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Steroidal glycosides from the aerial part of *Asclepias incarnata*

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Abstract

The aerial part of *Asclepias incarnata* afforded 34 pregnane glycosides. These were confirmed to have lineolon, isolineolon, ikemagenin, 12-*O*-nicotinoyllineolon, deacylmetaplexigenin, metaplexigenin, rostratamine, 12-*O*-acetyllineolon, 15β-hydroxylineolon and 15β-hydroxyisolineolon moieties as their aglycones, and 2,6-dideoxyhexopyranose, glucopyranose and allopyranose as the corresponding sugar constituents. Their structures were determined using both spectroscopic and chemical methods.

Keywords: *Asclepias incarnata*; Asclepiadaceae; Pregnane glycoside; 2,6-Dideoxyhexopyranose

1. Introduction

In connection with a study on the constituents of plants in the Asclepiadaceae family, we have investigated the aerial part of *Asclepias incarnata* L. The present paper describes the isolation and structural elucidation of 34 new oxypregnane glycosides.

2. Results and discussion

The MeOH extract obtained from the dried aerial part of *A. incarnata* L. (1.3 kg) was suspended in water. The suspension was then extracted with diethyl ether and partitioned into an ether layer and a H_2O layer. The H_2O layer was directly passed through a Mitsubishi Diaion HP-20 column and the adsorbed material was subsequently eluted with 50% MeOH in water, 70% MeOH in water, and MeOH. The Et_2O layer and MeOH eluates of the HP-20 column were concentrated, and the residues were rechromatographed on a silica gel column and on semi-preparative HPLC, respectively, to give oxypregnane glycosides (**1**–**34**).

To determine the component sugars, a mixture of pregnane glycosides was subjected to acid hydrolysis. The acquired component sugars were fractionated by silica gel column chromatography, and cymarose, oleandrose and digitoxose were obtained (See chart 2). The absolute configurations of cymarose, oleandrose and digitoxose were believed to be in the D-form based on their optical rotation values (Tsukamoto, Hayashi, Kaneko & Mitsuhashi, 1986; Nakagawa, Hayashi, Wada & Mitsuhashi, 1983; Abe, Mohri, Okabe & Yamauchi, 1994).

Glucose in the sugar chain of each compound was deduced to be in the D-form based on the reaction with D-cysteine methyl ester hydrochloride followed by GC analysis (see Section 3).

Compound **1** was suggested to have the molecular formula, $C_{42}H_{66}O_{16}$, based on FABMS [positive FABMS ion at *m/z* 827 $[M + H]^+$]. This molecular formula was supported by the ^{13}C-NMR spectrum, in which 42 carbon signals were observed and these signals consisted of eight methyl carbon signals, 10 methylene carbon ones, 16 methine carbon ones and eight quaternary carbon ones. In the ^{13}C-NMR spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those due to metaplexigenin (**39**) (Mitsuhashi & Nomura, 1965) within the range of glycosylation shifts at C-3 (+6.1 ppm), C-2 (−2.2 ppm) and

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C-4 (−4.0 ppm). In the ^{1}H- and ^{13}C-NMR spectra, three anomeric proton and carbon signals were observed at δ 5.46, 5.39, 4.79 and δ 96.4, 99.8, 101.6. Thus, compound **1** was believed to be metaplexigenin 3-*O*-trioside. On acid hydrolysis of **1** with 0.05 N HCl, metaplexigenin, digitoxose and oleandrose were obtained as the component aglycone and sugars. These sugars were identified as β-D-digitoxopyranose and β-D-oleandropyranose, as judged from the coupling constant of each anomeric proton signal ($J = 9.5$, 2.0 Hz). Furthermore, the presence of the characteristic H-3 signals of digitoxose [δ 4.63 (1H, *br s*) and 4.66 (1H, *br s*)] and one methoxyl proton signal [δ 3.46 (3H, *s*)] in the ^{1}H-NMR spectrum suggested that the sugar moiety included two digitoxopyranose and one oleandropyranose. The ^{1}H–^{1}H COSY experiment allowed the sequential assignments of the proton signals for each sugar as shown in Table 3, starting from each anomeric proton signal. For the sugar linkage, the difference nuclear Overhauser effect (NOE) spectra were measured on irradiation of the anomeric proton signals, and NOEs were observed as follows, δ 5.46 (1H, *dd*, J = 9.5, 2.0 Hz, H-1′ of β-D-digitoxopyranose) and δ 3.85 (1H, *m*, H-3 of the aglycone), δ 5.39 (1H, *dd*, J = 9.5, 2.0 Hz, H-1″ of β-D-digitoxopyranose) and δ 3.50 (1H, *dd*, J = 9.5, 3.0 Hz, H-4′ of β-D-digitoxopyranose), and δ 4.79 (1H, *dd*, J = 9.5, 2.0 Hz, H-1‴ of β-D-oleandropyranose) and δ 3.47 (1H, *dd*, J = 9.5, 3.0 Hz, H-4″ of β-D-digitoxopyranose). Hence, the structure of **1** was established as metaplexigenin 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-*D*-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Compound **2** had the molecular formula, $C_{48}H_{76}O_{19}$, by observating of $[M + H]^+$ ion peaks on FABMS and the consequences of the ^{1}H-, ^{13}C-NMR spectra and the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiment. On acid hydrolysis, **2** afforded metaplexigenin (**39**) as the aglycone moiety, and digitoxose and oleandrose were obtained as the component sugars. In the ^{1}H- and ^{13}C-NMR spectra which was measured in $CDCl_3$ solution, four anomeric proton and carbon signals were shown at δ 4.92 (1H, *dd*, J = 9.5, 2.0 Hz), 4.88 (1H, *dd*, J = 9.5, 2.0 Hz), 4.90 (1H, *dd*, J = 9.5, 2.0 Hz), 4.55 (1H, *dd*, J = 9.5, 2.0 Hz) and δ 95.9, 98.3 × 2, 100.4. The ^{13}C-NMR spectral comparison of **2** with that of metaplexigenin showed glycosylation shifts were for the C-3, C-2 and C-4 signals (in pyridine-d_5 solution). Thus, compound **2** was considered as metaplexigenin 3-*O*-tetraoside. As the ^{1}H-NMR spectrum of **2** exhibited one methoxyl proton signal at δ 3.40 (3H, *s*), the sugar sequence of **2** consisted of three digitoxopyranose and one oleandropyranose moiety. The coupling constant of each sugar indicated that these sugars had a β-glycosidic linkage. The proton signals for each sugar were assigned as shown in Table 4 on the basis of the ^{1}H–^{1}H COSY spectrum. Determination of the sugar sequence was carried out by the results of the difference NOE experiment irradiating the anomeric proton signals. NOEs were observed between δ 4.92 (H-1′ of β-D-digitoxopyranose)/3.57 (H-3 of the aglycone); 4.88 (H-1″ of β-D-digitoxopyranose)/3.22 (H-4′ of β-D-digitoxopyranose); 4.90 (H-1‴ of β-D-digitoxopyranose)/3.20 (H-4″ of β-D-digitoxopyranose); 4.55 (H-1⁗ of β-D-oleandropyranose)/3.20 (H-4‴ of β-D-digitoxopyranose). Furthermore, the $^3J_{COCH^s}$ were detected as follows in the ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum, δ 95.9 (C-1′ of β-D-digitoxopyranose) and 3.57 (H-3 of the aglycone); δ 98.3 (C-1″ of β-D-digitoxopyranose) and 3.22 (H-4′ of β-D-digitoxopyranose); δ 98.3 (C-3‴ of β-D-digitoxopyranose) and 3.20 (H-4″ of β-D-digitoxopyranose); δ 100.4 (C-1⁗ of β-D-oleandropyranoside) and 3.20 (H-4‴ of β-D-digitoxopyranose). Based on the above evidence, the structure of **2** was determined to be metaplexigenin 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The molecular formulae of compounds **3**–**10** were $C_{46}H_{74}O_{17}$, $C_{46}H_{74}O_{17}$, $C_{48}H_{76}O_{18}$, $C_{55}H_{80}O_{18}$, $C_{52}H_{77}NO_{18}$, $C_{46}H_{74}O_{18}$, $C_{52}H_{77}NO_{19}$ and $C_{46}H_{74}O_{18}$, respectively, based on observation of $[M + H]^+$ and/or $[M + Na]^+$ ion peaks in FABMS and comparison of each ^{1}H- and ^{13}C-NMR spectral data with those of **2** and each aglycone. On acid hydrolysis, **3**–**10** afforded lineolon (**35**) (Yamagishi, Hayashi, Mitsuhashi, Inamori & Matsushita, 1973a, 1973b; Warashina & Noro, 1994), isolineolon (**36**) (Yamagishi et al., 1973a, Yamagishi et al., 1973b), **37**, ikemagenin (Abe et al., 1994; Yamagishi & Mitsuhashi, 1972), 12-*O*-nicotinoyllineolon (Warashina & Noro, 1996), deacylmetaplexigenin (**38**)(Tsukamoto, Hayashi & Mitsuhashi, 1985; Mitsuhashi & Nomura, 1965), rostratamine (Gellert & Summons, 1973) and **40** as the aglycone moiety, respectively, and digitoxose and oleandrose as the sugar moiety. Because of the consistency of their ^{1}H- and ^{13}C-NMR spectral data with those of **2**, it was shown that **3**–**10** possessed the same sugar sequences in the oligosaccharide moiety as that of **2**, with the sugar moiety attached to the C-3 position of each aglycone. Thus, structures of **3**–**10** were elucidated as shown.

