Pergamon

0031-9422(94)00830-2

Phytochemistry, Vol. 38, No. 6, pp. 1431–1435, 1995

0031-9422/95 $9.50 + 0.00

ALANGIONOSIDES C–F, MEGASTIGMANE GLYCOSIDES FROM *ALANGIUM PREMNIFOLIUM*

HIDEAKI OTSUKA,* KENJI KAMADA, MASAMI YAO, KAORI YUASA, IKUKO KIDA and YOSHIO TAKEDA†

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima 734, Japan: †Faculty of Integrated Arts and Sciences, The University of Tokushima, 1-1 Minamijosanjima-cho, Tokushima 770, Japan

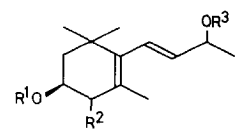
(*Received in revised form* 3 *October* 1994)

Key Word Index—*Alangium premnifolium*; Alangiaceae; leaves; megastigmane glycoside; ionol glycoside; alangionosides C–F; plucheoside B.

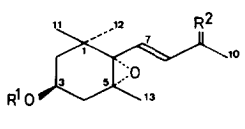
Abstract—From a methanol extract of leaves of *Alangium premnifolium*, four new megastigmane glycosides, alangionosides C–F, together with a known compound, plucheoside B, were isolated. The structures of the new compounds were elucidated from spectroscopic and chemical evidence.

INTRODUCTION

Recently, a variety of megastigmane (ionol) glycosides have been isolated from various plant families, including Vitaceae [1], Apocynaceae [2] and Beriberidaceae [3], and the number of families with this skeleton is increasing. An alangiaceous plant, *Alangium premnifolium*, was also found to be a good source of megastigmane glycosides, such as alangionosides A and B [4]. Further phytochemical investigation of this species has resulted in the isolation of four new megastigmane glycosides, along with a known ionol glucoside, plucheoside B.



	R^1	R^2	R^3
1	Glu	β-OH	H
2	H	β-OGlu	H
3	Glu	α-OH	H
9	Glu(OAc)$_4$	β-OAc	Ac
10	Glu(OAc)$_4$	α-OAc	Ac



	R^1	R^2
4	Glu	$\angle^{OH}_{H}$
5	Glu	=O
6	Glu(6-1)Xyl	$\angle^{OH}_{H}$
7	H	$\angle^{OGlu}_{H}$
8	H	$\angle^{OH}_{H}$
11	Glu(OAc)$_4$	$\angle^{OAc}_{H}$
12	Ac	$\angle^{OAc}_{H}$

RESULTS AND DISCUSSION

A *n*-butanol fraction was obtained by solvent partitioning of a methanol extract of the title plant. Through a combination of various kinds of column chromatography, five megastigmane glycosides were isolated.

Compound **1** was obtained as an amorphous powder and characterized as plucheoside B, previously isolated from *Pluchea indica* [5], based on the spectroscopic evidence. Although its absolute stereochemistry was not fully assigned, the structures of plucheoside B and related compounds are tentatively depicted in the formulae as 3*S*-forms.

Alangionoside C (**2**) was obtained as an amorphous powder and its elemental composition, $C_{19}H_{32}O_8$, determined by HR-FAB-mass spectrometry, was the same as that of plucheoside B (**1**); its UV absorption maximum (222 nm) was also similar to that of **1**. In addition to the signals owing to β-glucopyranose, the ^{13}C NMR spectrum showed the presence of three aliphatic methyl carbons, two of which appeared as singlets and the remaining one as a doublet (J = 6 Hz) in the ^1H NMR spectrum, one allylic methyl group (δ1.88), three methines with oxygen functions, one methylene group and disubstituted and tetrasubstituted double bonds which were conjugated to each other. These spectral data suggested that the aglycone portion of **2** consisted of a megastigmane skeleton with the same location of the double bonds as in **1**. In the ^1H–^1H COSY spectrum, H-4 (δ3.99) long-range coupled with the allylic methyl proton, correlated to H-3, which in turn showed cross-peaks with methylene protons at the 2-position. This indicated the planar structure of the ionone framework to be the same as **1**. The essentially similar coupling constants between H-3 and H-4 in **1** (J = 4 Hz) and **2** (J = 3 Hz) indicated that the vicinal hydroxyl substituents in **2** were placed in a cisoid relationship. Thus, **2** was considered to be a positional isomer of **1** with regard to the glucopyranose moiety. Since the C-4 (δ84.3) was obviously affected by the glucosylation-induced downfield shift in the

*Author to whom correspondence should be addressed.

