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Affinosides L_a—L_e, Major Cardenolide Glycosides from the Leaves of *Anodendron affine* (Anodendron. VII[1])

FUMIKO ABE and TATSUO YAMAUCHI*

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

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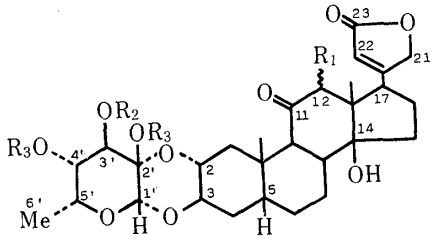
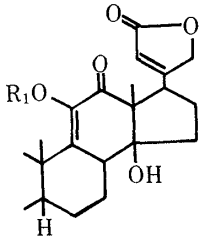
Affinosides L_a—L_e, major cardenolide glycosides which have oxygen functions at C-11 and C-12 or at C-11 of the aglycone moiety, and retain double linkages between the 2α,3β-dihydroxyl groups of the aglycone and 6-deoxy-, or 6-deoxy-3-*O*-methylgulosulose, were isolated from the leaves of *Anodendron affine*, and the structures were determined on the basis of spectral and chemical evidence.

Keywords——Apocynaceae; *Anodendron affine*; affinoside; 11-oxocardenolide; 11-oxo-12-hydroxy-cardenolide; 6-deoxygulosuloside; 6-deoxy-3-*O*-methylgulosuloside; 6-deoxy-3-*O*-methyl-idofuranose; doubly linked cardenolide glycoside

In the course of studies on the constituents of *Anodendron affine* DRUCE, eleven cardenolide glycosides with double linkages between the aglycone and 4,6-dideoxy-3-*O*-methylgulosulose have been isolated from the caules[2] and seeds,[3] and named affinosides A—H, J, K, and M. From the caules and leaves, eight normal-type glycosides,[4] affinosides S-I—S-VIII, were obtained in addition to six free cardenolides.[5] This paper deals with the isolation and structure elucidation of affinosides L_a—L_e, the major glycosides containing 6-deoxy-3-*O*-methyl-D-gulosulose or 6-deoxy-D-gulosulose as a component sugar, from the leaves.





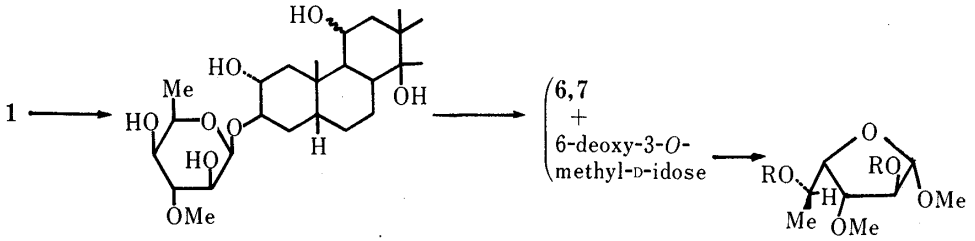
1: R_1=H, R_2=CH_3, R_3=H
2: R_1=H, R_2=CH_3, R_3=Ac
12: R_1=α-OH, R_2=CH_3, R_3=H
13: R_1=α-OAc, R_2=CH_3, R_3=H
14: R_1=α-OAc, R_2=CH_3, R_3=Ac
15: R_1=β-OH, R_2=CH_3, R_3=H
16: R_1=β-OAc, R_2=CH_3, R_3=H
17: R_1=β-OAc, R_2=CH_3, R_3=Ac
18: R_1=R_2=R_3=H
19: R_1=H, R_2=R_3=Ac
10: R_1=H, R_2=CH_3, R_3=H
11: R_1=Ac, R_2=CH_3, R_3=Ac

Chart 1

The glycosides were extracted from the methanol percolate of the fresh leaves, principally by the same procedure as used in the case of the caules. The 50% methanolic solution of the concentrated methanol percolate was extracted with benzene, $CHCl_3$, and then *n*-BuOH. Affinosides L_a—L_e were found in the $CHCl_3$ and *n*-BuOH extracts. From the benzene and $CHCl_3$ extracts, three unknown glycosides with less polar behavior on thin layer chromatography (TLC) (affinosides I, N, and O) and one free cardenolide (affinogenin D-VI) were obtained in small amounts in addition to the known glycosides, affinosides A, F, H,[2] and

affinogenin D-V.[5] Each of affinosides L_a—L_e was isolated by successive column chromatographies with MCI-gel and silica gel columns, and most of them were crystallized. The BuOH extract was subjected to chromatographic fractionation and affinosides L_e and P were isolated along with known glycosides, affinosides S-I, S-II, S-III, S-VIII, and affinogenins C, D-I, D-II, and D-III.

