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Studies on Sesquiterpenes from *Macroclinidium trilobum* MAKINO. I

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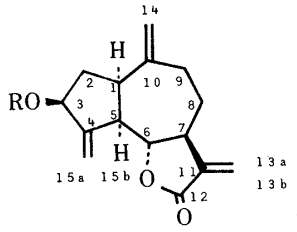
Five new sesquiterpene glycosides, macroclinisides A (II), B (III), C (IV), D (V), E (VI), have been isolated from *Macroclinidium trilobum* MAKINO together with glucozaluzanin C (I). The structures were determined on the basis of chemical and spectral data. Macrocliniside C has antitumor activity in mice.

Keywords——*Macroclinidium trilobum*; Compositae; sesquiterpene glycoside; antitumor activity; macrocliniside A; macrocliniside B; macrocliniside C; macrocliniside D; macroliniside E; glucozaluzanin C

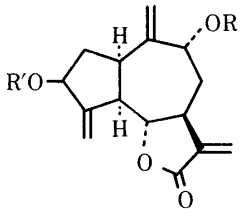
In connection with a study on the sesquiterpene glycosides of some plants in Compositae, we have also investigated *Macroclinidium trilobum* MAKINO (Syn. *Pertya triloba* MAKINO) and isolated five new guaianolide-type sesquiterpene glycosides, macroclinisides A—E, and glucozaluzanin C (a known compound). The structures of the five new glycosides were determined on the basis of some chemical transformations and spectroscopic studies using mainly carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra.

Glucozaluzanin C (I): $C_{21}H_{28}O_8 \cdot H_2O$, mp 103—105 °C. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited doublets at δ 5.38 (1H, J=3.3 Hz) and 6.22 (1H, J=3.3 Hz), which are characteristic of exocyclic α-methylene-γ-lactone. Acetylation of I afforded a tetraacetate (Ia), $C_{29}H_{36}O_{12}$, mp 160—162 °C. From these results and the spectral data, I was assumed to be glucozaluzanin C, previously isolated from *Ainsliae acerifolia* SCH. BIP.[1] The identity of I was established by direct comparison [thin layer chromatography (TLC), infrared (IR), ^{1}H-NMR, mixed mp] with an authentic sample.

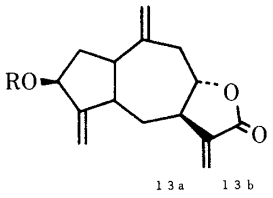
Macrocliniside A (II): $C_{21}H_{28}O_9 \cdot 1/2H_2O$, $[\alpha]_D$ −8.8°. The field desorption mass spectrum (FD-MS) exhibited an ion peak at m/z 447 $(M+Na)^+$. The IR spectrum suggested the presence of hydroxyl groups (3400 cm^{-1}), an unsaturated γ-lactone (1750 cm^{-1}) and double bonds (1660, 1635 cm^{-1}). The ^{1}H-NMR spectrum exhibited signals due to three exomethylene groups at δ 5.18 (2H, s), 5.48 (1H, d, J=3.1 Hz); 6.26 (1H, d, J=3.3 Hz) and 5.56 (1H, br s); 5.96 (1H, br s), suggesting that II had a guaianolide-type skeleton, like glucozaluzanin C (I). The ^{13}C-NMR spectrum also indicated the presence of three exomethylene groups: δ 111.1, 112.2, 118.9 (each triplet); 141.2, 150.9, 153.3 (each singlet). Acetylation of II afforded a pentaacetate (IIa), $C_{31}H_{38}O_{14}$, mp 215—216.5 °C. Acid hydrolysis of II afforded glucose as the sugar moiety. In the ^{13}C-NMR spectrum of II, various signals showed shifts as compared with those of I: C-1 and C-7 (each γ-position) at δ 41.6 (Δ −3.3 ppm) and 37.0 (Δ −8.3 ppm), respectively, C-8 and C-10 (each β-position) at δ 39.8 (Δ +9.1 ppm) and 153.3 (Δ +4.3 ppm), respectively, and C-9 (α-position) at δ 72.3 (Δ +38.1 ppm). Thus, Compound II was assumed to be a glucozaluzanin C analog having a hydroxyl group at C-9. The stereochemistry at C-9 was determined from the fact that II gave ixerin F (IIc) upon sodium borohydride reduction,[2] and the stereochemistry of the anomeric center was deduced from the $J_{C_1-H_1}$ coupling constant (157 Hz).[3] These results led us to conclude the structure of macrocliniside A to be II.



