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Affinosides M and K, Cardenolide Glycosides from the Seeds of *Anodendron affine* (Anodendron. V)[1)]

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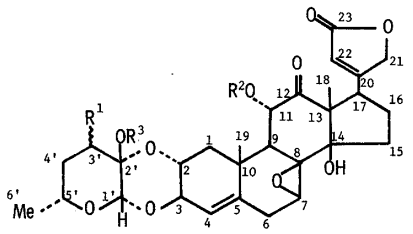
In addition to affinoside A, 3′-*epi*-affinoside A (affinoside M) and 3′-demethylaffinoside A (affinoside K) were isolated from the seeds of *Anodendron affine* DRUCE.

Keywords——cardenolide glycoside; affinoside A; 3′-*epi*-affinoside A; 3′-demethylaffinoside A; doubly linked glycoside; 4,6-dideoxy-3-*O*-methyl-hexosuloside; *Anodendron affine*; Apocynaceae

Plants from the genus *Anodendron* (Apocynaceae) are known to contain cardenolide glycosides with double linkages between the aglycone and sugar moieties, as do those from the genera *Asclepias*, *Gomphocarpus*, and *Calotropis* in Asclepiadaceae[2)] or *Elaeodendron* in Celastraceae.[3)] In the preceding papers of this series, we described the structures of affinosides A—H and J with the double linkages,[1b)] in addition to those of affinosides S-I—S-VIII, with a usual glycosidic linkage,[1a)] and cardenolides free from the component sugar such as affinogenins C and D-I—D-V,[1c)] from the caules and leaves of *A. affine* DRUCE. This paper deals with affinosides M and K, cardenolide glycosides homologous to affinoside A, from the seeds.



affinoside A (I):
$R^1=\beta$-OCH_3, $R^2=R^3=H$
affinoside M (II):
$R^1=\alpha$-OCH_3, $R^2=R^3=H$
affinoside M-monoacetate (IIa):
$R^1=\alpha$-OCH_3, $R^2=Ac$, $R^3=H$
affinoside M-diacetate (IIb):
$R^1=\alpha$-OCH_3, $R^2=R^3=Ac$
affinoside K (III):
$R^1=\beta$-OH, $R^2=R^3=H$

Chart 1

From the MeOH percolate of the seeds, three substances (I, II, and III) reacting positively to Kedde's reagent were isolated in yields of 0.1, 0.027, and 0.017%, respectively. Substance I was identified as affinoside A (I). Substances II and III are new glycosides, although II was present in a small amount in the extract from the caules, and they were named affinosides M and K, respectively.

Affinoside M (II), mp 233—240 °C, $[\alpha]_D^{15}$ −19.1 °, has the same molecular formula as I on the basis of elementary analysis and the field desorption mass spectrum (FD-MS). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, II also showed a similar pattern to I as regards 4-H, 7α-H, 11β-H, 1′-H, 3′-OCH_3, and 6′-CH_3, but not the 3′-carbinyl proton, which was observed as a doublet of doublets (J=4 and 12 Hz), whereas it gave a broad singlet in the spectrum of I. Upon acetylation with Ac_2O and pyridine, II afforded a monoacetate

TABLE I. ^{1}H Chemical Shifts of Affinosides A, M, K, and Their Derivatives[a]

