Studies on the Constituents of *Actinostemma lobatum* MAXIM. IV.[1] Structures of Lobatosides C, D and H, the Dicrotalic Acid Esters of Bayogenin Bisdesmosides Isolated from the Herb

Toshihiro FUJIOKA, Masayo IWAMOTO, Yukiko IWASE, Shizuko HACHIYAMA, Hikaru OKABE, Tatsuo YAMAUCHI and Kunihide MIHASHI*

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka 814-01, Japan. *Received January 9, 1989*

Eight bayogenin glycosides, lobatosides A—H, were isolated from the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae). The isolation of lobatosides A—H and the structures of lobatosides A, C, D and H are described.

Lobatoside A is 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid). Lobatosides C, D and H are dicrotalic acid (3-hydroxy-3-methylglutaric acid) esters of the 28-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, 28-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester and 28-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester of lobatoside A, respectively. Dicrotalic acid is linked at one end to the C_4-hydroxyl group of the α-L-arabinopyranosyl group in the C_3-linked sugar moiety, and at the other end to the C_4-hydroxyl group of the α-L-rhamnopyranosyl group in the ester-linked sugar moiety to form a macrocyclic structure ("cyclic bisdesmoside"). Lobatoside H was proved to be identical with tubeimoside I isolated from the tuber of *Bolbostemma paniculatum* (MAXIM.) FRANQUET (Cucurbitaceae).

Keywords *Actinostemma lobatum*; Cucurbitaceae; oleanane-type triterpene glycoside; bayogenin; 2β,3β,23-trihydroxyolean-12-en-28-oic acid; lobatoside; tubeimoside I; cyclic bisdesmoside; dicrotalic acid; 3-hydroxy-3-methylglutaric acid

As reported in the first paper[2a] of this series, the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae) contains a number of triterpene glycosides. They were divided into two groups, actinostemmosides and lobatosides, according to the violet and dark blue colorations on a thin-layer chromatography (TLC) plate stained by heating after spraying sulfuric acid. The less polar glycoside fraction mainly contained actinostemmosides which stained violet, and from this fraction six dammarane-type triterpene glycosides were isolated (actinostemmosides A, B, C, D, G and H) together with two baccharane-type triterpene glycosides, actinostemmosides E and F. Their structures were reported in the preceding papers.[2] Further examination of the less polar glycoside fraction has resulted in the isolation of one triterpene glycoside which stained dark blue, and this compound was named lobatoside A. The polar glycoside fraction contained lobatosides which stained dark blue and this fraction has provided seven triterpene glycosides named lobatosides B—H. This paper deals with the structures of lobatosides A, C, D and H.

Lobatoside A (I) was obtained as colorless needles from aqueous MeOH and the fast atom bombardment mass spectrum (FAB-MS) showed an $[M+Na]^+$ ion at m/z 805 and an $[M-H]^-$ ion at m/z 781, indicating the molecular weight to be 782. The results of the elemental analysis were consistent with the molecular formula $C_{41}H_{66}O_{14}\cdot 5/2H_2O$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Tables I and II) of I showed signals of six methyl groups on saturated quaternary carbons, a proton (δ 5.48, br s) on a trisubstituted olefin and two anomeric protons (δ 5.14, d, $J=7$ Hz; δ 5.08, d, $J=8$ Hz) together with signals of hydroxymethine groups and hydroxymethylene groups. The carbon-13 NMR (^{13}C-NMR) spectrum (Tables III and IV) showed the signals of six C–C bonded quaternary carbons, trisubstituted olefinic carbons, a carboxylic acid carbon, two anomeric carbons and three oxymethylene carbons. These spectral data and the molecular formula suggested I to be an oleanane-type triterpene acid digly-

TABLE I. ^{1}H-NMR Chemical Shifts[a] of Aglycone Moieties of I—IV, VII, VIII and X

	I	II	III	IV	VII	VIII	X
H2	4.78 br s	4.52 br s	4.60 br s	4.79 br s	4.61 br s	4.79 br s	4.62 br s
H3	4.18 d (3)	4.27 d (4)	4.32 d (3)	4.14 d (3)	4.30 d (3)	4.19 d (3)	4.30 d (3)
H12	5.48 br s	5.52 t-like	5.47 br s	5.48 br s	*ca.* 5.45	*ca.* 5.45	5.43 br s
H18	3.24 dd (4, 14)	3.31 dd (4, 14)	3.15 dd (4, 14)	3.22 dd (4, 14)	3.14 dd (4, 14)	3.23 dd (4, 14)	3.15 dd (4, 14)
H23	3.62 d (12)	3.72 d (11)	3.55 d (11)	3.59 d (11)	3.67 d (11)	3.63 d (12)	3.67 d (12)
	4.35 d (12)	4.17 d (11)	4.18 d (11)	4.31 d (11)	*ca.* 4.28	4.33 d (12)	*ca.* 4.27
H24	1.33 s	1.37 s	1.48 s	1.29 s	1.52 s	1.34 s	1.52 s
H25	1.50 s	1.61 s	1.56 s	1.50 s	1.59 s	1.54 s	1.62 s
H26	1.03 s	1.12 s	1.03 s	1.08 s	1.11 s	1.14 s	1.12 s
H27	1.28 s	1.27 s	1.25 s	1.25 s	1.26 s	1.26 s	1.27 s
H29	0.94 s	0.93 s	0.92 s	0.92 s	0.91 s	0.92 s	0.92 s
H30	1.00 s	1.01 s	0.94 s	0.99 s	0.93 s	0.99 s	0.93 s
Dicrotalic acid moiety							
2′(4′)			3.06 d (16)		3.09 d (16)		3.08 d (15)
			3.24 d (16)		3.41 d (16)		3.45 d (15)
4′(2′)			3.13 d (15)		3.10 d (16)		3.12 d (15)
			3.20 d (15)		3.22 d (16)		3.28 d (15)
6′			1.91 s		1.98 s		2.01 s

