Studies on the Chemical Modification of Monensin. IV. Synthesis, Sodium Ion Permeability, and Biological Activity of 7-*O*-Acyl- and 7-*O*-Alkylmonensins

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7-*O*-Acyl-(4a—e) and 7-*O*-alkylmonensins (5a—d) were prepared from monensin (1). Their lipophilicity, sodium ion permeability in human erythrocytes, antibacterial activity and effect on rat tail artery were examined. There was a correlation between lipophilicity and sodium ion permeability as well as between lipophilicity and antibacterial activity. We also found that the compound having larger sodium ion permeability, showed stronger contraction of rat tail artery. 7-*O*-Benzylmonensin (5c) exhibited higher lipophilicity and larger sodium ion permeability than monensin (1) among the tested monensin derivatives. In addition, antibacterial activity and contractile effect on rat tail artery of 5c were comparable to those of 1.

Keywords monensin; 7-*O*-acylmonensin; 7-*O*-alkylmonensin; lipophilicity; Na^+ ion permeability; antibacterial activity; contractile effect; 7-*O*-benzylmonensin

Monensin (**1**, Fig. 1) is a monovalent carboxylic polyether ionophor. The structure of the Na^+ salt of monensin (**1**) has a lipophilic exterior and a hydrophilic central cavity lined with oxygen atoms which serve as ligands for the encapsuled Na^+ ion. The molecule as a whole is therefore neutral and lipophilic enough to transport Na^+ ions across lipophilic biomembranes.[1] These abilities result in a variety of biological activities such as antibiotic activity,[2] anticoccidial activity,[3] neurogenic contractile effect on smooth muscle,[4] and vascular smooth muscle relaxation.[5]

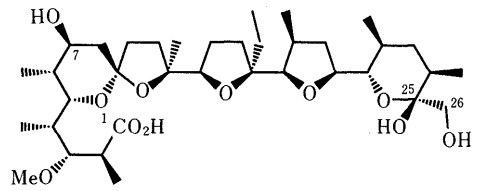


Fig. 1. Chemical Structure of Monensin (**1**)

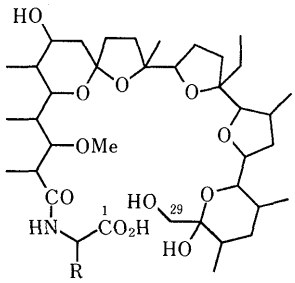
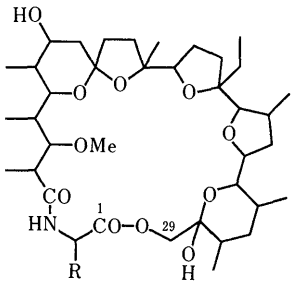


Fig. 2. Monensylamino Acids (**2**)

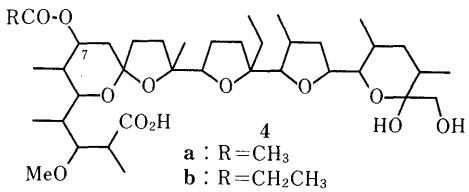


3

a : R=H
b : R=CH_3
c : R=CH_2Ph
d : R=$CH_2C_6H_4OH$
e : R=CH_2CO_2H
f : R=$CH_2CH_2CO_2H$

Chart 1

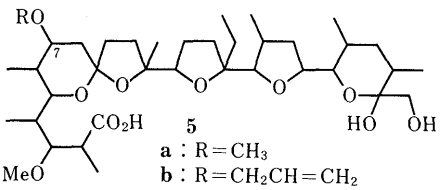
We have recently reported the chemical modification of monensin (**1**) to monensylamino acids (**2**, Fig. 2),[6] which show smaller Na^+ ion permeability than **1** due to their lower lipophilicity.[7] We then synthesized macrocyclic monensylamino acid-1,29-lactones (**3**, Chart 1).[8] Although compounds **3a—d** were more lipophilic than **1**, they had no Na^+ ion permeability. In contrast, the Na^+ ion transport activity of compound **3e** was greater than that of **1** in a $CHCl_3$ liquid membrane, though **3e** did not exhibit any Na^+ ion permeability or biological activity, probably due to its less lipophilic character. These findings suggested that the preparation of more lipophilic monensin analogues whose carboxylic group remains unchanged, might lead to the enhancement of Na^+ ion permeability and biological activity. From the X-ray analysis of the NaBr complex of monensin, the hydroxy group at C-7 is known to be appended to the periphery of the macrocyclic molecule.[9] Thus we have been interested in the chemical modification of the C-7 hydroxy group with a variety of lipophilic acyl and alkyl groups. In this paper, we will report the synthesis, Na^+ ion permeability,



4

a : R=CH_3
b : R=CH_2CH_3
c : R=$CH_2CH_2CH_3$
d : R=$(CH_2)_6CH_3$
e : R=Ph

Chart 2



5

a : R=CH_3
b : R=$CH_2CH=CH_2$
c : R=CH_2Ph
d : R=$CH_2CH_2CH_3$

Chart 3

and biological activity of 7-*O*-acyl-(**4a**—**e**) and 7-*O*-alkylmonensins (**5a**—**d**) as shown in Charts 2 and 3.

Results

Chemistry The general synthetic pathway to 7-*O*-acylmonensins (**4a**—**e**) is outlined in Chart 4. The primary and tertiary hydroxy groups at C-26 and C-25 of **1** were protected simultaneously as the *p*-methoxybenzylideneacetal according to Oikawa's method[10] to give 25,26-(4-methoxybenzylidene)monensin (**6**) in 95% yield. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **6** revealed that it was a 3:1 diastereomeric mixture at the asymmetric acetal carbon. The two isomers could be separated by high performance liquid chromatography (HPLC). The acylation of C-7 hydroxy group, however, was carried out on the mixture of the two isomers, because the 25,26-*O*,*O*-acetal group is to be removed at the final step of the synthetic pathway.