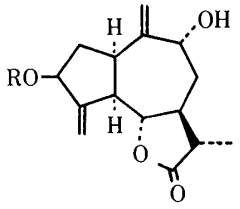
I: R=β-glc
Ia: R=(β-glc)Ac_4
Ib: R=H
III: R=β-glc$^{3\,1}$—β-glc



II: R=H, R′=β-glc
IIa: R=$COCH_3$, R′=(β-glc)Ac_4
IIb: R=H, R′=H



IV: R=β-glc
IVa: R=(β-glc)Ac_4
IVb: R=H
V: R=β-glc$^{3\,1}$—β-glc
Va: R=(β-glc$^{3\,1}$—β-glc)Ac_7
VI: R=β-glc$^{3\,1}$—β-glc



IIc: R=β-glc

Chart 1

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

Proton No.	I	II	III
13a	5.38 (1H, d, J=3.3 Hz)	5.48 (1H, d, J=3.1 Hz)	5.41 (1H, d, J=3.1 Hz)
13b	6.22 (1H, d, J=3.3 Hz)	6.26 (1H, d, J=3.3 Hz)	6.23 (1H, d, J=3.2 Hz)
14	4.85 (1H, s) 5.02 (1H, s)	5.18 (2H, s)	4.85 (1H, s) 5.00 (1H, s)
15a	5.85 (1H, br s)	5.96 (1H, br s)	5.83 (1H, br s)
15b	5.53 (1H, br s)	5.56 (1H, br s)	5.53 (1H, br s)

Proton No.	IV	V	VI
13a	5.44 (1H, d, J=3.1 Hz)	5.44 (1H, d, J=3.1 Hz)	5.47 (1H, d, J=3.1 Hz)
13b	6.20 (1H, d, J=3.4 Hz)	6.18 (1H, d, J=3.2 Hz)	6.23 (1H, d, J=3.2 Hz)
14	4.92 (1H, s) 5.04[a]	4.91 (1H, s) 5.02[a]	4.94 (1H, s) 5.06[a]
15a	5.79 (1H, br s)	5.76 (1H, br s)	5.82 (1H, br s)
15b	5.04[a]	5.02[a]	5.06[a]

Run at 89.55 MHz in pyridine-d_5 solution.
a) Overlapped each other (in each column).

Macrocliniside B (III): $C_{27}H_{38}O_{13}\cdot H_2O$, $[\alpha]_D$ −18.3°. The FD-MS exhibited an ion peak at *m/z* 593 $(M+Na)^+$. The IR spectrum suggested the presence of hydroxyl groups (3400 cm^{-1}), an unsaturated γ-lactone (1760 cm^{-1}) and double bonds (1660, 1635 cm^{-1}). The ^{1}H-NMR spectrum was similar to that of I, exhibiting three exomethylene groups at δ 4.85 (1H, s); 5.00 (1H, s), 5.41 (1H, d, J=3.1 Hz); 6.23 (1H, d, J=3.2 Hz) and 5.53 (1H, br s); 5.83 (1H, br s). Enzymatic hydrolysis of III afforded zaluzanin C (Ib) as aglycone, and acid hydrolysis afforded glucose as the sugar moiety. The ^{13}C-NMR spectrum of III showed almost the same signals in the aglycone region as that of I, but in the sugar region six more signals were observed. Therefore, III was assumed to be a diglucoside of zaluzanin C (Ib). The C-3′ of glucose showed a downfield shift in the ^{13}C-NMR, at δ 88.7 (Δ +10.1 ppm), and the C-2′ and C-4′ showed highfield shifts at δ 73.9 (Δ −1.5 ppm) and 69.9 (Δ −2.1 ppm), respectively. Thus, a glucose was attached to C-3′ of another glucose, so that the structure of

