Studies on Chemical Modification of Monensin. VI.[1] Preparation of C-7 Modified Monensins *via* Protected 7-Oxomonensin and Evaluation of Their Ion Transport Activity

Akito NAGATSU (née NAKAMURA),[a] Yuichiro TABUNOKI,[a] Shin-ichi NAGAI,[a] Taisei UEDA,[a] Jinsaku SAKAKIBARA,*,[a] and Hiroyoshi HIDAKA[b]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] Tanabe-dori, Mizuho-ku, Nagoya 467, Japan and Nagoya University School of Medicine,[b] Tsurumai, Shouwa-ku, Nagoya 466, Japan.
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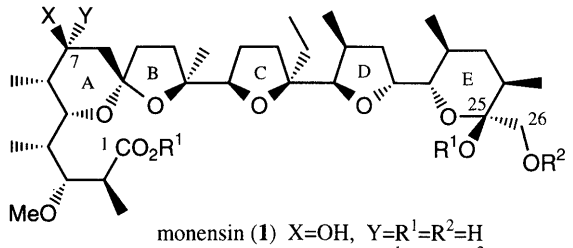
7-Carboxymethylmonensin (3), 7-aminomonensin (4), and 7-oxomonensin (5) were synthesized *via* protected 7-oxomonensin (2), which is expected to be a useful intermediate for the preparation of various monensin derivatives. Compounds 3—5 exhibited smaller ion transport activity than monensin (1).

Key words monensin; 7-oxomonensin; dicarboxylic monensin; aminomonensin; ion transport activity; ionophore

Monensin (**1**, Chart 1) is one of the monovalent polyether ionophores, which selectively transport Na^+ ion through biomembranes and exhibit various biological activities, such as antibacterial,[2] anticoccidial,[3] and cardiovascular[4] activities. In the course of chemical modifications of monensin (**1**), we have performed condensation of amino acids on the carboxyl group, macrolactonization, and substitution of the hydroxy group at C-7,[1,5] and obtained a compound which transported Na^+ ions through erythrocyte membrane twice as efficiently as **1**.[1] Tsukube *et al.* also synthesized monensin amides and their macrolactones as chiral receptors.[6] Modification of 26-OH was reported by Westley *et al.* to give 26-urethane derivatives.[7] However, more extensive structural modifications should give further information on the structure–ion transport and/or biological activity relationships of these classes of pseudocyclic ionophores. We therefore planned to convert the hydroxy group(s) in the molecule to carbonyl group(s), which would undergo various reactions. We describe here the preparation of protected 7-oxomonensin (**2**) as the key intermediate, and the reaction of **2** with a Wittig reagent, an amine, or aqueous alkali, affording 7-carboxymethylmonensin (**3**), 7-aminomonensin (**4**), and 7-oxomonensin (**5**), respectively. Since Na^+ and Ca^{2+} ions are similar to each other in size and we are interested in calcium ionophores, we also evaluated Ca^{2+} ion transport activity of the dicarboxylic derivative **3**.



monensin (**1**) X=OH, Y=R^1=R^2=H
protected 7-oxomonensin (**2**) X=Y=O, R^1=Me, R^2= Ac
7-carboxymethylmonensin (**3**) X=CH_2CO_2H, Y= R^1=R^2=H
7-aminomonensin (**4**) X=NH_2, Y= R^1=R^2=H
7-oxomonensin (**5**) X=Y=O, R^1=R^2=H

Chart 1

Chemistry

The synthetic course to protected 7-oxomonensin (**2**) is summarized in Chart 2. Monensin (**1**) was treated with MeOH in the presence of LiBr[8] and *n*-Bu_4NBr at room temperature to give the 25-methoxy derivative (**6**) and unreacted **1**. The ^{1}H-NMR spectrum of **6** showed the methyl signal of 25-OMe at δ 3.28 ppm. As compound **6** was difficult to separate from **1**, the esterification of the carboxyl group of the mixture was carried out by using MeI in the presence of K_2CO_3. In this reaction, only **6** was esterified to **7**, which was easily separated from the

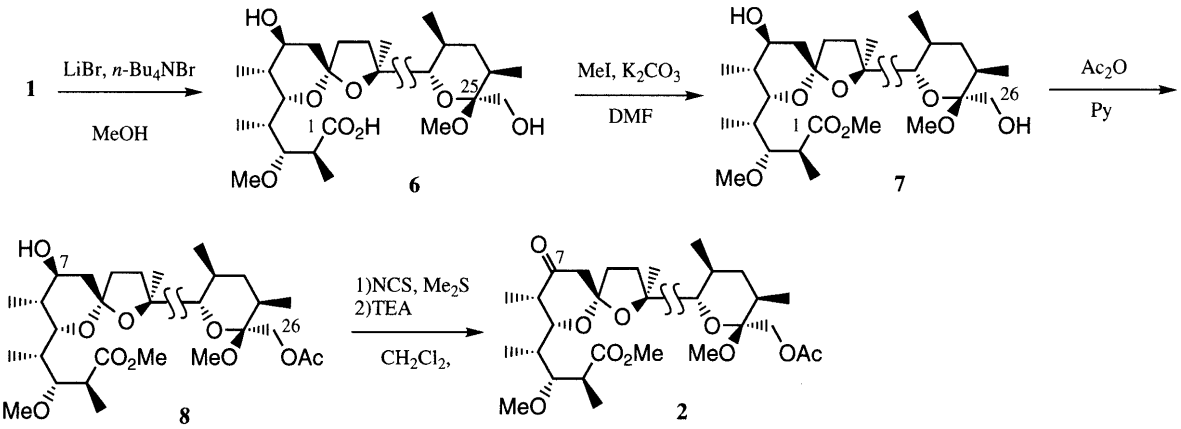


Chart 2

* To whom correspondence should be addressed.