In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, affinoside L_a (**1**), mp 198—202 °C, $[\alpha]_D$ +55.9 °, showed characteristic peaks ascribable to methoxyl and 6′-methyl groups, besides the signals due to the functions of the aglycone moiety, such as 21-methylene and 22-olefin protons. On the bases of the molecular ion (M^+) peak at *m/z* 562 in the field desorption (FD)-mass spectrum (MS) and elementary analysis, the molecular formula was determined to be $C_{30}H_{42}O_{10}$. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum suggested the presence of one carbonyl group, probably in ring C (δ 212.5). All signals due to the aglycone moiety were in good agreement with those of affinoside C, indicating that **1** is a glycoside of affinogenin C (=2α,3β,14-trihydroxy-11-oxo-5β,14β-card-20(22)-enolide). Upon acetylation with pyridine and Ac_2O, **1** afforded a diacetate (**2**). In the ^{1}H-NMR spectra, H-4′ at δ 3.28 in **1**, which was assigned by means of the decoupling procedure initiated from 6′-CH_3, was shifted downfield to δ 5.25 by acetylation. Therefore, one of the two acylable hydroxyl groups is located at C-4′ of the sugar moiety, and the methoxyl group was supposed to be at C-3′. The C–H coupling constant of C-1′ ($J_{C1'-H1'}$)[6] (J=167 Hz) showed the same value as that of affinoside A,[3] indicating the same binding mode of the sugar moieties in the two glycosides. The 3′-OMe group was confirmed to retain β-orientation on the basis of 17% nuclear Overhauser effect (NOE) between the 3′-OMe protons and the anomeric proton. The orientation of the 4′-hydroxyl group was assigned as α (axial), since the coupling constants of H-4′/H-5′ and H-4′/H-3′ were observed to be 2 and 4 Hz, respectively, in **2**. The component sugar of **1** was therefore considered to be 6-deoxy-3-*O*-methyl-D-gulosulose.



4: 11α-OH
5: 11β-OH

8: R=H
9: R=Ac

Chart 2

On reaction with $NaBH_4$, **1** afforded a dihydro derivative (**3**), or two tetrahydro derivatives (**4** and **5**) at longer reaction time. The splitting of the C_2–O–$C_{2'}$ linkage in these derivatives was apparent from the ^{1}H- and ^{13}C-NMR spectra, since the sharp singlet peak due to the anomeric proton was transformed into a broad singlet (**3**) or a doublet (J=3 Hz) (acetates of **4** and **5**), and one *sec*-carbinyl carbon (δ 72.3) was seen in place of the 2′-acetal carbon (δ 93.3). The $J_{C1'-H1'}$ value of **3** (J=154 Hz) showed the glycosidic linkage to be β.[3,6] The orientation of H-2′ was assigned as *cis* to H-1′, based on the coupling constant of H-1′. Substances **4** and **5** were found to be 11α- and 11β-hydroxy derivatives of **3**, respectively. When a mixture of **4** and **5** was subjected to hydrolysis with 1% HCl in acetone, two aglycones (**6** and **7**) were isolated and determined to be 11-hydroxy derivatives of affinogenin C by comparisons with authentic samples. After refluxing with 1% HCl in methanol, the component sugar was isolated as a methyl glycoside (**8**), from the water-soluble portion of the

TABLE I. ^{1}H Chemical Shifts of Affinoside L_a and Its Derivatives[a)]

	1	2	4-Acetate	5-Acetate	9
18,19-H_3	0.91, 1.04	0.92, 1.05	0.97, 1.25	1.14, 1.24	
21-H_2	4.78 (d, 2)	4.75 (d, 2)	4.81 (br s)	4.70 (dd, 18, 2), 4.96 (dd, 18, 2)	
22-H	5.90 (t, 2)	5.86 (t, 2)	5.82 (br s)	5.83 (t, 2)	
1′-H	4.59[b)]	5.35	5.34 (d, 3)	5.36 (d, 3)	4.83 (br s)
2′-H			5.14 (br s)	5.18 (dd, 1, 3)	5.21 (d, 4)
3′-H	3.21 (d, 4)	4.41 (d, 4)	4.51 (br s)	4.51 (br s)	3.86 (t, 4)
4′-H	3.28 (d, 4)	5.25 (dd, 4, 2)		5.06 (br s)	3.91 (dd, 4, 1)
5′-H	3.58 (q, 6)	3.87 (dq, 2, 6)	3.60 (dq, 1, 6)	3.61 (dq, 1, 6)	5.02 (dq, 1, 6)
6′-H_3	1.39 (d, 6)	1.26 (d, 6)	1.25 (d, 6)	1.25 (d, 6)	1.24 (d, 6)
3′-OMe	3.51[b)]	3.40	3.36	3.35	3.34
Others		2.14 (×2, –OAc)	1.99, 2.05, 2.14 (×2, –OAc), 3.86 (br s, 3α-H)	1.97, 2.14 (×2, –OAc), 3.83 (br s, 3α-H)	2.06, 2.13 (–OAc), 3.38 (1′-OMe)

a) δ (ppm) in $CDCl_3$ from tetramethylsilane (TMS) (*J*/Hz values in parentheses). *b*) On irradiation of 3′-OMe, 17% NOE was observed at 1′-H.

