Steroidal Glycosides from Roots of *Cynanchum caudatum* M. III

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The roots of *Cynanchum caudatum* M. (Asclepiadaceae) afforded 21 new pregnane glycosides which had sibirigenin, cynanchogenin, gagaminin, 12-*O*-nicotinoyllineolon and rostratamine as the aglycone moiety and 2,6-dideoxy-3-*O*-methylhexopyranoses as component sugars. The structures of these compounds were elucidated by spectroscopic methods and chemical evidence.

Key words *Cynanchum caudatum* M.; Asclepiadaceae; esterified pregnane glycoside; 2,6-dideoxy-3-*O*-methylhexopyranose

We have previously reported esterified pregnane glycosides which were obtained from the aerial part and roots of *Cynanchum caudatum* M. (Asclepiadaceae).[1-3] As 21 pregnane glycosides could be newly procured from the roots of this plant at this time, we now report the structural elucidation of these glycosides as part of our research on the steroidal glycosides of Asclepiadaceous plants.

Extraction and isolation of the roots of *C. caudatum* M. were described in a previous report.[2] A crude pregnane glycoside fraction afforded 21 new glycosides (compounds **1**—**20** and **21**).

Compound **1** was suggested to have the molecular formula $C_{49}H_{78}O_{15}$, based on FAB-MS. This formula was consistent with that of cynanchoside C_2,[4] and comparison of the ^{1}H- and ^{13}C-NMR spectral data of **1** with those of cynanchoside C_2 showed that **1** had the same sugar sequence, β-D-oleandropyranosyl-(1→4)-β-D--cymaropyranosyl-(1→4)-β-D-cymaropyranoside, as that of cynanchoside C_2, but the aglycone moiety was not cynanchogenin. In the ^{13}C-NMR spectrum, C-12, 18 and 20 signals were observed at δ 73.9, 12.0 and 217.5, which was consistent with those of sibirigenin[5]; thus, the aglycone moiety of **1** was identified as sibirigenin. Moreover, as a glycosylation shift was observed at around C-3 in the aglycone, the linked location of the sugar sequence was decided to be at the C-3 position of sibirigenin. On the basis of these arguments, its structure was determined to be sibirigenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compounds **2**, **3** and **4** were also pregnane 3-*O*-triglycosides which had the same sugar sequence as that of **1**, because of the consistency of their ^{1}H and ^{13}C-NMR signals. Each aglycone moiety of **2**, **3** and **4** was determined to be 12-*O*-nicotinoyllineolon,[6] rostratamine[7] and gagaminin,[8] respectively, on acid hydrolysis (see Experimental). Consequently, the structures of these compounds were determined as shown in Chart 1.

Compounds **5** and **6** were also 3-*O*-triglycosides which had 12-*O*-nicotinoyllineolon and gagaminin as the aglycone moiety, respectively. On acid hydrolysis followed by $NaBH_4$ reduction and acetylation (see Experimental) of both compounds, only cymaritol acetate was detected; thus, the sugar sequence consisted of three cymaroses. The magnitude of the (H, H) coupling constants indicated that one anomeric proton (δ 4.79) formed a part of α-cymaropyranose whose configuration was determined to be L-form based on the chemical shift of C-2,[9] and other anomeric protons (δ 4.84, 4.76) formed a part of β-cymaropyranoses which were determined to have a D-configuration. Subsequently, to determine the sugar sequence, the nuclear Overhauser effect (NOE) difference spectra were examined in **6**. Irradiation at the anomeric proton signal of the first β-D-cymaropyranose at δ 4.84 caused emhancement of the signal intensity at δ 3.55 (H-3 of aglycone). Accordingly, β-D-cymaropyranose was attached to C-3 of the aglycone moiety. Similarly, NOEs were observed between δ 4.76 (H-1 of the second β-D-cymaropyranose)/3.21 (H-4 of the first β-D-cymaropyranose) and δ 4.79 (H-1 of α-L-cymaropyranose)/3.23 (H-4 of the second β-D-cymaropyranose), and preceding H-4 signals were assignable on the result of the correlation spectroscopy (COSY) experiment. Based on the above evidence, the sugar sequence was identified as 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, and then, the structures of **5** and **6** were determined as shown.

Compounds **7** and **8** was also considered to be gagaminin 3-*O*-triglycosides because of the consistency of the ^{1}H- and ^{13}C-NMR spectra. In regard to the sugar sequence, ^{1}H- and ^{13}C-NMR spectral data of **7** and **8** were consistent with those of cynanchogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside[2] and cynanchogenin 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside, respectively.[3] Based on the above evidence, the structures of **7** and **8** were elucidated as shown.