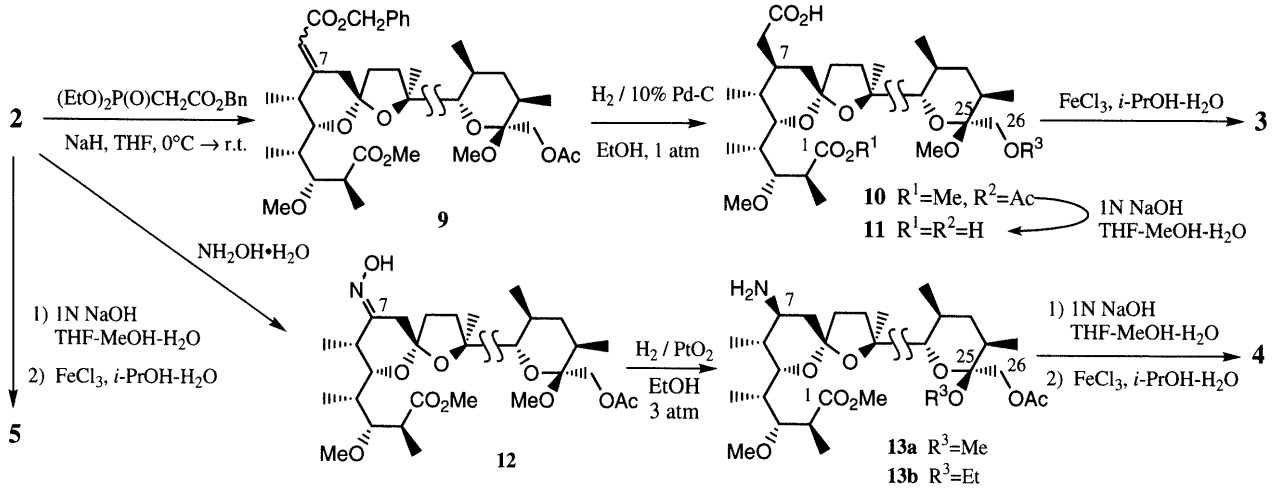


Chart 3

unreacted **1**. Then, 26-OH of **7** was protected as the acetate. The oxidation of the 7-OH group of **8** was successfully achieved by the Corey–Kim method.[9] *N*-Chlorosuccinimide (NCS) was reacted with dimethylsulfide in CH_2Cl_2 at 0 °C, followed by addition of **8** at −25 °C. After having been stirred for 3 h, the mixture was treated with triethylamine (TEA) to give the 7-oxo derivative (**2**) in 78% yield. In the ^{1}H-NMR spectrum of **2**, the signals of 6-CH and 8-CH_2 appeared downfield relative to those of **8**. The IR spectrum showed the absorption of ketone at 1650 cm^{-1}, and FAB-MS also supported the structure. This protected 7-oxomonensin (**2**) was used as the key compound in the following reactions.

The route to 7-carboxymethylmonensin (**3**) is shown in Chart 3. Wittig-Horner reaction of **2** with benzyl diethylphosphonoacetate afforded the olefinic derivative (**9**) as a mixture of *E* and *Z* isomers. In the ^{1}H-NMR spectrum of **9**, olefinic proton signals appeared at δ 5.60 and δ 5.80. Catalytic hydrogenation of olefin and hydrogenolysis of the benzyl ester of **9** gave **10** as a single isomer, which was confirmed by both TLC and ^{1}H-NMR examination. The methyl ester and the acetate were cleaved by alkaline hydrolysis to afford **11** (98%). The methyl signals of both ester and acetyl groups were absent in the ^{1}H-NMR spectrum of **11**. The acetal at the 25 position was changed to a hemiacetal (**3**) in 99% yield, affording the desired 7-carboxymethylmonensin (**3**).

7-Aminomonensin (**4**) was also prepared as shown in Chart 3. Compound **2** was treated with hydroxylamine to give the oxime (**12**). In the IR spectrum of **12**, an absorption due to the C=N bond appeared at 1520 cm^{-1}. Then the catalytic reduction of **12** in the presence of PtO_2 under 3 atm of hydrogen gave a mixture of **13a** and **13b**, which showed a positive ninhydrin test. Compound **13b** was supposed to have been formed by the replacement of the 25-OMe group with an ethoxyl group in the presence of EtOH. The FAB-MS of the mixture showed pseudomolecular peaks $(M+H)^+$ at 740 and 754, which supported the structures of **13a** and **13b**, respectively. The cleavage of the protecting groups at the 1, 25, and 26 positions of **13** was carried out as described for the deprotection of **8** to give the 7-amino derivative (**4**) as a single compound. The 7-H signal (δ 3.76) in the ^{1}H-NMR spectrum of **4** has the same shape as that of monensin (**1**), and C7 of **4** was finally confirmed to have identical configuration and conformation with those of **1**.