The acylation of the C-7 hydroxy group was easily accomplished by treatment of **6** with the corresponding acid anhydride in the presence of dimethylaminopyridine (DMAP) to provide 7-*O*-acylated derivatives (**7a**—**d**) in 86—92% yields (Table I). In the ^{1}H-NMR spectrum, the signal of 7-H appeared 0.97—1.00 ppm downfield relative to that of **6**, suggesting the complete acylation of the C-7 hydroxy group. A similar acylation of **6** with benzoic anhydride, however, failed to give 7-*O*-benzoylmonensin (**7e**). Thus the C-1 carboxylic group was converted to the benzyl ester (**8**), which was treated with benzoyl chloride followed by catalytic debenzylation of **9** to yield 7-*O*-benzoylmonensin (**7e**) in an overall yield of 86% from **6**. The *p*-methoxybenzylidene acetals of **7a**—**e** were readily cleaved by acid catalyst to give 7-*O*-acylmonensins (**4a**—**e**) in 67—75% yield. The structures of **4a**—**e** were confirmed by the ^{1}H-NMR spectra, from which the signals due to the acetals had disappeared completely.

Table I. 7-*O*-Acyl-25,26-(4-methoxybenzylidene)monensins (**7**) and 7-*O*-Acylmonensins (**4**)

	$(RCO)_2O$ or RCOCl	Reaction time (h)	Yield (%)	
			7	**4**
a	Ac_2O	7	92	72
b	$(CH_3CH_2CO)_2O$	7	90	70
c	$(CH_3CH_2CH_2CO)_2O$	8	86	75
d	$(CH_3(CH_2)_6CO)_2O$	8	90	70
e	PhCOCl	12[a]	86[b]	67

a) Reaction time of acylation from **8** to **9**. *b*) Yield from **6** *via* **8** and **9**.

Synthesis of 7-*O*-alkylmonensins (**5a**—**d**) was carried out as outlined in Chart 5. Compound **6** was converted to the corresponding methyl ester (**10**) in 92% yield, and **10** was heated with NaH at 50 °C for 30 min followed by treatment with MeI at room temperature to yield a mixture of the desired 7-*O*-methyl derivative (**11a**) and the olefinic derivative (**12a**) in a ratio of 10:1. In the ^{1}H-NMR spectrum of **11a**, a new signal due to the 7-*O*-methoxy group appeared at δ 3.26 ppm, and the 7-H signal was observed 0.49 ppm upfield compared with that of **10**. In contrast, the ^{1}H-NMR spectrum of **12a** showed the disappearance of 2-H and the appearance of the olefinic 3-H at δ 6.60, which indicated that **12a** is the *E* isomer. Since it was difficult to separate **11a** and **12a** by column chromatography, hydrolysis of the methyl ester of the mixture followed by cleavage of the *p*-methoxybenzylidene acetal group gave the desired 7-*O*-methylmonensin (**5a**) after separation of the reaction mixture by HPLC. 7-*O*-Allylation and benzylation of **10** were similarly performed using the corresponding bromides and iodides. Allylation of **10** with allyl iodide in place of allyl bromide was found to proceed in a shorter reaction time and in better yield (Table II) to give the 7-*O*-allyl derivatives (**11b**). In the case of preparation of 7-*O*-benzyl derivatives (**11c**), benzyl iodide also gave a better result than benzyl bromide. 7-*O*-Propylmonensin was obtained quantitatively by the catalytic hydrogenation of 7-*O*-allylmonensin (**5b**).

We finally measured the Rm_{50} values[9] of monensin (**1**) and 7-*O*-substituted monensins (**4a**—**e**, **5a**—**d**) as parameters of lipophilicity. The compounds having bulky acyl and alkyl groups showed increased lipophilicity. In particular, compound **5c** was more lipophilic than monensin (**1**), as shown in Table III.

Na^+ Ion Permeability We determined the Na^+ ion permeability of the synthesized compounds using human erythrocytes suspended in a medium containing Na^+ (140 mM), K^+ (5 mM), and test compound (10^{-5} mM). The