Compounds **9**—**17** and **18** were deduced to be esterified pregnane 3-*O*-tetraglycosides, because four anomeric carbon and proton signals were observed in addition to the signals of each aglycone moiety in NMR spectra. Acid hydrolysis, followed by $NaBH_4$ reduction and acetylation, showed that component sugars of the sugar sequences in all compounds were cymarose and oleandrose, according to the detection of cymaritol acetate and oleandritol acetate with GLC analysis, and comparison of the peak area of cymaritol acetate and oleandritol acetate suggested that the relative ratio of cymarose and oleandrose in **9** and **14**—**17** was 3:1, **10**—**13** was 1:1, and **18** was 1:3. Thus, each sugar sequence of **9**, **14**—**17** consisted of three

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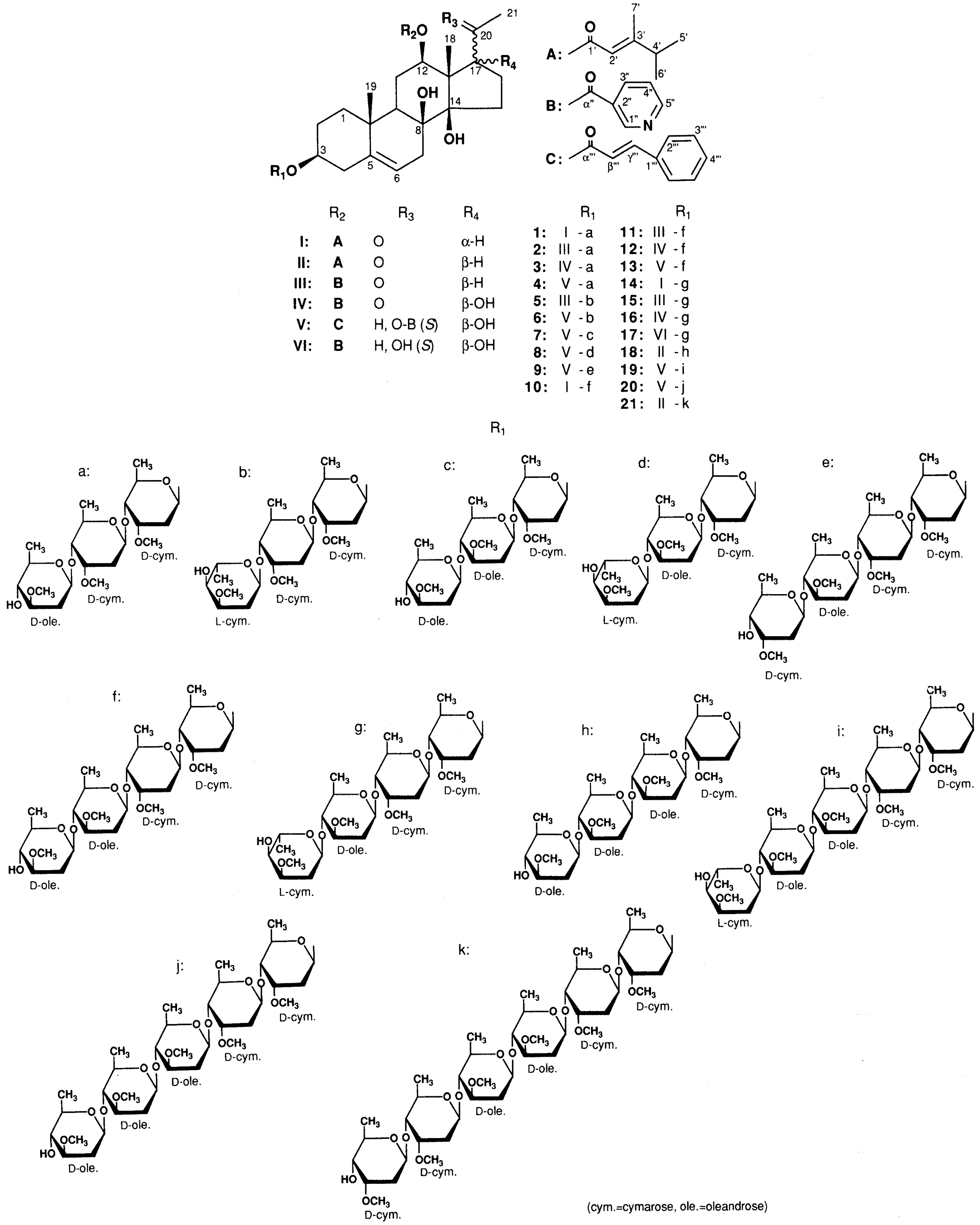

	R_2	R_3	R_4
I:	A	O	α-H
II:	A	O	β-H
III:	B	O	β-H
IV:	B	O	β-OH
V:	C	H, O-B (*S*)	β-OH
VI:	B	H, OH (*S*)	β-OH

	R_1		R_1
1:	I -a	11:	III -f
2:	III -a	12:	IV -f
3:	IV -a	13:	V -f
4:	V -a	14:	I -g
5:	III -b	15:	III -g
6:	V -b	16:	IV -g
7:	V -c	17:	VI -g
8:	V -d	18:	II -h
9:	V -e	19:	V -i
10:	I -f	20:	V -j
		21:	II -k

(cym.=cymarose, ole.=oleandrose)

