Studies on the Constituents of *Actinostemma lobatum* MAXIM. V.[1] Structures of Lobatosides B, E, F and G, the Dicrotalic Acid Esters of Bayogenin Bisdesmosides Isolated from the Herb

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The structures of lobatosides B, E, F and G, the dicrotalic acid esters of bayogenin bisdesmosides isolated from the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae), were determined on the basis of chemical and spectral evidence.

Lobatoside B is the dicrotalic acid (3-hydroxy-3-methylglutaric acid) ester of 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin 28-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester. Dicrotalic acid is linked at one end to the C_6-hydroxyl group of the terminal β-D-glucopyranosyl 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).

Lobatosides E, F and G are cyclic bisdesmosides of bayogenin similar to lobatoside B, but different in the numbers and species of the component sugars and positions of the ester linkages of the dicrotalic acid.

Keywords *Actinostemma lobatum*; Cucurbitaceae; lobatoside; bayogenin glycoside; cyclic bisdesmoside; dicrotalic acid; NOE difference spectrum; decoupling difference spectrum

In the preceding paper[2] of this series, we reported the isolation of eight oleanane-type triterpene glycosides, lobatosides A—H, from the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae) and the structures of lobatosides A, C, D and H.

Lobatoside A (I) was determined to be a 3-*O*-glycoside of bayogenin, and C (II), D (III) and H (IV) were elucidated to be the 3,28-*O*-bisdesmosides of bayogenin having a dicrotalic acid ester bridge between the two sugar moieties. Lobatosides B (V), E (VI), F (VII) and G (VIII) were proved to be the cyclic bisdesmosides of bayogenin similar to lobatosides C, D and H, and the present paper deals with their structures.

Lobatoside B (V) was obtained as colorless needles from aqueous MeOH. The positive fast atom bombardment mass spectra (FAB-MS) showed an $[M+Na]^+$ ion at *m/z* 1239 and negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 1215, indicating the molecular weight to be 1216. The results of the elemental analysis were consistent with the molecular formula $C_{59}H_{92}O_{26}\cdot 4H_2O$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Tables I and II) of V showed the signals of the aglycone moiety to be similar to those of lobatoside C (II) and revealed four anomeric protons (δ 5.04, d, $J=8$ Hz; 5.29, d, $J=8$ Hz; 5.89, d, $J=8$ Hz; 6.35, br s). Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Tables III and IV) of V with that of II indicated that V is a 3,28-*O*-bisdesmoside of bayogenin and the ^{13}C-NMR signals (δ 26.5, 46.4, 70.1, 171.7 and 172.0) suggested the presence of ester-linked dicrotalic acid (3-hydroxy-3-methylglutaric acid).

Compound V yielded a desacylated compound (IX) and a dicrotalic acid (X) on treatment with 0.5% KOH. Compound IX showed an $[M+Na]^+$ ion at *m/z* 1113 in positive FAB-MS and an $[M-H]^-$ ion at *m/z* 1089 in negative FAB-MS, and it gave bayogenin (XI), D-glucose, L-arabinose and L-rhamnose on acid hydrolysis.

The selective cleavage of the ester glycoside linkage of IX

TABLE I. ^{1}H-NMR Chemical Shifts[a] of Aglycone Moieties of Lobatosides and Their Degradation Products

	V	IX	XII	VI	XIV	XV	VII	VIII
H-2	4.78 br s	4.80 br s	4.81 br s	4.85 br s	4.78 br s	4.78 br s	4.61 br s	4.60 br s
H-3	4.17 d (3.5)	4.16 d (3)	4.19 d (3)	4.31 d (3)	4.16 d (3)	4.17 d (3.5)	4.33 d (3)	4.32 d (3)
H-12	5.47 br s	5.48 br s	5.49 br s	*ca.* 5.45	*ca.* 5.50	*ca.* 5.45	5.41 br s	5.44 br s
H-18	3.15 dd (4, 14)	3.24 dd (4, 14)	3.26 dd (4, 14)	3.12 dd (4, 14)	3.23 dd (4, 14)	3.25 dd (4, 14)	3.14 dd (5, 14)	3.15 dd (4, 13)
H-23	3.52 d (12)	3.64 d (11)	3.68 d (11)	3.91 d (11)	3.66 d (11)	3.66 d (11)	3.72 d (11)	3.60 d (11)
	4.34 d (12)	4.32 d (11)	4.35 d (11)	4.41 d (11)	4.35 d (11)	4.35 d (11)	4.41 d (11)	4.29 d (11)
H-24	1.35 s	1.34 s	1.38 s	1.53 s	1.34 s	1.34 s	1.63 s	1.54 s
H-25	1.53 s	1.51 s	1.51 s	1.65 s	1.51 s	1.51 s	1.63 s	1.58 s
H-26	1.13 s	1.09 s	1.04 s	1.23 s	1.13 s	1.13 s	1.09 s	1.05 s
H-27	1.32 s	1.24 s	1.27 s	1.35 s	1.25 s	1.25 s	1.23 s	1.25 s
H-29	0.93 s	0.91 s	0.93 s	0.92 s	0.92 s	0.92 s	0.91 s	0.93 s
H-30	0.88 s	0.99 s	1.00 s	0.88 s	0.98 s	0.98 s	0.91 s	0.92 s
Dicrotalic acid moiety								
2′ (4′)	2.89 d (17)			3.01 d (16)			3.06 d (16)	3.00 d (16)
	3.19 d (17)			3.30 d (16)			3.42 d (16)	3.19 d (16)
4′ (2′)	2.90 d (15)			3.19 d (17)			3.14 d (15)	3.18 s-like
	3.52 d (15)			3.32 d (17)			3.22 d (15)	
6′	1.77 s			2.01 s			2.10 s	1.98 s

a) The spectra were measured in pyridine-d_5 containing D_2O (the spectrum of VII was measured in pyridine-d_5). The values in parentheses are coupling constants in Hz.

