Chem. Pharm. Bull.
31(4)1199—1206(1983)

Affinosides S-I—S-VIII, Cardenolide Glycosides with a Usual Glycosidic Linkage, from *Anodendron affine* (Anodendron. IV)[1]

FUMIKO ABE and TATSUO YAMAUCHI*

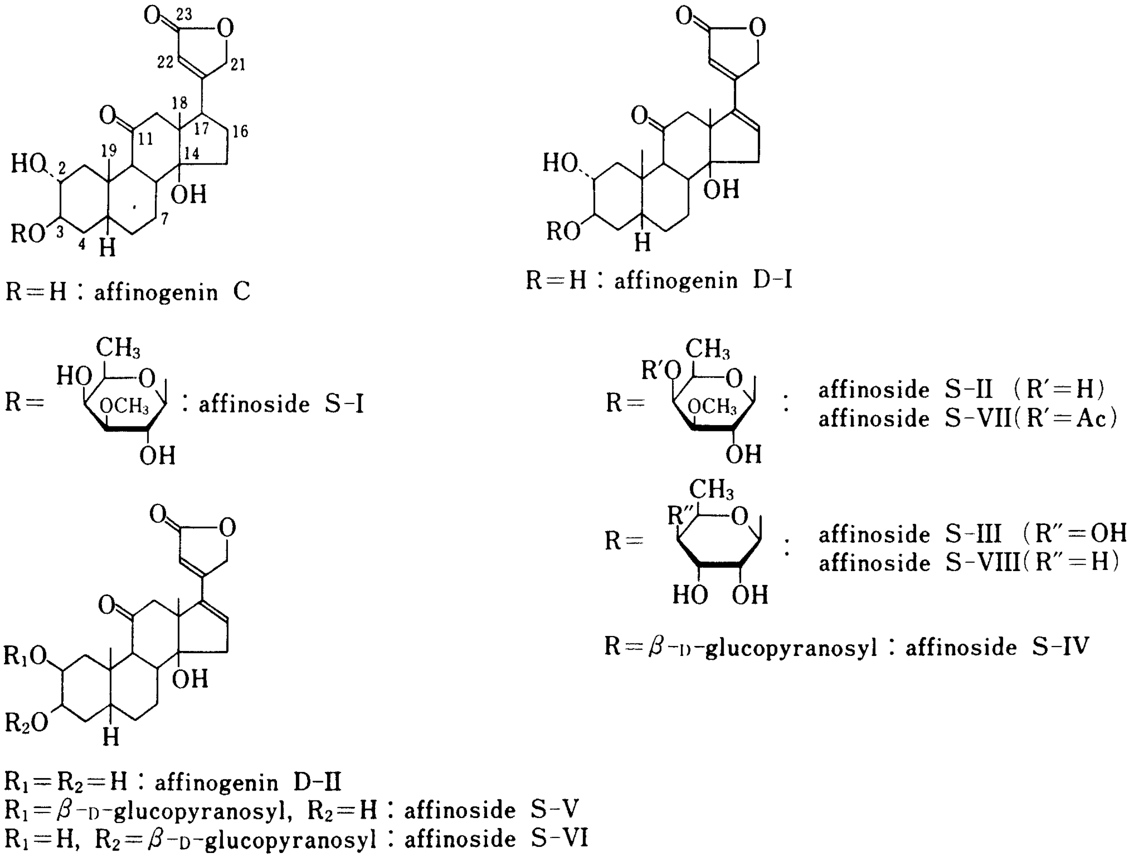
Faculty of Pharmaceutical Sciences, Fukuoka University,
8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-01, Japan

(Received September 22, 1982)

Affinosides S-I—S-VIII were isolated from the caules and leaves of *Anodendron affine* DRUCE, and identified as glycosides of affinogenin C, D-I, and D-II, each having a single glycosidic linkage with D-digitalose, 6-deoxy-D-gulose, 4,6-dideoxy-D-gulose, or D-glucose.

Keywords——*Anodendron*; Apocynaceae; affinoside; affinogenin; cardiac glycoside; 2,3,14-trihydroxy-11-oxo-cardenolide glycoside; 6-deoxyguloside; 4,6-dideoxyguloside; 2-*O*-glucosylcardenolide

Many cardenolide glycosides having unusual double linkages between the aglycone and a deoxy-hexosulose[2,4] (or deoxy-pentosulose)[3] moiety have been found from plants belonging to the genera *Asclepias*, *Calotropis*, *Pergularia* (Asclepiadaceae),[2] *Elaeodendron* (Celastraceae)[3] and *Anodendron* (Apocynaceae).[4] In the preceding papers of this series, we have described affinosides A—J, cardenolide glycosides of the unusual type,[4b] and affinogenins C and D-I—V, cardenolides free from the component sugar, from *Anodendron affine* DRUCE.[1] This paper deals with the structures of affinosides S-I—VIII (I—VIII), glycosides with a usual glycosidic linkage.



R=H : affinogenin C

R=H : affinogenin D-I

R= : affinoside S-I

R= : affinoside S-II (R'=H)
affinoside S-VII (R'=Ac)

R= : affinoside S-III (R''=OH)
affinoside S-VIII (R''=H)

R=β-D-glucopyranosyl : affinoside S-IV

$R_1=R_2=H$: affinogenin D-II
$R_1=\beta$-D-glucopyranosyl, $R_2=H$: affinoside S-V
$R_1=H$, $R_2=\beta$-D-glucopyranosyl : affinoside S-VI