^{13}C NMR spectrum, the structure of **2** was elucidated to be that shown in the formulae.

Alangionoside D (**3**) was obtained as an amorphous powder, whose elemental composition was the same as that of the above compounds. The ^{1}H and ^{13}C NMR spectra also indicated that **3** had the same functionality. Upon irradiation of the 13-methyl proton signal in the difference NOE experiment, significant enhancement of the signal intensity of δ3.90 revealed the same disposition of substituents on the six-membered ring as those in **1** and **2**. Alangionoside D was considered to be an epimer of a hydroxyl substituent at the 4-position in **1** and **2** based on the following three reasons. First, the hydroxyl group at the 3-position must be in an equatorial orientation ($J_{H-2ax-H-3}$ = 13 Hz). Second, the equatorial proton at the 2-position lost long-range coupling via the *W* interaction with H-4, which was seen in plucheoside B and alangionoside C. Third, the coupling constant between the protons at the 3- and 4-positions was 7 Hz, which indicated that two hydroxyl substituents were in a *trans*-relationship. Since the ^{13}C NMR chemical shifts of the side-chain were virtually the same as those in **1** and **2**, the β-glucopyranose moiety should be linked to the hydroxyl group at the 3- or 4-positions. On acetylation of **3**, the corresponding hexaacetate (**10**) was obtained. The ^{1}H NMR spectrum of this acetate clearly disclosed the site of glucosylation to be at C-4, as judged from the fact that the proton at the 4-position was obviously shifted downfield (δ_H 3.90 → 5.41) by acylation, whereas that at the 3-position remained almost intact (δ_H 3.83 → 3.89). Thus, the structure of alangionoside D was determined to be 4-epi-plucheoside B, shown as **3** in the formulae.

Alangionoside E (**4**) was obtained as an amorphous powder, whose elemental composition was determined to be $C_{19}H_{32}O_8$ by HR-FAB-mass spectroscopy. The ^{13}C NMR spectrum showed the presence of a β-glucopyranose moiety and the remaining 13 signals, including those of four methyl groups, indicating that the aglycone skeleton is a derivative of megastigmane. A disubstituted *trans*-double bond must be present at $\Delta^{7,8}$ in the side-chain and two methine signals with a hydroxyl substituent were assigned to the 3- and 9-positions on the basis of a ^{1}H–^{1}H COSY experiment. Only two locations, the 5- and 6-positions, were thus available for two quaternary carbons with an oxygen function. Finally, one more double bond or an equivalent system, as demanded by the elemental composition, must be satisfied by the formation of an epoxy ring between the 5- and 6-positions. This was supported by comparison with the ^{13}C NMR data for a similar compound, icariside B_2 (**5**), obtained from *Epimedium grandiflorum* var. *thunbergianum* (Table 1) [3]. The planar structure of the aglycone, thus elucidated, was the same as that of epoxyactinidioionoside (**7**), obtained from *Actinidia polygama* [6] and flue-cured tobacco [7]. The location of the glucopyranose moiety in **4** was determined to be at the 3-position, based on the evidence that the ^{13}C NMR chemical shift of the 9-position (δ68.7) was essentially the same as that of alangionoside C and, on acetylation of **4**, the 9-position shifted downfield (δ4.28 → 5.36), whereas the 3-position remained intact (δ3.89 → 3.80) in the ^{1}H NMR spectrum. This was also supported by the ^{13}C NMR spectrum of the aglycone (**8**) obtained by enzymatic hydrolysis of **4** [$\Delta\delta_{C-3(8)} - \delta_{C-3(5)} = -8.4$]. The absolute configurations were assigned as 3*S*, 5*R* and 6*S*, since dichlorodicyanobenzoquinone (DDQ) oxidation of the allyl alcohol of **4** gave a 9-oxo compound, namely icariside B_2 (**5**) [3]. Therefore, the structure of alangionoside E was determined to be **4**, as shown in the formulae. However, the absolute configuration of the 9-position still remains to be determined.

