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# Synthesis and antimicrobial properties of Monensin A esters

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**Abstract**—The esters (**2**–**10**) of the ionophore antibiotic Monensin (**1**) were synthesized by four different methods, which are discussed in detail. These new esters were characterized by various spectroscopic techniques and subsequently tested in the face of their antimicrobial properties. Three derivatives (**3**, **8** and **10**) showed activity against Gram-positive bacteria. Additionally derivative (**10**) exhibited a relatively low antifungal activity against *Candida* in contrast to Monensin A.


Monensin A (**1**, Fig. 1) isolated from *Streptomyces cinnamonensis* is a well-known polyether antibiotic, capable to transport monovalent and divalent metal cations across lipid membranes. Therefore, it belongs to a group of highly bioactive molecules.[1,2] Monensin exhibits antibiotic,[3] coccidiostatic,[4] cardiovascular[5] and other important biological and medical properties.[6] Only recently it was shown that Monensin A is also a highly effective ionophore for $Li^+$, $Rb^+$ as well as for $Pb^{2+}$ cations.[7] These properties are the basis of many biological and pharmaceutical fields of application of this compound and new ones can be expected for the future.

Up to now various derivatives of Monensin were synthesized in order to reduce its toxicity and to extend its fields of application.[8] In previous publications we reported on the synthesis and the physicochemical properties of several new Monensin A esters.[9] The complexation of mono- and divalent metal cations by Monensin A esters and the properties of these complexes have been described in detail. These esters show especially high affinity towards $Na^+$ and $Ca^{2+}$ cations.[9]

From the literature data it is known that the complexation ability towards $Na^+$ and $Ca^{2+}$ cations is often related with a strong antifungal and/or antibacterial activity.[10,11] Furthermore, our previous results demonstrated that the esterification of Monensin A influences its physicochemical properties and consequently these Monensin derivates have the potential to change the mode of action.[12] These interesting results have motivated our group to optimize the synthesis methods and to study the biological activities of these new compounds. In the present contribution, we compare four methods of Monensin A ester synthesis. Furthermore, we compare the antimicrobial activity of the new ester with the activity of unmodified Monensin A.

The synthesis of carboxylic esters is one of the most fundamental methods in organic chemistry to obtain useful natural and synthetic compounds. However, most esterification procedures require rather harsh conditions such as the presence of strong acids, bases or other catalysts. Furthermore, the reactions of this type often proceed only at high temperatures. However, Monensin A is very sensitive to acidic conditions and heating. For this reason we attempted to choose the reaction conditions for the esterification as mild as possible. The new esters of Monensin A (**2**–**10**) were prepared according to four different methods. All esters used for our experiments including the acyloxy esters are stable under the experimental conditions. This was checked by several spectroscopic and spectrometric methods such as FT-IR, NMR and ESI-MS.

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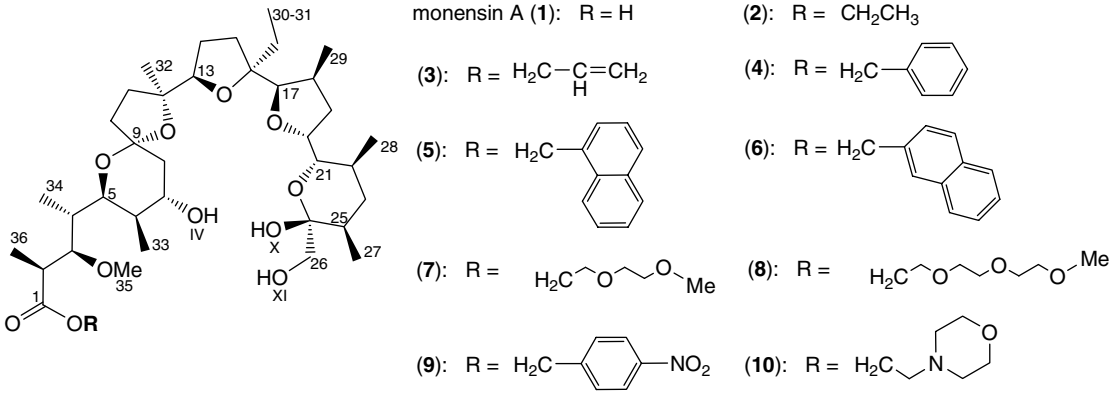