TABLE II. ^{13}C Chemical Shifts of Affinosides L_a—L_e and Their Derivatives, δ (ppm) from TMS in Pyridine-d_5

Carbon	1	3	10	12	15	18	20
C-1	40.6	37.2	33.8[a)]	40.2	41.7	40.6	37.1
C-2	68.0	69.6	68.6	68.3	67.0	68.2	69.6
C-3	69.7	77.6	69.6	69.6	69.7	69.7	77.6
C-4	31.9	27.2[a)]	31.2	32.2	30.8	31.9	27.0[a)]
C-5	38.5	37.7	36.6	34.7	37.3	38.5	37.6
C-6	25.0[a)]	27.0[a)]	24.6	24.7	25.4[a)]	25.0[a)]	27.0[a)]
C-7	24.5[a)]	23.1	22.2	24.7	24.1[a)]	24.5[a)]	23.1
C-8	38.5	43.3	39.0	38.8	38.6	38.5	43.2
C-9	56.9	50.5[b)]	137.3	56.1	54.0	56.9	50.3
C-10	38.3	35.3	40.1	38.8	36.6	38.3	35.2
C-11	212.5	211.2	142.1	213.9	209.8	212.4	211.0
C-12	54.7	55.5	199.3	82.7	79.6	54.7	55.4
C-13	51.2	53.4	60.6	56.1	59.8	51.2	53.4
C-14	83.3	83.7	82.2	83.8	83.2	83.2	83.6
C-15	34.8	33.6	33.5[a)]	34.2	33.9	34.8	33.6
C-16	26.6	26.7[a)]	26.7	27.8	27.3	26.6[a)]	26.7[a)]
C-17	49.9	50.3[b)]	43.3	45.6	46.2	49.9	50.3
C-18	20.0	17.6	15.9	19.6	11.1	19.8	17.5
C-19	21.6	24.0	23.0	21.1	22.0	21.6	24.0
C-20	171.9	174.3	174.1	173.2	175.0	171.6	174.1
C-21	73.6	73.6	73.6	75.3	73.7	73.6	73.3
C-22	117.4	118.1	118.7	117.5	118.1	117.4	118.0
C-23	174.0	174.1	174.1	174.4	174.4	173.8	174.1
C-1′	96.9[d)]	100.1[e)]	97.1	96.8	96.9	97.2[f)]	100.1[g)]
C-2′	93.3	72.3	93.5	93.2	93.2	93.6	73.6[b)]
C-3′	83.3	79.3	83.3	83.2	83.2	75.0	73.3[b)]
C-4′	68.0	70.1[c)]	68.0	68.0	68.0	71.7	72.1
C-5′	71.6	69.8[c)]	71.7	71.6	71.6	73.2	70.7
C-6′	17.4	17.2	17.3	17.3	17.2	17.2	17.2
3′-OMe	56.9	55.2	56.9	56.8	56.9		

a—*c*) Signal assignments marked *a*), *b*) or *c*) in each column may be reversed.
d) $J_{C1'-H1'}$ = 167 Hz. *e*) $J_{C1'-H1'}$ = 154 Hz. *f*) $J_{C1'-H1'}$ = 166 Hz. *g*) $J_{C1'-H1'}$ = 152 Hz.

hydrolyzate. The peracetate of **8** (**9**) showed an anomeric proton signal as broad singlet, and the coupling constants of H-2′/H-3′, H-3′/H-4′, and H-4′/H-5′ as 4, 4, and 1 Hz, respectively. Deshielding of the H-5′ by acetylation (δ 5.02), in comparison with that of **5** (δ 3.60), indicated that **9** retains the furanoside structure. On the basis of the coupling pattern between H-1′ and H-5′, **9** was determined to be methyl 6-deoxy-2,5-di-*O*-acetyl-3-*O*-methyl-α-D-idofuranoside. The structure of **1** was thus established to be as shown in Chart 1.

Upon usual acetylation, affinoside L_b (**10**), mp 248—253 °C, $[\alpha]_D$ +30.1 °, afforded a triacetate (**11**). The same ultraviolet (UV) absorption maximum as that of affinoside B at 285 nm[2a] suggested the presence of a diosphenol moiety in ring C. Therefore, one of the three acylable hydroxyl groups was assigned as the C_{11}-enolic group and the others should be on the sugar moiety. The resonances due to the aglycone and the sugar moiety were identical to those of affinoside B and the sugar moiety of **1**, respectively, in the ^{13}C-NMR spectra.

Affinoside L_c (**12**) was obtained as prisms. Upon acetylation, **12** afforded a monoacetate (**13**) and a triacetate (**14**). A singlet peak at δ 4.43 in **12** showed deshielding in **13** (δ 5.03) and **14** (δ 5.10), suggesting the presence of acylable hydroxyl group in the aglycone. In the ^{13}C-NMR spectrum, the peak of the carbonyl carbon at δ 213.9 was shielded (−6.1 ppm) by acetylation. The carbonyl carbon was therefore considered to be C-11, and the carbinyl carbon, C-12. In fact, the signal patterns due to the aglycone and the sugar moiety were superimposable on those of affinoside E with the 11-oxo-12α-hydroxyl function,[2b] and the sugar moiety of **1**, respectively, so that the structure was assignable. Finally, **12** was transformed into **10** by means of pyridinium chlorochromate (PCC) oxidation, so that the structure of **12** was established.

