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**Studies on the Constituents of *Actinostemma lobatum* MAXIM. I.¹⁾
Structures of Actinostemmosides A, B, C and D, Dammarane
Triterpene Glycosides Isolated from the Herb**

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From the dried herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae), four dammarane-type triterpene glycosides named actinostemmosides A, B, C and D were isolated and their structures were elucidated on the basis of chemical and spectral evidence. Actinostemmosides A, B and C were identified as 20-*O*- β -D-glucopyranosides of 3 β ,6 α ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene, 3 β ,7 β ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene and 3 β ,7 β ,18,20,27-pentahydroxy-(20*S*)-dammar-24-ene, respectively, and actinostemmoside D, as the α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside of 3 β ,6 α ,20,27-tetrahydroxy-(20*R*)-dammar-24-ene.

Keywords—*Actinostemma lobatum*; Cucurbitaceae; dammarane; triterpene glycoside; 3 β ,6 α ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene; 3 β ,7 β ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene; 3 β ,7 β ,18,20,27-pentahydroxy-(20*S*)-dammar-24-ene; 3 β ,6 α ,20,27-tetrahydroxy-(20*R*)-dammar-24-ene; ¹³C-NMR spectrometry; FAB-MS

Actinostemma lobatum MAXIM. (Cucurbitaceae) is a vine which grows in the area from China to Japan. The herb has been traditionally used in China, though not in Japan, as a diuretic for the treatment of nephrotic edema, and as an antidote (applied externally) for poisonous snake bite.²⁾ In the course of screening of Cucurbitaceous plants for saponin constituents, it was found that the seeds and herb of the title plant contain a considerable amount of saponins. The preliminary check by thin-layer chromatography (TLC) showed that the MeOH extract of the herb contains a large amount of polar saponins (tentatively named lobatosides) which stain dark blue when the plate is sprayed with sulfuric acid followed by heating, and a small amount of less polar saponins (named actinostemmosides) which strain violet. This paper deals with isolation and characterization of four actinostemmosides.

The extraction and fractionation are summarized in Chart 1 and described in the experimental section.

Actinostemmoside A (I), C₃₆H₆₂O₉, was obtained as colorless needles in the yield of 0.00085%. The fast atom bombardment mass spectrum (FAB-MS) of I showed the [M+Na]⁺ ion at *m/z* 661. The enzymatic hydrolysis of I with cellulase gave the aglycone (II) and D-glucose. The FAB-MS of II showed the [M+Na]⁺ ion at *m/z* 499. The proton nuclear magnetic resonance (¹H-NMR) spectrum exhibited signals due to seven methyl groups (δ 0.97, 0.97, 1.08, 1.40, 1.48, 2.01 and 2.04, all singlets), one hydroxymethylene group (δ 4.54, s-like) linked to an olefinic carbon, two hydroxymethine groups (δ 3.56, dd, *J* = 11, 6 Hz; δ 4.40, ddd, *J* = 10, 10, 4 Hz) and a proton (δ 5.50, t, *J* = 7 Hz) on a trisubstituted double bond next to a methylene group (δ 2.55, m). The ¹³C-NMR spectrum showed the signals of four C—C bonded quaternary carbons (δ 39.5, 40.4, 41.8 and 50.6), one oxygenated quaternary carbon (δ 74.0) and the functional groups assigned on the basis of the ¹H-NMR spectrum. These spectral data

Chart 1. Fraction and Isolation of Glycosides

strongly suggest that II is a tetrahydroxy-(20*S*)-dammarene having two secondary hydroxyl groups in the saturated tetracyclic moiety, and a tertiary hydroxyl group, a primary hydroxyl group and a trisubstituted double bond in the side chain.

The ^{13}C -NMR spectrum of II was quite similar to that of 3 β ,6 α ,20,26-tetrahydroxy-(20*S*)-dammar-24-ene (III), the aglycone of Kizuta saponin K₉ (IV), which was isolated from *Hedera rhombea* BEAN (Araliaceae) by Tomimori *et al.*³⁾ The ^{13}C -NMR spectral differences between II and III are that the signals of C₂₇ (δ 14.0) and C₂₆ (δ 68.2) of III were not observed, and instead, the signals of a methyl group (δ 21.8) and a hydroxymethylene group (δ 60.9) were observed in the spectrum of II. The two-dimensional ^1H -NMR spectrum (^1H - ^1H shift-correlated spectrum) measured in the NOESY mode showed the cross-peak arising from the nuclear Overhauser effect (NOE) between the olefinic proton and methyl protons on an olefinic carbon, indicating that the olefinic proton and the methyl group are in the *cis* configuration. These spectral data unequivocally show that II is 3 β ,6 α ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene.

