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Studies on chemical modification of monensin VIII. Synthesis of 7-*O*-substituted-25-carboxymonensins and their Ca^{2+} ion transport activity

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Abstract—Monensin (**1**), an ionophore antibiotic agent, was converted to 25-carboxymonensin (**2**) and 7-*O*-substituted-25-carboxyl derivatives (**3a**–**d**), which have two carboxyl groups at both ends of the molecule. Ca^{2+} ion transport activity of the dicarboxylic monensins was evaluated by the $CHCl_3$ liquid membrane method, and 25-carboxymonensins (**2** and **3a**–**d**) were shown to transport the Ca^{2+} ion through the $CHCl_3$ liquid membrane.

Monensin (**1**, Fig. 1), isolated from *Streptomyses cinnamonensis*, is a representative of polyether antibiotics such as X-206, nigericin and septamycin, and is well known as a Na^+ ionophore.[1] Monensin has attracted much attention because of its unique structure with 17 asymmetric centers and contiguous tetrahydrofuran and tetrahydropyran rings. X-Ray crystal structure analysis of the free acid and many cation complexes clarified that monensin (**1**) formed a lipophilic exterior and a hydrophilic cavity lined with oxygen atoms which serve as ligands for the encapsulated Na^+ ion.[2] The molecule as a whole is, therefore, lipophilic enough to transport the Na^+ ion across lipophilic biological membranes depending on the density gradient.[3] This ability results in a variety of biological activities such as antibiotic[4] and anticoccidial[5] activities. In spite of such chemically and biologically unique features, monensin is now used only for veterinary medicine.

Calcium ionophores[6] are widely used as important reagents in the field of biological research in order to clarify the mechanisms of various phenomena including signal transduction across membranes. There are only few natural ionophores such as lasalocid A and A-23187, which transport one Ca^{2+} ion by two molecules. Some synthetic 'non-cyclic crown ethers' having carboxyl groups at both terminals were reported to form a 1:1 complex with the Ca^{2+} ion.[7] As a diameter of the Na^+ ion (0.97 Å) is close to that of the Ca^{2+} ion (0.99 Å), we expected the transformation of monensin (**1**) to the divalent molecule led to a Ca^{2+} ionophore, which transports one Ca^{2+} ion by one molecule utilizing the structural nature of monensin.

We reported that 7-*O*-benzylmonensins[8] were more lipophilic and much more potent Na^+ ionophores than monensin (**1**), indicating that lipophilicity of the molecule is an important factor for ion transport through the membrane. Furthermore, in a previous communication we described the conversion of monensin (**1**) to 25-carboxymonensin (**2**), and clarified that the Ca^{2+} ion transport activity of **2** was about threefold higher than that of lasalocid A.[9] This current work in our laboratory led us to plan the preparation of more lipophilic 25-carboxymonensin

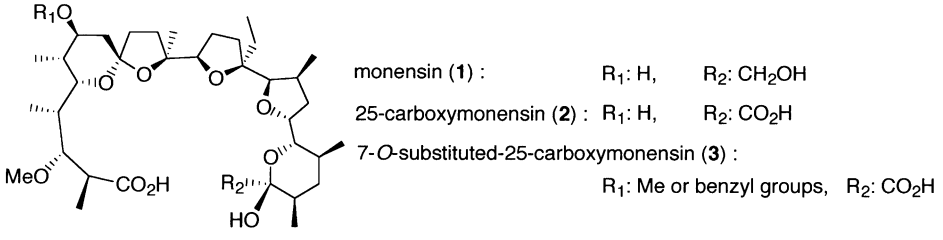

Figure 1. Chemical structure of monensin and its derivatives.

Keywords: monensin; calcium ionophore; ion transport activity; 25-carboxymonensin.
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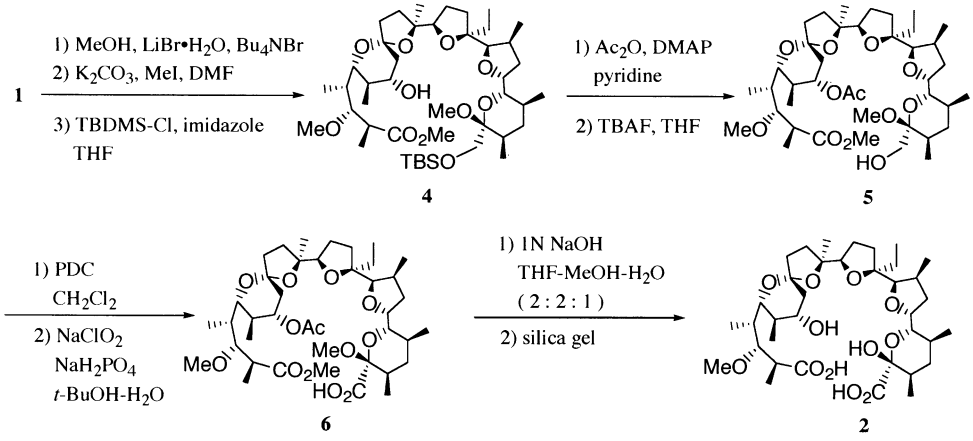
derivatives, 7-*O*-substituted-25-carboxymonensins (**3a**–**d**), to obtain potent calcium ionophores. In this paper, we describe the preparation of 7-*O*-substituted-25-carboxymonensins (**3a**–**d**) together with 25-carboxymonensin (**2**) in detail and the evaluation of their calcium ion transport activity.

1. Result and discussion

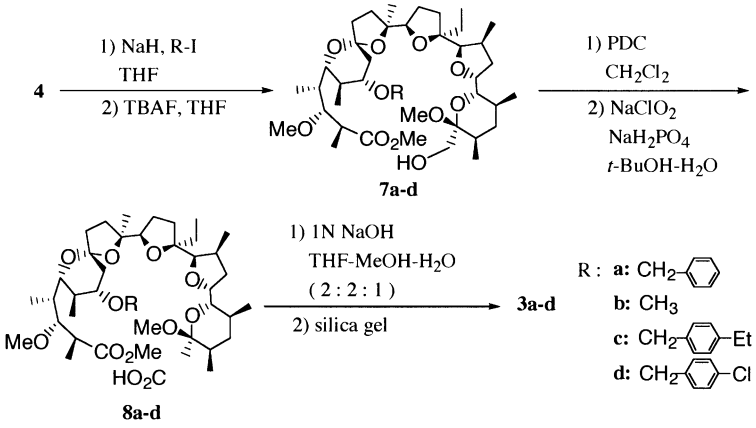
1.1. Chemistry

The synthetic course to **2** is summarized in Scheme 1. The hydroxy group at 25-C and the carboxyl group were protected as methyl ether and methyl ester, respectively,[10] followed by silylation of 26-OH to yield **4**. Then, 7-OH in **4** was converted to acetate, followed by cleavage of silyl ether to give **5**. In the ^{1}H NMR spectrum of **5**, the methyl signals due to the acetyl group (δ 2.06) and two methoxy groups (δ 3.28, 3.35) appeared. The signal of 7-H appeared at δ 4.72 which shifted downfield relative to monensin (**1**). Compound **5** was treated with PDC to give the aldehyde. The ^{1}H NMR spectrum of the resulting compound showed the signal of the formyl proton at δ 9.38. The aldehyde was further oxidized successively to the carboxylic acid (**6**) by Lindgren's method.[11] In the ^{1}H NMR spectrum of **6**, the signal due to the formyl group disappeared and a signal of the second carboxyl group appeared at δ_c175.9 in the ^{13}C NMR spectrum. The FABMS of **6** also supported the structure. Then the methyl ester was hydrolyzed in aqueous alkaline to give 25-*O*-methyl-25-carboxymonensin. The exchange of 25-OMe with OH was achieved by adsorption on a precoated SiO_2 plate for a week, followed by elution and purification to give the desired dicarboxylic monensin derivative (**2**).