Table 1. ^{13}C NMR spectral data for compounds **1**–**6** (CD_3OD and/or pyridine-d_5 in parentheses, 100 MHz)

C	1	2	3	5†	4	6
1	37.9 (36.9)*	37.9	37.7	(35.1)	35.9	35.9
2	40.0 (39.7)	42.9	44.8	(44.8)	45.8	45.7
3	76.1 (74.7)	67.5	81.9	(71.5)	73.0	73.2
4	70.1 (68.8)	84.3	77.0	(37.8)	38.5	38.5
5	127.9 (125.0)	127.6	129.6	(67.1)	67.7	67.7
6	143.0 (141.3)	143.1	141.2	(69.9)	71.4	71.4
7	126.7 (127.9)	126.6	127.0	(143.0)	125.8	125.8
8	140.7 (141.3)	140.9	140.7	(133.3)	139.1	139.1
9	69.5 (68.2)	69.5	69.5	(197.1)	68.7	68.7
10	23.8 (24.7)	23.9	23.9	(27.7)	23.8	23.8
11	27.8 (27.4)	27.5	28.1	(29.0)	25.4	25.3
12	30.3 (30.0)	30.3	30.6	(22.5)	29.8	29.8
13	19.9 (20.2)	19.9	17.0	(20.2)	20.3	20.3
1′	102.7 (101.6)	106.1	105.3	(103.2)	102.9	103.4
2′	75.3 (75.6)	75.2	75.7	(75.3)	75.2	74.5
3′	78.1 (79.0)	78.2	78.1	(78.7)	78.2	77.7
4′	71.6 (71.7)	71.5	71.7	(71.8)	71.6	71.5
5′	78.0 (78.7)	78.0	78.0	(78.4)	77.8	76.8
6′	62.7 (62.8)	62.6	62.8	(62.8)	62.7	69.8
1″						105.5
2″						75.1
3″						78.0
4″						71.2
5″						66.9

*Data taken from ref. [5] (22.5 MHz).
†Data taken from ref. [3] (22.5 MHz).

Alangionoside F (**6**) was also obtained as an amorphous powder. The ^{13}C NMR spectrum showed that the aglycone portion was essentially the same as that in alangionoside E, but one more sugar unit was present. On comparison of the signals in the sugar region with reported data [8], five typical signals [δ_C 105.5 (*d*), 75.1 (*d*), 76.8 (*d*) 71.5 (*d*), 66.9 (*t*)] were reasonably assigned as those from a β-xylopyranose moiety. The remaining six signals were those from a 6-glycosylated β-glucopyranose moiety. Thus, the structure of alangionoside F was established to be 6′-β-D-xylopyranosyl alangionoside E (**6**), as shown in the formulae.

EXPERIMENTAL

General. Mp: uncorr. ^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively, with TMS

as the int. standard. The droplet counter-current chromatograph (DCCC) was equipped with 500 glass columns (40 cm length; 2 mm i.d.). The ascending method was used with $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) and 5 g frs were collected from the beginning of elution with the mobile phase. Reverse phase gravity column chromatography (RPCC) was performed with ODS (Cosmosil $75C_{18}$ – OPN; 55 × 250 mm) and a linear solvent gradient from 10% (1 l) to 50% MeOH (1 l); 10 g frs were collected. Prep. reverse-phase HPLC was performed with ODS (Inertsil; 20 × 250 mm, flow rate, 6 ml min^{-1}). Emulsin was purchased from Sigma.

Extraction and isolation procedures. The plant material was the same as that used in the previous study [4]; part of the extraction and fractionation was also described. The residue of the 40% MeOH eluate (fr. 5, 18.4 g) on Diaion CC was sepd by silica gel CC (450 g) with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$, (2 l), $CHCl_3$–MeOH (100:1, 3 l), (50:1, 6 l), (97:3, 6 l), (24:1, 6 l), (47:3, 6 l), (23:2, 6 l), (9:1, 6 l), (7:1, 6 l) and (17:5, 6 l)], frs of 500 ml being collected. The residue of the 6% MeOH eluate (0.93 g) was purified by RPCC (frs 93–101, 185 mg), followed by DCCC (frs 51–81) to give 100 mg of alangionoside E (**4**). The residue of the 8% MeOH eluate (1.35 g) was purified by RPCC (frs 116–125, 116 mg), followed by HPLC (MeOH–H_2O, 1:3) to give 32 mg of plucheoside B (**1**). The residue of the 12.5% MeOH eluate (1.79 g) was purified by RPCC (frs 125–134, 166 mg), followed by DCCC (frs 28–32) to give 86 mg of crude alangionoside F (**6**), which was finally purified by HPLC (MeOH–H_2O, 3:7) to yield **6** in a pure state (64 mg).

The residues of the 40 and 60% MeOH eluates (frs 5–8) on Diaion CC were sepd by silica gel CC in a similar way. The residue of the 10% MeOH eluate (2 g out of 3.18 g) was sepd by RPCC (frs 150–160, 150 mg) and then by DCCC. The residue of frs 44–50 (25 mg) was purified by HPLC (MeOH–H_2O, 3:7) to give 4.4 mg of alangionoside D (3). Purification of the residue of the DCCC fraction (frs 51–62, 34 mg) by HPLC (MeOH–H_2O, 7:13) afforded 14 mg of alangionoside C (**2**).