a) The spectra were measured in pyridine-d_5 containing D_2O. The values in parentheses are coupling constants in hertz (Hz).

TABLE II. ^{1}H-NMR Chemical Shifts[a] of Sugar Moieties of I, III, IV, VII, VIII and X

	I	III	IV	VII	VIII	X
3Glc-1	5.08 d (8)	5.06 d (7.5)	5.08 d (8)	5.02 d (8)	5.08 d (8)	5.03 d (7)
2	4.04 dd (8, 9)	4.23 dd (7.5, 9)	4.06 dd (8, 9)	4.21 dd (8, 9)	4.07 dd (8, 9)	4.23 dd (7, 9)
3	4.21 t (9)	4.29 t (9)	4.23 t (9)	4.13 t (9)	4.22 t (9)	4.08 t (9)
4	4.08 t (9)	4.09 t (9)	4.09 t (9)	4.07 t (9)	4.09 t (9)	*ca.* 4.05
5	3.82 ddd (2, 6, 9)	3.85 ddd (2, 6, 9)	3.84 ddd (2, 5, 9)	*ca.* 3.80	3.83 ddd (3, 6, 9)	*ca.* 3.81
6	4.20 dd (6, 12)	4.22 dd (6, 12)	4.20 dd (5, 12)	*ca.* 4.20	*ca.* 4.20	*ca.* 4.20
	4.38 dd (2, 12)	4.41 dd (2, 12)	4.38 dd (2, 12)	*ca.* 4.40	*ca.* 4.40	*ca.* 4.40
3Ara-1	5.14 d (7)	5.43 d (7.5)	5.14 d (7)	5.42 d (8)	5.16 d (7)	5.48 d (8)
2	4.50 dd (7, 9)	4.38 dd (7.5, 10)	4.48 dd (7, 9)	4.38 dd (8, 10)	4.51 dd (7, 9)	4.39 dd (8, 9)
3	4.15 dd (3, 9)	4.25 dd (3, 10)	4.15 dd (3, 9)	4.23 dd (3, 10)	4.15 dd (4, 9)	4.22 dd (3, 9)
4	4.31 brs	5.55 brs	4.32 brs	5.52 brd (3)	4.31 brs	5.52 brs
5	3.75 brd (12)	3.67 brd (13)	3.76 brd (11)	3.62 brd (12)	3.76 brd (12)	3.63 brd (12)
	4.36 brd (12)	4.15 brd (13)	4.33 brd (11)	4.13 brd (12)	4.35 dd (3, 12)	4.13 brd (12)
28Ara-1		6.14 brs	6.42 (3)	6.13 d (4)	6.42 brs	6.13 d (4)
2		4.58 brs	4.49 dd (3, 4)	4.58 dd (4, 5)	*ca.* 4.49	4.61 dd (4, 5)
3		*ca.* 4.41	*ca.* 4.45	*ca.* 4.52	*ca.* 4.40—4.50	*ca.* 4.49
4		*ca.* 4.45	*ca.* 4.45	*ca.* 4.34	*ca.* 4.40—4.50	*ca.* 4.40
5		*ca.* 3.97	*ca.* 3.93	*ca.* 3.88	*ca.* 3.90	*ca.* 3.84
		ca. 4.46	*ca.* 4.45	*ca.* 4.35	*ca.* 4.45—4.50	*ca.* 4.40
28Rha-1		5.96 brs	5.69 brs	5.96 brs	5.65 brs	6.05 brs
2		4.68 dd (1.5, 3)	4.54 dd (1.5, 3)	5.01 dd (1.5, 3)	4.87 dd (1.5, 3)	4.89 dd (1.5, 3)
3		4.44 dd (3, 10)	4.43 dd (3, 9)	4.55 dd (3, 10)	4.57 dd (3, 9)	4.53 dd (3, 10)
4		5.83 t (10)	4.26 t (9)	5.96 t (10)	4.39 t (9)	6.01 t (10)
5		4.20 dq (10, 6)	4.37 dq (9, 6)	4.29 dq (10, 6)	4.44 dq (9, 6)	*ca.* 4.30
6		1.53 d (6)	1.68 d (6)	1.51 d (6)	1.67 d (6)	1.52 d (6)
28Glc-1				5.02 d (8)	5.21 d (8)	
2				3.82 dd (8, 9)	4.05 dd (8, 9)	
3				4.23 t (9)	4.18 t (9)	
4				4.07 t (9)	4.11 t (9)	
5				*ca.* 3.80	3.81 ddd (3, 6, 9)	
6				*ca.* 4.20	*ca.* 4.20	
				ca. 4.40	*ca.* 4.40	
28Xyl-1						4.98 d (8)
2						3.81 dd (8, 9)
3						4.24 t (9)
4						*ca.* 4.10
5						*ca.* 3.60
						ca. 4.05

a) The spectra were measured in pyridine-d_5 containing D_2O. The values in parentheses are coupling constants in hertz (Hz). Abbreviations: Glc, glucose; Ara, arabinose; Rha, rhamnose; Xyl, xylose, all in a pyranose form. 3Glc-1 means the H_1 of the glucopyranosyl group in a sugar moiety which is linked to C_3 of the aglycone.