Compounds **11**–**15** had the molecular formulae, $C_{52}H_{84}O_{22}$, $C_{52}H_{84}O_{22}$, $C_{52}H_{86}O_{23}$, $C_{54}H_{86}O_{24}$ and $C_{52}H_{84}O_{23}$, respectively, based on FABMS and the ^{1}H- and ^{13}C-NMR spectra. On acid hydrolysis with 0.05 N HCl and the ^{1}H- and ^{13}C-NMR spectra, **11**–**15** were determined to be pregnane 3-*O*-pentaosides whose aglycones were lineolon (**35**) for **11**, isolineolon (**36**) for **12**, **37** for **13**, metaplexigenin (**39**) for **14** and **40** for **15**. The sugar sequences of these compounds were the same based on the ^{1}H- and ^{13}C-NMR spectral

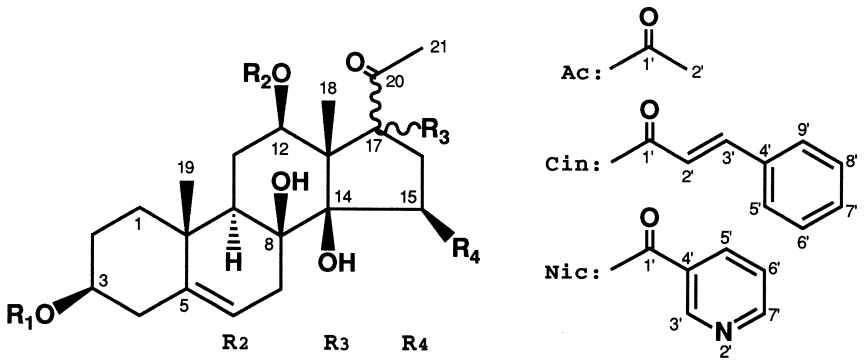
data. On acid hydrolysis of the above, digitoxose, oleandrose and glucose were liberated with each aglycone. In the ^{1}H-NMR spectrum of **13**, the characteristic H-3 signals of digitoxopyranose were observed at δ 4.58 (1H, *q*, J = 3.0 Hz), 4.62 (1H, *q*, J = 3.0 Hz) and 4.63 (1H, *q*, J = 3.0 Hz) and one methoxyl signal of oleandropyranose was observed at δ 3.52 (3H, *s*). Thus, the oligosaccharide moieties of **11**–**15** were composed of three digitoxopyranose, one oleandropyranose and one glucopyranose whose glycosidic linkages were deduced to be in a β-orientation based on the coupling constants of the anomeric proton signals.

Enzymatic hydrolysis of **13** with cellulase produced compound **5**, which was confirmed by comparison of the ^{1}H-NMR spectral data and HPLC analysis with data of an authentic sample. Moreover, in the difference NOE experiment of **13**, irradiation of the anomeric proton signal of glucopyranose at δ 5.10 (1H, *d*, J = 8.0 Hz) caused enhancement of the signal intensity at δ 3.63 (H-4‴ of β-D-oleandropyranose). So, β-D-glucopyranose was attached to the C-4 position of β-D-oleandropyranose, and the sugar sequence of **13** was decided as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranoside. Similarly, the structures of compounds **11**, **12**, **14** and **15** were elucidated as shown.

Compound **16** had the molecular formula, $C_{52}H_{84}O_{23}$, based on FABMS and the ^{1}H- and ^{13}C-NMR spectra. As the ^{1}H- and ^{13}C-NMR spectra were almost identical to those of **15**, the structure of **16** was considered to be similar to that of **15**, but the signals due to an unidentified sugar appeared instead of the signals due to the terminal β-D-glucopyranose. On acid hydrolysis of **16**, D-allose was obtained, together with digitoxose and oleandrose as the sugar moiety (see Section 3). Therefore, this unidentified sugar was determined to be β-D-allopyranose in consideration of the coupling constant of the H-1 signal, and the structure of **16** was elucidated as presented.

The molecular formulae of compounds **17**–**24** were suggested to be $C_{47}H_{76}O_{17}$, $C_{47}H_{76}O_{17}$, $C_{49}H_{78}O_{18}$, $C_{56}H_{82}O_{18}$, $C_{53}H_{79}NO_{18}$, $C_{49}H_{78}O_{19}$, $C_{47}H_{76}O_{18}$ and $C_{47}H_{76}O_{18}$, respectively, based on FABMS and the ^{1}H- and ^{13}C-NMR spectra. From the results of acid hydrolysis and the ^{1}H- and ^{13}C-NMR spectral data, these compounds were identified to be pregnane 3-*O*-tetraosides whose aglycones were lineolon (**35**) for **17**, isolineolon (**36**) for **18**, **37** for **19**, ikemagenin for **20**, 12-*O*-nicotinoyllineolon for **21**, metaplexigenin (**39**) for **22**, **40** for **23** and **41** for **24**. Compounds **17**–**24** possessed the same sugar sequences based on the ^{1}H- and ^{13}C-NMR spectral data of the oligosaccharide moieties. On acid hydrolysis, compounds **17**–**24** yielded cymarose, digitoxose and oleandrose as the constituents of each oligosaccharide moiety, and these monosaccharides retained β-glycosidic linkage as shown by the coupling constants of the H-1 signals. In the ^{1}H-NMR spectrum of **19**, the characteristic H-3 signals of cymaropyranose and digitoxopyranose were observed at δ 4.09 (1H, *q*, J = 3.0 Hz), 4.63 (1H, *br s*) × 2, and two methoxyl proton signals were observed at δ 3.46 (3H, *s*) and 3.62 (3H, *s*). The signals of the terminal oleandrose were also observed in the ^{13}C-NMR spectrum. Therefore, one methoxyl proton signal at δ 3.46 belonged to C-3-OMe of β-D-oleandropyranose and another methoxyl signal at δ 3.62 was due to C-3-OMe of β-D-cymaropyranose. Based on analysis of the HMQC and HMBC spectra, the methoxyl proton signal at δ 3.62 showed a $^3J_{HCOC}$ to the C-3 signal at δ 78.1, and this C-3 signal had a $^1J_{CH}$ to the signal at δ 4.09. The carbon and proton signals at δ 78.1 and 4.09 were assigned to C-3 and H-3 of β-D-cymaropyranose; the remaining signals at δ 4.63 × 2 corresponded to H-3 of β-D-digitoxopyranose. Using the ^{1}H–^{1}H COSY experiment, the assignments of the proton signals in the oligosaccharide moiety were carried out as shown in Table 3. In the difference NOE experiment, upon irradiation of the anomeric proton signals, NOEs were observed as follows: δ 5.26 (H-1′ of β-D-cymaropyranose) and δ 3.82 (H-3 of the aglycone); δ 5.32 (H-1″ of β-D-digitoxopyranose) and δ 3.52 (H-4′ of β-D-cymaropyranose); δ 5.38 (H-1‴ of β-D-digitoxopyranose) and δ 3.48 (H-4″ of β-D-digitoxopyranose); δ 4.80 (H-1⁗ of β-D-oleandropyranose) and δ 3.48 (H-4‴ of β-D-digitoxopyranose). Consequently, the sugar linkage of **19** was found to be 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranoside. This sugar linkage was supported by a long-range correlation between the anomeric carbon signals and the H-4 signals of monosaccharides in the HMBC spectrum. Similarly, the structures of **17**, **18**, **20**–**23** and **24** were established as shown.

Compounds **25**–**29** had the molecular formulae, $C_{53}H_{86}O_{22}$, $C_{53}H_{86}O_{22}$, $C_{55}H_{88}O_{23}$, $C_{59}H_{89}NO_{23}$ and $C_{55}H_{88}O_{24}$, respectively, based on the results of FABMS, the ^{1}H- and ^{13}C-NMR spectra. Analyzing the ^{1}H- and ^{13}C-NMR spectral data, **25**–**29** were found to be pregnane 3-*O*-pentaosides with identical sugar sequences. On acid hydrolysis of **25**–**29**, cymarose, digitoxose, oleandrose and glucose were shown to constitute the sugar moieties, and the aglycones were determined to be lineolon (**35**) for **25**, isolineolon (**36**) for **26**, **37** for **27**, 12-*O*-nicotinoyllineolon for **28** and metaplexigenin (**39**) for **29**. Compounds **25**–**29** displayed the signals of a terminal β-D-glucopyranose as did **11**–**15**, as shown by the ^{1}H- and ^{13}C-NMR spectra. Enzymatic hydrolysis with cellulase produced **17** from **25**, **18** from **26**, **19** from **27**, **21** from **28** and **22** from **29**. The difference NOE spectrum of **28** showed, upon irradiation of the H-1 signal of β-D-glucopyranose [δ