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

Carbon No.	I	II	III	IV	V	VI
Aglycone moiety						
1	44.9[a]	41.6	44.8[b]	45.1[c]	45.2[d]	45.2[e]
2	38.2	37.5	38.0	37.5	37.4	37.6
3	80.6	80.8	80.5	80.3	80.4	80.6
4	150.6	150.9	150.2	153.4	154.2	154.4
5	50.4	49.5	50.3	47.6	47.7	47.7
6	83.7	84.4	83.5	28.6	28.6	28.7
7	45.3[a]	37.0	45.3[b]	44.8[c]	44.8[d]	44.9[e]
8	30.7	39.8	30.7	85.0	84.9	85.0
9	34.2	72.3	34.1	42.3	42.4	42.4
10	149.0	153.3	148.8	144.4	144.4	144.5
11	141.1	141.2	140.9	140.7	140.8	140.8
12	170.0	170.1	169.8	170.0	170.0	170.1
13	119.4	118.9	119.4	118.1	118.0	118.0
14	114.0	111.1	114.0	116.4	116.4	116.5
15	112.5	112.2	112.5	107.7	107.7	107.8
Sugar moiety						
1′	104.1	104.4 (157 Hz)	103.5 (157 Hz)	104.8 (156 Hz)	104.4 (156 Hz)	104.7 (155 Hz)[f]
2′	75.4	75.3	73.9	75.1	73.9	74.8
3′	78.6	78.6	88.7	78.5	88.9	76.4
4′	72.0	71.9	69.9	71.7	69.8	81.4
5′	78.3	78.2	77.7	78.1	77.9	76.8
6′	63.1	63.0	62.5	62.9	62.5	62.5
1″			105.7 (159 Hz)		105.9 (160 Hz)	104.9 (158 Hz)[f]
2″			75.4		75.4	74.8
3″			78.4		78.5	78.3[g]
4″			71.6		71.6	71.6
5″			78.0		78.1	78.2[g]
6″			62.5		62.5	62.5

Run at 22.5 MHz in pyridine-d_5 solution.
a—g) Assignments may be interchanged in each column.

macrocliniside B is III.[4] The stereochemistry of the two anomeric centers was deduced to be β from the $J_{C_1-H_1}$ coupling constants (157, 159 Hz).[3]

Macrocliniside C (IV): $C_{21}H_{28}O_8 \cdot 1/2H_2O$, $[\alpha]_D$ +13.6°. The FD-MS exhibited an ion peak at *m/z* 409 $(M+1)^+$. The IR spectrum suggested the presence of hydroxyl groups (3420 cm^{-1}), an unsaturated γ-lactone (1762 cm^{-1}) and double bonds (1660, 1635 cm^{-1}). The ^{1}H-NMR spectrum showed doublets at δ 5.44 (1H, d, J=3.1 Hz) and 6.20 (1H, d, J=3.4 Hz), which were characteristic of exocyclic α-methylene-γ-lactone. The allylic coupling constants were both larger than 3.0 Hz, so the γ-lactone ring was suggested to be *trans*-fused.[5] Furthermore, two exomethylene groups were observed at δ 4.92 (1H, s); 5.04 (overlapped) and 5.04 (overlapped); 5.79 (1H, br s). Acetylation of IV afforded a tetraacetate (IVa) $C_{29}H_{36}O_{12}$, mp 162—163 °C. Acid hydrolysis of IV gave glucose as the sugar moiety and enzymatic hydrolysis gave an unstable aglycone (IVb). The MS of IVb showed a molecular ion peak at *m/z* 246 in agreement with the molecular formula $C_{15}H_{18}O_3$. The ^{1}H-NMR spectrum established IVb as a sesquiterpene lactone[6] exhibiting a carbinol proton signal at δ 4.56 (1H, tt, J=2.5, 9.5 Hz) which was long-range-coupled with two olefinic protons at δ 4.98 and 5.20 (C-15 methylene protons), and a lactonized carbinol proton signal at δ 3.75 (1H, ddd, J=5, 12, 13 Hz). The circular dichroism (CD) spectrum of IV showed a positive Cotton effect $[\theta]_{268}$ +169, suggesting that the γ-lactone ring fusion is 7β,8α-*trans*.[7] The ^{13}C-NMR spectrum of IV

