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Studies on the Constituents of *Actinostemma lobatum* MAXIM. III.[1] Structures of Actinostemmosides E and F, New Baccharane-Type Triterpene Glycosides Isolated from the Herb

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From the dried herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae), two new baccharane-type triterpene glycosides, actinostemmosides E and F, were isolated. The structure of actinostemmoside F was elucidated mainly on the basis of ^{13}C–^{13}C-nuclear magnetic resonance (NMR) correlation spectrometry as the 3-*O*-*β*-D-galactopyranosyl-(1→2)-α-L-arabinopyranoside of 3*β*,17*β*,21,26,30-pentahydroxybacchar-24-ene. The structure of actinostemmoside E was determined as the 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranoside of 3*β*,17*β*,21,26-tetrahydroxybacchar-24-ene by comparison of the ^1H- and ^{13}C-NMR spectra with those of actinostemmoside F.

Keywords——*Actinostemma lobatum*; Cucurbitaceae; actinostemmoside; triterpene glycoside; baccharane-type triterpene; 3*β*,17*β*,21,26,30-pentahydroxybacchar-24-ene; 3*β*,17*β*,21,26-tetrahydroxybacchar-24-ene; 2D-incredible natural abundance double quantum transfer experiment

In the preceding papers[2] of this series, we reported the isolation of eight triterpene glycosides from the less polar glycoside fraction of the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae), and structure determination of six dammarane-type triterpene glycosides, actinostemmosides A, B, C, D,[2a] G and H.[2b] This paper deals with the structures of the remaining two glycosides, actinostemmosides E and F.

Actinostemmoside F (I) was obtained as colorless needles (yield; 637 mg from 6.5 kg of dried herb). The fast atom bombardment mass spectrum (FAB-MS) showed an $[M+Na]^+$ ion at *m/z* 809 and an $[M-H]^-$ ion at *m/z* 785, and analyzed as $C_{41}H_{70}O_{14}\cdot 3H_2O$. The ^1H-nuclear magnetic resonance (^1H-NMR) spectrum (Table I) of I showed signals of four methyl groups on saturated quaternary carbons, one methyl group on an olefinic carbon, a proton (δ 5.41, t, $J=7$ Hz) of a trisubstituted double bond and two anomeric protons (δ 4.96, d, $J=5.5$ Hz; δ 5.02, d, $J=8$ Hz) together with signals of hydroxymethine groups and hydroxymethylene groups, indicating I to be a triterpene bioside. The enzymatic hydrolysis of I with cellulase gave an aglycone (II) and a sugar fraction, which was proved to be a mixture of D-galactose and L-arabinose.

The high-resolution FAB-MS of II showed an $[M+Na]^+$ ion at *m/z* 515.369, from which the molecular formula $C_{30}H_{52}O_5$ was deduced. The ^1H-NMR spectrum showed the signals of two hydroxymethylene groups on saturated quaternary carbons, one hydroxymethylene group on an olefinic carbon and two hydroxymethine groups which were masked by signals of the sugar moiety in the ^1H-NMR spectrum of I. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table II) indicated the presence of five quaternary carbons and the functional groups predicted by the ^1H-NMR spectrum, suggesting that II is a tetracyclic triterpene having two hydroxymethine groups, two hydroxymethylene groups and a hydroxylated isopropylidene group.

