# Steroidal Glycosides from *Asclepias fruticosa* L.

Tsutomu WARASHINA* and Tadataka NORO

*Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1, Yada, Shizuoka 422, Japan.*
Received July 19, 1993; accepted September 13, 1993

**Five novel steroidal glycosides 2—6 were isolated from the whole plant of *Asclepias fruticosa* L. (Asclepiadaceae). The structures of these steroidal glycosides were determined on the basis of spectral and chemical evidence. All of these glycosides contain 2,6-dideoxyhexopyranoses as component sugars and their structures were elucidated as polyoxypregnane-type glycosides, which have lineolon as the aglycone moiety.**

**Keywords** *Asclepias fruticosa*; Asclepiadaceae; polyoxypregnane glycoside; 2,6-dideoxyhexopyranose

*Asclepias fruticosa* L. (Asclepiadaceae) is indigenous to southern Africa. Asclepiadaceous plants are known to contain significant quantities of cardenolides. Since some phytochemical investigations have already been reported on this plant, we decided to investigate its constituents as part of our research on steroidal glycosides of the Asclepiadaceous plant. This paper describes the isolation and structural determination of five new polyoxypregnane glycosides.

The methanol extract of the whole plant was partitioned between ether and water, the water layer was passed through a Mitsubishi Diaion HP-20 column, and the absorbed material was eluted with 50% methanol in water and methanol. The methanol eluate was chromatographed on a silica gel column to give a steroidal glycoside fraction from which five new polyoxypregnane glycosides (compounds **2**—**5** and **6**) were obtained. These pregnane glycosides gave compound **1** as an aglycone moiety by acid hydrolysis, which was determined to be lineolon by comparison of the $^{13}C$- and $^{1}H$-NMR spectral data with previously reported data.[1)]

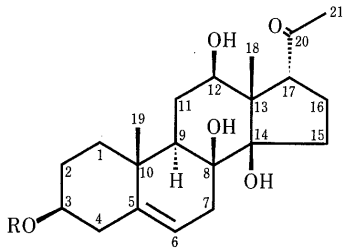
Compound **2** showed four anomeric proton signals [$\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz), 4.55 (1H, dd, $J$=9.5, 2.0 Hz), 4.83 (1H, dd, $J$=9.5, 2.0 Hz), 4.75 (1H, dd, $J$=9.5, 2.0 Hz)] in the $^{1}H$-NMR spectrum. In the $^{13}C$-NMR spectrum, four anomeric carbon signals were observed at $\delta$ 95.8, 100.3, 99.2 and 98.1. The signal due to the C-3 of the aglycone at $\delta$ 78.0 shifted downfield in comparison with that of lineolon, which was a glycosylation shift, indicating that the sugar chain was attached at the C-3 position. Acid hydrolysis gave digitoxose, olivose and cymarose as the sugar moiety, and analysis by GC showed that the relative ratio of these monosaccharides was two digitoxose to one olivose and one cymarose (see Experimental). The FAB-MS spectrum of **2** revealed a $[M+Na]^+$ ion peak at $m/z$ 921, which was larger by 534 mass units than that of lineolon, and the extra mass units corresponded to the total molecular weight of these four monosaccharides. The $^{13}C$–$^{1}H$ correlation spectroscopy (C–H COSY) spectrum of compound **2** revealed cross peaks between the anomeric carbon signals at $\delta$ 95.8, 100.3, 99.2, 98.1 and the anomeric proton signals at $\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz), 4.55 (1H, dd, $J$=9.5, 2.0 Hz), 4.83 (1H, dd, $J$=9.5, 2.0 Hz), 4.75 (1H, dd, $J$=9.5, 2.0 Hz), respectively. In the $^{1}H$-NMR spectrum, the characteristic signals of H-3$\beta$ in digitoxopyranose and cymaropyranose were observed at $\delta$ 4.22 (1H, q, $J$=3.0 Hz), 4.25 (1H, q, $J$=3.0 Hz) and 3.64 (1H, q, $J$=3.0 Hz), and one methoxyl proton signal was observed at $\delta$ 3.44 (3H, s). In the heteronuclear multiple bond connectivity (HMBC) spectrum, $^{3}J_{CCCH}$s were confirmed between these characteristic H-3 signals and the anomeric carbon signals as follows, $\delta$ 4.22 (1H, q, $J$=3.0 Hz) and $\delta$ 95.8, $\delta$ 4.25 (1H, q, $J$=3.0 Hz) and $\delta$ 99.2, $\delta$ 3.64 (1H, q, $J$=3.0 Hz) and $\delta$ 98.1. Similarly, between the methoxyl carbon signal at $\delta$ 57.4 and the H-3 signal at $\delta$ 3.64 (1H, q, $J$=3.0 Hz), a $^{3}J_{COCH}$ was observed in the HMBC spectrum. From the above results, the signals at $\delta$ 98.1 and $\delta$ 4.75 (1H, dd, $J$=9.5, 2.0 Hz) were assigned to the anomeric carbon and proton of cymaropyranose, and two pairs of the signals at $\delta$ 95.8 and $\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz), and $\delta$ 99.2 and $\delta$ 4.83 (1H, dd, $J$=9.5, 2.0 Hz) were assigned to the anomeric carbons and protons of two digitoxopyranoses. Thus, the remaining anomeric signals at $\delta$ 100.3 and $\delta$ 4.55 (1H, dd, $J$=9.5, 2.0 Hz) could belong to olivopyranose. In addition, the HMBC spectrum gave us information about the sugar linkage. The signal at $\delta$ 95.8, due to the anomeric carbon of digitoxopyranose, showed a $^{3}J_{COCH}$ to the signal due to the H-3 of lineolon [$\delta$ 3.56 (1H, m)], and the C-3 signal of lineolon exhibited a $^{3}J_{COCH}$ to the anomeric proton signal at $\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz) of digitoxopyranose. Accordingly, the sugar which was attached at the C-3 position of aglycone was decided to be digitoxopyranose. Secondly, the anomeric proton and carbon signals of olivopyranose [$\delta$ 4.55 (1H, dd, $J$=9.5, 2.0 Hz) and $\delta$ 100.3] showed $^{3}J_{COCH}$s to the C-4 and H-4 signals of the above digitoxopyranose [$\delta$ 82.7 and $\delta$ 3.21 (1H, dd, $J$=9.5, 3.0 Hz)]. Thirdly, the C-4 and H-4 signals of olivopyranose [$\delta$ 87.9 and $\delta$ 2.97 (1H, t, $J$=9.5 Hz)] displayed $^{3}J_{COCH}$s to the anomeric proton and carbon signals of another digitoxopyranose [$\delta$ 4.83 (1H, dd, $J$=9.5, 2.0 Hz) and $\delta$ 99.2], in a similar manner. And finally, between the C-4 and H-4 signals of this digitoxopyranose [$\delta$ 81.9 and $\delta$ 3.23 (1H, dd, $J$=9.5, 3.0 Hz)] and the anomeric signals of cymaropyranose [$\delta$ 4.75 (1H, dd, $J$=9.5, 3.0 Hz), 98.1], $^{3}J_{COCH}$s were observed. Moreover, in the difference nuclear Overhauser effect (NOE) spectra, irradiation at each anomeric proton signal of the four monosaccharides brought about the NOEs shown in Chart 3. Consequently, the sugar linkage was determined as shown in Chart 3. Based on the above evidence, the structure of compound