The chemical shift of the anomeric carbon (δ 98.6), the glycosylation shift (48.2 ppm) of

Chart 2

the oxygenated quaternary carbon and the anomeric proton signal (δ 5.03, d, $J=8$ Hz) indicate that D-glucose is linked to the C₂₀-hydroxyl group in the β -pyranoside form. Thus, actinostemmoside A (I) is the 20-*O*- β -D-glucopyranoside of 3 β ,6 α ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene.

Actinostemmoside B (V), C₃₆H₆₂O₉ · 1.5H₂O, was obtained as colorless needles (yield: 0.001%). The FAB-MS of V showed the $[M + Na]^+$ ion at m/z 661. Enzymatic hydrolysis of V gave D-glucose and the aglycone (VI), FAB-MS: m/z 499 ($[M + Na]^+$). The ¹³C- and ¹H-NMR data of VI indicated the presence of the same functional groups as those of II. Comparison of the ¹³C-NMR spectra of II and VI showed that II and VI have the same side-chain carbon signals, indicating that they have the same side-chain structure. The other carbon signals were considerably different from those of II. The ¹H-NMR signals of two hydroxymethine protons appeared at δ 3.48 (t, $J=8$ Hz) and δ 4.08 (dd, $J=11, 5$ Hz). The former is the signal of C₃-H, and the latter should be that of the proton adjacent to the unlocated hydroxyl group. The splitting pattern indicates that the hydroxymethine group is between a methylene group and a quaternary carbon atom. The positions which satisfy this requirement are C₁, C₇ and C₁₅, among which the C₁ is ruled out because the proton signal (δ 1.9—2.0) of the methylene group next to the hydroxymethine group in question was not influenced by irradiation at the frequency of C₃-H.

The ¹³C-NMR spectra of VI and dammarenediol II (VII)⁴⁾ were compared. Compound VI exhibited quaternary carbon signals at δ 37.4, 39.4, 46.6 and 50.4, and the first, second and fourth signals correspond to those of C₁₀ (δ 37.4), C₄ (δ 39.5) and C₁₄ (δ 50.6) of VII, but the third one, which should be that of C₈, is shifted downfield by 5.9 ppm compared to that of VII (δ 40.7). If the hydroxyl group is at C₁₅, the downfield shift of the C₈ signal and unchanged chemical shift of C₁₄ cannot be explained. If the hydroxyl group is located at C₇, the downfield shifts of the C₆ signal (Δ 10.6 ppm) and the C₈ signal (Δ 5.9 ppm) compared to those of VII, and upfield shifts of signals due to C₅ (Δ 2.5 ppm) and one of the methyl carbons on quaternary carbon (Δ 5.3—6.0 ppm) are well rationalized. The anomalous downfield shift of

TABLE I. ^{13}C -NMR Chemical Shifts of Actinostemmosides and Their Aglycones^{a)}