TABLE I. ^{1}H-NMR Chemical Shifts[a] of I, II, IV, V

	I	II	IV	V
H1	*ca.* 0.80	0.98 dt (5, 13)	*ca.* 0.78	*ca.* 0.95
	ca. 1.55	*ca.* 1.70	*ca.* 1.48	*ca.* 1.65
H2	*ca.* 1.85	*ca.* 1.90	*ca.* 1.85	*ca.* 1.85
	ca. 2.05		*ca.* 2.25	
H3	3.19 dd (5, 12)	3.40 dd (5, 11)	3.31 dd (5, 12)	3.45 dd (6, 11)
H5	*ca.* 0.80	0.94 dd (3, 13)	0.67 br d (11)	0.82 br d (10)
H6	*ca.* 1.28	1.44 dq (3, 13)	*ca.* 1.30	*ca.* 1.40
	ca. 1.48	*ca.* 1.60	*ca.* 1.48	*ca.* 1.46
H7	*ca.* 1.55	*ca.* 1.70	*ca.* 1.30	*ca.* 1.35
	ca. 2.15	2.29 dt (3, 13)		
H9	*ca.* 1.55	*ca.* 1.70	*ca.* 1.30	*ca.* 1.40
H11	*ca.* 1.18	1.31 dq (4, 13)	*ca.* 1.15	*ca.* 1.28
	ca. 1.55	*ca.* 1.70	*ca.* 1.48	*ca.* 1.60
H12	*ca.* 1.18	1.24 dq (4, 13)	*ca.* 1.25	*ca.* 1.28
	ca. 2.40	*ca.* 2.45	*ca.* 2.40	*ca.* 2.45
H13	*ca.* 2.15	2.23 ddd (4, 12, 13)	2.05 dt (2, 12)	2.10 dt (3, 11)
H15	*ca.* 1.55	*ca.* 1.60	*ca.* 0.95	*ca.* 0.95
	ca. 2.05	2.13 br d (13)	*ca.* 1.55	*ca.* 1.65
H16	*ca.* 1.78	1.75 br d (13)	*ca.* 1.30	1.50 dd (4, 13)
	ca. 1.95	1.95 dt (4, 13)	*ca.* 1.55	*ca.* 1.85
H17	3.97 d (12)	3.97 d (12)	3.68 d (12)	3.67 d (11)
H18	1.01 s	1.09 s	0.94 s	1.01 s
H19	0.82 s	0.93 s	0.77 s	0.88 s
H21	3.96 d (12)	3.92 d (11)	3.87 d (11)	3.83 d (10)
	4.55 d (12)	4.64 d (11)	4.49 d (11)	4.53 d (10)
H22	*ca.* 1.90	*ca.* 1.90	*ca.* 1.85	*ca.* 1.85
	ca. 2.23	2.34 dt (5, 13)	*ca.* 2.25	2.29 ddd (5, 12, 13)
H23	*ca.* 2.45	*ca.* 2.45	2.44 m	*ca.* 2.45
H24	5.41 t (7)	5.45 t (7)	5.48 t (7)	5.51 t (7)
H26	4.45 s-like	4.48 s-like	4.53 s-like	4.52 s-like
H27	1.97 s	1.99 s	2.02 s	2.02 s
H28	1.12 s	1.17 s	1.29 s	1.24 s
H29	1.01 s	1.05 s	1.11 s	1.04 s
H30	4.05 d (12)	4.08 d (12)	0.98 s	0.98 s
	4.47 d (12)	4.54 d (12)		
Ara 1	4.96 d (5.5)		Glc 1	4.92 d (8)
Ara 2	4.53 dd (5.5, 8)		Glc 2	4.20 dd (8, 9)
Ara 3	4.38 dd (3, 8)		Glc 3	4.31 t (9)
Ara 4	4.42 ddd (2, 3, 5)		Glc 4	4.13 t (9)
Ara 5	3.85 dd (2, 12)		Glc 5	3.93 ddd (2, 6, 9)
	4.32 dd (5, 12)		Glc 6	4.32 dd (6, 12)
				4.52 dd (2, 12)
Gal 1	5.02 d (8)		Gal 1	5.20 d (8)
Gal 2	4.44 dd (8, 9)		Gal 2	4.55 dd (8, 9)
Gal 3	4.08 dd (3, 9)		Gal 3	4.15 dd (3, 9)
Gal 4	4.58 dd (3, 1)		Gal 4	4.65 dd (1, 3)
Gal 5	3.94 ddd (6, 8, 1)		Gal 5	4.03 ddd (1, 5, 7)
Gal 6	4.30 dd (6, 12)		Gal 6	4.40 dd (5, 11)
	4.42 dd (8, 12)			4.52 dd (7, 11)

a) The spectra of I and IV were measured in pyridine-d_5 containing D_2O. The spectra of II and V were measured in pyridine-d_5. The values in parentheses are coupling constants in Hz. Abbreviations: Ara, arabinose; Gal, galactose; Glc, glucose.