**2** was elucidated to be lineolon 3-*O*-*β*-cymaropyranosyl-(1→4)-*β*-digitoxopyranosyl-(1→4)-*β*-olivopyranosyl-(1→4)-*β*-digitoxopyranoside.

Compound **3** exhibited signals of lineolon and four monosaccharides as did **2**, but one more methoxyl signal was observed, at *δ* 56.7 and 3.41 (3H, s) in the $^{13}$C- and $^{1}$H-NMR spectra. Since acid hydrolysis allowed us to determine that these four monosaccharides were two digitoxopyranoses, one oleandropyranose and one cymaropyranose, by comparison with authentic samples, this methoxyl signal was deduced to belong to oleandropyranose. In the long range C–H COSY spectrum, the anomeric carbon signals at *δ* 95.8, 98.5 and 98.1 showed $^{3}J_{CCCH}$s to the H-3*β* ones at *δ* 4.24 (1H, q, *J*=3.0 Hz), 4.24 (1H, q, *J*=3.0 Hz) and 3.63 (1H, q, *J*=3.0 Hz), respectively, which were characteristic of digitoxopyranose and cymaropyranose, and the carbon signal at *δ* 77.3, which had a $^{1}J_{CH}$ with a signal at *δ* 3.63 (1H, q, *J*=3.0 Hz), exhibited a $^{3}J_{COCH}$ with the methoxyl proton signal at *δ* 3.45 (3H, s). From the above results, the anomeric carbon signals at *δ* 95.8 and 98.5 were assigned to two digitoxopyranoses, and the carbon signal at *δ* 98.1 to cymaropyranose. Since, however, the remaining anomeric carbon signal at *δ* 100.1 did not reveal a $^{3}J_{CCCH}$ in the long range C–H COSY spectrum, it was determined to belong to oleandropyranose. The anomeric proton signals of each monosaccharide were decided in Table III by the C–H COSY spectrum. In the difference NOE spectra, irradiation at the anomeric proton signal of digitoxopyranose at *δ* 4.93 (1H, dd, *J*=9.5, 2.0 Hz) revealed an NOE to the signal due to the H-3 [*δ* 3.56 (1H, m)] of the aglycone. Accordingly, this digitoxopyranose was attached to the C-3 position of the aglycone. Similarly, irradiation at the anomeric proton signals of oleandropyranose [*δ* 4.50 (1H, dd, *J*=9.5, 2.0 Hz)], another digitoxopyranose [*δ* 5.00 (1H, dd, *J*=9.5, 2.0 Hz)] and cymaropyranose [*δ* 4.74 (1H, dd, *J*=9.5, 2.0 Hz)] showed NOEs to the signals due to H-4 [*δ* 3.21 (1H, dd, *J*=9.5, 3.0 Hz)] of digitoxopyranose, which was attached to the C-3 of aglycone, H-4 [*δ* 3.18 (1H, t, *J*=9.5 Hz)] of oleandropyranose and H-4 [*δ* 3.21 (1H, dd, *J*=9.5, 3.0 Hz)] of another digitoxopyranose, respectively. Based on the above evidence, the structure of **3** was determined to be lineolon 3-*O*-*β*-cymaropyranosyl-(1→4)-*β*-digitoxopyranosyl-(1→4)-*β*-oleandropyranosyl-(1→4)-*β*-digitoxopyranoside.