coside. Compound I gave an aglycone (II), D-glucose and L-arabinose on acid hydrolysis. The high-resolution FAB-MS of II showed an $[M+Na]^+$ ion at *m/z* 511.336, from which the molecular formula $C_{30}H_{48}O_5$ was deduced. The electron impact (EI) MS showed the fragment ions at *m/z* 248 and 203 (248 − COOH), showing the presence of three hydroxyl groups at ring(s) A and/or B, and a carboxylic acid group at ring D or E of an olean-12-ene. The ^{1}H-NMR spectrum showed the signals of six methyl groups, an olefinic proton (δ 5.52, t-like), one hydroxymethylene group (δ 3.72, d, $J=11$ Hz; δ 4.17, d, $J=11$ Hz) on a quaternary carbon and two hydroxymethine groups (δ 4.52, brs; δ 4.27, d, $J=4$ Hz). The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum indicated that the two hydroxyl groups are vicinal and are located on carbons between a quaternary carbon and a methylene group. The ^{13}C-NMR spectrum revealed the presence of six C–C bonded saturated quaternary carbons, one trisubstituted olefin group, a carboxylic group, two hydroxymethine groups and one hydroxymethylene group. These spectral data suggested that II is bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid), and its identity was established by comparison of the spectral data with those of the aglycone of tubeimoside I isolated from the tuber of *Bolbostemma paniculatum* (MAXIM.) FRANQUET.[3]

The structure of the sugar moiety of I was determined as α-L-arabinopyranosyl-(1→2)-β-D-glucopyranose by assignments of the ^{1}H-NMR and ^{13}C-NMR signals with the aid of the ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra. The position of the sugar linkage to the aglycone was determined as C_3 from the glycosylation shift of the C_3 signal and also from the presence of nuclear Overhauser effect (NOE) between the C_3-H of the aglycone and the anomeric proton of the glucopyranosyl group. Accordingly, lobatoside A is 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin.

Lobatoside C (III) was obtained as a white amorphous powder and its FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1209 and an $[M-H]^-$ ion at *m/z* 1185, showing the molecular weight to be 1186. The analysis data were consistent with $C_{58}H_{90}O_{25}\cdot 7H_2O$. The ^{1}H-NMR spectrum showed the signals of seven methyl groups on saturated quaternary carbons, an olefinic proton (δ 5.47, brs) and four anomeric protons (δ 5.06, d, $J=7.5$ Hz; δ 5.43, d, $J=7.5$ Hz; δ 5.96, brs; δ 6.14, brs). The ^{13}C-NMR spectrum showed signals of six C–C bonded quaternary carbons, one oxygenated quaternary carbon (δ 70.0), a pair of olefinic

Table III. ^{13}C-NMR Chemical Shifts[a] of Aglycone Moieties of I—IV, VII, VIII and X

	I	II	III	IV	VII	VIII	X
C1	44.0	44.8	44.4	44.1	44.3	44.1	44.2
C2	70.5	71.6	68.9	70.5	68.8	70.5	69.2
C3	82.6	73.1	84.0	83.5	83.5	82.6	83.1
C4	42.7	42.3[b]	43.4	42.7	43.3	42.7	43.1
C5	47.7	48.2	47.0	47.7	47.3	47.8	47.4
C6	17.9	18.3	18.8	18.0	18.8	18.0	18.6
C7	33.3	33.1[c]	33.1	33.0	33.1	33.0[b]	33.2
C8	39.9	39.8	40.1	40.0	40.1	40.1	40.1
C9	48.6	48.5	48.5	48.5	48.6	48.6	48.6
C10	36.9	37.2	37.3	36.9	37.3	36.9	37.2
C11	23.7	23.9	23.9	24.0	24.0	24.0	23.9
C12	122.7	122.7	123.2	123.1	123.3	123.1	123.2
C13	144.9	144.8	144.1	144.3	144.2	144.2	144.2
C14	42.3	42.4[b]	42.0	42.3	42.0	42.3	41.9
C15	28.3	28.2	28.9	28.3	28.9	28.3	29.2
C16	24.0	23.7	22.9	23.2	22.8	23.2	22.8
C17	46.7	46.6	47.0	47.3	47.1	47.3	47.1
C18	42.0	42.0	41.5	41.7	41.4	41.7	41.3
C19	46.4	46.4	46.0	46.3	46.1	46.3	46.0
C20	30.9	30.9	30.7	30.9	30.8	30.8	30.7
C21	34.3	34.2	34.0	34.2	34.0	34.2	34.0
C22	33.0	33.0[c]	32.2	32.7	32.3	32.7[b]	32.2
C23	64.7	67.8	65.8	64.7	65.4	65.4	64.7
C24	14.6	14.5	15.8	14.6	15.6	14.6	15.5
C25	17.2	17.2	17.8	17.3	17.8	17.3	17.7
C26	17.5	17.5	17.6	17.5	17.6	17.5	17.7
C27	26.3	26.2	26.3	26.1	26.4	26.1	26.4
C28	180.1	180.1	176.1	176.2	176.1	176.2	176.0
C29	33.3	33.1	33.1	33.1	33.1	33.1	33.1
C30	23.8	23.8	23.6	23.7	23.7	23.7	23.7
Dicrotalic acid moiety							
1′			171.3		171.2[b]		171.2[b]
2′			46.9[b]		46.5[c]		46.4[c]
3′			70.0		70.2		70.1
4′			47.8[b]		47.8[c]		47.8[c]
5′			171.3		171.4[b]		171.4[b]
6′			26.4		26.2		26.0