	I	II	III ^{b)}	V	VI	VII ^{b)}	XI	XII	X	VIII	IX	XIII ^{b)}
C-1	39.6	39.5	39.5	39.6	39.5	39.5	39.5	39.5	39.1	39.8	39.8	39.5
C-2	27.9	28.0	28.1	28.4	28.4	28.3	27.5	28.0	28.2	28.5	28.5	28.1
C-3	78.5	78.5	78.6	78.1	78.0	78.0	78.5	78.5	78.0	78.0	78.0	78.0
C-4	40.4	40.4	40.4	39.4	39.4	39.5	40.4	40.3	39.4	39.5	39.5	39.5
C-5	61.9	61.9	62.0	54.1	54.0	56.5	61.8	61.9	53.1	54.6	54.7	56.5
C-6	67.7	67.7	67.9	29.4	29.4	18.8	67.8	67.7	29.2	30.3	30.4	18.8
C-7	48.1	48.1	48.1	74.8	74.8	35.7	48.1	48.1	74.0	77.8	78.2	35.7
C-8	41.8	41.8	41.9	46.6	46.6	40.7	41.9	41.8	45.2	49.2	49.3	40.8
C-9	50.8	50.7	50.9	51.0	51.0	51.1	50.9	50.7	51.5	52.2	52.2	51.2
C-10	39.5	39.5	39.5	37.0	37.4	37.4	39.5	39.5	37.0	37.8	37.9	37.4
C-11	22.0	21.9	22.0	22.2	22.1	21.9	21.8	21.8	21.1	22.9	22.9	21.9
C-12	25.5	25.3	25.4	26.1	25.8	25.8	25.3	25.7	25.7	25.8	25.7	25.8
C-13	42.2	42.1	42.2	43.7	43.6	42.6	42.4	42.2	43.9	44.4	44.6	42.6
C-14	50.7	50.6	50.7	50.4	50.4	50.6	50.2	50.4	50.5	50.4	50.3	50.4
C-15	31.5	31.6	31.7	35.4	35.5	31.7	31.2	31.5	36.3	36.5	36.5	31.5
C-16	28.2	28.2	28.2	28.1	28.2	28.1	28.2	28.2	28.2	28.2	28.2	28.1
C-17	48.4	50.4	50.5	47.6	49.8	50.3	48.1	50.0	49.7	47.4	49.5	49.9
C-18	17.7 ^{b)}	17.6 ^{b)}	17.7 ^{b)}	10.5	10.5	16.5 ^{b)}	17.6 ^{b)}	17.6 ^{b)}	59.6	62.0	61.7	16.5 ^{b)}
C-19	17.4 ^{b)}	17.3 ^{b)}	17.4 ^{b)}	16.7 ^{b)}	16.6 ^{b)}	16.3 ^{b)}	17.3 ^{b)}	17.6 ^{b)}	14.4 ^{b)}	16.9 ^{b)}	16.9 ^{b)}	16.3 ^{b)}
C-20	82.2	74.0	74.2	82.5	74.2	74.0	82.6	74.3	74.4	82.4	74.4	74.4
C-21	21.4	26.1	26.1	21.8	26.2	25.3	22.7	24.4	26.3	21.9	26.4	24.5
C-22	40.7	42.1	41.8	40.5	42.0	41.9	38.9	43.2	42.1	40.9	41.8	42.9
C-23	22.7	22.8	22.9	22.7	22.9	23.3	22.2	22.6	22.9	22.9	22.9	23.0
C-24	127.7	127.7	125.7	127.9	127.7	126.0	127.8	127.6	127.6	127.8	127.8	126.0
C-25	136.0	136.1	136.3	135.9	136.1	130.6	136.1	136.1	136.2	136.0	136.1	130.6
C-26	21.8	21.8	68.2	21.4	21.8	26.1	21.9	21.8	21.8	21.8	21.9	25.8
C-27	61.0	60.9	14.0	61.1	60.9	17.7	61.1	60.9	60.9	61.0	60.9	17.7
C-28	32.0	32.0	32.0	28.7	28.6	28.7	32.0	32.0	28.5	28.7	28.8	28.7
C-29	16.5 ^{c)}	16.5 ^{c)}	16.5	16.5 ^{b)}	16.5 ^{b)}	15.8 ^{b)}	16.5 ^{c)}	16.5 ^{c)}	16.0 ^{b)}	16.6 ^{b)}	16.7 ^{b)}	15.8 ^{b)}
C-30	16.8 ^{c)}	16.8 ^{c)}	16.8	16.8 ^{b)}	16.7 ^{b)}	16.9	16.9 ^{c)}	16.7 ^{c)}	17.3 ^{b)}	17.1 ^{b)}	16.9 ^{b)}	16.8
$\begin{array}{c} \text{Me} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{Me} \end{array}$									26.7			
									31.2			

a) Assignments are essentially based on the reports by Tanaka *et al.*⁴⁾ and Tomimori *et al.*³⁾ The chemical shifts of the sugar moieties of the glycosides are shown in the experimental section. b, c) Values in each column may be interchangeable.

the C₁₅ signal (Δ 3.8 ppm) can be explained in terms of the δ_1 -shift which was reported by Eggert *et al.*⁵⁾ in the case of several hydroxylated steroids. The ^1H - ^1H shift-correlated spectrum measured in the NOESY mode revealed the presence of NOE between the hydroxymethine proton and a methyl group on C₁₄ indicating that the hydroxyl group is linked to C₇ in the β -configuration. The splitting pattern (dd, $J=11$, 5 Hz) of the hydroxymethine proton supports this configuration.

The glucose linkage was judged to be at the C₂₀-hydroxyl group in the β -configuration for the same reasons as in the case of I. Thus, actinostemmoside B (V) was determined to be the 20-*O*- β -D-glucopyranoside of 3 β ,7 β ,20,27-tetrahydroxy-(20*S*)-dammar-24-ene.

Actinostemmoside C (VIII), C₃₆H₆₂O₁₀·1/2H₂O, was obtained as colorless needles (yield: 0.027%). The FAB-MS showed the $[\text{M} + \text{Na}]^+$ ion at m/z 677. Enzymatic hydrolysis gave D-glucose and the aglycone (IX). The FAB-MS of IX showed the $[\text{M} + \text{Na}]^+$ ion at m/z 515, 16 mass units more than that of VI. When the ^{13}C - and ^1H -NMR spectra of IX were compared with those of VI, it was found that IX has one more hydroxymethylene group and one less methyl group on a quaternary carbon atom than VI. The ^{13}C -NMR spectrum indicated that IX has the same side chain, and therefore the new hydroxymethylene group

