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Structures of Affinogenin C and Affinogenins D-I—D-V from *Anodendron affine* (*Anodendron*. III)[1]

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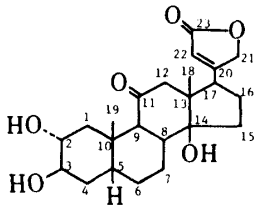
Affinogenin C and affinogenins D-I—V, cardenolides free from the component sugar, were isolated from the caules and leaves of *Anodendron affine* Druce, and their structures were established on the basis of spectrometric and chemical evidence.

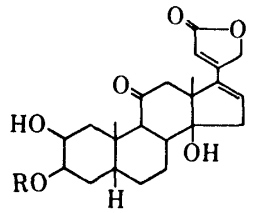
Keywords——Apocynaceae; *Anodendron*; 5β-cardenolide; 2,3,14-trihydroxy-11-oxo-cardenolide; 3,14-dihydroxy-2,11-dioxo-cardenolide; Δ^{16}-cardenolide; 2-seco-cardenolide; ^{13}C-NMR

In the preceding paper of this series, we described the structures of affinosides A—J, cardenolide glycosides having double linkages between an aglycone and a 4,6-dideoxy-3-*O*-methyl-2-hexosulopyranose moiety, isolated from *Anodendron affine* Druce.[1] In further studies on the more polar fraction from the methanol percolate, six cardenolides free from the component sugar, and nine cardenolide glycosides with a normal glycosidic linkage were isolated. They were named affinogenin C (Cg), affinogenins D-I—V (Dg-I—V, respectively), and affinosides S-I—IX (S-I—IX, respectively). The present paper describes their isolation and the structure elucidation of the affinogenins (Chart 1).

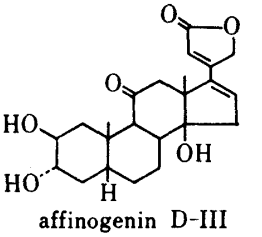
From the $CHCl_3$–EtOH (2: 1) extractives of the MeOH percolate from the caules, four cardenolides, Dg-V, -I, -II, and -III, and four cardenolide glycosides, S-VII, -VIII, -II, and -III, showing similar polarity on thin layer chromatographies (TLC), were isolated in order of increasing polarity by successive silica gel column chromatography and droplet counter current chromatography (DCCC), while S-IV—VI were isolated from the BuOH extractives. Cg, Dg-IV, S-I, and S-IX were isolated from the $CHCl_3$ and the $CHCl_3$–EtOH (2: 1) extractives of the leaves in addition to Dg-I, -II, -III and several cardenolide glycosides bearing 6-deoxy-3-*O*-methyl-2-hexosulopyranose as a component sugar.[2]



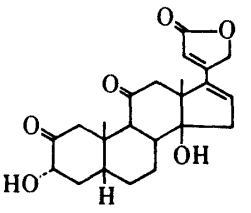
affinogenin C
affinogenin D-I(Δ^{16})



affinogenin D-II : R=H
affinogenin D-IV : R=Ac



affinogenin D-III



affinogenin D-V

Chart 1

TABLE I. ^{13}C-Chemical Shifts of Affinogenins and Their Acetates, δ (ppm) from TMS in Pyridine-d_5

Carbon	Cg	Dg-I	Dg-I -1	Dg-I -2	Dg-II	Dg-II -1	Dg-IV	Dg-II -2	Dg-III	Dg-III -1a	Dg-III -1b	Dg-III -2	Dg-V	Dg-V -1
1	37.0	37.3[a)]	34.1	34.7	39.0	34.9	40.0	35.7	44.5	43.1	44.8	40.6	49.6	49.7
2	70.5	70.4	73.9	69.7[a)]	67.9	72.7	65.9	69.3[a)]	71.7	74.9	67.9	70.7	211.5[a)]	204.6
3	71.7	71.6	66.8	69.9[a)]	70.1	66.8	73.6	69.7[a)]	76.5	72.9	79.2	75.1	75.1	76.3
4	30.2	30.2	30.8	27.7	33.7	33.8	31.2	30.7	35.4	35.6	32.0	31.7	38.1	33.6
5	37.4	37.5[a)]	36.7	37.3	36.6	36.5	37.4	37.3	42.8	41.2[a)]	42.3	42.0	41.9	42.4
6	27.1	27.1	27.0	26.7	26.5	26.3	26.2	25.9	26.7	26.3	26.4	26.2	25.5	25.5
7	23.1	23.0	22.4	21.9	22.1	22.4	22.1	22.1	22.2	22.4	22.1	22.3	22.2	22.2
8	43.3	43.0	43.0	42.8	43.1	43.2	42.9	43.0	43.0	42.5	43.0	43.1	43.1	43.1
9	50.3[a)]	50.2	49.5	49.3	48.6	48.3	48.5	48.2	49.0	48.5	48.9	48.4	48.8	48.7
10	35.6	35.7	35.9	35.6	38.0	37.8	37.8	37.6	37.9	37.3	37.6	37.2	41.6	41.7
11	211.4	211.9	211.6	211.6	211.5	210.6	211.4	210.7	211.3	209.8	211.1	209.7	208.9[a)]	208.6
12	55.5	52.8	52.1	51.8	51.8	52.1	51.8	51.9	51.8	52.2	51.7	52.1	52.4	52.3
13	53.4	57.1	56.8	56.7	57.0	57.1	56.9	57.0	56.8	57.0	56.8	57.0	57.1	57.1
14	83.6	83.6	83.1	82.8	83.0	83.0	82.8	82.8	82.9	83.0	82.8	82.9	83.0	83.0
15	33.6	41.5	42.0	42.1	41.8	41.6	41.8	41.6	41.6	40.8[a)]	41.7	41.4	40.9	41.0
16	27.1	135.2	134.9	135.8	135.3	135.3	135.5	135.8	135.5	135.2	135.3	135.1	135.1	134.9
17	50.5[a)]	142.2	142.0	141.7	141.4	141.6	141.3	141.4	141.3	141.8	141.4	141.9	142.2	142.2
18	17.6	18.6	19.6	19.7	19.8	19.2	19.7	19.4	19.5	18.7	19.6	18.8	18.1	18.0
19	24.3	24.2	24.0	23.7	24.2	24.0	24.0	23.8	23.6	23.3	23.5	23.1	23.0	22.9
20	174.0	158.7	158.4	158.7	158.5	158.5	158.5	158.4	158.7	158.7	158.5	158.4	158.6	158.4
21	73.6	71.9	71.9	71.9	71.9	71.9	71.9	71.9	72.0	72.0	71.9	71.8	71.9	71.8
22	118.0	112.4	112.9	112.9	112.6	112.6	112.6	112.6	112.4	112.4	112.6	112.7	112.4	112.6
23	174.2	174.5	174.5	174.5	174.5	174.5	174.5	174.5	174.6	174.6	174.5	174.5	174.6	174.4
-OAc			21.5	21.0		21.1	21.4	21.0		21.3	21.3	21.0		20.6
			169.6	21.3		170.4	170.7	21.0		170.7	170.7	21.0		169.6
				169.0				170.2				170.1		
				169.8				170.3				170.2		