TABLE II. ^{1}H-NMR Chemical Shifts[a] of Sugar Moieties of Lobatosides and Their Degradation Products

	V	IX	XII	VI	XIV	XV	VII	VIII
3Glc-1	5.04 d (8)	5.11 d (8)	5.13 d (8)	5.12 d (8)	5.08 d (8)	5.08 d (8)	5.07 d (8)	5.04 d (8)
3Glc-2	4.07 dd (8, 9)	4.22 dd (8, 9)	4.21 dd (8, 9)	4.25 dd (8, 9)	4.15 dd (8, 9)	4.15 dd (8, 9)	4.44 dd (8, 9)	4.48 dd (8, 9)
3Glc-3	4.25 t (9)	4.29 t (9)	4.29 t (9)	4.18 t (9)	4.25 t (9)	4.24 t (9)	4.18 t (9)	4.25 t (9)
3Glc-4	4.12 t (9)	4.09 t (9)	4.12 t (9)	4.02 t (9)	4.08 t (9)	4.09 t (9)	*ca.* 4.06	4.05 t (9)
3Glc-5	3.87 ddd (3, 6, 9)	3.86 ddd (3, 6, 9)	3.88 ddd (2, 5, 9)	3.79 ddd (3, 6, 9)	3.84 ddd (3, 6, 9)	3.83 ddd (3, 5, 9)	*ca.* 3.80	3.81 ddd (3, 6, 9)
3Glc-6	4.24 dd (6, 12)	4.23 dd (6, 12)	4.23 dd (5, 12)	4.20 dd (6, 12)	*ca.* 4.20	4.21 dd (5, 12)	*ca.* 4.20	4.22 dd (6, 12)
	4.42 dd (3, 12)	4.40 dd (3, 12)	4.42 dd (2, 12)	4.38 dd (3, 12)	*ca.* 4.35	4.39 dd (3, 12)	*ca.* 4.35	4.42 dd (3, 12)
3Glc′-1	5.29 d (8)	5.38 d (8)	5.39 d (8)					
3Glc′-2	4.08 dd (8, 9)	4.09 dd (8, 9)	4.11 dd (8, 9)					
3Glc′-3	4.20 t (9)	4.21 t (9)	4.22 t (9)					
3Glc′-4	4.13 t (9)	4.15 t (9)	4.18 t (9)					
3Glc′-5	4.00 br d (9)	3.94 ddd (3, 6, 9)	3.95 ddd (3, 5, 9)					
3Glc′-6	4.69 dd (4, 12)	4.33 dd (6, 12)	4.34 dd (5, 12)					
	4.93 br d (12)	4.51 dd (3, 12)	4.52 dd (3, 12)					
3Gal-1				5.07 d (7.5)	5.26 d (8)	5.26 d (8)	5.75 d (7)	5.73 d (7.5)
3Gal-2				4.51 dd (7.5, 9)	4.51 dd (8, 10)	4.52 dd (8, 10)	4.47 dd (7, 9)	4.40 dd (7.5, 9)
3Gal-3				4.06 dd (3, 9)	4.10 dd (3, 10)	4.11 dd (3, 10)	4.30 dd (3, 9)	4.27 dd (3, 9)
3Gal-4				4.28 br d (3)	4.57 br d (3)	4.57 br d (3)	6.01 br d (3)	5.97 br d (3)
3Gal-5				*ca.* 4.08	4.03 br t (6)	4.04 br t (6)	4.05 br t (6)	4.04 br t (6)
3Gal-6				4.73 dd (2, 12)	*ca.* 4.40	4.42 dd (6, 11)	*ca.* 4.15	4.12 dd (6, 12)
				4.82 dd (7, 12)	*ca.* 4.40	4.47 dd (6, 11)	*ca.* 4.30	*ca.* 4.24
28Ara-1	5.89 d (8)	6.44 d (3)		6.01 d (6)	6.41 br s		6.17 d (4)	6.13 d (4)
28Ara-2	4.68 dd (8, 9)	*ca.* 4.53		4.60 t (6)	*ca.* 4.48		4.59 dd (4, 5)	4.60 dd (4, 6)
28Ara-3	4.23 dd (3, 9)	*ca.* 4.53		*ca.* 4.32	*ca.* 4.45		4.53 dd (3, 5)	4.52 dd (3, 6)
28Ara-4	*ca.* 4.29	*ca.* 4.53		*ca.* 4.28	*ca.* 4.45		*ca.* 4.30	*ca.* 4.38
28Ara-5	3.88 br d (12)	*ca.* 3.93		*ca.* 3.83	*ca.* 3.91		*ca.* 3.87	*ca.* 3.93
	4.27 br d (12)	*ca.* 4.41		*ca.* 4.26	*ca.* 4.45		*ca.* 4.30	*ca.* 4.38
28Rha-1	6.35 br s	5.72 br s		6.09 br s	5.63 br s		5.98 br s	6.03 br s
28Rha-2	4.87 dd (1.5, 3)	4.56 dd (1.5, 3)		5.15 dd (1.5, 3)	4.88 dd (1.5, 3)		5.08 dd (1.5, 3)	4.70 dd (1.5, 3)
28Rha-3	4.52 dd (3, 10)	4.47 dd (3, 9)		4.61 dd (3, 10)	4.58 dd (3, 9)		4.62 dd (3, 9)	4.45 dd (3, 10)
28Rha-4	5.88 t (10)	4.28 t (9)		6.00 t (10)	4.38 t (9)		6.02 t (9)	5.83 t (10)
28Rha-5	4.42 dq (10, 6)	4.38 dq (9, 6)		4.41 dq (10, 6)	4.43 dq (9, 6)		4.31 dq (9, 6)	4.22 dq (10, 6)
28Rha-6	1.52 d (6)	1.70 d (6)		1.70 d (6)	1.67 d (6)		1.51 d (6)	1.50 d (6)
28Glc-1				5.02 d (8)	5.19 d (8)		5.09 d (8)	
28Glc-2				3.83 dd (8, 9)	4.06 dd (8, 9)		3.80 dd (8, 9)	
28Glc-3				4.09 t (9)	4.17 t (9)		4.10 t (9)	
28Glc-4				4.01 t (9)	4.10 t (9)		*ca.* 4.06	
28Glc-5				3.75 ddd (3, 6, 9)	3.82 ddd (3, 6, 9)		*ca.* 3.80	
28Glc-6				4.20 dd (6, 12)	*ca.* 4.20		*ca.* 4.20	
				4.32 dd (3, 12)	*ca.* 4.35		*ca.* 4.35	