	18-, 19-H_3	21-H_2	22-H	3α-H	4-H	11β-H	1′-H	3′-H	3′-OMe	6′-Me	Others
I	1.07 1.42	4.82 (br s)	5.98	4.50 (9, 2, 1)	5.33	4.82 (11, 2)	4.68	3.28 (br s)	3.39	1.20 (6)	3.47 (7α-H) (5) 3.63 (-OH)
II	1.07 1.43	4.83	5.98	4.56 (9, 2, 2)	5.35	4.81 (12, 2)	4.62	3.30 (12, 4)	3.45	1.30 (6)	3.47 (7α-H) (5)
IIa	1.14 1.26	4.76	5.94	4.52 (br d, 9)	5.36	5.71 (14)	4.58[c]	3.29[c] (12, 4)	3.44	1.31 (6)	3.53 (7α-H) (4) 2.27 (-OAc)
IIb	1.14 1.24	4.76	5.93	4.60 (br d, 9)	5.33	5.70 (13)	5.40[d]	4.35 (12, 4)	3.38	1.30 (6)	3.52 (7α-H) (5) 3.82[e] (5′-H) (m) 2.13[d] 2.24 (-OAc)
III[b]	1.23 1.49	5.04	6.23	4.97 (br d, 9)	5.55	5.22 (13)	5.48	3.50 (t, 2)		1.31 (6)	2.36 (9-H) (13) 3.74 (7α-H) (5)
IIIa	1.08 1.26	4.82	5.99	4.35 (br d, 9)	5.34	4.82 (14)	4.67			1.26 (6)	1.47 -O⟩Me (6×H) -O⟩Me 3.47 (7α-H) (5)
Ia[b]	1.15 1.59	5.12	6.10		5.48				3.50	1.18 (6)	3.63 (7α-H) (5)
Ib[b]	1.29 1.60	5.12	6.21		5.50				3.50	1.18 (6)	
Ic	1.07 1.19	4.79 (br s)	5.90	4.24	5.34	5.42 (11, 2)	4.89 (8)		3.38	1.19 (6)	1.98, 2.06, 2.12, 2.18 (4×-OAc) 4.56 (2′-H) (8, 3) 5.23 (12β-H) (2)
Id	1.20 1.22	4.70 (br s)	5.75	4.17	5.28	5.42 (t, 10)	4.85 (8)		3.35	1.17 (6)	1.97, 2.03, 2.10 (4×-OAc) 4.53 (2′-H) (8, 3) 4.93 (12α-H) (10)
IIc[b]	1.18 1.60	5.12	6.11		5.47				3.42	1.21 (6)	3.70 (7α-H) (5) 4.72 (2′-H) (br s)
IId[b]	1.30 1.61	5.12	6.22		5.50				3.40	1.23 (6)	3.63 (7α-H) (5) 4.79 (2′-H) (br s)
IIe	1.06 1.18	4.77 (br s)	5.91		5.37	5.42 (11, 3)	4.51 (br s)		3.34	1.30 (6)	1.98, 2.08, 2.13, 2.18 (4×-OAc) 5.22 (12β-H) (3)
IIf	1.24 (6H)	4.72 (br s)	5.80		5.35	5.48 (t, 10)	4.53 (br s)		3.35	1.28 (6)	1.99, 2.04, 2.09, 2.13 (4×-OAc) 4.96 (12α-H) (10)

a) δ (ppm) in $CDCl_3$ from TMS unless otherwise mentioned (J/Hz values in parentheses). The abbreviations br s, br d, t, and m, mean broad singlet, broad doublet, triplet, and multiplet, respectively.
b) Dissolved in pyridine-d_5.
c) On irradiation of 3′β-H (δ 3.29), 14% NOE was observed at 1′-H.
d) On irradiation of 1′-H (δ 5.40), 13.7% NOE was observed at 2′-OAc.
e) Doublet of doublets (6, 13 Hz) on irradiation of 6′-Me.