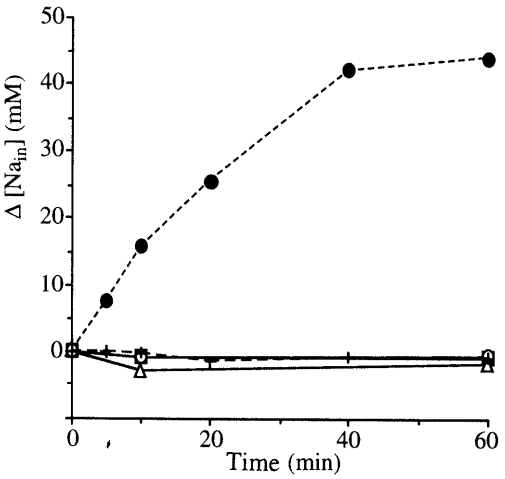


Fig. 1. Time Course of Increase of Intracellular Na^+ Ion Concentration ($[\Delta Na_{in}]$) Induced by **1** and **3**—**5**

---●---, **1**; —△—, **3**; —○—, **4**; —□—, **5**; --+--, DMSO (control).

Deprotection of **2** was also carried out to obtain 7-oxomonensin (**5**) by a similar method. The alkaline hydrolysis of the methyl ester and acetate, followed by deprotection of the methoxy group at the 25 position, however, led to **5** in low yield (6.5% from **2**).

Ion Transport Activity

The Na^+ ion transport activity of the monocarboxylic acids (**1**, **4**, **5**) through a biological membrane was elucidated by using the ^{23}Na-NMR spectroscopic method described before.[1,10] As shown in Fig. 1, compounds **4** and **5** transported only a little Na^+ ion at the concentration of 10^{-6} M.

The Ca^{2+} ion transport activity of the dicarboxylic acid (**3**) was measured by using the $CHCl_3$ liquid membrane system.[11] We used the U-tube system shown in Fig. 2, and the amount of Ca^{2+} ion transported from the ion-containing water phase into the pure water phase was determined by measuring the UV absorption of picrate, which was transported as the counter ion of Ca^{2+} ion.

We also measured the Ca^{2+} ion transport activity of lasalocid A (Fig. 3), a commercially available calcium ionophore, as a positive control. As shown in Fig. 4, the Ca^{2+} ion transport activity of 7-carboxymethylmonensin (**3**) was much lower than that of lasalocid A or monensin (**1**).

Discussion

We prepared protected 7-oxomonensin (**2**) in good yield as a synthetically useful intermediate of 7-substituted monensins. As described above, compound **2** can be used as a substrate of the Wittig reaction and gives the olefin in high yield. Since the reaction of **2** with hydroxylamine also proceeded smoothly to give the oxime, compound **2** seems to react easily with various primary amines. It is noteworthy that the reduction of the olefin and Schiff base gave only a single isomer. The stereoselectivity is probably a result of the pseudocyclic structure of the monensin skeleton and the chair conformation of A ring. As indicated in Fig. 5, the catalyst can probably approach only from the direction indicated by the arrow "a". Therefore, the reduction gave single isomers, such as **10** and **13**.

Compound **2**, however, was unstable under alkaline conditions. Presumably enolization of the 7-oxo group occurs, followed by cleavage of the spiroketal.

The ion transport activities of **3**—**5** were weaker than that of monensin (**1**), probably because of the lower lipophilicity of the molecules as compared with monensin (**1**) and lasalocid A. The *Rf* values of **3**—**5** were much smaller than those of **1** and lasalocid A. In the cases of **3** and **4**, the low lipophilicity is probably due to additional hydrophilic substituents (carboxyl or amino groups) at the periphery of the pseudocyclic conformation. In the case of 7-oxomonensin (**5**), the carbonyl group at C7 should cause conformational change of the A ring relative to monensin (**1**). As this change alters the direction of the B ring and the acyclic C1—C5 segment, it would lead to conformational change of the pseudocyclic structure, which may expose hydrophilic functions such as the terminal hydroxy and/or carboxyl groups to the outside. Thus, compound **5** shows lower lipophilicity.

In conclusion, we prepared the dicarboxylic compound 7-carboxymethylmonensin (**3**), as well as 7-aminomonensin (**4**) and 7-oxomonensin (**5**) *via* protected 7-oxomonensin (**2**) as the key intermediate. Although **3**—**5** showed low ion transport activity, compound **2** will be a useful intermediate to a large variety of monensin derivatives. Attempts to obtain other series of monensin derivatives are in progress.