TABLE II. ^1H -NMR Chemical Shifts of Actinostemmosides

	I	V	VIII	XI	
H-3	3.53 dd (6, 11)	3.46 t (8)	3.45 t (8)	3.50 dd (7, 10)	
H-5	1.21 d (11)			1.17 d (11)	
H-6	4.38 ddd (11, 11, 4)			4.35 ddd (11, 11, 3)	
H-7		4.05 dd (11, 4)	4.11 dd (11, 3)		
H-13			2.33 ddd (11, 11, 2)		
H-17	2.15 ddd (11, 11, 6)	2.17 m		2.22 ddd (11, 11, 6)	
H-18	0.96 s ^{a)}	1.26 s	4.49 d, 4.68 d (12) (12)	0.85 s ^{a)}	
H-19	1.10 s ^{a)}	0.88 s ^{a)}	1.05 s ^{a)}	1.08 s ^{a)}	
H-21	1.47 s	1.50 s	1.52 s	1.49 s	
H-23	2.47 m, 2.53 m	2.52 m, 2.62 m		2.45 m, 2.70 m	
H-24	5.50 t (7)	5.50 t (7)	5.45 t (7)	5.50 t (7)	
H-26	2.02 s	2.02 s	1.99 s	2.00 s	
H-27	4.49 d, 4.57 d (12) (12)	4.50 d, 4.57 d (12) (12)	4.47 d, 4.52 d (12) (12)	4.54 d, 4.64 d (12) (12)	
H-28	1.97 s	1.20 s	1.20 s	1.92 s	
H-29	1.44 s ^{b)}	1.05 s ^{a)}	1.02 s ^{a)}	1.42 s ^{b)}	
H-30	0.94 s ^{b)}	1.13 s ^{a)}	1.16 s ^{a)}	1.03 s ^{b)}	
	Glc	Glc	Glc	Glc	Rha
Sug-1	5.03 d (8)	5.03 d (8)	5.02 d (8)	4.98 d (8)	6.40 d (s-like)
Sug-2	3.97 dd (8, 9)	3.97 dd (8, 9)	3.92 dd (8, 9)	4.13 dd (8, 10)	4.77 t (3)
Sug-3	4.26 t (9)	4.26 t (9)	4.20 t (9)	4.25 t (10)	4.57 dd (3, 9)
Sug-4	4.17 t (9)	4.17 t (9)	4.11 t (9)	3.99 t (10)	4.28 t (9)
Sug-5	3.88 ddd (9, 5, 3)	3.88 ddd (9, 5, 3)	3.84 ddd (9, 5, 3)	3.87 ddd (10, 7, 2)	4.65 dd (9, 6)
Sug-6	4.29 dd (5, 12)	4.29 dd (5, 12)	4.25 dd (5, 12)	4.18 dd (7, 12)	1.76 d (6)
	4.46 dd (3, 12)	4.46 dd (3, 12)	4.41 dd (2, 12)	4.45 dd (2, 12)	

The spectra were measured in pyridine- d_5 + D₂O, and the numbers in parentheses are coupling constants in Hz. a, b) Values in each column may be interchangeable, although those given here are preferred.

should be at the tetracyclic moiety. Among the signals of the quaternary carbons (δ 37.9, 39.5, 49.3 and 50.3), the first, second and fourth signals have almost the same chemical shifts as those of C₁₀, C₄ and C₁₄ of VI, whereas the signal of the third quaternary carbon is shifted downfield by 2.7 ppm from the C₈ signal (δ 46.6) of VI. These spectral data suggested that IX is 18-hydroxy-VI, namely 3 β ,7 β ,18,20,27-pentahydroxy-(20S)-dammar-24-ene. When IX was treated with acetone in the presence of anhydrous CuSO₄, it gave, as expected, an acetonide (X). The glucose linkage was determined to be at the C₂₀-hydroxyl group, in the β -configuration, on the same bases as in the case of V. Thus, actinostemmoside C (VIII) was

identified as the 20-*O*- β -D-glucopyranoside of 3 β ,7 β ,18,20,27-pentahydroxy-(20*S*)-dammar-24-ene.

Actinostemmoside D (XI), C₄₂H₇₂O₁₃·H₂O, was obtained as colorless needles in the yield of 0.0038%. The FAB-MS of XI showed the [M+Na]⁺ ion at *m/z* 807. The NMR spectra exhibited two anomeric carbon signals at δ 97.4 and 101.5 ppm and two anomeric proton signals at δ 4.98 (d, *J* = 8 Hz) and δ 6.40 (s-like), and the C–H shift correlated spectrum revealed that the former proton is on the anomeric carbon which appeared at δ 97.4 and the latter proton is on the carbon having the chemical shift δ 101.5.

On heating in 10% acetic acid, XI gave the aglycone (XII) and a biose; the latter furnished methyl glycosides of α -L-rhamnopyranose and α -D-glucopyranose on methanolysis. The ¹³C-NMR chemical shifts of the oxygenated quaternary carbon (δ 82.6 ppm) shifted upfield (δ 74.3 ppm) on going from XI to XII. These data show that XI is a glycoside having a rhamnosylglucose moiety linked to the tertiary hydroxyl group of the aglycone.