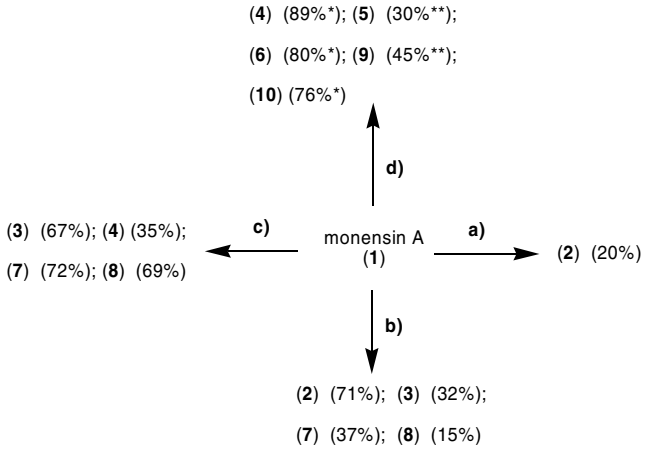

**Figure 1.** Chemical structure of Monensin A and its esters.

These synthesis pathways summarized in Scheme 1 are the only efficient ones, albeit with different yields.

In the first method DCC (*N*,*N*′-dicyclohexylcarbodiimide) was used as a coupling agent (Scheme 1, method a).[13,14] This procedure results in low to moderate chemical yields, for example, the yield of Monensin ethyl ester (**2**) was only 20%.

When PPy (4-pyrrolidinopyridine), a very effective acylation catalyst, was additionally used in catalytic amounts, the yield of compound (**2**) could be drastically increased to 71%. However, for the synthesis of the other Monensin esters such as (**3**), (**7**) and (**8**), this esterification method was unsatisfactory because only low yields of these compounds could be achieved (Scheme 1, method b).[14]

The third method for the synthesis of new Monensin esters (Scheme 1, method c) is based on the reaction between Monensin A and the appropriate alcohol in the presence of DCC, PPy and *p*-TSA (*p*-toluenesulfonic acid monohydrate). This method was quite efficient and esters (**3**), (**7**) and (**8**) could be obtained in high yields (up to 70%). However, under the same reaction conditions, the Monensin benzyl ester (**4**) was only obtained with a yield of 35%.[14]




**Scheme 1.** Synthetic access to Monensin A ester derivatives. Reagents and conditions: (a) EtOH, DCC (*N*,*N*′-dicyclohexylcarbodiimide), $CH_2Cl_2$, 0 °C to rt; (b) R-OH (corresponding alcohols), DCC, PPy (4-pyrrolidinopyridine), $CH_2Cl_2$, 0 °C to rt; (c) R-OH, DCC, PPy, *p*-TSA (*p*-toluenesulfonic acid monohydrate), $CH_2Cl_2$, 0 °C to rt; (d) R-X (bromides* or chlorides**), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), toluene, 90–100 °C, 5 h.

The fourth reliable strategy of Monensin (**1**) esterification is based on the direct alkylation of carboxylate ions. This method uses the corresponding alkyl bromides with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as an effective nucleophilic catalysts. Under these reaction conditions the yield was above 75% (**4**, **6**). Note that the use of alkyl bromides instead of alkyl chlorides significantly increases the yields of the respective esters. Interestingly, this esterification method shows also a remarkable solvent dependence. Toluene was the most appropriate of all solvents tested, probably because of an optimal solubility of reactants and products in this solvent (Scheme 1, method d).[14]

All esters (**2**–**10**) can easily be purified by column chromatography on silica gel. The structures of the esters were determined on the basis on elemental analysis, FT-IR, $^{1}H$, $^{13}C$ NMR, ESI-MS and semiempirical (PM5) methods.[14,15]

Monensin A (**1**) as well as the new esters of Monensin A (**2**–**10**) were tested in vitro in the face of their antibacterial and antifungal activity. The Gram-positive cocci, Gram-negative rods and yeasts-like micro-organisms used in the tests are collected in Table 1.

Hospital strains of *S. aureus* were isolated from different biological materials of patients of the Warsaw Medical University Hospital. 10 of these strains were *methicillin-susceptible* (MSSA) and 10 other strains were *methicillin-resistant* (MRSA). Due to this resistance these strains were tested concerning their sensitivity towards the new Monensin A esters. The other micro-organisms used here were provided by the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.[16,17]

The data concerning the antimicrobial activity of the compounds are summarized in Table 1.