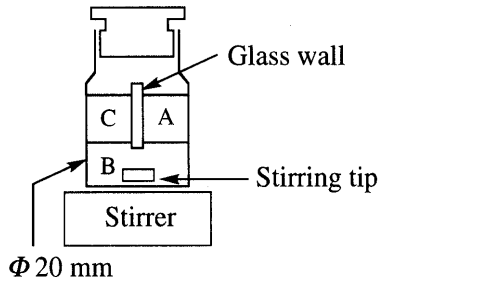


Fig. 2. Apparatus for Measurement of Ion Transport Activity by the $CHCl_3$ Liquid Membrane Method

The apparatus consists of a glass tube (20 mm i.d.) partly divided by a glass wall. The tube contained 1 ml of aqueous 7 mM calcium picrate. (A), 3.5 ml of 1 mM test compound solution in water-saturated $CHCl_3$ (B), and 1 ml of distilled water (C).

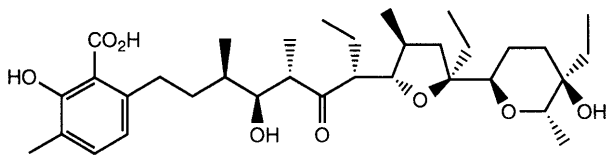


Fig. 3. Chemical Structure of Lasalocid A

Experimental

General All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The FAB-MS and high-resolution (HR) FAB-MS were measured with a 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, GSX-400 or α-500 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t,

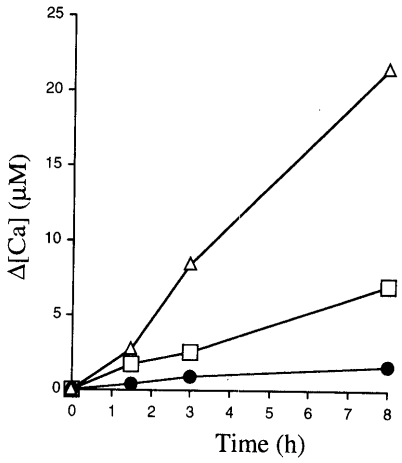


Fig. 4. Ca^{2+} Ion Transport Activity of **1**, **3**, and Lasalocid A —□—, **1**; —●—, **3**; —△—, lasalocid A.

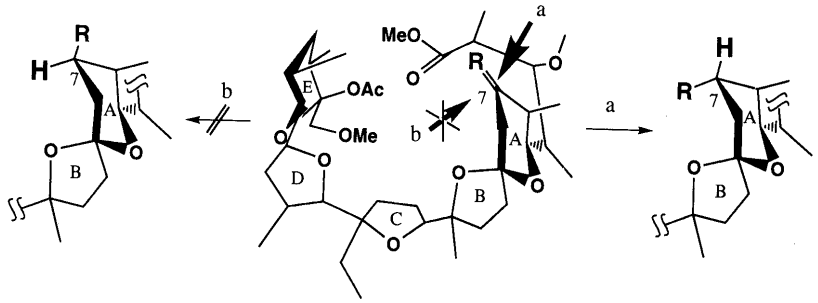


Fig. 5. Putative Reaction Face in Catalytic Hydrogenation of **8** and **12**

triplet; dd, doublet-of-doublets; td, triplet-of-doublets; qd, quartet-of-doublets; ddd, doublet-of-doublets-of-doublets; m, multiplet. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Medium-pressure liquid chromatography was carried out on a C.I.G. ODS-C_{18}-10/20 (22 mm i.d. × 100 mm, Kusano Kagakukikai Co.) using a Kusano KPW-20 pump and KU-331 UV detector. TLC was carried out on precoated plates (Kieselgel $60F_{254}$, 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 Davison Chemicals Co., Ltd.).

25-*O*-Methylmonensin Methyl Ester (7) A mixture of monensin (**1**, 1704 mg), $LiBr \cdot H_2O$ (26 mg), and *n*-Bu_4NBr (41 mg) in MeOH (75 ml) was stirred under an Ar atmosphere at room temperature. After 48 h, the reaction mixture was concentrated *in vacuo*, and the residue was chromatographed on silica gel ($CHCl_3$–acetone (4 : 1)) to give a mixture (1716 mg) of 25-*O*-methylmonensin (**6**) and unreacted **1**. A DMF solution (20 ml) of the mixture was treated with K_2CO_3 (380 mg) and methyl iodide (0.2 ml) and the whole was stirred for 70 min at room temperature. The reaction mixture was poured into $Na_2S_2O_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the residue, which was chromatographed on silica gel ($CHCl_3$–EtOAc (4 : 1)) to give **7**. (1285 mg, 74% from **1**).

6: Colorless needles, mp 168—169 °C (ether–hexane). $[\alpha]_D^{23}$: +48.6° (c = 0.25, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1710 (C=O). FAB-MS (m/z): 707 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.65 (1H, qd, J = 5.1, 7.0 Hz, 2-H), 3.28 (3H, s, 25-OCH_3), 3.37 (3H, s, 3-OCH_3), 3.48 (1H, dd, J = 3.1, 9.9 Hz, 3-H), 3.53, 3.70 (each 1H, both d, J = 11 Hz, 26-H_2), 3.56 (1H, t, J = 4.8 Hz, 13-H), 3.67 (1H, dd, J = 6.2, 9.2 Hz, 21-H), 3.78 (1H, m, 7-H), 3.93 (1H, d, J = 4.4 Hz, 17-H), 4.04 (1H, dd, J = 2.2, 9.5 Hz, 5-H), 4.30 (1H, td, J = 5.1, 8.8 Hz, 20-H).