Plucheoside B (**1**). Amorphous powder. $[\alpha]_D^{23}$ – 65.2° (MeOH; *c* 1.43). UV λ_{max}^{MeOH} nm (log ε): 206 (3.78), 223 (3.73). ^{1}H NMR (CD_3OD): δ 1.04 (eq), 1.08 (ax) (3H each, each *s*, H_3-11 and H_3-12), 1.26 (3H, *d*, *J* = 7 Hz, H_3-10), 1.58 (H, *ddd*, *J* = 1, 3, 12 Hz, H-2eq), 1.84 (3H, *d*, *J* = 1 Hz), 1.88 (H, *t*, *J* = 12 Hz, H-2ax), 3.23 (H, *dd*, *J* = 8, 9 Hz, H-2′), 3.39 (H, *t*, *J* = 9 Hz, H-3′), 3.68 (H, *dd*, *J* = 5, 12 Hz, H-6′a), 3.86 (H, *dd*, *J* = 2, 12 Hz, H-6′b), 3.98 (H, *td*, *J* = 4, 13 Hz, H-3), 4.08 (H, *br d*, *J* = 4 Hz, H-4), 4.30 (H, *dq*, *J* = 1, 7 Hz, H-9) 4.50 (H, *d*, *J* = 8 Hz, H-1′), 5.52 (H, *dd*, *J* = 6, 16 Hz, H-8), 6.03 (H, *dd*, *J* = 1, 16 Hz, H-7). ^{13}C NMR (CD_3OD): see Table 1; ^{13}C NMR (pyridine-d_5): see Table 1, essentially indistinguishable from reported data [5]. HR-FAB-MS (negative centroid) *m/z*: 387.1996 $[M - H]^-$ ($C_{19}H_{31}O_8$ requires 387.2019).

Alangionoside C (**2**). Amorphous powder. $[\alpha]_D^{23}$ – 58.5° (MeOH; *c* 0.91). UV λ_{max}^{MeOH} nm (log ε): 207 (3.71), 222 (3.72). ^{1}H NMR (CD_3OD): δ 1.00 (eq), 1.07 (ax) (3H each, each *s*, H_3-11 and H_3-12), 1.26 (3H, *d*, *J* = 6 Hz, H_3-10), 1.48 (H, *ddd*, *J* = 1, 4, 13, H-2eq), 1.79 (H, *t*, *J* = 13 Hz, H-2ax), 1.88 (3H, *d*, *J* = 1 Hz, H_3-13), 3.25 (H, *t*, *J* = 8 Hz, H-2′), 3.67 (H, *dd*, *J* = 5, 12 Hz, H-6′a), 3.79 (H, *td*, *J* = 3, 13 Hz, H-3), 3.87 (H, *dd*, *J* = 2, 12 Hz, H-6′b), 3.99 (H, *br d*, *J* = 3 Hz, H-4), 4.30 (H, *d quin*, *J* = 1, 6 Hz, H-9), 4.43 (H, *d*, *J* = 8 Hz, H-1′) 5.53 (H, *dd*, *J* = 6, 16 Hz, H-8), 6.01 (H, *br d*, *J* = 16 Hz, H-7). ^{13}C NMR: see Table 1. HR-FAB-MS (negative centroid) *m/z*: 387.2039 $[M - H]^-$ ($C_{19}H_{31}O_8$ requires 387.2019).

Alangionoside D (**3**). Amorphous powder. $[\alpha]_D$ – 61.4° (MeOH; *c* 0.29). UV λ_{max}^{MeOH} nm (log ε): 207 (3.79), 227 inf. (3.63). ^{1}H NMR (CD_3OD): δ 1.02 (eq), 1.09 (ax) (3H each, each *s*, H_3-11 and H_3-12), 1.26 (3H, *d*, *J* = 6 Hz, H_3-10), 1.75 (3H, *t*, *J* = 1 Hz, H_3-13), 1.56 (H, *t*, *J* = 13 Hz, H-2ax), 1.98 (H, *dd*, *J* = 4, 13 Hz, H-2eq), 3.22 (H, *dd*, *J* = 8, 9 Hz, H-2′), 3.68 (H, *dd*, *J* = 6, 12 Hz, H-6′a), 3.83 (H, *ddd*, *J* = 4, 7, 13 Hz, H-3), 3.86 (H, *dd*, *J* = 2, 12 Hz, H-6′b), 3.90 (H, *qd*, *J* = 1, 7 Hz, H-4), 4.29 (H, *d quin*, *J* = 1, 6 Hz, H-9), 4.56 (H, *d*, *J* = 8 Hz, H-1′), 5.49 (H, *dd*, *J* = 6, 16 Hz, H-8), 6.02 (H, *d quin*, *J* = 1, 16 Hz, H-7). ^{13}C NMR: see Table 1. HR-FAB-MS (negative centroid) *m/z*: 387.2044 $[M - H]^-$ ($C_{19}H_{31}O_8$ requires 387.2019).