**Table 1.** Antimicrobial activity of Monensin A (**1**) and its esters: (**3**), (**8**), (**10**); diameter of the growth inhibition zone (GIZ) [mm] and minimal inhibitory concentration (MIC) [μg/ml][16,17]

| | Growth inhibition zone (GIZ) [mm] and Minimal inhibitory concentration (MIC) [μg/ml] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (**1**) | | (**3**) | | (**8**) | | (**10**) | |
| | GIZ | MIC | GIZ | MIC | GIZ | MIC | GIZ | MIC |
| *S. aureus* NCTC 4163 | 22 | 2 | 13 | 100 | 19 | 100 | 23 | 12.5 |
| *S. aureus* ATCC 25923 | 22 | 1 | 11 | 100 | 19 | 50 | 20 | 6.25 |
| *S. aureus* ATCC 6538 | 20 | 2 | 13 | 100 | 17 | 100 | 23 | 12.5 |
| *S. aureus* ATCC 29213 | 18 | 1 | 13 | 100 | 17 | 50 | 25 | 6.25 |
| *S. epidermidis* ATCC 12228 | 15 | 2 | — | 100 | 13 | 100 | 24 | 12.5 |
| *B. subtilis* ATCC 6633 | 22 | 1 | 15 | 12.5 | 20 | 25 | 27 | 6.25 |
| *B. cereus* ATCC 11778 | 18 | 2 | 14 | 12.5 | 19 | 50 | 25 | 6.25 |
| *E. hirae* ATCC 10541 | — | 12.5 | — | >400 | — | >400 | 13 | 50 |
| *M. luteus* ATCC 9341 | 12 | 4 | — | 100 | 11 | 200 | 22 | 25 |
| *M. luteus* ATCC 10240 | 12 | 2 | 11 | 50 | 13 | 50 | 18 | 12.5 |
| *E. coli* ATCC 10538 | na | | na | | na | | na | |
| *E. coli* ATCC 25922 | na | | na | | na | | na | |
| *E. coli* NCTC 8196 | na | | na | | na | | na | |
| *P. vulgaris* NCTC 4635 | na | | na | | na | | na | |
| *P. aeruginosa* ATCC 15442 | na | | na | | na | | na | |
| *P. aeruginosa* NCTC 6749 | na | | na | | na | | na | |
| *P. aeruginosa* ATCC 27853 | na | | na | | na | | na | |
| *B. bronchiseptica* ATCC 4617 | na | | na | | na | | na | |
| *C. albicans* ATCC 10231 | na | | na | | na | | 18 | 200 |
| *C. albicans* ATCC 90028 | na | | na | | na | | 17 | 200 |
| *C. parapsilosis* ATCC 22019 | na | | na | | na | | 14 | 400 |

na, no activity in disc diffusion test—denotes lack of the growth inhibition zone.

Among the tested compounds (Table 1), only Monensin A (**1**) as well as three ester derivatives (**3**, **8** and **10**) showed detectable but different activities against Gram-positive bacteria. Only one of the derivatives (**10**) exhibited relatively low antifungal activity against *Candida* while Monensin A was inactive against these fungi. Compounds (**2**, **4**–**7** and **9**) were inactive towards all micro-organisms tested.[16]

The interactions between the oxygen atoms of Monensin A or of the Monensin A esters with mono- and di-valent metal cations lead to the formation of pseudo-cyclic structures which are additionally stabilized by intramolecular hydrogen bonds.[2,7,9] In previous investigations we could show that the mode of complex formation with $Na^+$ cations is very similar for the majority of Monensin A derivatives and rather independent of the nature of the respective ester groups. This suggests that the ester groups are not engaged in the coordination process.[7,9] Thus, the differences in the biological activities between Monensin A and the Monensin A derivatives described here are not based on a different capability of complex formation but on other parameters such as size and chemical nature of the substituent. One of these parameters is potentially the lipophilic character of the substituent, which evokes lower solubility in aqueous solutions. Furthermore, the presence of aromatic substituent in the ester group, such as phenyl (compounds **4** and **9**) or naphthalene rings (compounds **5** and **6**), might decrease the mobility of Monensin A esters in the lipid bilayers.