	R2	R3	R4	
I:	H	β-H	H	(lineolon)
II:	H	α-H	H	(isolineolon)
III:	**Ac**	β-H	H	(12-*O*-acetyllineolon)
IV:	**Cin**	β-H	H	(ikemagenin)
V:	**Nic**	β-H	H	(12-*O*-nicotinoyllineolon)
VI:	H	β-OH	H	(deacylmetaplexigenin)
VII:	**Ac**	β-OH	H	(metaplexigenin)
VIII:	**Nic**	β-OH	H	(rostratamine)
IX:	H	β-H	OH	(15β-hydroxylineolon)
X:	H	α-H	OH	(15β-hydroxyisolineolon)

R1

a: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole
b: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole
c: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole $\xrightarrow{(4\leftarrow1)}$ β-D-Glc
d: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole $\xrightarrow{(4\leftarrow1)}$ β-D-All
e: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole
f: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole $\xrightarrow{(4\leftarrow1)}$ β-D-Glc
g: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole
h: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Dig $\xrightarrow{(4\leftarrow1)}$ β-D-Ole $\xrightarrow{(4\leftarrow1)}$ β-D-Glc
i: (agly.$\xrightarrow{(3\leftarrow1)}$) β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Cym $\xrightarrow{(4\leftarrow1)}$ β-D-Ole $\xrightarrow{(4\leftarrow1)}$ β-D-Ole

(agly.: aglycone, Dig: digitoxopyranosyl, Cym: cymaropyranosyl, Ole: oleandropyranosyl, Glc: glucopyranosyl, All: allopyranosyl)

	R1		R1		R1		R1
1:	**VII -a**	**11:**	**I -c**	**21:**	**V -e**	**31:**	**II -g**
2:	**VII -b**	**12:**	**II -c**	**22:**	**VII -e**	**32:**	**III -g**
3:	**I -b**	**13:**	**III -c**	**23:**	**IX -e**	**33:**	**IX -h**
4:	**II -b**	**14:**	**VII -c**	**24:**	**X -e**	**34:**	**IX -i**
5:	**III -b**	**15:**	**IX -c**	**25:**	**I -f**	**35:**	**I -H**
6:	**IV -b**	**16:**	**IX -d**	**26:**	**II -f**	**36:**	**II -H**
7:	**V -b**	**17:**	**I -e**	**27:**	**III -f**	**37:**	**III -H**
8:	**VI -b**	**18:**	**II -e**	**28:**	**V -f**	**38:**	**VI -H**
9:	**VIII -b**	**19:**	**III -e**	**29:**	**VII -f**	**39:**	**VII -H**
10:	**IX -b**	**20:**	**IV -e**	**30:**	**I -g**	**40:**	**IX -H**
						41:	**X -H**

Chart 1

5.10 (1H, *d*, *J* = 8.0 Hz)], an NOE to the H-4 signal of β-D-oleandropyranose [δ 3.62 (overlapping with other signals)]. Accordingly, it was concluded that the β-D-glucopyranose was attached to the C-4 position of β-D-oleandropyranose. Thus, the structures of **25**–**29** were elucidated.

By observation of the $[M + H]^+$ ion peak in FABMS, the molecular formulae of compounds **30**–**32** were deduced to be $C_{48}H_{78}O_{17}$, $C_{48}H_{78}O_{17}$ and $C_{50}H_{80}O_{18}$, respectively. On acid hydrolysis of **30**–**32**, cymarose, digitoxose and oleandrose were obtained along with the aglycones lineolon (**35**), isolineolon (**36**) and **37**, respectively. As evident from the ^{1}H-NMR spectra, each sugar moiety was composed of two β-D-cymaropyranose, one β-D-digitoxopyranose and other β-D-oleandropyranose. Their sugar sequences were determined to be 3-*O*-β-D-oleandropyanosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside, using the same difference NOE procedure used for **31**, i.e. irradiation of the H-1 signal of each monosaccharide. Thus, the structures of **30**–**32** were elucidated as shown.

Compound **33** was presumed to be a pregnane 3-*O*-pentaoside based on the ^{1}H- and ^{13}C-NMR spectral data. Compound **33** was hydrolyzed to yield **40**, cymarose, digitoxose, oleandrose and glucose, and comparison of the NMR spectral data with those of **15** suggested that β-D-glucopyranose was the terminal sugar in the oligosaccharide sequence. The ^{1}H-NMR spectral data of the oligosaccharide sequence of the product of enzymatic hydrolysis of **33** were consistent with those of **30**–**32**. Therefore, the sugar sequence of **33** was determined to be 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Compound **34** was presumed to be a pregnane 3-*O*-tetraoside, with the molecular formula $C_{49}H_{80}O_{18}$. Acid hydrolysis of **34** afforded **40** as the aglycone moiety and cymarose and oleandrose as the sugar moieties. The ^{1}H- and ^{13}C-NMR spectral data of **34** were in good agreement with those of calotroposide E (Shibuya, Zhang, Park, Beak & Takeda, 1992). The sugar sequence of **34** was determined to be 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside; therefore, the structure of **34** was determined as shown in Chart 1.

The aglycones obtained by acid hydrolysis of a mixture of pregnane glycosides together with sugars were identified as lineolon (**35**), isolineolon (**36**), deacylmetaplexigenin (**38**), metaplexigenin (**39**) based on the ^{1}H- and ^{13}C-NMR spectral data and $[\alpha]_D$ values. In addition, one new acylated pregnane (**37**) and two new oxypregnanes (**40** and **41**) were obtained.

Compound **37** was presumed to have molecular formula, $C_{23}H_{34}O_6$, based on the FABMS spectrum. (Positive FABMS ion peak at *m*/*z* 407 $[M + H]^+$). This molecular formula was supported by the ^{1}H- and ^{13}C-NMR spectra, in which the signals of an acetyl group were observed at δ 2.05 (3H, *s*, $COCH_3^*$) and δ 167.0 (C^*OCH_3), 20.8 (COC^*H_3), in addition to the signals of lineolon. On comparing the NMR spectral data of **37** with those of lineolon, acylation shifts were displayed at C-11 (−4.6 ppm), C-12 (+4.2 ppm) and C-13 (−2.3 ppm). Furthermore, the H-12 signal was shifted downfield to δ 5.05. These facts demonstrated that the acetyl group was attached to C-12-OH of lineolon, and **37** was determined to be 12-*O*-acetyllineolon.

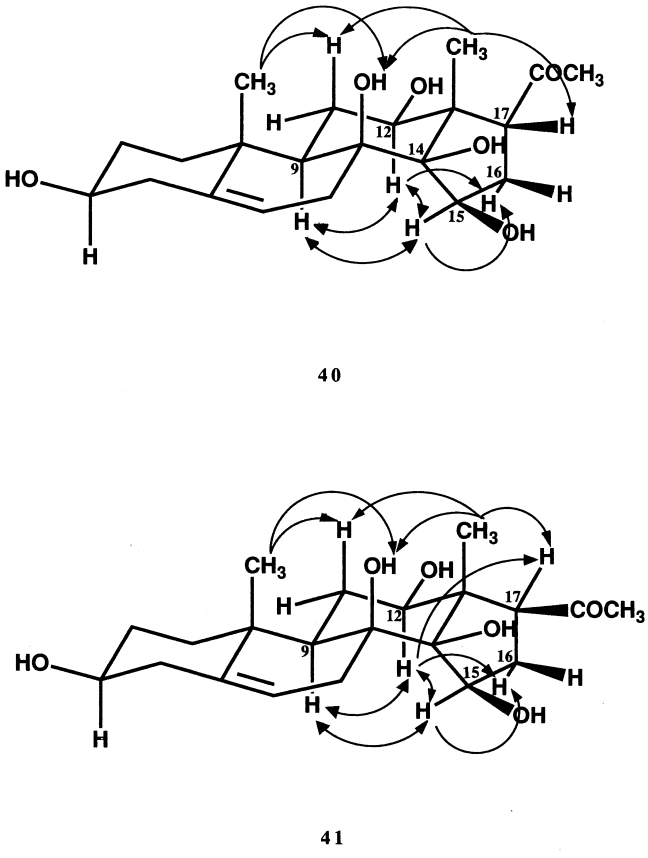


Fig. 1. Observed NOE correlations in compounds **40** and **41**.

In the FABMS spectrum, compound **40** showed an $[M + H]^+$ ion peak at *m*/*z* 381, 16 mass units larger than that of lineolon, suggesting the molecular formula $C_{21}H_{32}O_6$. Upon comparison of the ^{13}C-NMR spectral data of **40** with that of lineolon, a new carbinyl carbon signal was observed at δ 71.7 instead of a methylene carbon signal. In the HMQC spectrum, this carbinyl carbon signal showed $^1J_{CH}$ to δ 4.54 (1H, *m*), and in the HMBC spectrum, $^3J_{CCCH^s}$ and a $^2J_{CCH}$ were observed δ 59.4 (C-17) and δ 4.54; δ 71.7 and δ 5.07 (1H, *s*, 14-OH*); δ 71.7 and δ 3.33 (1H, *dt*, *J* = 14.5,

10.0 Hz, H-16). Thus, this carbinyl carbon and proton signals were due to C-15 and H-15, and the hydroxyl group was attached to C-15. The orientation of the hydroxyl group was presumed to be β, based on comparison of the C-15 signal to that of 17-*epi*-hancogenine (Konda, Toda & Harigaya, 1992). This was proved using a difference NOE experiment, wherein irradiation at δ 4.03 (1H, *dd*, J = 12.0, 4.0 Hz, H-12) and δ 1.67 (1H, *dd*, J = 13.5, 2.5 Hz, H-9) showed NOEs to δ 4.54 (1H, *m*, H-15), and irradiation at δ 4.54 (H-15) exhibited NOEs to δ 4.03 (H-12) and 1.67 (H-9) (see fig. 1). Thus, the orientation of H-15 was decided to be α, and in conclusion, this hydroxyl group had β-orientation. Based on the above arguments, the structure of **40** was elucidated as 15β-hydroxylineolon.

