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Studies on chemical modification of monensin. Part 7: Synthesis and Ca^{2+} ion transport activity of 25-carboxylmonensin[1]

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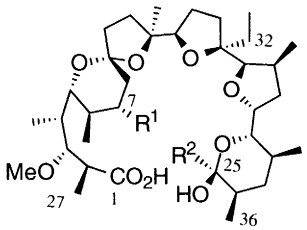
Abstract

Ionophore antibiotic monensin (**1**) was converted to 25-carboxylmonensin (**3**), which has two carboxyl groups at both ends of the molecule. Ca^{2+} ion transport activity of 25-carboxylmonensin (**3**) was evaluated by the $CHCl_3$ liquid membrane method, and the activity was 3.1 times the value of the calcium ionophore, lasalocid A.

Keywords: monensin; calcium ionophore; ion transport activity; 25-carboxylmonensin.

Monensin (**1**, Fig. 1), obtained from *Streptomyces cinnamonensis* in large amounts, is representative of many monovalent acid ionophores, and selectively transports the Na^+ ion through a biological membrane.[2] Therefore, monensin (**1**) reveals various biological activities, but is now used only in veterinary medicines.



monensin (**1**): R^1 = OH, R^2 = $^{26}CH_2OH$
7-carboxylmethylmonensin (**2**): R^1 = CH_2CO_2H, R^2 = $^{26}CH_2OH$
25-carboxylmonensin (**3**): R^1 = OH, R^2 = $^{26}CO_2H$

Fig. 1.

Calcium ionophores are widely used as important reagents in the field of biological research in order to clarify the mechanisms of various phenomena occurring inside the cells and/or on the cell membrane. However, only a few calcium ionophores such as lasalocid A and A-23187 are practically used. As the

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diameter of the Na^+ ion (0.97 Å) is close to that of the Ca^{2+} ion (0.99 Å), modification of monensin (**1**) to divalent molecules should convert **1** to calcium ionophores. Some papers have reported the preparation of dicarboxylic crown ethers,[3] but there have been few reports on the conversion of natural ionophores to the divalent molecules. Thus, we attempted to synthesize the divalent monensin derivatives to create new calcium ionophores. In our previous paper,[1] we reported the synthesis of 7-carboxylmethylmonensin (**2**) and showed that **2** had no Ca^{2+} ion transport activity, probably due to its low lipophilicity. In order to obtain a more lipophilic molecule, we planned to introduce the second carboxyl group at C-25 of monensin (**1**). The carboxyl groups at both ends of the molecule should associate with each other, which should reduce the hydrophilicity of the carboxyl groups. In this paper, we describe the synthesis and Ca^{2+} ion transport activity of 25-carboxylmonensin (**3**).

The synthetic course to **3** is summarized in Fig. 2. The hydroxy groups at C-26 and C-7 of **4**[1] were protected as *t*-butyldimethylsilyl (TBS) ether and acetate, respectively, followed by cleavage of the silyl ether at the 26-*O*-position to yield the alcohol (**5**).[4] The FAB-MS spectrum of **5** showed a quasi-molecular ion peak at 763 [$(M+Na)^+$]. The ^1H NMR spectrum of **5** exhibited two methyl signals due to the methoxy groups (δ 3.28 and 3.35) and one due to the acetyl group (δ 2.06). The signal of 7-H appeared at δ 4.72, which shifted downfield relative to monensin (**1**). Compound **5** was then oxidized in the presence of pyridinium dichromate (PDC) to give the aldehyde. The ^1H NMR spectrum of the compound showed the signal of a formyl proton at δ 9.38. This aldehyde was further oxidized to the carboxylic acid (**6**)[5] by Lindgren's method.[6] In the ^1H NMR spectrum of **6**, the signal due to the aldehyde disappeared, and a signal of the second carboxyl group appeared at δ_C 175.9 in the ^{13}C NMR spectrum. The quasi-molecular ion peak at 777 [$(M+Na)^+$] in the FAB-MS spectrum also supported the structure. The acetyl group and methyl ester of **6** were cleaved by treatment with aqueous NaOH, followed by exchange of 25-OMe to OH on the precoated SiO_2 plate to obtain the desired dicarboxylic monensin (**3**).[7] No signals due to the methoxy groups other than 3-OMe were observed in the ^1H NMR spectrum of **3**. The high-resolution (HR) FAB-MS spectrum of **3** also supported the structure. Thus, we established an efficient method for preparation of 25-carboxylmonensin (**3**) by a combination of mild and essential reactions. Only 33% of monensin was lost through nine steps of the reactions from **1**.