The highest antibacterial activity of compound **10** among the ester derivates and its slight antifungal activity is probably related to the presence of the morpholine ring moiety, since it was previously observed that various compounds containing this substituent show moderate antibacterial and strong antifungal activities.[18] The low antibacterial activity of compound **3** is probably connected with the presence of allyl group, which has a significant influence on the antimicrobial activity.[19]

Drug-resistant Gram-positive bacterial pathogens including *methicillin-resistant S. aureus* (MRSA) cause serious chemotherapeutic problems in hospitals.[20] Our studies reveal that Monensin A clearly shows antimicrobial activity against both MRSA (*methicillin-resistant*) and MSSA (*methicillin-susceptible*) strains of *Staphylococcus aureus* at doses MIC = 1–2 μg/ml, whereas from among the Monensin A esters studied only compound **10** shows interesting activity at the doses MIC = 6.25–12.5 μg/ml (Supplementary material, Table 1S).

In the present work, we synthesized nine new esters (**2**–**10**) of Monensin A using four different synthesis pathways. We provide evidence that three Monensin esters (**3**, **8** and **10**) show antibacterial activity against human pathogenic bacteria, including antibiotic-resistant *S. aureus*. Concerning the Monensin A esters, only compound (**10**) shows relatively low antifungal activity.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.038.

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12. Huczyński, A.; Przybylski, P.; Brzezinski, B.; Bartl, F. *J. Phys. Chem. B* **2006**, *110*, 15615.
13. Synthesis of Monensin A (**1**): Monensin A sodium salt **(Fluka)** was dissolved in dichloromethane and stirred vigorously with a layer of aqueous sulphuric acid (pH 1.5). The organic layer containing MONA was washed with distilled water, and dichloromethane was evaporated under reduced pressure to dryness.
14. (a) General procedure for the synthesis of Monensin A esters (**2**–**10**): (*method a*, Scheme 1): To a mixture of (**1**) (500 mg, 0.75 mmol) in dichloromethane (15 ml) the following compounds were added: DCC (206 mg, 1.0 mmol), EtOH (5 mmol). The mixture was first stirred at a temperature below 0 °C for 24 h and then for further 24 h at room temperature. Subsequently, the solvent was evaporated under reduced pressure to dryness. The residue was then suspended in hexane and filtered off. The filtrate was evaporated under reduced pressure and the residue purified chromatographically on silica gel (Fluka type 60) to give (**2**) (20% yield) as a colourless oil showing a tendency to form the glass state; (b) (*method b*, Scheme 1): To a mixture of MONA (500 mg, 0.75 mmol) in dichloromethane (15 ml) the following compounds were added: DCC (206 mg, 1.0 mmol), PPy (50 mg, 0.33 mmol), corresponding alcohol (5.0 mmol). The mixture was first stirred at a temperature below 0 °C for 24 h and then for further 24 h at room temperature. After this time the solvent was evaporated under reduced pressure to dryness. The residue was suspended in hexane and filtered off. The filtrate was evaporated under reduced pressure and the residue was purified chromatographically on silica gel (Fluka type 60) to give corresponding esters (**2**–**3**, **7**–**8**) (yield from 15% to 71%) as a colourless oil showing a tendency to form the glass state; (c) (*method c*, Scheme 1): To a mixture of MONA (500 mg, 0.75 mmol) in dichloromethane (15 ml) the following compounds were added: DCC (206 mg, 1.0 mmol), PPy (50 mg, 0.33 mmol), corresponding alcohol (5 mmol) and *p*-TSA (28.5 mg, 0.15 mmol). The mixture was first stirred at a temperature below 0 °C for 24 h and then for further 24 h at room temperature. The solvent was subsequently evaporated

under reduced pressure to dryness. The residue was suspended in hexane and filtered off. The filtrate was evaporated under reduced pressure and the residue was purified chromatographically on silica gel (Fluka type 60) to give (**3**–**4**, **7**–**8**) (yield from 35% to 73%) as a colourless oil showing a tendency to form the glass state; (d) (*method d*, Scheme 1): A mixture of alkyl bromide or chloride (1.45 mmol), MONA (500 mg, 0.75 mmol), and DBU (175 mg, 1.15 mmol) and 40 ml toluene was heated at 90 °C for 5 h. After cooling, the precipitate DBU-hydrohalide (DBUHX) was filtered and washed hexane. The filtrate and the washing were combined and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (Fluka type 60) to give the corresponding ester (**4**–**6**, **9**–**10**) (yield from 30% to 89%) as a colourless oil showing a tendency to form the glass state.