As in the case of L_c, affinoside L_d (**15**) furnished a monoacetate (**16**) and a triacetate (**17**) upon usual acetylation, and a proton attached to the carbon bearing the acetoxyl group was observed as a singlet at δ 4.84 in **16** and δ 4.78 in **17**, suggesting that **15** also has one acylable hydroxyl group in the aglycone moiety, possibly at C-12. Since the signals of carbonyl carbon (δ 209.8) and carbinyl carbon (δ 79.6) as well as C-18 (δ 11.1) were observed at higher field than those of **12**, **15** was considered to be the 11-oxo-12β-hydroxy compound. Affinoside F[2b] was previously reported to retain the 11-oxo-12β-hydroxyl moiety in addition to the Δ^4-

TABLE III. ^{1}H Chemical Shifts of Affinosides L_b—L_e and Their Acetates[a]

	10	**12**[b]	**14**	**15**[b]	**17**	**18**[b]	**19**
18,19-H_3	0.98, 1.42	1.37	1.03, 1.13	1.02, 1.12	0.80, 1.26	0.93, 1.05	0.93, 1.08
21-H_2	4.89 (d, 2)	4.79 (dd, 19, 2), 5.43 (dd, 19, 2)	4.68 (dd, 18, 2), 4.97 (dd, 18, 2)	4.99 (dd, 18, 2), 5.24 (dd, 18, 2)	4.82 (br s)	4.97 (br s)	4.78 (br s)
22-H	6.03 (t, 2)	6.10 (t, 2)	5.92 (br s)	6.21 (br s)	5.90 (br s)	6.09 (br s)	5.89 (br s)
1′-H	4.60	5.02	5.37	5.00	5.36	5.08	5.57
3′-H	3.22 (d, 3)	3.57 (d, 2)	4.43 (d, 4)		4.42 (d, 4)		5.93 (d, 4)
4′-H	3.30 (dd, 1, 3)	3.98 (d, 2)	5.26 (dd, 1, 4)		5.23 (br d, 4)		5.11 (dd, 2, 4)
5′-H	3.61 (dq, 1, 6)	3.76 (q, 6)	3.88 (dq, 1, 6)				4.00 (dq, 2, 6)
6′-H_3	1.39 (d, 6)	1.55 (d, 6)	1.23 (d, 6)	1.46 (d, 6)	1.25 (d, 6)	1.50 (d, 6)	1.25 (d, 6)
3′-OMe	3.50	3.50	3.40	3.48	3.38		
Others		4.43 (s, 12β-H)	5.10 (s, 12β-H), 2.11, 2.15, 2.19 (–OAc)	4.16 (s, 12α-H)	2.08, 2.14, 2.16 (–OAc), 4.78 (s, 12α-H)		2.07 (×2), 2.15 (–OAc)

a) δ (ppm) in $CDCl_3$ from TMS, unless otherwise mentioned (*J*/Hz values in parentheses). *b*) Dissolved in pyridine-d_5.

function. In the ^{13}C-NMR spectrum of **15**, C-11, C-12, C-13, and C-18 were observed at almost the same chemical shifts as in the case of affinoside F. Since the PCC oxidation of **15** provided **10**, as in the case of **12**, the structure was confirmed.

Affioside L_e (**18**), mp 238—246 °C, $[\alpha]_D$ −21.7°, showed the most polar chromatographic behavior among the five glycosides. The aglycone of **18** was found to be affinogenin C by comparison of the ^{13}C-NMR spectrum with that of **1**. Unlike other affinosides described above, **18** showed no methoxyl signal in the ^{1}H- and ^{13}C-NMR spectra. Upon acetylation, a triacetate (**19**) was obtained. Since the anomeric proton was observed as a singlet at δ 5.08, and the 6′-methyl group at δ 1.50 (d, J=6 Hz), the component sugar was considered to be a 6-deoxy-hexosulose, possibly 6-deoxy-D-gulosulose, in view of the nature of the other glycosides in the leaves. The linking mode as well as the conformation of the sugar moiety appeared to be the same as those of the other glycosides, on the basis of the similar $J_{C1'-H1'}$ value (J=166 Hz) to that of **1**. Since the coupling constants between H-6′/H-5′, H-5′/H-4′, and H-4′/H-3′ in **19** were observed as 6, 2, and 4 Hz, respectively, the 4′-hydroxyl group seemed to retain α-orientation. When **18** was reacted with phenylhydrazine and HOAc,[3,7] the sugar moiety formed an osazone, and the aglycone was determined to be affinogenin C. On reduction with $NaBH_4$, **18** afforded a dihydrate (**20**) having a usual glycosidic linkage. The $J_{C1'-H1'}$ value (J=152 Hz) in **20** indicated the glycosidic linkage to be β. Since the anomeric proton was observed as a singlet peak, the orientation of the newly formed C-2′ hydroxyl group was assigned as axial, as in the case of **1**. In order to compare the structures of **18** and **1**, **18** was subjected to partial methylation according to the procedure of Aritomi and Kawasaki.[8] When **18** was treated with CH_2N_2 in MeOH–$CHCl_3$ mixture containing $SnCl_2$, the methylation proceeded at the 3′-hydroxyl group and the product was determined to be **1** by direct comparison. The sugar moiety of **18** was thus established as 6-deoxy-D-gulosulose.