Chart 1

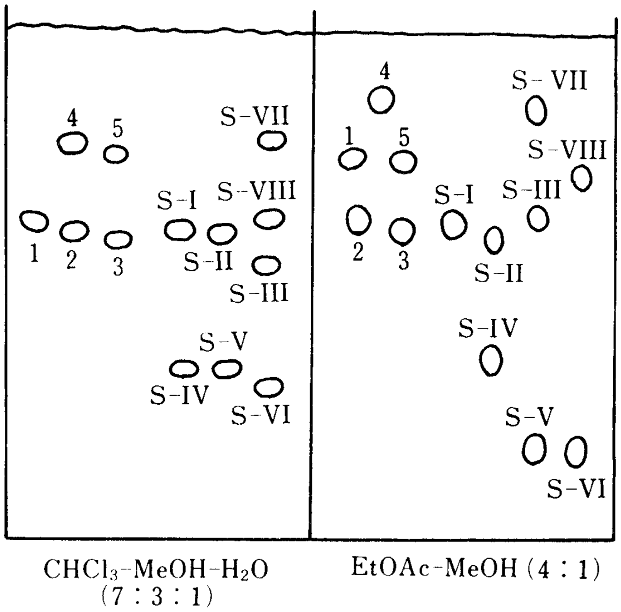


Fig. 1. Thin Layer Chromatograms of Affinosides S-I—VIII and Affinogenin D-I—V on Silica Gel Plates

1: affinogenin D-I 2: affinogenin D-II 3: affinogenin D-III
4: affinogenin D-IV 5: affinogenin D-V

Compounds I and II were obtained as crystals from the leaves and caules, respectively, following the affinogenins on column chromatography, as mentioned in the preceding paper.[1] In the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra of I and II, the signals due to the sugar moieties were superimposable on each other, being identical with those of D-digitalopyranoside (*e.g.* odoroside H).[5] In the ^{1}H-NMR spectrum, II showed a triplet at δ 6.18 due to a 16-olefinic proton, together with 18- and 19-methyl, 21-methylene, and 22-olefinic protons (these appeared in both I and II). From the ^{13}C-NMR spectra, the aglycone moieties of I and II were considered to be affinogenin C (Cg) and affinogenin D-I (Dg-I), respectively, (Chart 1) based on the glycosylation shifts of the carbinyl carbon at C-3 and of the neighboring C-2 and C-4[6] caused by the 3-*O*-digitalosyl residue ($\delta_{\text{I-Cg}}$: +6.7 ppm at C-3, −0.6 ppm at C-2, −3.0 ppm at C-4; $\delta_{\text{II-Dg-I}}$: +6.6 ppm at C-3, −0.7 ppm at C-2, −3.5 ppm at C-4). Upon hydrolysis of I and II with acid, the aglycones and sugars were identified by comparison with authentic samples. Since the D-form of digitalose has been found in the cardiac glycosides of Apocynaceae, the sugars in I and II are tentatively regarded as being D-digitalose. The anomeric protons were both observed at δ 4.80 with coupling constants of 8 Hz, suggesting the glycosidic linkages to be β. Thus, the structures of I and II were established to be 3-*O*-(β-D-digitalopyranosyl)-2α,3β,14-trihydroxy-11-oxo-5β,14β-card-20(22)-enolide and its Δ^{16}-derivative, respectively (Chart 1).

Compound VII was obtained from the extract of the caules after affinogenin D-V (3α, 14-dihydroxy-2,11-dioxo-5β,14β-carda-16,20(22)-dienolide) on the column chromatography.[1] One acetyl residue was apparent from the ^{1}H-NMR spectrum (δ 2.00) in addition to 6′-methyl protons, 3′-*O*-methyl protons and resonances due to the aglycone moiety. Since the ^{13}C-NMR spectrum due to the aglycone moiety was almost superimposable on that of II, the acetyl group seemed to be linked to the sugar moiety. Although alkaline deacetylation of VII was unsuccessful, a peracetate of VII (VII-1) was identified as II-triacetate (II-1). Consequently, VII was considered to be the 2′- or 4′-acetate of II. In a comparison of the ^{1}H-NMR spectrum of VII with those of 2′-*O*-acetyl and 4′-*O*-acetyl odoroside H (digitoxigenin β-D-digitalopyranoside), a peak of doublet of doublets, corresponding to 4′-H of 4′-*O*-acetyl odoroside H at δ 5.28 (in $CDCl_3$), was observed at δ 5.54 with coupling constants of 3 and 1 Hz. In a ^{13}C-NMR comparison of VII with 4′-*O*-acetyl odoroside H, the signals due to the sugar moieties of the two samples were in good agreement. VII was therefore considered to be the 4′-acetate of II.

Compound III was isolated as a solid from the fraction following affinogenin D-III (2β,3α, 14-trihydroxy-5β,14β-carda-16,20(22)-dienolide. Compound III afforded a tetraacetate (III-1) upon acetylation with Ac_2O and pyridine, and was hydrolyzed to Dg-I and a sugar. In the ^{1}H-NMR spectrum of III, a doublet of one proton at δ 5.37 with a coupling constant of 8 Hz was ascribed to the anomeric proton. The glycosidic linkage was therefore considered to be β, and the 2′-OH, α. With 1% hydrochloric acid in acetone, III formed an acetonide (III-2), which was further acetylated with Ac_2O and pyridine to yield a diacetate (III-3). The peaks of 2′-, 3′-, 4′-, and 5′-H in III-2 and III-3 were assigned by decoupling experiments based on the peaks of the anomeric and 6′-methyl protons. In the ^1H-NMR spectra of III-2 and III-3,

TABLE I. ^1H Chemical Shifts of Affinosides and Their Derivatives[a]