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

according to the method reported by Ohtani *et al.*[3] provided a monodesmoside (XII) and an anomeric mixture (XIII) of methyl glycosides. Compound XII showed an $[M+Na]^+$ ion at *m/z* 835 in positive FAB-MS and an $[M-H]^-$ ion at *m/z* 811 in negative FAB-MS. The ^{1}H-NMR spectrum showed two anomeric proton signals at δ 5.13 (d, $J=8$ Hz) and 5.39 (d, $J=8$ Hz). It gave D-glucose on acid hydrolysis, and its permethylate gave methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose and 3,4,6-tri-*O*-methyl-D-glucopyranose on methanolysis. Therefore, XII is bayogenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

The negative FAB-MS of XIII showed an $[M-H]^-$ ion at *m/z* 309 and a fragment ion at *m/z* 163 ($[M-H-146]^-$), and its ^{1}H-NMR spectrum showed four anomeric proton signals at δ 4.58 (d, $J=6$ Hz), 5.34 (d, $J=3.5$ Hz), 5.69 (d, $J=1.5$ Hz) and 6.02 (d, $J=1$ Hz). It gave L-arabinose and L-rhamnose on acid hydrolysis, and the permethylate of XIII gave methyl glycosides of 2,3,4-tri-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis, thus indicating that XIII is a mixture of methyl α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer. The α-configuration of the L-rhamnosyl group is postulated from the $J_{H1,H2}$ values (1, 1.5 Hz), supposing that the L-rhamnosyl group is in the $^1C_4$ conformation. The α-configuration and $^1C_4$-conformation of the L-rhamnopyranosyl group in IX were determined from the $J_{C1,H1}$ value (169 Hz)[4] and the splitting patterns of the oxymethine protons of the rhamnopyranosyl group. Consequently, the structure of IX formulated to be 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin 28-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.

The anomeric configuration of the L-arabinopyranosyl unit in IX was determined to be α from the $J_{C1,H1}$ and $J_{H1,H2}$ values of the ester-linked arabinopyranosyl group in the original glycoside (V). The $J_{C1,H1}$ value (162 Hz) and J values of H_1 (d, $J=8$ Hz), H_2 (dd, $J=8$, 9 Hz) and H_3 (dd, $J=9$, 3 Hz) of the L-arabinopyranosyl group in V clearly indicate that the sugar has an α-configuration in the $^4C_1$ conformation.[4] The configuration of the sugar linkage would not be changed by the mild alkaline hydrolysis of the

TABLE III. ^{13}C-NMR Chemical Shifts[a] of Aglycone Moieties of Lobatosides and Their Degradation Products