Alangionoside E (**4**). Amorphous powder. $[\alpha]_D^{23}$ – 50.5° (MeOH; *c* 1.33). ^{1}H NMR (CD_3OD): δ 0.96 (3H, *s*, H_3-12ax), 1.14 (3H, *s*, H_3-11eq), 1.19 (3H, *s*, H-13), 1.22 (3H, *d*, *J* = 6 Hz, H_3-10), 1.35 (H, *dd*, *J* = 10, 13 Hz, H-2ax), 1.72 (H, *ddd*, *J* = 2, 3, 13 Hz, H-2eq), 1.74 (H, *dd*, *J* = 7, 14 Hz, H-4ax), 2.39 (H, *ddd*, *J* = 2, 5, 14 Hz, H-4eq), 3.11 (H, *dd*, *J* = 8, 9 Hz, H-2′), 3.66 (H, *dd*, *J* = 5, 12 Hz, H-6′a), 3.84 (H, *dd*, *J* = 2, 12 Hz, H-6′b), 3.89 (H, *dddd*, *J* = 3, 5, 7, 10 Hz, H-3), 4.28 (H, *d quin*, *J* = 1, 6 Hz, H-9), 4.33 (H, *d*, *J* = 8 Hz, H-1′), 5.66 (H, *dd*, *J* = 6, 16 Hz, H-8), 5.91 (H, *dd*, *J* = 1, 16 Hz, H-7). ^{13}C NMR: see Table 1. HR-FAB-MS (negative centroid) *m/z*: 387.2006 $[M - H]^-$ ($C_{19}H_{31}O_8$ requires 387.2019).

Alangionoside F (**6**). Amorphous powder. $[\alpha]_D^{23}$ – 85.7° (MeOH; *c* 1.40). ^{1}H NMR (CD_3OD): δ 0.97 (3H, *s*, H_3-12ax), 1.14 (3H, *s*, H_3-11eq), 1.19 (3H, *s*, H_3-13), 1.22 (3H, *d*, *J* = 6 Hz, H_3-10), 1.35 (H, *dd*, *J* = 10, 13 Hz, H-2ax), 1.72 (H, *ddd*, *J* = 2, 3, 13 Hz, H-2eq), 1.75 (H, *dd*, *J* = 8, 14 Hz, H-4ax), 2.39 (H, *ddd*, *J* = 2, 5, 14 Hz, H-4eq), 3.12 (H, *dd*, *J* = 8, 9 Hz, H-2″), 3.20 (H, *dd*, *J* = 10, 11 Hz, H-5″a), 3.21 (H, *dd*, *J* = 7, 9 Hz, H-2′), 3.42 (H, *m*, H-5′), 3.49 (H, *ddd*, *J* = 5, 9, 10 Hz, H-3), 3.75 (H, *dd*, *J* = 6, 12 Hz, H-6a), 3.87 (H, *dd*, *J* = 5, 11 Hz, H-5″b), 3.89 (H, *m*, H-3). 4.05 (H, *dd*, *J* = 2, 12 Hz, H-6b), 4.28 (H, *d quin*, *J* = 1, 6 Hz, H-9), 4.31 (H, *d*, *J* = 7 Hz, H-1′), 4.33 (H, *d*, *J* = 8 Hz, H-1″), 5.66 (H, *dd*, *J* = 6, 16 Hz, H-8), 5.91 (H, *dd*, *J* = 1, 16 Hz, H-7). ^{13}C NMR: see Table 1. HR-FAB-MS (negative centroid) *m/z*: 519.2405 $[M - H]^-$ ($C_{24}H_{39}O_{12}$ requires 519.2442).

Enzymatic hydrolysis of alangionoside E (**4**). Alangionoside E (25 mg) was hydrolysed with 15 mg of emulsin at 37° for 6 hr in 1 ml of H_2O. The hydrolysate was sepd by silica gel CC [15 × 200 mm, C_6H_6–$CHCl_3$, (1:1, 100 ml), (3:7, 100 ml) and (1:9, 100 ml), $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (9:1, 100 ml) and (7:3, 200 m), frs of 15 g being collected] to give 12.5 mg of the aglycone (frs

39–42) (**8**, 86%) and 7.5 mg of D-glucose (frs 52–55) (65%).