Chart 1

cymaroses and one oleandrose, **10**—**13** of two cymaroses and two oleandroses, **18** of one cymarose and three oleandroses. The sugar linkages of all the compounds were determinded as shown in Chart 1 on the basis of consistency of the ^{1}H- and ^{13}C-NMR spectra with those of previously isolated compounds.[2,3] Moreover, the aglycone moiety of **9**—**16** and **18** were determined by comparing the residues which were obtained by acid hydrolysis with authentic samples by HPLC. The aglycone moiety of **17** was determined to be 12-*O*-nicoti-

Table 1. ^{13}C-NMR Spectral Data of Aglycone Moiety

	I	II	III	IV	V	VI
1	39.0	38.8	38.8	38.8	38.8	38.8[a]
2	29.1	28.9	28.9	28.9	28.9	29.0
3	78.0	77.9	77.8	77.8	77.8	77.9
4	38.8	38.8	38.8	38.8	38.8	38.9[a]
5	139.3	141.3	141.3	140.9	140.3	140.0
6	118.7	117.4	117.3	117.4	117.8	118.1
7	37.2	34.5	34.5	34.2	34.3	34.4
8	74.9	74.8	74.8	74.4	74.3	74.1
9	44.5	44.2	44.1	43.7	43.4	43.5
10	37.0	37.3	37.3	37.2	37.0	37.1
11	24.1	24.4	24.4	24.4	24.9	24.8
12	73.9	71.1[h]	73.0	73.7	73.4	75.0
13	54.1	55.1	55.3	58.1	56.5	56.4[i]
14	86.3	86.7	86.6	88.2	87.9	87.8[b]
15	35.6	33.3	33.3	32.9	33.0	33.1
16	24.7	21.2	21.6	32.4	32.7	31.9
17	57.7	60.0	59.5	91.6	87.3	87.7[b]
18	12.0	15.0	15.0	9.6	10.4	10.9
19	18.2[g]	18.9	18.9	18.7	18.3[g]	18.8
20	217.5	209.2	209.7	209.7	75.7	70.9
21	32.9	31.8	32.3	27.6	15.0	18.4[g]
1′	167.0	166.1	—	—	—	—
2′	113.4	113.1	—	—	—	—
3′	166.9	165.7	—	—	—	—
4′	38.3	38.1	—	—	—	—
5′	20.9	20.8[a]	—	—	—	—
6′	20.9	20.9[a]	—	—	—	—
7′	16.5	16.5	—	—	—	—
α″	—	—	163.9	163.9	163.7	164.9
1″	—	—	150.6	150.7	150.8	151.2
2″	—	—	126.2	126.0	126.3	126.3
3″	—	—	137.1	137.0	137.3	137.2
4″	—	—	123.3	123.3	123.2	123.4
5″	—	—	153.3	153.5	153.2	153.6
α‴	—	—	—	—	166.2	—
β‴	—	—	—	—	118.8	—
γ‴	—	—	—	—	144.2	—
1‴	—	—	—	—	134.1	—
2‴	—	—	—	—	128.0	—
3‴	—	—	—	—	128.7	—
4‴	—	—	—	—	130.2	—

Measured at 100.40 MHz in $CDCl_3$ solution at 35 °C. *a, b*) Assignments may be interchanged in each column. *g, h, i*) Assignments may be interchanged between Tables 1 and 2.

noylsarcostin, because the ^{13}C- and ^{1}H-NMR spectra of **17** suggested the existence of nicotinic acid and sarcostin, and the signal of H-12 in sarcostin was shifted downfield at δ 4.89. Accordingly, the structures of **9**—**17** and **18** were elucidated as shown.

Compounds **19** and **20** were suggested to be gagaminin 3-*O*-pentaglycosides, whose sugar sequences were deduced to be α-L-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside by consistency of the ^{1}H- and ^{13}C-NMR spectral data with those of known compounds.[2,3] Moreover, the sugar linkages were confirmed by irradiation of each anomeric proton in the NOE difference spectra.

Compound **21** was considered to be cynanchogenin 3-*O*-hexaglycoside based on the ^{1}H- and ^{13}C-NMR spectra and FAB-MS. Acid hydrolysis followed by $NaBH_4$ reduction and acetylation and GLC analysis of the sugar part suggested that the sugars consisted of four cymaroses and two oleandroses because the relative ratio of cymarose and oleandrose was 2 : 1 by comparison of the peak area of cymaritol acetate and oleandritol acetate. Then, the sugar linkage was determined by irradiation at each anomeric proton signal in NOE difference spectra. NOEs were observed between δ 4.84 (H-1 of the first cymaropyranose)/3.55 (H-3 of aglycone), δ 4.75 (H-1 of the second cymaropyranose)/3.21 (H-4 of the first cymaropyranose), δ 4.44 (H-1 of the first oleandropyranose)/3.21 (H-4 of the second cymaropyranose), δ 4.66 (H-1 of the second oleandropyranose)/3.15 (H-4 of the first oleandropyranose), δ 4.94 (H-1 of the third cymaropyranose)/3.17 (H-4 of the second oleandropyranose) and δ 4.67 (H-1 of the fourth cymaropyranose)/3.21 (H-4 of the third cymaropyranose). The preceding H-4 signal assignments were done on the basis of comparison of the ^{1}H-NMR spectrum with that of cynanchogenin 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside[2] and the correlation with H-3 and H-5 signals of each monosaccharide in the COSY spectrum. Hence, the structure of **21** was elucidated as shown.