	V	IX	XI	XII	VI	XIV	XV	VII	VIII
C-1	44.1	44.0	44.8	43.9	44.2	44.0	43.9	44.0	44.4
C-2	69.4	70.3	71.5	70.3	70.7	70.4	70.3	70.0	69.0
C-3	83.1	83.1	73.1	83.0	83.2	82.6	82.5	83.4	84.2
C-4	42.2[b]	42.7	42.3	42.7	42.7	42.7	42.6	43.4	43.5
C-5	48.7	48.5	48.5	48.5	48.5	48.5	48.5	48.4	48.5
C-6	18.0	18.1	18.3	18.0	18.8	18.1	18.0	18.7	19.0
C-7	33.7	33.0	33.0	33.2	33.4	33.0	33.0	32.9	33.1
C-8	40.1	40.0	39.9	39.8	40.0	40.0	39.9	40.0	40.1
C-9	47.7	48.2	48.2	48.2	47.6	48.0	47.9	47.1	47.0
C-10	36.9	36.9	37.2	36.9	37.0	36.9	36.9	37.2	37.4
C-11	24.0	24.0	23.9	24.0	24.0	24.0	24.0	23.9	23.9
C-12	122.9	123.1	122.7	122.6	123.2	123.1	121.7	124.0	123.2
C-13	144.2	144.3	144.8	144.9	144.1	144.2	144.8	144.1	144.0
C-14	42.1[b]	42.3	42.3	42.3	42.3	42.3	42.3	41.8	42.1
C-15	29.3	28.3	28.2	28.2	28.9	28.3	28.2	28.8	28.9
C-16	22.6	23.2	23.7	23.7	22.9	23.2	23.7	22.8	22.9
C-17	47.3	47.4	46.6	46.7	47.2	47.3	46.6	47.0	47.2
C-18	41.5	41.7	42.0	42.0	41.6	41.7	42.0	41.5	41.6
C-19	46.2	46.3	46.4	46.4	46.2	46.3	46.4	45.9	46.1
C-20	30.7	30.9	30.9	30.9	30.7	30.8	30.9	30.8	30.7
C-21	34.1	34.2	34.2	34.2	34.1	34.2	34.2	34.0	34.1
C-22	32.3	32.7	33.1	33.0	32.3	32.7	33.2	32.3	32.3
C-23	64.0	65.8	67.8	65.8	64.7	65.4	65.3	65.3	66.0
C-24	15.0	14.8	14.4	14.7	15.4	14.9	14.8	16.0	16.2
C-25	17.3	17.2	17.2	17.1	17.4	17.3	17.1	17.7	17.6
C-26	17.4	17.5	17.5	17.5	17.7	17.5	17.4	17.9	18.1
C-27	26.2	26.1	26.2	26.2	25.6	26.1	26.2	26.1	26.2
C-28	176.3	176.2	180.1	180.3	176.2	176.2	180.1	176.1	176.2
C-29	33.1	33.1	33.1	33.2	33.1	33.1	33.2	33.2	33.1
C-30	23.6	23.7	23.7	23.7	23.5	23.7	23.7	23.7	23.6
Dicrotalic acid moiety									
1′	172.0[c]				171.3[b]			170.9[b]	171.0[b]
2′	46.1[d]				47.0[c]			46.8[c]	47.3[c]
3′	70.1				70.3			70.2	70.1
4′	46.4[d]				47.5[c]			48.9[c]	48.7[c]
5′	171.7[c]				170.9[b]			171.4[b]	171.1[b]
6′	26.5				26.1			26.3	26.1

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

TABLE IV. ^{13}C-NMR Chemical Shifts[a] of Sugar Moieties of Lobatosides and Their Degradation Products

	V	IX	VII	VI	XIV	XV	VII	VIII
3Glc-1	102.4	103.0	102.9	105.0	103.1	103.0	104.1	103.7
3Glc-2	85.3	83.5	83.6	82.3	83.4[b]	83.4	78.4	78.4
3Glc-3	78.5	78.3[b]	78.0[b]	77.2[b]	78.1[c]	78.2	78.9	79.1
3Glc-4	70.0[b]	71.1	71.0	71.6[c]	71.2	71.2	71.4[b]	71.5
3Glc-5	78.3	78.0	78.0	77.6[d]	77.9[c]	77.9	78.1[c]	78.2
3Glc-6	62.5	62.4	62.4	62.5[e]	62.5	62.5	62.5[d]	62.5
3Glc′-1	105.8	105.7	105.7					
3Glc′-2	76.7	76.7	76.7					
3Glc′-3	77.7	78.0[b]	78.2[b,c]					
3Glc′-4	70.4[b]	71.4	71.3					
3Glc′-5	75.5	78.4	78.4[c]					
3Glc′-6	64.2	62.6	62.5					
3Gal-1				106.1	106.3	106.3	103.8	103.7
3Gal-2				75.0	74.4	74.3	73.8	73.7
3Gal-3				73.4	74.9	74.9	73.2	73.1
3Gal-4				70.9	69.8	69.8	71.5[b]	71.5
3Gal-5				75.1	77.0	77.0	74.9	74.9
3Gal-6				66.6	61.7	61.7	61.5[d]	61.3
28Ara-1	94.8	93.5		94.5	93.6		94.1	94.3
28Ara-2	75.7	75.2		76.6	75.3		75.4	74.8
28Ara-3	75.8[c]	70.4		73.6	70.4[d]		70.3	71.4
28Ara-4	70.5[b]	66.3		68.8	66.2		67.2	67.7
28Ara-5	67.9	63.1		66.5	63.2		64.3	65.0
28Rha-1	102.2	101.4		101.9	101.5		100.5	100.4
28Rha-2	72.4	72.3		72.1	71.5		72.0	72.4
28Rha-3	76.6[b]	72.6		79.1	83.5[b]		78.9	70.2
28Rha-4	76.0[c]	73.8		73.2	72.7		73.2	75.3
28Rha-5	67.3	70.4		67.9	70.2[d]		68.1	67.8
28Rha-6	18.2	18.5		18.4	18.5		18.5	18.3
28Glc-1				105.7	106.5		105.8	
28Glc-2				74.9	75.9		74.8	
28Glc-3				77.9[b]	78.3[c]		78.1	
28Glc-4				71.7[c]	71.5		71.6[b]	
28Glc-5				77.7[d]	78.1[c]		77.7[c]	
28Glc-6				62.7[e]	62.5		62.5[d]	

a) The spectra were measured in pyridine-d_5 and chemical shifts were expressed in δ values. Abbreviations: Glc, glucose; Glc′, glucose at the terminal of the C_3-linked sugar moiety; Gal, galactose; Ara, arabinose; Rha, rhamnose, 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. b—e) The values in each column may be interchanged.