Aglycone. Liquid. $[\alpha]_D^{21}$ −74.4° (MeOH; *c* 0.83). ^{1}H NMR (CD_3OD): δ0.95, 1.12 (3H each, each *s*, H_3-11 and H_3-12), 1.18 (3H, *s*, H_3-13), 1.22 (3H, *d*, *J* = 6 Hz, H-9), 1.55 (H, *ddd*, *J* = 2, 3, 13 Hz, H-2eq), 1.60 (H, *dd*, *J* = 9, 14 Hz, H-4ax), 2.26 (H, *ddd*, *J* = 2, 5, 14 Hz, H4-eq), 3.74 (H, *dddd*, *J* = 3, 5, 9, 14 Hz, H-3), 4.28 (H, *d quin*, *J* = 1, 6 Hz, H-9), 5.66 (H, *dd*, *J* = 6, 15 Hz, H-8), 5.90 (H, *dd*, *J* = 1, 15 Hz, H-7), the H-2ax signal was embedded in the envelopes of methyl signals. ^{13}C NMR (CD_3OD): δ20.2 (C-13), 23.8 (C-10), 25.0 (C-11), 30.0 (C-12), 35.9 (C-1), 41.6 (C-4), 48.1 (C-2), 64.6 (C-3), 68.0 (C-5), 68.7 (C-9), 71.1 (C-6), 125.9 (C-7), 139.1 (C-8). HR-EIMS (70 eV) *m/z*: 226.1631 $[M]^+$ ($C_{13}H_{22}O_3$ requires 226.1529), 208.1490 $[M - H_2O]^+$ ($C_{13}H_{20}O_2$ requires 208.1463), 190.1331 $[M - H_2O \times 2]^+$ ($C_{13}H_{18}O$ requires 190.1357). D-glucose: $[\alpha]_D^{21}$ +44.0° (after 24 hr being dissolved in H_2O; *c* 0.5).

DDQ oxidation of compound **4** *to* **5**. Alangionoside E (20 mg) was oxidized with 25 mg of DDQ in 2 ml of dioxane at 75° for 20 hr. The oxidation product was purified by silica gel CC [15 × 200 mm, $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19:1, 100 ml; 9:1, 100 ml; 17:3, 100 ml; 4:1, 100 ml), frs of 15 g being collected] to give 10.3 mg of 9-oxo-alangionoside E in frs 28–31 (69%). A small portion was recrystallized from MeOH–EtOAc to give needles, mp 162–164°. $[\alpha]_D^{28}$ −97.1° (MeOH; *c* 0.69). UV λ_{max}^{MeOH} 232 (3.99). HR-FAB-MS (negative centroid) *m/z*: 385.1876 $[M - H]^-$ ($C_{24}H_{39}O_{12}$ requires 385.1862). ^{1}H and ^{13}C NMR (pyridine-d_5) essentially the same as those reported for icariside B_2 [3].