	18,19-Me	21-CH_2	22-H	2,3-H	16-H	1′-H	2′-H	3′-H	4′-H	5′-H	6′-CH	Others
I[b]	1.03 1.21	4.97 5.22 (dd, 17, 2)	6.10 (t, 2)	4.50 (2H, bs)		4.80 (d, 8)	4.37 (dd, 8, 9)	3.48 (dd, 9, 3)		3.74 (dq, 1, 6)	1.50 (d,6)	3.57 (3′-OMe)
II[b]	1.23 1.55	4.78 5.03 (dd, 16, 2)	6.38 (bs)	4.47 (2H, bs)	6.18 (t, 2)	4.80 (d, 8)	4.34 (dd, 8, 9)	3.47 (dd, 9, 3)		3.74 (dq, 1, 6)	1.49 (d, 6)	3.57 (3′-OMe)
II-1	1.11 1.30	4.92 (2H, d, 2)	5.97 (bs)	3.79 (bs,3-H) 5.10 (m, 2-H)	6.14 (bs)	4.42 (d,8)	5.10 (m)		5.30 (bd,3)	3.68 (q, 6)	1.20 (d, 6)	3.34 (3′-OMe) 1.88, 2.07, 2.18 (-OAc)
VII[b]	1.23 1.56	4.78 5.02 (d, 16)	6.40 (bs)	4.50 (2H, bs)	6.16 (bs)	4.85 (d, 8)	4.23 (dd, 8, 10)		5.54 (dd, 3, 1)		1.26 (d, 6)	3.51 (3′-OMe) 2.00 (-OAc)
H-2′-OAc[c]						4.37 (d, 8)	5.06 (dd, 8, 9)	3.30 (dd, 9, 3)	3.87 (dd, 3, 1)	3.56 (dq, 1, 6)	1.34 (d, 6)	3.43 (3′-OMe) 2.04 (-OAc)
H-4′-OAc						4.30 (d, 8)	3.65 (dd, 8, 9)	3.24 (dd, 9, 3)	5.28 (dd, 3, 1)	3.67 (dq, 1, 6)	1.18 (d,6)	3.42 (3′-OMe) 2.11 (-OAc)
III[b]	1.24 1.54	4.77 5.03 (dd,16,2)	6.37 (bs)		6.17 (bs)	5.37 (d,8)			4.73 (t, 3)		1.48 (d,6)	
III-1	1.11 1.29	4.91 (2H, bs)	5.97 (bs)	3.82 (bs, 3-H) 5.10 (bs, 2-H)	6.13 (t,3)	4.87 (d,8)		4.77 (dd, 5, 2)	5.31 (t, 2)	4.10 (dq, 2, 6)	1.14 (d, 6)	1.88, 1.99, 2.12 2.16 (-OAc)
III-2	1.06 1.48	4.91 (2H, bs)	5.93 (bs)	4.94 (2H, bs)	6.09 (bs)	4.70[d] (d,5)	4.05[e] (dd, 5, 6)	4.36[f] (dd, 6, 2)		3.70[g] (dq, 2, 6)	1.26 (d,6)	1.36 (6H, (Me)$_2$C<)
III-3	1.12 1.49	4.88 (2H,bs)	5.93 (bs)	3.83 (bs, 3-H) 5.08 (bs, 2-H)	6.13 (t, 3)	4.48[h] (d, 6)	3.90[i] (dd, 6, 5)	4.15 (dd, 5, 2)	5.01[j] (t, 2)	3.87[k] (dq, 2, 6)	1.18[l] (d, 6)	1.31, 1.36 ((Me)$_2$C<) 1.87, 2.15 (-OAc)
VIII[b]	1.27 1.56	4.81 5.05 (dd, 16, 2)	6.39 (t, 2)	4.50 (2H, bs)	6.17 (bs)	5.30 (d, 8)					1.21 (d, 6)	
VIII-1	1.09 1.29	4.90 (2H,bs)	5.96 (bs)	3.80 (bs, 3-H) 5.09 (bs, 2-H)	6.13 (bs)			5.38 (m)			1.19 (d,6)	1.87, 1.98, 2.08 (-OAc)
VIII-2	1.04 1.21	4.90 (2H, bs)	5.94 (bs)		6.08 (t, 2)	4.50 (d, 7)		4.39 (m)			1.19 (d,6)	1.33 (6H, (Me)$_2$C<)
VIII-3	1.10 1.23	4.87 (2H, bs)	5.89 (bs)	3.83 (m, 3-H)[m] 5.08 (bd, 2, 2-H)	6.08 (bs)	4.54[n] (d, 7)	3.83[m] (m)	4.40[o] (m)		3.83[m] (m)	1.20 (d, 6)	1.28, 1.32 ((Me)$_2$C<) 1.89 (-OAc)
VIII-4	1.07 1.37	4.93 (2H, bs)	5.97 (bs)	3.90 (bs) 4.00 (bs)	6.10 (t, 2)	4.69 (d, 8)	3.00 (dd, 8, 3)	4.19 (m)			1.17 (d, 6)	3.51(2′-OMe)
VIII-5	1.25 1.31	4.93 (2H, bs)	5.95 (bs)	3.80 (bs, 3-H) 5.12 (bs, 2-H)	6.15 (bs)	4.65[p] (d, 8)	3.02[q] (dd, 8, 3)	5.38[r] (bd, 3)			1.19 (d, 6)	1.89, 2.10 (-OAc) 3.43(2′-OMe)
IV[b]	1.24 1.54	4.79 5.04 (dd, 16, 2)	6.37 (t, 2)		6.19 (bs)	4.99 (d, 8)						
V[b]	1.27 1.57	4.82 5.05 (d, 16)	6.38 (bs)		6.20 (bs)	5.06 (d, 8)						
VI[b]	1.16 1.57	4.79 5.04 (dd, 16, 2)	6.42 (t, 2)		6.23 (bs)	4.98 (d, 8)						

a) δ(ppm) in $CDCl_3$, unless otherwise mentioned (*J*/Hz values in parentheses).
b) Dissolved in pyridine-d_5.
c) H=odoroside H (=digitoxigenin β-D-digitaloside); signals due to the aglycone moiety are omitted.
d) s by irradiation of 2′-H.
e) d of 6Hz by irradiation of 1′-H.
f) d of 2Hz by irradiation of 2′-H.
g) d of 2Hz by irradiation of 6′-CH_3.
h) s by irradiation of 2′-H.
i) d of 5Hz by irradiation of 1′-H.
j) d of 2Hz by irradiation of 3′-H.
k) q of 6Hz by irradiation of 4′-H.
l) s by irradiation of 5′-H.
m) bd by irradiation of 1′-H.
n) s by irradiation of 2′-H.
o) bs by irradiation of 2′-H.
p) s by irradiation of 2′-H.
q) d of 3Hz by irradiation of 1′-H, d of 8Hz by irradiation of 3′-H.
r) bs by irradiation of 2′-H.

