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Sesquiterpene Lactones from *Diaspananthus uniflorus* (SCH. BIP.) KITAM.

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Five new guaiane-type sesquiterpene lactones, diaspanolides A (V) and B (VI) and diaspanosides A (VIII), B (IX) and C (X), have been isolated from *Diaspananthus uniflorus* (SCH. BIP.) KITAM. (Compositae), together with five known compounds, costunolide (I), reynosin (II), dehydrocostuslactone (III), 11,13α-dihydrozaluzanin C (IV) and glucozaluzanin C (VII). The structures were determined on the basis of spectral data and some chemical transformations.

Keywords——*Diaspananthus uniflorus*; Compositae; sesquiterpene lactone; sesquiterpene glucoside; diaspanolide A; diaspanolide B; diaspanoside A; diaspanoside B; diaspanoside C

In a previous paper, we reported that macrocliniside C, a sesquiterpene glucoside, had considerable antitumor activity in mice.[1] Thus, we have been continuing to search for sesquiterpene glycosides with antitumor activity in Compositae plants. We have investigated the constituents of *Diaspananthus uniflorus* (SCH. BIP.) KITAM., and isolated two new lactones, diaspanolide A (V) and diaspanolide B (VI), in addition to three new glycosides diaspanoside A (VIII), diaspanoside B (IX) and diaspanoside C (X), along with the known compounds costunolide (I), reynosin (II), dehydrocostuslactone (III), 11,13α-dihydrozaluzanin C (IV) and glucozaluzanin C (VII). The structures of these compounds were elucidated on the basis of spectroscopic studies and some chemical transformations.

Costunolide (I),[2] reynosin (II),[3] dehydrocostuslactone (III),[4] 11,13α-dihydrozaluzanin C (IV)[5] and glucozaluzanin C (VII)[6] were identified by direct comparison with authentic samples.

Diaspanolide A (V): $C_{20}H_{28}O_4$, $[\alpha]_D$ +373.8°. The infrared (IR) spectrum suggested the presence of a γ-lactone group (1770 cm^{-1}) and an ester group (1730 cm^{-1}). Its proton nuclear magnetic resonance (^{1}H-NMR) spectrum was quite similar to that of IV, except for the additional presence of a doublet methyl signal at δ 0.99 (6H, d, J=6 Hz) characteristic of an isopropyl residue. Furthermore, the signal of H-3 was shifted downfield 1.03 ppm in comparison with that of IV.

In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, twenty signals were observed, including three methyl signals at δ 22.3 ($CH_3 \times 2$) and 13.2 ppm, and two carbonyl signals at δ 172.7 and 178.3 ppm, so we assumed that isovaleric acid was esterified at C-3 of IV. Thus, V was identified by direct comparison [^{1}H-NMR, ^{13}C-NMR, IR] with an authentic sample synthesized from 11,13α-dihydrozaluzanin C (IV) and isovaleric acid. These results led us to conclude that diaspanolide A has the structure V.

Diaspanolide B (VI): $C_{20}H_{26}O_4$, $[\alpha]_D$ +235.3°. The IR spectrum showed γ-lactone (1770 cm^{-1}) and ester group (1730 cm^{-1}) absorptions. The ^{1}H-NMR spectrum was similar to that of V except for the appearance of two doublet signals at δ 5.52 (1H, d, J=3.3 Hz) and 6.25 (1H, d, J=3.5 Hz), characteristic of exocyclic methylene protons of the α-methylene-γ-lactone grouping (common in sesquiterpene lactones), instead of a doublet signal due to methyl protons.

A comparison of the ^{13}C-NMR spectrum of VI with that of V showed an upfield shift of 8.6 ppm at C-12 (lactone carbonyl), suggesting that the methyl group at the γ-lactone ring had been converted to an exocyclic methylene. Thus, VI was identified by direct comparison [^{1}H-NMR, ^{13}C-NMR, IR] with an authentic sample synthesized from zaluzanin C (VIIa) and isovaleric acid. These results led us to conclude that diaspanolide B has the structure VI.