Experimental

General Procedure Instrumental analysis were carried out as described previously.[2]

Isolation The procedure of extracting and isolating pregnane glycosides was described in a previous report.[2] Isolated pregnane glycoside fraction afforded compounds **1** (7 mg), **2** (16 mg), **3** (12 mg), **4** (20 mg), **5** (3 mg), **6** (4 mg), **7** (8 mg), **8** (8 mg), **9** (3 mg), **10** (5 mg), **11** (9 mg), **12** (4 mg), **13** (7 mg), **14** (4 mg), **15** (9 mg), **16** (6 mg), **17** (6 mg), **18** (3 mg), **19** (7 mg), **20** (6 mg) and **21** (5 mg).

Compound **1**: Amorphous powder, $[\alpha]_D^{20}$ +30.9° (c=0.70, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{15}\cdot 1/2H_2O$: C, 64.24; H, 8.69. Found:C, 64.04; H, 8.74. FAB-MS *m/z*: 907 $[M+H]^+$, 929 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **2**: Amorphous powder, $[\alpha]_D^{20}$ −20.7° (c=1.6, MeOH). *Anal.* Calcd for $C_{49}H_{71}NO_{15}\cdot 2H_2O$: C, 61.46; H, 8.06; N, 1.49. Found: C, 61.44; H, 8.04; N, 1.51. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 219 (4.12), 263 (3.58), 269 (sh). FAB-MS *m/z*: 902 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **3**: Amorphous powder, $[\alpha]_D^{20}$ −3.8° (c=1.2, MeOH). *Anal.* Calcd for $C_{48}H_{71}NO_{16}\cdot 2H_2O$: C, 60.43; H, 7.92; N, 1.47. Found: C, 60.50; H, 7.93; N, 1.43. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 218 (4.00), 263 (3.49), 270 (sh). FAB-MS *m/z*: 918 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **4**: Amorphous powder, $[\alpha]_D^{20}$ +127° (c=2.0, MeOH). *Anal.* Calcd for $C_{57}H_{79}NO_{17}\cdot 3/2H_2O$: C, 63.55; H, 7.67; N, 1.30. Found: C, 63.37; H, 7.73; N, 1.28. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 218 (4.34), 222 (sh), 281 (4.26). FAB-MS *m/z*: 1051 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **5**: Amorphous powder, FAB-MS *m/z*: 902 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **6**: Amorphous powder, $[\alpha]_D^{20}$ +111° (c=0.41, MeOH). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 218 (4.45), 222 (sh), 282 (4.37). FAB-MS *m/z*: 1050 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **7**: Amorphous powder, $[\alpha]_D^{20}$ +96.0° (c=0.75, MeOH). *Anal.* Calcd for $C_{57}H_{79}NO_{17}\cdot 5/2H_2O$: C, 62.51; H, 7.73; N, 1.28. Found: C, 62.47; H, 7.77; N, 1.15. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 217 (4.39), 223 (sh), 273 (4.30), 282 (4.31). FAB-MS *m/z*: 1050 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **8**: Amorphous powder, $[\alpha]_D^{20}$ +86.8° (c=0.82, MeOH). *Anal.* Calcd for $C_{57}H_{79}NO_{17}\cdot 2H_2O$: C, 63.03; H, 7.70; N, 1.29. Found: C, 63.18; H, 7.76; N, 1.28. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 217 (4.43), 222 (sh), 273 (4.35), 282 (4.36). FAB-MS *m/z*: 1050 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Table 2. ^{13}C-NMR Spectral Data of Sugar Moiety