The FAB-MS of XII exhibited the [M+Na]⁺ ion at *m/z* 499. The ¹³C- and ¹H-NMR spectra of XII are quite similar to those of II indicating that XII is a compound closely related to II. The differences in the ¹³C-NMR chemical shifts are that the C₂₃, C₂₁, C₁₇ and C₁₄ signals are shifted upfield by 0.2, 1.7, 0.4 and 0.2 ppm, respectively, and C₁₂, C₂₂ and C₂₀ signals are shifted downfield by 0.4, 1.1 and 0.3 ppm, respectively. The same differences in the chemical shifts were observed between VII and its (20*R*)-epimer, dammarediol I (XIII).⁴⁾ These data indicate that XII is 3 β ,6 α ,20,27-tetrahydroxy-(20*R*)-dammar-24-ene.

Compound XI was enzymatically hydrolyzed with crude hesperidinase in a neutral medium and the ¹³C-NMR spectrum of the aglycone was measured without crystallization. The ¹³C-NMR spectrum was superimposable on that of XII obtained by acetic acid hydrolysis, and no sign of the presence of the (20*S*)-epimer was observed.

Compound XI was fully methylated by the modified Hakomori's method and the product was methanolized to give methyl glycosides of 2,3,4-tri-*O*-methyl- α -L-rhamnopyranose and 3,4,6-tri-*O*-methyl- α -D-glucopyranose. From the ¹³C-NMR chemical shifts of the anomeric carbons and ¹H-NMR coupling constants of the component sugars, coupled with the glycosylation shift of the oxygenated quaternary carbon, the position of the biose linkage was determined to be at the C₂₀-hydroxyl group and the configurations of the component sugars were identified as α for rhamnose and β for glucose. Thus, the structure of actinostemmoside D was determined to be the 20-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside of 3 β ,6 α ,20,27-tetrahydroxy-(20*R*)-dammar-24-ene.

It is well-known that a 20-*O*-glycosylated dammarane triterpene is readily hydrolyzed even under mild acidic conditions to give a C₂₀-epimeric mixture of the corresponding sapogenin. (20*R*)-Dammarane saponins have been reported as constituents of red ginseng,⁶⁾ but are generally considered to be artifacts formed during the processing procedure.

Actinostemmoside D gave only a (20*R*)-epimer on both acid and enzymatic hydrolyses. This shows that XII is the genuine aglycone and thus, actinostemmoside D is the first naturally occurring (20*R*)-dammarane glycoside.

Actinostemmosides E and F seem to have similar partial structures to above-mentioned actinostemmosides, but they are different in that they have no tertiary hydroxyl group and that the C₃-hydroxyl group seems to be glycosylated. Characterization of their structures is in progress.

Experimental⁷⁾

Extraction and Isolation of Actinostemmosides A–F—The air-dried herb (6.5 kg) of *Actinostemma lobatum* MAXIM. collected in the suburbs of Fukuoka city in September 1984 was packed in a glass tube and percolated with MeOH (60 l). Water (6 l) was added to the MeOH solution and the MeOH was evaporated off. The aqueous solution

was set aside, and the water-insoluble dark resinous material was dissolved in CHCl_3 (1 l). This solution was washed with water (1 l). The CHCl_3 layer was evaporated *in vacuo* to give a dark resin (170 g). The aqueous solution and the washing were combined and 1/7 of it was passed through an MCI gel (polystyrene gel) column (300 ml). The column was washed with water (1 l) and then eluted with 1 l each of aqueous acetone solutions containing increasing proportions of acetone. The rest of the aqueous solution was treated in the same manner. The yield of each fraction is shown in Chart 1.

The 40% acetone eluate contained actinostemmosides and lobatosides, and this fraction was roughly fractionated by silica gel (10 times the weight of the material) column chromatography, first eluted with CHCl_3 -MeOH- H_2O (7:3:0.5) (frs. 1–4) and then with CHCl_3 -MeOH (1:1) (frs. 5–6). Actinostemmosides were contained in frs. 2 (3 g) and 4 (2.5 g). Fraction 2 was repeatedly chromatographed on silica gel using CHCl_3 -MeOH (9:1) and CHCl_3 -EtOH (17:3) to separate two fractions (frs. 2a and 2b). Separation of the glycosides was monitored by TLC (CHCl_3 -MeOH- H_2O , 7:3:0.5). Fraction 2a contained actinostemmosides A (I) and B (V). Fraction 2b was a thin-layer chromatographically homogeneous sample of actinostemmoside C (VIII, 1.9 g). Fraction 2a was chromatographed on silica gel (Lobar column, 31×2.5 cm i.d.; three columns were connected) using EtOAc-PrOH- H_2O (20:3:0.3) to give I (55 mg) and V (68 mg).