a) The spectra were measured in pyridine-d_5 and chemical shifts are expressed in δ values. b, c) The values in each column may be interchanged.

Table IV. ^{13}C-NMR Chemical Shifts[a] of Sugar Moieties of I, III, IV, VII, VIII and X

	I	III	IV	VII	VIII	X
3Glc-1	103.5	103.2	106.5	103.1	103.4	102.9
2	83.5	80.3	82.5	80.3	83.5	80.0
3	78.0	78.9	78.0	78.9[b]	78.3	78.9
4	71.3	71.4	71.3	71.4[c]	71.3[b]	71.4[b]
5	77.9	78.2	77.9	78.1	78.1[c]	78.2
6	62.5	62.5	62.5	62.5[d]	62.5	62.5
3Ara-1	106.5	104.7	103.5	104.7	106.4	104.5
2	73.8	73.7	73.8	73.8	73.8	73.7
3	74.3	72.5	74.3	72.5	74.3	72.5
4	69.2	72.4	69.2	72.3	69.2	72.4
5	67.2	64.4	67.2	64.4	67.2	64.3
28Ara-1		94.3	93.4	94.2	93.5	94.1
2		74.9	75.2	75.1	75.3	74.7
3		71.4	70.2	71.2	70.1	70.9
4		67.6[b]	66.1	67.6	66.2	67.6
5		64.9	63.0	64.8	63.2	64.7
28Rha-1		100.6	101.4	100.7	101.5	100.6
2		72.4	72.3	72.1	71.5[b]	72.3
3		70.2	72.6	79.0[b]	83.5	78.6
4		75.5	73.8	73.3	72.7	73.2
5		67.8[b]	70.4	67.9	70.1	67.9
6		18.4	18.5	18.3	18.4	18.2
28Glc-1				105.8	106.4	
2				74.9	75.9	
3				78.1	78.0[c]	
4				71.5[c]	71.5[b]	
5				77.7	77.9[c]	
6				62.6[d]	62.5	
28Xyl-1						106.5
2						74.7
3						78.0
4						70.9[b]
5						66.9

a) The spectra were measured in pyridine-d_5 and chemical shifts are expressed in δ values. b—d) The values in each column may be interchanged. Abbreviations: Ara, arabinose; Glc, glucose; Rha, rhamnose; Xyl, xylose, all in a pyranose form. 3Glc-1 means the C_1 of the glucopyranosyl group in a sugar moiety which is linked to C_3 of the aglycone.

carbons (δ 123.2, 144.1), four anomeric carbons (δ 94.3, 100.6, 103.2, 104.7) and three ester carbons (δ 176.1, 171.3×2). These spectral data suggested that III is a bayogenin tetraglycoside having two acylated hydroxyl groups. On treatment with 0.5% KOH, III yielded a deacylated compound (IV) and a carboxylic acid (V). Compound V showed an $[M+Na]^+$ ion at m/z 185 in its FAB-MS. The ^{1}H-NMR spectrum showed two singlets at δ 1.84 (3H) and δ 3.28 (4H), and the ^{13}C-NMR spectrum showed signals of an oxygenated quaternary carbon at δ 70.0, a methylene carbon at δ 46.5, a methyl carbon at δ 28.2 and a carboxylic acid carbon at δ 174.7. These spectral data suggested V to be a symmetric dicarboxylic acid, and V was identified as 3-hydroxy-3-methylglutaric acid (dicrotalic acid) by comparison of the ^{1}H-NMR chemical shifts with those reported.[3b]

Compound IV gave II, D-glucose, L-arabinose and L-rhamnose on acid hydrolysis. The ^{13}C-NMR chemical shift (δ 93.4) of one of four anomeric carbon signals indicated that one sugar moiety is linked to the C_{28} of bayogenin by an ester linkage, and the glycosylation shifts observed at the signals of C_2 (−1.1 ppm), C_3 (+10.4 ppm) and C_4 (+0.4 ppm) compared with II clearly indicated that another sugar moiety is linked to the C_3-hydroxyl group of the aglycone.