15. Selected spectra data for (**5**): ESI-MS (*m*/*z*): 834 ($M+Na^+$); $^1H$ NMR ($\delta$ ppm in $CD_3CN$): 2.69 (1H, m, 2-H), 2.78 (1H, t, $J$ = 6.6 Hz, O(11)-H), 3.11 (3H, s, 35-H), 3.33(2H, lt, $J$ = 6.6 Hz, 26-H), 3.50 (1H, t, $J$ = 4.3 Hz, 3-H), 3.58 (1H, overlapped, 3-H), 3.60 (1H, overlapped, 13-H), 3.62 (1H, overlapped, 21-H), 3.84 (1H, d, $J$ = 4.4 Hz, 17-H), 3.92 (1H, s, O(10)-H), 4.0 (1H, dd, $J$ = 2.2 Hz, 7.1 Hz, 5-H), 4.18 (1H, d, $J$ = 8.8 Hz, O(4)-H), 4.21 (1H, m, 20H), 5.57, 5.64 (each 1H, both d, $J$ = 12.6 Hz, $OCH_2$-Ar), 7.45–8.11 (9H, Ar), 0.70–2.28 pattern of 45 protons; $^{13}C$ NMR ($\delta$ ppm in $CD_3CN$): 175.9, 134.6, 132.8, 132.4, 129.9, 129.4, 128.3, 127.5, 126.9, 126.3, 127.7, 108.5, 97.9, 88.1, 86.9, 86.4, 84.4, 81.8, 77.9, 77.3, 71.9, 68.7, 67.5, 65.2, 58.2, 41.5, 39.7, 37.7, 36.8, 35.9, 35.1, 34.9, 34.6, 34.0, 32.6, 32.0, 30.1, 28.4, 26.3, 17.8, 16.5, 16.1, 12.5, 12.2, 11.1, 8.3; IR(KBr): 1734 $cm^{-1}$ ($\nu$C=O); Elemental analysis: (%): Calcd for $C_{47}H_{70}O_{11}$: C, 69.60; H, 8.70; Found: C, 69.40; H, 8.89. Compounds (**3**–**4** and **7**) as well as their complexes with monovalent cations were characterized by us in Refs. 9c–e, respectively.
16. Antimicrobial activity was examined by the disc diffusion and MIC method under standard conditions using Mueller–Hinton II agar medium (Becton Dickinson) for bacteria and RPMI agar with 2% glucose (Sigma) for yeasts, according to CLSI (previously NCCLS) guidelines.[17]
    The compounds giving some growth inhibition zone in disc diffusion assay were tested by the twofold serial agar dilution technique to determine their minimal inhibitory concentration (MIC) values.
    For the disc diffusion method, sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatography paper) were dripped with the compound solutions tested (in ethanol) to load 400 μg of a given compound per disc. Dry discs were placed on the surface of an appropriate agar medium. The results (diameter of the growth inhibition zone) were read after 18 h of incubation at 35 °C.
    For MICs determination, all compounds were dissolved in DMSO. Concentrations of the agents tested in solid medium ranged from 3.125 to 400 μg/ml. The final inoculum of all organisms studied was $10^4$ CFU $mL^{-1}$ (colony forming units per ml), except the final inoculums for *E. hirae*ATCC 10541, which was $10^5$ CFU $mL^{-1}$. A control test was also performed for DMSO which was found inactive in the culture medium. Minimal inhibitory concentrations were read off after 18 h (for bacteria) and 24 h (for yeasts) of incubation at 35 °C. Ionophore antibiotic—Monensin A was used as a control for bacteria and fluconazole (for the disc diffusion method 25 μg per disc has been used) for yeast (*C. albicans* ATCC 10231 GIZ = 22 mm, MIC = 1 μg/ml; *C. albicans* ATCC 90028 GIZ = 32 mm, MIC = 1 μg/ml; *C. parapsilosis* ATCC 22019 GIZ = 22 mm, MIC = 2 μg/ml).
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