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# FT-IR, $^{1}H$, $^{13}C$ NMR, ESI–MS and semiempirical investigation of the structures of Monensin phenyl urethane complexes with the sodium cation

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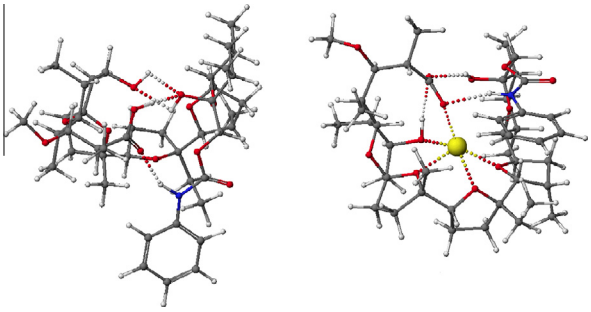
## HIGHLIGHTS

- Three forms of the urethane derivative of Monensin are studied.
- In the structure of Monensin urethane $Na^+$ salt the C=O urethane group does not coordinate $Na^+$ cation.
- In the 1:1 Monensin urethane acid complex with $Na^+$ an equilibrium is observed.
- Structures of Monensin urethane complexes were visualised using PM5 semiempirical method.

## GRAPHICAL ABSTRACT



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## ABSTRACT

In this paper three forms of phenyl urethane of Monensin i.e. its acid form (H–MU) and its 1:1 complex with $NaClO_4$ (H–MU–Na) and its sodium salt (Na–MU) were obtained and their structures were studied by FT-IR, $^{1}H$ and $^{13}C$ NMR, ESI MS and PM5 methods. The FT-IR data of Na–MU complexes demonstrate that the C=O urethane group is not engaged in the complexation of the sodium cation. However spectroscopic studies of H–MU–Na complex show that the structure in which this C=O urethane groups participate in the complexation is also present, but it is in the minority. The PM5 semiempirical calculations allow visualisation of all structures and determination of the hydrogen bond parameters.



## Introduction

The carboxylic polyether antibiotics have been recently very intensively studied because of their biological properties including antibacterial, antiviral and anticancer activity [1,2]. Monensin A (Scheme S1, Supplementary material) is one of the most commonly studied natural ionophores and it is currently very widely used in veterinary medicine as a coccidiostatic and non-hormonal growth-promoting agent [3]. The biological activity of Monensin and its mode of action are strictly connected with its ionophore properties. Monensin has high preferences to form complexes with $Na^+$ cation and is able to transport it across cell membranes disturbing the natural $Na^+/K^+$ concentration gradient and leading to death of bacteria [4–6]. The chemical modification of naturally occurring antibiotics is one of convenient ways to obtain less toxic compounds of improved antimicrobial activity [7,8]. Up to now various modifications of monensin such as esters [9,10], amides [11–13] and urethanes [14–20] have been developed to obtain less toxic derivatives, enabling expansion of their applications. The urethane derivatives of Monensin are very interesting compounds especially from the biochemical and pharmaceutical point of view and for the studies on correlation between structure and activity (*Structure–Activity Relationship*, SAR). The influence of urethane

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residues in the respective derivatives of Monensin A on their antibacterial, anticoccidial, antiparasitic and antimalarial activity has been studied [14–19]. It has been shown that several urethanes of Monensin A exhibit higher antibacterial activity than pure monensin. Other studies have shown that the urethane derivatives of Monensin A demonstrate antihypertensive and antimalarial activity and are used in the treatment of swine dysentery and are very useful growth promoting agents in ruminants [18,19]. Many explanations have been proposed of the higher antibacterial activity of urethane derivatives of Monensin in comparison with those of unmodified Monensin. It has been demonstrated that chemical modifications of the C(26)—OH hydroxyl group by the urethane function changes $Na^+$ transport through the membrane [16]. All these explications are linked to the structure of the Monensin A urethane complex with sodium cation. Therefore, the structural studies of biologically active Monensin urethanes are very important to understand the relation between their structure and high antibacterial activity. Unfortunately, Westley et al. [14] and Tanaka et al. [17] have proposed two mutually exclusive structures of Monensin urethane sodium salt. Therefore, to explain the real structure of Monensin urethane the detailed structural and spectroscopic studies of C(26)-*O*-phenylurethane of monensin A sodium salt (Na–MU) have been performed by us [20]. The results of these studies were quite surprising as it turned out that both structures proposed were false. Our studies have shown that the interactions between the etheric oxygen atoms of Monensin and its phenyl urethane with sodium cation lead to the formation of the pseudo-cyclic structures which are stabilized by intramolecular hydrogen bonds. We have proved that the oxygen atom of the C=O urethane group is not engaged in the coordination of the sodium cations as was postulated by Westley et al. The X-ray and spectroscopic studies show clearly that the system of intramolecular hydrogen bonds present in the molecular structure of Na–MU is also different from that proposed by Tanaka et al. We have also provided evidence that phenyl urethane of monensin shows higher antibacterial activity against human pathogenic bacteria, including antibiotic-resistant *S. aureus* and *S. epidermidis* compared to the parent unmodified Monensin [20].