supported the presence of a 7,8-lactone ring; C-9 (β-position) was downfield shifted at δ 42.3 (Δ +8.1 ppm) and C-10 (γ-position) was highfield shifted at δ 144.4 (Δ −4.6 ppm) as compared with the signals of a 6,7-lactonized guaianolide, glucozaluzanin C (I). Thus, IVb was assumed to be a 7,8-*trans*-lactonized guaianolide having a hydroxyl group at C-3. The stereochemistry at C-3 was established to be *S* by Horeau's method.[8] If the assumption is made that the absolute configuration of the C-7 side chain is as shown (as in all other known sesquiterpene lactones of authenticated stereochemistry), the structure of macrocliniside C can be concluded to be IV.

Macrocliniside D (V): $C_{27}H_{38}O_{13}$, mp 238—241 °C, $[\alpha]_D$ −4.9°. The FD-MS exhibited an ion peak at *m/z* 593 $(M+Na)^+$. The ^{1}H-NMR spectrum was very similar to that of IV and exhibited two olefinic proton signals due to exocyclic α-methylene-γ-lactone at δ 5.44 (1H, d, J=3.1 Hz) and 6.18 (1H, d, J=3.2 Hz) and two exomethylene signals at δ 4.91 (1H, s); 5.02 (overlapped) and 5.02 (overlapped); 5.76 (1H, br s). Acetylation of V gave a heptaacetate (Va), $C_{41}H_{52}O_{20}$, mp 181—185 °C. Enzymatic hydrolysis of V afforded IVb as an aglycone and acid hydrolysis afforded glucose as the sugar moiety. The ^{13}C-NMR spectrum of V showed almost the same signals in the aglycone region as that of IV, but in the sugar region six more signals were observed to be nearly superimposable on those of III. These results led us to conclude the structure of macrocliniside D to be V.

Macrocliniside E (VI): $C_{27}H_{38}O_{13}$, $[\alpha]_D$ +25.7°. The FD-MS exhibited an ion peak at *m/z* 571 $(M+1)^+$. The IR and ^{1}H-NMR spectra were similar to those of V and the ^{13}C-NMR spectrum suggested that VI was also a diglucoside of IVb. In the sugar region, C-4′ of glucose was found to be shifted downfield to δ 81.4 (Δ +9.7 ppm) and C-3′ and C-5′ to be shifted highfield to δ 76.4 (Δ −2.1 ppm) and 76.8 (Δ −1.3 ppm), respectively, suggesting that a glucose is attached to C-4′ of another glucose. The stereochemistry of the two anomeric centers was shown to be both β by the $J_{C_1-H_1}$ coupling constants (155, 158 Hz).[3] From these results, the structure of macrocliniside E was concluded to be VI.

Macroclinisides B, D and E are the first reported examples of diglycosides of a guaianolide-type sesquiterpene. Macrocliniside C (IV) was active in the sarcoma 180 Std: ddY mice survival system at 150 mg/kg/d (T/C > 300%).