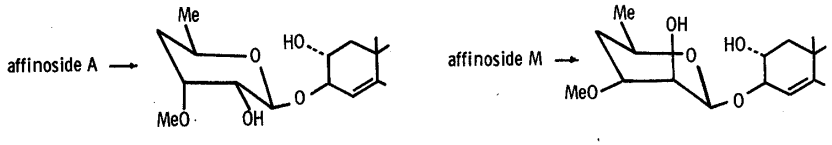
(IIa) and a diacetate (IIb). In the ^{1}H-NMR spectrum of IIb, irradiation of a doublet at δ 1.30, assignable to 6′-methyl protons, transformed a multiplet at δ 3.82, due to 5′-H, into a doublet of doublets with coupling constants of 6 and 13 Hz, suggesting that the 6′-CH_3 group retains equatorial orientation, if the sugar moiety takes the same conformation as in I, and thus the component sugar of II is a D-sugar as in the case of I. Substance II was therefore considered to have a similar structure to I except for the epimeric orientation of the 3′-methoxyl group. The fact that I and II afforded different osazones on reaction with phenylhydrazine,[3,4] also indicates a difference of the C-3′ configuration in the two glycosides. In the carbon-13 nuclear

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

	I	II	III	Ia	Ib	IIc	IId
C-1	44.7	44.3	44.6	46.8	47.3	46.7	47.5
C-2	67.3	66.7[a]	67.6	67.1[a]	67.1[a]	67.4[a]	67.4[a]
C-3	70.5	70.3	70.4	68.7[a]	68.8[a]	69.0[a]	69.0[a]
C-4	123.2	122.7	123.2	123.3	123.8	122.9	122.9
C-5	140.0	139.7	140.0	141.0	140.8	141.6	141.5
C-6	30.3	30.1	30.3	30.5[b]	30.5	30.6[b]	30.6
C-7	53.9	53.7	53.8	52.8	52.4	52.8	52.4
C-8	64.6	64.3	64.6	64.3	64.1	64.4	64.1
C-9	49.1	48.9	49.0	46.0	48.1	46.0	48.0
C-10	41.3	40.9	41.2	39.3	40.0	39.3	40.0
C-11	74.6	74.2	74.8	70.8	72.0	70.8	72.0
C-12	212.7	212.0	212.6	79.7	80.3	79.6	80.2
C-13	63.6	63.3	63.5	55.1	57.8	55.1	57.8
C-14	82.0	81.7	81.9	81.1	81.3	81.1	81.3
C-15	36.5	36.3	36.5	36.9	35.7	36.9	35.7
C-16	28.6	28.4	28.6	30.8[b]	29.0	30.8[b]	29.0
C-17	42.4	42.2	42.5	44.8	47.5	44.8	47.5
C-18	18.5	18.3	18.5	18.2	11.9	18.2	11.9
C-19	21.3	21.1[b]	21.3[a]	21.0[c]	21.1[b]	21.1[c]	21.0[b]
C-20	173.1	172.6	173.0	175.4	174.8	175.4	174.8
C-21	73.9	73.7	73.9	74.1	73.8	74.1	73.8
C-22	118.7	118.3	118.7	117.7	117.7	117.7	117.7
C-23	174.1	173.7	174.0	174.1	174.3	174.1	174.3
C-1′	96.2	97.1	95.9	101.8	101.8	101.3	100.5
C-2′	92.0	92.6	92.1	78.9	78.9	78.7	78.7
C-3′	81.4	82.6	71.6	83.2	83.2	83.6	82.5
C-4′	35.1	35.0	38.1	37.2	37.2	33.4	33.3
C-5′	66.4	68.1[a]	66.5	72.2	72.2	68.6[a]	68.6[a]
C-6′	21.3	21.4[b]	21.5[a]	21.3[c]	21.3[b]	21.2[c]	21.3[b]
3′-OMe	58.3	57.7		58.2	58.2	55.2	55.3

a—c) Signal assignments marked *a*), *b*) or *c*) in each column may be reversed.

magnetic resonance (^{13}C-NMR) spectrum of II, carbon signals due to the aglycone were identical with those of I within 0.7 ppm, except for C-1′, C-3′, and C-5′ of II, which were at lower field than those of I by +0.9, +1.2, and +1.7 ppm, respectively. These deshieldings are consistent with equatorial orientation of the 3′-methoxyl group in II.