a) Signal assignments in each column may be reversed.

Among the six affinogenins, Cg and Dg-V were crystallized, whereas the others were obtained as solids. Dg-I—V have ultraviolet (UV) absorption at 265 nm showing the presence of a Δ^{16}-function conjugated to the unsaturated linkage in the butenolide ring. All of these cardenolides are considered to retain a 5β-framework on the basis of the C-19 signal at δ 23.0—24.3 in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I). Upon partial acetylation, Dg-I and Dg-II each gave one monoacetate (Dg-I-1 and Dg-II-1, respectively), while Dg-III afforded two monoacetates (Dg-III-1a and Dg-III-1b). Diacetates were obtained from the less polar fraction on partial acetylation, or by acetylation for 24 h (Dg-I-2, Dg-II-2, Dg-III-2, and Cg-1).

The ^{13}C-NMR spectra of Dg-I, Dg-II, and Dg-III indicated, along with the signals due to the butenolide ring, the presence of one carbonyl carbon at δ 211.3—211.9, and three carbons bearing oxygen, one of which at δ 82.9—83.6 (s) is assignable to C-14. Accordingly, the remain-

Table II. ^{1}H-Chemical Shifts of Affinogenins and Their Acetates, δ (ppm) from TMS in $CDCl_3$

Compound	18,19-Me	21-CH_2	22-H	16-H	2-H	3-H	Others
Cg[a]	1.06	4.94	6.09		4.37 (br s)		
	1.37	5.21	(t, 2)		4.45 (br s)		
		(18, 2)[b]					
Cg-1	0.87	4.71	5.84		4.86 (br s)		1.95 (−OAc)
	1.14	4.90	(br s)				2.06 (−OAc)
		(17, 1)					
Dg-I[a]	1.42	4.79	6.38	6.20	4.34 (br s)		
	1.58	5.04	(t, 2)	(t, 2)	4.44 (br s)		
		(16, 2)					
Dg-I-1	1.14	4.92	5.95	6.14	3.87 (br s)		1.88 (−OAc)
	1.31	(2)	(2)	(t, 3)	4.81 (br s)		
Dg-I-2	1.15	4.91	5.95	6.19	4.91 (br s)		1.88 (−OAc)
	1.30	(2)	(2)	(t, 3)			2.07 (−OAc)
Dg-II[a]	1.29	4.93	6.43	6.23	4.38	4.44	
	1.59	(br s)	(br s)	(t, 2)	(3, 4, 12)	(br s)	
Dg-II-1	1.18	4.94	5.91	6.13	4.84[c]	4.12	2.03 (−OAc)
	1.31	(br s)	(br s)	(t, 3)	(3, 4, 12)	(br s)	
Dg-IV[a]	1.29	4.81	6.42	6.22	4.32	5.63	2.09 (−OAc)
	1.59	5.03	(br s)	(t, 2)	(3, 4, 12)	(br s)	
		(16, 1)					
Dg-II-2	1.20	4.94	5.95	6.15	4.85[c]	5.35[d]	1.94 (−OAc)
	1.30	(br s)	(br s)	(t, 3)	(3, 4, 12)	(br s)	2.07 (−OAc)
Dg-III[a]	1.24	4.82	6.42	6.18	4.00—4.50		
	1.56	5.03	(2)	(t, 3)	(m)		
		(16, 2)					
Dg-III-1a	1.16	4.95	5.94	6.18	4.52—4.97[e]	3.38—3.85[e]	2.05 (−OAc)
	1.29	(2)	(2)	(t, 2)	(m)	(m)	
Dg-III-1b	1.16	4.95	5.99	6.16	3.52—3.97	4.50—4.90	2.08 (−OAc)
	1.32	(2)	(2)	(t, 2)	(m)	(m)	
Dg-III-2	1.18	4.94	5.96	6.18	4.70—5.20		1.98 (−OAc)
	1.29	(br s)	(br s)	(t, 3)	(m)		2.00 (−OAc)
Dg-V[a]	1.37	4.77	6.41	6.17		4.52	3.34 (1-Ha)
	1.51	5.08	(2)	(t, 3)		(t, 9)	(13)
		(15, 2)					
Dg-V-1[a]	1.39	4.74	6.38	6.19		5.54	2.15 (−OAc)
	1.51	5.05	(t, 2)	(t, 3)		(12, 8)	3.35 (1-Ha)
		(16, 2)					(13)

a) Dissolved in pyridine-d_5.
b) Figures in parentheses indicate coupling constants (Hz).
c) Transformed into a doublet of doublets (4, 12 Hz) by irradiation of the 3-H peak.
d) Transformed into a triplet (3 Hz) by irradiation of the 2-H peak.
e) Transformed into a triplet (8 Hz) by irradiation of the 2-H peak.