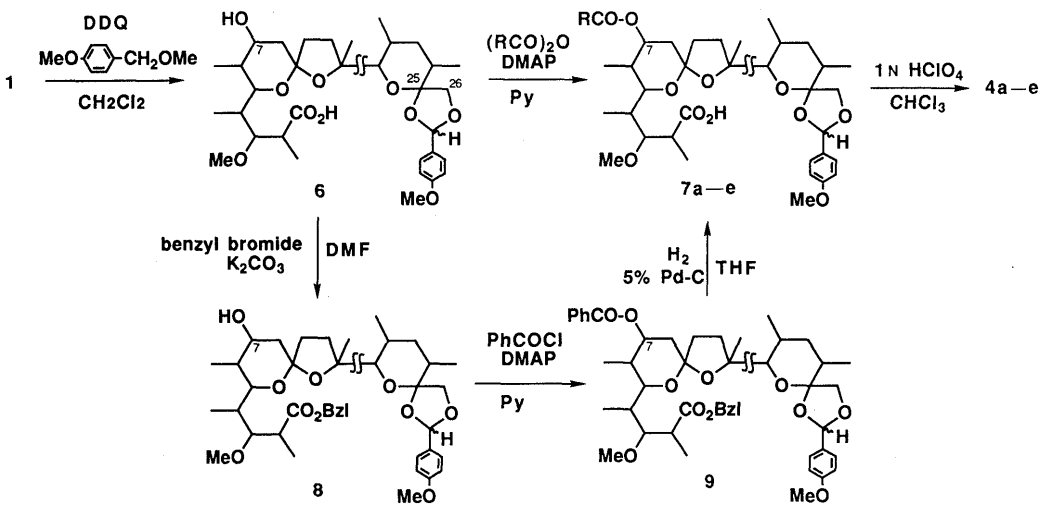


Chart 4

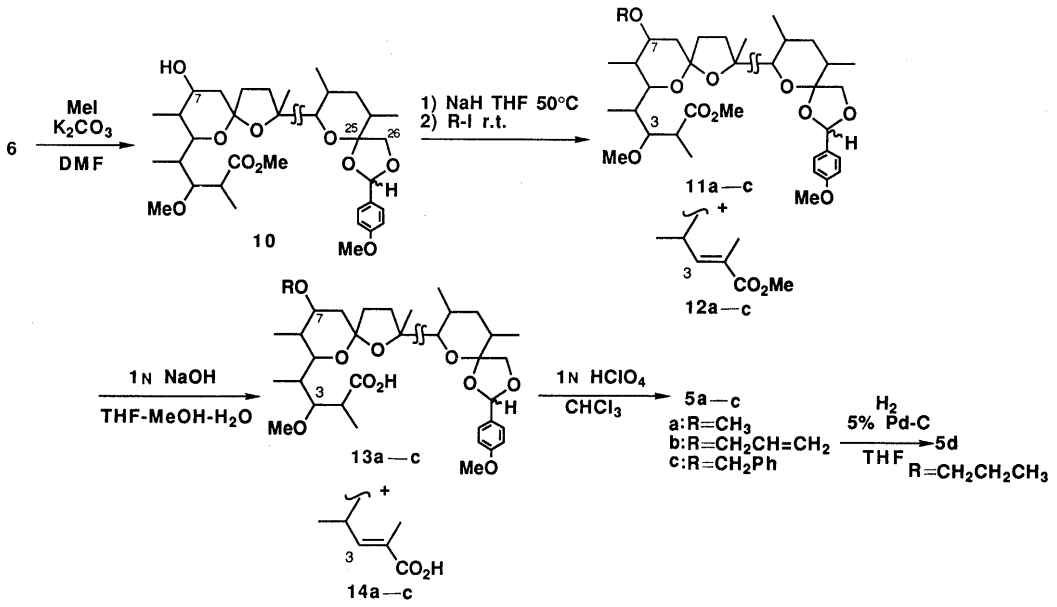


Chart 5

Table II. Reaction Times and Yields of 7-*O*-Alkylation

R–X	Reaction time (h)	Yield of **11**+**12** (%)	Ratio[a] of **11**:**12**
Me–I	3	91	10:1
Allyl–I	3	88	7:1
Allyl–Br	17	83	3:1
Benzyl–I	4	86	7:1
Benzyl–Br	24	80	2:1

a) The ratio was determined by ^{1}H-NMR.

Table III. Rm_{50} Values of Monensin (**1**), 7-*O*-Acylmonensins (**4**) and 7-*O*-Alkylmonensins (**5**)

Compound	Rm_{50}	Compound	Rm_{50}
1	1.83		
4a	0.62	**5a**	1.09
4b	0.77	**5b**	1.55
4c	0.92	**5c**	2.01
4d	1.55	**5d**	1.45
4e	1.01		

intracellular Na^+ ion concentration ($[Na^+_{in}]$) was measured by the ^{23}Na-NMR spectroscopic method developed in our laboratory.[7] Dimethylsulfoxide (DMSO) used as a solvent for the test compounds had no influence on $[Na^+_{in}]$. Although **4b** showed the greatest increase of $[Na^+_{in}]$ among the 7-*O*-acylmonensins (**4a**—**e**), monensin (**1**) and 7-*O*-alkylmonensins (**5a**—**d**) exhibited much larger rates of initial increase of $[Na^+_{in}]$ within 0—5 min (Fig. 3). So we selected monensin (**1**) and compounds **5a**—**d**, and measured $[Na^+_{in}]$ in a medium containing 10^{-6} M test compound. As shown in Fig. 4, 7-*O*-benzylmonensin (**5c**) gave a much larger initial increase than monensin (**1**).

Biological Activity The values of minimum inhibitory concentration against various bacteria were measured. Compounds **4d** and **5a**—**d** showed antibacterial activity (Table IV). 7-*O*-Benzylmonensin (**5c**) was most active, its activity being comparable to that of monensin (**1**).

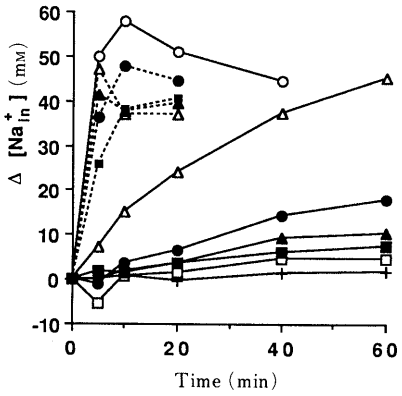


Fig. 3. Time Course of $\Delta[Na^+_{in}]$ Induced by 10^{-5} M Monensin (**1**), 7-*O*-Acylmonensins (**4**), and 7-*O*-Alkylmonensins (**5**)

1, —○—; DMSO, —+—; **4a**, —●—; **4b**, —△—; **4c**, —▲—; **4d**, —□—; **4e**, —■—; **5a**, --●--; **5b**, --△--; **5c**, --▲--; **5d**, --□--.

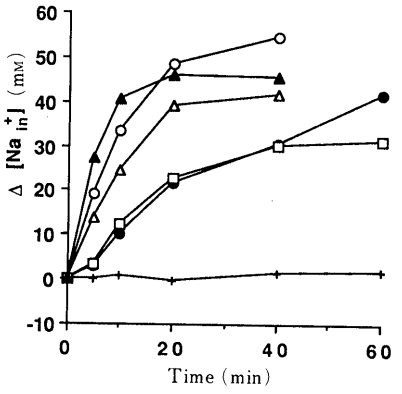


Fig. 4. Time Course of $\Delta[Na^+_{in}]$ Induced by 10^{-6} M Monensin (**1**) and 7-*O*-Alkylmonensins (**5**)

1, ○; DMSO, +; **5a**, ●; **5b**, △; **5c**, ▲; **5d**, □.

