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Studies on Sesquiterpenes from *Macroclinidium trilobum* MAKINO. II[1)]

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Four new guaiane-type sesquiterpene glycosides, macroclinisides F(II), G(III), H(IV) and I(V), and a new phenyl propanoid glycoside, 4-allyl-2,6-dimethoxyphenol glucoside (VI), have been isolated from *Macroclinidium trilobum* MAKINO together with isolipidiol (I). The structures were determined on the basis of chemical and spectral data.

Keywords——*Macroclinidium trilobum*; Compositae; sesquiterpene glycoside; macrocliniside F; macrocliniside G; macrocliniside H; macrocliniside I; ^{1}H-NMR; ^{13}C-NMR

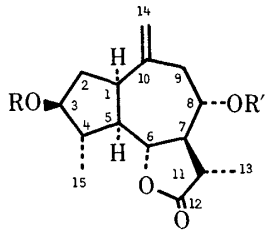
In a previous paper[1)] on sesquiterpene glycosides from *Macroclinidium trilobum* MAKINO (syn. *Pertya triloba* MAKINO), we reported the structures of macroclinisides A—E and the antitumor activity of macrocliniside C (VII). Now we wish to report the structures of four new sesquiterpene glycosides, macroclinisides F (II), G (III), H (IV) and I (V), and a new phenyl propanoid glycoside, 4-allyl-2,6-dimethoxyphenol glucoside (VI), which were isolated together with a known sesquiterpene, isolipidiol (I), from the methanol extract of *M. trilobum* MAKINO. The structures of these compounds were determined on the basis of chemical transformations and spectroscopic studies.

Isolipidiol (I), mp 167—168.5 °C, $[\alpha]_D$ +41.3°, gave a diacetate (Ia) on acetylation. The physical and spectral data of I were identical with those of isolipidiol.[2)]

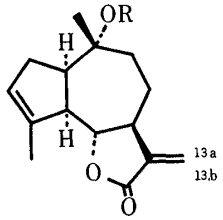
Macrocliniside F (II) was isolated as an acetate (IIa), $C_{31}H_{42}O_{14}$, mp 197—198 °C, $[\alpha]_D$ +16.7°. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of IIa exhibited two doublet methyl signals at δ 1.24 (3H, J=6 Hz); 1.35 (3H, J=7 Hz), exomethylene signals at δ 5.00 (1H, s); 5.07 (1H, s), and five alcoholic acetoxyl groups at δ 2.03 (9H, s); 2.07 (3H, s); 2.11 (3H, s). Saponification of IIa gave pure II as an amorphous powder. Acid hydrolysis of IIa gave glucose as the sugar moiety and enzymatic hydrolysis of II afforded isolipidiol (I) as an aglycone. Thus, II was assumed to be a glucoside of I. The ^{1}H-NMR spectral data of I and II were compared with those of Ia and IIa to decide the position of glycosidation, because I has two hydroxyl groups in the molecule. In the ^{1}H-NMR spectrum of Ia, the methyl signals at C-13 (δ 1.34, d, J=7 Hz) and C-15 (δ 1.17, d, J=6 Hz) were shifted upfield by 0.35 and 0.28 ppm, respectively. However, in the ^{1}H-NMR spectrum of IIa, the methyl signal at C-13 (δ 1.35, d, J=7 Hz) was shifted upfield by 0.29 ppm, while that at C-15 (δ 1.24, d, J=6 Hz) was shifted upfield by only 0.15 ppm. Thus, a hydroxyl group of II must be at C-8, affecting the chemical shift of the methyl group at C-13. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of IIa, C-7 (δ 55.2) and C-9 (δ 43.0) were shifted upfield by 3.3 and 5.2 ppm, respectively, compared with those of II. From these data, the glycosidation position was decided to be at C-3. In the ^{1}H-NMR spectrum of IIa in deuterochloroform, an anomeric proton was observed at δ 4.56 (1H, d, J=8 Hz), indicating a β glycosidic linkage. These results led us to conclude the structure of macrocliniside F to be II.