ing two seem to be the acylable carbinyl carbons. In the ^{13}C-NMR spectra of the monoacetates, a downfield shift of the carbinyl carbon bearing the *O*-acetyl function was seen along with shielding of the other carbinyl carbons in comparison with those of the original cardenolides: from δ 70.4 and 71.6 (Dg-I) to δ 73.9 and 66.8 (Dg-I-1); from δ 67.9 and 70.1 (Dg-II) to δ 72.7 and 66.8 (Dg-II-1); from δ 71.7 and 76.5 (Dg-III) to δ 74.9 and 72.9 (Dg-III-1a) or δ 67.9 and 79.2 (Dg-III-1b). Two hydroxyl groups are therefore considered to form a glycol.

In the ^{1}H-NMR, two carbinyl protons of Dg-I are observed as broad singlets and those of Dg-III as ambiguous multiplets, while Dg-II and its acetates show two carbinyl protons as a doublet of double doublets (J=3, 4, 12 Hz) and a broad singlet, respectively (Table II). The doublet of double doublets in Dg-II-1 centered at δ 4.84 is transformed into a doublet of doublets (J=4, 12 Hz) by irradiation of the peak at δ 4.12 (br s). In Dg-II-2, these two signals are observed at δ 4.85 (ddd) and 5.35 (br s), the latter of which forms a triplet of 3 Hz on irradiation of the former peak. Among Dg-I, -II, and -III, only Dg-II affords an acetonide (Dg-II-3), showing it to have a *cis* glycol moiety, possibly at C-2 and C-3, since the aglycones of the cardenolide glycosides so far isolated from this plant have a 2α,3β-dihydroxyl structure.[1] The location of the glycol moieties in Dg-I and Dg-III was tentatively considered to be the same as in Dg-II, on the basis of the ^{13}C-NMR similarity of the carbons due to rings C and D in these three cardenolides.

The carbonyl group in Dg-I, -II, and -III is suggested to be located in the same position, C-11 or C-12, since the chemical shifts of the carbonyl carbons are nearly identical, and the naturally occurring oxo-cardenolides retain the carbonyl moiety in ring C. Consequently, they seem to be isomers in regard to the orientation of the two hydroxyl groups.

On $NaIO_4$ oxidation, Dg-I, -II, and -III produced the same bis-formyl compound (Dg-I-3, Dg-II-4, or Dg-III-3). In the ^{1}H-NMR, Dg-I-3 shows two formyl protons as a triplet (J=2 Hz) at δ 9.56 and 9.73, indicating the presence of methylene protons adjacent to the formyl groups, and the cleavage is considered to have occurred at the linkage between C-2 and C-3. The glycol moiety is therefore assigned to C-2 and C-3. Each bis-formyl compound from these cardenolides was then led to the same crystalline carbinyl compound (Dg-I-4) by brief reduction with $NaBH_4$. In the ^{13}C-NMR of Dg-I-4, the appearance of one singlet peak at δ 95.4, instead of the peak due to the carbonyl carbon, suggests that the carbonyl residue forms a hemiacetal structure in which the C-2 carbinol is linked to one of the acetal hydroxyl groups. The resonance of the carbonyl carbon is again observed at δ 209.7 in the spectrum of Dg-I-4 diacetate (Dg-I-5). The hemiacetal structure of the 11-carbonyl group in Dg-I-4 is also supported by shieldings of the C-1 (−4.8 ppm), C-9 (−7.7 ppm), and C-12 (−2.8 ppm) signals by comparison with those of Dg-I-5. The downfield shifts of C-18 and C-19 seen in Dg-I-4 are known as the δ_1 effect in the 11β-hydroxy-steroids[3] (Chart 2).

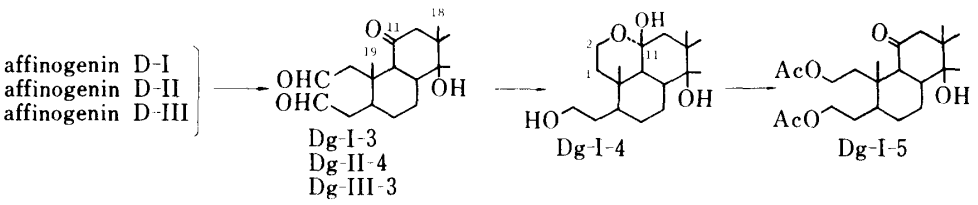


Chart 2

$NaBH_4$ reduction of Dg-I yielded a dihydro derivative (Dg-I-6), in which the newly formed carbinyl proton is found as a broad singlet at δ 4.50, duplicating the 2-H and 3-H. Since Dg-I-6 afforded a diacetate (Dg-I-7) upon 24 h acetylation with Ac_2O and pyridine, the new hydroxyl group is considered to retain 11β-orientation. Thus, the original carbonyl group in the three cardenolides is assigned to C-11. As a result of the formation of the same 2-seco derivative, Dg-I, -II, and -III were proved to be 2,3-dihydroxy-11-oxo compounds.