The course to **3a**–**d** is indicated in Scheme 2. Compound **4** was treated with benzyl iodide in the presence of NaH to give a 7-*O*-benzyl derivative in 57% yield. In the ^{1}H NMR spectrum of the resulting compound, the signals due to the benzyl position appeared at δ 4.45 and 4.66. The FABMS of this compound also supported the structure. After cleavage of the silyl group to give **7a**, stepwise oxidation of the resulting OH group at C-26 of **7a** was carried out in the same manner as **5** to yield a 25-carboxyl derivative (**8a**). In the ^{13}C NMR spectrum of **8a**, the signal due to the 25-carboxyl group appeared at 168.9 ppm. Then the methyl ester was hydrolyzed in aqueous alkaline to give 7-*O*-benzyl-25-*O*-methyl-25-carboxymonensin. The conversion of 25-OMe to OH was achieved in the same manner to **2**, yielding the desired compound **3a**. In the ^{1}H NMR spectrum of **3a**, the signal due to 25-OMe disappeared. The high-resolution (HR) FABMS spectrum of **3a** also supported the structure. Other 7-*O*-susbstituted-25-carboxymonensins



Scheme 1.



Scheme 2.

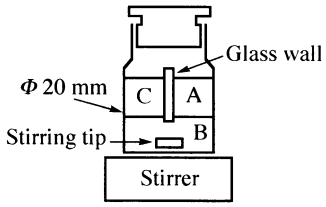


Figure 2. U-Tube system for measurement of ion transport activity through $CHCl_3$ liquid membrane. The tube contained 1 mL of 10 mM calcium or sodium chloride in 25 mM tricine buffer (A), 3.5 mL of 0.25 mM test compound solution in water-saturated $CHCl_3$ (B) and 1 mL of 50 mM citric acid solution (pH 5, C). The pH of the aqueous phases were adjusted by addition of Me_4NOH.

(**3b**–**d**) were similarly prepared from monensin as **3a**. Thus, we established an efficient method for the preparation of 25-carboxymonensin (**2**) and 7-*O*-substituted-25-carboxymonensins (**3a**–**d**) by a combination of mild and essential reactions. We obtained 25-formyl derivatives as the intermediate, and the formyl derivative will probably become a synthetically useful intermediate of 26-substituted monensins.

1.2. Ca^{2+} ion transport activity

Ca^{2+} ion transport activity of the monensin derivatives (**2**, **3a**–**d**) was evaluated by a $CHCl_3$ liquid membrane method using a U-tube system (Fig. 2). In the previous letter, the ion transport activity was evaluated using a calcium or sodium picrate solution as ion donor aqueous phase (A) and distilled water as the receiving aqueous phase (C). This time, we used the buffer solutions to set the pH of the donor phase (A) containing $CaCl_2$ or NaCl and the receiving phase (C) at 7.4 and 5.0, respectively. The amount of Ca^{2+} ion transported from phase A to phase C through the $CHCl_3$ liquid membrane (B) for 2.0 h at 31°C was 0.53 μmol for **3a**, 0.55 μmol for **3b**, 0.52 μmol for **3c**, 0.58 μmol for **3d** and 0.61 μmol for **2**, while monensin (**1**) showed no Ca^{2+} ion transport activity (Table 1). These data indicate that the introduction of the second carboxyl group at C-25 gave the Ca^{2+} ion transport activity to the molecules. Ca^{2+} ion transport activity of **2** and **3a**–**d** was 1.6–1.8-fold higher than that of lasalocid A, a well-known calcium ionophore. Judging from the R_f values in TLC, the lipophilicities of **3a**–**d** were higher than, or similar to, that of **2**. Nevertheless, the Ca^{2+} ion transport activities of **3a**–**d** were similar to or less than that of **2**. This fact suggested that the 7-OH group is important for trapping and releasing of Ca^{2+} ion and/or stability of the resulting complex with Ca^{2+}

Table 1. Ca^{2+} and Na^+ ion transport activities of monensin and its derivatives at pH 7.4 (μmol, mean±SE, n=3)

	Ca^{2+}		Na^+
	1 h	2 h	2 h
Monensin (**1**)		0.01±0.00	2.73±0.07
2	0.36±0.04	0.61±0.04	0.80±0.03
3a	0.25±0.03	0.53±0.01	1.05±0.06
3b	0.26±0.02	0.55±0.06	1.01±0.05
3c	0.26±0.04	0.52±0.02	0.93±0.11
3d	0.24±0.02	0.58±0.02	0.95±0.08
Lasalocid A	0.15±0.02	0.33±0.02	0.21±0.00

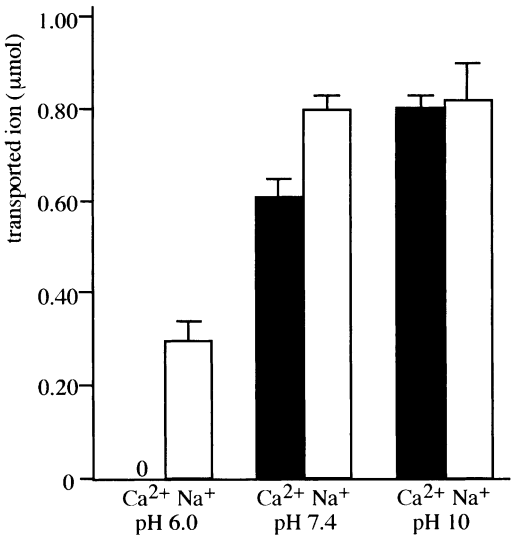


Figure 3. Ca^{2+} and Na^+ ion transport activity of **2** in the different pH.

ion than higher lipophilicity due to the substituents on the 7-*O*-position.

We also measured Na ion transport activity of **2** and **3a**–**d** using the same system. The Na^+ ion transport activity of **2** and **3a**–**d** was much lower than that of monensin (**1**), but **2** and **3a**–**d** transported Na^+ ions more than Ca^{2+} ions in this condition. The ratio of Ca^{2+}/Na^+ ion transport of **3a**–**d** was less than that of **2**. These data imply that 7-*O*-substitution could not contribute to the increase of Ca^{2+} ion transport activity and should reduce the Ca^{2+} ion selectivity.

We evaluated the Ca^{2+} ion transport activity of **2** by setting the pH of the ionic water phase at 6.0 and 10.0. Compound **2** transported no ions at pH 6.0, while **2** transported 0.80 μmol at pH 10.0, which was 1.3-fold larger than the value at pH 7.4. (Fig. 3). The Na^+ ion transport activity of **2** at pH 6.0 and 10.0 was also measured. Compound **2** transported 0.30 μmol at pH 6.0 and 0.82 μmol at pH 10 which is the same as the value at pH 7.4. These data suggested that **2** transported Ca^{2+} ion mainly as the dicarboxylate form and Na^+ ion as the monocarboxylate form.