TABLE II. ^{13}C-NMR Chemical Shifts[a] of I—V

	I	II	III	IV	V
C1	39.3	39.3	39.5	39.2	39.3
C2	26.7	28.3	28.3	26.8	28.3
C3	88.8	78.2	78.0	89.0	78.1
C4	39.7	39.7	39.5	39.7	39.5
C5	56.2	56.2	56.5	56.1	56.0
C6	18.6	18.6	18.8	18.4	18.7
C7	35.7	35.7	35.7	33.9	33.9
C8	41.9	41.9	40.7	41.0	41.0
C9	52.4	52.4	51.1	51.1	51.2
C10	37.4	37.4	37.4	37.1	37.5
C11	21.6	21.6	21.9	21.2	21.2
C12	24.8	24.8	25.8	25.3	25.3
C13	41.6	41.6	42.6	40.6	40.5
C14	47.0	47.0	50.6	42.6	42.6
C15	20.7	20.7	31.7	26.8	26.8
C16	29.0	29.0	28.1	28.3	28.3
C17	77.2	77.3	50.3	77.3	77.2
C18	16.4	16.4	16.5[b]	15.9	15.9
C19	17.0	17.0	16.3[b]	16.6	16.3
C20	41.4	41.4	74.0	41.6	41.6
C21	65.5	65.5	25.3	65.2	65.1
C22	38.1	38.1	41.9	38.0	38.0
C23	22.1	22.1	23.3	22.0	21.9
C24	128.1	128.0	126.0	127.9	127.8
C25	135.8	135.8	130.6	136.1	136.1
C26	60.9	60.8	26.1	60.9	60.9
C27	21.8	21.8	17.7	21.9	21.8
C28	28.1	28.6	28.7	28.1	28.6
C29	16.6	16.3	15.8[b]	16.6	16.6
C30	59.4	59.4	16.9	15.0	15.0
Ara 1	104.6 (163)		Glc 1	105.1 (159)	
Ara 2	81.3		Glc 2	84.4	
Ara 3	73.1		Glc 3	78.3	
Ara 3	68.0		Glc 4	71.7	
Ara 4	64.5		Glc 5	78.0	
			Glc 6	62.9	
Gal 1	106.8 (157)		Gal 1	107.1 (159)	
Gal 2	73.8		Gal 2	74.7	
Gal 3	75.2		Gal 3	75.0	
Gal 4	69.6		Gal 4	69.5	
Gal 5	76.8		Gal 5	76.9	
Gal 6	61.5		Gal 6	61.3	

a) The spectra were measured in pyridine-d_5 and chemical shifts were expressed in δ values. The values in parentheses are J_{CH} values. The signals of methyl groups were assigned on the basis of ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectra. *b*) The values in the vertical columns may be interchanged. Abbreviations: Ara, arabinose; Gal, galactose; Glc, glucose.

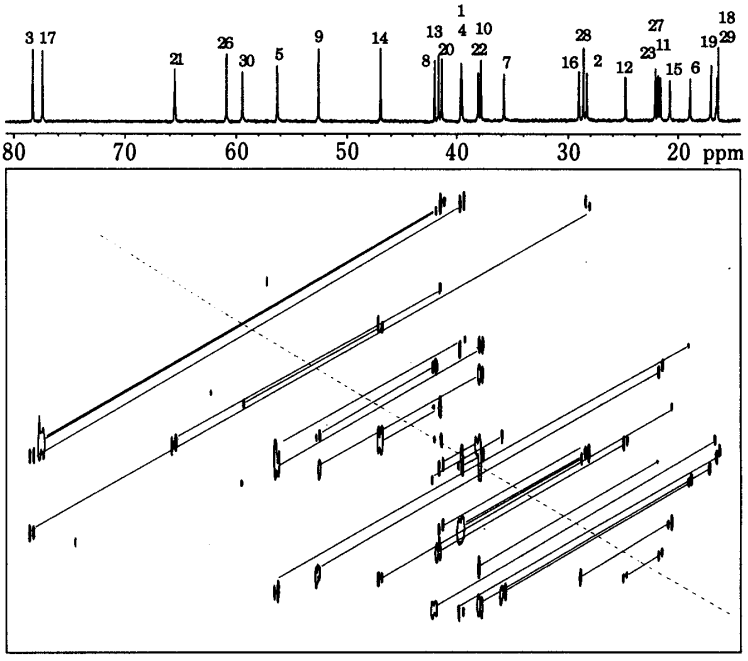
Compound I was first presumed to be a glycoside of a dammarane-type triterpene because it showed the same violet coloration on thin layer chromatography (TLC) as other actinostemmosides on heating of the plate after spraying sulfuric acid, and it has the same hydroxylated isopropylidene groups, and also because the ^{13}C-NMR spectrum of II showed carbon signals in accordance with the A/B ring carbons of (20*S*)-dammarenediol (III)[3] (Table II).