As compound **41** showed the same $[M + H]^+$ ion peak in FABMS spectrum as did **40**, the molecular formula of **41** was presumed to be $C_{21}H_{32}O_6$. The ^{13}C-NMR spectrum was similar to that of **40**; **41** was deduced to have a hydroxyl group at C-15β the same as did **40**. This was confirmed by the analysis of the NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC and difference NOE). In the ^{13}C-NMR spectrum, the C-20 signal shown at δ 215.3 was in good agreement with that of isolineolon. Thus, **41** was determined to be 15β-hydroxyisolineolon.

3. Experimental

Optical rotations were determined using a JASCO-DIP1000 digital polarimeter. FABMS spectra were collected on a JEOL JMS-SX120 spectrometer in *m*-nitrobenzyl alcohol, whereas both ^{1}H- and ^{13}C-NMR were recorded in pyridine-d_5 and/or $CDCl_3$ solutions on a JOEL JNM A-400 (400 and 100.40 MHz, respectively) spectrometer. Chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. UV spectra were measured with a Beckman UV640 spectrophotometer. GC utilised a Hitachi G-3000 gas chromatograph, and HPLC analyses employed a JASCO 800 system instrument.

3.1. Plant material

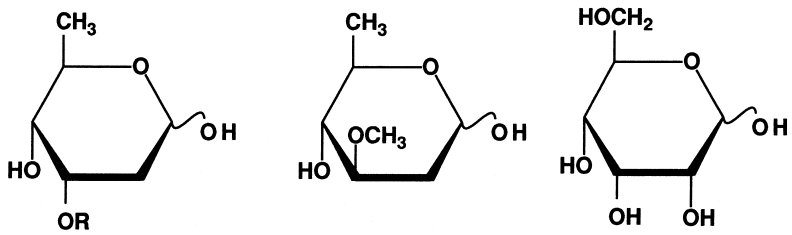
Asclepias incarnata L. was cultivated in the botanical garden and harvested on July 1996 in Shizuoka, Japan and identified by Professor Noro (University of Shizuoka).

3.2. Extraction and isolation

The air dried aerial part of *A. incarnata* L. (1.3 kg) was extracted twice with MeOH, heated until reflux. The resulting extracts were combined, then concentrated under reduced pressure with the resulting residue next suspended in H_2O (4 l). This suspension was extracted with Et_2O (1.5 l). The H_2O layer was directly passed through a Mitsubishi Diaion HP-20 column and the adsorbed material was subsequently eluted with 50% MeOH in water, 70% MeOH in water, and MeOH. The Et_2O layer and MeOH eluates of the HP-20 column were concentrated, respectively, and each residue was rechromatographed on a silica gel column with $CHCl_3$–MeOH as eluant system, and on semi-preparative HPLC (Develosil-ODS, PhA., C-8 and YMC-ODS, eluted with MeCN–H_2O (22.5–42.5% MeCN in water) and/or MeOH–H_2O (55–80% MeOH in water) systems to give compounds **1** (18 mg), **2** (37 mg), **3** (12 mg), **4** (15 mg), **5** (12 mg), **6** (5 mg), **7** (5 mg), **8** (20 mg), **9** (6 mg), **10** (30 mg), **11** (17 mg), **12** (11 mg), **13** (15 mg), **14** (10 mg), **15** (8 mg), **16** (4 mg), **17** (10 mg), **18** (15 mg), **19** (17 mg), **20** (10 mg), **21** (3 mg), **22** (15 mg), **23** (18 mg), **24** (4 mg), **25** (8 mg), **26** (9 mg), **27** (10 mg), **28** (5 mg), **29** (7 mg), **30** (5 mg), **31** (5 mg), **32** (2 mg), **33** (6 mg) and **34** (8 mg).

Compound **1**. Amorphous powder; $[\alpha]_D^{23}$ −9.7° (MeOH; *c* 1.02); FABMS *m*/*z*: 827 $[M + H]^+$, 849 $[M + Na]^+$; ^{13}C- and ^{1}H-NMR spectral data: see Tables 1, 2 and 3.

Compound **2**. Amorphous powder; $[\alpha]_D^{24}$ −4.5° (MeOH; *c* 1.04); FABMS *m*/*z*: 957 $[M + H]^+$, 979 [M



R=H: D-Digitoxopyranose D-Oleandropyranose D-Allopyranose
R=CH_3: D-Cymaropyranose

Chart 2

Table 1
^{13}C-NMR spectral data of the aglycone moiety (in pyridine-d_5 solution at 35°C)

	3	4	5	6	7	8	1	9	10	24
Carbon No.										
C-1	39.1	39.2	39.0	39.0	39.0	39.1	39.0	39.0	39.0	39.2
C-2	30.0	30.0	29.9	29.9	29.9	30.0	29.9	29.9	30.0	30.1
C-3	77.8	77.8	77.7	77.7	77.7	77.7	77.7	77.6	77.8	77.8
C-4	39.4	39.4	39.3	39.3	39.3	39.4	39.3	39.3	39.4	39.4
C-5	139.6	139.5	139.5	139.5	139.5	139.5	139.4	139.4	138.9	138.4
C-6	119.4	119.7	119.1	119.1	119.1	119.4	119.1	119.1	119.7	120.2
C-7	34.5	37.3	34.1	34.2	34.2	34.2	34.7	34.7	35.4	35.1
C-8	74.6	74.5	74.6	74.6	74.5[a]	74.3	74.3	74.3[a]	74.7	74.9
C-9	45.2	45.6	44.8	44.8	44.7	45.0	44.6	44.5	45.3	45.9
C-10	37.5	37.6	37.5	37.6	37.6	37.4	37.4	37.4	37.6	37.7
C-11	29.4	28.5	24.8	25.0	25.0	29.4	24.8	25.1	29.2	28.5
C-12	69.0	74.0	73.2	73.4	74.3[a]	69.0	73.6	74.7[a]	68.7	73.5
C-13	58.0	56.7	55.7	55.9	56.1	60.5	57.9	58.3	58.2	56.3
C-14	87.4	86.6	87.5	87.5	87.5	89.3	89.4	89.5	86.3	84.7
C-15	35.4	36.2	35.1	35.2	35.1	35.1	32.9	33.8	71.7	74.9
C-16	22.2	24.6	21.8	22.0	22.2	32.8	33.7	33.3	34.5	36.0
C-17	61.5	58.7	60.5	60.5	60.2	92.6	92.4	92.5	59.4	56.3
C-18	14.8	11.6	15.6	15.8	15.8	9.4	10.4	10.7	15.2	12.5
C-19	18.4	18.7	18.2	18.2	18.2	18.4	18.2	18.2	18.7	18.7
C-20	210.4	216.8	209.6	209.3	209.8	209.6	210.1	210.5	209.7	215.2
C-21	32.1	32.2	32.3	32.2	32.4	27.8	27.6	27.9	32.1	31.9
Ester										
C-1′	–	–	169.9	165.9	164.5	–	169.8	164.3	–	–
C-2′	–	–	20.8	119.4	–	–	20.8	–	–	–
C-3′	–	–	–	144.8	153.8	–	–	153.8	–	–
C-4′	–	–	–	135.1	127.0	–	–	126.9	–	–
C-5′	–	–	–	128.6	137.1	–	–	137.1	–	–
C-6′	–	–	–	129.3	123.4	–	–	123.5	–	–
C-7′	–	–	–	130.5	151.1	–	–	151.5	–	–
C-8′	–	–	–	129.3	–	–	–	–	–	–
C-9′	–	–	–	128.6	–	–	–	–	–	–

[a] Interchangeable within each column.

+ Na]$^+$; ^{13}C- and ^{1}H-NMR spectral data: see Tables 2–4. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **1**.