In summary, 25-carboxymonensin (**2**) and its 7-*O*-substituted derivatives (**3a**–**d**) were prepared and were shown to transport the Ca^{2+} ion through the $CHCl_3$ liquid membrane in much higher efficiency with lower activity for Na^+ than monensin, but the ratio of Ca^{2+} and Na^+ ion transport activity was less than 1 at pH 7.4 in this condition. As **2** transported more Ca^{2+} ions at pH 10 than at pH 7.4, the dicarboxylic compounds were suggested to transport the Ca^{2+} ions as the dicarboxylate form. The investigation of the conformations of dicarboxyl derivatives in the solvent together with Job's method is now in progress.

2. Experimental

2.1. General

All the melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The

FABMS and high-resolution (HR) FABMS were measured with JEOL JMS DX-505 or SX-102 mass spectrometer, and the IR spectra with a JASCO IRA-2 spectrometer. The ^{1}H NMR spectra were measured with a JEOL EX-270, Lambda-400 or 500 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, doublet-of-doublets; td, triplet-of-doublets; qd, quartet of doublets; ddd, doublet-of-doublets-of-doublets; m, multiplet; br, broad. Optical rotations were measured on a JASCO DIP-140 or DIP-1000 digital polarimeter. TLC was carried out on precoated plates (Kieselgel 60 F254, 0.25 mm thick, Merck no. 5715), and spots were detected by illumination with an ultraviolet lamp or by spraying 1% $Ce(SO_4)_2$–10% H_2SO_4, followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Devison Chemicals).

2.1.1. 25-*O*-Methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester (4). A solution of 25-*O*-methylmonensin methyl ester[10] (1807 mg), imidazole (1409 mg) and TBDMS–Cl (2540 mg) in THF (80 mL) was stirred under Ar atmosphere at room temperature for 22 h. The mixture was poured into brine and extracted with EtOAc. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=6:1) to give **4** (2069 mg, 98%) as a colorless syrup: $[\alpha]_D^{25}$=+57 (*c* 0.30, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 1735 (C=O); ^{1}H NMR ($CDCl_3$, δ): 0.00 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, $SiC(CH_3)_3$), 2.64 (1H, qd, *J*=6.9, 3.5 Hz, 2-H), 3.20 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.41 (1H, dd, *J*=4.7, 3.5 Hz, 3-H), 3.52 (1H, t, *J*=5.0 Hz, 13-H), 3.64 (1H, m, 21-H), 3.71 (3H, s, CO_2CH_3), 3.66–3.75 (1H, m, 7-H), 3.93 (1H, dd, *J*=2.0, 9.6 Hz, 5-H), 4.02 (1H, d, *J*=4.3 Hz, 17-H), 4.24 (1H, td, *J*=3.0, 6.3 Hz, 20-H). FABMS (*m*/*z*): 835 $(M+Na)^+$. HRFABMS: Calcd for $C_{44}H_{80}O_{11}SiNa$: 835.5368 $(M+Na)^+$. Found: 835.5355.

2.1.2. 7-*O*-Acetyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. To a solution of **4** (631 mg) in pyridine (3.0 mL) and benzene (5.0 mL) was added Ac_2O (3.0 mL) and DMAP (9.4 mg). The mixture was stirred under Ar atmosphere at room temperature for 15 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc=9:1) to give 7-*O*-acetyl derivative (630 mg, 95%) as a colorless syrup: $[\alpha]_D^{25}$=+90 (*c* 0.26, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1732 (C=O); ^{1}H NMR ($CDCl_3$, δ): 0.04 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, $C(CH_3)_3$), 2.06 (3H, s, CH_3CO), 2.64 (1H, quintet-like, *J*=6.8 Hz, 2-H), 3.21 (3H, s, 25-OMe), 3.35 (3H, s, 3-OMe), 3.36 (1H, t, *J*=4.4 Hz, 13-H), 3.41 (1H, dd, *J*=3.2, 9.5 Hz, 21-H), 3.55 (1H, dd, *J*=6.8, 8.2 Hz, 3-H), 3.60 (2H, s, 26-H_2), 3.74 (3H, s, CO_2CH_3), 3.89 (1H, dd, *J*=2.0, 9.8 Hz, 5-H), 4.00 (1H, d, *J*=4.2 Hz, 17-H), 4.23 (1H, ddd, *J*=3.6, 6.5, 9.5 Hz, 20-H), 4.73 (1H, d, *J*=2.9 Hz, 7-H). FABMS (*m*/*z*): 855 $(M+1)^+$, 878 $(M+Na+1)^+$. HRFABMS: Calcd for $C_{46}H_{82}O_{12}SiNa$: 877.5473 $(M+Na)^+$. Found: 877.5519.

2.1.3. 7-*O*-Acetyl-25-*O*-methylmonensin methyl ester (5). A mixture of 7-*O*-acetyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester (325 mg) and TBAF·3H_2O (240 mg) in THF (8.5 mL) was stirred under Ar atmosphere at room temperature for 3.5 h. The reaction mixture was poured into brine, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=3:1) to give **5** (273 mg, 97%) as a colorless syrup: $[\alpha]_D^{25}$=+81 (*c* 0.23, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1732 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.06 (3H, s, CH_3CO), 2.63 (1H, quintet-like, *J*=6.8 Hz, 2-H), 3.28 (3H, s, 25-OCH_3), 3.35 (4H, 3-OCH_3 and 3-H, overlapped), 3.45 (1H, dd, *J*=3.5, 9.6 Hz, 21-H), 3.54 (1H, dd, *J*=6.8, 11.1 Hz, 26-H_a), 3.55 (1H, dd, *J*=6.6, 8.3 Hz, 13-H), 3.70 (1H, dd, *J*=5.0, 11.2 Hz, 26-H_b), 3.74 (3H, s, CO_2CH_3), 3.88 (1H, dd, *J*=2.0, 9.9 Hz, 5-H), 3.95 (1H, d, *J*=4.2 Hz, 17-H), 4.26 (1H, ddd, *J*=3.5, 6.6, 11.7 Hz, 20-H), 4.72 (1H, dd, *J*=2.7, 5.6 Hz, 7-H). Anal (%): Calcd for $C_{40}H_{68}O_{12}$: C, 64.84; H, 9.25. Found: C, 64.59; H, 9.29.

2.1.4. 7-*O*-Acetyl-25-*O*-methyl-25-formylmonensin methyl ester. To a solution of **5** (221 mg) in CH_2Cl_2 (9.0 mL) was added PDC (280 mg), MS4A (160 mg), and Celite (400 mg). The mixture was stirred under Ar atmosphere at room temperature for 4.5 h. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=6:1) to give the aldehyde (200 mg, 91%) as a colorless syrup: $[\alpha]_D^{25}$=+100 (*c* 0.21, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.06 (3H, s, CH_3CO), 2.64 (1H, quintet-like, *J*=6.8 Hz, 2-H), 3.27 (3H, s, 25-OMe), 3.35 (1H, t, *J*=5.5 Hz, 13-H), 3.47 (1H, dd, *J*=3.5, 10.1 Hz, 21-H), 3.54 (1H, dd, *J*=6.8, 8.4 Hz, 3-H), 3.74 (3H, s, CO_2CH_3), 3.88 (1H, dd, *J*=1.8, 9.9 Hz, 5-H), 3.97 (1H, d, *J*=3.9 Hz, 17-H), 4.30 (1H, ddd, *J*=3.5, 8.7, 8.5 Hz, 20-H), 4.72 (1H, d, *J*=2.9 Hz, 7-H), 9.38 (1H, s, CHO). FABMS (*m*/*z*): 738 $(M)^+$, 762 $(M+Na+1)^+$, HRFABMS: Calcd for $C_{40}H_{66}O_{12}Na$: 761.4452 $(M+Na)^+$. Found: 761.4435.