7: Colorless syrup. $[\alpha]_D^{23}$: +68.8° (c = 0.26, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1720 (C=O). FAB-MS (m/z): 721 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.64 (1H, qd, J = 5.0, 6.9 Hz, 2-H), 3.27 (3H, s, 25-OCH_3), 3.34 (3H, s, 3-OCH_3), 3.47 (1H, dd, J = 5.0, 11.2 Hz, 3-H), 3.43—3.55 (3H, m, 3-H, 13-H, 26-H_2), 3.66 (1H, dd, J = 6.1, 9.1 Hz, 21-H), 3.71 (3H, s, 1-OCH_3), 3.75 (1H, m, 7-H), 3.91 (1H, d, J = 4.3 Hz, 17-H), 3.95 (1H, dd, J = 2.3, 9.2 Hz, 5-H), 4.07 (1H, d, J = 9.6 Hz, 26-H_2), 4.30 (1H, m, 20-H).

26-*O*-Acetyl-25-*O*-methylmonensin Methyl Ester (8) Acetic anhydride (1.7 ml) was added to a solution of **7** (1634 mg) in pyridine (20 ml) at 0 °C. After having been stirred for 24 h, the reaction mixture was diluted with AcOEt, and the organic layer was washed with 5% HCl, 10% $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on silica gel (ether–hexane (1 : 1)) to give **8** (1589 mg, 92%).

8: Colorless syrup. $[\alpha]_D^{23}$: +76.6° (c = 0.26, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1725 (C=O). FAB-MS (m/z): 763 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, 26-$OCOCH_3$), 2.65 (1H, qd, J = 5.3, 6.9 Hz, 2-H), 3.27 (3H, s, 25-OCH_3), 3.34 (3H, s, 3-OCH_3), 3.40 (1H, dd, J = 3.0, 9.6 Hz, 3-H), 3.51 (1H, t, J = 5.0 Hz, 13-H), 3.64 (1H, dd, J = 6.6, 8.6 Hz, 21-H), 3.71 (3H, s, 1-OCH_3), 3.75 (1H, m, 7-H), 3.93 (1H, dd, J = 2.0, 9.6 Hz, 5-H), 3.97 (1H, d, J = 4.0 Hz, 17-H), 4.08, 4.19 (each 1H, both d, J = 11.6 Hz, 26-H_2), 4.24 (1H, td, J = 3.3, 8.3 Hz, 20-H).

Oxidation of 8 Dimethylsulfide (0.85 ml) was added to a stirred solution of NCS (1275 mg) in CH_2Cl_2 at 0 °C and the mixture was cooled to −25 °C. After 5 min, a solution of **8** (1419 mg) in CH_2Cl_2 (15 ml) was added to the mixture over 40 min at −25 °C and the mixture was stirred at the same temperature. After 3 h, Et_3N (0.7 ml) was added, and stirring was continued for an additional 20 min. Then the cooling bath was removed and Et_2O (40 ml) was added to the mixture. After 20 min, the resulting mixture was poured into Et_2O, washed with 5% HCl and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane–Et_2O (1 : 1)) to give **2** (1102 mg, 78%).

26-*O*-Acetyl-7-dehydroxy-25-*O*-methyl-7-oxomonensin Methyl Ester (**2**): Colorless syrup. $[\alpha]_D^{23}$: +103.7° (c = 0.29, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1730, 1650 (C=O). FAB-MS (m/z): 761 $(M+Na)^+$. HR FAB-MS: Calcd for $C_{40}H_{66}NaO_{12}$: 761.4452 $(M+Na)^+$. Found: 761.4445. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, 26-$OCOCH_3$), 2.36, 2.75 (each 1H, both d, J = 14.5 Hz, 8-H_2), 2.60 (1H, qd, J = 5.0, 6.9 Hz, 2-H), 2.70 (1H, qd, J = 4.0, 8.6 Hz, 6-H), 3.26 (3H, s, 25-OCH_3), 3.34 (3H, s, 3-OCH_3), 3.39 (1H, dd, J = 3.0, 9.2 Hz, 3-H), 3.46 (1H, t, J = 5.9 Hz, 13-H), 3.62 (1H, dd, J = 6.3, 8.6 Hz, 21-H), 3.68 (3H, s, 1-OCH_3), 3.96 (1H, d, J = 4.0 Hz, 17-H), 4.01 (1H, dd, J = 3.6, 7.9 Hz, 5-H), 4.09, 4.19 (each 1H, both d, J = 11.6 Hz, 26-H_2), 4.22 (1H, td, J = 3.6, 8.9 Hz, 20-H).