Acetylation of compounds **1**, **3**, **4** *and* **8**. Plucheoside B (**1**, 11 mg) was acetylated with 0.5 ml each of Ac_2O and pyridine at 20° for 18 hr. The reaction mixt. was evapd under a stream of N_2 and the residue obtained purified by prep. TLC on silica gel with C_6H_6–Me_2CO (4:1) for 9 cm and eluted with $CHCl_3$–MeOH (9:1) to give 7.5 mg (42%) of the hexaacetate (**9**) as an amorphous powder, $[\alpha]_D^{21}$ −58.0° ($CHCl_3$; *c* 0.50). ^{1}H NMR ($CDCl_3$): δ 1.04, 1.045 (3H each, each *s*, H_3-11 and H_3-12), 1.35 (3H, *d*, *J* = 6 Hz, H_3-10), 1.65 (3H, *d*, *J* = 1 Hz, H_3-13), 1.72 (H, *ddd*, *J* = 1, 4, 13 Hz, H-2eq), 1.90 (H, *t*, *J* = 13 Hz, H-2ax), 1.99, 2.02, 2.04, 2.05, 2.06, 2.08 (3H each, each *s*, MeCO– × 6), 3.71 (H, *ddd*, *J* = 3, 5, 10 Hz, H-5′), 3.89 (H, *td*, *J* = 4, 13 Hz, H-3), 4.17 (2H, *m*, H_2-6), 4.65 (H, *d*, *J* = 8 Hz, H-1′), 4.96 (H, *dd*, *J* = 8, 9 Hz, H-2′), 5.02 (H, *dd*, *J* = 9, 10 Hz, H-3′), 5.19 (H, *t*, *J* = 10 Hz, H-4′), 5.37 (H, *d quin*, *J* = 1, 7 Hz, H-9), 5.45 (H, *br d*, *J* = 4 Hz, H-4), 5.46 (H, *dd*, *J* = 7, 16 Hz, H-8), 6.05 (H, *qd*, *J* = 1, 16 Hz, H-7). ^{13}C NMR ($CDCl_3$): δ18.6 (C-13), 20.4, 20.59, 20.62, 20.7, 20.8, 20.9 (Me_3CO– × 6), 21.3 (C-10), 27.0 (C-11), 30.0 (C-12), 36.8 (C-1), 40.3 (C-2), 62.4 (C-6′), 68.8 (C-4′), 69.9 (C-4)[a], 71.05 (C-9)[a], 71.13 (C-2′), 71.7 (C-5′), 72.9 (C-3′), 74.5 (C-3), 100.8 (C-1′), 124.3 (C-5), 128.0 (C-7), 134.9 (C-8), 143.5 (C-6), 169.5, 169.6, 170.2, 170.3, 170.5, 171.1 (MeCO– × 6); assignments with the same superscripts may be interchangeable. FAB-MS (*m*-nitrobenzyl alcohol) *m/z* (rel. int.): 621 (100) $[M + Na]^+$, 561 (18) $[M + Na - HOAc]^+$, 331 (7) [Glc $(OAc)_4$ oxonium ion]$^+$ (+ NaI), 637 (100) $[M + K]^+$, 577 (5) $[M + K - HOAc]^+$, 331 (17) [Glc $(OAc)_4$ oxonium ion]$^+$ (+ KI).

Alangionoside D (**3**) (1.6 mg) was acetylated with 100 μl each of Ac_2O and pyridine. The reaction mixt. was concd and the residue purified by prep. TLC on silica gel to give 1.9 mg (73%) of the hexaacetate (**10**). ^{1}H NMR ($CDCl_3$): δ1.02, 1.06 (3H each, each *s*, H_3-11 and H_3-12), 1.35 (3H, *d*, *J* = 6 Hz, H_3-10), 1.55 (3H, *br d*, H_3-13), 1.85 (H, *dd*, *J* = 4, 13 Hz, H-2eq), 2.00, 2.03, 2.038, 2.043, 2.07, 2.12 (3H each, each *s*, MeCO– × 6), 3.70 (H, *ddd*, *J* = 3, 6, 10 Hz, H-5′), 3.89 (H, *ddd*, *J* = 4, 8, 12 Hz, H-3), 4.14 (H, *dd*, *J* = 3, 12 Hz, H-6′a), 4.20 (H, *dd*, *J* = 6, 12 Hz, H-6′b), 4.66 (H, *d*, *J* = 8 Hz, H-1′), 4.95 (H, *dd*, *J* = 8, 9 Hz, H-2′), 5.03 (H, *t*, *J* = 10 Hz, H-4′), 5.18 (H, *t*, *J* = 10 Hz, H-3′), 5.37 (H, *d quin*, *J* = 1, 8 Hz, H-9), 5.41 (H, *br d*, *J* = 8 Hz, H-4), 5.43 (H, *dd*, *J* = 8, 16 Hz, H-8), 6.03 (H, *br d*, *J* = 16 Hz, H-7). FAB-MS (*m*-nitrobenzyl alcohol) *m/z* (rel. int.): 663 (100) $[M + Na]^+$, 603 (20) $[M + Na - HOAc]^+$, 543 (10) $[M + K - HOAc \times 2]^+$, 331 (5) [Glc $(OAc)_4$ oxonium ion]$^+$ (+ NaI), 679 (100) $[M + K]^+$, 619 (13) $[M + K - HOAc]^+$, 331 (17) [Glc $(OAc)_4$ oxonium ion]$^+$ (+ KI).