2.1.5. 7-*O*-Acetyl-25-*O*-methyl-25-carboxymonensin methyl ester (6). To a solution of 7-*O*-acetyl-25-*O*-methyl-25-formylmonensin methylester (148 mg) in *t*-BuOH–H_2O (1:1, 15.0 mL), NaH_2PO_4·2H_2O (47 mg) in H_2O (1.0 mL) and $NaClO_2$ (136 mg) in H_2O (2.0 mL) were added. The mixture was stirred at room temperature for 2 h, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=1:1) to give **6** (150 mg, 99%) as a colorless syrup: $[\alpha]_D^{25}$=+93 (*c* 0.26, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1732 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.06 (3H, s, CH_3CO), 2.64 (1H, qd, *J*=7.0, 6.4 Hz, 2-H), 3.30 (3H, s, 25-OCH_3), 3.35 (3H, s, 3-OCH_3), 3.36 (1H, t-like, *J*=4.3 Hz, 13-H), 3.52 (1H, dd, *J*=3.8, 10.2 Hz, 21-H), 3.56 (1H, dd, *J*=6.4, 8.5 Hz, 3-H), 3.75 (3H, s, CO_2CH_3), 3.89 (1H, dd, *J*=2.0, 9.9 Hz, 5-H), 3.94 (1H, d, *J*=4.3 Hz, 17-H), 4.31 (1H, m, 20-H), 4.72 (1H, dd, *J*=2.9, 5.9 Hz, 7-H), ^{13}C NMR ($CDCl_3$, δ): 169.0, 170.8, 175.9 (C=O). FABMS (*m*/*z*): 777 $(M+Na)^+$. HRFABMS: Calcd for $C_{40}H_{66}O_{13}Na$: 777.4401 $(M+Na)^+$. Found: 777.4393.

2.1.6. 25-*O*-Methyl-25-carboxymonensin. To a solution of **6** (46 mg) in MeOH–THF (1:1, 4.0 mL) was added 5 mol/L

NaOH (1.0 mL). The mixture was stirred at room temperature for 4 h, neutralized with 5% aquerous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$/MeOH=100:1) to give 25-*O*-methyl-25-carboxymonensin (39 mg, 91%) as a colorless syrup: $[\alpha]_D^{25}$=+40 (*c* 0.28, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720, 1745 (C=O); ^1H NMR ($CDCl_3$, δ): 2.66 (1H, qd, *J*=6.8, 6.1 Hz, 2-H), 3.33 (3H, s, 25-OMe), 3.38 (3H, s, 3-OMe), 3.47 (1H, br, 13-H), 3.54 (1H, dd, *J*=2.8, 10.1 Hz, 21-H), 3.72 (1H, dd, *J*=6.1, 8.5 Hz, 3-H), 3.77 (1H, br, 7-H), 3.91 (1H, d, *J*=4.1 Hz, 17-H), 4.01 (1H, dd, *J*=1.6, 9.9 Hz, 5-H), 4.32 (1H, td, *J*=2.8, 7.8 Hz, 20-H). HRFABMS: Calcd for $C_{37}H_{62}O_{12}Na$: 721.4139 $(M+Na)^+$. Found: 721.4164.

2.1.7. 25-Carboxymonensin (2). 25-*O*-Methyl-25-carboxymonensin (52 mg) was adsorbed on a silica gel plate for 7 days. The silica gel was eluted with $CHCl_3$/MeOH=10:1. The effluent was evaporated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$/MeOH=80:1) to give **2** (25 mg, 50%) with recovery (50%) as colorless syrup: $[\alpha]_D^{25}$=+95 (*c* 0.20, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (1H, qd, *J*=6.6, 10.0 Hz, 2-H), 3.13 (1H, dd, *J*=1.8, 10.0 Hz, 3-H), 3.37 (3H, s, 3-OMe), 3.52 (1H, dd, *J*=4.6, 10.7 Hz, 13-H), 3.84 (1H, d, *J*=3.1 Hz, 17-H), 3.87 (1H, dd, *J*=4.0, 10.4 Hz, 21-H), 3.90 (1H, dd, *J*=2.0, 11.4 Hz, 5-H), 3.95 (1H, d, *J*=1.5 Hz, 7-H), 4.39 (1H, m, 20-H). ^{13}C NMR ($CDCl_3$, δ): 173.7, 180.4 (C=O). HRFABMS: Calcd for $C_{36}H_{60}O_{12}Na$: 707.3982 $(M+Na)^+$. Found: 707.3952.

2.1.8. 7-*O*-Substituted-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. To a solution of **4** (352 mg) in THF (7.5 mL), NaH (26 mg), benzyl bromide (0.36 mL) and *n*-Bu_4NI (160 mg) were added. The mixture was stirred under N_2 atmosphere at room temperature for 2 h, quenched by the addition of NH_4Cl solution and diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane/EtOAc=10:1) to give 7-*O*-benzyl derivative (231 mg, 57%) as colorless syrup. 7-*O*-methyl, 7-*O*-*p*-ethylbenzyl and 7-*O*-*p*-chlorobenzyl derivatives were similarly prepared from **4**, using the corresponding bromide instead of benzyl bromide.

7-*O*-Benzyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. $[\alpha]_D^{25}$=+56 (*c* 0.23, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^1H NMR ($CDCl_3$, δ): 0.04 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, $SiC(CH_3)_3$), 2.64 (1H, qd, *J*=6.9, 5.6 Hz, 2-H), 3.21 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.37–3.49 (3H, m, 3-H, 13-H and 26-H_2), 3.54 (3H, s, CO_2CH_3), 3.57–3.71 (2H, m, 7-H and 26-H_2), 3.67 (1H, dd, *J*=4.3, 6.3 Hz, 21-H), 4.03 (1H, d, *J*=4.0 Hz, 17-H), 4.04 (1H, dd. *J*=1.7, 4.5 Hz, 5-H), 4.25 (1H, td, *J*=4.3, 3.6 Hz, 20-H), 4.45, 4.66 (each 1H, both d, *J*=12.0 Hz, O*CH*$_2$Ar), 7.19–7.42 (5H, m, Ar); FABMS (*m*/*z*): 926 $(M+Na+1)^+$. HRFABMS: Calcd for $C_{51}H_{86}O_{11}SiNa$: 925.5837 $(M+Na)^+$. Found: 925.5830.