Macrocliniside G (III), $C_{21}H_{30}O_8 \cdot H_2O$, mp 128—129 °C, $[\alpha]_D$ +39.8°. The ^{1}H-NMR

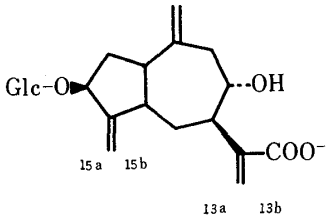
spectrum exhibited two doublet signals at δ 5.30 (1H, J=3.1 Hz); 6.12 (1H, J=3.6 Hz), which are characteristic of exocyclic α-methylene-γ-lactone, as well as two methyl signals at δ 1.40 (3H, s); 1.96 (3H, br s) and an olefinic proton at δ 5.43 (1H, br s). This olefinic proton signal was long-range-coupled with a methyl signal at δ 1.96. The circular dichroism (CD) spectrum showed a negative Cotton effect $[\theta]_{257}$ −4112, suggesting that the γ-lactone ring fusion is 6,7-*trans* or 7,8-*cis*.[3] Enzymatic hydrolysis of III afforded IIIa as an aglycone and acid hydrolysis afforded glucose as the sugar moiety. The mass spectrum (MS) of IIIa showed a molecular ion peak at m/z 248, in agreement with the molecular formula $C_{15}H_{20}O_3$. The ^{1}H-NMR spectrum of IIIa exhibited a triplet signal at δ 4.13 (1H, J=10 Hz) assignable to H-6 of 6,7-*trans*-lactonized guaianolide,[4] in addition to two methyl signals at δ 1.20 (3H, s); 1.88 (3H, br s), exocyclic methylene signals at δ 5.43 (1H, J=3.3 Hz); 6.16 (1H, d, J=3.6 Hz), an olefinic proton signal at δ 5.46 (1H, br s) and a methine signal at δ 3.2 (1H, m, $W_{1/2}$=22 Hz). The downfield shift of H-7 can be explained in terms of a 10α-hydroxyl group.[5] From these spectral data, the aglycone IIIa was assumed to be 8-deoxycumambrin B,[6] and this was confirmed by comparison of the spectral data. Thus, the structure of macrocliniside G was decided to be III. The stereochemistry of the anomeric center of III was deduced to be β from the $J_{C_1-H_1}$ coupling constant (155 Hz).[7]



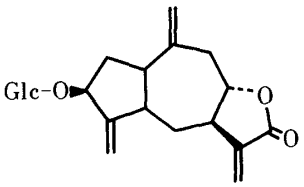
I : R=H R′=H
Ia : R=Ac R′=Ac
II : R=β-Glc R′=H
IIa : R=(β-Glc)Ac$_4$ R′=Ac



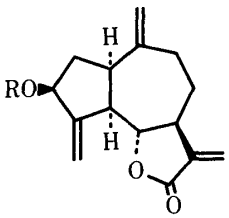
III : R=β-Glc
IIIa : R=H



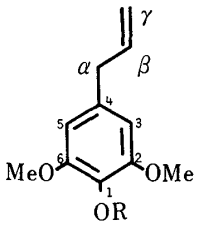
IV



VII



V : R=β-Glc $\overset{3}{\underset{1}{-}}$$\beta$-Glc $\overset{4}{\underset{1}{-}}$$\beta$-Glc
VIII : R=β-Glc $\overset{3}{\underset{1}{-}}$$\beta$-Glc
IX : R=β-Glc
X : R=H



VI : R=β-Glc
VIa : R=H

Chart 1

Macrocliniside H (IV), $[\alpha]_D$ +20.5°, was obtained as an amorphous powder and was so polar that it was soluble only in water. In the ^{1}H-NMR spectrum, three exomethylene signals were observed at δ 4.99 (1H, s), 5.07 (1H, s), 5.18 (1H, br s), 5.32 (2H, br s), 5.78 (1H, s). The infrared (IR) spectrum suggested the presence of hydroxyl groups (3400 cm^{-1}), a double bond (1640 cm^{-1}) and a carboxylate (1550, 1410 cm^{-1}). In the ^{13}C-NMR spectrum, twenty-one signals were observed, six of which were assigned to the carbons of a glucopyranosyl moiety. From these data, IV was assumed to be sesquiterpenic carboxylate having a glucopyranosyl residue. This assumption was confirmed by the formation of IV from macrocliniside C (VII), which was the main component of this plant, on treatment with sodium hydroxide. The

