Studies on Chemical Modification of Monensin IX. Synthesis of 26-Substituted Monensins and Their Na⁺ Ion Transport Activity

Rie Tanaka, Akito Nagatsu,* Hajime Mizukami, Yukio Oghara, and Jinsaku Sakakibara

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan.

Received January 11, 2001; accepted February 27, 2001

The C-26 modified monensin derivatives, 26-O-benzoylmonensin (3), 26-O-benzylmonensin (4) and 26-phenylaminomonensin (5) were prepared from monensin (1). Na⁺ ion transport activity through biological membrane and antibacterial activity of 3—5 were evaluated and compared with the activities reported for a 26-phenylurethane derivative (2). Among these compounds, 5 showed the largest Na⁺ ion transport and antibacterial activities. In these compounds, the formation of head-to-tail hydrogen bonds was suggested to be an important factor for Na⁺ ion transport and antibacterial activities.

Key words C-26 modified monensin; sodium ion transport activity; antibacterial activity; 26-phenylaminomonensin

Monensin (1), a polyether isolated from Streptomyces cinna nomensis, is a potent antibiotic and anticoiccial(1) agent. These features are believed to be caused by its Na⁺ ion transport activity. Various chemical modifications of monensin have been carried out by substitution or oxidation of 7-OH group and condensation of the carboxyl group with amino acid or alcohols. However, there are few examples of modification at the C-26 position, except for the preparation of 26-O-urethane derivatives reported by Westley et al.(5) They reported that 26-O-phenylcarbamoyl monensin (2) showed strong antibacterial and anticoiccial activities. The aromatic ring is reported to contribute to trapping and/or stabilizing the guest ions in the host molecules. In addition, the phenyl group usually increases the lipophilicity of the molecule, and the higher lipophilicity of ionophores is believed to induce more potent biological activities. So, the activity of 2 was recognized as quite reasonable. But no one has confirmed whether or not urethane is the most suitable linkage between the C-26 position and phenyl group to increase the antibacterial activities. Thus we attempted to introduce a phenyl moiety at C-26 of monensin as ether, ester and amine. In this paper, we report the preparation of novel C-26 modified monensins having a phenyl group, 26-O-benzoylmonensin (3), 26-O-benzylmonensin (4) and 26-phenylaminomonensin (5), and the comparison of their Na⁺ ion transport and antibacterial activities with those of 2.

Results

Chemistry The route to 3 is shown in Chart 1. The direct esterification of 26-O position by anhydrides was unsuccessful, probably due to the presence of hydrogen bonds between carboxyl group and 1,2-diol at C-25 and C-26. Thus the carboxyl group of 1 was protected as benzyl ester by treatment with benzyl bromide in the presence of NaH and K₂CO₃ to give 6. The acylation of 26-OH was performed by treatment of 6 with benzoic anhydride in pyridine. In the ¹H-NMR spectrum of the product, the signals of 26-H appeared downfield relative to those of 6. Cleavage of the benzyl ester of the compound by catalytic hydrogenation gave 3. The FABMS of 3 supported the structure.

The route to 4 is shown in Chart 2. The 7-OH of 7 was protected as benzoate followed by cleavage of the silyl ether at the 26-O position to give 8. Compound 8 was then treated with benzyl bromide in the presence of NaH and n-Bu₄NI to give 26-O-benzyl derivative (9) in 62.3% yield. In the ¹H-NMR spectrum of 9, the signals due to benzyl position appeared at δ 4.52 and 4.65. Then the methyl and benzyl esters were hydrolyzed in aqueous alkaline followed by the exchange of 25-OMe to OH in 0.01 mol/l FeCl₃ in iso-ProOH-H₂O to yield 4. In the ¹H-NMR spectrum of 4, the methyl signals due to 25-OMe and methyl ester and aromatic proton signals due to benzoate disappeared.