dicrotalic acid moiety, and therefore the configuration of the L-arabinopyranosyl group in IX should be also the α. The anomalous small $J_{H1,H2}$ value (3 Hz) of the α-L-arabinopyranosyl group in IX indicates that the $^4C_1$ conformation of the α-L-arabinopyranosyl group in V has changed to $^1C_4$ as a result of the splitting of the dicrotalic acid moiety. The $J_{C1,H1}$ value (169 Hz) of the α-L-arabinopyranosyl group in IX supports the $^1C_4$ conformation.[4]

The last problem concerning the structure of V is the positions of the ester linkages of dicrotalic acid. This problem was solved by NMR spectroscopic methods. All proton signals of the sugar moieties of V and IX were assigned as summarized in Table II using the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), the nuclear Overhauser effect (NOE) difference spectroscopy and the decoupling difference spectroscopy techniques. Thus, when the anomeric proton (G1, δ 5.04) of one glucopyranosyl group was irradiated, NOE was observed at the signals of C_2-H and C_3-H of the aglycone moiety (Fig. 1). Therefore, G1 is the anomeric proton of the inner glucopyranosyl group. Irradiation at the anomeric proton (G′1, δ 5.29) of the other (terminal) glucopyranosyl group showed up the signals of C_2-H (G′2), C_3-H (G′3), C_4-H (G′4) and C_5-H

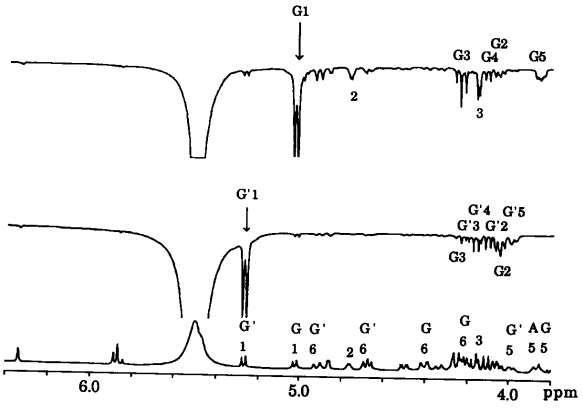
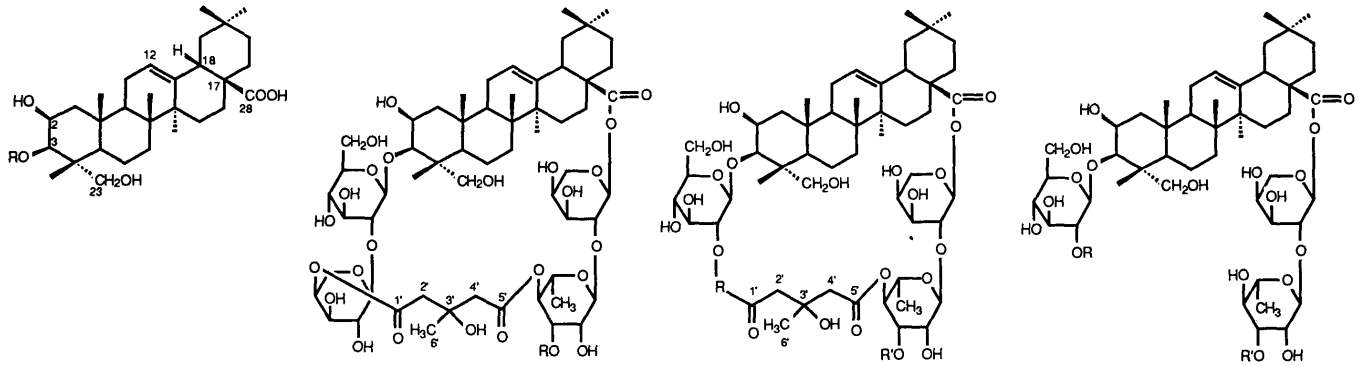


Fig. 1. NOE Difference Spectra of Lobatoside B (V)
G, inner glucose; G′, terminal glucose.

(G′5). The signals of the C_6-H (G′6) were assigned at δ 4.69 and 4.93 by ^{1}H–^{1}H COSY. The ^{1}H-NMR signals of the rhamnopyranosyl group were identified using the decoupling difference spectroscopy technique as shown in Fig. 2. The chemical shifts of protons of V and IX were compared.