Compound **3**. Amorphous powder; $[\alpha]_D^{24}$ +3.2° (MeOH; *c* 1.24); FABMS *m/z*: 899 [M + H]$^+$, 921 [M + Na]$^+$; ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **4**. Amorphous powder; $[\alpha]_D^{24}$ +30.9° (MeOH; *c* 1.14); FABMS *m/z*: 899 [M + H]$^+$, 921 [M + Na]$^+$; ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **5**. Amorphous powder; $[\alpha]_D^{24}$ +21.5° (MeOH; *c* 0.59); FABMS *m/z*: 941 [M + H]$^+$, 963 [M + Na]$^+$; ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **6**. Amorphous powder; $[\alpha]_D^{27}$ +12.1° (MeOH; *c* 0.52); FABMS *m/z*: 1051 [M + Na]$^+$; UV λ_{max}^{MeOH} nm (log ε): 217 (4.14), 223 (4.08), 278 (4.33); ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **7**. Amorphous powder; $[\alpha]_D^{24}$ −19.1° (MeOH; *c* 0.57); FABMS *m/z*: 1004 [M + H]$^+$; UV λ_{max}^{MeOH} nm (log ε): 219 (4.00), 258 (3.46), 263 (3.49), 269 (sh); ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **8**. Amorphous powder; $[\alpha]_D^{27}$ +18.6° (MeOH; *c* 1.17); FABMS *m/z*: 915 [M+H]$^+$, 937 [M+Na]$^+$; ^{13}C NMR spectral data: see Table 1. The ^{13}C and ^{1}H NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **9**. Amorphous powder; $[\alpha]_D^{24}$ −4.6° (MeOH; *c* 0.61); FABMS *m/z*: 1020 [M + H]$^+$; UV λ_{max}^{MeOH}nm (log ε): 218 (3.97), 258 (3.39), 263 (3.42), 270 (sh); ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **10**. Amorphous powder; $[\alpha]_D^{27}$ −0.53° (MeOH; *c* 1.23); FABMS *m/z*: 915 [M + H]$^+$, 937 [M + Na]$^+$; ^{13}C-NMR spectral data: see Table 1. The

Table 2
^{13}C-NMR spectral data of the sugar moiety (in pyridine-d_5 solution at 35°C)[a]

Carbon No.	1	2	13	16	19	28	31	33	34
	Dig	Dig	Dig	Dig	Cym	Cym	Cym	Cym	Cym
C-1′	96.4	96.4	96.4	96.4	96.5	96.5	96.4	96.5	96.4
C-2′	38.7[b]	38.7[b]	38.7[b]	38.7[b]	37.3[b]	37.4[b]	37.3[b]	37.3[b]	37.6[b]
C-3′	67.4[c]	67.5[c]	67.5[c]	67.5[c]	78.1	78.2	78.1[c]	78.0	78.1[c]
C-4′	83.4[d]	83.5[d]	83.5[d]	83.5[d]	83.4[c]	83.4[c]	83.4[d]	83.4[c]	83.4[d]
C-5′	68.7	68.6e	68.6[e]	68.6	69.1	69.1	69.0	69.0	69.0[e]
OMe	–	–	–	–	58.9	58.9	59.0[e]	59.0[d]	58.9[f]
	Dig	Dig	Dig	Dig	Dig	Dig	Cym	Cym	Cym
C-1″	99.8	99.9	99.8	99.8	100.5	100.5	100.5	100.5	100.3
C-2″	39.0[b]	39.1[b]	39.1[b]	39.1[b]	38.8[b]	38.9[b]	37.1[b]	37.3[b]	37.4[b]
C-3″	67.6[c]	67.4[c]	67.4[c]	67.4[c]	67.5[d]	67.5[d]	78.0[c]	78.0	77.8[c]
C-4″	83.3[d]	83.3[d]	83.2[d]	83.2[d]	83.3[c]	83.2[c]	83.3[d]	83.2[c]	83.2[d]
C-5″	68.7	68.7[e]	68.6[e]	68.6	68.7[e]	68.6	69.0	69.0	68.9[e]
OMe	–	–	–	–	–	–	58.9[e]	58.9[d]	58.8[f]
	Ole	Dig	Dig	Dig	Dig	Dig	Dig	Dig	Ole
C-1‴	101.6	99.9	99.8	99.8	99.9	99.9	100.6	100.5	102.0
C-2‴	37.0	38.5[b]	38.6[b]	38.6[b]	38.7	38.7	38.9	38.9	37.2[b]
C-3‴	81.4	67.4[c]	67.4[c]	67.4[c]	67.4[d]	67.5[d]	67.5	67.5	79.1
C-4‴	76.2	83.1[d]	83.1[d]	83.1[d]	83.1[c]	83.1[c]	83.2[d]	83.2[c]	82.7[d]
C-5‴	73.0	68.7[e]	68.7[e]	68.6	68.5[e]	68.6	68.6	68.5	71.7
OMe	57.0	–	–	–	–	–	–	–	57.3[g]
	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole
C-1⁗	–	101.6	101.4	101.4	101.6	101.4	101.6	101.4	100.5
C-2⁗	–	37.0	37.2	37.2	37.0[b]	37.2[b]	37.0	37.1[b]	37.1[b]
C-3⁗	–	81.4	79.3	79.3	81.4	79.3	81.4	79.3	81.7
C-4⁗	–	76.2	83.1	83.1	76.2	83.1[c]	76.2	83.2[c]	76.4
C-5⁗	–	73.0	72.1	72.1	73.0	72.1	73.0	72.1	73.0
OMe	–	57.0	57.2	57.2	57.0	57.2	57.0	57.2	57.1[g]
			Glc	All		Glc		Glc	
C-1⁗′	–	–	104.5	102.1	–	104.5	–	104.5	–
C-2⁗′	–	–	75.7	72.8	–	75.7	–	75.8	–
C-3⁗′	–	–	78.7	73.2	–	78.7	–	78.7	–
C-4⁗′	–	–	72.1	69.5	–	72.1	–	72.1	–
C-5⁗′	–	–	78.2	75.7	–	78.1	–	78.2	–
C-6⁗′	–	–	63.2	63.4	–	63.2	–	63.2	–
C-6s	18.5	18.5	18.5 × 2	18.5 × 2	18.5 × 2	18.5 × 2	18.4	18.2	18.5
	18.6	18.6 × 2	18.7	18.7	18.6 × 2	18.6	18.5	18.5	18.6
	18.7	18.7	18.8	18.9		18.8	18.6 × 2	18.6	18.7
								18.7	18.8

[a] Dig: β-D-digitoxopyransyl, Cym: β-D-cymaropyranosyl, Ole: β-D-oleandropyranosyl, Glc: β-D-glucopyranosyl, All: β-D-allopyranosyl.
[b–g] Interchangeable within each column.

^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **2**.

Compound **11**. Amorphous powder; $[\alpha]_D^{27}$ +6.5° (MeOH; *c* 0.73); FABMS *m/z*: 1083 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone moiety and sugar moiety were in good agreement with those of **3** and **13**, respectively.

Compound **12**. Amorphous powder; $[\alpha]_D^{27}$ +28.3° (MeOH; *c* 1.12); FABMS *m/z*: 1083 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **4** and **13**, respectively.

Compound **13**. Amorphous powder; $[\alpha]_D^{27}$ −13.8° (MeOH; *c* 1.47); FABMS *m/z*: 1125 $[M + Na]^+$; ^{13}C- and ^{1}H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **5**.

Compound **14**. Amorphous powder; $[\alpha]_D^{27}$ −0.83° (MeOH; *c* 1.03); FABMS *m/z*: 1141 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **1** and **13**, respectively.

Compound **15**. Amorphous powder;$[\alpha]_D^{21}$ +1.9° (MeOH; *c* 0.84); FABMS *m/z*: 1099 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **10** and **13**, respectively.

Compound **16**. Amorphous powder; $[\alpha]_D^{27}$ +1.6° (MeOH; *c* 0.41); FABMS *m/z*: 1099 $[M + Na]^+$; ^{13}C- and ^{1}H-NMR NMR spectral data: see Tables 2 and 3.

Table 3
^{1}H-NMR spectral data of the sugar moiety (in pyridine-d_5 solution at 35°C)[a]