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

Proton No.	I[a]	Ia[a]	II[a]	IIa[a]
6		3.95 (1H, t, J=10 Hz)		
13	1.69 (3H, d, J=7 Hz)	1.34 (3H, d, J=7 Hz) ($\delta_{I\text{-}Ia}$=0.35 ppm)	1.64 (3H, d, J=7 Hz)	1.35 (3H, d, J=7 Hz) ($\delta_{II\text{-}IIa}$=0.29 ppm)
14	5.01 (1H, s)	5.00 (1H, s)		5.00 (1H, s)
	5.09 (1H, s)	5.07 (1H, s)		5.07 (1H, s)
15	1.45 (3H, d, J=6 Hz)	1.17 (3H, d, J=6 Hz) ($\delta_{I\text{-}Ia}$=0.28 ppm)	1.39 (3H, d, J=6 Hz)	1.24 (3H, d, J=6 Hz) ($\delta_{II\text{-}IIa}$=0.15 ppm)
Ac		2.06 (3H, s)		2.03 (9H, s)
		2.07 (3H, s)		2.07 (3H, s)
				2.11 (3H, s)

Proton No.	III[a]	IIIa[b]	IV[c]	V[a]
3	5.43 (1H, br s)	5.46 (1H, br s)		
6		4.13 (1H, t, J=10 Hz)		
7		3.2 (m) $W_{1/2}$=22 Hz		
13a	5.30 (1H, d, J=3.1 Hz)	5.43 (1H, d, J=3.3 Hz)	5.32[d]	5.40 (1H, d, J=3.1 Hz)
13b	6.12 (1H, d, J=3.6 Hz)	6.16 (1H, d, J=3.6 Hz)	5.78 (1H, s)	6.23 (1H, d, J=3.4 Hz)
14	1.40 (3H, s)	1.20 (3H, s)	4.99 (1H, s)	4.85 (1H, s)
			5.07 (1H, s)	5.01 (1H, s)
15a	1.96 (3H, br s)	1.88 (3H, d, J=1.5 Hz)	5.18 (1H, br s)	5.85 (1H, br s)
15b			5.32[d]	5.55 (1H, br s)
Anomeric	5.01 (1H, d, J=8 Hz)		4.66 (1H, d, J=7 Hz)	

a) In pyridine-d_5 solution. *b*) In $CDCl_3$ solution. *c*) In D_2O solution (tetramethylsilane as an external standard). *d*) Overlapped.

cations were detected by atomic absorption spectrometry and fast atom bombardment mass spectrometry (FAB-MS), which showed ion peaks at m/z 449 $(C_{21}H_{29}NaO_9+H)^+$, 465 $(C_{21}H_{29}KO_9+H)^+$, 471 $(C_{21}H_{29}NaO_9+Na)^+$, 487 $(C_{21}H_{29}KO_9+Na)^+$. These results led us to conclude the structure of macrocliniside H to be IV. This compound was detected in the water extract obtained under mild conditions, so we concluded that IV was a naturally occurring substance and not an artifact formed during the extraction and isolation procedures.

Macrocliniside I (V), $C_{33}H_{48}O_{18}\cdot 2H_2O$, $[\alpha]_D$ $-13.0°$. The FAB-MS exhibited an ion peak at m/z 755 $(M+Na)^+$. The ^{1}H-NMR spectrum exhibited three exomethylene signals at δ 4.85 (1H, s); 5.01 (1H, s), 5.55 (1H, br s); 5.85 (1H, br s), 5.40 (1H, d, J=3.1 Hz); 6.23 (1H, d, J=3.4 Hz). These two doublet signals are characteristic of exocyclic α-methylene-γ-lactone. In the ^{13}C-NMR spectrum, three anomeric carbon signals were observed at δ 103.5; 104.8; 105.4 having $J_{C_1-H_1}$ 157 Hz; 159 Hz; 161 Hz, respectively. Acid hydrolysis of V afforded glucose as the sugar moiety and enzymatic hydrolysis with maltase afforded macrocliniside B (VIII),[1] glucozaluzanin C (IX)[1] and zaluzanin C (X).[1,8] Then, the ^{13}C-NMR spectrum of V was compared with that of VIII to decide the position of the third glucose. In the ^{13}C-NMR spectrum of V, C-4″ (δ 80.9) of glucose was shifted downfield by 9.3 ppm and C-3″ (δ 76.3) and C-5″ (δ 76.6) of glucose were shifted upfield by 2.1 and 1.4 ppm, respectively. Based on the above evidence, the structure of macrocliniside I was concluded to be V. This is the first reported example of the triglycoside of a guaiane-type sesquiterpene.