TABLE II. ^{13}C Chemical Shifts of I, II and VII, and Their Aglycones, δ (ppm) from TMS in pyridine-d_5

	I	Cg[a]	II	VII	Dg-I[b]	H-2′-OAc[c]	H-4′-OAc[c]
C-1	37.3	37.0	37.3	37.3	37.3[d]		
C-2	69.9	70.5	69.7	69.6	70.4		
C-3	78.4	71.7	78.2	78.6	71.6		
C-4	27.2[d]	30.2	26.7[d]	26.9	30.2		
C-5	37.3	37.4	37.3	37.3	37.5[d]		
C-6	27.2[d]	27.1	26.9[d]	26.9	27.1		
C-7	23.1	23.1	22.9	22.9	23.0		
C-8	43.3	43.3	43.0	43.1	43.0		
C-9	50.4[e]	50.3	50.1	50.1	50.2		
C-10	35.3	35.0	35.2	35.3	35.7		
C-11	211.2	211.4	211.5	211.5	211.9		
C-12	55.6	55.5	52.8	52.8	52.8		
C-13	53.4	53.4	57.1	57.1	57.1		
C-14	83.7	83.6	83.5	83.6	83.6		
C-15	33.6	33.6	41.4	41.4	41.5		
C-16	26.8[d]	27.1	135.2	135.2	135.2		
C-17	50.6[e]	50.5	142.3	142.6	142.2		
C-18	17.6	17.6	18.4	18.5	18.6		
C-19	23.9	24.3	23.8	23.8	24.2		
C-20	174.1	174.0	158.6	158.6	158.7		
C-21	73.6	73.6	71.8	71.9	71.9		
C-22	118.0	118.0	112.4	112.4	112.4		
C-23	174.2	174.2	174.2	174.5	174.5		
C-1′	103.7		103.7	103.5		99.7	103.3
C-2′	70.7		70.7	71.0		71.9	71.0
C-3′	85.0		84.9	82.9		82.2	82.9
C-4′	68.6		68.5	70.1		68.0	70.1
C-5′	71.1		71.1	69.3		71.2	69.1
C-6′	17.4		17.4	16.9		17.3	17.0
3′-OMe	57.2		57.1	58.2		56.5	58.1
-OAc				20.7		21.2	20.7
				170.7		169.6	170.7

a) Cg=affinogenin C.
b) Dg-I=affinogenin D-I.
c) H=odoroside H(=digitoxigenin β-D-digitaloside); signals due to the aglycone moiety are omitted.
d,e) Signal assignments marked *d*) or *e*) in each column may be reversed.

2′-H was observed at similar chemical shifts, δ 4.05 in III-2 and δ 3.90 in III-3, suggesting that the isopropylidene residue is attached at the 2′- and 3′-hydroxyl groups, and one of the two acetyl residues in III-3 is linked to the 4′-hydroxyl group. The 2′- and 3′- hydroxyl groups were tentatively considered to be *cis* on the bases of the ease of acetal formation, and the coupling constants between 3′-H and 4′-H, and 4′-H and 5′-H were both 2 Hz, suggesting that the 3′- and 4′-hydroxyl groups retain α and β configuration, respectively, if the component sugar takes the C1 conformation.

The evidence from the ^{1}H-NMR spectra suggests the component sugar to be 6-deoxygulose (antiarose) among 6-deoxyhexoses so far obtained in nature, and this was finally confirmed by paper chromatographic (PC)[7] and thin layer chromatographic (TLC)[8] comparisons with authentic 6-deoxy-D-gulose. Since the ^{13}C-NMR peaks due to the aglycone moiety of III were in good agreement with those of II, the sugar moiety is linked to the 3-hydroxyl group of Dg-I. Since only 6-deoxy-D-gulose has been found in nature, the component sugar of III is considered to belong to the D-series. The structure of III was thus established to be 3-*O*-(β-6-deoxy-D-gulopyranosyl)-affinogenin D-I.

Compound VIII shows similar polarity to Dg-I and is less polar than III on TLC and

TABLE III. ^{13}C Shifts of III—VI and VIII, and Their Derivatives, δ (ppm) from TMS in pyridine-d_5