However, there were difficulties in assignments of other carbon signals to the C/D ring carbons of a dammarane-type triterpene, and the expected hydroxylated quaternary carbon (C_{20}) signal could not be observed. These discrepancies indicated that II might in fact be a baccharane-type triterpene, which has been postulated to be biogenetically derived from dammarane by fission of the C_{20}-hydroxyl group followed by migration of the C_{16}–C_{17} linkage to the C_{20} carbonium cation.[4]

The occurrence of a baccharane-type triterpene in plants is very rare, and the isolation of hosenkol A[5] and hosenkosides[6] from the seeds of *Impatiens balsamina* L. (Balsaminaceae) is the only instance so far reported. No published ^{13}C-NMR data could be found to compare with the data for I and II. Comparison of the NMR spectral data with those of various kinds of dammarane-type triterpenes did not give valid assignments of the ^{13}C-NMR signals leading to the unambiguous confirmation of the baccharane structure for II. Therefore, the ^{13}C–^{13}C-NMR correlation spectrum of II was measured using the two dimensional-incredible natural abundance double quantum transfer experiment (2D-INADEQUATE) technique. A part of the spectrum is presented in the figure.

All C–C linkages were traced starting from quaternary carbons and oxygenated carbons with consideration of other spectral information such as the chemical shifts and multiplicities of the carbon signals, except for the connectivity of C_{24} to C_{25}. Thus, the plane structure of a postulated baccharane skeleton having hydroxyl groups at C_3, C_{17}, C_{21}, C_{30} and C_{26} or C_{27} was established.

The presence of the nuclear Overhauser effect (NOE) (observed in the NOE difference spectrum) between C_{21}-H and C_{13}-H, C_{13}-H and C_{18}-H, C_{18}-H and C_{19}-H, C_{19}-H and C_{29}-H,



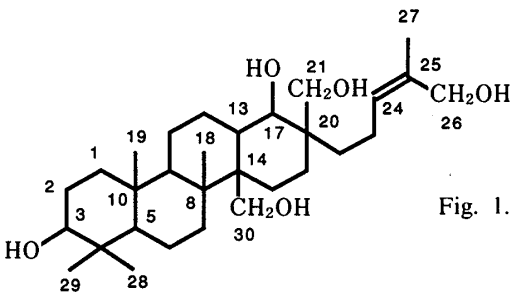


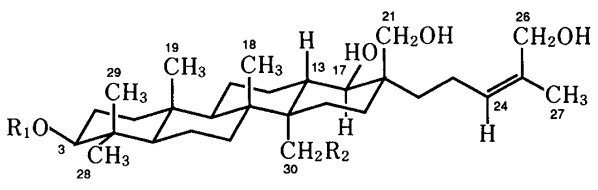
Fig. 1. The 2D-INADEQUATE Spectrum of II

and C_{30}-H and C_{17}-H unambiguously indicates that the conformations of all rings are chair form, and the ring junctures are all *trans*. These results are consistent with the baccharane structure which has been predicted on biogenetic grounds. The presence of the NOE between C_{30}-H and C_{17}-H indicates that the configurations of the hydroxymethylene groups at C_{20} and C_{14} are β and α, respectively.

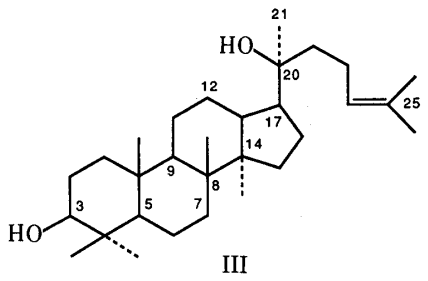
The configurations of the hydroxyl groups at C_3 and C_{17} were determined as β from the splitting patterns and coupling constants of the methine protons (C_3-H: δ 3.40, dd, $J=5$, 11 Hz; C_{17}-H: δ 3.97, d, $J=12$ Hz). The location of the last hydroxyl group was determined at C_{26} from the fact that NOE was observed between the olefinic proton and the methyl protons.

The structure of the sugar moiety of I was determined as β-D-galactopyranosyl-(1→2)-α-L-arabinopyranose by ordinary NMR spectral analyses. The site of the sugar linkage to the aglycone was determined from the observed glycosylation shift of the C_3 signal and also from the presence of the NOE between C_3-H and the anomeric proton of the arabinopyranosyl group.

Accordingly, actinostemmoside F is the 3-*O*-β-D-galactopyranosyl-(1→2)-α-L-arabinopyranoside of 3β,17β,21,26,30-pentahydroxybacchar-24-ene.