26-*O*-Acetyl-7-benzyloxycarbonylmethylene-7-dehydroxy-25-*O*-methylmonensin Methyl Ester (9) A suspension of NaH (17 mg) and benzyl diethylphosphonoacetate (241 mg) in THF (2 ml) was stirred at 0 °C. After 30 min, a solution of **2** (104 mg) in THF (2 ml) was added to the suspension at 0 °C. Then the ice bath was removed and the mixture was stirred at room temperature for 18 h. It was poured into water and extracted with Et_2O. The Et_2O layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane–Et_2O (1 : 2)) to give **9** (117 mg, 95%) as a 5 : 2 mixture of geometrical isomers.

9: Colorless syrup. IR ($CHCl_3$, cm^{-1}): 1735 (C=O). FAB-MS (m/z): 893 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s, 26-$OCOCH_3$, major), 2.08 (3H, s, 26-$OCOCH_3$, minor), 3.26 (3H, s, 25-OCH_3, major), 3.27 (3H, s, 25-OCH_3, minor), 3.33 (3H, s, 3-OCH_3, major + minor), 3.62 (3H, s, 1-OCH_3, major), 3.66 (3H, s, 1-OCH_3, minor), 5.11 (2H, s, 7″-OCH_2, major), 5.14 (2H, s, 7″-OCH_2, minor), 5.60 (1H, d, J = 1.7 Hz, 7′-H, major), 5.80 (1H, d, J = 1.7 Hz, 7′-H, minor), 7.28—7.40 (5H, m, Ar-H, major + minor).

26-*O*-Acetyl-7-carboxymethyl-7-dehydroxy-25-*O*-methylmonensin Methyl Ester (10) A mixture of **9** (12.3 mg) and 10% Pd–C (15 mg) in EtOH (2 ml) was stirred under an H_2 atmosphere at room temperature and 1 atm for 3 h. The mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2–EtOAc (2 : 1)) to yield **10** (10.5 mg, 95%).

10: Colorless syrup. $[\alpha]_D^{23}$: +77.8° (c = 0.26, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1720 (C=O). FAB-MS (m/z): 805 $(M+Na)^+$, 827 $(M+2Na-1)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s, 26-$OCOCH_3$), 2.51—2.65 (2H, m, 2-H, 7′-H_2), 2.94 (1H, dd, J = 9.6, 14.85 Hz, 7′-H_2), 3.26 (3H, s, 25-OCH_3), 3.34 (3H, s, 3-OCH_3), 3.37—3.44 (2H, m, 3-H, 13-H), 3.62—3.67 (2H, m, 5-H, 21-H), 3.71 (3H, s, 1-OCH_3), 3.97 (1H, d, J = 3.6 Hz, 17-H), 4.10, 4.19 (26-H, each 1H, both d, J = 11.2 Hz), 4.23 (1H, m, 20-H).

Deprotection of 10 to 3 A solution of **10** (94 mg) in MeOH–THF (1 : 1, 6 ml) was treated with 5 N NaOH, and the mixture was stirred for 2 h at room temperature, then neutralized with 5% aqueous citric acid and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (MeOH–$CHCl_3$ (1 : 20)) to give **11** (87 mg, 98%).

Compound **11** (24 mg) was added to 0.01 mol/l $FeCl_3$ in iso-PrOH–H_2O (1 : 1), and the solution was stirred for 2 h at room temperature. The resulting solution was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (MeOH–$CHCl_3$ (1 : 10)) to give **3** (24.5 mg, 99%).

7-Carboxymethyl-7-dehydroxy-25-*O*-methylmonensin (**11**): Colorless crystalline powder, mp 72—75 °C (iso-Pr_2O–hexane). $[\alpha]_D^{23}$: +48.6° (c = 0.27, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1700 (C=O). FAB-MS (m/z): 749 $(M+Na)^+$, 771 $(M+2Na-1)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.43 (1H, dd, J = 3.1, 16.6 Hz, 7′-H_2), 2.53 (1H, m, 2-H), 3.11 (1H, dd, J = 11.5, 16.6 Hz, 7′-H_2), 3.28 (3H, s, 25-OCH_3), 3.34 (3H, s, 3-OCH_3), 3.47 (1H, dd, J = 3.5, 9.9 Hz, 3-H), 3.55, 3.70 (each 1H, both d, J = 11.2 Hz, 26-H_2), 3.62 (1H, dd, J = 6.8, 8.6 Hz, 13-H), 3.71—3.76 (2H, m, 5-H, 21-H), 3.95 (1H, d, J = 4.0 Hz, 17-H), 4.28 (1H, ddd, J = 3.7, 7.0, 9.0 Hz, 20-H).