7-*O*-Methyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. Colorless syrup, yield; 63%; $[\alpha]_D^{25}$=+75 (*c* 0.27, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1740 (C=O); ^1H NMR ($CDCl_3$, δ): 0.04 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, $SiC(CH_3)_3$), 2.64 (1H, qd, *J*=7.0, 6.3 Hz, 2-H), 3.21 (3H, s, 25-OCH_3), 3.29 (1H, dd, *J*=2.6, 6.3 Hz, 3-H), 3.31 (3H, s, 7-OCH_3), 3.32 (3H, s, 3-OCH_3), 3.41 (1H, dd, *J*=3.3, 9.4 Hz, 7-H), 3.51 (1H, t, *J*=5.0 Hz, 13-H), 3.60 (2H, s, 26-H_2), 3.67 (1H, dd, *J*=6.3, 8.9 Hz, 21-H), 3.70 (3H, s, CO_2CH_3), 3.92 (1H, dd, *J*=1.7, 10.3 Hz, 5-H), 3.99 (1H, d, *J*=4.0 Hz, 17-H), 4.24 (1H, ddd, *J*=3.4, 7.3, 8.9 Hz, 20-H); FABMS (*m*/*z*): 850 $(M+Na+1)^+$. HRFABMS: Calcd for $C_{45}H_{82}O_{11}SiNa$: 849.5524 $(M+Na)^+$. Found: 849.5518.

7-*O*-*p*-Ethylbenzyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. Colorless syrup, yield; 62%; $[\alpha]_D^{25}$=+87 (*c* 0.32, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^1H NMR ($CDCl_3$, δ): 0.04 (6H, s, $Si(CH_3)_2$), 0.89 (9H, s, $SiC(CH_3)_3$), 2.58–2.67 (3H, m, 2-H, CH_3CH_2Ar), 3.21 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.41–3.49 (2H, m, 13-H and 26-H_a), 3.54 (3H, s, CO_2CH_3), 3.61–3.71 (3H, m, 7-H, 21-H and 26-H_b), 4.02 (1H, dd, *J*=2.0, 8.9 Hz, 5-H), 4.03 (1H, d, *J*=4.0 Hz, 17-H), 4.25 (1H, m, 20-H), 4.41, 4.62 (each 1H, both d, *J*=12.0 Hz, O*CH*$_2$Ar), 7.11–7.32 (4H, m, Ar); FABMS (*m*/*z*): 953 $(M+Na)^+$. HRFABMS: Calcd for $C_{53}H_{90}O_{11}SiNa$: 953.6150 $(M+Na)^+$. Found: 953.6202.

7-*O*-*p*-Chlorobenzyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester. Colorless syrup, yield; 50%; $[\alpha]_D^{25}$=+44 (*c* 0.27, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^1H NMR ($CDCl_3$, δ): 0.03 (6H, s, $Si(CH_3)_2$), 0.87 (9H, s, $SiC(CH_3)_3$), 2.61 (1H, qd, *J*=6.6, 6.6 Hz, 2-H), 3.20 (3H, s, 25-OCH_3), 3.32 (3H, s, 3-OCH_3), 3.40–3.45 (3H, m, 13-H, 26-H_a and 3-H), 3.51 (3H, s, CO_2CH_3), 3.53–3.69 (3H, m, 21-H, 7-H and 26-H_b), 3.99 (1H, br d, *J*=7.3 Hz, 5-H), 4.01 (1H, d, *J*=3.6 Hz, 17-H), 4.23 (1H, td, *J*=6.3, 3.3 Hz, 20-H), 4.40, 4.60 (each 1H, both d, *J*=12.0 Hz, O*CH*$_2$Ar), 7.20–7.36 (4H, m, Ar); FABMS (*m*/*z*): 959 $(M+Na)^+$. HRFABMS: Calcd for $C_{51}H_{85}O_{11}ClSiNa$: 959.5447 $(M+Na)^+$. Found: 959.5454.

2.1.9. 7-*O*-Substituted-25-*O*-methylmonensin methyl ester (7a–d). A mixture of 7-*O*-benzyl-25-*O*-methyl-26-*O*-*t*-butyldimethylsilylmonensin methyl ester (207 mg) and TBAF·$3H_2O$ (140 mg) in THF (5.0 mL) was stirred under Ar atmosphere at room temperature for 4 h. The reaction mixture was poured into brine, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=5:1) to give **7a** (175 mg, 99%) as a colorless syrup. Compounds **7b–d** were similarly obtained from the corresponding 26-*O*-silyl derivatives.

7a. $[\alpha]_D^{25}$=+34 (*c* 0.27, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (1H, qd, *J*=6.8, 6.2 Hz, 2-H), 3.28 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.47–3.58 (3H, m, 3-H and 13-H, 26-H_a), 3.54 (3H, s, CO_2CH_3), 3.64–3.74 (3H, m, 26-H_b, 7-H and 21-H), 3.96 (1H, d, *J*=4.0 Hz, 17-H), 4.02 (1H, dd, *J*=1.8, 9.9 Hz, 5-H), 4.27 (1H, ddd, *J*=3.7, 6.2, 9.2 Hz, 20-H), 4.45, 4.65 (each 1H, both d, *J*=12.0 Hz, O*CH*$_2$Ar), 7.19–7.42 (5H, m, Ar); FABMS (*m*/*z*): 811 $(M+Na)^+$. HRFABMS: Calcd for $C_{45}H_{72}O_{11}Na$: 811.4972 $(M+Na)^+$. Found: 811.4969.

7b. Colorless syrup, yield; 99%; $[\alpha]_D^{25}$=+83 (*c* 0.22,

$CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (1H, qd, J=6.9, 5.1 Hz, 2-H), 3.27 (3H, s, 25-OCH_3), 3.30 (3H, s, 7-OCH_3), 3.32 (3H, s, 3-OCH_3), 3.46–3.57 (3H, m, 13-H, 26-H_a, 7-H), 3.64–3.72 (2H, m, 21-H, 26-H_b), 3.70 (3H, s, CO_2CH_3), 3.91 (1H, dd, J=1.7, 10.0 Hz, 5-H), 3.94 (1H, d, J=4.3 Hz, 17-H), 4.27 (1H, ddd, J=3.6, 4.8, 9.6 Hz, 20-H); FABMS (m/z): 735 $(M+Na)^+$. HRFABMS: Calcd for $C_{39}H_{68}O_{11}Na$: 735.4659 $(M+Na)^+$. Found: 735.4698.

7c. Colorless syrup, yield; 95%; $[\alpha]_D^{25}$=+57 (c 0.38, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (2H, q, J=7.6 Hz, CH_3CH_2Ar), 2.65 (1H, m, 2-H), 3.28 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.46 (1H, m, 13-H), 3.48 (1H, dd, J=4.1, 8.7 Hz, 7-H), 3.54 (3H, s, CO_2CH_3), 3.56 (1H, m, 26-H_a), 3.68 (1H, dd, J=6.2, 8.4 Hz, 21-H), 3.70 (1H, dd, J=5.1, 10.5 Hz, 26-H_b), 3.97 (1H, d, J=4.0 Hz, 17-H), 4.01 (1H, br d, J=9.9 Hz, 5-H), 4.27 (1H, ddd, J=3.4, 9.5, 6.2 Hz, 20-H), 4.41, 4.62 (each 1H, both d, J=11.9 Hz, OCH_2Ar), 7.11–7.32 (4H, m, Ar); FABMS (m/z): 840 $(M+Na+1)^+$. HRFABMS: Calcd for $C_{47}H_{76}O_{11}Na$: 839.5285 $(M+Na)^+$. Found: 839.5274.