On the basis of ^{13}C- and ^{1}H-NMR considerations, Cg and Dg-I appeared to have a similar structure except for the Δ^{16}-function in the latter. Upon $NaBH_4$ reduction, Cg was transformed into two dihydro derivatives (Cg-2a and Cg-2b). Cg-2b, the more polar of them, yielded a diacetate in the same way as Dg-I-6, while Cg-2a provided a triacetate, indicating Cg-2b to be the 11β-hydroxyl derivative. When Dg-I was subjected to catalytic reduction, a small amount of Cg was formed along with the 17α-derivative (Dg-I-8) as a major product[4] which was also prepared from Cg by isomerization of the 17β-side chain.[5]

In the ^{13}C-NMR of Cg, Dg-I, -II, and -III, C-1 and C-5 in Cg (δ 37.0 and 37.4), Dg-I (δ 37.3 and 37.5), and Dg-II (δ 39.0 and 36.6) are found at higher field than in the case of Dg-III (δ 44.5 and 42.8), suggesting that the former three compounds have 3β (ax)- and Dg-III has a 3α (eq)-hydroxyl residue. Further, shieldings of C-4 and C-10 are seen in Cg (δ 30.2 and 35.6) and in Dg-I (δ 30.2 and 35.7) by comparison with Dg-II (δ 33.7 and 38.0) and Dg-III (δ 35.4 and 37.4), indicating the orientation of the C-2 hydroxyl groups of Cg and Dg-I to be α, and that of the groups of Dg-II and Dg-III, to be β. In Dg-III, chemical shifts of the resonances due to ring A are consistent with the ^{13}C-NMR of a 2β,3α-dihydroxy-5β-spirostane, *e.g.* yonogenin or neoyonogenin, recently presented by Tori *et al.*[6] Consequently, Cg was identified as 2α,3β,14-trihydroxy-11-oxo-5β,14β-card-20(22)-enolide, and Dg-I as the Δ^{16}-derivative of Cg. Dg-II and Dg-III are respectively the 2β,3β- and 2β,3α-isomers of Dg-I. Additional evidence on the structure of Cg was furnished by the fact that affinoside C was hydrolyzed to Cg by the preliminary reductive cleavage of the 2-*O*-2′ linkage, followed by acid hydrolysis.[1]

Dg-IV was considered to be a monoacetate of Δ^{16}-cardenolide on the basis of one acetyl signal at δ 2.09 and one triplet peak centered at δ 6.22 due to a 16-olefinic proton in the ^{1}H-NMR spectrum. Upon alkaline hydrolysis, it afforded Dg-II, showing it to be the 2- or 3-acetate of Dg-II. In a comparison of the ^{13}C-NMR of Dg-IV with that of Dg-II-1, the former shows shielding of C-4, while C-1 is shielded in the latter. Dg-IV was therefore determined to be the 3-acetate of Dg-II, and Dg-II-1, the 2-acetate. Among other monoacetates, Dg-I-1 was concluded to be the 2-acetate since shieldings were found at C-1 (−3.2 ppm) and C-3 (−4.8 ppm), in comparison with Dg-I. Dg-III-1a and Dg-III-1b were identified as the 2- and 3-acetates, respectively, from the shielding and deshielding of C-1—C-5 in the ^{13}C-NMR.

In the ^{13}C-NMR spectrum, Dg-V shows the presence of two carbonyl groups at δ 208.9 and 211.5, and two carbinyl carbons at δ 75.1 (d) and 83.0 (s), of which the latter signal is assigned to C-14. Upon acetylation, it afforded only a monoacetate (Dg-V-1). The finding that one of the carbonyl carbons is shifted to δ 204.6 in Dg-V-1 suggests that the carbonyl group forms an α-ketol. Since the chemical shifts due to rings B, C, and D are identical with those of Dg-I—III, the α-ketol moiety seems to be located in ring A, probably at C-2 and C-3. Among the oxidation products of the monoacetates of Dg-I, Dg-II, and Dg-III, 3α-acetoxy-2,11-dioxo-5β,14β-carda-16,20(22)-dienolide, prepared from Dg-III-1b, was identical with Dg-V-1, and hence the structure of Dg-V was established.

The structure of six cardenolides was thus elucidated. The presence of three isomers of the 2,3-dihydroxyl groups may be explained biogenetically by the co-occurrence of the 2-oxo-3-hydroxy-cardenolide such as Dg-V. The structures of the glycosides with normal glycosidic linkage, S-I—IX will be presented in the following paper.

Experimental

Melting points were measured on a Kofler block and are uncorrected. ^{1}H-NMR measurements were obtained with Hitachi R-22 and JEOL FX-100 spectrometers. The samples for ^{13}C-NMR were dissolved in pyridine-d_5 and the spectra were measured with the JEOL FX-100. Chemical shifts are given in δ values referred to internal tetramethylsilane, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, br s=broad singlet. Mass spectra (MS) and field desorption (FD)-MS were recorded on JEOL 01SG and D-300 FD spectrometers. UV and IR spectra were taken on a Shimadzu 200S double beam spectrophotometer and a Hitachi EPI-G3 spectrophotometer, respectively.

The following solvent systems were used for silica gel column chromatographies and DCCC; solv. 1: benzene–acetone, solv. 2: hexane–EtOAc, solv. 3: EtOAc–MeOH (9: 1 and 4: 1, for TLC), solv. 4: $CHCl_3$–MeOH–H_2O (bottom layer for column and TLC), solv. 5: (same system as solv. 4; 5: 6: 4, for DCCC, ascending and descending), solv. 6: EtOAc–MeOH–H_2O (top layer). As spray reagents, a) a 1: 1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH (Kedde reagent) and b) 5% H_2SO_4 were used. When b) was applied, the plate was heated until the spots were detected.