The aglycones of the major glycosides isolated from the leaves are characterized by the 11-oxo-, $\Delta^{9(11)}$-12-oxo-11-hydroxyl-, 11-oxo-12α-hydroxyl-, and 11-oxo-12β-hydroxyl functions in ring C, as described in the case of the glycosides from the caules.[2] Unlike the glycosides with 4,6-dideoxy-3-*O*-methylgulosulose from the caules and seeds, however, the major glycosides from the leaves appeared to have 6-deoxy-D-gulosulose and its 3-*O*-methylate as a component sugar.

Experimental

Melting points were measured on a Kofler block and are uncorrected. ^{1}H-, ^{13}C-NMR, MS, and UV measurements were conducted in the same manner as described in the preceding papers.[2,3,5] NMR chemical shifts are given in δ values referred to internal TMS, and the following abbreviations are used: s=singlet, br s=broad singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, dq=doublet of quartets, m=multiplet. For TLC and column chromatographies, the following solvent systems were applied; solv. 1, benzene–acetone; solv. 2, $CHCl_3$–MeOH–H_2O (bottom layer); solv. 3, EtOAc–MeOH–H_2O (top layer). Each spot on TLC plates (Kiesel gel 60 F_{254}, Merck) was detected by spraying 10% H_2SO_4 and heating the plates, or by spraying a 1:1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH (Kedde's reagent).

Extraction and Isolation of Glycosides——The leaves were collected in December of 1983 at Sata Experimental Plantation of Kagoshima University. The fresh leaves (22 kg) were homogenized with MeOH, and the methanolic solution was concentrated to 5 l *in vacuo*. Next, 5 l of H_2O was added to the concentrate, and the mixture was extracted with benzene and $CHCl_3$ successively, then the H_2O layer was concentrated to 2 l. The concentrated H_2O layer was extracted with *n*-BuOH saturated with H_2O. The benzene layer (ext. 10 g) was subjected to column chromatography over silica gel with solv. 1 (7:1), and affinoside A was obtained as prisms (60 mg) after crystallization from EtOAc–hexane.

The $CHCl_3$ layer (40 g) was chromatographed on a silica gel column with solv. 1 (7:1—3:1), and solv. 2 (7:1:3—7:1:1). The following substances were obtained: affinosides L_a (100 mg), L_b (50 mg), L_c (500 mg), L_d (130 mg), L_e (275 mg), I (40 mg), N (20 mg), and O (15 mg) and affinogenin D-VI (20 mg), in addition to the known glycosides, affinosides A (180 mg), F (12 mg), H (135 mg), and free cardenolide, affinogenin D-V (10 mg). From the BuOH extract (131 g), affinosides L_e (80 mg) and P (20 mg) were isolated along with the known cardenolides,

affinogenins C (160 mg), D-I (130 mg), D-II (160 mg), and D-III (190 mg) and cardenolide glycosides, affinosides S-I (48 mg), S-II (40 mg), S-III (80 mg), and S-VIII (80 mg) by successive column chromatographies on an MCI-gel column (solv. H_2O–MeOH), and a silica gel column (solv. 1, 5:1—3:1; solv. 2, 7:2:1—7:3:1; solv. 3, 5:1:4—3:1:2).

Affinoside L_a (1)——On crystallization from EtOAc–hexane, **1** afforded prisms, mp 198—202 °C, $[\alpha]_D^{23}$ +55.9° (c=0.73, MeOH). FD-MS m/z: 562 (M^+, $C_{30}H_{42}O_{10}$), 544, 414, 386, 112. *Anal.* Calcd for $C_{30}H_{42}O_{10}\cdot 2H_2O$: C, 60.19; H, 7.74. Found: C, 60.39; H, 7.54. Ac_2O (2 ml) was added to a solution of **1** (100 mg) in pyridine (3 ml), and the mixture was allowed to stand at room temperature for 2 d, then poured into ice–H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on a silica gel column with solv. 1 (5:1). A diacetate of **1** (**2**) (85 mg) was obtained as a solid.