We have also examined the effects of the synthesized compounds on rat tail artery preparation, which seemed to be most suitable for the observation of contraction and

TABLE IV. Minimum Inhibitory Concentrations of Monensin (**1**), 7-*O*-Acylmonensins (**4**), and 7-*O*-Alkylmonensins (**5**)

Organisms	MIC (ppm)									
	1	**4a**	**4b**	**4c**	**4d**	**4e**	**5a**	**5b**	**5c**	**5d**
Anaerobic bacteria										
Peptococcus anaerobius B-40	0.78	6.25	25	3.13	3.13	3.13	1.56	1.56	3.13	6.25
Peptostreptococcus anaerobius B-30	3.13	50	>50	25	6.25	12.5	12.5	12.5	6.25	12.5
Propionibacterium acnes ATCC 11828	3.13	50	>50	12.5	6.25	12.5	6.25	6.25	3.13	12.5
Eubacterium entum Beerens 515	1.56	25	>50	12.5	6.25	6.25	6.25	6.25	3.13	12.5
Lactobacillus acidophilus ATCC 4356	3.13	>50	>50	25	25	25	6.25	12.5	6.25	25
Lactobacillus saivarius ATCC 11742	6.25	>50	>50	12.5	6.25	6.25	6.25	12.5	6.25	12.5
Clostridium perfringens 7-heart	12.5	>50	>50	>50	6.25	12.5	25	25	6.25	50
Aerobic bacteria										
Staphylococcus aureus FDA 209P	3.13	>50	>50	50	12.5	50	12.5	12.5	6.25	25
Staphylococcus aureus 308A-1	3.13	>50	>50	50	12.5	>50	12.5	12.5	6.25	25
Staphylococcus epidermidis IFO 3762	3.13	>50	>50	>50	12.5	>50	12.5	25	12.5	25
Staphylococcus epidermidis IFO 12993	6.25	>50	>50	50	6.25	>50	12.5	12.5	12.5	25
Bacillus subtilis ATCC 6633	25	>50	>50	50	12.5	50	25	25	6.25	25
Bacillus subtilis PCI 219	25	>50	>50	50	12.5	50	25	25	6.25	25

MIC, minimum inhibitory concentration.

TABLE V. Contraction of Rat Tail Artery Induced by Monensin (**1**), 7-*O*-Propanoylmonensin (**4b**), and 7-*O*-Alkylmonensins (**5a—d**)

Compound	*n*	% of maximum contraction
1	10	42.1±4.0[a]
4b	2	23.6, 20.7
5a	3	42.4±4.3[a]
5b	3	45.9±1.3[a]
5c	3	37.2±0.8[a]
5d	3	27.4±4.3[a]

a) Each value represents the mean±S.E.

relaxation of smooth muscle. Monensin (**1**) caused 42.1% of the maximum contraction, and this effect was comparable to those of **5a—c** (Table V). Compounds **4b** and **5d** exhibited weaker contraction than **1** and **5a—c**. None of the compounds showed a marked relaxing effect on the rat tail artery.

Discussion

The synthesis of 7-*O*-acyl- (**4a—e**) and 7-*O*-alkylmonensins (**5a—d**) was accomplished in satisfactory yield by employing a combination of protecting groups for the hydroxy groups. The use of alkyl iodides as the alkylating reagent for **10** was found to complete the reaction in a shorter time and therefore to decrease the formation of undesired olefinic monensins (**12a—c**).

Lipophilicity is known to play an important role in the penetration of a compound into the cell membrane. Consequently, we investigated the correlation between Rm_{50} values and logarithmic initial increase of $[Na^+_{in}]$ (V_i) of **1**, **4b**, and **5a—d**, which caused large changes of $[Na^+_{in}]$. As can be seen from Fig. 5, a good correlation was observed, suggesting that higher lipophilicity of the compounds could result in faster Na^+ ion influx into the cells.

There was also a correlation between lipophilicity and antibacterial activities. Lipophilicity decreased in the order **5c**>**1**>**4d** and **5b**>**5d**>**5a**>**4e**>**4c**>**4b**>**4a**, while antibacterial activity decreased in the order **5c** and **1**>**4d** and **5a, b, d**>**4c, e**>**4a, b**. In contrast, there was no correlation between Na^+ ion permeability and antibacterial activity.

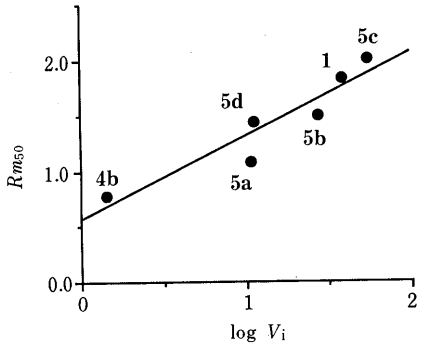


Fig. 5. Relation between Rm_{50} and $\log V_i$ for Monensin (**1**), 7-*O*-Propanoylmonensin (**4b**), and 7-*O*-Alkylmonensins (**5a—d**)

Values of $\log V_i$ of **1** and **5b, c** within 0—5 min and of **4b** and **5a, d** within 0—20 min are indicated. The line shows the correlation for **1**, **4b**, and **5a—d**. $Rm_{50}=0.75\log V_i+0.57$ ($r=0.937$, $p<0.05$).

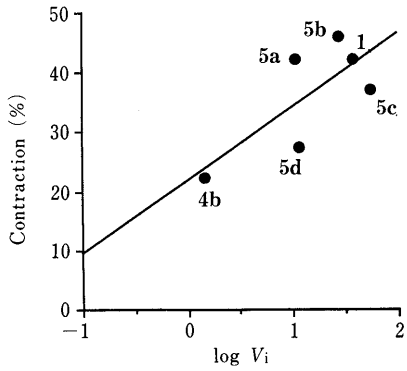


Fig. 6. Relation between $\log V_i$ and Contraction (% of Maximum Contraction) of Rat Tail Artery

Contraction (%)$=12.32\log V_i+21.82$ ($r=0.75$, $p<0.1$)

Furthermore, we investigated the correlation between V_i values and the contraction of rat tail artery. Figure 6 illustrates that a larger V_i value tends to be associated with increased contraction. Compounds **4a, c—e** having smaller V_i value than **4b**, did not cause contraction. These findings

suggest that the contractile effect might depend on the initial rate of increase of $[Na^+_{in}]$. This contractile effect of monensin (**1**) was not observed when the artery was pretreated with phentolamine (10^{-5} M), implying that contraction may have been induced by norepinephrine released from sympathetic nerve terminals by virtue of the increase of intracellular Na^+ ion.