Our recent studies have proved that Monensin acid and its complexes are very good models for the study of the electrogenic transport of sodium cations through the membranes [21]. The structures of the complexes of Monensin acid with $H_2O$, NaCl and $NaClO_4$ have been studied by X-ray and spectroscopic methods showing that Monensin is able to form stable complexes with metal cation not only as Monensin salt complexes but also as 1:1 complexes of Monensin acid with sodium salts [21].

As a continuation of these studies, phenylurethane of Monensin acid (H–MU) and its 1:1 complex with $NaClO_4$ (H–MU–Na) as well as phenylurethane of Monensin sodium salt (Na–MU) have been obtained and studied by FT-IR, ESI–MS, $^1H$ and $^{13}C$ NMR spectroscopic methods and PM5 semiempirical calculations.

Detailed spectroscopic investigation and semiempirical calculation of Monensin urethane in its three forms (acid, sodium salt and acid-sodium cation complex) should provide new information on the structure of Monensin urethane which can be very useful when describing their antimicrobial properties as well as for structural activity relationship analysis (SAR) and related investigation.

## Experimental

Phenyl isocyanate, $NaClO_4$ and solvents were obtained from Aldrich or Fluka and were used without further purification. $CH_3CN$, $CD_3CN$ as well as $CH_2Cl_2$ and $CD_2Cl_2$ spectral-grade solvents were stored over 3Å molecular sieves for several days. All manipulations with the substances were performed in a carefully dried and $CO_2$-free glove box.

### *Isolation of Monensin A sodium salt (NaM)*

Monensin sodium salt was isolated from Coxidin® 200 microGranulate an anticoccidial feed additive distributed by Huvepharma (Poland). 100 g of permix was dissolved in $CH_2Cl_2$. The solvent was evaporated under reduced pressure and the crude product obtained was purified by dry-column flash chromatography (gradient solvent mixture hexane/$CH_2Cl_2$) giving 12 g of pure NaM. The spectroscopic data of NaM are in agreement with previously published assignments [6].

### *Synthesis of Na–MU*

Na–MU was obtained according to our method described previously [20]. The purity of this compound was controlled by elemental analysis, FT-IR, $^1H$ and $^{13}C$ NMR spectroscopic methods.

### *Synthesis of H–MU*

Phenylurethane of monensin A sodium salt (Na–MU) was dissolved in $CH_2Cl_2$ and stirred vigorously with a layer of aqueous sulphuric acid (pH = 1.5). The organic layer containing MONA was washed with distilled water, and $CH_2Cl_2$ evaporated under reduced pressure to dryness to produce the acidic form of Monensin phenylurethane (H–MU).

### *Synthesis of H–MU complex with $NaClO_4$*

The solutions of the 1:1 complexes of H–MU with $NaClO_4$ were obtained by adding equimolar amounts of $NaClO_4$ dissolved in $CH_3CN$ to an $CH_3CN$ solution of H–MU. The solvent was evaporated under reduced pressure to dryness and the residue was dissolved in an appropriate volume of dry $CH_3CN$ and $CD_3CN$ to obtain the complex of the 0.07 mol $dm^{-3}$ concentration.