I : R_1=β-D-Gal-(1→2)-α-L-Ara, R_2=OH
II : R_1=H, R_2=OH
IV : R_1=β-D-Gal-(1→2)-β-D-Glc, R_2=H
V : R_1=H, R_2=H



III

Actinostemmoside E (IV) was obtained as colorless needles (130 mg from 6.5 kg of dried herb) and the FAB-MS showed an $[M+Na]^+$ ion at *m/z* 823 and an $[M-H]^-$ ion at *m/z* 799. It was analyzed as $C_{42}H_{72}O_{14}\cdot 2H_2O$. The ^{1}H-NMR spectrum (Table I) showed the signals of five methyl groups on quaternary carbons, a methyl group on an olefinic carbon, an olefinic proton (δ 5.48, t, $J=7$ Hz) and two anomeric protons (δ 4.92, d, $J=8$ Hz; δ 5.20, d, $J=8$ Hz) together with signals due to hydroxymethine groups and hydroxymethylene groups.

On mild acid methanolysis followed by enzymatic hydrolysis with cellulase, IV gave an aglycone (V), $C_{30}H_{52}O_4$, D-glucose and D-galactose. The ^{1}H-NMR spectrum of V showed two hydroxymethylene groups and two hydroxymethine groups. Compound V showed a similar ^{13}C-NMR spectrum to II except that the signal of one hydroxymethylene group (δ 59.4, C_{30} of II) was replaced by a methyl carbon signal (δ 15.0), and that a downfield shift of the C_{15} signal (Δ 6.1 ppm) and upfield shifts of the signals of C_7 (Δ 1.8 ppm), C_8 (Δ 0.9 ppm), C_9 (Δ 1.2 ppm), C_{13} (Δ 1.1 ppm), C_{14} (Δ 4.4 ppm) and C_{16} (Δ 0.7 ppm) were observed. These spectral differences indicated that V is 3β,17β,21,26-tetrahydroxybacchar-24-ene.

The structure of the sugar moiety was determined as β-D-galactopyranosyl-(1→2)-β-D-glucopyranose by detailed examination of the NMR signals of the sugar moiety, and the site of the sugar linkage was determined as C_3 on the same basis as described for I.

Accordingly, actinostemmoside E is the 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucopyranoside of 3β,17β,21,26-tetrahydroxybacchar-24-ene.

The aglycone of actinostemmoside E is the 28-deoxy compound of the aglycone of hosenkosides C and D isolated from the seeds of *Impatiens balsamina* L. by Shoji *et al.*[6] *Actinostemma lobatum* MAXIM. is the second plant from which baccharane-type triterpene glycosides have been isolated, and is the first among Cucurbitaceous plants.

Experimental[7]

Isolation of I and IV——The procedures for isolation of I and IV were described in the first paper[2a] of this series.

I: Colorless needles from aqueous MeOH, mp 193—196 °C. $[\alpha]_D^{19}$ +3.6° (c=0.5, MeOH). *Anal.* Calcd for $C_{41}H_{70}O_{14}\cdot 3H_2O$: C, 58.55; H, 9.11. Found: C, 58.61; H, 9.19. Positive FAB-MS m/z: 809 $[M+Na]^+$. Negative FAB-MS m/z: 785 $[M-H]^-$.

IV: Colorless needles from aqueous MeOH, mp 244—247 °C. $[\alpha]_D^{19}$ +6.1° (c=0.45, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{14}\cdot 2H_2O$: C, 60.27; H, 9.15. Found: C, 60.59; H, 8.95. Positive FAB-MS m/z: 823 $[M+Na]^+$. Negative FAB-MS m/z: 799 $[M-H]^-$.

Hydrolysis of I and IV——Compound I (120 mg) and cellulase (type I, 200 mg) were dissolved in 20% MeOH (50 ml) and the solution was stirred at 38 °C for 3 d, then concentrated and extracted with AcOEt. The organic layer was evaporated *in vacuo* and the residue was subjected to silica gel chromatography [benzene–acetone (2 : 1)] to give an aglycone (II, 40 mg). Colorless needles from aqueous acetone, mp 138—140 °C. $[\alpha]_D^{20}$ +14.4° (c=0.27, MeOH). FAB-MS m/z: 515.369. $C_{30}H_{52}NaO_5$ requires m/z 515.371.