Diaspanoside A (VIII): $C_{21}H_{28}O_9 \cdot 1/2\ H_2O$, $[\alpha]_D$ $-10.9°$. The IR spectrum showed hydroxyl group (3450 cm^{-1}) and γ-lactone (1765 cm^{-1}) absorptions. The ^{1}H-NMR spectrum exhibited signals due to three exomethylene groups at δ 5.50 (overlapped) and 6.28 (1H, d, J=3.3 Hz), at δ 5.42 (1H, s) and 5.83 (1H, s), and at δ 5.50 (overlapped) and 6.00 (1H, br s), suggesting that VIII has a guaianolide-type skeleton like that of glucozaluzanin C (VII).

The ^{13}C-NMR spectrum exhibited twenty-one signals including six signals due to a glucopyranosyl moiety, and also indicated the presence of three exomethylene groups. In a comparison of the ^{13}C-NMR spectrum of VIII with that of VII, various signals showed shifts: C-1 and C-7 (each γ to C-9) at δ 42.9 (Δ −1.9 ppm) and 41.4 (Δ −4.0 ppm), respectively, C-8 and C-10 (each β to C-9) at δ 40.7 (Δ +9.9 ppm) and 154.3 (Δ +5.3 ppm), respectively, and C-9 (α-position) at δ 74.6 (Δ +40.2 ppm). Thus, compound VIII was assumd to be a glucozaluzanin C analog having a hydroxyl group at C-9.

Acid hydrolysis of VIII afforded glucose as the sugar moiety, and enzymatic hydrolysis with crude hesperidinase afforded an aglycone (VIIIa). The mass spectrum (MS) of VIIIa showed a molecular ion peak at m/z 262 in agreement with the molecular formula $C_{15}H_{18}O_4$. The ^{1}H-NMR spectrum of VIIIa also exhibited three exomethylene signals at δ 5.55 (overlapped) and 6.32 (1H, d, J=3.3 Hz), at δ 5.55 (overlapped) and 5.92 (1H, s), and at δ 5.55 (overlapped) and 5.75 (1H, br s). A lactonic proton signal was observed at δ 4.26 as a triplet having a coupling constant of 9 Hz, so H-5, H-6 and H-7 are in anti-diaxial relationships.[7] Irradiation at a carbinyl proton signal at δ 4.44 affected an exomethylene proton signals at δ 5.55 and 5.92 (H-14), so we could assign the carbinyl proton to H-9. Furthermore, the carbinyl proton signal showed coupling constants of 5 and 11 Hz, so we assumed that H-9 was α-oriented (pseudo-axial), and thus the hydroxyl group at C-9 was β-oriented. In a nuclear Overhauser effect (NOE) experiment on VIIIa, irradiation of the H-2β signal increased the intensity of the H-14a signal by about 7%, so we decided that H-1 is α-oriented (H-1, H-5 *cis*) (Chart 2).

The circular dichroism (CD) spectrum of VIII showed a negative Cotton effect $[\theta]_{261}$ −1029, suggesting that the γ-lactone ring fusion is 6α,7β-*trans*.[8] A comparison of the ^{13}C-NMR spectrum of VIII with that of VIIIa revealed glycosidation shifts at C-3 (Δ +8.0 ppm), C-2 (Δ −1.5 ppm) and C-4 (Δ −4.1 ppm), so the glycosidic position was at C-3.[9] These results led us to conclude that diaspanoside A has the structure VIII. The stereochemistry of the anomeric center was deduced to be β from the $J_{H_{1'}-H_{2'}}$ coupling constant (7 Hz).

Diaspanoside B (IX): $C_{21}H_{28}O_8 \cdot 1/2\ H_2O$, $[\alpha]_D$ $-55.0°$. The IR spectrum suggested the presence of hydroxyl groups (3450 cm^{-1}), and a γ-lactone ring (1770 cm^{-1}). In the ^{1}H-NMR spectrum, three exomethylene groups were observed at δ 5.36 (overlapped) and 6.25 (1H, d, J=3.3 Hz), at δ 5.00 (1H, s) and 6.19 (1H, s), and at δ 5.05 (1H, br s) and 5.36 (overlapped).