In conclusion, we found that there was a correlation between lipophilicity and Na^+ ion permeability as well as between lipophilicity and antibacterial activity. The lipophilicity, therefore, seems a very convenient parameter to predict antibacterial activities of various monensin derivatives. Moreover, the V_i value of the monensin derivatives can be used to predict the contractile effect on the rat tail artery. Of the 7-*O*-substituted monensins evaluated for various activities, 7-*O*-benzylmonensin (**5c**) showed a higher lipophilicity and larger V_i value than monensin (**1**), resulting in the appearance of antibacterial and contractile activities comparable to those of **1**. Our forthcoming paper will focus on further elaboration of **5c** analogues.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The fast atom bombardment mass spectra (FAB-MS) were measured with a JEOL DX-300 mass spectrometer and high-resolution (HR) FAB-MS with a JEOL JMS-AX505HA. The infrared (IR) spectra were measured with a JASCO IRA-2 spectrometer. The ^{1}H-NMR spectra were recorded with a JEOL GSX-400 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dq, quartet of doublets; m, multiplet. Column chromatography was carried out on Silica gel BW-200 (Fuji Davison Chemicals, Ltd.). High performance liquid chromatography (HPLC) was carried out on C.I.G. ODS-C_{18}-10/20 (22 mm i.d. × 100 mm, Kusano Kagakukikai Co.).

25,26-(4-Methoxybenzylidene)monensin (6) A solution of monensin (**1**, 300 mg), *p*-methoxybenzyl methyl ether (400 mg) and 2,3-dichloro-5,6-dicyanobenzoquinone (300 mg) in CH_2Cl_2 (5 ml) was stirred for 1 h at room temperature and filtered. The filtrate was washed with 10% $NaHCO_3$ solution and NaCl solution. After being dried over $MgSO_4$, the CH_2Cl_2 layer was evaporated to dryness. The residue was chromatographed on silica gel (hexane–acetone) to give a syrup (335 mg, 95%) as a 3 : 1 diastereomeric mixture of **6** (free acid), which was subsequently separated by HPLC. **6**: Major syrup: $[\alpha]_D^{27}$ +30.1° (c=0.31, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1720 (C=O). HRFAB-MS m/z: 811.4590 $(M+Na)^+$, Calcd for $C_{44}H_{68}NaO_{12}$: 811.4610. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.65 (1H, dq, J=6.6 Hz, 2-H), 3.37 (3H, s, 3-OCH_3), 3.75 (1H, m, 7-H), 3.79, 4.09 (each 1H, both d, J=9.0 Hz, 26-H_2), 3.81 (3H, s, Ar-OCH_3), 5.88 (1H, s, >CH-Ar), 6.90, 7.44 (each 2H, both d, J=9 Hz, Ar-H). Minor syrup: $[\alpha]_D^{27}$ +46.6° (c=0.30, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1720 (C=O). FAB-MS m/z: 811 $(M+Na)^+$, 833 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.67 (1H, dq, J=6.7 Hz, 2-H), 3.37 (3H, s, 3-OCH_3), 3.75 (1H, m, 7-H), 3.81 (3H, s, Ar-OCH_3), 3.97, 4.00 (each 1H, both d, J=9.0 Hz, 26-H_2), 5.95 (1H, s, >CH-Ar), 6.68, 7.48 (each 2H, both d, J=9.0 Hz, Ar-H).

General Procedure for Preparations of 7-*O*-Acyl-25,26-(4-methoxybenzylidene)monensins (7a—d) To a solution of **6** (a 3 : 1 diastereomeric mixture, 50 mg) in pyridine (1.5 ml) were added a acid anhydride (0.1 ml) and DMAP (1 mg). The mixture was stirred at room temperature. After addition of 10% Na_2CO_3 solution, the mixture was extracted with AcOEt. The extract was washed with 1 N HCl, 10% $NaHCO_3$ and NaCl solution, then dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel (hexane–acetone) to give the free acids of **7a—d** as syrups. Reaction times and yields are summarized in Table I. **7a**: 7-*O*-Acetyl-25,26-(4-methoxybenzylidene)monensin, IR (KBr) ν_{max} cm^{-1}: 1710, 1730 (C=O). FAB-MS m/z: 853 $(M+Na)^+$, 875 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 4.72 (1H, m, 7-H). **7b**: 7-*O*-Propanoyl-25,26-(4-methoxybenzylidene)monensin, IR (KBr) ν_{max} cm^{-1}: 1710, 1730 (C=O). FAB-MS m/z: 867 $(M+Na)^+$, 889 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 4.73 (1H, m, 7-H). **7c**: 7-*O*-Butanoyl-25,26-(4-methoxybenzylidene)monensin, IR (KBr) ν_{max} cm^{-1}: 1710, 1730 (C=O). FAB-MS m/z: 881 $(M+Na)^+$, 9.03 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 4.75 (1H, m, 7-H). **7d**: 7-*O*-Octanoyl-25,26-(4-methoxybenzylidene)-monensin, IR (KBr) ν_{max} cm^{-1}: 1700, 1730 (C=O). FAB-MS m/z: 937 $(M+Na)^+$, 959 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 4.73 (1H, m, 7-H).