On the selective cleavage of the ester glycoside linkage according to the method reported by Ohtani *et al.*,[4] IV gave a monodesmoside and an anomeric mixture of methyl glycosides (VI). The former was identified as lobatoside A (I) by comparison of the NMR spectra. The latter gave L-arabinose and L-rhamnose on acid hydrolysis, and its permethylate gave methyl glycosides of 2,3,4-tri-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis, indicating that VI is a mixture of methyl α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer. The NMR data of the ester-linked sugar moiety of V are in good agreement with those reported for that of 3-*O*-acetyloleanolic acid 28-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.[5] Consequently, the structure of IV is formulated to be 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin 28-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.

In order to determine the positions of the ester linkage of dicrotalic acid (V), the ^{1}H-NMR spectra of III and its deacylated compound IV were examined in detail and all proton signals of the sugar moieties were assigned using ^{1}H–^{1}H COSY, NOE difference spectroscopy (Fig. 1) and

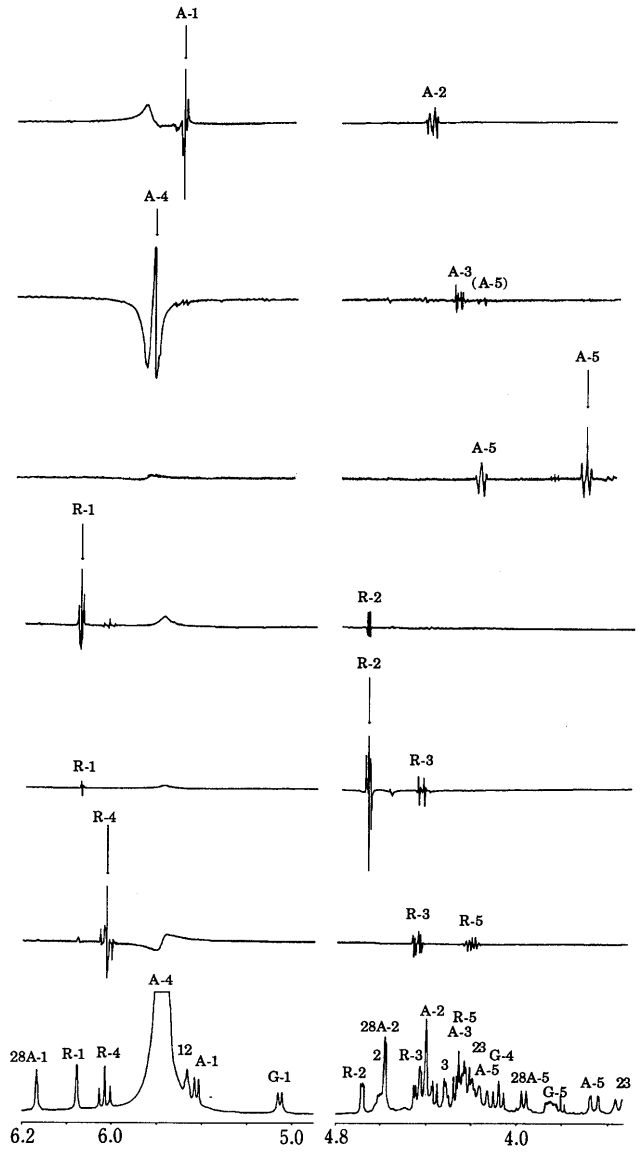


Fig. 1. Decoupling Difference Spectra of Lobatoside C (III)

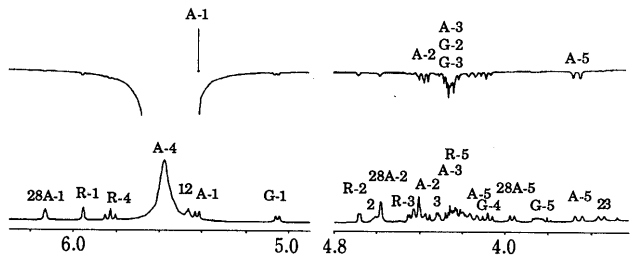
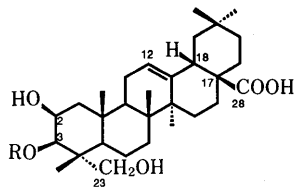
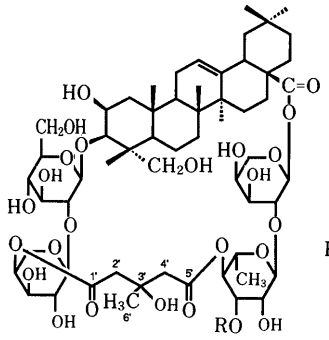


Fig. 2. NOE Difference Spectra of Lobatoside C (III)

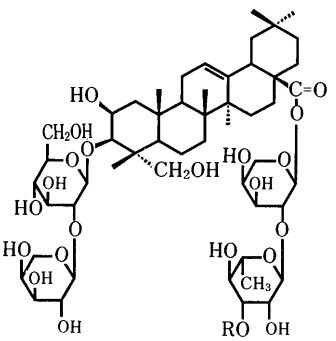
decoupling difference spectroscopy (Fig. 2) techniques, and the results are summarized in Table II. There were significant up-field shifts of the C_4-H signals of the rhamnopyranosyl unit (1.57 ppm) and of the terminal arabinopyranosyl unit (1.23 ppm) on going from III to IV. These up-field shifts clearly indicate that the dicrotalic acid is linked to the C_4-OH of the rhamnopyranosyl unit at one end and to the C_4-OH of the arabinopyranosyl unit at the other end to form a macrocyclic structure as shown. Lobatoside C (III) was proved to be identical with a prosapogenin which was obtained by the enzymatic hydrolysis of tubeimoside I.[3c]