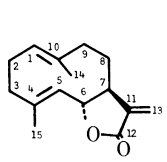
Acid hydrolysis of IX afforded glucose as the sugar moiety, and enzymatic hydrolysis with crude hesperidinase afforded an aglycone (IXa). The MS of IXa showed a molecular ion peak at m/z 246 in agreement with the molecular formula $C_{15}H_{18}O_3$. In the ^{1}H-NMR spectrum of IXa, a lactonic proton signal was observed at δ 4.06 as a triplet having a coupling constant of 9 Hz, so H-5, H-6 and H-7 are in anti-diaxial relationships. Irradiation at a carbinyl proton signal at δ 4.39 affected exomethylene signals at δ 4.99 and 5.82 (H-14), so we could assign the carbinyl proton to H-9. The stereochemistry of the hydroxyl group at C-9 was determined as in the case of VIIIa from the fact that the carbinyl proton signal was observed as a double doublet having coupling constants of 5 and 11 Hz, so we assumed that

the H-9 proton was α-oriented (pseudo-axial), and thus the hydroxyl group was β-oriented. In the high-resolution ^{1}H-NMR difference spectrum, NOE was observed between H-14a and H-2β, so we concluded that H-1 is α-oriented (H-1, H-5 *cis*) (Chart 2).

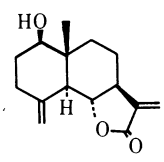
The CD spectrum of IX showed a negative Cotton effect $[\theta]_{258}$ −1002, suggesting that the γ-lactone ring fusion is 6α,7β-*trans*. In the ^{13}C-NMR spectrum of IXa, various signals showed shifts as compared with those of VIIIa: C-1 and C-5 (each γ to C-3) at δ 43.9 (Δ +1.8 ppm) and 52.2 (Δ +2.6 ppm), respectively, C-2 and C-4 (each β to C-3) at δ 33.4 (Δ −6.2 ppm) and 152.7 (Δ −2.5 ppm), respectively, and C-3 (α-position) at δ 30.7 (Δ −42.0 ppm). Thus, compound IXa was lacking a hydroxyl group at C-3. A comparison of the ^{13}C-NMR spectrum of IX with that of JXa revealed glycosidation shifts at C-9 (Δ +4.6 ppm), C-8 (Δ −1.5 ppm), and C-10 (Δ −5.1 ppm). Thus, we concluded that diaspanoside B has the structure IX. The stereochemistry of the anomeric center was deduced to be β from the $J_{C_1-H_1}$ coupling constant (159 Hz).[10]

Diaspanoside C (X): $C_{21}H_{30}O_9 \cdot 1/2\ H_2O$, $[\alpha]_D$ −22.1°. The IR spectrum suggested the presence of hydroxyl groups (3450 cm^{-1}), and a γ-lactone ring (1770 cm^{-1}). The ^{1}H-NMR spectrum exhibited a singlet methyl signal at δ 1.66 (3H, s) and two exomethylene group signals at δ 5.45 (1H, d, J=3.1 Hz); 6.28 (1H, d, J=3.3 Hz) due to exocyclic methylene protons of the α-methylene-γ-lactone group (H-13), and at δ 4.95 (2H, s).

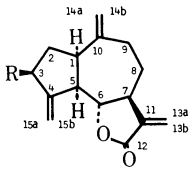
The ^{13}C-NMR spectrum was similar to that of VII, except for the appearance of a methyl carbon at δ 17.9 and a quaternary carbon at δ 80.7 having a hydroxyl group, instead of exomethylene carbons.



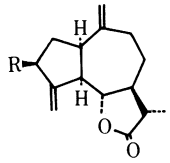
I



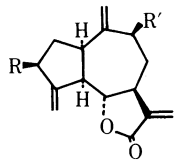
II



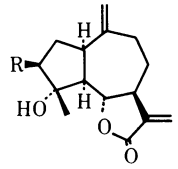
III : R=H
VI : R=O-C(=O)-CH$_2$-CH(CH$_3$)$_2$
VII : R=O-glc
VIIa : R=OH
Xb : R=O-C(=O)-CH$_3$



IV : R=OH
V : R=O-C(=O)-CH$_2$-CH(CH$_3$)$_2$



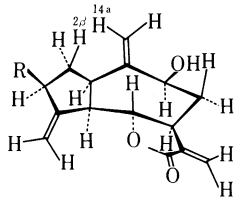
VIII : R=O-glc, R′=OH
VIIIa : R=OH, R′=OH
IX : R=H, R′=O-glc
IXa : R=H, R′=OH



X : R=O-glc
Xa : R=OH

Chart 1

NOE



VIIIa : R=OH
IXa : R=H

Chart 2

Acid hydrolysis of X afforded glucose as the sugar moiety, and enzymatic hydrolysis with crude hesperidinase afforded an aglycone (Xa). The MS of Xa showed a molecular ion peak at *m/z* 264 in agreement with the molecular formula $C_{15}H_{20}O_4$. Treatment of Xa with pyridine and acetic anhydride gave a monoacetate and successive dehydration with phosphorus oxychloride and pyridine afforded 3-acetylzaluzanin C (Xb), which was identified by direct comparison [high-performance liquid chromatography (HPLC)] with an authentic sample synthesized from glucozaluzanin C (VII).