Alangionoside E (**4**) (25 mg) was acetylated in a similar manner to alangionoside E. The reaction mixt. was poured into ice-water and then triturated to give a ppt. The ppt. was collected by filtration and then the filtrate was extracted with Et_2O. The Et_2O extract was washed with H_2O, 6 M HCl, 5% $NaHCO_3$ and H_2O, successively, and then dried with Na_2SO_4. The ppt. and the residue of the extract were purified by prep. TLC, as described earlier (20 × 10 cm), to give 20.5 mg (53%) of the pentaacetate (**11**). An amorphous powder, $[\alpha]_D^{21}$ −17.6° ($CHCl_3$; *c* 1.37). ^{1}H NMR ($CDCl_3$): δ0.94, 1.09 (3H each, each *s*, H_3-11 and H_3-12), 1.16 (3H, *s*, H_3-13), 1.31 (3H, *d*, *J* = 6 Hz, H_3-10), 1.33 (H, *dd*, *J* = 10, 12 Hz, H-2ax), 1.62 (H, *dd*, *J* = 8, 12 Hz, H-4ax), 1.70 (H, *ddd*, *J* = 2, 3, 13 Hz, H-2eq), 2.0, 2.02, 2.04, 2.05, 2.07 (3H each, *s* each, MeCO – × 5), 2.27 (H, *ddd*, *J* = 1, 5, 14 Hz, H-4eq), 3.68 (H, *ddd*, *J* = 2, 5, 9 Hz, H-5′), 3.80 (H, *m*, H-3), 4.11 (H, *dd*, *J* = 2, 12 Hz, H-6′a), 4.23 (H, *dd*, *J* = 5, 12 Hz, H-6′b), 4.54 (H, *d*, *J* = 8 Hz, H-1′), 4.93 (H, *dd*, *J* = 8, 9 Hz, H-2′), 5.04 (H, *t*, *J* = 9 Hz, H-4′), 5.19 (H, *t*, *J* = 9 Hz, H-3′), 5.36 (H, *d quin*, *J* = 1, 6 Hz, H-9), 5.65 (H, *dd*, *J* = 6, 15 Hz, H-8), 5.89 (H, *dd*, *J* = 1, 15 Hz, H-7). ^{13}C NMR ($CDCl_3$): δ19.8 (C-13), 20.4, 20.60, 20.62, 20.67, 20.74, (MeCO– × 5), 21.3 (C-10), 24.8 (C-11 or C-12), 29.1 (C-12 or C-11), 34.7 (C-1), 37.7 (C-4), 44.2 (C-2), 62.3 (C-6′), 65.3 (C-5), 68.6 (C-4′), 69.6 (C-6), 70.3 (C-9), 71.5 (C-2′), 71.8 (C-5′), 72.9 (C-3′), 73.2 (C-3), 100.0 (C-1′), 127.1, (C-7), 133.4 (C-8), 169.18, 169.43, 170.24, 170.28, 170.61 (MeCO- × 5). FAB-MS (*m*-nitrobenzyl alcohol) *m/z* (rel. int.): 663 (100) $[M + Na]^+$, 603 (10) $[M + Na - HOAc]^+$, 543 (11) $[M + Na - HOAc \times 2]^+$, 331 (32) [Glc $(OAc)_4$ oxonium ion]$^+$ (+ NaI), 679 (100) $[M + K]^+$, 619 (9) $[M + K - HOAc]^+$, 331 (10) [Glu $(OAc)_4$ oxonium ion]$^+$ (+ KI).

The aglycone of alangionoside E (**8**) (2.5 mg) was acetylated in a similar manner to **3**. The product was purified by prep. TLC on silica gel with C_6H_6–Me_2CO

(9:1) and eluted with C_6H_6–Me_2CO (4:1) to give 1.6 mg of **12** (47%). ^{1}H NMR ($CDCl_3$): δ0.97, 1.13, 1.17 (3H each, each *s*, H_3-11, H_3-12 and H_3-13), 1.31 (3H, *d*, J = 6 Hz, H_3-10), 1.34 (H, *dd*, J = 9, 13 Hz, H-2ax), 1.64 (H, *ddd*, J = 1, 3, 13 Hz, H-2eq), 1.75 (H, *dd*, J = 7, 15 Hz, H-4ax), 2.01, 2.05 (3H each, each *s*, MeCO– × 2), 2.38 (H, *ddd*, J = 1, 6, 15 Hz, H-4eq), 4.92 (H, *dddd*, J = 3, 6, 7, 9 Hz, H-3), 5.37 (H, *d quin*, J = 1, 6 Hz, H-9), 5.68 (H, *dd*, J = 6, 15 Hz, H-8), 5.92 (H, *dd*, J = 1, 15 Hz, H-7). HR-EIMS m/z: 250.1615 $[M - HOAc]^+$ ($C_{15}H_{22}O_3$ reqires 250.1569), 190.1336 $[M - HOAc \times 2]^+$ ($C_{13}H_{18}O$ requires 190.1357).

Acknowledgement—The authors are grateful to Prof. F. Murai (Aichi Medical University) for NMR spectral data of epoxyactinidioionoside pentaacetate.

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