4-Allyl-2,6-dimethoxyphenol glucoside (VI), $C_{17}H_{24}O_8$, mp 159—161 °C, $[\alpha]_D$ $-21.7°$. The IR spectrum suggested the presence of hydroxyl groups (3400 cm^{-1}) and an aromatic ring (1590, 1508 cm^{-1}). The ^{1}H-NMR spectrum indicated the presence of two methoxyl groups

TABLE II. ^{13}C-NMR Chemical Shifts and Coupling Constants

Carbon No.		I[a]	Ia[a]	II[a]	IIa[a]	III[a]	IIIa[a]	IV[b]	V[a]
Aglycone moiety	1	43.0[c]	41.3[d]	43.1[e]	41.4[g]	54.8[h]	55.0	45.2[j]	44.8
	2	39.4	35.6	38.0	37.4	31.0	34.1	37.8[k]	33.0
	3	78.0	79.8	87.0	87.7	125.4	126.4	78.7	80.5[l]
	4	47.7	44.2[d]	45.3	45.1[g]	143.9	142.8[i]	157.2	150.3
	5	51.6	50.7	51.0	50.7	53.4[h]	55.0	52.0	50.4
	6	82.2	80.9	81.8	81.0	84.3	84.6	37.0[k]	83.6
	7	58.9	55.2	58.5	55.2	43.1	44.2	44.8[j]	45.3
	8	76.1	76.7	75.8[f]	76.8	24.8	25.5	83.3	30.7
	9	48.7	42.9	48.3	43.0	34.1	35.7	44.4[j]	34.2
	10	145.8	143.4	145.3	143.3	80.7	73.0	148.2	148.8
	11	42.6[c]	43.0[d]	42.4[e]	42.6[g]	142.7	142.2[i]	151.8	141.0
	12	179.0	177.5	178.7	177.5	170.1	170.0	178.3	169.9
	13	16.7	15.7	16.5	15.7	118.2	118.4	119.8	119.3
	14	113.9	115.7	114.1	115.8	28.1	30.2	114.3	114.0
	15	18.6	18.1	18.7	18.3	17.8	17.4	110.3	112.5
Sugar moiety	1′			105.6	102.0	98.3 (155 Hz)		104.2	103.5 (157 Hz)
	2′			75.3[f]	72.2	75.1		74.8	74.1[m]
	3′			78.5	73.5	78.7		77.4	88.5
	4′			72.0	69.5	71.9		71.2	69.8
	5′			78.1	72.2	77.6		77.2	77.8
	6′			63.1	62.6	63.0		62.3	62.5[n]
	1″								105.4 (161 Hz)
	2″								74.7[m]
	3″								76.3
	4″								80.9[l]
	5″								76.6
	6″								61.8[n]
	1‴								104.8 (159 Hz)
	2‴								75.1[m]
	3‴								78.3
	4‴								71.5
	5‴								78.1
	6‴								62.5[n]
Acetyl moiety	CH_3		20.9 × 2		20.4 × 4 20.9				
	C=O		169.9 170.6		169.4, 169.6 169.8, 170.1 170.2				

a) In pyridine-d_5 solution. *b*) In D_2O solution (tetramethylsilane as an external standard). *c*—*n*) Assignments may be interchanged in each column.

[δ 3.75 (6H, s)], an allyl group [δ 3.34 (2H, d, J=7 Hz, –CH_2–), 5.1 (2H, m, =CH_2), 6.0 (1H, m, –CH=)],[9] two aromatic protons [δ 6.60 (2H, s)] and an anomeric proton [δ 5.67 (1H, d, J=7 Hz)]. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded an aglycone (VIa). The MS of VIa showed a molecular ion peak at m/z 194 in agreement with the molecular formula $C_{11}H_{14}O_3$. In the ^{13}C-NMR spectrum of VIa, C-1 (δ 131.2) is at very high field for an aromatic carbon with an attached hydroxyl group. This is considered to result from the steric shift of the two *ortho* methoxyl groups. From these data, VIa was assumed to be 4-allyl-2,6-dimethoxyphenol. This was confirmed by direct comparison with a synthetic sample.[10] Thus, the structure of 4-allyl-2,6-dimethoxyphenol glucoside was decided to be VI.