7-Carboxymethyl-7-dehydroxymonensin (**3**): Colorless plates, mp 85—87 °C (iso-Pr_2O–hexane). $[\alpha]_D^{23}$: +63.8° (c = 0.29, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1700 (C=O). FAB-MS (m/z): 735 $(M+Na)^+$, 757 $(M+2Na-1)^+$, 779 $(M+3Na-2)^+$. HR-FAB-MS: Calcd for $C_{38}H_{64}NaO_{12}$: 735.4296 $(M+Na)^+$. Found: 735.4296. ^{1}H-NMR ($CDCl_3$) δ: 2.56 (1H, dd, J = 4.9, 14.3 Hz, 7′-H_2), 2.63 (1H, m, 2-H), 3.03 (1H, dd, J = 10.3, 14.3 Hz, 7′-H_2), 3.23 (1H, d like, 13-H), 3.38 (3H, s, 3-OCH_3), 3.54 (1H, dd, J = 6.1, 9.8 Hz, 3-H), 3.60, 3.62 (each 1H, both d, J = 11.0 Hz, 26-H_2), 3.73 (1H, dd, J = 2.1, 11.3 Hz, 5-H), 3.89 (1H, dd, J = 3.5, 10.4 Hz, 21-H), 4.09 (1H, d, J = 3.7 Hz, 17-H), 4.42 (1H, ddd, J = 2.1, 6.1, 10.4 Hz, 20-H).

26-*O*-Acetyl-7-dehydroxy-25-*O*-methyl-7-hydroxyiminomonensin Methyl Ester (12) A solution of **8** (56 mg), $NH_2OH \cdot HCl$ (8 mg) and anhydrous NaOAc (12.5 mg) in EtOH–H_2O (10 : 1, 11 ml) was refluxed for 2.5 h, then poured into the water and extracted with AcOEt. The AcOEt layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (AcOEt–$CHCl_3$ (1 : 5)) to give **12** (55 mg, 96%) as a 3 : 2 mixture of

geometrical isomers.

12: Colorless syrup. IR ($CHCl_3$, cm^{-1}): 1725 (C=O), 1520 (C=N). FAB-MS (*m/z*): 776 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s, 26-$OCOCH_3$, major), 2.08 (3H, s, 26-$OCOCH_3$, minor), 2.60 (1H, m, 2-H), 3.28 (3H, s, 3-OCH_3, major), 3.29 (3H, s, 25-OCH_3, minor), 3.33 (3H, s, 3-OCH_3, major), 3.36 (3H, s, 3-OCH_3, minor), 3.68 (3H, s, 1-OCH_3, minor), 3.70 (3H, s, 1-OCH_3, major), 3.92 (1H, d, J=4.6 Hz, 17-H, minor), 4.07 (1H, d, J=4.9 Hz, 17-H, major), 4.09, 4.21 (each 1H, both d, J=11.6 Hz, 26-H_2, major), 4.10, 4.19 (each 1H, both d, J=11.6 Hz, 26-H_2, minor), 4.23 (1H, m, 20H).

26-*O*-Acetyl-7-amino-7-dehydroxy-25-*O*-methylmonensin Methyl Ester (13a) and 26-*O*-Acetyl-7-amino-7-dehydroxy-25-*O*-ethylmonensin Methyl Ester (13b) A mixture of **12** (56 mg) and PtO_2 (60 mg) in EtOH (12 ml) was stirred under an H_2 atmosphere at 3 atm and room temperature for 24 h. The mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2–EtOAc (2:1)) to yield a 7:2 mixture of **13a** and **13b** (45 mg, 82%).

13a and **13b**: Colorless syrup. IR ($CHCl_3$, cm^{-1}): 3205 (N−H), 1725 (C=O). FAB-MS (*m/z*): 740 [**13a**: $(M+H)^+$], 754 [**13b**: $(M+H)^+$]. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s, 26-$OCOCH_3$ of **13a**), 2.08 (3H, s, 26-$OCOCH_3$ of **13b**), 2.68 (2H, m, 2H of **13b** and **13b**), 3.24 (3H, s, 25-OCH_3 of **13a**), 3.26 (3H, s, 25-OCH_3 of **13b**), 3.35 (3H, s, 3-OCH_3 of **13b**), 3.37 (3H, s, 3-OCH_3 of **13a**), 3.70 (3H, s, 1-OCH_3 of **13b**), 3.72 (3H, s, 1-OCH_3 of **13a**), 3.80 (1H, dd, J=1.7, 8.3 Hz, 5-H of **13a**), 3.96 (1H, d, J=3.6 Hz, 17-H of **13a**), 3.99 (1H, d, J=3.6 Hz, 17-H of **13b**), 4.04, 4.23 (each 1H, both d, J=11.5 Hz, 26-H_2 of **13a**), 4.05 (1H, d, J=11.5 Hz, 26-H_2 of **13b**), 4.19—4.27 (2H, m, 20-H of **13b** and 26-H_2 of **13b**).

Deprotection of 13 to 4 Compound **13** was similarly hydrolyzed by the procedure of deprotection of **10** to give **3** *via* a mixture of 7-amino-7-dehydroxy-25-*O*-methylmonensin (major) and 7-amino-7-dehydroxy-25-*O*-ethylmonensin (minor).

7-Amino-7-dehydroxy-25-*O*-methylmonensin: Yield, 55%. Colorless syrup. IR ($CHCl_3$, cm^{-1}): 3200 (N-H), 1710 (C=O). FAB-MS (*m/z*): 684 [major: $(M+H)^+$], 698 [minor: $(M+H)^+$], 706 [major: $(M+Na)^+$], 720 [minor: $(M+Na)^+$]. ^{1}H-NMR ($CDCl_3$) δ: 2.53 (1H, m, 2-H), 3.27 (3H, s, 25-OCH_3, major), 3.35 (3H, s, 3-OCH_3), 3.93 (1H, d, J=4.0 Hz, 17-H, minor), 3.96 (1H, d, J=4.0 Hz, 17-H, major), 4.49 (1H, m, 20-H).