In a comparison of the ^{13}C-NMR spectrum of X with that of VII, C-1 and C-2 exhibited upfield shifts of 6.1 and 5.3 ppm (each γ to C-4), and C-3 and C-5 exhibited downfield shifts of

TABLE I. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	IV	V	VI
Aglycone moiety			
3	4.56 (1H, br t, J=7 Hz)	5.59 (1H, dd, J=6, 8 Hz)	5.60[a]
6	4.04 (1H, t, J=9 Hz)	4.01 (1H, t, J=9 Hz)	4.08 (1H, t, J=9 Hz)
13a	1.24 (3H, d, J=7 Hz)	1.25 (3H, d, J=7 Hz)	5.52 (1H, d, J=3.3 Hz)
13b			6.25 (1H, d, J=3.5 Hz)
14	4.96 (2H, s)	4.95 (2H, s)	4.97 (2H, s)
15	5.32 (1H, br s)	5.28 (1H, br t, J=2 Hz)	5.30 (1H, br s)
	5.40 (1H, br s)	5.43 (1H, br t, J=2 Hz)	5.50 (1H, br s)
Ester moiety			
4, 5		0.99 (6H, d, J=6 Hz)	0.99 (6H, d, J=6 Hz)

Run at 89.55 MHz in $CDCl_3$ solution. *a*) Overlapped.

TABLE II. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	VII	VIII	IX	X
Aglycone moiety				
13a	5.38 (1H, d, J=3.3 Hz)	5.50[a]	5.36[a]	5.45 (1H, d, J=3.1 Hz)
13b	6.22 (1H, d, J=3.3 Hz)	6.28 (1H, d, J=3.3 Hz)	6.25 (1H, d, J=3.3 Hz)	6.28 (1H, d, J=3.3 Hz)
14a	5.02 (1H, s)	5.42 (1H, s)	5.00 (1H, s)	4.95 (2H, s)
14b	4.85 (1H, s)	5.83 (1H, s)	6.19 (1H, s)	
15a	5.85 (1H, br s)	6.00 (1H, br s)	5.05 (1H, br s)	1.66 (3H, s)
15b	5.53 (1H, br s)	5.50[a]	5.36[a]	
Sugar moiety				
1	5.05[a]	5.03 (1H, d, J=7 Hz)		5.56 (1H, d, J=7 Hz)

Proton No.	VIIIa	IXa	Xa
3	4.90 (1H, br t, J=10 Hz)		4.51 (1H, br t, J=9 Hz)
6	4.26 (1H, t, J=9 Hz)	4.06 (1H, t, J=9 Hz)	4.24 (1H, dd, J=9, 11 Hz)
9	4.44 (1H, dd, J=5, 11 Hz)	4.39 (1H, dd, J=5, 11 Hz)	
13a	5.55[a]	5.53 (1H, d, J=3.1 Hz)	5.45 (1H, d, J=3.1 Hz)
13b	6.32 (1H, d, J=3.3 Hz)	6.31 (1H, d, J=3.4 Hz)	6.29 (1H, d, J=3.6 Hz)
14a	5.55[a]	4.99 (1H, s)	5.08 (1H, s)
14b	5.92 (1H, s)	5.82 (1H, s)	4.99 (1H, s)
15a	5.75 (1H, br s)	5.12 (1H, br s)	1.62 (3H, s)
15b	5.55[a]	5.43 (1H, br s)	

Run at 89.55 MHz in pyridine-d_5 solution. *a*) Overlapped.