Ia and Ib IIc and IId

Chart 2

Since the C–H coupling constant of the C-1′ signal ($^1J_{C1'-H1'}$) of II was 168 Hz, which is the same as that of I, the state of the anomeric carbon was expected to be the same in the two glycosides. In order to confirm the glycosidic linkage of II to be β, the reductive cleavage of

C_2–O–$C_{2'}$ was attempted. When I and II were reacted with $NaBH_4$ in MeOH, two major products (Ia and Ib; IIc and IId) were obtained in each case. The ^{1}H-NMR spectra of these compounds indicated that the reduction proceeded at the C-2′ hemiacetal linkage as well as the 12-oxo function to afford 12α-hydroxy (Ia and IIc) and 12β-hydroxy (Ib and IId) derivatives. The identities of the sugar moieties in Ia and Ib, and IIc and IId were confirmed by analysis of the ^{13}C-NMR spectra, and by direct comparisons of the sugars from Ia and Ib, and those from IIc and IId by means of thin layer chromatography (TLC) and paper chromatography (PC). The $^1J_{C1'-H1'}$ values of Ia and Ib, in which the glycosidic linkages are already known to be β, were 159.5 and 160.2 Hz, respectively, and those of IIc and IId were both 157.2 Hz, suggesting β-glycosidic linkages. Since the anomeric protons of Ia and Ib appeared as a doublet of 8 Hz, while those of IIc and IId appeared as a broad singlet, the orientation of the C-2′ hydroxyl group in the former two compounds is concluded to be equatorial and that in the latter two, to be axial. The sugar moieties of Ia and Ib were therefore assigned as 4,6-dideoxy-3-*O*-methylalloside, and those of IIc and IId were considered to be 4,6-dideoxy-3-*O*-methylmannoside. In fact, the $^1J_{C1'-H1'}$ values of IIc and IId are in good agreement with the value of *ca.* 156 Hz of β-D-mannoside,[5] while those of Ia and Ib are close to that of *ca.* 160 Hz of β-D-glucoside.[6]

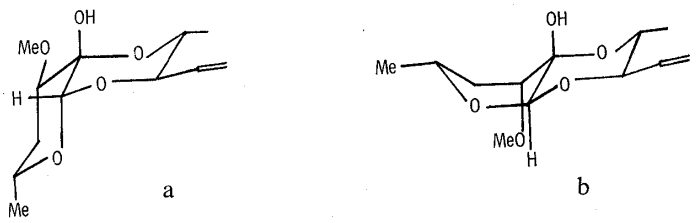


Chart 3

There are two possible structures, a and b, when the orientations of the two oxygen atoms linked to C-1′ of II are the same as in I. Since the doubly linked glycosides so far known in nature take the conformation a, II was also expected to retain the form a. On irradiation of 3′-H in IIa, 14% nuclear Overhauser effect (NOE) was found at 1′-H, while 13.7% NOE was observed at 2′-OAc on irradiation of 1′-H in IIb. The structure of II was thus determined to be 3′-*epi*-I, retaining the same conformation as I.

Affinoside K (III) was obtained as a solid. Unlike affinosides A—J from the caules, III showed no methoxyl protons in the ^{1}H-NMR spectrum, although other signals due to the aglycone moiety of I, such as 4-H, 7α-H, 11β-H, were observed. In the sugar moiety, 3α-H appeared as a triplet of 2 Hz, in addition to three protons of the 6′-methyl group and a characteristic sharp singlet of an anomeric proton, and these resonances suggested that III is 3′-demethyl-I. The sugar moiety of III showed ^{13}C-NMR signals similar to those of gomphoside,[2] which is a doubly linked cardenolide glycoside with a 4,6-dideoxy-allosulose moiety, although the measurements were taken in different solvents. When III was reacted with acetone containing H_2SO_4, an acetonide (IIIa) was formed, so that the presence of a 2′, 3′-glycol moiety in III was confirmed.