Fraction 4 was repeatedly chromatographed on silica gel using CHCl_3 -MeOH- H_2O (32:8:1) and EtOAc-MeOH (4:1) and separated into two fractions (frs. 4a and 4b) on the basis of TLC monitoring. Fraction 4a was chromatographed on silica gel (CHCl_3 -MeOH-EtOAc- H_2O , 3:3:4:1.5, bottom layer) and then on an RP-18 column (70% MeOH) to give thin-layer chromatographically homogeneous XI (250 mg). Fraction 4b was chromatographed on silica gel (CHCl_3 -MeOH-EtOAc- H_2O , 3:3:4:1.5, bottom layer) to give two fractions (frs. 4b-1 and 4b-2). Both fractions were passed through the RP-18 column using 65% MeOH as the eluant to give actinostemmoside E (130 mg) from fr. 4b-1, and F (150 mg) from fr. 4b-2.

Actinostemmoside A (I): Colorless needles from dil. EtOH, mp 125–130 °C, $[\alpha]_D^{16} + 32.3^\circ$ ($c=0.3$, MeOH). FAB-MS m/z : 661.428 ($[\text{M} + \text{Na}]^+$). $\text{C}_{36}\text{H}_{62}\text{NaO}_9$ requires m/z 661.429. $^1\text{H-NMR}$: shown in Table II. $^{13}\text{C-NMR}$: sugar moiety; 98.6 (1), 75.6 (2), 79.0 (3), 71.9 (4), 77.9 (5), 63.0 (6).

Actinostemmoside B (V): Colorless needles from dil. EtOH, mp 142–145 °C, $[\alpha]_D^{19} + 15.4^\circ$ ($c=0.5$, MeOH). Anal. Calcd for $\text{C}_{36}\text{H}_{62}\text{O}_9 \cdot 1.5\text{H}_2\text{O}$: C, 64.93; H, 9.84. Found: C, 65.01; H, 9.80. FAB-MS m/z : 661 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$: shown in Table II. $^{13}\text{C-NMR}$: sugar moiety; 98.6 (1), 75.6 (2), 79.0 (3), 71.9 (4), 77.9 (5), 63.0 (6).

Actinostemmoside C (VIII): Colorless needles from dil. MeOH, mp 194–197 °C, $[\alpha]_D^{17} + 3.3^\circ$ ($c=1.0$, MeOH). Anal. Calcd for $\text{C}_{36}\text{H}_{62}\text{O}_{10} \cdot 1/2\text{H}_2\text{O}$: C, 65.13; H, 9.57. Found: C, 64.93; H, 9.99. FAB-MS m/z : 661 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$: shown in Table II. $^{13}\text{C-NMR}$: sugar moiety; 98.7 (1), 75.6 (2), 78.9 (3), 71.9 (4), 77.9 (5), 62.9 (6).

Actinostemmoside D (XI): Colorless needles from dil. EtOH, mp 168–171 °C, $[\alpha]_D^{17} - 2.2^\circ$ ($c=1.0$, MeOH). Anal. Calcd for $\text{C}_{42}\text{H}_{72}\text{O}_{13} \cdot \text{H}_2\text{O}$: C, 62.82; H, 9.29. Found: C, 62.40; H, 9.53. FAB-MS m/z : 807 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$: shown in Table II. $^{13}\text{C-NMR}$: sugar moiety; 97.4 (G-1), 77.9 (G-2), 80.2 (G-3), 72.7 (G-4), 77.3 (G-5), 63.2 (G-6), 101.5 (R-1), 72.3 (R-2), 72.4 (R-3), 74.2 (R-4), 69.6 (R-5), 19.4 (R-6).

Enzymatic Hydrolysis of I, V and VIII—Compound I (40 mg) was suspended in 20% MeOH (50 ml). After addition of cellulase (100 mg), the mixture was stirred at 38 °C for 40 h. MeOH was evaporated off, and the aqueous solution was extracted with EtOAc. The EtOAc extract was purified by silica gel column chromatography (CHCl_3 -MeOH- H_2O , 32:8:1; benzene-acetone, 3:1) followed by crystallization of the product from ether to give II (20 mg): Colorless needles, mp 148–150 °C, $[\alpha]_D^{20} + 52.3^\circ$ ($c=0.13$, MeOH). FAB-MS m/z : 499 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$ (pyridine- d_5 + D_2O) δ : 3.56 (H, dd, $J=11$, 6 Hz, C_3 -H), 4.40 (H, ddd, $J=10$, 10, 4 Hz, C_6 -H), 2.55 (2H, m, C_{23} -H), 5.50 (H, t, $J=7$ Hz, C_{24} -H), 4.54 (2H, s-like, C_{27} -H). Methyl signals: 0.97, 0.97, 1.08, 1.40, 1.48, 2.01, 2.04. $^{13}\text{C-NMR}$: shown in Table I. The aqueous layer was evaporated to dryness and then extracted with MeOH. The MeOH extract was dissolved in 1 N HCl-MeOH and the solution was refluxed for 1 h. The HCl was neutralized by adding Ag_2CO_3 , the precipitates were removed by filtration, and the MeOH was evaporated off. The residue was acetylated in the usual manner and purified by silica gel chromatography (hexane-AcOEt, 2:1) to give a syrup (10 mg); $[\alpha]_D^{21} + 104.8^\circ$ ($c=0.5$, CHCl_3). The $^1\text{H-NMR}$ spectrum was superimposable on that of methyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside.