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. IR spectra were run on a JASCO A-202 IR spectrophotometer, while MS were measured on a JEOL JMS-D 100 and FD-MS on a JEOL JMS-D 300 mass spectrometer. CD spectra were recorded on a JASCO J-40 spectropolarometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX-90Q NMR spectrometer (89.55 and 22.5 Hz, respectively). Chemical shifts are given on a δ scale with tetramethylsilane as an internal standard (s, singlet; d, double; 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.

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) using a chloroform–methanol system with Silica gel 60 and a water–acetonitrile system with silanized silica gel, six sesquiterpene glycosides were isolated.

Glucozaluzanin C (I)——Recrystallization from water gave colorless needles (0.3 g), mp 103—105 °C. *Anal.* Calcd for $C_{21}H_{28}O_8 \cdot H_2O$: C, 59.14; H, 7.09. Found: C, 59.28; H, 6.86. IR ν_{max}^{KBr} cm^{-1}: 3400, 1755, 1660, 1635. ^{1}H-NMR and ^{13}C-NMR δ: Table I, II. This was identified by direct comparison (mixed mp, IR, ^{1}H-NMR, TLC) with an authentic sample.

Macrocliniside A (II)——Amorphous powder (0.1 g). $[\alpha]_D^{24}$ −8.8° (c=1.14, methanol). *Anal.* Calcd for $C_{21}H_{28}O_9 \cdot 1/2H_2O$: C, 58.19; H, 6.74. Found: C, 57.92; H, 6.70. IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1660, 1635. FD-MS *m/z*: 447 $(M+Na)^+$. ^{1}H-NMR and ^{13}C-NMR δ: Tables I, II.

Macrocliniside B (III)——Amorphous powder (0.2 g). $[\alpha]_D^{25}$ −18.3° (c=1.15, water). FD-MS *m/z*: 593

$(M+Na)^+$. *Anal.* Calcd for $C_{27}H_{38}O_{13}\cdot H_2O$: C, 55.09; H, 6.85. Found: C, 55.14; H, 6.69. IR $\nu_{max}^{KBr}cm^{-1}$: 3400, 1760, 1660, 1635. ^{1}H-NMR and ^{13}C-NMR δ: Tables I, II.

Macrocliniside C (IV)——Amorphous powder (10 g). $[\alpha]_D^{25}$ +13.6° ($c=1.10$, water). *Anal.* Calcd for $C_{21}H_{28}O_8\cdot 1/2H_2O$: C, 60.42; H, 7.00. Found: C, 60.31; H, 7.00. IR $\nu_{max}^{KBr}cm^{-1}$: 3420, 1762, 1660, 1635. FD-MS *m/z*: 409 $(M+1)^+$. CD ($c=0.48$, methanol) [θ] (nm): +169 (268). ^{1}H-NMR and ^{13}C-NMR δ: Tables I, II.

Macrocliniside D (V)——Recrystallization from methanol gave colorless needles (1 g), mp 238—241 °C. $[\alpha]_D^{25}$ −4.9° ($c=0.41$, water). *Anal.* Calcd for $C_{27}H_{38}O_{13}$: C, 56.83; H, 6.71. Found: C, 56.68; H, 6.68. IR $\nu_{max}^{KBr}cm^{-1}$: 3350, 1758, 1660, 1635. FD-MS *m/z*: 593 $(M+Na)^+$. CD ($c=0.33$, water) [θ] (nm): +170 (266). ^{1}H-NMR and ^{13}C-NMR δ: Tables I, II.

Macrocliniside E (VI)——Amorphous powder (0.1 g). $[\alpha]_D^{25}$ +25.7° ($c=0.53$, water). *Anal.* Calcd for $C_{27}H_{38}O_{13}\cdot H_2O$: C, 55.09; H, 6.85. Found: C, 54.86; H, 6.67. IR $\nu_{max}^{KBr}cm^{-1}$: 3420, 1760, 1660, 1630. FD-MS *m/z*: 571 $(M+1)^+$. CD ($c=0.20$, water) [θ] (nm): +88 (263). ^{1}H-NMR and ^{13}C-NMR δ: Tables I, II.