	a	b	c	d	e	f	g	h	i	j	k
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
1	96.1	96.1	96.1	96.1	96.1	96.0	96.0	96.1	96.1	96.1	96.1
2	35.6$^{a)}$	35.8$^{a)}$	35.9	36.2	35.7	35.6$^{a)}$	35.6	35.5$^{a)}$	35.7$^{a)}$	35.7$^{a)}$	35.7$^{a)}$
3	77.0$^{b)}$	77.2	77.1	77.2	77.1	77.1$^{b)}$	77.1	77.1	77.1	77.1	77.1
4	82.5$^{c)}$	82.5	82.7	82.6	82.6	82.6	82.5	82.7$^{b)}$	82.5$^{b)}$	82.5$^{b)}$	82.5$^{b)}$
5	68.3$^{d)}$	68.6$^{b)}$	68.4	68.4	68.4$^{a)}$	68.3$^{c)}$	68.4$^{a)}$	68.5	68.3$^{c)}$	68.3$^{c)}$	68.3$^{c)}$
	D-cym.	D-cym.	D-ole.	D-ole.	D-cym.	D-cym.	D-cym.	D-ole.	D-cym.	D-cym.	D-cym.
1	99.7	99.7	101.4	101.4	99.7	99.7	99.7	101.4	99.7	99.7	99.7
2	35.6$^{a)}$	36.0$^{a)}$	36.4	36.2	35.7	35.6$^{a)}$	35.6	36.5	35.6$^{a)}$	35.7$^{a)}$	35.7$^{a)}$
3	77.1$^{b)}$	77.2	79.2	78.8	77.1	77.2$^{b)}$	77.1	79.1$^{c)}$	77.1	77.1	77.1
4	82.7$^{c)}$	81.6	82.3	81.4	82.6	82.6	82.5	82.4$^{b)}$	82.6$^{b)}$	82.5	82.5$^{b)}$
5	68.5$^{d)}$	68.7$^{b)}$	71.0	71.7	68.6$^{a)}$	68.5$^{c)}$	68.6$^{a)}$	71.0$^{h)}$	68.5$^{c)}$	68.6$^{c)}$	68.6$^{c)}$
	D-ole.	L-cym.	D-ole.	L-cym.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.
1	101.5	98.2	100.2	96.9	101.4	101.4	101.4	100.1$^{d)}$	101.4	101.4	101.4
2	35.4$^{a)}$	31.0	35.5	31.0	36.4	36.4	36.1	36.5	36.5$^{d)}$	36.4$^{d)}$	36.5$^{d)}$
3	80.6	74.8	80.8	75.0	79.0	79.2	78.9	79.3$^{b)}$	79.3$^{e)}$	79.2$^{e)}$	79.0$^{e)}$
4	75.4	72.2	75.5	72.2	82.3	82.3	81.4	82.5$^{c)}$	82.3$^{b)}$	82.4$^{b)}$	82.4$^{b)}$
5	71.6	65.7	71.7	65.2	71.1	71.0	71.7	71.0$^{h)}$	71.0	71.1	70.7$^{f,h)}$
					D-cym.	D-ole.	L-cym.	D-ole.	D-ole.	D-ole.	D-ole.
1	—	—	—	—	98.2	100.2	96.9	100.3$^{d)}$	100.2	100.1$^{f)}$	100.2
2	—	—	—	—	33.9	35.5$^{a)}$	31.0	35.9$^{a)}$	36.1$^{d)}$	36.5$^{d)}$	36.6$^{d)}$
3	—	—	—	—	77.6	80.8	75.1	80.8	79.0$^{e)}$	79.3$^{e)}$	79.2$^{e)}$
4	—	—	—	—	72.5	75.5	72.2	75.5	81.6	82.6$^{b)}$	82.4$^{b)}$
5	—	—	—	—	71.1	71.7	65.3	71.7	71.9	71.2	71.0$^{f)}$
									L-cym.	D-ole.	D-cym.
1	—	—	—	—	—	—	—	—	96.9	100.2$^{f)}$	98.5
2	—	—	—	—	—	—	—	—	31.0	35.6$^{a)}$	35.4$^{a)}$
3	—	—	—	—	—	—	—	—	75.1	80.8	77.1
4	—	—	—	—	—	—	—	—	72.2	75.5	82.5$^{b)}$
5	—	—	—	—	—	—	—	—	65.2	71.7	68.8$^{c)}$
											D-cym.
1	—	—	—	—	—	—	—	—	—	—	99.5
2	—	—	—	—	—	—	—	—	—	—	33.8
3	—	—	—	—	—	—	—	—	—	—	77.5
4	—	—	—	—	—	—	—	—	—	—	72.5
5	—	—	—	—	—	—	—	—	—	—	71.4
6	18.0$^{g)}$	18.0$^{g)}$	18.0$^{g)}$	17.8	18.2$^{g)}$ × 2	18.0$^{g)}$	17.7	17.9	17.7	18.0$^{g)}$	18.2 × 3
	18.2$^{g)}$ × 2	18.2$^{g)}$	18.2$^{g)}$	18.2$^{g)}$	18.4$^{g)}$ × 2	18.2$^{g)}$ × 2	18.2$^{g)}$ × 2	18.2 × 2	18.2$^{g)}$ × 2	18.3$^{g)}$ × 2	18.3
		18.3$^{g)}$	18.4$^{g)}$	18.4$^{g)}$		18.4$^{g)}$	18.4$^{g)}$	18.4	18.4$^{g)}$ × 2	18.4$^{g)}$ × 2	18.4 × 2
OMe	56.2	56.2	56.3	56.2 × 2	56.5	56.3	56.2$^{i)}$	56.3	56.3	56.3	56.8 × 2
	58.1	58.1 × 2	56.6	58.4	57.1	56.7	56.3$^{i)}$	56.7	56.8 × 2	56.7	57.2
	58.2		58.2		58.1	58.0	58.0	56.8	58.1	56.8	57.9
					58.3	58.2	58.4	58.2	58.2	58.1 × 2	58.1
											58.3

Measured at 100.40 MHz in CDCl$_3$ solution at 35 °C. *a*—*f*) Assignments may be interchanged in each column. *g, h, i*) Assignments may be interchanged between Tables 1 and 2.