Experimental

Melting points were determined on a Yanaco MP-500 melting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. IR spectra were run on a JASCO A-202 IR spectrophotometer, while MS were measured on a JEOL JMS-D 100 and FAB-MS on JEOL JMS-DX 300. CD spectrum was recorded on a JASCO J-20A spectropolarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX-90Q NMR spectrometer (89.55 and 22.5 MHz, respectively). Chemical shifts are given on the δ scale with tetramethylsilane as an internal standard unless otherwise stated (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was done on a Hitachi K 53 gas chromatograph. High-performance liquid chromatography (HPLC) was done on a Kyowa Seimitsu model K 880 instrument. Atomic absorption spectrometry was done on a Hitachi 518 digital atomic absorption spectrometer.

Isolation——Air-dried whole herb of *M. trilobum* (5 kg) was extracted twice with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with ether and *n*-butanol, successively. After repeated chromatography of the *n*-butanol-soluble fraction (120 g) on Silica gel 60 with a chloroform–methanol system and on silanized silica gel with a water–acetonitrile system, five sesquiterpenes and a phenyl propanoid were isolated.

Isolipidiol (I)——Recrystallization from acetone gave colorless prisms (0.1 g), mp 167—168.5 °C (lit. mp 167—169 °C),[2] $[\alpha]_D^{25}$ +41.3° (c=0.78, chloroform). *Anal.* Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.51; H, 8.16. IR ν_{max}^{KBr} cm^{-1}: 3420, 3370, 1745, 1640, 1060, 1030, 980, 900. MS *m/z*: 266 (M^+, 3), 264 (3), 248 (9), 238 (7), 233 (7), 220 (12), 193 (100), 168 (86), 165 (38), 139 (35), 119 (39), 107 (49), 95 (63), 93 (50), 91 (56), 81 (66), 79 (64). ^{1}H-NMR and ^{13}C-NMR: Tables I and II. This compound was identified by comparison of the spectral and physical data with literature data.

Macrocliniside G (III)——Recrystallization from water–methanol gave colorless needles (0.1 g), mp 128—129 °C, $[\alpha]_D^{25}$ +39.8° (c=1.86, methanol). *Anal.* Calcd for $C_{21}H_{38}H_8 \cdot H_2O$: C, 58.87; H, 7.53. Found: C, 58.70; H, 7.35. IR ν_{max}^{KBr} cm^{-1}: 3430, 1730, 1640, 1083, 1030. CD (c=0.22, methanol) [θ] (nm): −4112 (257). ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

Macrocliniside H (IV)——Amorphous powder (0.05 g). $[\alpha]_D^{25}$ +20.5° (c=0.95, water). IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1550, 1410, 1070, 1020. FAB-MS *m/z*: 449 $(C_{21}H_{29}NaO_9+H)^+$, 465 $(C_{21}H_{29}KO_9+H)^+$, 471 $(C_{21}H_{29}NaO_9+Na)^+$, 487 $(C_{21}H_{29}KO_9+Na)^+$. Cations were detected by atomic absorption spectrometry (Na 27.4%, K 14.7%, Zn 15.4%, calculated). ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

Macrocliniside I (V)——Amorphous powder (0.05 g), $[\alpha]_D^{25}$ −13.0° (c=1.35, water). *Anal.* Calcd for $C_{33}H_{48}O_{18} \cdot 2H_2O$: C, 51.56; H, 6.82. Found: C, 51.37; H, 6.56. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1630, 1070, 1030. FAB-MS *m/z*: 755 $(M+Na)^+$, 733 $(M+H)^+$. ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