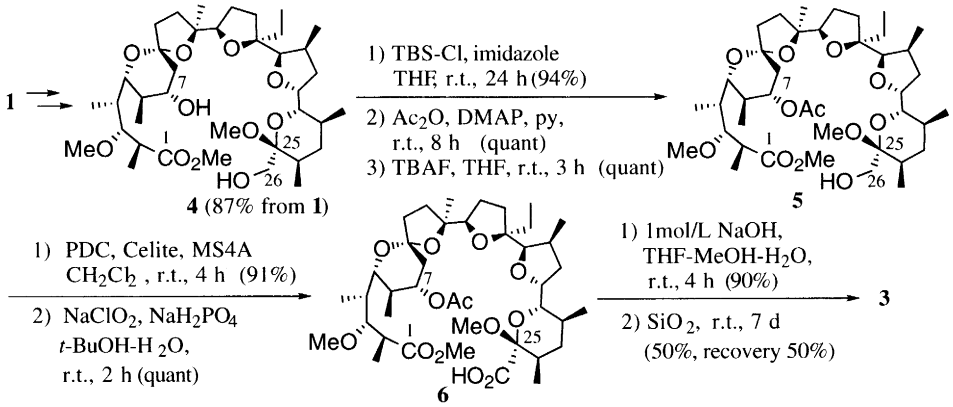

Fig. 2.

Ca^{2+} ion transport activity of **3** was determined by the $CHCl_3$ liquid membrane method[1,8] using the U-tube system indicated in Fig. 3.[1] In this system, the transported Ca^{2+} ion from the ionic water phase (A) into the pure water phase (C) through the ionophore solution in $CHCl_3$ (B) can be determined by measurement of the concentration of the cation in C using atomic absorption spectrometry.[9] Lasalocid A, one of the calcium ionophores, was used as a positive control. Compound **3** transported 253 nmol of

the Ca^{2+} ion within 6 h, whereas **1** and lasalocid A transported 43.7 nmol and 81.6 nmol, respectively, of the Ca^{2+} ion (Fig. 4A). We also tested Na^+ ion transport activity in the same manner. Monensin (**1**) transported a large amount of the Na^+ ion (307 nmol) within 6 h, but **3** transported only a small amount (88.2 nmol), which was less than that transported by lasalocid A (160 nmol) (Fig. 4B). The ratio of Ca^{2+}/Na^+ ion transport by 25-carboxylmonensin (**3**) was 2.87, and that of monensin (**1**) was 0.14. These data indicated that Na^+ ionophore monensin (**1**) was successfully converted to a Ca^{2+} ionophore by introducing the second carboxyl group at C-25 of **1**. Therefore, 25-carboxylmonensin (**3**) should be a good lead compound for developing new divalent ionophores. It is noteworthy that the known calcium ionophores such as lasalocid A and A-23187 are monovalent and transport one Ca^{2+} ion by two ionophore molecules, while **3** is divalent and apparently transports one Ca^{2+} ion by one molecule.

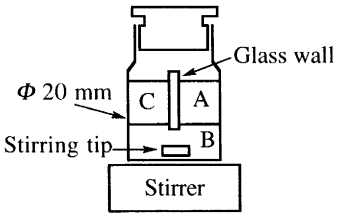

Fig. 3. U-Tube system for measurement of ion transport activity. The apparatus consists of a glass tube partly divided by a glass wall. The tube contained 1 ml of 7 mM calcium or sodium picrates (A), 3.5 ml of 0.5 mM test compound solution in water-saturated $CHCl_3$ (B) and 1 ml of distilled water (C)