### *Spectroscopic measurements*

The $^1H$, $^{13}C$ NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer. $^1H$ NMR measurements of samples (0.07 mol $dm^{-3}$) in $CD_2Cl_2$ or $CD_3CN$ were carried out at the operating frequency 600.055 MHz; flip angle, pw = 45°; spectral width, sw = 4500 Hz; acquisition time, at = 2.0 s; relaxation delay, $d_1$ = 1.0 s; $T$ = 293.0 K and using TMS as the internal standard. No window function or zero filling was used. Digital resolution was 0.2 Hz per point. The error of the chemical shift value was 0.01 ppm. The $^{13}C$ NMR spectra were recorded at the operating frequency 150.899 MHz; pw = 60°; sw = 19,000 Hz; at = 1.8 s; $d_1$ = 1.0 s; $T$ = 293.0 K and TMS as the internal standard. Line broadening parameters were 0.5 or 1 Hz. The error of chemical shift value was 0.1 ppm. All spectra were locked to deuterium resonance of respective solvent. The $^1H$ and $^{13}C$ NMR signals were assigned using 2-D spectra (COSY, HETCOR, NOESY, HMBC) shown in the Supplementary Materials. 2-D spectra were recorded using standard pulse sequences from Varian and Bruker pulse-sequence libraries. In the mid infrared region the FT-IR spectra of sample (0.07 mol $dm^{-3}$) were recorded in $CH_2Cl_2$ or $CH_3CN$ solution. A cell with Si windows and wedge-shaped layers was used to avoid interferences (mean layer thickness 170 μm). The spectra were taken with an IFS 113v FT-IR spectrophotometer (Bruker, Karlsruhe) equipped with a DTGS detector; resolution 2 $cm^{-1}$, NSS = 64. The Happ-Genzel apodization function was used.

### *ESI MS measurements*

The ESI (Electrospray Ionisation) mass spectra were recorded on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer

equipped with a Harvard Apparatus syringe pump. The sample was prepared in dry $CH_3CN$ ($5 \times 10^{-5}$ mol dm$^{-3}$). The sample was infused into the ESI source using a Harvard pump at a flow rate of 20 μl min$^{-1}$. The ESI source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V. The standard ESI mass spectra were recorded at the cone voltages: 10, 30, 50, 70, 90, 110 and 130 V. The source temperature was 120 °C and the desolvation temperature was 300 °C. Nitrogen was used as the nebulizing and desolvation gas at flow-rates of 100 dm$^3$ h$^{-1}$. Mass spectra were acquired in the positive ion detection mode with unit mass resolution at a step of 1 m/z unit. The mass range for ESI experiments was from $m/z$ = 300 to $m/z$ = 1100.

*Semiempirical calculations*

PM5 quantum calculations were performed using the Win Mopac 2003 program at the semiempirical level (Cache Work System Pro Version 5.04 – Fujitsu). For all calculated compounds the initial optimisation of the structures was carried out using the molecular mechanics – extensive global minimum energy conformation search with the Conflex/MM3 from WinMopac 2003 program. For the calculated the most energetically favourable structures of conformers corresponding the global minimum points, further calculation of heat of formation was calculated using the PM5 quantum semiempirical method [22,23].

## Results and discussion

The structures and the numbering of the atoms of Monensin and its phenyl urethane derivative are shown in Scheme S1 (Supplementary material).

*Spectroscopic studies*

In Fig. 1a, the FT-IR spectra of Na–MU (dashed-dotted line), H–MU (dashed line) both in $CH_2Cl_2$ solution and H–MU–Na (solid line) in $CH_3CN$, are compared. The same spectra with extended scales in the regions of the ν(O—H) and ν(N—H) as well as ν(C=O) vibrations are shown in Figs. 1b and 1c, respectively.

The previous studies of the Na–MU have shown that its structure in the crystal is also conserved in the $CH_2Cl_2$ solution and have been discussed in detail [20]. In the FT-IR of Na–MU the ν(C=O) (amide I) band of urethane and the $\nu_{as}(COO^-)$ band of the carboxylate group are observed at 1727 cm$^{-1}$and about 1572 cm$^{-1}$, respectively (Fig. 1c). The $\nu_{as}(COO^-)$ band is relatively broad due to its overlapping with the amide II band of the urethane group.

In the spectra of the acidic form of Monensin phenylurethane (H–MU) and its 1:1 complexes with $NaClO_4$ (H–MU–Na) (Fig. 1c, dashed and solid lines, respectively) the band assigned to $\nu_{as}(COO^-)$ vibration at 1572 cm$^{-1}$ is no longer observed, but a new complex band arises in the region above 1650 cm$^{-1}$. In the spectrum of H–MU a new broad band is observed at 1727 cm$^{-1}$. This new composite band is a superposition of the ν(C=O) vibrations of urethane and carboxylic groups and indicates the existence of COOH group.

In the FT-IR spectrum of the H–MU–Na three bands at 1680 cm$^{-1}$, 1714 cm$^{-1}$ and 1739 cm$^{-1}$ are observed instead of one broad band observed in the spectrum of H–MU indicating that one of carbonyl groups (from urethane or carboxylic moiety) is partially engaged in the coordination of the sodium cation. The unambiguous assignment of these bands is possible after analysis of the $^{13}$C NMR spectra.