$NaBH_4$ Reduction of 1——a) $NaBH_4$ (100 mg) was added portionwise to a solution of **1** (100 mg) in MeOH (10 ml) under stirring. After further stirring for 1 h at room temperature, the mixture was diluted with H_2O, and extracted with *n*-BuOH. The BuOH extract was purified on a silica gel column with solv. 2 (7:2:1.8) to give a dihydrate of **1** (**3**) as a solid. ^{1}H-NMR (pyridine-d_5): 1.05, 1.25 (3H each, s, 18,19-H_3), 1.50 (3H, d, J=6 Hz, 6′-H_3), 3.35 (1H, br s, 3′-H), 3.50 (3H, s, 3′-OMe), 3.76 (1H, q, J=6 Hz, 5′-H), 4.76 (1H, br s, 1′-H), 5.02 (1H, d, J=19 Hz, 21-H_a), 5.20 (1H, d, J=19 Hz, 21-H_b), 6.12 (1H, br s, 22-H).

b) $NaBH_4$ (70 mg) was added portionwise to a solution of **1** (60 mg) in EtOH (10 ml), and the mixture was stirred at room temperature for 2 h, then worked up in the same way as above. After chromatographic fractionation, two tetrahydro derivatives were isolated, each as a solid, 11α-hydroxyl derivative (**4**) (18 mg) and 11β-hydroxyl derivative (**5**) (26 mg). **5**: ^{1}H-NMR (pyridine-d_5): 1.51 (3H, d, J=6 Hz, 6′-H_3), 1.49, 1.57 (3H each, s, 18,19-H_3), 3.37 (1H, t, J=2 Hz, 3′-H), 3.60 (1H, q, J=6 Hz, 5′-H), 3.97 (1H, br s, 4′-H), 4.98 (1H, dd, J=18, 2 Hz, 21-H_a), 5.17 (1H, d, J=3 Hz, 1′-H), 5.33 (1H, dd, J=18, 2 Hz, 21-H_b), 6.07 (1H, t, J=2 Hz, 22-H). On acetylation of **4** and **5**, a tetraacetate and a triacetate were formed, respectively.

Hydrolysis of a Mixture of 4 and 5——$NaBH_4$ (150 mg) was added portionwise to a solution of **1** (150 mg) in MeOH (15 ml). The mixture was stirred at room temperature for 3 h, and worked up as described above. The BuOH extract was dissolved in 1% HCl in acetone (5 ml) and the solution was allowed to stand for 5 d at room temperature. The reaction mixture was diluted with MeOH, neutralized with IR-410, and the solvent was evaporated off *in vacuo*. The residue was partitioned between *n*-BuOH and H_2O. The BuOH layer was concentrated to dryness *in vacuo* and the residue was chromatographed on a silica gel column with solv. 2 (7:2:1.8) to yield two products, **6** and **7**. Compound **6** (24 mg) was crystallized from EtOAc–hexane to give prisms, mp 258—268 °C, and this product was identified as 2α,3β,11α,14-tetrahydroxy-5β,14β-card-20(22)-enolide by comparison with an authentic sample (melting point, *Rf*, ^{1}H-NMR). ^{1}H-NMR (pyridine-d_5): 1.09, 1.20 (3H each, s, 18,19-H_3), 4.00—4.45 (1H, m, 11β-H), 4.44 (2H, br s, 2β,3α-H), 4.97 (1H, dd, J=19, 2 Hz, 21-H_a), 5.28 (1H, dd, J=19, 2 Hz, 21-H_b), 6.07 (1H, t, J=2 Hz, 22-H). Upon usual acetylation of **6**, a triacetate was obtained. ^{1}H-NMR ($CDCl_3$): 0.99 (6H, s, 18,19-H_3), 1.98, 2.04, 2.06 (3H each, s, –OAc), 4.69 (1H, dd, J=19, 2 Hz, 21-H_a), 4.93 (2H, br s, 2β,3α-H), 4.96 (1H, dd, J=19, 2 Hz, 21-H_b). Compound **7** (24 mg) was crystallized from EtOAc–hexane to give prisms, mp 140—149 °C, and this product was identified as 2α,3β,11β,14-tetrahydroxy-5β,14β-card-20(22)-enolide by comparison with an authentic sample (melting point, *Rf*, ^{1}H-NMR). ^{1}H-NMR (pyridine-d_5): 1.59, 1.64 (3H each, s, 18,19-H_3), 4.49 (2H, br s, 2β,3α-H), 4.98 (1H, dd, J=19, 2 Hz, 21-H_a), 5.36 (1H, dd, J=19, 2 Hz, 21-H_b), 6.07 (1H, t, J=2 Hz, 22-H). Upon usual acetylation of **7**, followed by crystallization from EtOAc–hexane, a diacetate was obtained, mp 235—240 °C. ^{1}H-NMR ($CDCl_3$): 1.15, 1.24 (3H each, s, 18,19-H_3), 2.05, 2.12 (3H each, s, –OAc), 4.16 (1H, br s, 11α-H), 4.76 (1H, dd, J=18, 2 Hz, 21-H_a), 4.91 (2H, br s, 2β,3α-H), 5.01 (1H, dd, J=18, 2 Hz, 21-H_b), 5.88 (1H, t, J=2 Hz, 22-H).

The H_2O layer was concentrated *in vacuo*. The residue was refluxed with 1% HCl in MeOH (2 ml) for 1 h and the solution was neutralized with IR-410. The MeOH was evaporated off *in vacuo*, and the residue was chromatographed on a silica gel column with solv. 2 (7:1:2) to give 5 mg of methyl glycoside (**8**). After usual acetylation of **8**, the peracetate (**9**) was subjected to ^{1}H-NMR measurement.