I : R=α-L-ara-(1→2)-β-D-glc-
XI : R=H
XII : R=β-D-glc-(1→2)-β-D-glc-
XV : R=β-D-gal-(1→2)-β-D-glc-

II : R=H
III : R=β-D-glc-
IV : R=β-D-xyl-

V : R=β-D-glc(6)*, R′=H
VI : R=β-D-gal(6), R′=β-D-glc-
VII : R=β-D-gal(4), R′=β-D-glc-
VIII: R=β-D-gal(4), R′=H

IX : R=β-D-glc-, R′=H
XIV: R=β-D-gal-, R′=β-D-glc-

* glc(6) means that one carboxylic acid group of dicrotalic acid is linked to the C_6-hydroxyl group of the glucopyranosyl group

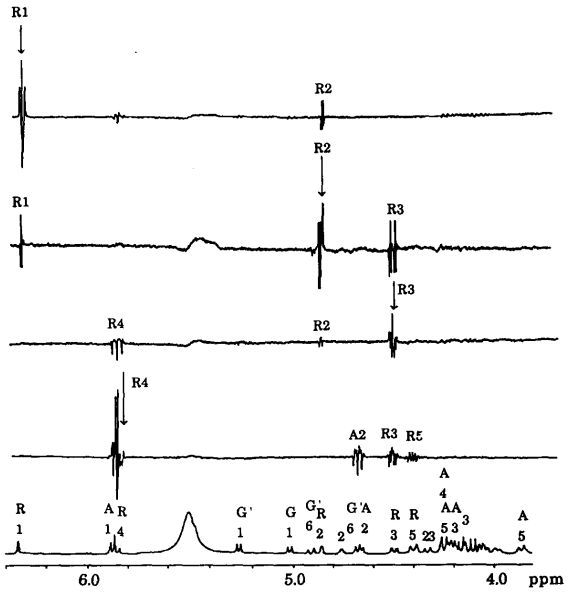


Fig. 2. Decoupling Difference Spectra of Lobatoside B (V)
G, inner glucose; G′, terminal glucose; A, arabinose; R, rhamnose.

There were significant upfield shifts of the C_4-H (R4) signal of the rhamnopyranosyl unit (1.60 ppm) and the C_6-H (G′6) signals of the terminal glucopyranosyl unit (0.36—0.42 ppm) on going from V to IX.

These upfield shifts indicated that dicrotalic acid is linked to the C_4-hydroxyl group of the rhamnopyranosyl unit at one end, and to the C_6-hydroxyl group of the terminal glucopyranosyl unit at the other end. From all the above evidence, the structure of V was concluded to be as shown.

Lobatoside E (VI), $C_{65}H_{102}O_{31}\cdot 3H_2O$, was obtained as colorless needles from aqueous MeOH. The positive FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1401 and negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 1377. The general feature of the NMR spectra suggested that VI is also a cyclic bisdesmoside of bayogenin similar to lobatoside B (V). Compound VI gave dicrotalic acid and a desacylated derivative (XIV) on mild alkaline hydrolysis. Compound XIV showed an $[M+Na]^+$ ion at *m/z* 1275 in positive FAB-MS and an $[M-H]^-$ ion at *m/z* 1251 in negative FAB-MS. The ^{1}H-NMR spectrum showed the signals of five anomeric protons at δ 5.08 (d, J=8 Hz), 5.26 (d, J=8 Hz), 6.41 (br s), 5.63 (br s) and 5.19 (d, J=8 Hz). Compound XIV gave XI, D-glucose, D-galactose, L-arabinose and L-rhamnose on acid hydrolysis. The selective cleavage of the ester glycoside linkage provided a monodesmoside (XV) and an anomeric mixture (XVI) of methyl glycosides.

Compound XV showed an $[M+Na]^+$ ion at *m/z* 835 in positive FAB-MS and an $[M-H]^-$ ion at *m/z* 811 in negative FAB-MS, and the ^{1}H-NMR spectrum showed the signals of the anomeric protons at δ 5.08 (d, J=8 Hz) and 5.26 (d, J=8 Hz). Compound XV gave D-glucose and D-galactose on acid hydrolysis, and its permethylate gave methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 3,4,6-tri-*O*-methyl-D-glucopyranose on methanolysis. Therefore, XV was concluded to be 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin.

Compound XVI 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 ($[M-H-162]^-$) and *m/z* 163 ($[M-H-308]^-$). These ions indicated that XVI is a methyl glucosyl-rhamnosyl-arabinoside. The permethylate of XVI 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 XIV is formulated to be 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucopyranosyl]bayogenin 28-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester. The configuration of the L-arabinopyranosyl group is considered to be α because the ^{1}H- and ^{13}C-NMR chemical shifts of the protons and carbons of the arabinopyranosyl unit were almost the same as those of IX. The positions of the ester linkages of dicrotalic acid in VI were determined to be the C_6-hydroxyl group of the galactopyranosyl group and the C_4-hydroxyl group of the rhamnopyranosyl group by comparison of the ^{1}H-NMR data (Table II) of VI and XIV, leading to the structure VI as shown.

Lobatoside F (VII) was obtained as a white powder. The positive FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1401 and negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 1377. The NMR spectra showed that VII is also a dicrotalic acid ester of a bayogenin bisdesmoside. Compound VII gave dicrotalic acid and a desacylated compound (XIV) on mild alkaline hydrolysis. The positions of the ester linkages of dicrotalic acid in VII were determined to be the C_4-hydroxyl groups of the galactopyranosyl group and the

rhamnopyranosyl group by comparison of the ^{1}H-NMR data (Table II) of VII and XIV. From all the above evidence, the structure of VII was determined to be as shown.