25,26-(4-Methoxybenzylidene)monensin Benzyl Ester (8) To a solution of **6** (a 3 : 1 diastereomeric mixture, 250 mg) in *N*,*N*-dimethylformamide (DMF) (5 ml), K_2CO_3 (88 mg) and benzyl bromide (50 μg) were added. The mixture was stirred at 50 °C for 3 h, and extracted with AcOEt. The extract was washed with NaCl solution, dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was chromatographed on silica gel

Table VI. Spectral Data for Sodium Salts of 7-*O*-Acylmonensins (**4**)

4	FAB-MS m/z	IR (KBr) cm^{-1} C=O	^{1}H-NMR ($CDCl_3$), δ ppm
a	735 $(M+Na)^+$ 757 $(M+2Na-1)^+$	1560, 1725	2.09 (3H, s, O-$COCH_3$), 2.51 (1H, dq, J=6.6, 10.0 Hz, 2-H), 3.28, 4.07 (each 1H, both d, J=12.0 Hz, 26-H_2), 3.41 (3H, s, 3-OCH_3), 4.40 (1H, ddd, J=4.7, 6.2, 11.3 Hz, 7-H)
b	749 $(M+Na)^+$ 771 $(M+2Na-1)^+$	1560, 1735	2.52 (1H, dq, J=6.8, 10.2 Hz, 2-H), 3.28, 4.07 (each 1H, both d, J=12.0 Hz, 26-H_2), 3.41 (3H, s, 3-OCH_3), 4.39 (1H, ddd, J=4.4, 6.9, 11.4 Hz, 7-H)
c	763 $(M+Na)^+$ 785 $(M+2Na-1)^+$	1560, 1725	2.52 (1H, dq, J=6.8, 10.2 Hz, 2-H), 3.27, 4.08 (each 1H, both d, J=11.7 Hz, 26-H_2), 3.41 (3H, s, 3-OCH_3), 4.35 (1H, ddd, J=4.6, 7.2, 12.1 Hz, 7-H)
d	819 $(M+Na)^+$ 841 $(M+2Na-1)^+$	1560, 1715	2.51 (1H, dq, J=6.7, 10.1 Hz, 2-H), 3.28, 4.07 (each 1H, both d, J=12.0 Hz, 26-H_2), 3.42 (3H, s, 3-OCH_3), 4.36 (1H, ddd, J=4.6, 7.0, 12.1 Hz, 7-H)
e	797 $(M+Na)^+$ 819 $(M+2Na-1)^+$	1565, 1715	2.55 (1H, dq, J=6.7, 10.2 Hz, 2-H), 3.28, 4.12 (each 1H, both d, J=11.9 Hz, 26-H_2), 3.43 (3H, s, 3-OCH_3), 4.36 (1H, ddd, J=4.6, 7.0, 11.6 Hz, 7-H)

Table VII. Physicochemical Data for Sodium Salts of 7-*O*-Acylmonensins (**4**)

4	$[\alpha]_D^{24}$ ($CHCl_3$)	mp (°C) (Solvent)	Formula	Analysis (%) Calcd C	Calcd H	Found C	Found H
a	+83.7 (0.26)	183—185[a] (Acetone–hexane)	$C_{38}H_{63}NaO_{12}$	62.11	8.64	61.92	8.67
b	+91.8 (0.26)	198—201[a] (Acetone–hexane)	$C_{39}H_{65}NaO_{12}$	62.55	8.75	62.51	8.82
c	+81.1 (0.33)	180—183[a] (Ether–hexane)	$C_{40}H_{67}NaO_{12}$	62.97	8.85	62.90	9.21
d	+79.0 (0.35)	Syrup	$C_{44}H_{75}NaO_{12}$	64.52	9.23	64.39	9.49
e	+53.3 (0.31)	145—148[b] (AcOEt)	$C_{43}H_{65}NaO_{12}$	64.80	8.22	64.85	8.51

a) Colorless prisms. b) Colorless needles.

(hexane–ether) to give a 3:1 diastereomeric mixture of **8** as a syrup (250 mg, 90%), which was subsequently separated by HPLC. **8**: Major syrup: $[\alpha]_D^{27}$ +35.5° (c=0.33, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1740 (C=O). FAB-MS m/z: 901 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.67 (1H, dq, J=4.6, 7.1 Hz, 2-H), 3.26 (3H, s, 3-OCH_3), 3.64 (1H, m, 7-H), 3.78, 4.09 (each 1H, both d, J=9.0 Hz, 26-H_2), 3.81 (3H, s, Ar-OCH_3), 5.14, 5.18 (each 1H, both d, J=12.5 Hz, OCH_2Ar), 5.88 (1H, s, >CH-Ar). Minor syrup: $[\alpha]_D^{27}$ +53.9° (c=0.30, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1740 (C=O). FAB-MS m/z: 901 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.68 (1H, dq, J=4.4, 7.0 Hz, 2-H), 3.26 (3H, s, 3-OCH_3), 3.67 (1H, m, 7-H), 3.98, 4.00 (each 1H, both d, J=9.0 Hz, 26-H_2), 3.81 (3H, s, Ar-OCH_3), 5.14, 5.18 (each 2H, both d, J=12.5 Hz, OCH_2Ar), 5.95 (1H, s, >CH-Ar).

7-*O*-Benzoyl-25,26-(4-methoxybenzylidene)monensin Benzyl Ester (9) To a solution of **8** (a 3:1 diastereomeric mixture, 53 mg) in pyridine, benzoyl chloride (0.5 ml) and DMAP (1 mg) were added. The mixture was stirred for 8 h at room temperature, poured into water, and extracted with AcOEt. After being washed with 1 N HCl, 10% $NaHCO_3$, and NaCl solution, the extract was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane–ether) to give **9** as a syrup (57 mg, 96%). **9**: IR (KBr) ν_{max} cm^{-1}: 1720 (C=O). FAB-MS m/z: 1005 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.63 (1H, dq, J=4.4, 7.0 Hz, 2-H), 3.23 (3H, s, 3-OCH_3), 4.90, 4.91 (each 2H, both d, J=12.5 Hz, OCH_2Ar), 4.99 (1H, m, 7-H).

7-*O*-Benzoyl-25,26-(4-methoxybenzylidene)monensin (7e) A solution of **9** (40 mg) in tetrahydrofuran (THF) was hydrogenated in the presence of 5% Pd–C (10 mg) at atmospheric pressure of hydrogen for 30 min. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH) to give the free acid of **7e** as a syrup (36 mg, 99%). IR (KBr) ν_{max} cm^{-1}: 1710, 1720 (C=O). FAB-MS m/z: 915 $(M+Na)^+$, 937 $(M+2Na-H)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 5.03 (1H, m, 7-H).