The synthetic course to 5 is summarized in Chart 3. Protected 25-formylmonensin (10) was reacted with aniline to give the Schiff base, which was reduced by NaBH₄CN to amine (11). The formation of amine was confirmed by ¹H-NMR spectrum and FAB-MS. Deprotection of 11 was carried out in the same manner as 4 to give desired 5.

Na⁺ Ion Transport Activity The Na⁺ ion transport activity of 1 and the C-26 modified compounds (2—5) through human erythrocyte membrane was determined by measuring intracellular Na⁺ ion concentration using the ²³Na-NMR method as described before. The Na⁺ ion transport activity of 2—5 at 10⁻⁶ M is shown in Fig. 2. Hemolysis induced by the tested samples was not observed during the assay.

Compounds 3 and 5 initially caused a rapid increase of the intracellular Na⁺ ion concentration ([Na⁺]c) and then the rate gradually decreased to reach a plateau. This alteration of [Na⁺]c was similar to that induced by 1. The increase of...
of compound 2 gave an approximately linear plot. The increase of $[\text{Na}^+]$ within the first 5 min ($D[\text{Na}^+]_{5}$) is believed to represent the initial rate of increase of $[\text{Na}^+]$, which is an important indicator of the Na$^+$ ion permeability of the compounds. Compound 5 induced almost the same $D[\text{Na}^+]_{5}$ as 1, 10.5 mM for 5 and 10.4 mM for 1. The $D[\text{Na}^+]_{5}$ value of the other compounds was 4.0 mM (2), 5.3 mM (3) and 2.3 mM (4).

**Antibacterial Activity** The values of minimum inhibitory concentration against various bacteria were measured by agar dilution method (Table 1). Vancomycin was used as a control for *Staphylococcus aureus* and *Clostridium perfringens*, rifampicin for *Streptococcus pneumoniae* and *Enterococcus faecalis*, cycloserine for *Mycobacterium intracellulare*, and tylosin for *Mycoplasma gallisepticum*. Compound 5 showed higher antibacterial activity than 2 against *S. aureus*, and *C. perfringens*. The activity of 5 against *S. aureus*, *M. intracellulare*, *E. faecalis* and *C. perfringens* was comparable to or stronger than that of the corresponding control compounds. Compound 3 showed the same antibacterial activity as 2, and that of compound 4 was the weakest among these monensin derivatives.

**Discussion** Among the derivatives (2—5), 5 showed the highest Na$^+$ ion transport activity, and the value of $D[\text{Na}^+]_{5}$ of 5 was 2.5 times larger than that of the phenylurethane derivative (2). The value of $D[\text{Na}^+]_{5}$ decreased in the order $5>3>2>4$. Compound 5 was potent against the tested bacteria and the antibacterial activity also decreased in the order $5>3>2>4$. This result implied that higher Na$^+$ ion permeability of the compounds tended to induce higher antibacterial activity, as we reported on 7-O-alkylmonensins. Westley et al. reported
that 2 exhibited higher antibacterial activity than \( ^{1}\text{Na}^{+} \) in spite of having lower \( \text{Na}^{+} \) ion permeability than 1. This is probably due to the presence of a phenyl group in monensin. Some monensin derivatives with a phenyl group showed potent antibacterial activities in spite of their relatively low \( \text{Na}^{+} \) ion permeability, suggesting that the phenyl group in these derivatives might enhance their antibacterial activities.

