=0.24, 24 h after dissolution in H_2O) (lit: $[\alpha]_D^{24}$ −15.9° (c=0.34, H_2O)[5]): D-digitoxose: $[\alpha]_D^{23}$ +39° (c=0.21, 24 h after dissolution in H_2O).

From the mixture of the H_2O layer of compounds **29** and **30**, cymarose, L-sarmentose and D-digitoxose were obtained. Cymarose: $[\alpha]_D^{23}$ +17.0° (c=1.10, 24 h after dissolution in H_2O): L-sarmentose: $[\alpha]_D^{23}$ −9.3° (c=0.27, 24 h after dissolution in H_2O): D-digitoxose: $[\alpha]_D^{23}$ +44° (c=0.35, 24 h after dissolution in H_2O).

Enzymatic Hydrolysis of Compounds 7, 8, 14—17, 32 and 33 Compounds **7**, **8**, **14**—**17**, **32** and **35** (*ca.* 1 mg) were dissolved in EtOH (30 μl) and H_2O (0.3 ml), respectively, then cellulase (Sigma Chem. Co.) (*ca.* 10 mg) was added to each solution. The mixtures were stirred at 40 °C for 1 d. After hydrolysis, the reaction mixtures were diluted with H_2O and extracted with EtOAc, and each EtOAc extract was analyzed by HPLC to identify *via* comparison with authentic samples. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; 82.5% MeOH in water; t_R, 18.0 min (**30**), 13.6 min (**29**), 12.2 min (**9**); 80% MeOH in water; t_R, 18.6 min (**13**), 11.8 min (**12**); 77.5% MeOH in water; t_R, 16.0 min (**10**), 12.8 min (**6**), 10.2 min (**5**). Compounds **5**, **6**, **10**, **9**, **12**, **13**, **29** and **30** were detected from the EtOAc extracts of **7**, **8**, **14**, **15**, **16**, **17**, **32** and **33**, respectively.

Enzymatic Hydrolysis of Compound 22 Compound **22** (11 mg) was dissolved in EtOH (0.2 ml) and H_2O (1.3 ml), and cellulase (Sigma Chem. Co.) (95 mg) was added into the solution. The mixtures were stirred at 40 °C for 2 d. The procedures after hydrolysis were described above. When the residue of the EtOAc extract was chromatographed by HPLC (column, YMC-ODS 20 mm×25 cm; solvent, 82.5% MeOH in water), **22a** (3 mg) was obtained.

Compound **22a**: Amorphous powder. $[\alpha]_D^{21}$ +10° (c=0.33, MeOH). FAB-MS *m/z*: 831 $[M+Na]^+$. HR-FAB-MS *m/z*: 831.4131 (Calcd for $C_{42}H_{64}O_{15}Na$: 831.4143). ^{13}C-NMR: shown in Table 2. The ^{13}C-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **4**. ^1H-NMR (pyridine-d_5 at 35 °C) δ: 5.19 (1H, dd, 9.5, 2.0, H-1′), 5.07 (1H, dd, 9.5, 2.0, H-1″), 4.98 (1H, dd, 4.5, 2.0, H-1‴), 4.53 (1H, dq, 9.5, 6.5, H-5‴), 4.21 (1H, dq, 9.5, 6.5, H-5′), 4.17 (1H, dq, 9.5, 6.5, H-5″), 4.05 (1H, q, 3.0, H-3′), 3.87 (1H, q, 3.0, H-3″), 3.71 (1H, q, 3.5, H-3‴), 3.61 (3H, s, C-3′-OMe), 3.56 (3H, s, C-3″-OMe), 3.44 (2H, dd, 9.5, 3.0, H-4′, -4″), 3.38 (3H, s, C-3‴-OMe), 1.52 (3H, d, 6.5, H-6‴), 1.37 (3H, d, 6.5, H-6″), 1.32 (3H, d, 6.5, H-6′). The ^1H-NMR spectroscopic data of the aglycone moiety were similar to those of **4**.

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