Isolation of Cardenolides and Glycosides——a) From the Caules: As described in the preceding paper,[1] the MeOH percolate of the caules was subjected to partition with $CHCl_3$, $CHCl_3$–EtOH (2: 1), and *n*-BuOH. The $CHCl_3$–EtOH (2: 1) fraction (158 g) was subjected to chromatography on an XAD-2 column. Elution with MeOH in H_2O (0—60%) afforded the following fractions; Fr. 1: Kedde-negative, Fr. 2: ext. 13 g, containing S-IV, -V, -VI; Fr. 3: 21 g, Dg-V, -I, -II, -III, S-II, -III, -IV; Fr. 4: 38 g, Dg-V, -I, -II, S-VII, affinosides E and G. Fr. 2, obtained with 15% MeOH, containing principally S-IV, -V, and -VI, was chromatographed on a silica gel column with solv. 6 (4: 1: 4) to fractionate S-IV, -V, and -VI from other Kedde-negative substances. The isolation of each glycoside was conducted by successive silica gel column chromatography with solv. 4 (7: 3: 1) and DCCC (ascending). The overall yield of each glycoside was approximately 0.0005—0.001%. Fr. 3, obtained by 30—40% MeOH elution, was chromatographed on a silica gel column with solv. 4 (7: 2: 1.8), solv. 1 (5: 1—3: 1), and DCCC (solv. 5, ascending), successively. Dg-I, -II, and -III were isolated in yields of 0.003% by successive chromatographies with solv. 1 (4: 1—3: 1). S-II was crystallized from EtOAc–hexane after silica gel column chromatography with solv. 1 (4: 1) (0.0002%). S-III and S-VIII showed polarities similar to those of Dg-III and Dg-I. They were isolated in yields of 0.0002 and 0.0001%, respectively, by ascending and descending DCCC and chromatographies on a silica gel column with solv. 1 (3: 1) and solv. 4 (7: 2: 1.8—7: 2: 1.5). Fr. 4, obtained by 50—60% MeOH elution, was subjected to successive chromatographies with solv. 6 (7: 1: 7) and with solv. 1 (5: 1). Small amounts of affinosides D, E, and G were found in the less polar fraction of the eluates. The fraction containing S-VII was purified by DCCC (ascending) to yield 50 mg of S-VII as a solid.

b) From the Leaves: Fresh leaves (20 kg), collected at Madarajima in Oct., 1980, were homogenized with MeOH and the mixture was filtered. The filtrate was concentrated to 10 l and extracted with benzene, $CHCl_3$, $CHCl_3$–EtOH (2: 1), and *n*-BuOH. The $CHCl_3$ fraction, principally containing the glycosides with the doubly linked sugar moiety, was chromatographed on a silica gel column with solv. 1. The fraction containing small amounts of affinosides A, F, *etc.*, was again chromatographed on a column with solv. 6 (5: 1: 5), followed by DCCC (descending) to yield S-IX (15 mg) and Dg-IV (25 mg).

The $CHCl_3$–EtOH (2: 1) fraction was chromatographed in the same manner as the 2: 1 fraction from the caules. S-IV (trace), S-V (0.0005%), and S-VI (trace) were isolated. Cg and S-I, showing the same *Rf* values as Dg-I and S-II, respectively, were obtained following affinosides L-I—IV,[2] on chromatography with solv. 4, and crystallized from EtOAc–hexane to give prisms (Cg: 0.0003%, S-I: 0.0002%).

Affinogenin D-I (Dg-I)——Solid, $[\alpha]_D^{25}$ +30.6° (c=0.12, MeOH), UV λ_{max}^{MeOH} nm (ε): 265 (14200), FD-MS m/z: 403 (M^++1, $C_{23}H_{30}O_6$).

Acetylation of Dg-I——A mixture of 70 mg of Dg-I in 2 ml of pyridine with 1 ml of Ac_2O was allowed to stand at room temp. for 40 min, diluted with ice-water, and extracted with *n*-BuOH. The *n*-BuOH ext. was subjected to silica gel column chromatography with solv. 1 (6: 1—4: 1) to give 35 mg of a diacetate (Dg-I-2), solid, $[\alpha]_D^{18}$ +15.8° (c=0.91, MeOH), FD-MS m/z: 487 (M^++1, $C_{27}H_{34}O_8$) and 15 mg of a monoacetate (Dg-I-1), solid, $[\alpha]_D^{18}$ −0.9° (c=1.60, MeOH), FD-MS m/z: 445 (M^++1, $C_{25}H_{32}O_7$).

$NaIO_4$ Oxidation of Dg-I——A mixture of 200 mg of Dg-I in 15 ml of MeOH with 140 mg of $NaIO_4$ in 4 ml of H_2O was allowed to stand at room temp. for 3 h, then diluted with H_2O and extracted with $CHCl_3$–EtOH (2: 1) mixture. The ext. (55 mg) was subjected to silica gel column chromatography with solv. 4 (7: 2: 2.6) to give 40 mg of the major product as a solid (Dg-I-3). ^{1}H-NMR ($CDCl_3$): 1.28, 1.48 (3H each, s, 18- and 19-CH_3), 4.96 (2H, d, J=2 Hz, 21-H_2), 5.94 (1H, br s, 22-H), 6.21 (1H, t, J=3 Hz, 16-H), 9.57 (1H, t, J=2 Hz, 2- or 3-H), 9.73 (1H, t, J=2 Hz, 2- or 3-H). $NaBH_4$ (40 mg) was added portionwise to a solution of 40 mg of Dg-I-3 in 5 ml of MeOH, and the mixture was stirred at room temp. for 30 min. The mixture was then diluted with H_2O and extracted with *n*-BuOH. The BuOH ext. was purified on a silica gel column with solv. 4 (7: 2: 2) to give Dg-I-4, which was crystallized from EtOAc–hexane to give prisms, mp 210—220°C, $[\alpha]_D^{23}$ +63.2° (c=0.25, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3540, 3360, 3240, 2950, 2880, 1820, 1740 (sh), 1730, 1670 1624. *Anal.* Calcd for $C_{23}H_{32}O_6 \cdot H_2O$: C, 65.37; H, 8.11. Found: C, 65.05; H, 8.55. ^{1}H-NMR (pyridine-d_5): 1.60, 1.87 (3H each, s, 18- and 19-CH_3), 3.60—4.19 (4H, m, 2×CH_2OR), 4.77, 5.06 (1H each, dd, J=2, 17 Hz, 21-H_2), 5.91 (1H, br s, 22-H), 6.19 (1H, t, J=3 Hz, 16-H). ^{13}C-NMR: 20.0 (q, C-18), 21.8 (t, C-7), 22.9 (q, C-19), 24.2 (t, C-6), 30.9 (t, C-1), 34.6 (s, C-10), 36.1 (t, C-4), 37.3 (d, C-5), 41.3 (t, C-15), 43.0 (d, C-8), 44.9 (d, C-9), 50.0 (t, C-12), 53.0 (s, C-13), 57.6 (t, C-2), 61.9 (t, C-3), 72.1 (t, C-21), 85.6 (s, C-14), 95.4 (s, C-11), 111.5 (d, C-22), 134.3 (d, C-16), 144.3 (s, C-17), 160.0 (s, C-20), 174.6 (s, C-23).