The $^{1}$H- and $^{13}$C-NMR spectra of compound **4** were similar to those of **3**, but three methoxyl signals were seen at *δ* 56.8, 57.8 and 57.2 in the $^{13}$C-NMR spectrum and at *δ* 3.40 (3H, s), 3.44 (3H, s) and 3.43 (3H, s) in the $^{1}$H-NMR spectrum. Acid hydrolysis of this compound gave digitoxose, oleandrose and cymarose, and the relative ratio of these monosaccharides was one digitoxose, one oleandrose and two cymaroses. In the difference NOE spectrum, irradiation at the anomeric proton signal at *δ* 4.92 (1H, dd, *J*=9.5, 2.0 Hz), which was assigned to digitoxopyranose, brought an NOE to the H-3 signal of the aglycone [*δ* 3.56 (1H, m)]. Similarly, NOEs were observed between the anomeric proton signal of oleandropyranose [*δ* 4.50 (1H, dd, *J*=9.5, 2.0 Hz)] and the H-4 one of digitoxopyranose [*δ* 3.21 (1H, dd, *J*=9.5, 3.0 Hz)], the H-1 one of cymaropyranose [*δ* 4.94 (1H, dd, *J*=9.5, 2.0 Hz)] and the H-4 one of oleandropyranose [*δ* 3.17 (1H, t, *J*=9.5 Hz)], and between the H-1 signal of another cymaropyranose [*δ* 4.68 (1H, dd, *J*=9.5, 2.0 Hz)] and the H-4 one of the above cymaropyranose [*δ* 3.22 (1H, dd, *J*=9.5, 3.0 Hz)]. Thus, the structure of **4** was determined to be lineolon 3-*O*-*β*-cymaropyranosyl-(1→4)-*β*-cymaropyranosyl-(1→4)-*β*-oleandropyranosyl-(1→4)-*β*-digitoxopyranoside.

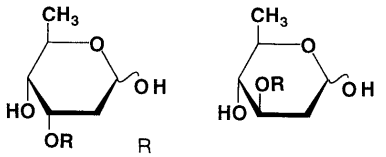
In the FAB-MS spectrum, compound **5** revealed a $[M+Na]^{+}$ ion peak at *m/z* 949, which was the same as that of **4**. Accordingly, the molecular formula of this compound was $C_{48}H_{78}O_{17}$. The $^{1}$H- and $^{13}$C-NMR spectra of **5** were similar to those of **4**, but the characteristic signals due to the H-3*β* and H-4*β* of the digitoxopyranose- and cymaropyranose-types were only seen at *δ* 4.22 (1H, q, *J*=3.0 Hz), 3.81 (1H, q, *J*=3.0 Hz), 3.21 (1H, dd, *J*=9.5, 3.0 Hz) and 3.23 (1H, dd, *J*=9.5, 3.0 Hz). On the other hand, the characteristic signals due to the H-4*β* of olivopyranose and oleandropyranose types were seen at *δ* 3.18 (1H, t, *J*=9.5 Hz) and 3.13 (1H, t, *J*=9.5 Hz). One of three methoxyl carbon signals at *δ* 58.0 showed a $^{3}J_{COCH}$ with the above characteristic proton signal at *δ* 3.81 (1H, q, *J*=3.0 Hz) in the HMBC spectrum. The remaining methoxyl proton signals at *δ* 3.40 (3H, s) and 3.39 (3H, s) showed $^{3}J_{COCH}$s to the C-3 signals at *δ* 78.7 and 80.6, which also had $^{2}J_{CCH}$s to the characteristic H-4 signals at *δ* 3.18 (1H, t, *J*=9.5 Hz) and 3.13 (1H, t, *J*=9.5 Hz), respectively. From the above results, the sugar moiety contained a digitoxopyranose, a cymaropyranose and two oleandropyranoses, which was also confirmed by acid hydrolysis in comparison with authentic samples. About the sugar linkage, $^{3}J_{COCH}$s were observed as follows in the HMBC spectrum, *δ* 95.8 [C-1 of digitoxopyranose] and *δ* 3.56 (1H, m) [H-3 of aglycone], *δ* 100.2 [C-1 of




R

**1**: H
**2**: digito.–(4→1)–olv.–(4→1)–digito.–(4→1)–cym.
**3**: digito.–(4→1)–ole.–(4→1)–digito.–(4→1)–cym.
**4**: digito.–(4→1)–ole.–(4→1)–cym.–(4→1)–cym.
**5**: digito.–(4→1)–ole.–(4→1)–cym.–(4→1)–ole.
**6**: digito.–(4→1)–ole.–(4→1)–ole.–(4→1)–cym.

Chart 1




D-digitoxopyranose : R = H
D-cymaropyranose : R = $CH_3$
D-olivopyranose : R = H
D-oleandropyranose : R = $CH_3$