	III	III-1	III-2	III-3	VIII	VIII-1	VIII-4	IV	V	VI	Dg-II[a]
C-1	37.3	34.1	37.3	34.1	37.4	34.1	37.4	37.4	36.6	40.0	39.0
C-2	69.9	71.6	69.7	69.7	69.8	71.7	69.7	69.8	74.9	67.2	67.9
C-3	78.1	75.7	79.1	75.8	78.0	75.2	77.3	78.4	66.5	74.6	70.1
C-4	26.9	28.1	27.0	27.3	26.8	28.0	26.8[b]	26.8[b]	32.5	31.8	33.7
C-5	37.3	37.0	37.3	36.6	37.4	36.9	37.6	37.4	37.0	36.7	36.6
C-6	26.9	26.9	27.0	26.9	26.8	26.9	26.4[b]	26.9[b]	26.3	26.2	26.5
C-7	22.9	22.2	23.0	22.1	22.9	22.1	22.8	22.9	22.0	22.0	22.1
C-8	42.9	42.9	43.1	42.9	43.0	42.9	42.9	43.0	43.1	43.0	43.1
C-9	50.1	49.4	50.1	49.3	50.1	49.4	50.0	50.2	48.5	48.4	48.6
C-10	35.3	35.7	35.4	35.7	35.2	35.7	35.2	35.3	37.8	37.8	38.9
C-11	211.8	212.0	211.5	211.9	211.5	211.7	211.6	212.0	212.0	211.2	211.5
C-12	52.8	51.8	52.8	51.8	52.8	51.8	52.7	52.8	51.8	51.8	51.8
C-13	57.1	56.8	57.2	56.7	57.1	56.8	57.0	57.1	57.0	56.9	57.0
C-14	83.6	82.9	83.6	82.9	83.5	82.9	83.5	83.5	82.9	82.9	83.0
C-15	41.4	42.1	41.4	42.1	41.4	42.2	41.3	41.5	41.7	41.7	41.8
C-16	135.5	134.8	135.2	134.9	135.2	134.9	135.4	135.2	135.4	135.4	135.3
C-17	142.1	141.8	142.4	141.8	142.2	141.8	142.0	142.2	141.4	141.3	141.4
C-18	18.5	19.7	18.5	19.7	18.4	19.7	18.4	18.5	19.5	19.7	20.0
C-19	23.8	23.9	24.1	23.8	23.8	23.7	24.2	23.8	24.1	23.8	24.2
C-20	158.9	158.4	158.7	158.4	158.7	158.4	158.7	158.7	158.6	158.7	158.5
C-21	71.9	71.9	71.9	71.9	71.8	71.9	71.9	71.9	71.9	72.0	72.0
C-22	112.2	113.0	112.5	112.9	112.4	112.9	112.2	112.4	112.5	112.5	112.6
C-23	174.7	174.7	174.6	174.4	174.5	174.4	174.6	174.5	174.5	174.5	174.5
C-1′	101.2	98.5	101.4	101.5	100.8	93.4	99.2	103.4	101.4	103.6	
C-2′	73.6	70.8[b]	75.2	74.6[b]	72.1	71.3	81.7	75.2	75.3	74.9	
C-3′	74.0	69.0[b]	77.4	74.7[b]	69.0	69.0	66.3	78.6	78.5	78.6	
C-4′	69.7	69.0[b]	70.6	71.8	40.7	37.4	40.4	71.6	71.5	71.6	
C-5′	69.3	68.9[b]	68.6	68.9	66.5	67.2	66.3	78.4	78.5	78.3	
C-6′	16.9	16.1	16.8	16.1	21.4	21.4	21.2	62.7	62.6	62.5	
		20.3	26.4	26.3		20.9	58.5				
		20.7	28.4	28.1		20.9	(-OMe)				
		20.7	109.0	109.5		20.9					
		21.4	((Me)$_2$C<)	((Me)$_2$C<)		168.4					
		169.2		20.6		169.2					
		169.5		21.4		169.8					
		169.7		169.2		(-OAc)					
		169.9		170.2							
		(-OAc)		(-OAc)							

a) Dg-II=affinogenin D-II.
b) Signal assignments marked *b*) in each column may be reversed.

column chromatography. In the ^{1}H-NMR and ^{13}C-NMR spectra, the aglycone moiety was found to be Dg-I, so that the sugar moiety is linked to the 3-hydroxyl group. In the ^{1}H-NMR of VIII, 6′-methyl protons at δ 1.21 (d, J=6 Hz) and an anomeric proton at δ 5.30 (d, J=8 Hz), but no methoxyl protons, were seen. In the ^{13}C-NMR spectrum (Table III), four doublets (δ 66.5, 69.0, 72.1, and 100.8) due to the sugar moiety were found, two of them being ascribable to C-1′ and C-5′. Compound VIII afforded a triacetate (VIII-1) with Ac$_2$O and pyridine, and a monoacetonide (VIII-2) with 1% hydrochloric acid in acetone. Usual acetylation of VIII-2 provided a monoacetate (VIII-3). In VIII-2 and VIII-3, anomeric protons were clearly observed as a doublet with a coupling constant of 7 Hz at δ 4.50 and δ 4.54, respectively. In VIII-3, only a proton attached to the carbon bearing an acetoxyl residue was found at δ 5.08 as a doublet; this is assignable to the 2β-H of the aglycone by comparison with that of VIII-2, and therefore, the component sugar was considered to be a dideoxyhexopyranose. When the anomeric proton at δ 4.54 in VIII-3 was irradiated, a multiplet centered at δ 3.83, including 2′-, 5′-, and 3α-H, was transformed into an unbalanced broad doublet. On the other hand, irradiation of the signal at δ 3.83 transformed the peak at δ 4.40 ascribable to 3′-H, and that of the anomeric proton at δ 4.54, into a broad singlet and a singlet, respectively, showing the isopropylidene residue to be attached to the 2′- and 3′-hydroxyl groups. In order to verify the configuration of the 2′- and 3′-hydroxyl groups, partial methylation of the sugar moiety was attempted by the procedure of Aritomi *et al.*[9] With diazomethane and $SnCl_2$ in MeOH, VIII afforded a monomethylate (VIII-4), which was further acetylated to yield a diacetate of VIII-4 (VIII-5). In the ^{1}H-NMR spectrum of VIII-5, a doublet of doublets with coupling constants of 3 and 8 Hz was observed at δ 3.02, close to that of VIII-4 at δ 3.00. The irradiation of the peak at δ 3.02 caused the transformation of the anomeric proton signal and a broad doublet at δ 5.38 (J=3 Hz) into a singlet and a broad singlet, respectively. Consequently, the peak at δ 3.02 was assigned to 2′-H and that at δ 5.38 to 3′-H. The chemical shifts of 2′-H and 3′-H indicate that *O*-methylation occurred at 2′-OH. The 1′-H/2′-H and 2′H/3′-H are considered to retain ***trans-diaxial*** and ***cis*** relations, respectively. On the bases of the ^{1}H-NMR and ^{13}C-NMR spectra of VIII, and its derivatives, the sugar moiety of VIII was considered to be 4,6-dideoxygulose, which is bound to the 3-hydroxyl group with a β-linkage. Affinosides A—J, glycosides from this plant, each have a 4,6-dideoxy-3-*O*-methyl-D-gulosulose moiety, which is a 2-dehydro-3-*O*-methyl derivative of 4,6-dideoxy-D-gulose. The component sugar of VIII, therefore, seems to belong to the D-series.