Compound **9**: Amorphous powder, $[\alpha]_D^{20}$ +130° (c=0.34, MeOH). UV λ_{max}^{MeOH} nm (log ε): 217 (4.33), 222 (sh), 281 (4.21). FAB-MS *m/z*: 1194 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **10**: Amorphous powder, $[\alpha]_D^{20}$ +29.6° (c=0.50, MeOH). FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **11**: Amorphous powder, $[\alpha]_D^{20}$ −26.9° (c=0.96, MeOH). *Anal.* Calcd for $C_{55}H_{83}NO_{18}\cdot 5/2H_2O$: C, 60.53; H, 8.13; N, 1.28. Found: C, 60.79; H, 8.17; N, 1.13. UV λ_{max}^{MeOH} nm (log ε): 219 (3.97), 257 (3.48), 263 (3.50), 270 (3.39). FAB-MS *m/z*: 1046 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **12**: Amorphous powder, $[\alpha]_D^{20}$ −6.1° (c=0.41, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.09), 264 (3.58), 269 (sh). FAB-MS *m/z*: 1062 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **13**: Amorphous powder, $[\alpha]_D^{20}$ +101° (c=0.70, MeOH). *Anal.* Calcd for $C_{64}H_{91}NO_{20}\cdot 2H_2O$: C, 62.47; H, 7.78; N, 1.14. Found: C, 62.28; H, 7.75; N, 0.98. UV λ_{max}^{MeOH} nm (log ε): 218 (4.39), 223 (sh), 274 (4.31), 281 (4.31). FAB-MS *m/z*: 1194 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **14**: Amorphous powder, $[\alpha]_D^{20}$ −6.1° (c=0.38, MeOH). FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **15**: Amorphous powder, $[\alpha]_D^{20}$ −51.8° (c=0.90, MeOH). *Anal.* Calcd for $C_{55}H_{83}NO_{18}\cdot 2H_2O$: C, 61.04; H, 8.10; N, 1.29. Found: C, 61.28; H, 8.11; N, 1.19. UV λ_{max}^{MeOH} nm (log ε): 219 (4.10), 258 (3.60), 263 (3.63), 269 (sh). FAB-MS *m/z*: 1046 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **16**: Amorphous powder, $[\alpha]_D^{20}$ −34.2° (c=0.59, MeOH). *Anal.* Calcd for $C_{55}H_{83}NO_{19}\cdot 2H_2O$: C, 60.15; H, 7.98; N, 1.28. Found: C, 60.05; H, 7.98; N, 1.19. UV λ_{max}^{MeOH} nm (log ε): 218 (4.05), 258 (3.52), 263 (3.55), 270 (3.45). FAB-MS *m/z*: 1062 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **17**: Amorphous powder, $[\alpha]_D^{20}$ −13.9° (c=0.61, MeOH). *Anal.* Calcd for $C_{55}H_{85}NO_{19}\cdot 3H_2O$: C, 59.07; H, 8.20; N, 1.25. Found: C, 59.21; H, 8.13; N, 1.13. UV λ_{max}^{MeOH} nm (log ε): 219 (4.03), 263 (3.54), 270 (sh). FAB-MS *m/z*: 1064 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **18**: Amorphous powder, $[\alpha]_D^{22}$ −28.7° (c=0.26, MeOH). FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **19**: Amorphous powder, $[\alpha]_D^{22}$ +74.1° (c=0.74, MeOH). *Anal.* Calcd for $C_{71}H_{103}NO_{23}\cdot 3H_2O$: C, 61.24; H, 7.89; N, 1.01. Found: C, 61.51; H, 7.83; N, 1.01. UV λ_{max}^{MeOH} nm (log ε): 218 (4.41), 222 (sh), 281 (4.32). FAB-MS *m/z*: 1338 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **20**: Amorphous powder, $[\alpha]_D^{22}$ +87.7° (c=0.59, MeOH).