Chart 2

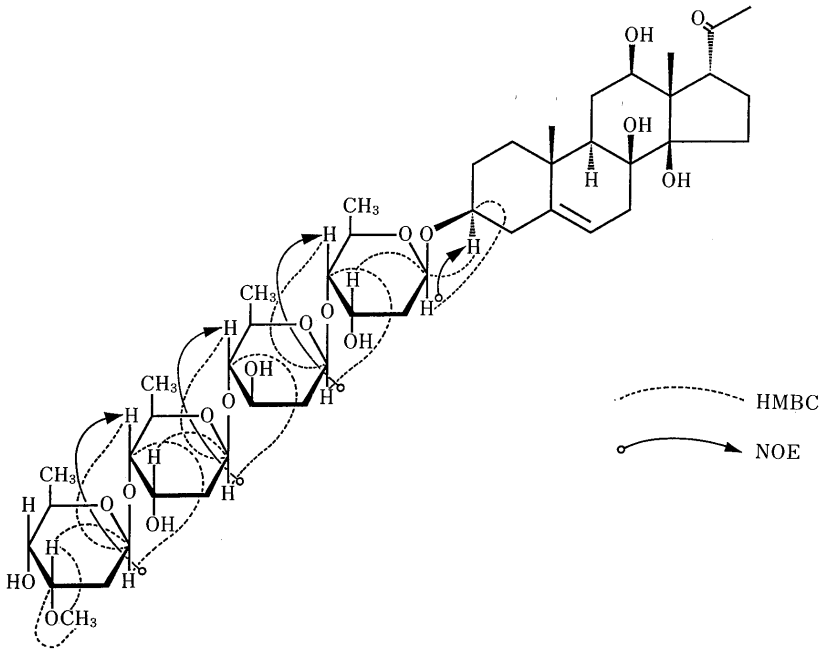



Chart 3. The Important $^3J$s in the HMBC Spectrum and NOEs in the Difference NOE Spectra on Compound **2**

TABLE I. $^{13}$C-NMR Spectral Data of Aglycone of Compounds **1** and **2**—**6**

| Carbon No. | **1**[a] | **2**—**6**[b] |
|---|---|---|
| C- 1 | 38.2 | 38.8 |
| C- 2 | 30.9 | 28.9 |
| C- 3 | 70.3 | 78.0 |
| C- 4 | 42.1 | 38.8 |
| C- 5 | 139.2 | 141.2 |
| C- 6 | 117.9 | 117.4 |
| C- 7 | 34.3 | 34.3 |
| C- 8 | 73.3 | 74.6 |
| C- 9 | 43.7 | 44.1 |
| C-10 | 36.3 | 37.1 |
| C-11 | 27.9 | 27.2 |
| C-12 | 67.8 | 68.3 |
| C-13 | 56.4 | 55.9 |
| C-14 | 86.4 | 85.7 |
| C-15 | 33.4 | 33.1 |
| C-16 | 20.8 | 23.2 |
| C-17 | 60.0 | 60.7 |
| C-18 | 13.8 | 13.0 |
| C-19 | 17.8 | 18.9 |
| C-20 | 209.2 | 214.7 |
| C-21 | 31.4 | 31.8 |

Run at 67.80 and 125.65 MHz. *a*) Measured in $(Me)_2SO$-$d_6$. *b*) Measured in $CDCl_3$.

TABLE II. $^{13}$C-NMR Spectral Data of Sugar Moiety of Compounds **2**—**6**

| Carbon No. | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|
| | Digito. | Digito. | Digito. | Digito. | Digito. |
| C-1 | 95.8 | 95.8 | 95.8 | 95.8 | 95.8 |
| C-2 | 37.1 | 37.0 | 37.1 | 37.1 | 37.2 |
| C-3 | 66.6 | 66.5 | 66.5 | 66.5 | 66.6 |
| C-4 | 82.7 | 82.7[a] | 82.7 | 82.7 | 82.8 |
| C-5 | 67.9 | 67.9 | 67.9 | 68.0 | 67.9 |
| C-6 | 18.2 | 18.2[b] | 18.2 | 18.2 | 18.2 |
| | Olv. | Ole. | Ole. | Ole. | Ole. |
| C-1 | 100.3 | 100.1 | 100.2 | 100.2 | 100.2 |
| C-2 | 38.4 | 36.2 | 36.3 | 36.3 | 36.3 |
| C-3 | 69.4 | 78.8 | 78.7 | 78.7 | 79.0 |
| C-4 | 87.9 | 82.3[a] | 82.0 | 82.0 | 82.2 |
| C-5 | 70.6 | 71.4 | 71.4 | 71.4 | 71.3 |
| C-6 | 17.8 | 18.3 | 18.3[c] | 18.3 | 18.4 |
| OMe | — | 56.7 | 56.8 | 56.8 | 56.9 |
| | Digito. | Digito. | Cym. | Cym. | Ole. |
| C-1 | 99.2 | 98.5 | 98.5 | 98.5 | 100.2 |
| C-2 | 36.5 | 37.0 | 35.3 | 35.5 | 36.4 |
| C-3 | 66.1 | 66.4 | 77.0 | 77.0 | 79.0 |
| C-4 | 81.9 | 82.4[a] | 82.4 | 82.6 | 82.4 |
| C-5 | 68.5 | 68.3 | 68.8 | 68.7 | 71.2 |
| C-6 | 17.8 | 18.2 | 18.2[c] | 18.2 | 18.4 |
| OMe | — | — | 57.8 | 58.0 | 56.6 |
| | Cym. | Cym. | Cym. | Ole. | Cym. |
| C-1 | 98.1 | 98.1 | 99.4 | 101.5 | 98.2 |
| C-2 | 33.8 | 33.8 | 33.7 | 35.3 | 33.8 |
| C-3 | 77.3 | 77.3 | 77.3 | 80.6 | 77.5 |
| C-4 | 72.2 | 72.3 | 72.4 | 75.4 | 72.4 |
| C-5 | 71.0 | 70.9 | 70.7 | 71.5 | 71.1 |
| C-6 | 18.2 | 18.3[b] | 18.3 | 18.0 | 18.3 |
| OMe | 57.4 | 57.4 | 57.2 | 56.2 | 57.1 |

Run at 67.80 and 125.65 MHz in $CDCl_3$ solution. *a*—*c*) Assignment may be interchanged in each column.