In the $^{13}$C NMR spectrum of Na–MU salt, the most characteristic signal of C(1) atom of carboxylate group was observed at 183.9 ppm, while the signal of C(1) atom of carboxyl group of

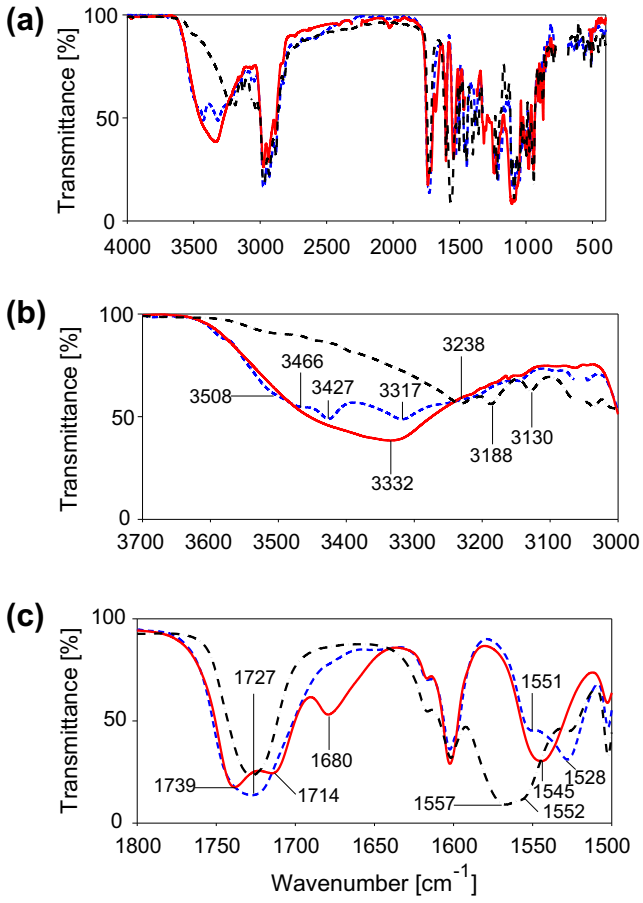


**Fig. 1.** The FT-IR spectra of: (—) H–MU–Na in $CH_3CN$, (- -) H–MU in $CH_2Cl_2$ and (– · –) Na–MU in $CH_2Cl_2$, in the ranges of: (a) 4000–400 cm$^{-1}$; (b) ν(O—H, N—H) and (c) ν(C=O) stretching vibrations.

Monensin phenylurethane acid (H–MU) was at 177.2 ppm. The position of this signal did not shift after the formation of the 1:1 complex with $NaClO_4$ (H–MU–Na), indicating that the oxygen atom of the carboxylic group of Monensin phenylurethane acid is not involved in the complexation of the sodium cation. The signal of carbon atom C(37) of the urethane group was observed at 154.5 ppm in the spectrum of Na–MU salt did not shift after the conversion of this salt to its acid form (H–MU). The signal of the carbon atom of the urethane group C(37) in the spectrum of H–MU–Na is observed at 156.5 ppm. This signal shifts toward higher ppm values in comparison to its position observed for both Na–MU salt and H–MU acid. Thus, the oxygen atom of urethane group should interact with $Na^+$ cation within the structure of H–MU–Na complex.

According to $^{13}$C NMR data discussed above, the band present in the in the FT-IR spectrum of H–MU–Na complex (Fig. 1c, solid line) at 1739 cm$^{-1}$ is assigned to the ν(C=O) stretching vibration of carboxylic group, while the band at 1714 cm$^{-1}$ is assigned to the ν (C=O) of the urethane group, respectively. In this spectrum, besides the band at 1714 cm$^{-1}$, new less intense bands appear at *ca.* 1680 cm$^{-1}$. The second band is assigned to the ν(C=O) vibrations of the urethane group which takes part in the complexation process of the sodium cation. The band at 1714 cm$^{-1}$ is assigned to the ν(C=O) vibration of urethane group in the structure of the complex in which this group is not engaged in the complexation process. The presence of both bands suggests existence of an equilibrium between these structures. However, the structure with the engaged carbonyl group does not play a significant role in the

$CH_3CN$ solution. This means that the $Na^+$ cation can fast fluctuate between the oxygen atoms of H–MU acid showing the so-called cation polarizability discussed extensively earlier by Brzezinski and Zundel [24,25].