7d. Colorless syrup, yield; 97%; $[\alpha]_D^{25}$=+45 (c 0.26, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^1H NMR ($CDCl_3$, δ): 2.62 (1H, qd, J=6.3, 6.5 Hz, 2-H), 3.28 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.44 (1H, dd, J=4.6, 6.1 Hz, 13-H), 3.46 (1H, dd, J=4.9, 8.1 Hz, 7-H), 3.53 (3H, s, CO_2CH_3), 3.55 (1H, dd, J=6.6, 11.2 Hz, 26-H_a), 3.63 (1H, dd, J=6.3, 8.8 Hz, 21-H), 3.70 (1H, dd, J=4.6, 11.0 Hz, 26-H_b), 3.95 (1H, d, J=4.0 Hz, 17-H), 4.00 (1H, dd, J=2.0, 10.0 Hz, 5-H), 4.27 (1H, ddd, J=3.4, 6.3, 9.3 Hz, 20-H), 4.42, 4.60 (each 1H, both d, J=12.0 Hz, OCH_2Ar), 7.21–7.38 (4H, m, Ar); FABMS (m/z): 845 $(M+Na)^+$. HRFABMS: Calcd for $C_{45}H_{71}O_{11}ClNa$: 845.4583 $(M+Na)^+$. Found: 845.4577.

2.1.10. 7-*O*-Substituted-25-*O*-methy-25-formylmonensin methyl ester. To a solution of **7a** (197 mg) in CH_2Cl_2 (9.0 mL), PDC (141 mg), MS4A (140 mg), and Celite (350 mg) were added. The mixture was stirred under Ar atmosphere at room temperature for 4.5 h. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=8:1) to give the aldehyde (164 mg, 84%) as colorless syrup. Compounds **7b–d** were similarly converted to the corresponding 25-folmyl derivatives.

7-*O*-Benzyl-25-*O*-methy-25-formylmonensin methyl ester. $[\alpha]_D^{13}$=+66 (c 0.38, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1740 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (1H, qd, J=6.3, 6.6 Hz, 2-H), 3.28 (3H, s, 25-OMe), 3.33 (3H, s, 3-OMe), 3.47–3.50 (3H, m, 13-H, 3-H and 7-H), 3.54 (3H, s, CO_2CH_3), 3.65 (1H, dd, J=4.3, 6.3 Hz, 21-H), 3.98 (1H, d, J=3.6 Hz, 17-H), 4.02 (1H, br d, J=10.0 Hz, 5-H), 4.31 (1H, m, 20-H), 4.45, 4.64 (each 1H, both d, J=12.0 Hz, CH_2–Ar), 7.19–7.41 (5H, m, benzyl), 9.39 (1H, s, 26-CHO). HRFABMS: Calcd for $C_{45}H_{70}O_{11}Na$: 809.4816 $(M+Na)^+$. Found: 809.4786.

7-*O*-Methyl-25-*O*-methy-25-formylmonensin methyl ester. Colorless syrup, yield; 85%; $[\alpha]_D^{13}$=+56 (c 0.37, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1740 (C=O); ^1H NMR ($CDCl_3$, δ): 2.64 (1H, qd, J=6.9, 6.1 Hz, 2-H), 3.28 (3H, s, 25-OMe), 3.30 (3H, s, 7-OMe), 3.33 (3H, s, 3-OMe), 3.45–3.52 (2H, m, 13-H and 7-H), 3.66 (1H, dd, J=6.6, 8.3 Hz, 21-H), 3.71 (3H, s, CO_2CH_3), 3.91 (1H, br d, J=9.9 Hz, 5-H), 3.97 (1H, d, J=3.3 Hz, 17-H), 4.30 (1H, m, 20-H), 9.38 (1H, s, 26-CHO). HRFABMS: Calcd for $C_{39}H_{66}O_{11}Na$: 733.4503 $(M+Na)^+$. Found: 733.4487.

7-*O*-*p*-Ethylbenzyl-25-*O*-methy-25-formylmonensin methyl ester. Colorless syrup, yield; 85%; $[\alpha]_D^{13}$=+71 (c 0.33, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1740 (C=O); ^1H NMR ($CDCl_3$, δ): 2.59–2.67 (3H, m, 2-H, CH_3CH_2Ar), 3.28 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.45–3.51 (2H, m, 13-H and 3-H), 3.54 (3H, s, CO_2CH_3), 3.64–3.71 (2H, m, 21-H and 7-H), 3.99–4.15 (2H, m, 17-H, 5-H), 4.32 (1H, m, 20-H), 4.41, 4.61 (each 1H, both d, J=11.9 Hz, OCH_2Ar), 7.11–7.32 (4H, m, Ar), 9.39 (1H, s, 26-CHO). HRFABMS: Calcd for $C_{47}H_{74}O_{11}Na$: 837.5129 $(M+Na)^+$. Found: 837.5141.

7-*O*-*p*-Chlorobenzyl-25-*O*-methy-25-formylmonensin methyl ester. Colorless syrup, yield; 69%; $[\alpha]_D^{13}$=+51 (c 0.24, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 1740 (C=O); ^1H NMR ($CDCl_3$, δ): 2.62 (1H, qd, J=6.6, 6.8 Hz, 2-H), 3.27 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.42–3.55 (3H, m, 3-H, 7-H and 13-H), 3.53 (3H, s, CO_2CH_3), 3.62 (1H, dd, J=6.6, 8.6 Hz, 21-H), 3.97 (1H, d, J=3.6 Hz, 17-H), 4.00 (1H, br d, J=7.9 Hz, 5-H), 4.30 (1H, m, 20-H), 4.41, 4.59 (each 1H, both d, J=12.0 Hz, OCH_2Ar), 7.25–7.37 (4H, m, Ar), 9.38 (1H, s, 26-CHO). HRFABMS: Calcd for $C_{45}H_{69}O_{11}ClNa$: 843.4426 $(M+Na)^+$. Found: 843.4426.

2.1.11. 7-*O*-Substituted-25-*O*-methyl-25-carboxymonensin methyl ester (8a–d). To a solution of 7-*O*-benzyl-25-*O*-methyl-25-formylmonensin methyl ester (164 mg) in *t*-BuOH–H_2O (1:1, 5.0 mL), $NaH_2PO_4 \cdot 2H_2O$ (23 mg) in H_2O (1.0 mL) and $NaClO_2$ (95 mg) in H_2O (2.0 mL) were added. The mixture was stirred at room temperature for 2 h, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc=1:1) to give **8a** (162 mg, 97%). Compounds **8b–d** were similarly obtained from the corresponding 25-formyl derivatives.

8a. $[\alpha]_D^{25}$=+70 (c 0.20, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^1H NMR ($CDCl_3$, δ): 2.63 (1H, qd, J=5.9, 6.9 Hz, 2-H), 3.29 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.45–3.57 (2H, m, 13-H, 3-H), 3.54 (3H, s, CO_2CH_3), 3.64–3.71 (2H, m, 21-H and 7-H), 3.95 (1H, d, J=4.3 Hz, 17-H), 4.02 (1H, dd, J=2.0, 9.9 Hz, 5-H), 4.31 (1H, m, 20-H), 4.45, 4.64 (each 1H, both d, J=12.0 Hz, OCH_2Ar), 7.19–7.41 (5H, m, Ar); ^{13}C NMR ($CDCl_3$, δ): 168.9 (25-CO_2H), 176.0 (CO_2CH_3); FABMS (m/z): 826 $(M+Na+1)^+$, 848 $(M+2Na)^+$. HRFABMS: Calcd for $C_{45}H_{70}O_{12}Na$: 825.4765 $(M+Na)^+$. Found: 825.4795.