Acetylation of Glucozaluzanin C (I), and Macroclinisides A (II), C (IV) and D (V)——I (30 mg), II (25 mg), IV (15 mg) and V (66 mg) were acetylated in the usual manner using acetic anhydride and pyridine to give the acetate Ia (20 mg), IIa (15 mg), IVa (10 mg) and Va (40 mg), respectively. Ia: Colorless needles, mp 160—162 °C (methanol). ^{1}H-NMR ($CDCl_3$) δ: 1.97, 2.00, 2.04, 2.10 (3H each, s, OAc), 4.54 (1H, br t, $J=6$ Hz, H-3), 5.47 (1H, d, $J=3.3$ Hz, H-13a), 6.17 (1H, d, $J=3.3$ Hz, H-13b). This was identified by direct comparison (mixed mp, ^{1}H-NMR) with an authentic sample.[1] IIa: Colorless needles, mp 215—216.5 °C (chloroform–methanol). ^{1}H-NMR ($CDCl_3$) δ: 1.96, 1.99, 2.03, 2.09, 2.10 (3H each, s, OAc), 6.23 (1H, d, $J=3.5$ Hz, H-13b). IVa: Colorless needles, mp 162—163 °C (methanol). *Anal.* Calcd for $C_{29}H_{36}O_{12}$: C, 60.41; H, 6.29. Found: C, 60.41; H, 6.18. ^{1}H-NMR ($CDCl_3$) δ: 2.02, 2.03, 2.04, 2.07 (3H each, s, OAc), 3.75 (1H, m, H-8), 4.46 (1H, br t, $J=7$ Hz, H-3), 5.47 (1H, d, $J=3.2$ Hz, H-13a), 6.17 (1H, d, $J=3.5$ Hz, H-13b). Va: Colorless needles, mp 181—185 °C (ethyl acetate–hexane). *Anal.* Calcd for $C_{41}H_{52}O_{20}$: C, 56.94; H, 6.06. Found: C, 56.66; H, 6.00. ^{1}H-NMR ($CDCl_3$) δ: 1.99, 2.04, 2.12 (3H each, s, OAc), 2.02, 2.08 (6H each, s, OAc × 2), 5.17 (1H, d, $J=3.1$ Hz, H-13a), 6.17 (1H, d, $J=3.3$ Hz, H-13b).

Acid Hydrolysis of Macroclinisides A (II), B (III), C (IV), D (V) and E (VI)——A solution of a glycoside (*ca.* 1 mg) in 10% sulfuric acid (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 eluate was concentrated to dryness. Boric acid was removed by codistillation 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 3.7 min.

Enzymatic Hydrolysis of Macroclinisides A (II), B (III), C (IV), D (V) and E (VI)——A glycoside (*ca.* 1 mg) was dissolved in water (0.2 ml), crude hesperidinase (*ca.* 1 mg) was added to the solution, and the reaction mixture was stirred for 1 h at 37 °C. The aglycone was extracted with ethyl acetate and detected by HPLC. Conditions: column, Lichrosorb RP-8, 4 mm × 25 cm; solvent, methanol–water (6 : 4); flow, 1.2 ml/min; detector, UV 220 nm; t_R 3.2 min (IIb from II), 4.7 min (IVb from IV, V and VI), 5.3 min (Ib from III).