Dg-I-4 diacetate (Dg-I-5) was obtained as a solid in the usual acetylation with pyridine and Ac_2O at room temp. ^{1}H-NMR (pyridine-d_5): 1.37, 1.57 (3H each, s, 18- and 19-CH_3), 1.98, 2.08 (3H each, s, CH_3CO–), 4.00—4.50 (4H, m, 2×–CH_2OAc), 4.78, 5.06 (1H each, dd, J=2, 16 Hz, 21-H_2), 6.30 (1H, br s, 16-H), 6.44 (1H, br s, 22-H). ^{13}C-NMR: 18.2 (q, C-18), 20.3 (q, C-19), 20.6, 20.8 (q, 2×CH_3CO–), 22.2 (t, C-7), 24.9 (t, C-6), 35.7 (t, C-1, C-4), 37.4 (s, C-10), 38.6 (d, C-5), 41.2 (t, C-15), 42.5 (d, C-8), 52.6 (d, C-9), 52.8 (t, C-12),

57.3 (s, C-13), 60.7 (t, C-2 or C-3), 63.1 (t, C-2 or C-3), 71.6 (t, C-21), 83.1 (s, C-14), 112.4 (d, C-22), 134.9 (d, C-16), 142.3 (s, C-17), 158.4 (s, C-20), 170.7 (s, $2\times CH_3\underline{C}O$–), 174.3 (s, C-23), 209.7 (s, C-11).

$NaBH_4$ Reduction of Dg-I——A soln. of 100 mg of Dg-I in 5 ml of MeOH was treated with 50 mg of $NaBH_4$, portionwise. The mixture was stirred for 1 h at room temp., diluted with H_2O, and extracted with *n*-BuOH. The BuOH ext. (80 mg) was subjected to chromatography on a silica gel column with solv. 4 (7: 2: 2). From the first fraction, Dg-I (50 mg) was recovered. A product showing a homogeneous spot on TLC was obtained from the more polar fraction as a solid (25 mg) (Dg-I-6; 2α,3β,11β,14-tetrahydroxy-5β,14β-carda-16,20(22)-dienolide). ^{1}H-NMR (pyridine-d_5): 1.63, 1.95 (3H each, s, 18 and 19-CH_3), 4.50 (3H, m, 2β-H, 3α-H, 11α-H), 4.79, 5.06 (1H each, dd, $J=2$, 16 Hz, 21-H_2), 6.08 (1H, br s, 16-H), 6.19 (1H, br s, 22-H). Upon acetylation for 24 h at room temp. with Ac_2O and pyridine, a diacetate was obtained as a solid (Dg-I-7). ^{1}H-NMR ($CDCl_3$): 1.26, 1.48 (3H each, s, 18- and 19-CH_3), 1.93, 2.08 (3H each, s, CH_3CO–), 4.16 (1H, br s, 11α-H), 4.96 (4H, br s, 21-H_2, 2β-H, 3α-H), 5.98 (1H, br s, 22-H), 6.09 (1H, t, $J=2$ Hz, 16-H).

Catalytic Reduction of Dg-I[4]——Dg-I (100 mg) was dissolved in 10 ml of MeOH, and shaken with 100 mg of Pd-carbon under an atmosphere of hydrogen for 2 h. The mixture was filtered and the filtrate was chromatographed on a silica gel column with solv. 4 (7: 2: 1.8). The first fraction was crystallized from EtOAc–hexane to give 1.7 mg of prisms, mp 262—268°C, which were identified as Cg by comparison (TLC and mixed fusion) with authentic Cg. A second product showing a homogeneous spot on TLC was obtained as a solid (Dg-I-8, 10 mg). A comparison of Dg-I-8 with 17α-Cg, prepared by the procedure described below,[5] showed in good agreement of TLC behavior and ^{1}H-NMR spectra. ^{1}H-NMR (pyridine-d_5): 1.20, 1.47 (3H each, s, 18- and 19-CH_3), 4.40 (2β-H, 3α-H), 4.66, 4.97 (1H each, dd, $J=2$, 18 Hz, 21-H_2), 5.98 (1H, d, $J=2$ Hz, 22-H). ^{13}C-NMR:[7] 18.9 (q, C-18), 22.9 (t, C-7), 24.3 (q, C-19), 25.1 (t, C-16), 27.1 (t, C-6), 30.1 (t, C-4), 31.7 (t, C-15), 35.3 (s, C-10), 36.9 (t, C-1), 37.5 (d, C-5), 43.8 (d, C-8), 48.2 (t, C-12; d, C-17), 50.6 (d, C-9), 53.7 (s, C-13), 70.7 (d, C-2), 71.7 (d, C-3), 74.0 (t, C-21), 84.2 (s, C-14), 117.0 (d, C-22), 171.4 (s, C-20), 173.6 (s, C-23), 210.8 (s, C-11).

Affinogenin D-II (Dg-II)——Solid, $[\alpha]_D^{25}$ +23.4° ($c=1.93$, MeOH), UV λ_{max}^{MeOH} nm (ε): 265 (14000), FD-MS *m/z*: 403 (M^++1, $C_{23}H_{30}O_6$).