8b. Colorless syrup, yield; 83%; $[\alpha]_D^{25}$=+63 (c 0.40, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^1H NMR ($CDCl_3$,δ): 2.64 (1H, qd, J=7.1, 5.1 Hz, 2-H), 3.28 (1H, dd, J=2.6, 7.1 Hz, 3-H), 3.30 (3H, s, 25-OCH_3), 3.31 (3H, s, 7-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.49–3.54 (2H, m, 13-H,

7-H), 3.65–3.74 (1H, m, 21-H), 3.71 (3H, s, CO_2CH_3), 3.90 (1H, dd, *J*=1.8, 9.0 Hz, 5-H), 3.94 (1H, d, *J*=4.4 Hz, 17-H), 4.32 (1H, m, 20-H); ^{13}C NMR ($CDCl_3$, δ): 169.3 (25-CO_2H), 176.1 (CO_2CH_3); FABMS (*m/z*): 749 $(M+Na)^+$, 771 $(M+2Na-1)^+$. HRFABMS: Calcd for $C_{39}H_{66}O_{12}Na$: 749.4452 $(M+Na)^+$. Found: 749.4427.

8c. Colorless syrup, yield; 84%; $[\alpha]_D^{25}=+55$ (*c* 0.39, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.62 (1H, m, 2-H), 2.63 (2H, q, *J*=7.6 Hz, CH_3CH_2Ar), 3.29 (3H, s, 25-OCH_3), 3.32 (1H, m, 3-H), 3.33 (3H, s, 3-OCH_3), 3.45–3.59 (1H, m, 13-H), 3.55 (3H, s, CO_2CH_3), 3.67 (1H, dd, *J*=6.3, 8.6 Hz, 21-H), 3.69–3.78 (1H, m, 7-H), 3.95 (1H, d, *J*=4.0 Hz, 17-H), 4.01 (1H, dd, *J*=2.0, 9.6 Hz, 5-H), 4.32 (1H, m, 20-H), 4.42, 4.61 (each 1H, both d, *J*=12.0 Hz, OCH_2Ar), 7.12–7.38 (4H, m, Ar); ^{13}C NMR ($CDCl_3$, δ): 169.2 (25-CO_2H), 176.0 (CO_2CH_3); FABMS (*m/z*): 853 $(M+Na)^+$. HRFABMS: Calcd for $C_{47}H_{74}O_{12}Na$: 853.5078 $(M+Na)^+$. Found: 853.5093.

8d. Colorless syrup, yield; 90%; $[\alpha]_D^{25}=+53$ (*c* 0.33, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1735 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.62 (1H, qd, *J*=6.6, 6.6 Hz, 2-H), 3.29 (3H, s, 25-OCH_3), 3.33 (3H, s, 3-OCH_3), 3.55–3.58 (2H, m, 13-H, 21-H), 3.53 (3H, s, CO_2CH_3), 3.63 (1H, dd, *J*=4.3, 6.6 Hz, 3-H), 3.94 (1H, d, *J*=4.3 Hz, 17-H), 3.99 (1H, dd, *J*=2.0, 9.9 Hz, 5-H), 4.31 (1H, td, *J*=9.3, 3.0 Hz, 20-H), 4.41, 4.59 (each 1H, both d, *J*=12.0 Hz, OCH_2Ar), 7.21–7.37 (4H, m, Ar); ^{13}C NMR ($CDCl_3$, δ): 169.2 (25-CO_2H), 176.0 (CO_2CH_3); FABMS (*m/z*): 860 $(M+Na+1)^+$. HRFABMS: Calcd for $C_{45}H_{69}O_{12}ClNa$: 859.4375 $(M+Na)^+$. Found: 859.4363.

2.1.12. 7-*O*-Alkyl-25-*O*-methyl-25-carboxymonensin. To a solution of **8a** (134 mg) in MeOH–THF (1:1, 4.0 mL) was added 5 mol/L NaOH (1.0 mL). The mixture was stirred at room temperature for 4 h, neutralized with 5% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$/MeOH=100:1) to give 7-*O*-benzyl-25-*O*-methyl-25-carboxylmonensin (50 mg, 57%). 7-*O*-methyl, 7-*O*-ethylbenzyl and 7-*O*-chlorobenzyl derivatives were similarly obtained from **8b–d**, respectively.

7-*O*-Benzyl-25-*O*-methyl-25-carboxymonensin. $[\alpha]_D^{25}=+45$ (*c* 0.27, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 1600 (C=O), 1700 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.92 (1H, dd, *J*=2.3, 10.2 Hz, 3-H), 3.16 (3H, s, 25-OCH_3), 3.27–3.39 (1H, m, 13-H), 3.46 (1H, br s, 7-H), 3.50 (3H, s, 3-OCH_3), 3.65–3.69 (1H, m, 21-H), 4.04 (1H, br s, 5-H), 4.09 (1H, d, *J*=4.3 Hz, 17-H), 4.34 (1H, m, 20-H), 4.31, 4.87 (each 1H, both d, *J*=14.3 Hz, OCH_2Ar), 7.26–7.41 (5H, m, Ar); FABMS (*m/z*): 811 $(M+Na)^+$, 833 $(M+2Na-1)^+$. HRFABMS: Calcd for $C_{44}H_{67}O_{12}Na_2$: 833.4428 $(M+2Na-H)^+$. Found: 833.4412.

7-*O*-Methyl-25-*O*-methyl-25-carboxymonensin. Colorless syrup, yield; 64%; $[\alpha]_D^{25}=+53$ (*c* 0.50, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1740 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.66 (1H, br s, 2-H), 3.31 (6H, s, 7-OCH_3, 25-OCH_3), 3.36 (3H, s, 3-OCH_3), 3.09–3.36 (1H, m, 3-H), 3.42–3.53 (2H, m, 7-H and 13-H), 3.67 (1H, dd, *J*=6.3, 8.6 Hz, 21-H), 3.95 (1H, br s, 17-H), 3.97 (1H, br d, *J*=10.6 Hz, 5-H), 4.30 (1H, m, 20-H), FABMS (*m/z*): 735 $(M+Na)^+$, 757 $(M+2Na-H)^+$. HRFABMS: Calcd for $C_{38}H_{64}O_{12}Na$: 735.4295 $(M+Na)^+$. Found: 735.4240.

7-*O*-*p*-Ethylbenzyl-25-*O*-methyl-25-carboxymonensin. Colorless syrup, yield; 58%; $[\alpha]_D^{25}=+51$ (*c* 0.26, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1730 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.59–2.76 (3H, m, 2-H, CH_3CH_2Ar), 3.30 (3H, s, 25-OCH_3), 3.33–3.55 (3H, m, 3-H, 13-H and 7-H), 3.37 (3H, s, 3-OCH_3), 3.67 (1H, dd, *J*=6.6, 8.3 Hz, 21-H), 3.95 (1H, d, *J*=4.0 Hz, 17-H), 4.12 (1H, br d, *J*=9.7 Hz, 5-H), 4.30 (1H, br s, 20-H), 4.46, 4.57 (each 1H, both d, *J*=12.0 Hz, OCH_2Ar), 7.13–7.30 (4H, m, Ar); FABMS (*m/z*): 839 $(M+Na)^+$, 861 $(M+2Na-H)^+$. HRFABMS: Calcd for $C_{46}H_{72}O_{12}Na$: 839.4921 $(M+Na)^+$. Found: 839.4902.