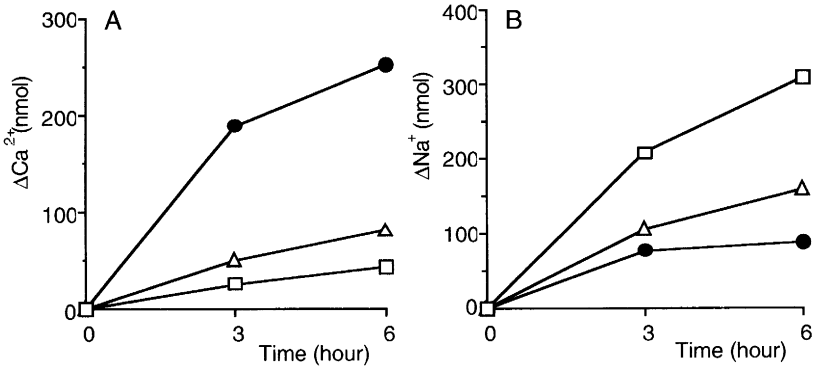

Fig. 4. Time course of the amounts of transported Ca^{2+} and Na^+ ion. The amounts of the Ca^{2+} (A) and the Na^+ (B) ions transported through the $CHCl_3$ layer including **1** (□), **3** (●), and lasalocid A (△) are indicated, respectively

Further investigation on the biological activity, the conformation of the Ca^{2+} complex and synthesis for more potent derivatives of **3** is now in progress.

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4. Data for **5**: $[\alpha]_D^{25}$ +80.5 (*c* 0.230, $CHCl_3$). FAB-MS (*m/z*): 763 $(M+Na)^+$. IR ($CHCl_3$) 1732 cm^{-1} (CO). ^1H NMR (δ ppm, in $CHCl_3$): 2.06 (3H, s, CH_3CO), 2.63 (1H, pentet-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*=3.7, 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.
5. Data for **6**: $[\alpha]_D^{25}$ +92.5 (*c* 0.255, $CHCl_3$). FAB-MS (*m/z*): 777 $(M+Na)^+$. IR ($CHCl_3$) 1732 cm^{-1} (CO). ^1H NMR (δ ppm, in $CHCl_3$): 2.06 (3H, s, CH_3CO), 2.64 (1H, qd, *J*=7.0, 6.7 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, 3-H), 3.56 (1H, dd, *J*=6.4, 8.5 Hz, 21-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 (δ ppm, in $CHCl_3$): 169.0, 170.8, 175.9 (CO).
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7. Data for **3**: $[\alpha]_D^{25}$ +94.5 (*c* 0.200, $CHCl_3$). FAB-MS (*m/z*): 707 $(M+Na)^+$. HR-FAB-MS (*m/z*): calcd for $C_{36}H_{60}NaO_{12}$: 707.3982 $(M+Na)^+$; found: 707.3952 $(M+Na)^+$. IR ($CHCl_3$) 1620, 1680 cm^{-1} (CO). ^1H NMR (δ ppm, in $CHCl_3$): 2.63 (1H, qd, *J*=3.9, 6.6 Hz, 2-H), 3.13 (1H, dd, *J*=1.8, 10.0 Hz, 3-H), 3.37 (3H, s, 3-OCH_3), 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), 3.87 (1H, d, *J*=1.5 Hz, 7-H), 4.39 (1H, m, 20-H). ^{13}C NMR (δ ppm, in $CHCl_3$): 180.4 (C-1), 41.0 (C-2), 81.9 (C-3), 37.1 (C-4), 67.6 (C-5), 34.9 (C-6), 70.5 (C-7), 33.5 (C-8), 107.0 (C-9), 39.1 (C-10), 33.2 (C-11), 85.3 (C-12), 82.5 (C-13), 27.4 (C-14), 29.9 (C-15), 86.0 (C-16), 85.1 (C-17), 34.1 (C-18), 33.3 (C-19), 76.5 (C-20), 74.8 (C-21), 31.5 (C-22), 35.9 (C-23), 34.8 (C-24), 97.5 (C-25), 173.6 (C-26), 15.9 (C-27), 58.1 (C-28), 10.8 (C-29), 10.4 (C-30), 27.4 (C-31), 30.7 (C-32), 8.2 (C-33), 14.6 (C-34), 16.9 (C-35), 16.7 (C-36).
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9. Atomic absorption was measured by a Shimadzu AA-660 spectrometer at 422.7 nm for the Ca^{2+} ion and at 589.0 nm for the Na^+ ion.