I : R=α-L-ara-(1→2)-β-D-glc-
II : R=H



III : R=H
VII : R=β-D-glc-
X : R=β-D-xyl-



IV : R=H
VIII : R=β-D-glc-

Lobatoside D (VII) was obtained as a white amorphous powder. The FAB-MS showed an $[M+Na]^+$ ion at m/z 1371 and an $[M-H]^-$ ion at m/z 1347. It gave analysis data consistent with the molecular formula $C_{64}H_{100}O_{30}\cdot 2H_2O$. The general features of the NMR spectra suggested that VII is also a cyclic bisdesmoside of bayogenin, like lobatoside C (III), but differing in the number of component monosaccharides. Compound VII gave V and a deacylated derivative (VIII) on mild alkaline hydrolysis, and the latter gave lobatoside A (I) and an anomeric mixture of methyl glycosides (IX) on the selective cleavage of the ester glycoside linkage. Compound IX provided L-arabinose, L-rhamnose and D-glucose on acid hydrolysis and the negative FAB-MS showed an $[M-H]^-$ ion at m/z 471 and fragment ions at m/z 309 ($[M-163]^-$) and 163 ($[M-309]^-$). These ions indicated that IX is a methyl glucosyl-rhamnosyl-arabinoside. The permethylate of IX gave methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, 2,4-di-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis. Therefore, VIII is formulated to be 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin 28-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]ester. The positions of the ester linkages of dicrotalic acid in VII were determined to be the C_4-hydroxyl groups of the terminal arabinosyl group and the rhamnopyranosyl group by comparison of the ^{1}H-NMR data of VII and VIII. From all the above evidence, VII was elucidated to be a β-D-glucopyranoside of lobatoside C (III) as shown. When VII was treated with cellulase, lobatoside C (III) was formed, as expected.

Lobatoside H (X) was obtained as a white amorphous powder. The FAB-MS spectra showed an $[M+Na]^+$ ion at m/z 1341 and an $[M-H]^-$ ion at m/z 1317, and the results of the elemental analysis were in good agreement with the molecular formula $C_{63}H_{98}O_{29}\cdot 4H_2O$. The NMR spectral features suggested X to be a xyloside of lobatoside C (III), and it was identified as tubeimoside I by comparison of the ^{13}C-NMR spectral data with those reported.[3]

Tubeimoside I was isolated from the tuber of *Bolbostemma paniculatum* (MAXIM.) FRANQUET independently by a Hiroshima University group in Japan[3a,b] and by a group at The Shanghai Institute of Materia Medica, China.[3c] It is a bayogenin bisdesmoside having a novel macrocyclic structure with a 3-hydroxy-3-methylglutarate bridge between the two sugar moieties, and Kasai *et al.*[3a] have proposed the name "cyclic bisdesmoside" for glycosides of this type. Both groups have reported the isolation of two additional cyclic bisdesmosides, tubeimosides II and III, which are polygalacic acid bisdesmosides having similar cyclic structures from the same source.[3b] Lobatosides C and D are further additions to "cyclic bisdesmosides" isolated from cucurbitaceous plants.

Experimental[6]

Isolation of Lobatosides The fraction (fr.) (300 mg) less polar than actinostemmoside E obtained from fr. 4b-1[2a] was chromatographed repeatedly on silica gel [solvents: AcOEt–MeOH–H_2O (35 : 4 : 3), $CHCl_3$–MeOH–H_2O (9 : 3 : 0.5) and AcOEt–PrOH–H_2O (5 : 1 : 0.5)] to give lobatoside A (I) (250 mg from 6.5 kg of dried herb). Fraction 5[2a] was repeatedly chromatographed on silica gel [solvents: $CHCl_3$–MeOH–H_2O (13 : 5 : 1), AcOEt–PrOH–H_2O (6 : 3 : 1), $CHCl_3$–MeOH–H_2O (6 : 4 : 1) and AcOEt–PrOH–H_2O (10 : 3 : 1)] and then on LiChroprep RP-18 (solvent: 70% MeOH) to give lobatosides B (539 mg), C (III, 4 g), G (33 mg), H (X, 15 mg). Fraction 6[2a] was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (28 : 15 : 3.5) and divided into two fractions, frs. 6a and 6b. Fraction 6a was chromatographed on LiChroprep RP-18 (solvent: 70% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) and then subjected to high-performance liquid chromatography (HPLC) on a reversed-phase column [Inertsil ODS (5 μm, Gasukuro Kogyo Inc.); 74% MeOH] to give lobatoside D (VII, 4.1 g) and lobatoside E (365 mg). Fraction 6b was subjected to HPLC on Inertsil ODS column (solvent: 74% MeOH) and then on a normal-phase column [Nucleosil (5 μm, Gasukuro Kogyo Inc.); $CHCl_3$–MeOH–H_2O (65 : 35 : 5)] to give lobatoside F (70 mg).