oleandropyranose] and $\delta$ 3.21 (1H, dd, $J$=9.5, 3.0 Hz) [H-4 of digitoxopyranose], $\delta$ 98.5 [C-1 of cymaropyranose] and $\delta$ 3.18 (1H, t, $J$=9.5 Hz) [H-4 of oleandropyranose], $\delta$ 101.5 [C-1 of another oleandropyranose] and $\delta$ 3.23 (1H, dd, $J$=9.5, 3.0 Hz) [H-4 of cymaropyranose]. And, in the difference NOE spectra, irradiation at the anomeric proton signal at $\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz), which was assigned to digitoxopyranose, revealed an NOE to the H-3 signal of the aglycone [$\delta$ 3.56 (1H, m)]. Similarly, NOEs were observed between the anomeric proton signal of oleandropyranose [$\delta$ 4.50 (1H, dd, $J$=9.5, 1.5 Hz)] and the H-4 signal of digitoxopyranose [$\delta$ 3.21 (1H, dd, $J$=9.5, 3.0 Hz)], the H-1 one of cymaropyranose [$\delta$ 4.95 (1H, dd, $J$=9.5, 2.0 Hz)] and the

TABLE III. $^1$H-NMR Spectral Data of Compounds **2**—**6**

| Proton No. | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|
| Aglycone | | | | | |
| H-3 | 3.56 (1H, m) | 3.56 (1H, m) | 3.56 (1H, m) | 3.56 (1H, m) | 3.56 (1H, m) |
| H-6 | 5.33 (1H, br s) | 5.34 (1H, br s) | 5.34 (1H, br s) | 5.34 (1H, br s) | 5.33 (1H, br s) |
| H-9 | 1.46 (1H, dd, $J$=13.0, 3.5 Hz) | 1.45 (1H, dd, $J$=13.5, 3.0 Hz) | 1.46 (1H, dd, $J$=13.5, 3.0 Hz) | 1.46 (1H, dd, $J$=13.5, 3.0 Hz) | 1.46 (1H, dd, $J$=13.5, 3.5 Hz) |
| H-12 | 3.70 (1H, dd, $J$=12.0, 4.0 Hz) | 3.70 (1H, dd, $J$=12.0, 4.0 Hz) | 3.70 (1H, dd, $J$=12.0, 3.5 Hz) | 3.70 (1H, dd, $J$=12.0, 4.0 Hz) | 3.71 (1H, dd, $J$=12.5, 3.5 Hz) |
| H-17 | 3.38 (1H, t. $J$=11.0 Hz) | 3.38 (1H, t, $J$=11.0 Hz) | 3.38 (1H, t, $J$=11.0 Hz) | 3.37 (1H, t, $J$=11.0 Hz) | 3.38 (1H, t, $J$=11.0 Hz) |
| H-18 | 1.26 (3H, s) | 1.27 (3H, s) | 1.26 (3H, s) | 1.26 (3H, s) | 1.27 (3H, s) |
| H-19 | 1.14 (3H, s) | 1.15 (3H, s) | 1.14 (3H, s) | 1.14 (3H, s) | 1.14 (3H, s) |
| H-21 | 2.24 (3H, s) | 2.25 (3H, s) | 2.24 (3H, s) | 2.25 (3H, s) | 2.24 (3H, s) |
| Sugar moiety | | | | | |
| | Digito. | Digito. | Digito. | Digito. | Digito. |
| H-1 | 4.93 (1H, dd, $J$=9.5, 2.0 Hz) | 4.93 (1H, dd, $J$=9.5, 2.0 Hz) | 4.92 (1H, dd, $J$=9.5, 2.0 Hz) | 4.93 (1H, dd, $J$=9.5, 2.0 Hz) | 4.93 (1H, dd, $J$=9.5, 2.0 Hz) |
| H-2a | 2.09 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.09 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.09 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.09 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.09 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) |
| H-2b | 1.70[a] | 1.72[a] | 1.73[a] | 1.72[a] | 1.73[a] |
| H-3 | 4.22 (1H, q, $J$=3.0 Hz) | 4.24 (1H, q, $J$=3.0 Hz) | 4.22 (1H, q, $J$=3.0 Hz) | 4.22 (1H, q, $J$=3.0 Hz) | 4.22 (1H, q, $J$=3.0 Hz) |
| H-4 | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) |
| H-5 | 3.80 (1H, dq, $J$=9.5, 6.5 Hz) | 3.79 (1H, dq, $J$=9.5, 6.5 Hz) | 3.79 (1H, dq, $J$=9.5, 6.5 Hz) | 3.79 (1H, dq, $J$=9.5, 6.5 Hz) | 3.79 (1H, dq, $J$=9.5, 6.5 Hz) |
| H-6 | 1.24 (3H, d, $J$=6.5 Hz) | 1.24 (3H, d, $J$=6.5 Hz) | 1.24 (3H, d, $J$=6.5 Hz) | 1.24 (3H, d, $J$=6.5 Hz) | 1.24 (3H, d, $J$=6.5 Hz) |
| | Olv. | Ole. | Ole. | Ole. | Ole. |
| H-1 | 4.55 (1H, dd, $J$=9.5, 2.0 Hz) | 4.50 (1H, dd, $J$=9.5, 2.0 Hz) | 4.50 (1H, dd, $J$=9.5, 2.0 Hz) | 4.50 (1H, dd, $J$=9.5, 1.5 Hz) | 4.50 (1H, dd, $J$=9.5, 2.0 Hz) |