Proton No.	**1**	**2**	**13**
	Dig	Dig	Dig
H-1′	5.46 (*dd*, 9.5, 2.0)	5.45 (*dd*, 9.5, 2.0)	5.45 (*dd*, 9.5, 2.0)
H-3′	4.66 (*br s*)	4.63 (*br s*)	4.63 (*q*, 3.0)
H-4′	3.50 (*dd*, 9.5, 3.0)	3.50 (*dd*, 9.5, 3.0)	3.51 (*dd*, 9.5, 3.0)
H-5′	4.28 (*dq*, 9.5, 6.5)	4.28 (*dq*, 9.5, 6.5)	4.28 (*dq*, 9.5, 6.5)
H-6′	1.43 (*d*, 6.5)	1.39 (*d*, 6.5)[b]	1.42 (*d*, 6.5)
OMe′	–	–	–
	Dig	Dig	Dig
H-1″	5.39 (*dd*, 9.5, 2.0)	5.36 (*dd*, 9.5, 2.0)	5.36 (*dd*, 9.5, 2.0)
H-3″	4.63 (*br* s)	4.63 (*br s*)	4.62 (*q*, 3.0)
H-4″	3.47 (*dd*, 9.5, 3.0)	3.44 (∗)[e]	3.44 (*dd*, 9.5, 3.0)
H-5″	4.30 (*dq*, 9.5, 6.5)	4.25 (*dq*, 9.5, 6.5)	4.25 (*dq*, 9.5, 6.5)
H-6″	1.40 (*d*, 6.5)	1.33 (*d*, 6.5)	1.33 (*d*, 6.5)
OMe″	–	–	–
	Ole	Dig	Dig
H-1‴	4.79 (*dd*, 9.5, 2.0)	5.36 (*dd*, 9.5, 2.0)	5.36 (*dd*, 9.5, 2.0)
H-3‴	3.45 (∗)[e]	4.63 (*br s*)	4.58 (*q*, 3.0)
H-4‴	3.43 (∗)[e]	3.47 (∗)[e]	3.41 (*dd*, 9.5, 3.0)
H-5‴	3.57 (*dq*, 8.5, 6.0)	4.28 (*dq*, 9.5, 6.5)	4.26 (*dq*, 9.5, 6.5)
H-6‴	1.49 (*d*, 6.0)	1.42 (*d*, 6.5)[b]	1.35 (*d*, 6.5)
OMe‴	3.46 (*s*)	–	–
		Ole	Ole
H-1⁗	–	4.79 (*dd*, 9.5, 2,0)	4.72 (*dd*, 9.5, 2.0)
H-3⁗	–	3.44 (∗)[e]	3.63 (∗)[e]
H-4⁗	–	3.41 (∗)[e]	3.63 (∗)[e]
H-5⁗	–	3.57 (*dq*, 8.5, 6.0)	3.64 (∗)[e]
H-6⁗	–	1.49 (*d*, 6.0)	1.64 (*d*, 6.0)
OMe⁗	–	3.46 (*s*)	3.52 (*s*)
			Glc
H-1′⁗	–	–	5.10 (*d*, 8.0)
H-2′⁗	–	–	3.98 (*t*, 8.0)
H-3′⁗	–	–	4.20 (*t*, 8.0)[b]
H-4′⁗	–	–	4.17 (*t*, 8.0)[b]
H-5′⁗	–	–	3.93 (*m*)
H-6′⁗	–	–	4.33 (*dd*, 11.5, 5.5)
			4.51 (*dd*, 11.5, 2.5)

Proton No.	**16**	**19**	**28**
	Dig	Cym	Cym
H-1′	5.45 (*dd*, 9.5, 2.0)	5.26 (*dd*, 9.5, 2.0)	5.28 (*dd*, 9.5, 2.0)
H-3′	4.62 (*br s*)	4.09 (*q*, 3.0)	4.09 (*q*, 3.0)
H-4′	3.49 (*dd*, 9.5, 3.0)	3.52 (*dd*, 9.5, 3.0)	3.52 (∗)[e]
H-5′	4.26 (*dq*, 9.5, 6.5)	4.21 (*dq*, 9.5, 6.5)	4.21 (∗)[e]
H-6′	1.39 (*d*, 6.5)	1.38 (*d*, 6.5)	1.38 (*d*, 6.5)[b]
OMe′	–	3.62 (*s*)	3.62 (*s*)
	Dig	Dig	Dig
H-1″	5.35 (*dd*, 9.5, 2.0)	5.32 (*dd*, 9.5, 2.0)	5.32 (*dd*, 9.5, 2.0)
H-3″	4.62 (*br s*)	4.63 (*br s*)	4.63 (*q*, 3.0)
H-4″	3.43 (*dd*, 9.5, 3.0)	3.48 (*dd*, 9.5, 3.0)	3.47 (*dd*, 9.5, 3.0)
H-5″	4.24 (*dq*, 9.5, 6.5)	4.24 (*dq*, 9.5, 6.5)	4.24 (*dq*, 9.5, 6.5)
H-6″	1.32 (*d*, 6.5)	1.38 (*d*, 6.5)	1.38 (*d*, 6.5)[b]
OMe″	–	–	–
	Dig	Dig	Dig
H-1‴	5.35 (*dd*, 9.5, 2.0)	5.38 (*dd*, 9.5, 2.0)	5.36 (*dd*, 9.5, 2.0)
H-3‴	4.62 (*br s*)	4.63 (*br s*)	4.59 (*q*, 3.0)
H-4‴	3.40 (*dd*, 9.5, 3.0)	3.48 (*dd*, 9.5, 3.0)	3.42 (*dd*, 9.5, 3.0)
H-5‴	4.26 (*dq*, 9.5, 6.5)	4.30 (*dq*, 9.5, 6.0)	4.28 (*dq*, 9.5, 6.5)

(continued on next page)

Table 3 (*continued*)

Proton No.	**16**	**19**	**28**
H-6‴	1.35 (*d*, 6.5)	1.41 (*d*, 6.0))	1.36 (*d*, 6.5)[b]
OMe‴	–	–	–
	Ole	Ole	Ole
H-1⁗	4.71 (*dd*, 9.5, 2.0)	4.80 (*dd*, 9.5, 2.0)	4.73 (*dd*, 9.5, 2.0)
H-3⁗		3.45 (∗)[e]	3.62 (∗)[e]
H-4⁗		3.43 (∗)[e]	3.62 (∗)[e]
H-5⁗	3.60 (∗)[e]	3.57 (*dq*, 8.5, 6.0)	3.65 (∗)[e]
H-6⁗	1.60 (*d*, 6.0)	1.50 (*d*, 6.0)	1.64 (*d*, 6.0)
OMe⁗	3.53 (*s*)	3.46 (*s*)	3.52 (*s*)
	All		Glc.
H-1′⁗	5.50 (*d*, 8.0)	–	5.10 (*d*, 8.0)
H-2′⁗	3.93 (*br s*)	–	3.99 (*t*, 8.0)
H-3′⁗	4.69 (*t*, 2.5)	–	4.20 (∗)[e]
H-4′⁗	4.17 (*br d*, 9.5)	–	4.17 (*t*, 8.5)
H-5′⁗	4.43 (∗)[e]	–	3.93 (*m*)
H-6′⁗	4.32 (∗)[e]	–	4.33 (*dd*, 12.0, 5.5)
	4.47 (*dd*, 11.5, 3.0)	–	4.52 (*dd*, 12.0, 2.0)

Proton No.	**31**	**33**	**34**
	Cym	Cym	Cym
H-1′	5.29 (*dd*, 9.5, 2.0)	5.25 (*dd*, 9.5, 2.0)	5.26 (*dd*, 9.5, 2.0)
H-3′	4.07 (*q*, 3.0)	4.06 (*q*, 3.0)	4.08 (*q*, 3.0)
H-4′	3.49 (*dd*, 9.5, 3.0)	3.48 (*dd*, 9.5, 3.0)	
H-5′	4.22 (*dq*, 9.5, 6.5)	4.19 (∗)[e]	4.17 (*dq*, 9.5, 6.5)
H-6′	1.38 (*d*, 6.5)	1.36 (*d*, 6.5)	1.36 (*d*, 6.5)[b]
OMe′	3.63 (*s*)	3.62 (*s*)	3.62 (*s*)[c]
	Cym	Cym	Cym
H-1″	5.12 (*dd*, 9.5, 2.0)	5.11 (∗)[e]	5.11 (*dd*, 9.5, 2.0)
H-3″	4.08 (*q*, 3.0)	4.07 (*q*, 3.0)	4.03 (*q*, 3.0)
H-4″	3.48 (*dd*, 9.5, 3.0)	3.47 (*dd*, 9.5, 3.0)	
H-5″	4.16 (*dq*, 9.5, 6.5)	4.15 (*dq*, 9.5, 6.5)	4.19 (*dq*, 9.5, 6.5)
H-6″	1.33 (*d*, 6.5)	1.33 (*d*, 6.5)	1.39 (*d*, 6.5)[b]
OMe″	3.62 (*s*)	3.61 (*s*)	3.59 (*s*)[c]
	Dig	Dig	Ole
H-1‴	5.32 (*dd*, 9.5, 2.0)	5.30 (*dd*, 9.5, 2.0)	4.71 (*dd*, 9.5, 2.0)
H-3‴	4.63 (*br s*)	4.59 (*q*, 3.0)	
H-4‴	3.51 (*dd*, 9.5, 3.0)	3.43 (*dd*, 9.5, 3.0)	
H-5‴	4.29 (*dq*, 9.5, 6.5)	4.27 (*dq*, 9.5, 6.5)	
H-6‴	1.46 (*d*, 6.5)	1.41 (*d*, 6,5)	1.48 (*d*, 6.0)
OMe‴	–	–	3.52 (*s*)[d]
	Ole	Ole	Ole
H-1⁗	4.82 (*dd*, 9.5, 2.0)	4.74 (*dd*, 9.5, 2.0)	4.99 (*dd*, 9.5, 2.0)
H-3⁗	3.46 (∗)[e]	3.63 (∗)[e]	
H-4⁗	3.42 (∗)[e]	3.65 (∗)[e]	
H-5⁗	3.59 (*dq*, 8.5, 6.0)	3.63 (∗)[e]	
H-6⁗	1.51 (*d*, 6,0)	1.65 (*d*, 6.0)	1.59 (*d*, 6.0)
OMe⁗	3.46 (*s*)	3.52 (*s*)	3.48 (*s*)[d]
		Glc	
H-1′⁗	–	5.10 (*d*, 8.0)	–
H-2′⁗	–	3.99 (*t*, 8.0)	–
H-3′⁗	–	4.19 (∗)[e]	–
H-4′⁗	–	4.17 (∗)[e]	–
H-5′⁗	–	3.93 (*m*)	–
H-6′⁗	–	4.33 (*dd*, 11.5, 4.5)	–
	–	4.52 (*dd*, 11.5, 2.5)	–

[a] Assignments were done based on the 2D-NMR (^{1}H–^{1}H COSY, HMQC and HMBC) spectra.
[b–d] Interchangeable within each column.
[e] (∗) Overlapping with other signals.