Compounds IV, V, and VI were hydrolyzed with β-glucosidase to yield glucose as a component sugar, together with Dg-I from IV, and Dg-II from V and VI. In the ^{1}H-NMR spectra, the anomeric protons of the three glucosides were found at δ 4.99—5.06 as doublets (J=8 Hz), showing the glucosidic linkage to be β. Compound IV was found to be 3-*O*-glucoside of Dg-I, and V and VI were both considered to be the 2- or 3-glucoside of Dg-II on the bases of the ^{13}C-NMR spectra. In a ^{13}C-NMR comparison of V and VI with Dg-II, V was found to be the 2-*O*-glucoside and VI to be the 3-*O*-glucoside, since deshielding was observed at C-2 of V (+7.2 ppm) and C-3 of VI (+4.5 ppm), while C-1 of V and C-4 of VI were shielded by −2.4 ppm and −1.9 ppm, respectively. The structures of IV, V, and VI were hence established to be as represented in Chart 1.

The structures of eight cardenolides were elucidated. The isolation of VIII is of interest, since this is the first glycoside having a 4,6-dideoxyhexose as a component sugar to be isolated from the higher plants, although many other cardenolide glycosides with a 4,6-dideoxyhexosulose in the sugar moiety have been found.[1-4] Compound V is the only cardenolide glycoside having a component sugar attached to a carbinol other than C-3.

Experimental

Melting points were measured on a hot stage and are uncorrected. ^{1}H-NMR, ^{13}C-NMR, field desorption mass spectra (FD-MS), and ultraviolet absorption spectra (UV) were obtained with the same machines as described in the preceding paper.[1] NMR chemical shifts are given in δ values referred to internal tetramethylsilane, and the following abbreviations are used: s=singlet, d=doublet, dd=doublet of doublets, t=triplet, m=multiplet, bs=broad singlet.

The following solvent systems were used for silica gel column chromatography and TLC: solv. 1, benzene–acetone (5: 1—1: 1); solv. 2, hexane–EtOAc (1: 3—1: 5); solv. 3, EtOAc–MeOH (9: 1—4: 1); solv. 4, $CHCl_3$–MeOH–H_2O (7: 1: 5—7: 3: 1, bottom layer); solv. 5, same system as solv. 4 (5: 6: 4, for droplet countercurrent chromatography (DCCC)); solv. 6, EtOAc–MeOH–H_2O (7: 1: 7—2: 1: 2, upper layer); solv. 7, methyl ethyl ketone satd. with H_2O (for PC);[7] solv. 8, *n*-BuOH–EtOH (4: 1) satd. with borate buffer (for PC);[7] solv. 9, EtOAc–iso-PrOH–MeOH (7: 1.5: 1.5);[8] solv. 10, *n*-BuOH–AcOH–H_2O (4: 1: 5, upper layer, for PC). As a spray reagent, a) 1: 1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH (Kedde's reagent), b) 5% H_2SO_4, and c) aniline hydrogen phthalate reagent (for sugar) were used. When b) or c) was applied, the plate or paper was heated until the spots developed.

The samples for elementary analysis were dried at room temp. *in vacuo* over P_2O_5.

Isolation of Glycosides——Procedure and yields were presented in the preceding paper.[1]

Acetylation of Glycosides——Glycoside (20—50 mg) was dissolved in 1 ml each of pyridine and Ac_2O, and the mixture was allowed to stand for 24 h at room temp., then diluted with ice water, and extracted with $CHCl_3$. The $CHCl_3$ layer, after being washed with H_2O, was dried over Na_2SO_4, and the solvent was evaporated off *in vacuo*. The residue was chromatographed on a silica gel column with solv. 1. In most cases, the acetate was obtained as a solid.

Affinoside S-I (I)——I was crystallized from EtOAc–hexane to give prisms, mp 150—155°C, $[\alpha]_{\mathrm{D}}^{23}$ +9.1° (c=0.57, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 214 (13000), *Anal.* Calcd for $C_{30}H_{44}O_{10}\cdot 3/2H_2O$: C, 60.89; H, 8.01. Found: C, 60.95; H, 8.24. I-acetate (I-1) was obtained as a crystalline powder from MeOH–H_2O, mp 158—161°C, $[\alpha]_{\mathrm{D}}^{24}$ −8.0° (c=0.43, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 215 (18600).

Hydrolysis of I——I (5 mg) was dissolved in 2 ml of 1% HCl in acetone (3 ml of conc.HCl in 97 ml of acetone) and the solution was allowed to stand at room temp. for 20 h. The mixture was diluted with MeOH and deacidified with Amberlite IR-410. The methanolic solution was concentrated *in vacuo* and partitioned into n-BuOH/H_2O. The BuOH layer, was concentrated and the residue was examined by TLC; the *Rf* values were in good agreement with those of authentic Cg (solv. 1, 1: 1; solv. 4, 7: 2: 0.8). The sugar in the water layer was examined by PC (solv. 10: *Rf* 0.40) and TLC (solv. 4, 7: 3: 1); the *Rf* values were in good agreement with those of authentic D-digitalose.