The typical glycosides from the caules retain a 4,6-dideoxy-3-*O*-methylhexosulose moiety, whereas those from the leaves are characterized by a 6-deoxy-3-*O*-methylhexosulose sugar moiety.[7] The glycosides from the seeds therefore seem to be rather similar to those from the caules.

Experimental

Melting points were measured on a Kofler block and are uncorrected. ^{1}H-, ^{13}C-NMR, and MS measurements

were taken in the same manner as described in the preceding papers.[1] For TLC and column chromatographies, the following solvent systems were applied: solv. 1, benzene–acetone; solv. 2, EtOAc–hexane; solv. 3, $CHCl_3$–MeOH–H_2O (7:2:1, bottom layer); solv. 4, EtOAt–MeOH–H_2O (5:1:4, top layer); solv. 5, EtOAc–MeOH–H_2O (4:1:0.5). Each spot on the TLC plates 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).

Isolation of Glycosides——The seeds were collected in March of 1981 and 1982 from plants cultivated in the medicinal plant garden of Fukuoka University. The seeds (300 g) were homogenized with MeOH. The MeOH homogenate was filtered and the filtrate was concentrated to 200 ml *in vacuo*, and diluted with water to 400 ml. The whole solution was partitioned with hexane, and then with $CHCl_3$. The water layer was concentrated to half the initial volume *in vacuo*, diluted with water, and then extracted with *n*-BuOH.[8] Among these fractions, only the $CHCl_3$ fraction (2.0 g) showed a positive reaction with Kedde's reagent on TLC, and was used for the isolation of the cardenolide glycosides. The $CHCl_3$ extract was chromatographed successively on a silica gel column with solv. 1 (7:1—3:1) and solv. 3 (7:1:3—7:1:1), and finally three substances were isolated as crystals (I and II), and a solid (III). Substance I (mp 250—255 °C) was identified as affinoside A by comparison of the melting point and *Rf* values with those of authentic I.

Affinoside M (II) (Substance II)——Affinoside M showed more polar *Rf* values than I on TLC with solv. 1 and the same as I with solv. 3. II was crystallized from EtOAt–hexane to give prisms, mp 233—240°C, $[\alpha]_D^{15}$ −19.1° (*c*=1.07, MeOH), (yield: 0.027%), *Anal.* Calcd for $C_{30}H_{38}O_{11} \cdot 1/2H_2O$: C, 61.74; H, 6.74. Found: C, 61.99; H, 6.61. FD-MS *m/z*: 574 (M^+). II (30 mg) was treated with 1 ml each of Ac_2O and pyridine at room temperature for 20 h, and the mixture was diluted with ice-water. The solution was extracted with $CHCl_3$ and the $CHCl_3$ extract was chromatographed on a silica gel column with solv. 1 (7:1). A monoacetate of II (IIa) was crystallized from EtOAc–hexane to give prisms, mp 215—218 °C. When II was reacted with Ac_2O and pyridine at 60 °C for 3 h, and then worked up in the same manner as in the preparation of IIa, a diacetate of II(IIb) was obtained as a solid.

An osazone from II was prepared with 8 mg of II in EtOH (1 ml) and 0.01 ml each of phenylhydrazine and HOAc. The mixture was refluxed for 1.5 h and the solution was concentrated *in vacuo*. The residue was chromatographed on a silica gel column with solv. 1 to give a yellow solid, MS *m/z*: 340 (M^+). An osazone from I was prepared by the same procedure as in the case of II, and the osazone was obtained as needles on crystallization from dil. EtOH, mp 147—150 °C, *m/z*: 340 (M^+). On TLC of the two osazones with solv. 1, 2, or 3, the former osazone showed slightly more polar behavior than the latter.