4-Allyl-2,6-dimethoxyphenol Glucoside (VI)——Recrystallized from methanol as colorless needles (0.15 g), mp 159—161 °C, $[\alpha]_D^{25}$ −21.7° (c=1.61, methanol). *Anal.* Calcd for $C_{17}H_{24}O_8$: C, 57.29; H, 6.79. Found: C, 57.22; H, 6.73. IR ν_{max}^{KBr} cm^{-1}: 3400, 1590, 1508, 1465, 1425, 1240, 1130, 1070. ^{1}H-NMR (pyridine-d_5) δ: 3.34 (2H, d, J=7 Hz, H_2-α), 3.75 (6H, s, OMe×2), 5.1 (2H, m, H_2-γ), 5.67 (1H, d, J=7 Hz, H-1′), 6.0 (1H, m, H-β), 6.60 (2H, s, H-3; H-5). ^{13}C-NMR (pyridine-d_5) δ: 40.4 (C-α), 56.7 (OMe×2), 62.8 (C-6′), 71.7 (C-4′), 76.0 (C-2′), 78.2 (C-5′), 78.3 (C-3′), 105.3 (C-1′), 107.5 (C-3; C-5), 115.9 (C-γ), 134.9 (C-4), 136.5 (C-1), 137.8 (C-β), 153.8 (C-2; C-6).

Acetylation of Isolipidiol (I) and Crude Macrocliniside F (II)——I (35 mg) and crude II (180 mg) were acetylated in the usual manner with acetic anhydride and pyridine to give the acetates Ia (25 mg) and IIa (50 mg), respectively. Ia: Colorless oil. ^{1}H-NMR: Table I. IIa: Colorless needles, mp 197—198 °C (methanol), $[\alpha]_D^{25}$ +16.7° (c=1.23, chloroform). *Anal.* Calcd for $C_{31}H_{42}O_{14}$: C, 58.30; H, 6.63. Found: C, 58.11; H, 6.59. IR ν_{max}^{KBr} cm^{-1}: 1780, 1760, 1750, 1640, 1370, 1230, 1210, 1165, 1085, 1065, 1025. ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

Saponification of Macrocliniside F Peracetate (IIa)——IIa (12 mg) was dissolved in dioxane (1 ml) and 10% aq. sodium hydroxide (1 ml), and the mixture was stirred for 2 h at room temperature under a nitrogen atmosphere. The solution was acidified with dilute hydrogen chloride and passed through an Amberlite XAD-2 column. The methanol eluate gave II (8 mg) as an amorphous powder. ^{1}H-NMR, ^{13}C-NMR: Tables I and II. IR ν_{max}^{KBr} cm^{-1}: 3430, 1760, 1640, 1070, 1030.

Enzymatic Hydrolysis of Crude Macrocliniside F (II)——Crude II (60 mg) was dissolved in water (2 ml), crude hesperidinase (40 mg) was added, and the reaction mixture was stirred for 3 h at 37 °C. The solution was passed through an Amberlite XAD-2 column and the absorbed material was eluted with methanol. The methanol eluate was purified by silica gel column chromatography using benzene–acetone (85 : 15) as an eluent to give I (13 mg), mp 162—165 °C. This was identified as isolipidiol by mixed mp and comparison of the spectral data with those of an authentic specimen.

Enzymatic Hydrolysis of Macrocliniside G (III)——III (40 mg) was hydrolyzed in the same way as II, and an aglycone (IIIa) (16 mg) was obtained after silica gel column chromatography of the methanol eluate using benzene–acetone (9 : 1) as an eluent. IIIa: Colorless powder. IR ν_{max}^{KBr} cm^{-1}: 1760, 1205, 1140, 995. MS *m/z*: 248 (M^+, 8), 230 (M^+-18, 85), 215 ($M^+-18-15$, 15), 107 (100), 93 (94). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, s, H_3-14), 1.88 (3H, d, J=1.5 Hz, H_3-15), 4.13 (1H, t, J=10 Hz, H-6), 5.43 (1H, d, J=3.3 Hz, H-13a), 5.46 (1H, br s, H-3), 6.16 (1H, d, J=

3.6 Hz, H-13b). This was assigned as 8-deoxycumambrin B[6] by comparison of spectral data with literature values.

Macrocliniside H (IV) from Macrocliniside C (VII)——VII (100 mg) was dissolved in 2% sodium hydroxide aq. solution and the solution was stirred for 1 h at room temperature. The reaction mixture was diluted with water and passed through an Amberlite XAD-2 column. The absorbed material was eluted with methanol to give IV as the sodium salt. Colorless powder. ^{1}H-NMR (D_2O) δ: 4.66 (1H, d, J=7 Hz, H-1′), 4.99; 5.07 (each 1H, s, H-14), 5.18 (1H, br s, H-15b), 5.32 (2H, s, H-13a; H-15a), 5.78 (1H, s, H-13b). The ^{1}H-NMR spectrum was superimposable on that of IV.