We also examined the lipophilicity of these derivatives on TLC (SiO\(_{2}\), chloroform : MeOH = 10 : 1). Compounds 2, 3 and 5 showed almost the same \( R' \) values as monensin (1) (\( R' \): 1 = 0.57, 2, 3, 5 = 0.55), while that of 4 was much lower (\( R' = 0.43 \)). The pseudocyclic conformation of monensin sodium is believed to be stabilized by two intracellular hydrogen bonds between two oxygens of the carbonylate at one end of the molecule and two OH groups on C-25 and C-26 at the other end. Compounds 2 and 5 could also form two hydrogen bonds, one between N–H at C-26 and O at the carbonylate and the second between OH at C-25 and the other O at the carbonylate (Figs. 3A, B). In the case of 3, the ester part should be unable to form a hydrogen bond with the carbonylate, but the carbonyl oxygen at the ester should contribute to coordination of \( \text{Na}^{+} \) ion and the ester should contribute to stability of the pseudocyclic complex together with the hydrogen bond between carbonylate and 25-0H (Fig. 3C). Thus, compounds 2, 3 and 5 could exhibit the lipophilicity comparable to 1. In contrast, 4 could form only one head-to-tail hydrogen bond between 25-0H and the carbonylate, and the 26-0-benzyl group could not contribute to the stability of the \( \text{Na}^{+} \) complex nor to the pseudocyclic conformation (Fig. 3D). Probably for this reason, 4 showed lower lipophilicity, which resulted in lower \( \text{Na}^{+} \) ion transport activity and lower antibacterial activity than the other derivatives.

In summary, we prepared the C-26 modified monensin derivatives, 3–5 and compared their Na\(^{+}\) ion transport and antibacterial activities with those of reported 26-phenylmonensin derivative (2). Among these compounds, 5 showed the largest \( \text{Na}^{+} \) ion transport activity and the highest antibacterial activity, and the formation of head-to-tail hydrogen bonds was suggested to be an important factor for \( \text{Na}^{+} \) ion transport and antibacterial activities. As 5 can be the lead compound for preparation of more potent C-26 modified monensins, additional investigation is now in progress.

**Experimental**

**General** The FABMS and high resolution (HR) FABMS were measured with a JEOL JMS DX-505 or a SX-102 mass spectrometer, and the IR spectra with a Shimadzu FTIR-8100 spectrometer. The \( ^{1}\text{H}-\text{NMR} \) spectra were measured with a JEOL EX-270, Lambda-400 or \( ^{0}\text{H}-\text{NMR} \) 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; dq, quartet-of-doublets; ddd, doublet-of-doublets-of-doublets; quin, quinete; 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 F\(_{254}\), 0.25 mm thick, Merck no. 5715) and spots were detected by illumination with an ultraviolet lamp or by spraying 1% \( \text{Ce(SO}_{4}\text{)}_{2}-10\% \text{H}_{2}\text{SO}_{4} \), followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Daisou Chemicals Co., Ltd.).

**Monensin Benzyl Ester (6)**

Compounds 6 was prepared under different conditions from the reported method. The solution of monensin (1, 700 mg), benzyl bromide (249 \( \mu \)l, 2 eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene (234 \( \mu \)l, 1.5 eq) in benzene (15 ml) was refluxed for 2.5 h, poured into brine, and extracted with EtOAc. The organic layer was dried over MgSO\(_{4}\), and evaporated in vacuo. The residue was chromatographed on silica gel (EtOAc : hexane = 1:2) to give 6 (673 mg, 82%). Colorless syrup. \( ^{1}\text{H}-\text{NMR} \) (CDCl\(_{3}\), 300 MHz): 7.40 (1H, d, \( J = 7.7 \) Hz, 4-H), 3.76 (1H, br, 7-H), 4.03 (1H, d, \( J = 3.9 \) Hz, 7-H), 3.90 (1H, d, \( J = 3.9 \) Hz, 7-H), 4.31 (1H, m, 20-H), 4.40, 4.53 (each 1H, both d, \( J = 2.2, 9.0 \) Hz, 5-H), 4.24 (1H, dd, \( J = 3.2, 8.8 \) Hz, 21-H), 4.25–4.28 (1H, overlapped, 20-H), 3.87 (1H, br, 7-H), 3.93 (1H, d-like, \( J = 4.8 \) Hz, 17-H), 3.99 (1H, d-like, \( J = 9.2 \) Hz, 5-H), 4.11 (1H, m, 20-H), 5.14, 5.19 (each 1H, both d, \( J = 12.5 \) Hz, OCH\(_{2}\)Ar), 7.31–7.40 (5H, m, Ar). FAB-MS (m/z): 783 (M+Na\(^{+}\)).