In the $^1H$ NMR spectra of Na–MU salt and H–MU acid and its complex with $NaClO_4$ H–MU–Na (Table 1), the signals of the protons of two OH hydroxyl groups and the proton of NH urethane group are separate, as illustrated in Fig. S1 (Supplementary material). In the spectrum of Na–MU salt the proton signals of O(3)H and O(10)H are observed at 3.81 ppm and 7.81 ppm, respectively, while the signal of the N(1)H proton at 9.62 ppm. The position of these signals shows that the protons of O(10)H and N(1)H groups are involved in relatively strong intramolecular hydrogen bonds with carboxylate group and the proton of the O(4)H group is involved in a rather weak hydrogen bond, which is consistent with the FT-IR of Na–MU data mentioned above. The position of OH and NH signal in the spectra of H–MU acid and its complexes with sodium cation are shifted in different directions depending on the structure of intramolecular hydrogen bond present in their structure (Fig. S1, Table 1). In the spectra of H–MU acid, the signals of the N(1)H and O(10)H protons are strongly shifted toward lower ppm values indicating that the hydrogen bonds, in which the N(1)H and O(10)H protons take part, are weaker than those observed in the structure of Na–MU salt.

As a result of complexation of the sodium cation by H–MU acid, significant shifts toward higher ppm values are observed for the signals of protons of O(10)H and NH groups. These shifts are caused by the involvement of the respective protons in the slightly stronger intramolecular hydrogen bonds in comparison with those existing in the H–MU molecule. In contrast, the signal of O(4)H proton is shifted toward lower ppm values indicating that this hydrogen bond, in which O(4)H proton takes part becomes slightly weaker. This demonstrates that depending on the chemical form of phenyl urethane of Monensin (salt, acid, acid–$Na^+$ complex), the respective hydroxyl groups form intramolecular hydrogen bonds of different strength and therefore the complexes should exhibit different structures.

Comparison of the FT-IR spectra of H–MU acid and Na–MU salt in the region of the ν(O—H) and ν(N—H) vibrations also indicates that different hydrogen bonds exist within their structures (Fig. 1b). Most characteristic in the FT-IR spectrum of H–MU acid (dashed line) are the bands at 3466 $cm^{-1}$ and 3427 $cm^{-1}$ assigned to the ν(OH) vibrations of O(4)H, O(10)H groups, respectively, and at 3317 $cm^{-1}$ assigned to the ν(NH) vibrations of N(1)H group. These new bands clearly demonstrate that in the structure of the acid there are significant weaker intramolecular hydrogen bonds than those present in the structure of Na–MU salt. This conclusion is in agreement with the NMR data discussed above.

In the FT-IR spectrum of Na–MU complex (Fig. 1b, solid line) a broad band in the region 3550–3200 $cm^{-1}$ arises. The maximum of this band at 3332 $cm^{-1}$ indicates that in the structure of the cation complex, the hydrogen bonds in which OH and NH groups are engaged become only slightly stronger when compared with those in the uncomplexed molecule. This observation is in agreement with the $^1H$ NMR data (Fig. S1, Table 1).

*Electrospray mass spectrometry studies*

The ESI mass spectra of the 1:1 complex of H–MU with $Na^+$ cation measured at various cone voltages (*cv*) are shown in Fig. S2 (Supplementary material). As follows from the presence of the signal at *m*/*z* = 812 in this spectra, the H–MU molecule forms exclusively 1:1 stoichiometry complex with $Na^+$ cation and this complex is stable up to about *cv* = 50 V. In the spectrum at *cv* = 70 V, besides the *m*/*z* signals corresponding to the complex of 1:1 stoichiometry, new *m*/*z* signals arise indicating the beginning of fragmentation process of this complex. The proposed fragmentation pathways starting from structure A are illustrated in Scheme S2 (Supplementary material). The first step of the fragmentation is the loss of one water molecule as a result of the loss of the O(10)H or O(4)H hydroxyl groups yielding B or C cations, respectively. The regioselectivity of the loss of the first water molecule, especially in formation of the cation C, can be explained by strong engagement of the electrons of O(4) atom in complexation

**Table 1**
The most important $^1H$ and $^{13}C$ NMR chemical shifts δ (ppm) of Na–MU and H–MU in $CD_2Cl_2$ and H–MU–Na in $CD_3CN$.