7-Amino7-dehydroxymonensin (**4**): Yield, 62%. Colorless crystalline powder, mp 107—109 °C (ether–hexane). $[\alpha]_D^{23}$: +101.5° (c=0.34, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 3200 (N−H), 1710 (C=O). FAB-MS (*m/z*): 692 $(M+Na)^+$, 714 $(M+2Na-1)^+$. HR-FAB-MS: Calcd for $C_{36}H_{63}NNaO_{10}$: 692.4349 $(M+Na)^+$. Found: 692.4354. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 2.61 (1H, qd, J=3.1, 6.8 Hz, 2-H), 3.23 (1H, dd, J=2.2, 9.9 Hz, 3-H), 3.38 (3H, s, 3-OCH_3), 3.49, 3.79 (each 1H, both d, J=11.5 Hz, 26-H_2), 3.57 (1H, dd, J=5.3, 10.3 Hz, 13-H), 3.76 (1H, m, 7-H), 3.89 (1H, dd, J=1.7, 10.8 Hz, 13-H), 3.94 (1H, dd, J=1.5, 8.6 Hz, 5-H), 4.14 (1H, d, J=8.6 Hz, 17-H), 4.44 (1H, ddd, J=2.7, 5.9, 10.6 Hz, 20-H).

Deprotection of 2 to 5 Compound **2** was similarly hydrolyzed by the same procedure as used for the deprotection of **10** to give **5** *via* 7-dehydroxy-25-*O*-methyl-7-oxomonensin.

7-Dehydroxy-25-*O*-methyl-7-oxomonensin: Yield, 12%. Colorless prisms, mp 83—85 °C (iso-Pr_2O–hexane). $[\alpha]_D^{23}$: +2.1° (c=0.20, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1720, 1660 (C=O). FAB-MS (*m/z*): 705 $(M+Na)^+$, 727 $(M+2Na-1)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.56, 2.64 (each 1H, both d, J=13.5 Hz, 8-H_2), 2.74 (1H, qd, J=2.0, 6.9 Hz, 6-H), 3.29 (3H, s, 26-OCH_3), 3.38 (3H, s, 3-OCH_3), 3.47 (1H, dd, J=3.6, 9.9 Hz, 3-H), 3.55, 3.73 (each 1H, both d, J=11.2 Hz, 26-H_2), 3.70 (1H, dd, J=5.8, 9.7 Hz, 13-H), 3.86 (1H, dd, J=2.0, 9.2 Hz, 21-H), 3.90 (1H, d, J=4.0 Hz, 17-H), 4.06 (1H, d like, 5-H), 4.29 (1H, m, 20-H).

7-Dehydroxy-7-oxomonensin (**5**): Yield, 55%. Colorless plates, mp 75—78 °C (iso-PrO_2–hexane). $[\alpha]_D^{23}$: +52.7° (c=0.18, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 1710, 1630 (C=O). FAB-MS (*m/z*): 691 $(M+Na)^+$. HR-FAB-MS: Calcd for $C_{36}H_{60}NaO_{11}$: 691.4034 $(M+Na)^+$. Found: 691.4058. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (1H, m, 2-H), 2.57, 2.65 (each 1H, both d, J=13.4 Hz, 8-H_2), 2.88 (1H, m, 6-H), 3.40 (3H, s, 3-OCH_3), 3.53 (1H, d, J=11.0 Hz, 26-H_2), 3.60—3.65 (2H, m, 3-H, 26-H_2), 3.79—3.87 (2H, m, 13-H, 21-H), 3.83 (1H, d, J=4.3 Hz, 17-H), 3.99 (1H, d like, 5-H), 4.28 (1H, m, 20-H).

Measurement of Na^+ Ion Transport Activity through Erythrocyte Membrane The measurement was performed using the method we described before,[1,10] except for the shift reagent. Dysprosium 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetramethylene-phosphonate was used as the shift reagent for the extracellular Na^+ ion signal.[12]

Measurement of Ca^{2+} Ion Transport Activity through $CHCl_3$ Liquid Membrane The experiment was performed essentially according to Cram *et al.*[11a] using a glass cell as shown in Fig. 2. The ionophore in $CHCl_3$ (1 mM, 3.5 ml) was placed in the bottom of the cell. (B) The calcium picrate solution in water (A, 7 mM, 1 ml) and distilled water (C, 1 ml) were placed on the $CHCl_3$ phase. The $CHCl_3$ phase was constantly stirred at 225 rpm and 26 °C. After 1.5, 3, and 8 h, 50 μl of each phase was sampled. The transported amount of calcium ion was determined by measuring the concentration of picrate in the receiving aqueous phase, based on the UV–VIS absorbancy at 380 nm of the sampled solution diluted 20 times. The extinction coefficient (ε) of calcium picrate was determined by using a 0.07 mM standard solution (ε 16600 $M^{-1}cm^{-1}$).

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