Table 4
^{13}C- and ^{1}H-NMR spectral data of compound **2** (in $CDCl_3$ solution at 35°C)[a]

Aglycone moiety			Sugar moiety		
C-1	38.8		Dig-1′	95.9	4.92 (*dd*, 9.5, 2.0)
C-2	28.8		Dig-2′	37.1[b]	
C-3	77.8	3.57 (*m*)	Dig-3′	66.4[c]	4.24 (∗)[f]
C-4	38.8		Dig-4′	82.2[d]	3.22 (*dd*, 9.5, 3.0)
C-5	141.1	–	Dig-5′	68.1[e]	3.78 (*dq*, 9.5, 6.5)
C-6	117.3	5.34 (br s)	Dig-6′	18.2	1.22 (*d*, 6.5)
C-7	34.1				
C-8	74.5	–	Dig-1″	98.3	4.88 (*dd*, 9.5, 2.0)
C-9	43.7		Dig-2″	36.7	
C-10	37.2[b]	–	Dig-3″	66.5[c]	4.24 (∗)[f]
C-11	24.2		Dig-4″	82.4[d]	3.20 (*dd*, 9.5, 3.0)
C-12	72.5	4.51 (*dd*, 10.5, 6.0)	Dig-5″	68.2[e]	3.81 (*dq*, 9.5, 6.5)
C-13	57.8	–	Dig-6″	18.2	1.23 (*d*, 6.5)
C-14	88.2	–			
C-15	32.6		Dig-1‴	98.3	4.90 (*dd*, 9.5, 2.0)
C-16	32.2		Dig-2‴	36.7	
C-17	91.7	–	Dig-3‴	66.5[c]	4.24 (∗)[f]
C-18	9.2	1.42 (s)	Dig-4‴	82.5[d]	3.20 (*dd*, 9.5, 3.0)
C-19	18.7	1.12 (s)	Dig-5‴	68.3[e]	3.84 (*dq*, 9.5, 6.5)
C-20	209.3	–	Dig-6‴	18.2	1.24 (*d*, 6.5)
C-21	27.7	2.24 (s)			
			Ole-1⁗	100.4	4.55 (*dd*, 9.5, 2.0)
			Ole-2⁗	35.3	
Ac-1′	169.8	–	Ole-3⁗	80.4	3.18 (∗)[f]
Ac-2′	20.7	1.94 (s)	Ole-4⁗	75.3	3.12 (*t*, 8.5)
			Ole-5⁗	71.9	3.33 (*dq*, 8.5, 6.0)
			Ole-6⁗	17.9	1.32 (*d*, 6.0)
			Ole-OMe⁗	56.4	3.40 (*s*)

[a] Dig: β-D-digitoxopyranosyl, Ole: β-D-oleandropyranosyl.
[b–e] Interchangeable within each coloumn.
[f] (∗): Overlapping with other signals.

The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **10**.

Compound **17**. Amorphous powder; $[\alpha]_D^{27}$ +8.9° (MeOH; *c* 0.97); FABMS *m/z*: 913 $[M + H]^+$, 935 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **3** and **19**, respectively.

Compound **18**. Amorphous powder; $[\alpha]_D^{20}$ +35.5° ($CHCl_3$; *c* 0.78); FABMS *m/z*: 913 $[M + H]^+$, 935 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **4** and **19**, respectively.

Compound **19**. Amorphous powder; $[\alpha]_D^{23}$ −16.7° (MeOH; *c* 0.66); FABMS *m/z*: 955 $[M + H]^+$, 977 $[M + Na]^+$; ^{13}C- and ^{1}H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **5**.

Compound **20**. Amorphous powder; $[\alpha]_D^{27}$ +14.4° (MeOH; *c* 0.89); FABMS *m/z*: 1065 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 217 (4.19), 222 (4.12), 278 (4.39). The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **6** and **19**, respectively.

Compound **21**. Amorphous powder; $[\alpha]_D^{21}$ −14.4° (MeOH; *c* 0.33); FABMS *m/z*: 1019 $[M + H]^+$; UV λ_{max}^{MeOH} nm (log ε): 219 (4.00), 263 (3.49). The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **7** and **19**, respectively.

Compound **22**. Amorphous powder; $[\alpha]_D^{27}$ +2.2° (MeOH; *c* 0.74); FABMS *m/z*: 971 $[M + H]^+$, 993 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **1** and **19**, respectively.

Compound **23**. Amorphous powder; $[\alpha]_D^{27}$ +3.7° (MeOH; *c* 0.79); FABMS *m/z*: 929 $[M + H]^+$, 951 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **10** and **19**, respectively.

Compound **24**. Amorphous powder; $[\alpha]_D^{27}$ +25.4° (MeOH; *c* 0.40); FABMS *m/z*: 929 $[M + H]^+$, 951 $[M + Na]^+$; ^{13}C-NMR spectral data: see Table 1. The ^{13}C- and ^{1}H-NMR spectral data of the sugar moiety were in good agreement with those of **19**.

Compound **25**. Amorphous powder; $[\alpha]_D^{27}$ +18.7° (MeOH; *c* 0.78); FABMS *m/z*: 1097 $[M + Na]^+$. The ^{13}C- and ^{1}H-NMR spectral data of the aglycone and

sugar moiety were in good agreement with those of **3** and **28**, respectively.

Compound **26**. Amorphous powder; $[\alpha]_D^{21}$ +32.1° (MeOH; *c* 0.90); FABMS *m/z*: 1097 $[M + Na]^+$. The ^{13}C- and ^1H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **4** and **28**, respectively.

Compound **27**. Amorphous powder; $[\alpha]_D^{27}$ −10.0° (MeOH; *c* 1.01); FABMS *m/z*: 1139 $[M + Na]^+$. The ^{13}C- and ^1H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **5** and **28**, respectively.

Compound **28**. Amorphous powder; $[\alpha]_D^{27}$ −12.1° (MeOH; *c* 0.45); FABMS *m/z*: 1180 $[M + H]^+$, 1202 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 219 (4.07), 259 (3.45), 263 (3.49), 270 (sh); ^{13}C- and ^1H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **7**.

Compound **29**. Amorphous powder; $[\alpha]_D^{27}$ +1.3° (MeOH; *c* 0.65); FABMS *m/z*: 1155 $[M + Na]^+$. The ^{13}C- and ^1H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **1** and **28**, respectively.

Compound **30**. Amorphous powder; $[\alpha]_D^{27}$ +14.2° (MeOH; *c* 0.46); FABMS *m/z*: 927 $[M + H]^+$, 949 $[M + Na]^+$. The ^{13}C- and ^1H-NMR spectral data of the aglycone and sugar moiety were in good agreement with those of **3** and **31**, respectively.

Compound **31**. Amorphous powder; $[\alpha]_D^{27}$ +46.2° (MeOH; *c* 0.50); FABMS *m/z*: 927 $[M + H]^+$, 949 $[M + Na]^+$; ^{13}C- and ^1H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **4**.

Compound **32**. Amorphous powder; $[\alpha]_D^{27}$ −2.7° (MeOH; *c* 0.17). FABMS *m/z*: 991 $[M + Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone and sugar moiety were in good agreement with those of **5** and **31**, respectively.

Compound **33**. Amorphous powder; $[\alpha]_D^{21}$ +7.9° (MeOH; *c* 0.63); FABMS *m/z*: 1127 $[M + Na]^+$; ^{13}C- and ^1H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **10**.

Compound **34**. Amorphous powder; $[\alpha]_D^{27}$ −2.2° (MeOH; *c* 0.80); FABMS *m/z*: 979 $[M + Na]^+$; ^{13}C- and ^1H-NMR spectral data: see Tables 2 and 3. The ^{13}C-NMR spectral data of the aglycone moiety were in good agreement with those of **10**.

3.3. Acid hydrolysis of a mixture of pregnane glycosides

A mixture of pregnane glycosides (510 mg) was heated at 60°C for 5 h with dioxane (8 ml) and 0.2 N H_2SO_4 (2 ml) to yield 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. Purification of the residue by HPLC (YMC-ODS, 30, 35, 52.5% MeOH in water and 25, 27.5% MeCN in water) afforded lineolon (**35**, 12 mg), isolineolon (**36**, 2 mg), 12-*O*-acetyllineolon (**37**, 2 mg), deacylmetaplexigenin (**38**, 4 mg), metaplexigenin (**39**, 5 mg) and 15β-hydroxylineolon (**40**, 28 mg) and 15β-hydroxyisolineolon (**41**, 5 mg).