Lobatoside A (I) Colorless needles from aqueous MeOH, mp 249—252 °C. $[\alpha]_D^{24}$ +49.4° (c=0.53, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{14}\cdot 5/2H_2O$: C, 59.63; H, 8.66. Found: C, 59.47; H, 8.64. FAB-MS m/z: 805 ($[M+Na]^+$), 781 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

Lobatoside C (III) A white amorphous powder from aqueous MeOH, mp 262—266 °C. $[\alpha]_D^{22}$ +17.1° (c=0.63, pyridine). *Anal.* Calcd for $C_{58}H_{90}O_{25}\cdot 7H_2O$: C, 53.04; H, 7.98. Found: C, 53.08; H, 8.20. FAB-MS m/z: 1209 ($[M+Na]^+$), 1185 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

Lobatoside D (VII) A white amorphous powder from aqueous MeOH, mp 240—244 °C. $[\alpha]_D^{22}$ +12.4° (c=0.91, pyridine). *Anal.* Calcd for $C_{64}H_{100}O_{30}\cdot 2H_2O$: C, 55.48; H, 7.57. Found: C, 55.35; H, 7.80. FAB-MS m/z: 1371 ($[M+Na]^+$), 1347 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

Lobatoside H (Tubeimoside I) (X) A white amorphous powder. mp 235—240 °C. $[\alpha]_D^{22}$ +15.5° (c=0.61, pyridine). *Anal.* Calcd for $C_{63}H_{98}O_{29}\cdot 4H_2O$: C, 54.38; H, 7.68. Found: C, 54.33; H, 8.17. FAB-MS m/z: 1341 ($[M+Na]^+$), 1317 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

Mild Alkaline Hydrolysis of III and VII Compound III (300 mg) was dissolved in 0.5% KOH solution and the solution was stirred at room temperature for 24 h, then neutralized with ion exchange resin (Amberlite IR-120) and evaporated. The residue was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] to give IV (119 mg) and V (1 mg). Both compounds were purified by chromatography on LH-20 (MeOH).

IV: A white amorphous powder. FAB-MS m/z: 1083 ($[M+Na]^+$), 1059 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

V: A yellow syrup. FAB-MS m/z: 185 ($[M+Na]^+$). ^{1}H-NMR (pyridine-d_5) δ: 1.84 s (CH_3), 3.28 s ($CH_2\times 2$). ^{13}C-NMR (pyridine-d_5) δ: 174.7 (C_1 and C_5), 70.0 (C_3), 46.5 (C_2 and C_4), 28.2 (C_6).

Compound VII (300 mg) was treated in the same manner to give V (1 mg) and VIII (143 mg).

VIII: A white amorphous powder. FAB-MS m/z: 1245 ($[M+Na]^+$), 1221 ($[M-H]^-$). ^{1}H-NMR: Tables I and II. ^{13}C-NMR: Tables III and IV.

Selective Cleavage of the Ester-Glycoside Linkages of IV and VIII Compound IV (20 mg) and LiI (60 mg) were dissolved in a mixture of 2,6-lutidine (2 ml) and dry MeOH (1 ml) and the solution was heated at 260—270 °C for 24 h. The reaction mixture was diluted with 50% MeOH, deionized with Amberlite MB-3 and evaporated to dryness. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (7 : 3 : 0 : 5) as an eluant to give I (5 mg) and VI (2 mg). Compound VIII (82 mg) was treated in the same manner to give I (14 mg) and IX (4 mg). Compound IX was subjected to HPLC [Nucleosil; $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give an α-anomer (IXα) and its β-anomer (IXβ).

VI: A colorless syrup. FAB-MS m/z: 309 ($[M-H]^-$). ^{1}H-NMR (pyridine-d_5) δ: anomeric H; 6.02 (s, J=1 Hz, Rha), 5.69 (d, J=1.5 Hz, Rha), 5.34 (d, J=3.5 Hz, β-Ara), 4.58 (d, J=6 Hz, α-Ara). ^{13}C-NMR δ: anomeric C; 103.7 (α-Ara), 102.3 (Rha), 101.2 (β-Ara), 104.4 (Rha).

IXα: A colorless syrup. FAB-MS m/z: 471 ($[M-H]^-$), 309 ($[M-163]^-$), 163 ($[M-309]^-$). ^{1}H-NMR (pyridine-d_5) δ: anomeric H; 5.99 (s, Rha), 4.52 (d, J=6 Hz, Ara), 5.38 (d, J=8 Hz, Glc). ^{13}C-NMR δ: 103.7 (Ara), 102.3 (Rha), 106.6 (Glc).

IXβ: A colorless syrup. FAB-MS m/z: 471 ($[M-H]^-$), 309 ($[M-163]^-$), 163 ($[M-309]^-$). ^{1}H-NMR (pyridine-d_5) δ: anomeric H; 5.63 (d, J=1 Hz, Rha), 5.32 (d, J=3.5 Hz, Ara), 5.17 (d, J=8 Hz, Glc). ^{13}C-NMR δ: anomeric C; 101.1 (Ara), 104.0 (Rha), 106.3 (Glc).