**26-O-Benzoylmonensin Benzyl Ester** Benzoic anhydride (7.4 mg) and 4-dimethylaminopyridine (DMAP) (1.0 mg) were added to a solution of monensin benzyl ester (6, 10.0 mg) in pyridine (0.5 ml) at room temperature. The mixture was stirred at this temperature for 18 h, diluted with EtOAc, then washed with 5% HCl, 10% NaHCO\(_{3}\), and brine. The organic layer was dried over MgSO\(_{4}\), and evaporated in vacuo. The residue was chromatographed on silica gel (hexane : EtOAc = 3:1) to give 26-O-benzoylmonensin benzyl ester (12.9 mg, 99%). Colorless syrup. \( ^{1}\text{H}-\text{NMR} \) (CDCl\(_{3}\), 300 MHz): 7.31–7.40 (5H, m, Ar). FAB-MS (m/z): 877 (M+Na\(^{+}\)).

**26-O-Benzoyl-25-O-t-butylidemethylsilylmonensin Benzyl Ester** A solution of 26-O-benzoyl-25-O-t-butylidemethylsilylmonensin benzyl ester (6, 61.0 mg) and 10% Pd–C (10.0 mg) in EtOH (2.5 ml) was stirred under H\(_{2}\) atmosphere at room temperature and 1 atm for 2.0 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform : EtOAc = 1:1) to yield 3 (46.6 mg, 84.6%). Colorless syrup. \( ^{1}\text{H}-\text{NMR} \) (CDCl\(_{3}\), 300 MHz): 7.40 (1H, d, \( J = 7.7 \) Hz, 4-H), 3.76 (1H, br, 7-H), 4.03 (1H, d, \( J = 4.1 \) Hz, 17-H), 4.10 (1H, dd, \( J = 2.1, 10.6 \) Hz, 5-H), 4.15 (1H, dd, \( J = 1.7, 11.5 \) Hz, 21-H), 4.31 (1H, m, 20-H), 4.40, 4.53 (each 1H, both d, \( J = 12.0 \) Hz, 26-H), 2.96 (2H, t-like, \( J = 7.6 \) Hz, 3-H), 3.85 (1H, d-like, \( J = 7.4 \) Hz, 4-H), 0.85 (2H, d, \( J = 7.3 \) Hz, 2-H). FAB-MS (m/z): 797 (M+Na\(^{+}\)).

Calcd for C\(_{43}\)H\(_{66}\)O\(_{12}\)Na: 797.4452 (M\(^{+}\)). Found: 797.4454.

**7-O-Benzoyl-25-O-methyl-26-O-tert-butylidemethylsilylmonensin Methyl Ester** A solution of 25-O-methyl-26-O-tert-butylidemethylsilylmonensin methyl ester (7, 246.5 mg) in pyridine (6.5 ml) was added benzoyl chloride (2.4 ml) and DMAP (6.5 mg). The mixture was stirred at room temperature for 3.0 h, diluted with EtOAc, washed with 5% HCl, 10% NaHCO\(_{3}\), and brine. The organic layer was dried over Na\(_{2}\)SO\(_{4}\), filtered and evaporated to dryness. The residue was chromatographed on silica gel (chloroform) to
give 7-O-benzoyl derivative (260.9 mg, 93.9%). Colorless syrup. [α]D25 +58° (c=0.46, CHCl3); IR (CHCl3, cm−1): 1720, 1740 (C=O); 1′-NMR (CDCl3, δ): 0.04 (6H, s, CH3(CH3)2), 0.89 (9H, s, CH(C2H5)2), 2.60 (1H, q, J=7.3, 6.7 Hz, 2-H), 3.21 (3H, s, 25-OMe), 3.32 (3H, s, 3-OMe), 4.00 (1H, dd, d,J=3.7, 9.8 Hz, 3-H), 3.46 (1H, t, J=4.9, 13.9 Hz, 13-H), 3.47 (1H, m, J=13.9, 7.5 Hz, 17-H), 4.21 (1H, d, J=4.9, 13.9 Hz, 17-H), 4.29 (1H, dd, J=7.3, 12.2 Hz, CH2Ar), 7.24—7.34 (5H, m, Ar). FAB-MS (m/z): 940 (M+Na)++.