| H-2a | 2.23[a] | 2.28 (1H, ddd, $J$=12.5, 4.0, 2.0 Hz) | 2.28 (1H, ddd, $J$=12.0, 4.0, 2.0 Hz) | 2.28[a] | 2.29[a] |
| H-2b | 1.60 (1H, dt, $J$=9.5, 12.5 Hz) | 1.55 (1H, dt, $J$=9.5, 12.5 Hz) | 1.55 (1H, dt, $J$=9.5, 12.0 Hz) | 1.55 (1H, dt, $J$=9.5, 12.5 Hz) | 1.52 (1H, dt, $J$=9.5, 12.5 Hz) |
| H-3 | 3.58[a] | 3.35[a] | 3.36[a] | 3.36[a] | 3.35[a] |
| H-4 | 2.97 (1H, t, $J$=9.5 Hz) | 3.18 (1H, t, $J$=9.5 Hz) | 3.17 (1H, t, $J$=9.5 Hz) | 3.18 (1H, t, $J$=9.5 Hz) | 3.15 (1H, t, $J$=9.5 Hz) |
| H-5 | 3.32 (1H, dq, $J$=9.5, 6.5 Hz) | 3.32 (1H, dq, $J$=9.5, 6.5 Hz) | 3.32 (1H, dq, $J$=9.5, 6.5 Hz) | 3.33 (1H, dq, $J$=9.5, 6.5 Hz) | 3.35 (1H, dq, $J$=9.5, 6.5 Hz) |
| H-6 | 1.26 (3H, d, $J$=6.5 Hz) | 1.28 (1H, d, $J$=6.5 Hz) | 1.28 (3H, d, $J$=6.5 Hz) | 1.28 (3H, d, $J$=6.5 Hz) | 1.29 (3H, d, $J$=6.5 Hz) |
| OMe | — | 3.41 (3H, s) | 3.40 (3H, s) | 3.40 (3H, s) | 3.42 (3H, s) |
| | Digito. | Digito. | Cym. | Cym. | Ole. |
| H-1 | 4.83 (1H, dd, $J$=9.5, 2.0 Hz) | 5.00 (1H, dd, $J$=9.5, 2.0 Hz) | 4.94 (1H, dd, $J$=9.5, 2.0 Hz) | 4.95 (1H, dd, $J$=9.5, 2.0 Hz) | 4.64 (1H, dd, $J$=9.5, 2.0 Hz) |
| H-2a | 2.18 (1H, ddd, $J$=13.0, 3.0, 2.0 Hz) | 2.12 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.13 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.13 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.25 (1H, ddd, $J$=12.5, 3.0, 2.0 Hz) |
| H-2b | 1.70[a] | 1.68[a] | 1.55 (1H, ddd, $J$=14.0, 9.5, 3.0 Hz) | 1.55[a] | 1.48 (1H, dt, $J$=9.5, 12.5 Hz) |
| H-3 | 4.25 (1H, q, $J$=3.0 Hz) | 4.24 (1H, q, $J$=3.0 Hz) | 3.81 (1H, q, $J$=3.0 Hz) | 3.81 (1H, q, $J$=3.0 Hz) | 3.35[a] |
| H-4 | 3.23 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.22 (1H, dd, $J$=9.5, 3.0 Hz) | 3.23 (1H, dd, $J$=9.5, 3.0 Hz) | 3.17 (1H, t, $J$=9.5 Hz) |
| H-5 | 3.92 (1H, dq, $J$=9.5, 6.5 Hz) | 3.82 (1H, dq, $J$=9.5, 6.5 Hz) | 3.88 (1H, dq, $J$=9.5, 6.5 Hz) | 3.90 (1H, dq, $J$=9.5, 6.5 Hz) | 3.32 (1H, dq, $J$=9.5, 6.5 Hz) |
| H-6 | 1.28 (3H, d, $J$=6.5 Hz) | 1.26 (3H, d, $J$=6.5 Hz) | 1.24 (3H, d, $J$=6.5 Hz) | 1.25 (3H, d, $J$=6.5 Hz) | 1.32 (3H, d, $J$=6.5 Hz) |
| OMe | — | — | 3.44 (3H, s) | 3.45 (3H, s) | 3.40 (3H, s) |
| | Cym. | Cym. | Cym. | Ole. | Cym. |
| H-1 | 4.75 (1H, dd, $J$=9.5, 2.0 Hz) | 4.74 (1H, dd, $J$=9.5, 2.0 Hz) | 4.68 (1H, dd, $J$=9.5, 2.0 Hz) | 4.50 (1H, dd, $J$=9.5, 2.0 Hz) | 4.87 (1H, dd, $J$=9.5, 2.0 Hz) |
| H-2a | 2.23[a] | 2.24 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.25 (1H, ddd, $J$=14.0, 3.0, 2.0 Hz) | 2.33 (1H, ddd, $J$=12.5, 4.5, 2.0 Hz) | 2.25[a] |
| H-2b | 1.61[a] | 1.61 (1H, ddd, $J$=14.0, 9.5, 3.0 Hz) | 1.63 (1H, ddd, $J$=14.0, 9.5, 3.0 Hz) | 1.50 (1H, dt, $J$=9.5, 12.5 Hz) | 1.56 (1H, ddd, $J$=14.0, 9.5, 3.0 Hz) |
| H-3 | 3.64 (1H, q, $J$=3.0 Hz) | 3.63 (1H, q, $J$=3.0 Hz) | 3.62 (1H, q, $J$=3 Hz) | 3.17[a] | 3.63 (1H, q, $J$=3.0 Hz) |
| H-4 | 3.18 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) | 3.21[a] | 3.13 (1H, t, $J$=9.5 Hz) | 3.21 (1H, dd, $J$=9.5, 3.0 Hz) |
| H-5 | 3.62 (1H, dq, $J$=9.5, 6.5 Hz) | 3.62 (1H, dq, $J$=9.5, 6.5 Hz) | 3.56 (1H, dq, $J$=9.5, 6.5 Hz) | 3.29 (1H, dq, $J$=9.5, 6.5 Hz) | 3.60 (1H, dq, $J$=9.5, 6.5 Hz) |
| H-6 | 1.27 (3H, d, $J$=6.5 Hz) | 1.26 (3H, q, $J$=6.5 Hz) | 1.27 (3H, d, $J$=6.5 Hz) | 1.32 (3H, d, $J$=6.5 Hz) | 1.30 (3H, d, $J$=6.5 Hz) |
| OMe | 3.44 (3H, s) | 3.45 (3H, s) | 3.43 (3H, s) | 3.39 (3H, s) | 3.43 (3H, s) |