Table 3. ^{1}H-NMR Spectral Data of Sugar Moiety

	a	b	c	d	e	f
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
1	4.85 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.85 (dd, 9.4, 2.0)	4.84 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)
3	3.80[a)]	3.79 (q, 3.0)	3.78 (q, 3.0)	3.78 (q, 3.0)	3.80 (q, 3.0)	3.80 (q, 3.0)
4	3.22 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.22 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.85 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)	3.87 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)
6	1.22 (d, 6.5)	1.20 (d, 6.5)	1.21 (d, 6.5)	1.22 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
	D-cym.	D-cym.	D-ole.	D-ole.	D-cym.	D-cym.
1	4.76 (dd, 9.5, 2.0)	4.76 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.46 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)
3	3.80[a)]	3.68 (q, 3.0)			3.78 (q, 3.0)	3.78 (q, 3.0)
4	3.23 (d, 9.5, 3.0)	3.23 (dd, 9.5, 3.0)	3.17 (t, 9.0)	3.11 (t, 9.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.87 (dq, 9.5, 6.5)	3.87 (dq, 9.5, 6.5)	3.30 (dq, 9.0, 6.5)	3.27 (dq, 9.0, 6.5)	3.85 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)
6	1.22 (d, 6.5)	1.20 (d, 6.5)	1.29 (d, 6.5)	1.27 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
	D-ole.	L-cym.	D-ole.	L-cym.	D-ole.	D-ole.
1	4.50 (dd, 9.5, 2.0)	4.79 (dd, 4.5, 1.5)	4.72 (dd, 9.5, 2.0)	4.87 (dd, 4.5, 1.5)	4.44 (dd, 9.5, 2.0)	4.45 (dd, 9.5, 2.0)
3	3.17[a)]	3.58 (q, 3.5)		3.57 (q, 3.5)	3.36[a)]	
4	3.12 (t, 9.0)	3.28 (dd, 9.5, 3.5)	3.13 (t, 9.0)		3.16 (t, 9.0)	3.17 (t, 9.0)
5	3.28 (dq, 9.0, 6.5)	4.04 (dq, 9.5, 6.5)	3.30 (dq, 9.0, 6.5)	4.09 (dq, 9.5, 6.5)	3.29 (dq, 9.0, 6.5)	3.30 (dq, 9.0, 6.5)
6	1.32 (d, 6.5)	1.26 (d, 6.5)	1.34 (d, 6.5)	1.24 (d, 6.5)	1.28 (d, 6.5)	1.29 (d, 6.5)
					D-cym.	D-ole.
1	—	—	—	—	4.88 (dd, 9.5, 2.0)	4.72 (dd, 9.5, 2.0)
3	—	—	—	—	3.62 (q, 3.0)	
4	—	—	—	—	3.21 (dd, 9.5, 3.0)	3.13 (t, 9.0)
5	—	—	—	—	3.59 (dq, 9.5, 6.5)	3.30 (dq, 9.0, 6.5)
6	—	—	—	—	1.29 (d, 6.5)	1.34 (d, 6.5)
1	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—
1	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—
OMe	3.39 (s) 3.45 (s) × 2	3.38 (s) 3.45 (s) 3.49 (s)	3.40 (s) × 2 3.45 (s)	3.35 (s) 3.38 (s) 3.45 (s)	3.40 (s) 3.42 (s) 3.44 (s) × 2	3.40 (s) × 2 3.44 (s) 3.45 (s)

Anal. Calcd for $C_{71}H_{103}NO_{23} \cdot 2H_2O$: C, 62.04; H, 7.85; N, 1.02. Found: C, 61.79; H, 7.86; N, 1.00. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 218 (4.44), 223 (sh), 281 (4.34). FAB-MS *m/z*: 1338 [M+H]$^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **21**: Amorphous powder, $[\alpha]_D^{20}$ +3.2° (c=0.51, MeOH). *Anal.* Calcd for $C_{70}H_{114}O_{24} \cdot 3/2H_2O$: C, 61.52; H, 8.63. Found: C, 61.64; H, 8.71. FAB-MS *m/z*: 1361 [M+Na]$^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

As compounds **6**, **9**, **10**, **12**, **14** and **18** could be acquired only in a small amount, elemental analyses of these compounds were not performed; and since **5** could not be thoroughly purified, $[\alpha]_D$ and the elemental analysis were not measured.

Acid Hydrolysis of a Mixture of Pregnane Glycoside A mixture (*ca.* 500 mg) was heated at 60 °C for 2 h with dioxane (6 ml) and 0.2 N H_2SO_4 (1.5 ml). After hydrolysis, the reaction mixture was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ layer gave 12-*O*-nicitinoyllineolon (2 mg, $[\alpha]_D^{25}$ −55.5° (c=0.19, $CHCl_3$)), rostratamine (2 mg, $[\alpha]_D^{24}$ −28.0° (c=0.15, MeOH)), gagaminin (14 mg, $[\alpha]_D^{25}$ +189° (c=1.4, $CHCl_3$)) and sibirigenin (1 mg, $[\alpha]_D^{26}$+ 42.7° (c=0.13, MeOH)) (Develosil ODS 20 mm × 25 cm, 45, 50, 62.5, 65% MeOH). The H_2O layer gave a D,L-cymarose mixture and D-oleandrose (13 mg, $[\alpha]_D^{24}$ −11.9° (c=1.34, 24 h after dissolution in H_2O)).[10)]

Acid Hydrolysis of Compounds 1—21 Compounds **1**—**21** (*ca.* 0.5 mg) dissolved in dioxane (4 drops) and 0.2 N H_2SO_4 (1 drop) were heated at 60 °C for 90 min. The procedure after hydrolysis and the identification of the aglycone and sugar moieties of each compound were described in our previous paper.[2,3)] [Conditions for detecting the aglycone moiety: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 75% MeOH in H_2O; t_R (min), sibirigenin 12.8, cynanchogenin 7.6, 50% MeOH in H_2O; t_R (min), 12-*O*-nicotinoyllineolon 9.6, rostratamine 11.2, 65% MeOH in H_2O; t_R (min), gagaminin 13.0, conditions for detecting the sugar moiety: column, Supelco SP-2380 capillary column 0.25 mm × 30 m; column temperature, 200 °C; carrier gas, N_2; t_R (min), cymaritol acetate 6.2, oleandritol acetate 7.0]. The relative ratio of each monosaccharide was determined based on the peak area.