Affinoside L_b (10)——Affinoside L_b was crystallized from EtOAc–hexane to give prisms, mp 248—253 °C, $[\alpha]_D^{18}$ +30.1° (c=0.78, MeOH). UV λ_{max}^{MeOH} nm (ε): 214 (17000), 285 (10600). FD-MS m/z: 576 (M^+, $C_{30}H_{40}O_{11}$), 558, 418, 112. *Anal.* Calcd for $C_{30}H_{40}O_{11}$: C, 62.48; H, 6.99. Found: C, 62.17; H, 7.01. Upon usual acetylation with Ac_2O and pyridine at room temperature, **10** afforded a triacetate (**11**) as a solid. ^{1}H-NMR ($CDCl_3$): 1.04, 1.22 (3H each, s, 18,19-H_3), 1.27 (3H, d, J=6 Hz, 6′-H_3), 2.08, 2.15, 2.34 (3H each, s, –OAc), 3.38 (3H, s, 3′-OMe), 3.87 (1H, dq, J=1, 6 Hz, 5′-H), 4.42 (1H, d, J=3 Hz, 3′-H), 4.83 (2H, br s, 21-H_2), 5.23 (1H, dd, J=3, 1 Hz, 4′-H), 5.35 (1H, br s, 22-H). ^{13}C-NMR (pyridine-d_5): 170.8 (2×–$\underline{C}OCH_3$), 168.9 (–$\underline{C}OCH_3$), 140.1 (C-9,11), 101.1 (C-2′), 93.1 (C-1′), 77.1 (C-3′), 69.9 (C-4′,5′), 58.5 (3′-OMe), 41.9 (C-17), 21.9 (2×–CO$\underline{C}H_3$), 20.4 (–CO$\underline{C}H_3$).

Affinoside L_c (12)——Affinoside L_c was crystallized from EtOAc–hexane to give prisms, mp 205—213 °C, $[\alpha]_D^{23}$ −24.0° (c=0.30, MeOH). FD-MS m/z: 579 (M^++1, $C_{30}H_{42}O_{11}$). *Anal.* Calcd for $C_{30}H_{42}O_{11}\cdot H_2O$: C, 60.39; H, 7.43. Found: C, 60.67; H, 7.60. A solution of **10** (95 mg) in 3 ml of pyridine was treated with 2 ml of Ac_2O, and the mixture was allowed to stand at room temperature for 1 d, then diluted with ice–H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on a silica gel column with solv. 1 (5:1—3.5:1). A triacetate of **12** (**14**) (25 mg, from the first fraction) was crystallized from MeOH to give needles, mp 218—223 °C, $[\alpha]_D^{23}$ −23.3° (c=0.22, MeOH).

^{13}C-NMR (pyridine-d_5): 207.8 (C-11), 171.8, 171.0, 169.8 (-$\underline{C}OCH_3$), 100.9 (C-2′), 93.0 (C-1′), 84.7 (C-12), 77.1 (C-3′), 69.6 (C-4′,5′), 58.6 (3′-OMe), 54.2 (C-13), 22.0, 21.7, 20.7 (-CO$\underline{C}H_3$). The second fraction contained a monoacetate of **10** (**13**) (20 mg), which was obtained as a solid. ^{1}H-NMR ($CDCl_3$): 1.02, 1.11 (3H each, s, 18,19-H_3), 1.36 (3H, d, J=6 Hz, 6′-H_3), 2.17 (3H, s, -OAc), 3.23 (1H, d, J=4 Hz, 3′-H), 3.48 (3H, s, 3′-OMe), 3.59 (1H, dq, J=1, 6 Hz, 5′-H), 4.54 (1H, s, 1′-H), 4.69 (1H, dd, J=18, 2 Hz, 21-H_a), 4.96 (1H, dd, J=18, 2 Hz, 21-H_b), 5.03 (1H, s, 12β-H), 5.90 (1H, br s, 22-H).

Conversion of 12 into 10——PCC (600 mg) was added portionwise to a solution of **12** (80 mg) in 10 ml of CH_2Cl_2 during 5 h under stirring at room temperature. The reaction mixture was then diluted with $CHCl_3$. The $CHCl_3$ extract was purified on a silica gel column with solv. 2 (7 : 1 : 2.4). The product with an *Rf* value corresponding to that of **10** on TLC was crystallized from EtOAc–hexane to give prisms (5 mg), mp 246—248 °C. On admixture with authentic **10**, no melting point depression was observed, and the *Rf* values of the two samples were in good agreement.