Run at 500 MHz in $CDCl_3$ solution. Signal assignments were done based on the consequences of 2D-NMR (C–H COSY and/or HMBC and/or long range C–H COSY) spectra and the decoupling experiments. *a*) Overlapping with other signals.

H-4 one of the above oleandropyranose [$\delta$ 3.18 (1H, t, $J$=9.5 Hz)], and between the H-1 signal of another oleandropyranose [$\delta$ 4.50 (1H, dd, $J$=9.5, 2.0 Hz)] and the H-4 one of cymaropyranose [$\delta$ 3.23 (1H, dd, $J$=9.5, 3.0 Hz)]. Thus, the structure of **5** was determined to be lineolon 3-*O*-$\beta$-oleandropyranosyl-(1→4)-$\beta$-cymaropyranosyl-(1→4)-$\beta$-oleandropyranosyl-(1→4)-$\beta$-dioxopyranoside.

Compound **6** showed similar $^1$H- and $^{13}$C-NMR spectra to those of **4**, and was confirmed by acid hydrolysis to have one digitoxopyranose, one cymaropyranose and two oleandropyranoses in the sugar moiety. From the results of the C–H COSY and the HMBC spectra, each anomeric carbon and proton signal was assigned as shown in Tables II and III. In the HMBC spectrum, the anomeric carbon signals of the digitoxopyranose [$\delta$ 95.8] and one oleandropyranose [$\delta$ 100.2] showed $^3J_{COCH}$s as did **5**, but the H-1 signal of another oleandropyranose [$\delta$ 4.64 (1H, dd, $J$=9.5, 2.0 Hz)] exhibited a $^3J_{COCH}$ with the C-4 signal [$\delta$ 82.2] of oleandropyranose which was attached to the C-4 position of digitoxopyranose. The C-1 signal of cymaropyranose [$\delta$ 98.2] showed a $^3J_{COCH}$ with the H-4 of oleandropyranose [$\delta$ 3.17 (1H, t, $J$=9.5 Hz)]. And in the difference NOE spectra, irradiation at the anomeric proton signals of the digitoxopyranose [$\delta$ 4.93 (1H, dd, $J$=9.5, 2.0 Hz)] and one oleandropyranose [$\delta$ 4.50 (1H, dd, $J$=9.5, 2.0 Hz)] showed NOEs similar to **5**, but NOEs were confirmed between the H-1 signal of another oleandropyranose [$\delta$ 4.64 (1H, dd, $J$=9.5, 2.0 Hz)] and the H-4 one of oleandropyranose [$\delta$ 3.15 (1H, t, $J$=9.5 Hz)], which was attached to the C-4 position of digitoxopyranose, and between the H-1 one of cymaropyranose [$\delta$ 4.87 (1H, dd, $J$=9.5, 2.0 Hz)] and the H-4 signal of oleandropyranose [$\delta$ 3.17 (1H, t, $J$=9.5 Hz)]. Consequently, the structure of **6** was determined to be lineolon 3-*O*-$\beta$-cymaropyranosyl-(1→4)-$\beta$-oleandropyranosyl-(1→4)-$\beta$-oleandropyranosyl-(1→4)-$\beta$-digitoxopyranoside.

Finally, the absolute configuration of each monosaccharide was not determined in all compounds.

## Experimental

Optical rotations were determined with a JASCO-360 digital polarimeter. FAB-MS spectra were taken on a JEOL JMS-SX102 spectrometer. $^1$H- and $^{13}$C-NMR were recorded on a JEOL GSX-500 (500 and 125.65 MHz, respectively) and JEOL GSX-270 (270 and 67.80 MHz, respectively) spectrometers. Chemical shifts were given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard. GC was run on a Hitachi G-3000 gas chromatograph. HPLC was run on a JASCO system 800 instrument.

**Isolation** The air dried whole plant of *A. fruticosa* L. (7.4 kg) was extracted twice 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 $H_2O$ layer was passed through a Mitsubishi Diaion HP-20 column, and the absorbed material was eluted with 50% MeOH in water and MeOH. The MeOH eluate was concentrated under reduced pressure. The residue (21.8 g) was rechromatographed on a silica gel column with a $CHCl_3$–MeOH system and semi-preparative HPLC [ODS: $CH_3CN$–$H_2O$ and MeOH–$H_2O$ systems] to give compounds **2** (12 mg), **3** (21 mg), **4** (15 mg), **5** (12 mg) and **6** (4 mg).