Acid Hydrolysis of I, IV and VIII, Preparation of II Compound I (40 mg) was dissolved in 2 N H_2SO_4 (3 ml) and the solution was heated at 90 °C for 10 h. After cooling to room temperature, precipitates were collected by filtration and subjected to silica gel chromatography (benzene–acetone, 2 : 1) to give II (2 mg): a white amorphous powder. mp >300 °C (dec.), $[\alpha]_D^{22}$ +81.5° (c=0.40, pyridine). FAB-MS m/z: 511.336 ($[M+Na]^+$). $C_{30}H_{52}NaO_5$ requires m/z 511.340. ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and III, respectively. The same treatment of IV and VIII gave II.

Identification of Component Monosaccharides A glycoside (5 mg) was dissolved in 1 N HCl–MeOH (0.5 ml) and heated at 90 °C for 1 h. The acidic solution was neutralized with ion exchange resin Amberlite IR-410 and concentrated *in vacuo*. The residue was trimethylsilylated with the trimethylsilylimidazole reagent and checked by gas liquid chromatography (GLC). Authentic sugar samples were treated in the same manner and t_R values were compared with those of unknown samples. The results are shown in the text. The absolute configurations of the component monosaccharides were determined according to the method reported by Hara *et al.*[7] Thus, a glycoside (5 mg) was hydrolyzed with 1 N HCl. After neutralization with Amberlite IR-410, the free sugar samples were converted into the thiazolidine derivatives and checked by GLC. The absolute configuration confirmed by this method is shown in the text.

Permethylation of the Glycosides and Identification of the Component Methylated Monosaccharides Methylation of the glycosides was performed according to the method reported by Hakomori,[8] and the product was purified by column chromatography on silica gel. The permethylates were methanolyzed in 1 N HCl–MeOH, and neutralized with Ag_2CO_3. The methanolysates were acetylated with Ac_2O–pyridine and checked by gas chromatography–chemical ionization (GC-CI)-MS. Methylated monosaccharides were identified by comparison of t_R values and CI-MS patterns with those of authentic sugar samples.

Enzymatic Hydrolysis of VII Compound VII (100 mg) and a cellulase (100 mg) (Sigma Co., type I) were dissolved in 20% aqueous EtOH and the solution was shaken for 7 d at 37 °C. The reaction mixture was diluted with H_2O and extracted with BuOH. The BuOH layer was washed with H_2O and evaporated. The residue was purified by HPLC on a Nucleosil column using $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) as the eluting solvent to give III (20 mg).

Acknowledgements The authors are grateful to Miss J. Honda for elemental analysis and to Mr. T. Nagao for identification of the component sugars.

References and Notes

1) This work was reported at the 108th Annual Meeting of The Pharmaceutical Society of Japan, Hiroshima, April 1987.
2) *a*) M. Iwamoto, T. Fujioka, H. Okabe, K. Mihashi and T. Yamauchi, *Chem. Pharm. Bull.*, **35**, 553 (1987); *b*) T. Fujioka, Y. Iwase, H. Okabe, K. Mihashi and T. Yamauchi, *ibid.*, **35**, 3870 (1987); *c*) T. Fujioka, M. Iwamoto, Y. Iwase, H. Okabe, K. Mihashi and T. Yamauchi, *ibid.*, **36**, 2772 (1988).
3) *a*) R. Kasai, M. Miyakoshi, K. Matsumoto, R.-L. Nie, J. Zhou, T. Morita and O. Tanaka, *Chem. Pharm. Bull.*, **34**, 3974 (1986); *b*) R. Kasai, M. Miyakoshi, R.-L. Nie, J. Zhou, K. Matsumoto, T. Morita,

M. Nishi, K. Miyahara and O. Tanaka, *Phytochemistry*, **27**, 1439 (1988); *c*) F.-H. Kong, D.-Y. Zhu, R.-S. Xu, Z.-C. Fu, L.-Y. Zhou, T. Iwashita and H. Komura, *Tetrahedron Lett.*, **27**, 5765 (1986).
4) K. Ohtani, K. Mizutani, R. Kasai and O. Tanaka, *Tetrahedron Lett.*, **25**, 4537 (1984).
5) K. Mizutani, K.Ohtani, R. Kasai, O. Tanaka and H. Matsuura, *Chem. Pharm. Bull.*, **33**, 2266 (1985).
6) The instruments and materials used in this work were the same as described in the preceding papers.[2a,b] The GLC and GC-CI-MS conditions are described in another paper[9] from this laboratory. The NMR data of all samples were obtained in both pyridine-d_5 and pyridine-d_5–D_2O solutions. The ^{1}H-NMR data shown in the text, tables and the experimental section are those obtained in pyridine-d_5–D_2O solutions and the ^{13}C-NMR data are those obtained in pyridine-d_5 solutions, unless otherwise specified. The signal assignment was essentially based on the data reported for compounds having similar structures, and confirmed with the aid of NMR spectral techniques (^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, NOE difference, decoupling difference and long-range ^{1}H–^{13}C COSY spectra).
7) S. Hara, H. Okabe and K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986); *idem*, *ibid.*, **35**, 501 (1987).
8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 255 (1964).
9) T. Nagao, H. Okabe, K. Mihashi and T. Yamauchi, *Chem. Pharm. Bull.*, **37**, 925 (1989).