Affinoside S-II (II)——II was crystallized from EtOAc–hexane to give prisms, mp 200—210°C, $[\alpha]_{\mathrm{D}}^{23}$ +30.0° (c=0.71, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 265 (16400), *Anal.* Calcd for $C_{30}H_{42}O_{10}\cdot H_2O$: C, 62.05; H, 7.64. Found: C, 61.64; H, 7.49. II-acetate (II-1) was crystallized from EtOAc–hexane to give needles, mp 270—272°C, $[\alpha]_{\mathrm{D}}^{24}$ −2.4° (c=0.17, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 264 (20000).

Hydrolysis of II——Hydrolysis was conducted in the same manner as for I with 1% HCl in acetone. The aglycone was compared with authentic Dg-I by TLC with solv. 1 (1: 1), solv. 4 (7: 2: 0.8), and solv. 3 (6: 1); the *Rf* values of two samples were in good agreement. The sugar moiety was subjected to PC with solv. 10 (*Rf* 0.40) and TLC with solv. 4 (7: 3: 1); the *Rf* values were in good agreement with those of authentic D-digitalose.

Affinoside S-VII (VII)——A solid, $[\alpha]_{\mathrm{D}}^{23}$ +29.4° (c=0.66, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 264 (18900), FD-MS m/z: 605 (M^++1, $C_{32}H_{44}O_{11}$). An aq. soln. of $KHCO_3$ (10 mg/2.5 ml) was added to a methanolic soln. of VII (10 mg/1 ml). The mixture was allowed to stand at room temp. for one month. During the first two weeks, VII but no II was detected on TLC. After one month, no Kedde-reactive spot was observed on TLC. VII (10 mg) was acetylated and the acetate (VII-1) was crystallized from EtOAc–hexane to give 3 mg of needles, mp 268—270°C. On admixture of the product with II-1, no melting point depression was observed, and the ^{1}H-NMR spectrum of VII-1 was in good agreement with that of II-1.

Affinoside S-III (III)——A solid, $[\alpha]_{\mathrm{D}}^{25}$ +27.2° (c=0.63, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 264 (20000), FD-MS m/z: 549 (M^++1, $C_{29}H_{40}O_{10}$). III-acetate: A solid, $[\alpha]_{\mathrm{D}}^{23}$ −5.3° (c=0.57, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 263 (13700), FD-MS m/z: 717 (M^++1, $C_{37}H_{48}O_{14}$).

III-Acetonide (III-2)——III (50 mg) was dissolved in 2 ml of 1% HCl in acetone. The mixture was allowed to stand at room temp. for 24 h, then it was diluted with MeOH. The diluted solution was deacidified with IR-410 and the solvent was evaporated off *in vacuo*. The residue was chromatographed on a silica gel column with solv. 1 (5: 1—4: 1) to give 26 mg of an acetonide of III (III-2) as a solid, $[\alpha]_{\mathrm{D}}^{24}$ +2.3° (c=1.2, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 265 (16800), FD-MS m/z: 589 (M^++1, $C_{32}H_{44}O_{10}$). III-2 was acetylated in the usual manner to give a diacetate of III-2 (III-3) as a solid, $[\alpha]_{\mathrm{D}}^{23}$ −27.7° (c=0.38, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 263 (15000), FD-MS m/z: 673 (M^++1, $C_{36}H_{48}O_{12}$).

Hydrolysis of III——III (30 mg) was refluxed with 2 ml of 0.5 N H_2SO_4–50%EtOH for 3 h. The mixture was diluted with MeOH, and the solution was deacidified with IR-410, then concentrated to dryness *in vacuo*. The residue was partitioned into n-BuOH/H_2O. From the BuOH layer, Dg-I was detected on TLC (solv. 1, 1 : 1; solv. 4, 7: 2: 0.8). Concentration of the water layer gave a residue, which was subjected to PC and TLC. PC (solv. 7): $R_{\mathrm{rham.}}$ 0.94 (L-rhamnose: 1.00, 6-deoxy-D-gulose: 0.94, 6-deoxy-D-allose: 1.03, D-quinovose: 0.82), PC (solv. 8): $R_{\mathrm{rham.}}$ 0.57 (L-rhamnose: 1.00, 6-deoxy-D-gulose: 0.57, 6-deoxy-D-allose: 0.41, L-fucose: 0.27, D-quinovose: 0.48). TLC (solv. 9 and solv. 4, 7: 3: 1): the sugar from III was identical with authentic 6-deoxy-D-gulose.

Affinoside VIII (VIII)——A solid, $[\alpha]_{\mathrm{D}}^{23}$ +13.3° (c=1.05, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 264 (14900), FD-MS m/z: 533 (M^++1, $C_{29}H_{40}O_9$). VIII-acetate (VIII-1): A solid, $[\alpha]_{\mathrm{D}}^{23}$ −15.5° (c=0.30, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 264 (17300), FD-MS m/z: 659 (M^++1, $C_{35}H_4\ O_{12}$).

VIII-Acetonide (VIII-2)——VIII (65 mg) was dissolved in 4 ml of 1% HCl in acetone. The mixture was allowed to stand at room temp. for 40 h, then diluted with MeOH. The methanolic soln. was deacidified with IR-410 and the solvent was evaporated off *in vacuo*. The residue was chromatographed on a silica gel column with solv. 1 (5: 1). From Fr. 1, VIII-2 was obtained as a solid (9 mg), $[\alpha]_{\mathrm{D}}^{24}$ −12.3° (c=0.13, MeOH), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm (ε): 265 (17200), FD-MS m/z: 573 (M^++1, $C_{32}H_{44}O_9$). From Fr. 2, Dg-I (under 10 mg) was obtained. From Fr. 3, VIII (10 mg) was recovered. Upon acetylation of VIII-2, a monoacetate of VIII-2 was obtained as a solid (VIII-3).