TABLE III. ^{13}C-NMR Chemical Shifts and Coupling Constant

Carbon No.	IV[a]	V[a]	VI[a]	VII[b]	VIII[b]	VIIIa[b]	IX[b]	IXa[b]	X[b]	Xa[b]
Aglycone moiety										
1	43.5	44.0	44.6	44.8[f]	42.9	42.1	43.8	43.9	38.7	38.8
2	38.7	36.4	36.6	38.2	38.1	39.6	33.4	33.4	32.9	34.3
3	73.4	74.3	74.3	80.7	80.7	72.7	30.7	30.7	85.1	78.3
4	153.2	148.6[c]	147.7	151.1	151.1	155.2	152.5	152.7	80.7	80.5
5	50.8	50.3	50.3	50.4	49.6	49.6	52.0	52.2	53.3	53.9
6	83.7	83.7	83.8	83.8	84.4	84.8	85.8	86.1	83.4	83.7
7	49.5	50.0	45.2	45.4[f]	41.4	40.9	41.8	41.7	47.5	47.4
8	32.3	32.3	30.6	30.8	40.7	40.7	39.1	40.6	31.7	31.6
9	35.9	36.1	34.5	34.4	74.6	74.6	79.0	74.4	39.2	39.1
10	148.7	148.4[c]	147.7	149.0	154.3	155.0	150.0	155.1	148.3	148.9
11	42.0	42.0	139.6	141.1	140.3	140.1	139.8	140.1	140.3	140.4
12	178.4	178.3	169.7	170.2	169.5	170.1	170.0	170.1	170.2	170.1
13	13.1	13.2	120.1	119.6	119.1	119.7	120.0	119.8	119.6	119.4
14	114.3	113.4[d]	114.3[e]	114.1	110.5	110.4	111.1	108.5	112.6	112.5
15	113.5	113.1[d]	113.4[e]	112.4	110.7	107.3	108.7	108.5	17.9	17.7
Sugar or ester moiety										
1		172.7	172.7	104.3	104.6		101.9 (159 Hz)		104.6	
2		43.6	43.6	75.4	75.3		75.5		75.9	
3		25.7	25.7	78.7[g]	78.5[h]		78.6		78.7[i]	
4		22.3	22.3	72.0	72.2		71.9		71.8	
5		22.3	22.3	78.5[g]	77.9[h]		78.6		78.5[i]	
6				63.0	63.3		63.0		62.9	

a) Run at 22.5 MHz in $CDCl_3$ solution. *b*) Run at 22.5 MHz in pyridine-d_5 solution. *c*—*i*) Assignments may be interchanged in each column.

4.4 and 2.9 ppm (each β to C-4), respectively, so we concluded that X has a quaternary carbon at C-4 with a methyl group and a hydroxyl group. In an NOE experiment on Xa, irradiation of the H-15 methyl signal increased the intensity of the H-6 lactonic proton signal by about 14%, so we concluded that the methyl group at C-4 was β-oriented, and thus the hydroxyl group was α-oriented. These results led us the conclusion that diaspanoside C has the structure X. The stereochemistry of the anomeric center was deduced to be β from the $J_{H_{1'}-H_{2'}}$ coupling constant (7 Hz).

Experimental

Melting points were determined on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. CD spectra were recorded with a JASCO J-20A automatic recording spectropolarimeter. IR spectra were taken on a JASCO A-202 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL FX-90Q (89.55 and 22.5 MHz, respectively) and JEOL GX-400 (399.65 MHz) spectrometers. Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was run on a Hitachi K 53 gas chromatograph. HPLC was run on a Kyowa Seimitsu model K880 instrument.

Isolation——Air-dried whole plant (590 g) of *D. uniflorus* collected in November 1985, in Shizuoka, Japan, was extracted three times with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with ether to give a gum (25 g). The water layer was passed through an Amberlite XAD-2 column and the absorbed material was eluted with methanol to give an amorphous powder (17 g). The ether layer and methanol eluate were each chromatographed on a silica gel column to give compounds I—VI and VII—X, respectively.

Costunolide (I)——Amorphous powder (150 mg). IR ν_{max}^{KBr} cm^{-1}: 1765, 1670. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, br s, H_3-14), 1.71 (3H, d, J=1 Hz, H_3-15), 4.60 (3H, m, H-1, H-5, H-6), 5.54 (1H, d, J=3.2 Hz, H-13a), 6.28 (1H, d,

J=3.6 Hz, H-13b). ^{13}C-NMR ($CDCl_3$) δ: 16.0 (C-14), 17.2 (C-15), 26.1 (C-2), 28.0 (C-8), 39.4 (C-3), 40.9 (C-9), 50.4 (C-7), 81.8 (C-6), 119.4 (C-13), 126.9 (C-1), 127.2 (C-5), 136.8 (C-11), 140.1 (C-4), 141.3 (C-10), 170.3 (C-12).