Acetylation of Dg-II——A mixture of 85 mg of Dg-II in 2 ml of pyridine with 1 ml of Ac_2O was allowed to stand at room temp. for 40 min. The mixture was then diluted with ice-water, and extracted with $CHCl_3$. The $CHCl_3$ ext. was subjected to chromatography on a silica gel column with solv. 1 (6: 1—4: 1). From the first fraction, 42 mg of a diacetate (Dg-II-2) was obtained as a solid, $[\alpha]_D^{18}$ +11.9° ($c=1.24$, MeOH), FD-MS *m/z*: 487 (M^++1, $C_{27}H_{34}O_8$). From the second fraction, 28 mg of a monoacetate (Dg-II-1) was obtained as a solid, $[\alpha]_D^{18}$ +32.3° ($c=2.10$, MeOH), FD-MS *m/z*: 445 (M^++1, $C_{25}H_{32}O_7$).

Dg-II Acetonide (Dg-II-3)——A soln. of 60 mg of Dg-II in 3 ml of acetone, together with a catalytic amount of H_2SO_4, was allowed to stand at room temp. for 1 h, then the acetone was evaporated off at room temp. The residue was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ ext. was subjected to chromatography on a silica gel column with solv. 1 (7: 1) and Dg-II acetonide (Dg-II-3) was obtained as a solid (52 mg). MS *m/z*: 442 (M^+), 427 (M^+-CH_3), 385 ($427-C_3H_6$), 367 ($385-H_2O$), 349 ($367-2\times H_2O$). ^{1}H-NMR ($CDCl_3$): 1.13 (3H, s), 1.31 (6H, s), 1.47 (3H, s), (18-, 19-CH_3, $(CH_3)_2C<$), 4.22 (2H, m, 2-H, 3-H), 4.92 (2H, d, $J=2$ Hz, 21-H_2), 5.96 (1H, br s, 22-H), 6.13 (1H, t, $J=3$ Hz, 16-H). ^{13}C-NMR: 26.2, 28.5 (q, $(\underline{C}H_3)_2\cdot C<$), 29.2 (t, C-4), 36.9 (s, C-10), 72.8 (d, C-2), 73.7 (d, C-3), 107.4 (s, $(CH_3)_2\cdot\underline{C}<$); the peaks of the remaining carbons were observed at almost the same chemical shifts as in the case of Dg-II.

$NaIO_4$ Oxidation of Dg-II——A mixture of 170 mg of Dg-II in 15 ml of MeOH with an aq. soln. of $NaIO_4$ (140 mg in 4 ml) was allowed to stand at room temp. for 2 h, then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ ext. was purified quickly on a silica gel column with solv. 4 (7: 2: 2.6). The fraction showing a homogeneous spot on TLC (Dg-II-4) was compared with Dg-I-3; their ^{1}H-NMR spectra were in good agreement.

Dg-II-4 (40 mg) was dissolved in 5 ml of MeOH and the solution was stirred at room temp. with 40 mg of $NaBH_4$ for 50 min. The product was purified on a silica gel column with solv. 4 (7: 2: 2) and crystallized from EtOAc–hexane to give 17 mg of prisms, mp 210—223°C. On admixture with Dg-I-4, no melting point depression was observed, and the IR and ^{1}H-NMR spectra of the two samples were in good agreement.

Affinogenin D-III (Dg-III)——Solid, $[\alpha]_D^{25}$ +44.4° ($c=0.10$, MeOH), UV λ_{max}^{MeOH} nm (ε): 265 (12000), FD-MS *m/z*: 402 (M^+, $C_{23}H_{30}O_6$).

Acetylation of Dg-III——A soln. of 90 mg of Dg-III in 1 ml of pyridine was treated with 1 ml of Ac_2O, and the mixture was worked up as described in the acetylation of Dg-II. On column chromatography with solv. 1 (6: 1—4: 1), two monoacetates, Dg-III-1a (2β-*O*-acetyl-Dg-III; 20 mg, $[\alpha]_D^{18}$ +45.0° ($c=2.14$, MeOH), FD-MS *m/z*: 445 (M^++1, $C_{25}H_{32}O_7$)), Dg-III-1b (3α-*O*-acetyl-Dg-III; 20 mg, $[\alpha]_D^{18}$ +56.8° ($c=2.07$, MeOH), FD-MS *m/z*: 445 (M^++1)), and a diacetate, Dg-III-2 (30 mg, $[\alpha]_D^{18}$ +80.0° ($c=0.20$, MeOH), FD-MS *m/z*: 486 (M^+, $C_{27}H_{34}O_8$)) were obtained, all as solids.

$NaIO_4$ Oxidation of Dg-III——Dg-III (150 mg) was dissolved in 15 ml of MeOH, and treated with $NaIO_4$ in the same manner as described above. A diformyl compound (Dg-III-3) corresponding to Dg-I-3 or Dg-II-4 was obtained; its ^{1}H-NMR was superimposable on that of Dg-I-3. Dg-III-3 was then subjected to $NaBH_4$ reduction, and Dg-I-4 was obtained as prisms after crystallization from EtOAc–hexane, mp 210—220°C. On admixture with Dg-I-4 prepared from Dg-I-3, no melting point depression was observed, and

the IR spectra of the two samples were in good agreement.

Oxidation of Dg-III-1b to Dg-V-1——A soln. of 15 mg of Dg-III-1b in 2 ml of CH_2Cl_2 was stirred at room temp. and 200 mg of pyridinium chlorochromate was added portionwise. The mixture was stirred for 4 h then diluted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and the solvent was evaporated off *in vacuo*. The residue was subjected to chromatography on a silica gel column with solv. 1 (4.5: 1). The product was crystallized from EtOAc–hexane to give 2 mg of prisms, mp 250—260°C (dec.). The melting point and IR spectrum of the product and those of Dg-V-1 were in good agreement.

Affinogenin C (Cg)——mp 278—285°C (from EtOAc–hexane), $[\alpha]_D^{23}$ +34.0° (c=0.57, MeOH), UV λ_{max}^{MeOH} nm (ε): 215 (16300), *Anal.* Calcd for $C_{23}H_{32}O_6$: C, 68.63; H, 7.51. Found: C, 68.33; H, 7.67.