VIII-Monomethylate (VIII-4)——VIII (20 mg) was dissolved in 2 ml of MeOH, and 3 mg of $SnCl_2$ and an excess amount of CH_2N_2 in ether were added. The mixture was allowed to stand at room temp. for 1.5 h, then the solvent was evaporated off *in vacuo*. The residue was purified by DCCC (solv. 5) and then by chromatography on a silica gel column with EtOAc as the eluent. VIII-monomethylate (VIII-4) was obtained as a solid (14 mg). VIII-4 was acetylated in the usual manner to give a diacetate as a solid (VIII-), $[\alpha]_{\mathrm{D}}^{23}$

$-8.7°$ ($c=0.28$, MeOH), λ_{max}^{MeOH} nm (ε): 263 (13500), FD-MS m/z: 653 (M^++Na, $C_{34}H_{46}O_{11}$).

Affinoside S-IV (IV)——A solid, $[\alpha]_D^{23}$ $-10.2°$ ($c=0.90$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (16300), FD-MS m/z: 565 (M^++1, $C_{29}H_{40}O_{11}$). IV-acetate (IV-1): A solid, $[\alpha]_D^{23}$ $-2.0°$ ($c=0.30$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (18000), FD-MS m/z: 775 (M^++1, $C_{39}H_{50}O_{16}$). IV (10 mg) was dissolved in 20% EtOH (2 ml) and shaken with snail enzyme[10] for 18 h at 38°C. The mixture was diluted with water and extracted with *n*-BuOH. The BuOH layer and water layer were each concentrated *in vacuo* and subjected to TLC or PC for the detection of the aglycone and sugar. The aglycone was identified as Dg-I by TLC with solv. 1 (1: 1) and solv. 4 (7: 2: 0.8). The sugar was identified as glucose by PC with solv. 10 (*Rf* 0.17).

Affinoside S-V (V)——V was obtained as a crystalline powder, mp 155—158°C, $[\alpha]_D^{23}$ $+4.5°$ ($c=0.89$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (15400), FD-MS m/z: 564 (M^+, $C_{29}H_{40}O_{11}$), *Anal.* Calcd for $C_{29}H_{40}O_{11}\cdot H_2O$: C, 59.78; H, 7.27. Found: C, 59.65; H, 7.50. Upon usual acetylation, an acetate (V-1) was obtained as a solid, $[\alpha]_D^{23}$ $+5.1°$ ($c=0.28$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (13700), FD-MS m/z: 775 (M^++1, $C_{39}H_{50}O_{16}$). Hydrolysis of V was conducted in the same manner as for IV. The aglycone was identified as Dg-II by TLC with solv. 1 (1: 1) and solv. 4 (7: 2: 0.8). The sugar was identified as glucose by PC with solv. 10 (*Rf* 0.17).

Affinoside VI (VI)——A solid, $[\alpha]_D^{23}$ $-1.4°$ ($c=0.70$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (11000), FD-MS m/z: 565 (M^++1, $C_{29}H_{40}O_{11}$). An acetate of VI (VI-1) was obtained as a crystalline powder, mp 174—177°C, $[\alpha]_D^{23}$ $-1.7°$ ($c=0.23$, MeOH), λ_{max}^{MeOH} nm (ε): 264 (12000), *Anal.* Calcd for $C_{39}H_{50}O_{16}\cdot 2H_2O$: C, 57.77; H, 6.71. Found: C, 58.05; H, 6.95. Hydrolysis of VI was conducted in the same manner as for IV, and Dg-II and glucose were detected from the aglycone and sugar fractions.

Acknowledgement The authors are grateful to Prof. T. Reichstein for the kind gift of samples of 6-deoxy-D-gulose and 6-deoxy-D-allose. Thanks are also due to the staff members of this university, Messrs. M. Nishi, Y. Furukawa, and S. Hara, for carrying out NMR, FD-MS, and elementary analyses. This work was supported in part by a grant from the Central Research Institute of this university.

References and Notes

1) Anodendron. III: F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **30**, 3897 (1982).
2) *a*) P. Brown, J.v. Euw, T. Reichstein, K. Stöckel, and T.R. Watson, *Helv. Chim. Acta*, **62**, 412 (1979); H.T.A. Cheung, T.R. Watson, N. Seiber, and C. Nelson, *J. Chem. Soc.* I, **1980**, 2169; *b*) H.T.A. Cheung, R.G. Coombe, W.T.L. Sidwell, and T.R. Watson, *J. Chem. Soc.* I, **1981**, 64; D.H.G. Crout, C.H. Hassall, and T.L. Jones, *J. Chem. Soc.*, **1964**, 2187; G. Hesse and G. Ludwig, *Ann.*, **632**, 158 (1959); and references cited therein.
3) *a*) K. Shimada, T. Kyuno, T. Nambara, and I. Uchida, *Heterocycles*, **15**, 355 (1981); and references cited therein; *b*) A.S.R. Anjaneyulu and M.N. Rao, *Ind. J. Chem.* **19B**, 944 (1980).
4) *a*) H. Lichti, J.v. Euw, J. Polonia, and T. Reichstein, *Helv. Chim. Acta*, **53**, 1696 (1970); *b*) T. Yamauchi, K. Miyahara, F. Abe, and T. Kawasaki, *Chem. Pharm. Bull.*, **27**, 2463 (1979), F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **30**, 1183 (1982).
5) F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **26**, 3023 (1978).
6) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
7) M.T. Krauss, H. Jäger, O. Schindler, and T. Reichstein, *J. Chromatog.*, **3**, 63 (1960).
8) H. Kaufmann, P. Mahlradt, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2287 (1967).
9) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 677 (1970).
10) Precipitated with acetone from snail digestive juice.