Reynosin (II)——Amorphous powder (20 mg). IR ν_{max}^{KBr} cm^{-1}: 3460, 1760, 1655. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s, H_3-14), 3.53 (1H, dd, J=5, 11 Hz, H-1), 4.04 (1H, t, J=11 Hz, H-6), 4.87 (1H, br s, H-15), 4.99 (1H, br s, H-15′), 5.42 (1H, d, J=3.1 Hz, H-13a), 6.10 (1H, d, J=3.2 Hz, H-13b).

Dehydrocostuslactone (III)——Amorphous powder (200 mg). IR ν_{max}^{KBr} cm^{-1}: 1770, 1645. ^{1}H-NMR ($CDCl_3$) δ: 3.98 (1H, t, J=9 Hz, H-6), 4.84 (1H, s, H-14), 4.91 (1H, s, H-14′), 5.09 (1H, br s, H-15), 5.29 (1H, br s, H-15′), 5.51 (1H, d, J=3.2 Hz, H-13a), 6.25 (1H, d, J=3.5 Hz, H-13b). ^{13}C-NMR ($CDCl_3$) δ: 30.2 (C-3), 30.8 (C-8), 32.5 (C-2), 36.1 (C-9), 45.1 (C-7), 47.6 (C-1), 52.0 (C-5), 85. 1 (C-6), 109.5 (C-15), 112.5 (C-14), 120.0 (C-13), 139.7 (C-11), 149.2 (C-10), 151.1 (C-4), 169.5 (C-12).

11,13α-Dihydrozaluzanin C (IV)——Amorphous powder (25 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770, 1460. ^{1}H- and ^{13}C-NMR: Tables I and III.

Diaspanolide A (V)——Colorless oil (60 mg). $[\alpha]_D^{27}$ +373.8° (c=0.011, chloroform). *Anal.* Calcd for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.35; H, 8.52. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1730, 1300. MS m/z: 332 (M^+, trace), 248 ($M^+ - C_5H_8O$, 42), 247 ($M^+ - C_5H_9O$, 44), 85 (C_5H_9O, 100). ^{1}H- and ^{13}C-NMR: Tables I and III.

Diaspanolide B (VI)——Colorless oil (300 mg), $[\alpha]_D^{27}$ +235.3° (c=0.034, chloroform). *Anal.* Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.91; H, 8.04. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1730, 1300, 1260. MS m/z: 330 (M^+, trace), 246 ($M^+ - C_5H_8O$, 35), 245 ($M^+ - C_5H_9O$, 40), 85 (C_5H_9O, 100). ^{1}H- and ^{13}C-NMR: Tables I and III.

Glucozaluzanin C (VII)——Recrystallized from water as colorless needles (2 g), mp 106—108 °C. IR ν_{max}^{KBr} cm^{-1}: 3425, 1745, 1640, 1270. ^{1}H- and ^{13}C-NMR: Tables II and III.

Diaspanoside A (VIII)——Amorphous powder (100 mg). $[\alpha]_D^{20}$ −10.9° (c=0.32, methanol). *Anal.* Calcd for $C_{21}H_{28}O_9 \cdot 1/2\ H_2O$: C, 58.19; H, 6.74. Found: C, 58.29; H, 6.63. IR ν_{max}^{KBr} cm^{-1}: 3450, 1765. CD (c=0.32, methanol) [θ] (nm): −1029 (261). ^{1}H- and ^{13}C-NMR: Tables II and III.

Diaspanoside B (IX)——Amorphous powder (300 mg). $[\alpha]_D^{20}$ −55.0° (c=0.30, methanol). *Anal.* Calcd for $C_{21}H_{28}O_8 \cdot 1/2\ H_2O$: C, 60.42; H, 7.00. Found: C, 60.23; H, 6.73. IR ν_{max}^{KBr} cm^{-1}: 3450, 1770. CD (c=0.30, methanol) [θ] (nm): −1002 (258). ^{1}H- and ^{13}C-NMR: Tables II and III.