Sodium Borohydride Reduction of Macrocliniside A (II)——A solution of macrocliniside A (II) (11 mg) in methanol (1 ml) was treated at 0 °C with sodium borohydride (10 mg) and the mixture was stirred for 10 min. The reaction mixture was acidified with acetic acid, diluted with water and then passed through an Amberlite XAD-2 column. The column was washed with water and the reaction product was eluted with methanol. Purification on a silica gel column using ethyl acetate–methanol–water (95 : 5 : 1.5) as the eluent, provided an amorphous powder. IR $\nu_{max}^{KBr}cm^{-1}$: 3400, 1755, 1655, 1635. ^{1}H-NMR (pyridine-d_5) δ 1.19 (3H, d, $J=7$ Hz, CH_3), 2.91 (1H, br t, $J=9$ Hz, H-5), 5.08 (2H, s, CH_2 of C-14), 5.47 (1H, br s, H-15a), 5.90 (1H, br s, H-15b). ^{13}C-NMR (pyridine-d_5) δ: 13.3 (C-13), 36.7 (C-7), 37.5 (C-2), 40.9 (C-8), 42.0 (C-1), 45.5 (C-11), 49.5 (C-5), 63.1 (C-6′), 72.0 (C-4′), 73.1 (C-9), 75.4 (C-2′), 78.2 (C-5′), 78.6 (C-3′), 80.8 (C-3), 84.0 (C-6), 104.5 (C-1′), 111.1 (C-14), 111.6 (C-15), 151.4 (C-4), 153.7 (C-10), 178.5 (C-12). This was identified as ixerin F by direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

Enzymatic Hydrolysis of Macrocliniside C (IV)——A solution of macrocliniside C (IV) (240 mg) in water (5 ml) was treated with crude hesperidinase (170 mg) at 37 °C overnight. The reaction mixture was extracted with ethyl acetate. The ethyl acetate extract was concentrated to give a residue, which yielded an unstable aglycone (IVb) (90 mg), mp 128—131 °C from methanol. MS *m/z*: 246 (M^+, 5), 228 (M^+-H_2O, 23), 202 (27), 188 (23), 109 (5), 105 (52), 91 (100). ^{1}H-NMR ($CDCl_3$) δ: 3.05 (1H, dd, $J=5$, 13 Hz, H-9), 3.75 (1H, ddd, $J=5$, 12, 13 Hz, H-8), 4.56 (1H, tt, $J=2.5$, 9.5 Hz, H-3), 4.98 (1H, t, $J=2.5$ Hz, H-15b), 5.07 (2H, s, CH_2 of C-14), 5.20 (1H, t, $J=2.5$ Hz, H-15a), 5.49 (1H, d, $J=3.7$ Hz, H-13a), 6.17 (1H, d, $J=4.1$ Hz, H-13b). ^{13}C-NMR (pyridine-d_5) δ: 28.4 (C-6), 30.9 (C-2), 42.6 (C-9), 44.4 (C-7), 45.2 (C-5), 48.3 (C-1), 72.6 (C-3), 85.4 (C-8), 105.3 (C-15), 116.3 (C-14), 118.0 (C-13), 140.8 (C-11), 144.8 (C-10), 158.4 (C-4), 170.2 (C-12).

Determination of the Configuration at C-3 of IVb by Horeau's Method[8]——Racemic α-phenylbutylic anhydride (210 mg, 0.677 mmol) and IVb (85.6 mg, 0.348 mmol) were dissolved in pyridine (1 ml). The solution was allowed to stand at room temperature overnight, then water was added to hydrolyze the excess anhydride. The aqueous solution was extracted 4 times with ethyl acetate. The ethyl acetate layer was extracted with 5% sodium bicarbonate solution,

washed with water and finally acidified with 3 N hydrogen chloride. The ethyl acetate solution was washed with water and dried over sodium sulfate and then concentrated *in vacuo* to a constant-weight residue, 122.3 mg. The ^{1}H-NMR spectrum of the residue indicated that IVb was totally esterified. The sodium bicarbonate extract obtained above was extracted with chloroform before being acidified with hydrogen chloride. A chloroform extract of the acidic solution, worked up in the usual manner, yielded a residue which was dried to constant-weight, yielding 123.0 mg of pure α-phenylbutyric acid. The purity was established by ^{1}H-NMR spectroscopy. The specific rotation of the acid was determined: $[\alpha]_D^{24}$ −9.6°. The optical yield was 29%. From this result, the configuration at C-3 of IVb was deduced to be *S*.

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