Lobatoside G (VIII), $C_{59}H_{92}O_{26}\cdot H_2O$, was obtained as a white powder. The positive FAB-MS showed and $[M+Na]^+$ ion at *m/z* 1239 and negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 1215. The ^{1}H-NMR spectrum showed the signals of four anomeric protons (δ 5.04, d, J= 8 Hz; 5.73, d, J=7.5 Hz; 6.03, br s; 6.13, d, J=4 Hz), and VIII gave D-glucose, D-galactose, L-arabinose and L-rhamnose on acid hydrolysis. The ^{13}C-NMR spectrum showed signals assignable to bayogenin and dicrotalic acid moieties. The structure of the sugar moiety and the positions of the ester linkages of dicrotalic acid were investigated by detailed examination of the NMR spectra and the structure was tentatively proposed to be as shown. The configuration of the ester-linked L-arabinopyranosyl group was supposed to be α from the facts that the NMR chemical shifts of the protons and carbons of the arabinopyranosyl group are almost the same as those of VII.

The conformations of the ester-linked α-L-arabinopyranosyl groups in VI, VII and VIII are not obvious because the splitting patterns of the protons of the arabinopyranosyl groups could not be clarified. However, judging only from the $J_{C1,H1}$ and $J_{H1,H2}$ values of the arabinopyranosyl groups of VI (166, 6 Hz), VII (169, 4 Hz) and VIII (166, 4 Hz), it may be supposed that the α-L-arabinopyranosyl group is present as an equilibrium mixture of the $^1C_4$ and $^4C_1$ conformers, in which the $^4C_1$ conformer predominates in VI, while the $^1C_4$ conformer is predominant in VII and VIII.

Experimental[5]

Isolation of Lobatosides The procedure for isolation of lobatosides was described in the previous paper[2] of this series. The NMR chemical shifts and assignments are summarized in Tables I—IV.

Lobatoside B (V): Colorless needles from aqueous MeOH, mp 274—278 °C. $[\alpha]_D^{22}$ +31.5° (c=0.59, pyridine). *Anal.* Calcd for $C_{59}H_{92}O_{26}\cdot 4H_2O$: C, 54.96; H, 7.82. Found: C, 55.05; H, 7.83. Positive FAB-MS *m/z*: 1239 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1215 ($[M-H]^-$).

Lobatoside E (VI): Colorless needles from aqueous MeOH, mp 255—260 °C. $[\alpha]_D^{22}$ +11.0° (c=0.48, pyridine). *Anal.* Calcd for $C_{65}H_{102}O_{31}\cdot 3H_2O$: C, 54.46; H, 7.59. Found: C, 54.34; H, 7.67. Positive FAB-MS *m/z*: 1401 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1377 ($[M-H]^-$).

Lobatoside F (VII): A white amorphous powder, mp 227—235 °C. $[\alpha]_D^{22}$ +7.04° (c=0.27, pyridine). Positive FAB-MS *m/z*: 1401 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1377 ($[M-H]^-$).

Lobatoside G (VIII): A white amorphous powder, mp 250—254 °C. $[\alpha]_D^{22}$ +8.64° (c=0.44, pyridine). *Anal.* Calcd for $C_{59}H_{92}O_{26}\cdot H_2O$: C, 57.36; H, 7.67. Found: C, 57.53; H, 7.62. Positive FAB-MS *m/z*: 1239 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1215 ($[M-H]^-$).

Mild Alkaline Hydrolysis of V, VI and VII Compound V (280 mg) was dissolved in 0.5% KOH (20 ml) and the solution was stirred at room temperature for 24 h. The reaction solution was neutralized with an ion exchange resin (Amberlite IR-120) and the solvent was evaporated off. The residue was chromatographed on silica gel [solvent, $CHCl_3$–MeOH–H_2O (7:3:0.5)] to give IX (170 mg) and X (1 mg). Both compounds were purified by chromatography on LH-20 (solvent, MeOH).

IX: A white amorphous powder. Positive FAB-MS *m/z*: 1113 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1089 ($[M-H]^-$). ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR: shown in Tables III and IV.

X: A yellow syrup. Positive 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 VI (200 mg) was treated in the same manner to give X (1 mg) and XIV (104 mg).

XIV: A white amorphous powder. Positive FAB-MS *m/z*: 1275 ($[M+Na]^+$). Negative FAB-MS *m/z*: 1251 ($[M-H]^-$). ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR: shown in Tables III and IV.

Compound VII (20 mg) was treated in the same manner to give X and XIV (13 mg).

Selective Cleavage of the Ester Glycoside Linkages of IX and XIV Compound IX (55 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, then deionized with Amberlite MB-3 and the solvent was evaporated off. The residue was chromatographed on silica gel [solvent, $CHCl_3$–MeOH–H_2O (7:3:0.5)] and on LH-20 (solvent, MeOH) to give XII (13 mg) and XIII (2 mg). Compound XIV (50 mg) was treated in the same manner to give XV (14 mg) and XVI (2 mg). Compound XVI was subjected to high performance liquid chromatography (HPLC) [Nucleosil, solvent, $CHCl_3$–MeOH–H_2O (6:4:1)] to give an α-anomer (XVIα) and its β-anomer (XVIβ).

XII: A white amorphous powder. Positive FAB-MS *m/z*: 835 ($[M+Na]^+$). Negative FAB-MS *m/z*: 811 ($[M-H]^-$). ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR: shown in Tables III and IV.