Diaspanoside C (X)——Amorphous powder (18 mg). $[\alpha]_D^{20}$ −22.1° (c=0.34, methanol). *Anal.* Calcd for $C_{21}H_{30}O_9 \cdot 1/2\ H_2O$: C, 57.92; H, 7.18. Found: C, 58.11; H, 6.99. IR ν_{max}^{KBr} cm^{-1}: 3450, 1770, 1650. ^{1}H- and ^{13}C-NMR: Tables II and III.

Synthesis of Diaspanolide A (V)——Isovaleric acid (1 ml) was mixed with thionyl chloride (1 ml) and stirred for 4 h at room temperature. The excess thionyl chloride was evaporated off *in vacuo*, and 11,13α-dihydrozaluzanin C (IV) (124 mg) dissolved in pyridine (3 ml) was added to the residue. The mixture was stirred for 12 h at room temperature, then the reaction product was purified by silica gel column to afford a colorless oil (V) (40 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1730, 1300. ^{1}H- and ^{13}C-NMR: Tables I and III.

Synthesis of Diaspanolide B (VI)——Zaluzanin C (VIIa) (45 mg) was also esterified in the same way as V, and the isovalerate was obtained as a colorless oil (VI) (21 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1730, 1300, 1260. ^{1}H- and ^{13}C-NMR: Tables I and III.

Enzymatic Hydrolysis of Diaspanoside A (VIII)——VIII (18 mg) was dissolved in water (1 ml) and stirred with crude hesperidinase (*ca.* 10 mg) for 5 h at 37 °C. After being diluted with water, the mixture was passed through an Amberlite XAD-2 column, which was washed with water. The methanol eluate was purified on a silica gel column to give the aglycone (VIIIa) (7 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770. MS m/z: 262 (M^+, 5), 244 ($M^+ - H_2O$, 7), 226 ($M^+ - 2H_2O$, 6), 149 (26), 33 (100). ^{1}H- and ^{13}C-NMR: Tables II and III.

Enzymatic Hydrolysis of Diaspanoside B (IX)——IX (11 mg) was hydrolyzed in the same way as VIII, giving the aglycone (IXa) (4 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770. MS m/z: 246 (M^+, 2), 228 ($M^+ - H_2O$, 16), 149 (47), 42 (100). ^{1}H- and ^{13}C-NMR: Tables II and III.

Enzymatic Hydrolysis of Diaspanoside C (X)——X (13 mg) was also hydrolyzed in the same way as VIII, giving the aglycone (Xa) (3 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770. MS m/z: 264 (M^+, 2), 246 ($M^+ - H_2O$, 6), 228 ($M^+ - 2H_2O$, 3), 149 (18), 33 (100). ^{1}H- and ^{13}C-NMR: Tables II and III.

Dehydration of Diaspanoside C (X)——X was hydrolyzed in the same way as VIII, then the aglycone (Xa) (*ca.* 0.1 mg) was dissolved in acetic anhydride and pyridine (1 drop each), and the mixture was left standing for 2 h at room temperature. The reaction product was evaporated to dryness, and phosphorus oxychloride and pyridine (each 2 drops) were added successively to the residue. The mixture was left to stand for 3 h at room temperature, then excess H_2O was added in order to destroy the reagent. The aqueous solution was extracted with *n*-BuOH 3 times. The residue was evaporated to dryness. This product was examined by HPLC and shown to be identical with authentic 3-acetylzaluzanin C (Xb). HPLC conditions: column, Develosil ODS-7, 4.6 mm × 25 cm; eluent, H_2O–CH_3CN (40 : 60); UV detector 205 nm; flow rate, 1.1 ml/min; t_R, 8.5 min.

Acid Hydrolysis of Diaspanosides A (VIII), B (IX) and C (X)——A solution of a glycoside (*ca.* 0.1 mg) in 10% H_2SO_4 (2 drops) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IR-45 column and concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 0.1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and concentrated to dryness. Boric acid was

removed by distillation with methanol and the residue was acetylated with acetic anhydride (1 drop) and pyridine (1 drop) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. Glucitol acetate was detected by GC. GC conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 200 °C; carrier gas, N_2; t_R, 4.0 min.

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