Compound V (40 mg) was treated in the same manner to give VI (13 mg) and methyl glycoside acetate (7 mg). VI: Colorless needles from ether, mp 180–183 °C, $[\alpha]_D^{21} + 24.5^\circ$ ($c=0.1$, MeOH). FAB-MS m/z : 499 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$ (pyridine- d_5 + D_2O): 0.90, 1.05, 1.14, 1.22, 1.27, 1.43, 2.04 (CH_3), 2.55 (2H, m, C_{23} -H), 3.48 (H, t, $J=8$ Hz, C_3 -H), 4.08 (H, dd, $J=11$, 5 Hz, C_7 -H), 4.54 (2H, s-like, C_{27} -H), 5.50 (H, t, $J=8$ Hz, C_{24} -H). $^{13}\text{C-NMR}$: shown in Table I.

Methyl glycoside acetate: $[\alpha]_D^{21} + 110.0^\circ$ ($c=0.3$, CHCl_3). The $^1\text{H-NMR}$ spectrum was the same as that of methyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside.

Compound VIII (300 mg) and cellulase (150 mg) were dissolved in 20% MeOH (20 ml) and the mixture was stirred at 38 °C for 4 d. After evaporation of the solvent, the residue was dissolved in dilute MeOH and filtered. The filtrate was evaporated and chromatographed on silica gel (20 g). Elution with CHCl_3 -MeOH- H_2O (32:8:1) gave the aglycone fraction (95 mg) and VIII (130 mg). Further elution with CHCl_3 -MeOH- H_2O (25:17:3) gave the sugar fraction (31 mg). Rechromatography of the aglycone fraction on silica gel (10 g) using benzene-acetone (2:1) gave

thin-layer chromatographically homogeneous IX (86 mg): colorless needles from CHCl_3 , mp 119–121 °C, $[\alpha]_D^{25} + 7.6^\circ$ ($c = 0.87$, MeOH). FAB-MS m/z : 515 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$ (pyridine- d_5 + D_2O) δ : 1.05, 1.05, 1.20, 1.22, 1.45, 2.02 (CH_3), 2.55 (2H, m, $\text{C}_{23}\text{-H}$), 3.49 (H, t, $J = 8$ Hz, $\text{C}_3\text{-H}$), 4.18 (H, dd, $J = 11$, 4 Hz, $\text{C}_7\text{-H}$), 4.54 (2H, s-like, $\text{C}_{27}\text{-H}$), 4.71, 4.48 (H each, d, $J = 12$ Hz, $\text{C}_{18}\text{-H}$), 5.50 (H, t, $J = 8$ Hz, $\text{C}_{24}\text{-H}$). $^{13}\text{C-NMR}$: shown in Table I.

The sugar fraction was treated with 1 N HCl–MeOH and then acetylated. The product ($[\alpha]_D^{25} + 111.1^\circ$ ($c = 1.3$, MeOH)) was identified as methyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside from the $^1\text{H-NMR}$ spectrum and specific rotation.

Treatment of IX with CuSO_4 in Acetone; Preparation of the Acetonide (X) from IX—Compound IX (50 mg) and anhydrous CuSO_4 (250 mg) were stirred in acetone (5 ml) at room temperature for 2 d. After filtration of CuSO_4 , the filtrate was concentrated to dryness and then the residue was chromatographed on silica gel (benzene–acetone, 4:1) to give the acetonide (X, 33 mg). Crystallization from dilute acetone gave colorless needles, mp 132–134 °C. FAB-MS m/z : 555 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$ (pyridine- d_5) δ : 1.00, 1.11, 1.12, 1.25, 1.41, 1.46, 1.66, 2.04 (CH_3), 3.98, 4.03 (H, each, d, $J = 12$ Hz, $\text{C}_{18}\text{-H}$), 4.07 (H, dd, $J = 12$, 6 Hz, $\text{C}_7\text{-H}$), 3.49 (H, m, $\text{C}_3\text{-H}$). $^{13}\text{C-NMR}$: shown in Table I.