7-*O*-*p*-Chlorobenzyl-25-*O*-methyl-25-carboxymonensin. Colorless syrup, yield; 50%; $[\alpha]_D^{25}=+36$ (*c* 0.37, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.67 (1H, m, 2-H), 3.29 (3H, s, 25-OCH_3), 3.37 (3H, s, 3-OCH_3), 3.45–3.52 (3H, m, 3-H, 13-H and 7-H), 3.65 (1H, dd, *J*=6.3, 4.5 Hz, 21-H), 3.94 (1H, d, *J*=4.3 Hz, 17-H), 4.12 (1H, br d, *J*=7.9 Hz, 5-H), 4.30 (1H, m, 20-H), 4.45, 4.56 (each 1H, both d, *J*=11.9 Hz, OCH_2Ar), 7.26–7.35 (4H, m, Ar); FABMS (*m/z*): 846 $(M+Na+1)^+$, 868 $(M+2Na)^+$. HRFABMS: Calcd for $C_{44}H_{67}O_{12}ClNa$: 845.4219 $(M+Na)^+$. Found: 845.4172.

2.1.13. 7-*O*-Substituted-25-carboxymonensin (3a–d). 7-*O*-Benzyl-25-*O*-methyl-25-carboxymonensin (54 mg) was adsorbed on a silica gel plate for 7 days. The silica gel was eluted with $CHCl_3$/MeOH=10:1. The effluent was evaporated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$/MeOH=80:1) to give **3a** (21 mg, 40%) with recovery of the starting material (55%). Compounds **3b–d** were similarly obtained from corresponding 25-*O*-methyl derivatives, respectively.

3a. $[\alpha]_D^{25}=+47$ (*c* 0.21, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720, 1735 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.67 (1H, qd, *J*=6.7, 10.1 Hz, 2-H), 3.17 (1H, dd, *J*=1.5, 10.1 Hz, 3-H), 3.38(1H, dd, *J*=2.1, 4.6 Hz, 7-H), 3.40 (3H, s, 3-OCH_3), 3.43 (1H, dd, *J*=4.6, 10.1 Hz, 21-H), 3.58 (1H, dd, *J*=6.1, 9.7 Hz, 13-H), 3.90 (1H, dd, *J*=2.1, 11.6 Hz, 5-H), 3.93 (1H, m, 20-H), 4.03 (1H, d, *J*=3.4 Hz, 17-H), 4.51, 5.91 (each 1H, both d, *J*=15.8 Hz, OCH_2Ar), 7.35–7.37 (5H, m, Ar). HRFABMS: Calcd for $C_{43}H_{65}O_{12}Na_2$: 819.4271 $(M+2Na-H)^+$. Found: 819.4252.

3b. Colorless syrup, yield; 45%; $[\alpha]_D^{25}=+39$ (*c* 0.30, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720, 1730 (C=O); ^{1}H NMR ($CDCl_3$, δ): 2.61(1H, qd, *J*=6.6, 10.5 Hz, 2-H), 3.10 (1H, dd, *J*=1.5, 10.5 Hz, 3-H), 3.28 (1H, t-like, *J*=2.2 Hz, 7-H), 3.38 (3H, s, 3-OCH_3), 3.45 (1H, dd, *J*=5.7, 10.1 Hz, 13-H), 3.65 (3H, s, 7-OMe), 3.77 (1H, dd, *J*=2.0, 11.2 Hz, 5-H), 3.90 (1H, dd, *J*=4.5, 10.1 Hz, 21-H), 4.03 (1H, d, *J*=3.2 Hz, 17-H), 4.39 (1H, ddd, *J*=4.6, 7.3, 9.0 Hz, 20-H); FABMS (*m/z*): 722 $(M+Na+1)^+$, 744 $(M+2Na)^+$. HRFABMS: Calcd for $C_{37}H_{61}O_{12}Na_2$: 743.3958 $(M+2Na-H)^+$. Found: 743.3983.

3c. Colorless syrup, yield; 50%; $[\alpha]_D^{25}=+49$ (*c* 0.22,

$CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720, 1735 (C=O); ^1H NMR ($CDCl_3$, δ): 2.59–2.70 (3H, m, 2-H, CH_3CH_2-Ar), 3.16 (1H, d-like, J=9.9 Hz, 3-H), 3.40 (3H, s, 3-OCH_3), 3.45–3.77 (3H, m, 13-H, 7-H and 21-H), 3.89 (1H, dd, J=1.7, 11.3 Hz, 5-H), 4.03 (1H, d, J=3.0 Hz, 17-H), 4.21 (1H, m, 20-H), 4.47, 5.83 (each 1H, both d, J=15.8 Hz, OCH_2Ar), 7.16–7.34 (4H, m, Ar). HRFABMS: Calcd for $C_{45}H_{69}O_{12}Na_2$: 847.4584 $(M+2Na-H)^+$. Found: 847.4561.

3d. Colorless syrup, yield; 55%; $[\alpha]_D^{25}$=+35 (c 0.20, $CHCl_3$); IR ($CHCl_3$, cm^{-1}):1720, 1735 (C=O); ^1H NMR ($CDCl_3$, δ): 2.66 (1H, qd, J=6.2, 10.2 Hz, 2-H), 3.14 (1H, d-like, J=10.2 Hz, 3-H), 3.32 (1H, t-like, J=2.2 Hz, 7-H), 3.39 (3H, s, 3-OCH_3), 3.43 (1H, dd, J=4.4, 10.0 Hz, 21-H), 3.57 (1H, dd, J=6.2, 9.9 Hz, 13-H), 3.88 (1H, dd, J=2.0, 11.5 Hz, 5-H), 3.98 (1H, m, 20-H), 4.03 (1H, d, J=2.9 Hz, 17-H), 4.47, 5.83 (each 1H, both d, J=15.9 Hz, CH_2Ar), 7.26–7.64 (5H, m, Ar); FABMS (m/z): 832 $(M+Na+1)^+$, 854 $(M+2Na)^+$. HRFABMS: Calcd for $C_{43}H_{65}O_{12}ClNa$: 831.4062 $(M+Na)^+$. Found: 831.4077.

2.1.14. Ion transport activity. The experiment was performed essentially according to the method reported by Pressman12a using a glass cell as shown in Fig. 2. The 3.5 mL of 0.25 mM test compound solution in water-saturated $CHCl_3$ was placed in the bottom of the cell (B). $CaCl_2$ or NaCl solution in 25 mM tricine buffer (A, 10 mM, 1.0 mL) at the corresponding pH and citrate solution (C, 10 mM, 1.0 mL) at pH 5.0 were placed on the $CHCl_3$. The pH of both water phase was adjusted by addition of Me_4NOH. The $CHCl_3$ layer was stirred at 200 rpm and 31°C. After 1.0 or 2.0 h, the solution in the layer C was taken, diluted 2.5 times for Ca^{2+} ion and 10 times for Na^+ ion with water, and measured the atomic absorption by Shimadzu AA-660 spectrometer at 422.7 nm for Ca^{2+} ion and 589.0 nm for Na^+ ion. The concentration of the Ca^{2+} and Na^+ ions was determined from the calibration lines made by measurement of the absorption of 1.0, 10, 20, 50, 80 and 100 μM $CaCl_2$ and NaCl solutions, respectively.

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