Partial Hydrolysis of Macrocliniside I (V) with Maltase——V (*ca.* 1 mg) was dissolved in water (0.2 ml), maltase (*ca.* 2 mg) was added, and the reaction mixture was stirred for 20 min at 30 °C. The reaction mixture was passed through an Amberlite XAD-2 column, and the absorbed material was eluted with methanol. The hydrolyzed products were detected by HPLC. Conditions: column, TSK GEL LS-410 AK, 4 mm × 30 cm; solvent, acetonitrile–water (30 : 70); flow rate, 1.3 ml/min; detector, UV 220 nm; t_R 4.4 min (V), 4.8 min (VIII), 5.6 min (IX), 14.3 min (X).

Acid Hydrolysis of Macrocliniside F Pentaacetate (IIa), Macroclinisides G (III), H (IV) and I (V), and 4-Allyl-2,6-dimethoxyphenol Glucoside (VI)——A solution of a glycoside (*ca.* 0.5 mg) in 10% sulfuric acid (0.1 ml) was heated in a boiling water bath for 20 min. The solution was passed through an Amberlite IRA-45 column and concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 2 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the residue was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From each glycoside, glucitol acetate was detected by GC. Conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 230 °C; carrier gas, N_2; t_R 4.25 min.

Enzymatic Hydrolysis of 4-Allyl-2,6-dimethoxyphenol Glucoside (VI)——VI (15 mg) was hydrolyzed in the same way as II and an aglycone (VIa) (4 mg) was obtained after silica gel column chromatography of the methanol eluate using benzene as an eluent. VIa: Colorless oil. IR ν_{max}^{KBr} cm^{-1}: 3550, 1620, 1518, 1462, 1330, 1208, 1115. MS *m/z*: 194 (M^+, 100), 179 (19), 167 (15), 163 (11), 161 (7), 151 (17), 147 (17), 133 (15), 131 (22), 119 (31). ^{1}H-NMR ($CDCl_3$) δ: 3.32 (2H, d, J=7 Hz, H_2-α), 3.88 (6H, s, OCH_3 × 2), 4.8—5.2 (2H, m, H_2-γ), 5.7—6.1 (1H, m, H-β), 6.41 (2H, s, H-2, H-6). ^{13}C-NMR ($CDCl_3$) δ: 40.4 (C-α), 56.4 (OCH_3 × 2), 105.6 (C-2, C-6), 115.7 (C-γ), 131.2 (C-1), 133.4 (C-4), 137.6 (C-β), 147.2 (C-3, C-5).

Synthesis of 4-Allyl-2,6-dimethoxyphenol (VIa)[10]——2,6-Dimethoxyphenol (1.5 g) was dissolved in anhydrous acetone (5 ml) and allyl bromide (1.2 g) was added to the solution. To this solution, anhydrous potassium carbonate (1.8 g) was added and the resulting mixture was refluxed for 10 h. Acetone was distilled off, then the residue was treated with water (30 ml) and extracted with ether. The ether layer was washed twice with dilute aqueous sodium hydroxide. After additional washings with water, the ether solution was dried over anhydrous sodium sulfate and then distilled *in vacuo* at 105 °C/1 mmHg to give 1-allyloxy-2,6-dimethoxybenzene (1.8 g). 1-Allyloxy-2,6-dimethoxybenzene (1.8 g) was refluxed at 240 °C/100—145 mmHg for 1 h, and then distilled at 123—125 °C/2 mmHg to give 4-allyl-2,6-dimethoxyphenol (1 g) as a colorless oil. This was identical with VIa in terms of ^{1}H-NMR, ^{13}C-NMR and HPLC properties. HPLC conditions: Column, YMC-Pack AM-312 ODS, 6 mm × 15 cm; solvent, acetonitrile–water (6 : 4); flow rate, 1.0 ml/min; detector, UV 220 nm; t_R 7.2 min.

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