Acid Hydrolysis of XI—Compound XI (95 mg) was suspended in 10% acetic acid (4 ml) and heated at 75 °C for 1 h. The solvent was evaporated off and the residue was chromatographed on silica gel. Elution with CHCl_3 –MeOH (3:1) gave an aglycone fraction (50 mg). Further elution with CHCl_3 –MeOH (1:1) gave a sugar fraction (40 mg). Rechromatography of the aglycone fraction on silica gel (benzene–acetone, 3:1) gave a thin-layer chromatographically homogeneous compound (48 mg). Crystallization from dilute MeOH gave colorless needles, mp 183–185 °C, $[\alpha]_D^{25} + 49.0^\circ$ ($c = 0.1$, MeOH). FAB-MS m/z : 499 ($[\text{M} + \text{Na}]^+$). $^1\text{H-NMR}$ (pyridine- d_5 + D_2O) δ : 0.96, 0.98, 1.10, 1.37, 1.47, 2.00, 2.04 (CH_3), 2.55 (2H, m, $\text{C}_{23}\text{-H}$), 3.60 (H, dd, $J = 11$, 6 Hz, $\text{C}_3\text{-H}$), 4.40 (H, ddd, $J = 10$, 10, 3 Hz, $\text{C}_6\text{-H}$), 4.53 (2H, s-like, $\text{C}_{27}\text{-H}$), 5.50 (H, t, $J = 7$ Hz, $\text{C}_{24}\text{-H}$). $^{13}\text{C-NMR}$: shown in Table I.

Treatment of the sugar fraction with 0.1 N HCl–MeOH at room temperature for 40 h and chromatography of the product on silica gel (CHCl_3 –MeOH– H_2O , 32:8:1) gave methyl glycoside-I (8 mg) and -II (8 mg). The acetates were identified as methyl 2,3,4-tri-*O*-acetyl- α -L-rhamnopyranoside and methyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside by comparison of the $^1\text{H-NMR}$ spectra and specific rotations with those of authentic samples. Acetate of methyl glycoside-I: $[\alpha]_D^{21} - 54.4^\circ$ ($c = 0.57$, CHCl_3). Acetate of methyl glycoside-II: $[\alpha]_D^{21} + 107.0^\circ$ ($c = 0.33$, CHCl_3).

Methylation of XI and Identification of Component Methylated Sugar—Compound XI (10 mg) and NaH (in oil, 50%) (30 mg) were stirred in freshly distilled tetrahydrofuran (2 ml) at room temperature for 10 min. CH_3I (2 ml) was added and the mixture was stirred at room temperature for 50 h. The reaction mixture was poured into water (5 ml) and extracted with CHCl_3 (5 ml). The CHCl_3 layer was chromatographed on silica gel (benzene–acetone, 9:1) to give a methylation product (5 mg). The product (2 mg) was dissolved in 1 N HCl–MeOH (1 ml) and this solution was refluxed for 3 h. The acid was neutralized by adding Ag_2CO_3 , the precipitate was filtered off, and the filtrate was concentrated to dryness. TLC (benzene–acetone, 2:1) showed two spots. The R_f values of the spots were same as those of authentic samples of methyl 2,3,4-tri-*O*-methyl- α -L-rhamnopyranoside and methyl 3,4,6-tri-*O*-methyl- α -D-glucopyranoside. The methanolysate was acetylated in a usual manner and the acetylation product was analyzed by gas chromatography-chemical ionization-MS (GC-CI-MS). The reconstructed GC-CI-MS chromatogram showed two peaks and the CI-MS of the corresponding peaks were identical with those of methyl 2,3,4-tri-*O*-methyl- α -L-rhamnopyranoside and methyl 2-*O*-acetyl-3,4,6-tri-*O*-methyl- α -D-glucopyranoside.

Enzymatic Hydrolysis of XI—Compound XI (70 mg) and crude hesperidinase (70 mg) were dissolved in water (10 ml) and stirred at 37 °C for 10 d. The reaction solution was passed through an Amberlite XAD-2 (5 ml) column. After being washed with water (20 ml), the column was eluted with MeOH (30 ml). The MeOH eluate was evaporated and the residue was chromatographed on silica gel with CHCl_3 –MeOH– H_2O (32:8:1) and 15 mg of a thin-layer chromatographically homogeneous compound was obtained. The ^1H - and ^{13}C -NMR spectra were superimposable on those of XII obtained by acid hydrolysis of XI.

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References and Notes

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- 7) The instruments and materials used in this work were as follows: Yanaco micro melting point apparatus

(melting point, uncorrected), JASCO DIP-4 digital polarimeter (specific rotations), JEOL JNM GX-400 spectrometer (100 MHz for ^{13}C -NMR spectra and 400 MHz for ^1H -NMR spectra), JEOL JMS DX-300 mass spectrometer (mass spectra), Auto GCMS-6020 with GC-MSPAC 500 FDG data analyzer (GC-CI-MS), Kieselgel 60 (70–230 mesh, E. Merck), LiChroprep RP-18 (25–40 μm , E. Merck), MCI Gel CHP 20P (150–300 μm , Mitsubishi Chemical Industries Ltd.), precoated Kieselgel 60 F₂₅₄ plate (E. Merck). The cellulase was Type II from *Aspergillus niger* (Sigma Chemical Co., Ltd.). The crude hesperidinase was a gift from Prof. T. Nohara of Kumamoto University. ^1H - and ^{13}C -NMR spectra were measured in pyridine- d_5 or pyridine- d_5 containing D_2O and chemical shifts were expressed on the δ scale using TMS as an internal standard. The FAB-MS were obtained in a glycerol matrix containing NaI.