XIII: A colorless syrup. Negative FAB-MS *m/z*: 309 ($[M-H]^-$). ^{1}H-NMR (pyridine-d_5) δ: anomeric H; 6.02 (d, J=1 Hz, Rha of α-anomer), 5.69 (d, J=1.5 Hz, Rha of the β-anomer), 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 of the α-anomer), 101.2 (β-Ara), 104.4 (Rha of the β-anomer).

XV: A white amorphous powder. Positive FAB-MS *m/z*: 835 ($[M+Na]^+$). Negative FAB-MS *m/z*: 811 ($[M-H]^-$). ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR: shown in Tables III and IV.

XIVα: A colorless syrup. Negative FAB-MS *m/z*: 471 ($[M-H]^-$), 309 ($[M-H-162]^-$), 163 ($[M-H-308]^-$). ^{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).

XVIβ: A colorless syrup. Negative FAB-MS *m/z*: 471, 309, 163. ^{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 IX and XIV, Identification of the Aglycone Compound IX (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, the precipitates were collected by filtration and subjected to silica gel chromatography [solvent, benzene–acetone (2:1)] to give XI (2 mg). A white amorphous powder, mp >300 °C (dec.), $[\alpha]_D^{22}$ +81.5° (c=0.40, pyridine). Positive FAB-MS *m/z*: 511.336 ($[M+Na]^+$). $C_{30}H_{52}NaO_5$ requires *m/z*: 511.340. The ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and III, respectively. Compound XIV (40 mg) was treated in the same manner to give XI (2 mg).

Identification of Component Monosaccharides of the Glycosides 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 an ion exchange resin (Amberlite IR-410) and concentrated *in vacuo*. The residue was trimethylsilylated 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 the tetramethylsilyl derivatives of the methanolysate of the glycoside.

The absolute configurations of the component monosaccharides were determined according to the method reported by Hara *et al.*[6] Thus, a glycoside (5 mg) was hydrolyzed with 1 N HCl. After neutralization with Amberlite IR-410, the free sugars in the hydrolysate were converted into the thiazolidine derivatives and checked by GLC after trimethylsilylation. Authentic sugar samples were treated in the same manner and the unknown sugar was identified by comparison of its t_R value with those of the authentic sugar derivatives.

Permethylation of the Glycosides and Identification of the Component Methylated Monosaccharides Compound IX (20 mg) was fully methylated according to the method reported by Hakomori,[7] and the product was purified by column chromatography on silica gel [solvent, hexane–AcOEt (1:1)]. The permethylate (5 mg) was dissolved in 1 N HCl–MeOH (1 ml) and the solution was refluxed for 3 h. After neutralization with Ag_2CO_3, the product was acetylated with Ac_2O–pyridine (1:1) (0.2 ml) at room temperature. The solvent was blown off by an N_2 stream and the residue was checked by gas-liquid chromatography-chemical ionization mass spectrometry (GC-CI-MS). Methyl glycosides of 3,4,6-tri-*O*-methyl-2-*O*-acetyl-D-glucopyranose, 3,4-di-*O*-methyl-2-*O*-acetyl-L-arabinopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose and 2,3,4-tri-*O*-methyl-L-

rhamnopyranose were identified by comparison of t_R values and the CI-MS patterns with those of authentic samples.

Compounds XII, XIV and XV (10 mg each) were fully methylated according to Hakomori's method, and each product was purified by HPLC [Nova-pak C_{18} Radial-PAK (Waters Ltd.); solvent, 95% MeOH]. The thin-layer-chromatographically homogeneous product was dissolved in 1 N HCl–MeOH (1 ml) and refluxed for 3 h. The reaction mixture was neutralized with Ag_2CO_3, acetylated with Ac_2O–pyridine and checked by GC-CI-MS. The permethylate of XII gave methyl glycosides of 3,4,6-tri-*O*-methyl-2-*O*-acetyl-D-glucopyranose and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose. The permethylate of XIV gave methyl glycosides of 3,4,6-tri-*O*-methyl-2-*O*-acetyl-D-glucopyranose, 3,4-di-*O*-methyl-2-*O*-acetyl-L-arabinopyranose, 2,4-di-*O*-methyl-3-*O*-acetyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose. The permethylate of XV gave methyl glycosides of 3,4,6-tri-*O*-methyl-2-*O*-acetyl-D-glucopyranose and 2,3,4,6-tetra-*O*-methyl-D-galactopyranose.

Compounds XIII and XVI (2 mg each) were treated in the same manner. The permethylate of XIII gave methyl glycosides of 3,4-di-*O*-methyl-2-*O*-acetyl-L-arabinopyranose, 2,3,4-tri-*O*-methyl-L-rhamnopyranose. The permethylate of XVI gave 3,4-di-*O*-methyl-2-*O*-acetyl-L-arabinopyranose, 2,4-di-*O*-methyl-3-*O*-acetyl-L-rhamnopyranose and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose.

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References and Notes

1) This work was reported at the 108th Annual Meeting of The Pharmaceutical Society of Japan, Hiroshima, April 1988.
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5) The instruments and materials used in this work were the same as described in the preceding paper.[2] The GLC and GC-CI-MS conditions are described in another paper[8] from this laboratory. The NMR data of all samples were obtained in both pyridine-d_5 and pyridine-d_5–D_2O solutions. Unless otherwise specified, the ^{1}H-NMR data 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. The signal assignments were essentially based on the reported data, and were confirmed by the aid of the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, NOE difference, decoupling difference and long-range ^{1}H–^{13}C COSY spectroscopy techniques.
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