Compound **37**. $[\alpha]_D^{21}$ −70.2° (MeOH; *c* 0.26); FABMS *m/z*: 407 $[M + H]^+$, 429 $[M + Na]^+$; ^{13}C-NMR spectral data (pyridine-d_5 at 35°C): δ 209.7 (C-20), 167.0 (C^*OCH_3), 140.5 (C-5), 118.5 (C-6), 87.5, (C-14), 74.7 (C-8), 73.3 (C-12), 71.6 (C-3), 60.5 (C-17), 55.7 (C-13), 44.9 (C-9), 43.3 (C-4), 39.3 (C-1), 37.5 (C-10), 35.2, 34.2 (C-7, 15), 32.3 (C-21), 32.0 (C-2), 24.9 (C-11), 21.8 (C-16), 20.8 (COC^*H_3), 18.4 (C-19), 15.7 (C-18). ^1H-NMR spectral data (pyridine-d_5 at 35°C): δ 5.32 (*br s*, H-6), 5.05 (*dd*, 12.0, 4.0, H-12), 3.87 (*m*, H-3), 3.49 (*t*, 9.5, H-17), 2.26 (*s*, H-21), 2.05 (*s*, $COCH_3^*$), 1.90 (*s*, H-18), 1.72 (*dd*, 12.5, 3.5, H-9), 1.40 (*s*, H-19).

Compound **40**. $[\alpha]_D^{21}$ −3.4° (MeOH; *c* 0.85); FABMS *m/z*: 381 $[M + H]^+$; ^{13}C-NMR spectral data (pyridine-d_5 at 35°C): δ 209.8 (C-20), 139.8 (C-5), 119.1 (C-6), 86.4 (C-14), 74.8 (C-8), 71.7 × 2 (C-3, 15), 68.7 (C-12), 59.4 (C-17), 58.2 (C-13), 45.3 (C-9), 43.5 (C-4), 39.3 (C-1), 37.6 (C-10), 35.4 (C-7), 34.5 (C-16), 32.2 (C-2), 32.1 (C-21), 29.3 (C-11), 18.8 (C-19), 15.2 (C-18). ^1H-NMR spectral data (pyridine-d_5 at 35°C): δ 5.45 (*br s*, H-6), 5.07 (*s*, 14-OH*), 4.31 (*s*, 8-OH*), 4.54 (*m*, H-15), 4.03 (*dd*, 12.0, 4.0, H-12), 3.86 (*m*, H-3), 3.53 (*t*, 9.5, H-17), 3.33 (*dt*, 14.5, 10.0, H-16), 2.42 (*s*, H-21), 2.03 (*s*, H-18), 1.67 (*dd*, 13.5, 2.5, H-9), 1.48 (*s*, H-19).

Compound **41**. $[\alpha]_D^{21}$ +55.9° (MeOH; *c* 0.47); FABMS *m/z*: 381 $[M + H]^+$, 403 $[M + Na]^+$; ^{13}C-NMR spectral data (pyridine-d_5 at 35°C): δ 215.3 (C-20), 139.3 (C-5), 119.5 (C-6), 84.7 (C-14), 75.0, 74.9 (C-8, 15), 73.5 (C-12), 71.7 (C-3), 56.3 × 2 (C-13, 17), 45.9 (C-9), 43.5 (C-4), 39.4 (C-1), 37.7 (C-10), 36.0 (C-16), 35.1 (C-7), 32.3 (C-2), 31.9 (C-21), 28.5 (C-11), 18.9 (C-19), 12.5 (C-18). ^1H-NMR spectral data (pyridine-d_5 at 35°C): δ 5.47 (*br s*, H-6), 5.30 (*s*, 14-OH*), 4.58 (*br s*, H-15), 4.23 (*s*, 8-OH*), 3.89 (*m*, H-3), 3.83 (*dd*, 12.0, 4.0, H-12), 3.78 (*dd*, 9.5, 5.0, H-17), 2.61 (*dt*, 14.0, 9.5, H-16), 2.32 (overlapped, H-16), 2.30 (*s*, H-21), 1.78 (*dd*, 13.5, 2.5, H-9), 1.66 (*s*, H-18), 1.50 (*s*, H-19).

The H_2O layer was neutralized with Amberlite IRA-60E and the eluate was concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (7:1:1.2 bottom layer) system to obtain cymarose (13 mg), oleandrose (37 mg), digitoxose (57 mg).

All these monosaccharides were believed to be of D-form based on their optical rotation values (Tsuka-

moto et al., 1986; Nakagawa et al., 1983; Abe et al., 1994).

D-Cymarose: $[\alpha]_D^{21}$ +51.7° (*c* = 1.29, 24 h after dissolution in H_2O).

D-Oleandrose: $[\alpha]_D^{21}$ −11.7° (*c* = 1.87, 24 h after dissolution in H_2O).

D-Digitoxose: $[\alpha]_D^{21}$ +45.0° (*c* = 1.04, 24 h after dissolution in H_2O).

3.4. Acid hydrolysis of a mixture of pregnane glycosides to determine the configuration of glucose

A mixture of pregnane glycosides (ca. 10 mg) was heated at 95°C for 1.5 h with dioxane and 0.05 N HCl (10 drops each). After hydrolysis, the reaction mixture was passed through an Amberlite IRA-60E column, and the eluate was evaporated under reduced pressure. The residue was partitioned between H_2O and EtOAc, and the H_2O layer was concentrated to dryness. This residue was stirred with D-cysteine methyl ester hydrochloride (3 mg) (Hara, Okabe & Mihashi, 1987) in pyridine (25 μl) at 60°C for 1.5 h. Subsequently, hexamethyldisilazane (10 μl) and trimethlsilylchloride (10 μl) were added to the solution, and stirring was continued at 60°C for another 30 min. The precipitate was removed with centrifugation, with the supernatant next subjected to GC analysis. GC conditions: column, GL Capillary Column TC-1 (GL Sciences) 0.32 mm × 30 m, carrier gas N_2, column temperature 210°C;R_t, D-glucose 18.8 min, L-glucose 17.5 min. D-Glucose was detected from the mixture of pregnane glycosides.

3.5. Acid hydrolysis of compounds **1**–**34**

Solutions of compounds **1**–**34** (ca. 0.5 mg) in dioxane and 0.05 N HCl (2 drops each) were heated at 95°C for 1.5 h. After hydrolysis, this solution was passed through an Amberlite IRA-60E column and concentrated to dryness. The residues from **1**–**34** were partitioned between EtOAc and H_2O, and the EtOAc extract was analyzed by HPLC to identify the aglycone via comparison with authentic samples (conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 30% MeOH in water;R_t, 15β-hydroxylineolon 12.0 min, 15β-hydroxyisolineolon 12.8 min, 35% MeOH in water;R_t, lineolon 14.6 min, deacylmetaplexigenin 12.8 min, 42.5% MeOH in water;R_t, isolineolon 12.8 min, 50% MeOH in water;R_t, 12-*O*-acetyllineolon 14.4 min, 12-*O*-nicotinolylineolon 15.6 min, rostratamine 17.2 min, 52.5% MeOH in water;R_t, metaplexigenin 15.6 min, 65% MeOH in water;R_t, ikemagenin 21.0 min).

Subsequently, the H_2O layer was 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 (4 drops each) at room temperature. After evaporation of the reagents under a stream of air, cymaritol acetate, oleandritol acetate, digitoxitol acetate, glucitol acetate and allitol acetate were detected by GC (conditions: column, Supelco SP-2380™ capillary column 0.25 mm × 30 m, carrier gas N_2, column temperature 200°C;R_t, cymaritol acetate 7.5 min, oleandritol acetate 8.5 min, digitoxitol acetate 10.9 min, column temperature 250°C;R_t, allitol acetate 9.4 min, glucitol acetate 12.4 min).

3.6. Acid hydrolysis of compound **16**

Acid hydrolysis of **16** (3 mg) with 0.05 N HCl and a reaction of the product following hydrolysis with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethlsilylchloride was carried out as described previously. GC analysis was used with the same conditions as for the detection of D-glucose. R_t, D-allose 19.0 min, L-allose 17.9 min. The R_t for L-allose was obtained from its enantiomer (D-allose + L-cysteine). D-Allose was detected from **16**.

3.7. Enzymatic hydrolysis of compounds **11**–**15**, **25**–**29** *and* **33**

Compounds **11**–**15**, **25**–**29** and **33** (ca. 2 mg) were dissolved in H_2O (0.7 ml), and cellulase (EC 3.2.1.4 from *Penicillium funiculosum*, 6.1 units/mg solid, Sigma) (ca. 20–30 mg) was added. The mixture was stirred at 40°C for 1 day. After hydrolysis, the reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extract of each compound except for **33** contained **3, 4, 5, 2, 10, 17, 18, 19, 21** and **22**, whose structures were confirmed by comparison of their ^{1}H-NMR spectra and HPLC retention times with those of authentic samples. In the ^{1}H-NMR spectrum of the hydrolysis product of **33**, the signals of the sugar moiety were consistent with those of **31** (HPLC conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 60% MeOH in water: R_t, **10** 14.2 min, 65% MeOH in water: R_t, **3** 13.6 min, 70% MeOH in water: R_t, **2** 17.2 min, **4** 11.2 min, **5** 13.8 min, **17** 11.4 min, 75% MeOH in water: R_t, **18** 10.0 min, **19** 12.4 min, **21** 10.8 min, **22** 16.4 min).

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