7-O-Benzyl-25-O-methylmonensin Methyl Ester (8) A mixture of 7-O-benzyl-25-O-methyl-26-O-tert-butylidemethylmonensin methyl ester (273.2 mg) and n-Bu4NF H2O (141.0 mg) in tetrahydrofuran (THF) (5.0 ml) was added under Ar atmosphere at room temperature for 2.5 h. The reaction mixture was poured into brine, and extracted with EtOAc. The organic layer was washed with 10% aqueous Na2CO3 solution and dried and evaporated. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane:EtOAc=8:1) to give 8 (226.8 mg, 94.7%). Colorless syrup. [α]D25 +24° (c=0.28, CHCl3); IR (CHCl3, cm−1): 1700, 1730 (C=O); 1′-NMR (CDCl3, δ): 2.60 (1H, q, J=6.6, 5.6 Hz, 2-H), 3.28 (3H, s, 25-OMe), 3.32 (3H, s, 3-OMe), 3.46 (1H, t, J=5.3, 13.5 Hz, 13-H), 3.48 (3H, s, 1- CO2Me), 3.51 (1H, m, 13-H), 3.55, 3.57 (each 1H, both d, J=1.1, 10.3 Hz, 5-H), 4.26 (1H, dd, J=8.9, 3.6 Hz, 20-H), 5.01 (1H, t, J=7.3, 5-H), 7.25—8.18 (5H, m, Ar). FAB-MS (m/z): 826 (M+Na)++.

7-O-Benzyl-25-O-methyl-26-benzylmonensin Methyl Ester (9) To a solution of 8 (249.0 mg) in THF (5.5 ml), NaH (18.6 mg, benzylobromine (0.26ml) and n-Bu4NF (114.5 mg) were added. The mixture was stirred under N2 atmosphere at room temperature for 80 h, quenched by the addition of NH4Cl solution and diluted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane:EtOAc=8:1) to give 9 (254.6 mg) in THF-MeOH (1:1, 4.0 ml) was added 5 mol/l NaOH (0.8 ml). The mixture was stirred at room temperature for 5.0 h, neutralized with 10% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform:MeOH=1:1) to give 8 (249.0 mg, THF (5.5 ml), NaH (18.6 mg), benzylobromine (0.26ml) and n-Bu4NF (114.5 mg) were added. The mixture was stirred under N2 atmosphere at room temperature for 80 h, quenched by the addition of NH4Cl solution and diluted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane:EtOAc=8:1) to give 9 (254.6 mg) in THF-MeOH (1:1, 4.0 ml) was added 5 mol/l NaOH (0.8 ml). The mixture was stirred at room temperature for 5.0 h, neutralized with 10% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform:MeOH=1:1) to give 8 (249.0 mg, THF (5.5 ml), NaH (18.6 mg), benzylobromine (0.26ml) and n-Bu4NF (114.5 mg) were added. The mixture was stirred under N2 atmosphere at room temperature for 80 h, quenched by the addition of NH4Cl solution and diluted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane:EtOAc=8:1) to give 9 (254.6 mg) in THF-MeOH (1:1, 4.0 ml) was added 5 mol/l NaOH (0.8 ml). The mixture was stirred at room temperature for 5.0 h, neutralized with 10% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform:MeOH=1:1) to give 8 (249.0 mg, THF (5.5 ml), NaH (18.6 mg), benzylobromine (0.26ml)