Affinoside L_d (15)——Affinoside L_d was obtained as a solid, $[\alpha]_D^{15}$ +22.2° (c=0.86, MeOH). FAB-MS *m/z*: 579 (M^++1, $C_{30}H_{42}O_{11}$), *Anal.* Calcd for $C_{30}H_{42}O_{11}\cdot 1/2H_2O$: C, 61.31; H, 7.38. Found: C, 61.54; H, 7.25. After acetylation of **15** (50 mg) with Ac_2O and pyridine for 24 h at room temperature, the products were fractionated on a silica gel column with solv. 1 (7 : 1—5 : 1). The first fraction afforded a triacetate (**17**) (4 mg) as a solid. The second fraction was found to be a mixture of diacetates. The third fraction afforded a monoacetate (**16**) (9 mg). ^{1}H-NMR ($CDCl_3$): 0.82, 1.26 (3H each, s, 18,19-H_3), 1.39 (3H, d, J=6 Hz, 6′-H_3), 2.20 (3H, s, -OAc), 3.23 (1H, d, J=4 Hz, 3′-H), 3.48 (3H, s, 3′-OMe), 4.56 (1H, s, 1′-H), 4.84 (3H, br s, 21-H_2, 12α-H), 5.90 (1H, br s, 22-H). As in the case of **12**, **15** (60 mg) was dissolved in 10 ml of CH_2Cl_2 and reacted with 300 mg of PCC for 5 h. The reaction mixture was worked up as described before, and the product was purified on a silica gel column, followed by crystallization from EtOAc–hexane to give prisms (2 mg), mp 245—248 °C. On admixture with authentic **10**, no melting point depression was observed, and the *Rf* values of the two samples were in good agreement.

Affinoside L_e (18)——Affinoside L_e was crystallized from EtOAc to give prisms, mp 238—246 °C, $[\alpha]_D^{19}$ −21.7° (c=1.49, pyridine). *Anal.* Calcd for $C_{29}H_{40}O_{10}$: C, 63.49; H, 7.35. Found: C, 63.18; H, 7.26. Upon usual acetylation at room temperature, a triacetate (**19**) was obtained as a solid.

Reaction of 18 with Phenylhydrazine——Phenylhydrazine (0.05 ml) and HOAc (0.02 ml) were added to a solution of **18** (50 mg) in 2 ml of EtOH, and the mixture was refluxed for 4 h, diluted with H_2O and then extracted with *n*-BuOH. The BuOH extract was chromatographed on a silica gel column with solv. 2 (7 : 1 : 2—7 : 1 : 1.8). The first fraction was crystallized from MeOH–H_2O to give yellow needles (6-deoxy-D-idose osazone) (29 mg), mp 178—180 °C (dec.). On TLC (solv. 2, 7 : 3 : 1), it showed an *Rf* value similar to that of D-fucose osazone, but slight melting point depression was observed on admixture. The second fraction was crystallized from EtOAc to give prisms, mp 272—277 °C. No melting point depression was observed on admixture with authentic affinogenin C.

$NaBH_4$ Reduction of 18——A solution of **18** (100 mg) in 60 ml of MeOH was treated portionwise with 200 mg of $NaBH_4$, and the mixture was stirred for 30 min, then diluted with H_2O and extracted with *n*-BuOH. The BuOH extract was subjected to droplet counter-current chromatography (DCCC) (solv. 2, 5 : 6 : 4, ascending), and the major product (dihydro derivative of **18**) (**20**) (45 mg) was obtained as a solid. ^{1}H-NMR (pyridine-d_5): 1.04, 1.21 (3H each, s, 18,19-H_3), 1.45 (3H, d, J=6 Hz, 6′-H_3), 3.65 (1H, q, J=6 Hz, 5′-H), 4.80 (1H, br s, 1′-H), 4.98 (1H, dd, J=18, 2 Hz, 21-H_a), 5.24 (1H, dd, J=18, 2 Hz, 21-H_b), 6.11 (1H, br s, 22-H). A solution of **20** (30 mg) in 2 ml of 1% HCl in acetone was allowed to stand for 3 d at room temperature, and then diluted with MeOH. The solution was deacidified with IR-410, and the solvent was evaporated off *in vacuo*. The residue was partitioned between *n*-BuOH and H_2O. The BuOH layer was concentrated to dryness *in vacuo*, and the residue was crystallized from EtOAc to give affinogenin C as prisms, mp 274—278 °C (15 mg). The H_2O layer was concentrated *in vacuo*, and the residue showed the lowest *Rf* value on TLC (solv. 2, 7 : 3 : 1) in comparison with D-quinovose, L-rhamnose, 6-deoxy-D-allose, 6-deoxy-D-gulose, and D-fucose.

Partial Methylation of 18[4,8]——$SnCl_2$ (50 mg) and CH_2N_2 in ether were added to a solution of **18** (100 mg) in 30 ml of MeOH–$CHCl_3$ (2 : 1), and the mixture was allowed to stand for 2 h at room temperature. The solvent was evaporated off *in vacuo*, and the residue was chromatographed on a silica gel column with solv. 2 (7 : 1 : 2) to give the major product (30 mg), which was crystallized from EtOAc–hexane as prisms, mp 198—203 °C. On admixture with **1**, no melting point depression was observed, and the *Rf* values were identical to those of **1** on TLC (solv. 2, 7 : 3 : 1, solv. 3, 5 : 1 : 4). The ^{1}H-NMR spectra of the two samples were superimposable.

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