$NaBH_4$ Reduction of II and I——a) II (150 mg) was dissolved in MeOH (20 ml). The solution was stirred at room temperature and 200 mg of $NaBH_4$ was added portionwise. After being stirred for 1 h, the reaction mixture was diluted with water and extracted with *n*-BuOH. The BuOH extract was purified by chromatography on a silica gel column with solv. 3 (7:2:1) to give two major products (IIc and IId), each as a solid. On TLC with solv. 3, IIc (33 mg), $[\alpha]_D^{17}$ −42.6° (*c*=1.69, MeOH), FD-MS *m/z*: 579 ($M^+ + 1$), showed a less polar *Rf* value than IId, and was assigned as 3-*O*-(4,6-dideoxy-3-*O*-methyl-D-mannosyl)-7β,8β-epoxy-2α,3β,11α,12α,14-pentahydroxy-14β-carda-4,20(22)-dienolide. IId (32 mg) was assigned as the 12β-hydroxy isomer of IIc; $[\alpha]_D^{17}$ −29.4° (*c*=1.63, MeOH), FD-MS *m/z*: 579 ($M^+ + 1$). The 2,11,12,2′-tetraacetate of IIc and IId were obtained in the usual manner, each as a solid (IIe and IIf, respectively).

b) $NaBH_4$ reduction of I was conducted in the same manner described for II with 150 mg of I and 200 mg of $NaBH_4$. The major products (Ia and Ib) were each obtained as a solid. On TLC with solv. 3, Ia (30 mg) showed a less polar *Rf* value than Ib, and was assigned as 3-*O*-(4,6-dideoxy-3-*O*-methyl-D-allosyl)-7β,8β-epoxy-2α,3β,11α,12α,14-pentahydroxy-14β-carda-4,20(22)-dienolide, $[\alpha]_D^{15}$ −52.2° (*c*=1.56, MeOH), FD-MS *m/z*: 579 ($M^+ + 1$). Ib (19 mg) was identified as the 12β-hydroxy isomer of Ia; $[\alpha]_D^{15}$ −55.8° (*c*=1.62, MeOH), FD-MS *m/z*: 579 ($M^+ + 1$). The acetates of Ia and Ib were each obtained as a solid after usual acetylation (Ic and Id, respectively).

Ia, Ib, IIc, and IId (30 mg each) were each hydrolyzed with 1 ml of 0.5 N H_2SO_4–50% EtOH at 100 °C for 1 h. The reaction mixtures were concentrated to half the initial volume, deacidified with IR-410, and then extracted with *n*-BuOH. After extraction with *n*-BuOH, the water layers were concentrated to dryness *in vacuo*, and the residues were examined by TLC and PC. On TLC with solv. 5, the sugars from Ia and Ib, and those from IIc and IId showed the same *Rf* values; those from Ia and Ib showed less polar behavior than those from IIc and IId. On PC with a solvent system of *n*-BuOH–EtOH–H_2O (5:1:4, top layer, 20 h, descending), the following $R_{rham.}$ values were obtained: sugar from IIc, 2.0; from IId, 2.0; from Ia, 1.9; from Ib, 1.9; L-oleandrose, 2.08; D-cymarose, 2.02; D-diginose, 1.86; D-digitoxose, 1.61; D-digitalose, 1.17; L-rhamnose, 1.00 (*Rf* 0.36).

Affinoside K (III) (Substance III)——Affinoside K was obtained as a solid, $[\alpha]_D^{27}$ +10.3° (*c*=1.36, MeOH) (yield: 0.017%), FD-MS *m/z*: 561 ($M^+ + 1$, $C_{29}H_{36}O_{11}$). III (15 mg) was reacted with 2 ml of acetone containing 1 mg of H_2SO_4 for 20 h at room temperature. The mixture was diluted with MeOH and deacidified with IR-410. The methanolic solution was concentrated to dryness *in vacuo*, and the residue was purified by chromatography on a silica gel column with solv. 1 (7·1) to give III-acetonide (IIIa) as a solid, $[\alpha]_D^{15}$ +0.73° (*c*=0.55, MeOH), FD-MS *m/z*: 600 (M^+, $C_{32}H_{40}O_{11}$).

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