Acetylation of Cg——A mixture of 30 mg of Cg in 2 ml of pyridine with 1 ml of Ac_2O was allowed to stand for 24 h at room temp., then diluted with ice-water. The mixture was extracted with $CHCl_3$. The $CHCl_3$ ext. was crystallized from EtOAc–hexane to give 26 mg of Cg-diacetate (Cg-1) as needles, mp 224—232°C.

$NaBH_4$ Reduction of Cg——A solution of 60 mg of Cg in 10 ml of EtOH was treated with 60 mg of $NaBH_4$, portionwise. The mixture was stirred for 4 h at room temp., diluted with H_2O, and then extracted with *n*-BuOH. The BuOH ext. was subjected to chromatography on a silica gel column with solv. 4 (7: 2: 2). The less polar product was crystallized from EtOAc–hexane to give 20 mg of prisms, mp 262—270°C (Cg-2a; 2α,3β,11α,14-tetrahydroxy-5β,14β-card-20(22)-enolide). ^{1}H-NMR (pyridine-d_5): 1.09, 1.20 (3H each, s, 18- and 19-CH_3), 4.32 (1H, m, 11β-H), 4.44 (2H, br s, 2β-H, 3α-H), 4.98, 5.28 (1H each, dd, J=2, 18 Hz, 21-H_2), 6.04 (1H, d, J=2 Hz, 22-H). MS m/z: 406 (M^+, $C_{23}H_{34}O_6$). The more polar product was crystallized from EtOAc–hexane to give 25 mg of prisms, mp 110—120~150°C (Cg-2b; 2α,3β,11β,14-tetrahydroxy-5β,14β-card-20(22)-enolide). ^{1}H-NMR (pyridine-d_5): 1.60, 1.65 (3H each, s, 18- and 19-CH_3), 4.51 (3H, br s, 2β-H, 3α-H, 11α-H), 4.99, 5.36 (1H each, dd, J=2, 18 Hz, 21-H_2), 6.07 (1H, br s, 22-H). MS m/z: 406 (M^+).

Acetylation of Cg-2a and Cg-2b——Cg-2a and Cg-2b were acetylated with Ac_2O and pyridine at room temp. for 24 h. The acetates were obtained respectively as a solid (Cg-3a) and as crystals, mp 235—240°C (Cg-3b), from EtOAc–hexane. Cg-3a, the 2α,3β,11α-triacetate of Cg-2a, ^{1}H-NMR ($CDCl_3$): 0.99 (6H, s, 18- and 19-CH_3), 1.98, 2.04, 2.06 (3H each, CH_3CO–), 4.69, 4.96 (2H each, dd, J=2, 19 Hz, 21-H_2), 4.73—5.13 (m, 11β-H), 4.93 (2H, br s, 2β-H, 3α-H), 5.81 (1H, br s, 22-H). Cg-3b, the 2α,3β-diacetate of Cg-2b, ^{1}H-NMR ($CDCl_3$): 1.15, 1.24 (3H each, s, 18- and 19-CH_3), 2.05, 2.12 (3H each, s, CH_3CO–), 4.16 (1H, br s, 11α-H), 4.78, 5.01 (2H each, dd, J=2, 18 Hz, 21-H_2), 4.91 (2H, br s, 2β-H, 3α-H), 5.88 (1H, br s, 22-H).

17α-Cg from Cg——Cg (50 mg) was dissolved in 4 ml of dimethylformamide and heated at 110°C for 24 h with 180 mg of NaOTs and 80 mg of NaOAc.[5] The mixture was then diluted with H_2O and extracted with *n*-BuOH. The BuOH ext. was subjected to chromatography on a silica gel column with solv. 4 (7: 1: 0.8). Unreacted Cg was recovered from the first fraction. A small amount of 17α-Cg was obtained as a solid from the second fraction. The ^{1}H-NMR spectrum of the product and that of Dg-I-8 were in good agreement.

Affinogenin D-IV (Dg-IV)——Solid, $[\alpha]_D^{18}$ +15.0° (c=1.32, MeOH), UV λ_{max}^{MeOH} nm (ε): 265 (12000), FD-MS m/z: 445 (M^++1, $C_{25}H_{32}O_7$).

Deacetylation of Dg-IV——Dg-IV (3 mg) was dissolved in 1 ml of MeOH and 0.25 ml of aq. $KHCO_3$ (0.1 g/2.5 ml) was added. The mixture was allowed to stand for a week at room temp., then diluted with H_2O, and extracted with *n*-BuOH. The BuOH ext. was examined on TLC, in parallel with authentic Dg-I, -II, -III with solv. 1 (1: 1). The product showed the same *Rf* value as Dg-II.

Affinogenin D-V (Dg-V)——mp 230—234°C, $[\alpha]_D^{18}$ +115.4° (c=0.26, MeOH), UV λ_{max}^{MeOH} nm (ε): 265 (25000), MS m/z: 400.1878 (Calcd for $C_{23}H_{28}O_6$: 400.1885). *Anal.* Calcd for $C_{23}H_{28}O_6 \cdot H_2O$: C, 67.46; H, 7.14. Found: C, 67.41; H, 6.98.

Affinogenin D-V Monoacetate (Dg-V-1)——Dg-V (50 mg) was acetylated for 20 h at room temp. with pyridine and Ac_2O, and a monoacetate was crystallized from EtOAc–hexane to give 15 mg of prisms, mp 250—260°C (dec.), $[\alpha]_D^{18}$ +90.0° (c=0.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3580, 3500, 3420, 3360, 2920, 2880, 1820, 1730 (br), 1625. *Anal.* Calcd for $C_{25}H_{30}O_7 \cdot H_2O$: C, 65.20; H, 7.00. Found: C, 65.52; H, 6.71.

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