The aqueous layer after AcOEt extraction was evaporated and the residue was suspended in 1 N HCl–MeOH (1 ml). After heating in a boiling water bath for 2 h, the reaction solution was neutralized with Ag_2CO_3. After removal of the precipitates by filtration and evaporation of the solvent, the residue was subjected to silica gel chromatography [$CHCl_3$–MeOH–H_2O (32 : 8 : 1)] to give methyl glycosides I and II, both of which were converted to the corresponding acetates and compared with authentic samples. The acetate of methyl glycoside I showed $[\alpha]_D^{21}$ +18.3° (c=0.47, $CHCl_3$) and its ^{1}H-NMR spectrum was superimposable on that of methyl tri-*O*-acetyl-α-L-arabinopyranoside ($[\alpha]_D$ +12.6°). The acetate of methyl glycoside II showed $[\alpha]_D^{21}$ +128.4° (c=0.5, $CHCl_3$) and was identified as methyl tetra-*O*-acetyl-α-D-galactopyranoside ($[\alpha]_D$ +133°).

Compound IV (70 mg) was treated with 1 N HCl–MeOH (5 ml) at room temperature for 24 h. The reaction solution was neutralized with Ag_2CO_3 and the precipitates were filtered off. The filtrate was concentrated *in vacuo* and the residue was subjected to silica gel chromatography [$CHCl_3$–MeOH–H_2O (32 : 8 : 1), $CHCl_3$–MeOH (9 : 1)] to give methyl glycoside III and a prosapogenin (30 mg). The methyl glycoside III was converted to a corresponding acetate ($[\alpha]_D^{21}$ +130.5° (c=0.1, $CHCl_3$), which was identified as methyl α-D-galactopyranoside tetra-*O*-acetate by comparison of its ^{1}H-NMR spectrum with that of an authentic sample ($[\alpha]_D$ +133°). The prosapogenin and cellulase (type-I, 100 mg) were dissolved in 20% MeOH (100 ml) and stirred at 38 °C for 3 d. The reaction solution was concentrated, and extracted with AcOEt and the extract was evaporated to dryness. The residue was purified by silica gel chromatography [benzene–acetone (3 : 1)] to give an aglycone (V, 20 mg). Colorless needles from benzene–acetone, mp 217—219 °C, $[\alpha]_D^{20}$ +26.0° (c=0.01, MeOH). FAB-MS m/z: 499.375. $C_{30}H_{52}NaO_4$ requires m/z 499.376.

The aqueous layer after AcOEt extraction was treated in the same manner as described for the methyl glycoside III to give an acetate of methyl glycoside (IV, 8 mg), $[\alpha]_D^{21}$ +117.0° (c=0.027, $CHCl_3$). It was identified as methyl-α-D-glucopyranoside tetra-*O*-acetate by ^{1}H-NMR spectrometry.

Measurement of NMR Spectra——Measurement of all NMR spectra was performed on a JEOL JNM GX-400 spectrometer. Spectra were measured at a probe temperature of 30 °C, and the concentrations of the solutes were 5—10% (w/v%).

The 2D-INADEQUATE spectrum of the aglycone (II) of actinostemmoside F was obtained by using a program, V2DINARLP. The delay time was 5.6 ms (J_{CC}=45 Hz), and the pulse delay time was 4 s. The concentration of the solute was 50% (w/v%) and the spectrum was measured at a probe temperature of 35 °C.

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References and Notes

1) This work was reported at the 34th Annual Meeting of the Japanese Society of Pharmacognosy, Osaka, October 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).
3) J. Asakawa, R. Kasai, K. Yamasaki and O. Tanaka, *Tetrahedron*, **33**, 1935 (1977).
4) F. Patil, G. Ourisson, Y. Tanahashi and T. Takahashi, *Bull. Soc. Chim. Fr.*, **1962**, 1422; Y. Tanahashi, Y. Moriyama, T. Takahashi, F. Patil, J.-F. Biellmann and G. Ourisson, *ibid.*, **1966**, 1670.
5) N. Shoji, A. Umeyama, Z. Taira, T. Takemoto, K. Nomoto, K. Mizukawa and Y. Ohizumi, *J. Chem. Soc., Chem. Commun.*, **1983**, 871.
6) N. Shoji, Y. Saito, A. Umeyama, T. Takemoto, K. Nomoto, Y. Ohizumi, reported at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.
7) The instruments and materials used in this work were the same as described in the preceding paper.[2b]