General Procedure for Preparation of 7-*O*-Acylmonensins (4a—e) A solution of **7a—e** (40 mg) in $CHCl_3$ (3 ml) and 1 N $HClO_4$ (3 ml) was stirred vigorously at room temperature for 2 h. After being washed with 4% $NaHCO_3$ and water, the $CHCl_3$ layer was dried over Na_2SO_4, and evaporated to dryness. The residue was purified by HPLC (MeOH–H_2O) to give the sodium salts of **4a—e**. The yields are summarized in Table I, and the spectral and physicochemical data are summarized in Tables VI and VII, respectively.

25,26-(4-Methoxybenzylidene)monensin Methyl Ester (10) To a solution of **6** (a 3:1 diastereomeric mixture, 250 mg) in DMF (5 ml), K_2CO_3 (88 mg) and methyl iodide (50 μg) were added. The mixture was stirred at room temperature for 1.5 h and extracted with AcOEt. After being washed with NaCl solution, and dried over $MgSO_4$, the extract was evaporated to dryness. The residue was chromatographed on silica gel (hexane–acetone) to give a 3:1 diastereomeric mixture of **10** as a syrup (234 mg, 92%), which was subsequently separated by HPLC. **10**: Major syrup: $[\alpha]_D^{27}$ +40.2° (c=0.35, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1740 (C=O). HRFAB-MS m/z: 825.4760 $(M+Na)^+$, Calcd for $C_{45}H_{70}NaO_{12}$: 825.4767. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.63 (1H, dq, J=5, 7 Hz, 2-H), 3.33 (3H, s, 3-OCH_3), 3.70 (3H, s, CO_2CH_3), 3.73 (1H, m, 7-H), 3.79, 4.09 (each 1H, both d, J=9 Hz, 26-H_2), 3.81 (3H, s, Ar-OCH_3), 5.88 (1H, s, >CH-Ar), 6.90, 7.44 (each 2H, both d, J=9 Hz, Ar-H). Minor syrup: $[\alpha]_D^{27}$ +54.6° (c=0.25, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 1740 (C=O). FAB-MS m/z: 825 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.63 (1H, dq, J=5.7 Hz, 2-H), 3.33 (3H, s, 3-OCH_3), 3.70 (3H, s, CO_2CH_3), 3.73 (1H, m, 7-H), 3.98, 4.40 (each 1H, both d, J=9.0 Hz, 26-H_2), 3.81 (3H, s, Ar-OCH_3), 5.95 (1H, s, >CH-Ar), 6.88, 7.48 (each 2H, both d, J=9.0 Hz, Ar-H).

General Procedure for Preparation of 7-*O*-Alkylmonensins (5a—c) A solution of **10** (a 3:1 diastereomeric mixture, 100 mg) in THF (3 ml) was stirred in the presence of NaH (5 eq) at 50 °C for 30 min. The mixture was cooled to room temperature, and alkyl iodide (5 eq) was added. The reaction mixture was stirred at room temperature for 3 h, quenched by the addition of NH_4Cl solution and diluted with AcOEt. The organic layer was washed with NaCl solution, dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was chromatographed on the silica gel (hexane–ether) to give a mixture of **11** and **12** as a syrup. The spectral data for **11** and **12** are summarized in Table VIII.

A mixture of **11** and **12** in 1 N NaOH (3 ml) containing THF–MeOH–H_2O (2:2:1) was stirred at room temperature for 6 h and extracted with AcOEt. After being washed with NaCl solution and dried over $MgSO_4$, the extract was evaporated to dryness. The residue was chromatographed on silica gel (hexane–acetone) to give a mixture of **13** and **14** as a syrup. The spectral data for the free acids of **13** and **14** are summarized in Table IX.

Table VIII. Spectral Data for **11**+**12**

11+12	IR (KBr) ν_{max} (cm^{-1}) C=O	^{1}H-NMR ($CDCl_3$), δ ppm
a	1735	3.26 (1H, m, 7-H), 3.31 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 3.70 (3H, s, CO–OCH_3), 3.81 (3H, s, ArOCH_3), 6.60 (d, 3-H of **12a**)
b	1735	3.34 (3H, s, 3-OCH_3), 3.40 (1H, m, 7-H), 3.69 (3H, s, CO–OCH_3), 3.81 (3H, s, ArOCH_3), 6.60 (d, 3-H of **12b**)
c	1730	3.33 (3H, s, 3-OCH_3), 3.45 (1H, m, 7-H), 3.52 (3H, s, CO–OCH_3), 3.81 (3H, s, ArOCH_3), 4.46, 4.65 (each 1H, both d, J=16.4 Hz, 7-OCH_2Ar), 6.58 (d, 3-H of **12c**)

Table IX. Spectral Data for the Free Acids of **13**+**14**

13+14	IR (KBr) ν_{max} (cm^{-1}) C=O	^{1}H-NMR ($CDCl_3$), δ ppm
a	1710	3.26 (1H, m, 7-H), 3.32 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 3.81 (3H, s, ArOCH_3), 6.70 (d, 3-H of **14a**)
b	1700	3.37 (3H, s, 3-OCH_3), 3.40 (1H, m, 7-H), 3.81 (3H, s, ArOCH_3), 6.71 (d, 3-H of **14b**)
c	1700	3.36 (3H, s, 3-OCH_3), 3.47 (1H, m, 7-H), 3.81 (3H, s, ArOCH_3), 4.50, 4.62 (each 1H, both d, J=16.4 Hz, 7-OCH_2Ar), 6.70 (d, 3-H of **14c**)

Table X. Spectral Data for the Sodium Salts of 7-*O*-Alkylmonensins (**5**)