The reaction mixture of **17** by acid hydrolysis, moreover, was refluxed with 5% NaOMe for 2 h, and the residue was passed through an Amberlite IR-120B column. Sarcostin was detected as part of the aglycone moiety of **17** in comparison with an authentic sample by HPLC [condition: YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 40% MeOH in H_2O; t_R (min) 12.0].

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Table 3. (continued)

	g	h	i	j	k
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
1	4.85 (dd, 9.5, 2.0)	4.85 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)
3	3.80 (q, 3.0)	3.79 (q, 3.0)	3.80 (q, 3.0)	3.80 (q, 3.0)	3.80 (q, 3.0)
4	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.20 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.84 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)	3.83 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)
6	1.21 (d, 6.5)	1.22 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
	D-cym.	D-ole.	D-cym.	D-cym.	D-cym.
1	4.75 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)	4.74 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)
3	3.78 (q, 3.0)	3.35$^{a)}$	3.78 (q, 3.0)	3.78 (q, 3.0)	3.77 (q, 3.0)
4	3.21 (dd, 9.5, 3.0)	3.15 (t, 9.0)	3.21 (dd, 9.5, 3.0)	3.20 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.86 (dq, 9.5, 6.5)	3.30$^{a)}$	3.86 (dq, 9.5, 6.5)	3.85 (dq, 9.5, 6.5)	3.85 (dq, 9.5, 6.5)
6	1.21 (d, 6.5)	1.28 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.
1	4.46 (dd, 9.5, 2.0)	4.66 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)
3	3.27$^{a)}$	3.35$^{a)}$	3.34$^{a)}$	3.36$^{a)}$	3.34$^{a)}$
4	3.12 (t, 9.0)	3.18 (t, 9.0)	3.15 (t, 9.0)	3.15 (t, 9.0)	3.15 (t, 9.0)
5	3.27 (dq, 9.0, 6.5)	3.33$^{a)}$	3.29 (dq, 9.0, 6.5)	3.29 (dq, 9.0, 6.5)	3.29 (dq, 9.5, 6.5)
6	1.27 (d, 6.5)	1.32 (d, 6.5)	1.29 (d, 6.5)	1.28 (d, 6.5)	1.28 (d, 6.5)
	L-cym.	D-ole.	D-ole.	D-ole.	D-ole.
1	4.88 (dd, 4.5, 1.5)	4.72 (dd, 9.5, 2.0)	4.68 (dd, 9.5, 2.0)	4.67 (dd, 9.5, 2.0)	4.66 (dd, 9.5, 2.0)
3	3.58 (q, 3.5)	3.16$^{a)}$	3.25$^{a)}$	3.36$^{a)}$	3.34$^{a)}$
4	3.27$^{a)}$	3.13$^{a)}$	3.12 (t, 9.0)	3.17 (t, 9.0)	3.17 (t, 9.0)
5	4.08 (dq, 9.5, 6.5)	3.31$^{a)}$	3.29 (dq, 9.0, 6.5)	3.33$^{a)}$	3.30 (dq, 9.0, 6.5)
6	1.24 (d, 6.5)	1.34 (d, 6.5)	1.30 (d, 6.5)	1.32 (d, 6.5)	1.30 (d, 6.5)
			L-cym.	D-ole.	D-cym.
1	—	—	4.88 (dd, 4.5, 1.5)	4.72 (dd, 9.5, 2.0)	4.94 (dd, 9.5, 2.0)
3	—	—	3.58 (q, 3.5)		3.80 (q, 3.0)
4	—	—	3.27$^{a)}$	3.13 (t, 9.0)	3.21 (dd, 9.5, 3.0)
5	—	—	4.08 (dq, 9.5, 6.5)	3.31 (dq, 9.0, 6.5)	3.88 (dq, 9.5, 6.5)
6	—	—	1.25 (d, 6.5)	1.34 (d, 6.5)	1.23 (d, 6.5)
					D-cym.
1	—	—	—	—	4.67 (dd, 9.5, 2.0)
3	—	—	—	—	3.61 (q, 3.0)
4	—	—	—	—	3.18 (dd, 9.5, 3.0)
5	—	—	—	—	3.56 (dq, 9.5, 6.5)
6	—	—	—	—	1.26 (d, 6.5)
OMe	3.35 (s)	3.39 (s)	3.36 (s)	3.38 (s)	3.39 (s)
	3.38 (s)	3.40 (s)	3.38 (s)	3.40 (s)	3.41 (s)
	3.44 (s) × 2	3.41 (s)	3.40 (s)	3.41 (s)	3.42 (s)
		3.45 (s)	3.44 (s) × 2	3.44 (s) × 2	3.43 (s)
					3.44 (s)
					3.45 (s)

Measured at 400 MHz in $CDCl_3$ solution at 35 °C. *a*) Overlapping with other signals.

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