**Compound 2** Amorphous powder. $[\alpha]_D^{22}$ +13.4° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{17}\cdot 5/2H_2O$: C, 58.52; H, 8.43. Found: C, 58.82; H, 8.42. FAB-MS $m/z$: 921 $[M+Na]^+$. $^1$H- and $^{13}$C-NMR: Tables I—III.

**Compound 3** Amorphous powder. $[\alpha]_D^{22}$ +8.9° ($c$=0.95, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{17}\cdot 2H_2O$: C, 59.48; H, 8.50. Found: C, 59.50; H, 8.55. FAB-MS $m/z$: 935 $[M+Na]^+$. $^1$H- and $^{13}$C-NMR: Tables I—III.

**Compound 4** Amorphous powder. $[\alpha]_D^{22}$ +16.0° ($c$=1.2, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot H_2O$: C, 60.10; H, 8.53. Found: C, 60.10; H, 8.68. FAB-MS $m/z$: 949 $[M+Na]^+$. $^1$H- and $^{13}$C-NMR: Tables I—III.

**Compound 5** Amorphous powder. $[\alpha]_D^{22}$ −3.3° ($c$=0.80, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot 5/2H_2O$: C, 59.30; H, 8.61. Found: C, 59.48; H, 8.58. FAB-MS $m/z$: 949 $[M+Na]^+$. $^1$H- and $^{13}$C-NMR: Tables I—III.

**Compound 6** Amorphous powder. $[\alpha]_D^{22}$ 0° ($c$=0.40, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot 5/2H_2O$: C, 59.30; H, 8.61. Found: C, 59.34; H, 8.34. FAB-MS $m/z$: 949 $[M+Na]^+$. $^1$H- and $^{13}$C-NMR: Tables I—III.

**Degradation of the Crude Pregnane Glycoside** The MeOH eluate on the Diaion HP-20 column (see isolation) was chromatographed on a silica gel column, and the fraction eluted with $CHCl_3$–MeOH (93–7) on this silica gel column was rechromatographed on semi-preparative HPLC (ODS: gradient $CH_3CN$–$H_2O$ system). The crude pregnane glycoside fraction was eluted with 29—31% $CH_3CN$ on this preparative HPLC. This crude pregnane glycoside fraction (10 mg) was dissolved in dioxane (1 ml) and 0.2 N $H_2SO_4$ (5 drops), and the solution was heated at 60 °C for 90 min. After the addition of $H_2O$ (1 ml) to this reaction mixture, dioxane was removed by blowing air in a hot water bath for a short time. Then, this solution was heated at 60 °C for 90 min once more. After hydrolysis, this solution was passed through a Mitsubishi Diaion HP-20 column and the absorbed material was eluted with MeOH. The MeOH eluate was concentrated to dryness and the residue was recrystallized with MeOH to give pure lineolon (**1**) (1 mg), mp 237—244 °C. $[\alpha]_D^{22}$ +12.7° ($c$=0.38, MeOH) (lit. mp 233—239 °C, $[\alpha]_D$ +13°; MeOH).[2] $^{13}$C-NMR: Table I. $^1$H-NMR [$(CD_3)_2$ SO] $\delta$: 1.07 (3H, s) (H-19), 1.28 (3H, s) (H-18), 2.10 (3H, s) (H-21), 3.34 (1H, m) (H-3), 3.30[a] (H-12 or 17), 5.19 (1H, br s) (H-6). *a*) Overlapping with other signals.

**Acid Hydrolysis of Compounds 2—6** Compounds **2**—**6** (*ca.* 0.1 mg) dissolved in dioxane (4 drops) and 0.2 N $H_2SO_4$ (1 drop) were heated at 60 °C for 90 min. After the addition of $H_2O$ (3 drops) in this reaction mixture, dioxane was removed by blowing air in a hot water bath for a short time. Then, this solution was heated at 60 °C for 90 min once more. After hydrolysis, this solution was passed through a Mitsubishi Diaion HP-20 column and eluted with $H_2O$ and MeOH. The MeOH eluate was concentrated to dryness and the residual aglycone was identified by HPLC with an authentic sample. (Conditions: column ODS; flow rate 1.0 ml/min; 40% MeOH in water; $t_R$ lineolon 9.4 min). For sugar analysis, the $H_2O$ eluate was passed through an Amberlite IR-60E column and the eluate was concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with MeOH, and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From each glycoside, cymaritol acetate, oleandritol acetate, digitoxitol acetate and olivitol acetate were detected by GC. [Conditions: column Supelco SP-2380 capillary column (0.25 mm × 30 m); column temperature 200 °C; carrier gas $N_2$; $t_R$ cymaritol acetate (6.8 min), oleandritol acetate (7.6 min), digitoxitol acetate (9.6 min), olivitol acetate (10.8 min)]. The relative ratio of each monosaccharide was determined based on the peak area.

**Acknowledgement** We thank the staff of the Central Analytical Laboratory of this university for elemental analysis and measurement of MS, and wish to acknowledge the technical support and discussion of Dr. Toshio Miyase.

## References and Notes

1) T. Yamagishi, K. Hayashi, H. Mitsuhashi, M. Imanari, K. Matsushita, *Tetrahedron Lett.*, **1973**, 3527.
2) D. Deepak, A. Khare, M. P. Khare, *Phytochemistry*, **28**, 3255 (1989).