5	FAB-MS m/z	IR (KBr) cm^{-1} C=O	^{1}H-NMR ($CDCl_3$), δ ppm
a	707 $(M+Na)^+$ 729 $(M+2Na-1)^+$	1570	2.55 (1H, dq, J=6.6, 10.0 Hz, 2-H), 3.25 (1H, m, 7-H), 3.27, 3.92 (each 1H, both d, J=11.7 Hz, 26-H_2), 3.39 (3H, s, 3-OCH_3), 3.64 (3H, s, 7-OCH_3)
b	733 $(M+Na)^+$ 755 $(M+2Na-1)^+$	1560	2.56 (1H, dq, J=6.6, 10.1 Hz, 2-H), 3.27, 3.92 (each 1H, both d, J=11.7 Hz, 26-H_2), 3.33 (1H, m, 7-H), 3.39 (3H, s, 3-OCH_3)
c	783 $(M+Na)^+$ 805 $(M+2Na-1)^+$	1560	2.56 (1H, dq, J=6.6, 10.1 Hz, 2-H), 3.27, 3.92 (each 1H, both d, J=11.7 Hz, 26-H_2), 3.33 (1H, m, 7-H), 3.41 (3H, s, 3-OCH_3), 4.48, 6.04 (each 1H, both d, J=16.4 Hz, 7-OCH_2Ar)
d	735 $(M+Na)^+$ 757 $(M+2Na-1)^+$	1560	2.56 (1H, dq, J=6.8, 10.2 Hz, 2-H), 3.27, 3.92 (each 1H, both d, J=11.7 Hz, 26-H_2), 3.33, 4.46 (each 1H, m, 7-OCH_2–), 3.37 (1H, m, 7-H), 3.39 (3H, s, 3-OCH_3)

A mixture of **13** and **14** in $CHCl_3$ (3 ml) and 1 N $HClO_4$ was stirred vigorously at room temperature for 2 h. The $CHCl_3$ layer was shaken with 4% $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH) followed by HPLC (MeOH–H_2O) to give the sodium salts of **5a—c** (60—68% from **10**) as syrups. The spectral and physicochemical data for the sodium salts of **5a—c** are summarized in Tables X and XI. **5a**: 7-*O*-Methylmonensin; **5b**: 7-*O*-allylmonensin; **5c**: 7-*O*-benzylmonensin.

7-*O*-Propylmonensin (5d) A solution of **5b** (50 mg) in THF (3 ml) was hydrogenated in the presence of 5% Pd–C (8 mg) at atmospheric pressure of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH) to give **5d** as a syrup (49 mg, 98%). The spectral and physicochemical data for the sodium salt of **5d** was shown in Tables X and XI.

***Rm* Values** The *Rm* values were measured by the reported method.[11]

TABLE XI. Physicochemical Data for the Sodium Salts of 7-*O*-Alkylmonensins (**5**)

5	$[\alpha]_D^{24}$ ($CHCl_3$)	mp (°C) (Solvent)	Formula	Analysis (%) Calcd C	Calcd H	Found C	Found H
a	+62.5 (0.25)	243—245[a] (Benzene–hexane)	$C_{37}H_{63}NaO_{11}$	62.78	8.97	62.51	9.06
b	+66.9 (0.25)	243—245[a] (Acetone–hexane)	$C_{39}H_{65}NaO_{11}$	63.91	8.94	63.62	9.30
c	+50.1 (0.30)	243—245[a] (Ether–hexane)	$C_{43}H_{67}NaO_{11}$	65.96	8.62	65.86	8.75
d	+56.3 (0.33)	251—252[a] (Acetone–hexane)	$C_{39}H_{67}NaO_{11}$	63.74	9.19	63.94	9.49

a) Colorless prisms.

An MeOH solution of a compound was spotted on precoated thin-layer chromatography (TLC) plates of Silica gel 60 F_{254} silanized (layer thickness 0.25 mm, Merck No. 5747), and developed with 50, 55 and 60% (v/v) aqueous MeOH solutions. The *Rm* values were calculated from *Rf* values by means of the following equation.

$$Rm = \log(1/Rf - 1)$$

Determination of Na^+ Ion Permeability in Erythrocyte Membrane The Na^+ ion permeability in human erythrocyte membrane was measured by essentially the same method as we have reported, using ^{23}Na-NMR.[7] The ^{23}Na-NMR spectra were recorded using a JEOL EX-270 spectrometer at 71.32 MHz and 37 °C. The tube combination (1 mm o.d. tube inside a 5 mm o.d. NMR tube) contained 0.45 ml of human erythrocyte suspension, 0.05 ml of 100 mM dysprosium triethylenetetramine hexaacetate in water, and 5 μl of a monensin derivative in DMSO in the annular space. The inner tube contained an external reference (20 mM dysprosium sodium triphosphate, $Na_7Dy(PPPi)\cdot 3NaCl$). The human erythrocyte suspension medium contained 140 mM NaCl and 5 mM KCl at pH 7.4, and hematocrit was set to 0.4. The intracellular Na^+ ion concentrations were calculated by uing the reported equation.[7]

Effect of the Compounds on Arterial Smooth Muscle Contraction Helical strips (0.7 mm in width and 6 mm in length) of tail arteries isolated from 13-week-old Wistar–Kyoto rats were prepared according to the method described by Asano *et al.*[12] The strips were mounted for isometric recording of tension in a water-jacketed (37±0.5 °C) tissue bath containing 20 ml of oxygenated Krebs' bicarbonate solution. The effects of the compounds on arterial smooth muscle contraction were examined by means of the procedures described by Asano and Hidaka.[13] Briefly, the strips were maximally activated by 65.9 mM KCl, and after the washout, the strips were contracted with 35.9 mM KCl. After the contraction had reached a plateau, one of the test compounds at a concentration of 1 μM was added. The arterial contraction induced by the compound is expressed as a percentage of the maximum contraction developed by 65.9 mM KCl. When the compounds relaxed the strips, the relaxation was expressed as a percentage of the contraction developed by 35.9 mM KCl. The compounds were dissolved in 99.5% ethanol to make a stock solution of 4 mM.

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