| Position | Na–MU (salt)[a] | | H–MU (acid)[b] | | H–MU–Na (acid–$Na^+$ complex)[b] | |
|---|---|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) | $\delta_C$ (ppm) | $\delta_H$ (ppm) | $\delta_C$ (ppm) | $\delta_H$ (ppm) |
| 1 C=O | 183.9 | – | 177.2 | – | 177.2 | – |
| 2 (CH) | 44.8 | 2.55 (qd, *J* = 10.2, 5.5, 6.7) | 41.4 | 2.59 (p, *J* = 6.8) | 41.2 | 2.65 (dq, *J* = 7.0, 5.8) |
| 3 (CH) | 83.5 | 3.26 | 84.2 | 3.58 (dd, *J* = 9.9, 5.4) | 84.0 | 3.57 (dd, *J* = 11.1, 4.4) |
| 5 (CH) | 68.3 | 4.45(dd, *J* = 12.0, 1.4) | 68.3 | 4.15 (d, *J* = 11.2) | 68.3 | 4.08 (dd, *J* = 9.6, 2.0) |
| 7 (CH) | 71.5 | 3.91 (d, *J* = 4.7) | 72.0 | 3.69 | 70.6 | 3.96 |
| 9 (C) | 108.6 | – | 108.6 | – | 108.3 | – |
| 12 (C) | 85.8 | – | 87.2 | – | 87.2 | – |
| 13 (CH) | 83.0 | 3.49 (dd, *J* = 10.2, 5.5) | 82.3 | 3.46 (dd, *J* = 5.7, 4.5) | 82.4 | 3.52 (dd, *J* = 5.4, 4.8) |
| 16 (C) | 87.1 | – | 87.7 | – | 86.2 | – |
| 17 (CH) | 84.7 | 3.91 (d, *J* = 4.7) | 86.3 | 3.91 (d, *J* = 4.2) | 84.0 | 3.67 |
| 20 (CH) | 78.6 | 4.33 (ddd, *J* = 10.7, 5.5, 2.4) | 77.9 | 4.19 (ddd, *J* = 9.6, 6.4, 3.2) | 78.3 | 4.38 (ddd, *J* = 11.1, 5.4, 3.8) |
| 21 (CH) | 74.5 | 3.89 (dd, *J* = 8.7, 3.4) | 77.2 | 3.71 | 75.5 | 3.81 (dd, *J* = 10.3, 3.6) |
| 25 (C) | 96.7 | – | 97.5 | – | 99.2 | – |
| 26 ($CH_2$) | 66.6 | 4.31 (d, *J* = 11.0, 1H), 4.07 (d, *J* = 11.0, 1H) | 68.3 | 4.07 (t, *J* = 11.0) | 65.2 | 4.64 (d, *J* = 12.4) 3.96 (d, *J* = 12.2) |
| 35($CH_3$) | 57.9 | 3.32 (s) | 58.5 | 3.33 (s) | 58.7 | 3.34 (s) |
| 37 C=O | 154.5 | – | 154.5 | – | 156.5 | – |
| 38 (C) | 140.2 | – | 139.8 | – | 138.8 | – |
| 39 and 43 (CH) | 118.3 | 7.52 (d, *J* = 8.3) | 119.5 | 7.44 (d, *J* = 7.9) | 120.0 | 7.42 (d, *J* = 7.9) |
| 40 and 42 (CH) | 122.3 | 7.21 (t, *J* = 8.0) | 123.8 | 7.28 (t, *J* = 8.0) | 124.7 | 7.31 (t, *J* = 8.0) |
| 41 (CH) | 129.0 | 6.92 (t, *J* = 7.4) | 129.7 | 7.03 (t, *J* = 7.4) | 129.9 | 7.07 (t, *J* = 7.4) |
| O(4)H | – | 3.81 (s) | – | 4.33 (s) | – | 4.78(bs) |
| O(10)H | – | 7.81 (s) | – | 4.59 (s) | – | 4.78(bs) |
| N(1)H | – | 9.62 (s) | – | 7.93(s) | – | 8.18 (s) |
| COOH | – | – | – | 9.89(bs) | – | 10.0–10.5 (vbr) |

[a] Data from [20].
[b] NMR studies from this work.

**Table 2**
The coordination distances (Å) between oxygen atoms and the sodium cation in the structure of Na–MU salt and H–MU–Na complex calculated by PM5 method (WinMopac 2003).

| Coordinating oxygen atom | Distance (Å) between coordinating oxygen atom and $Na^+$ cation | | |
|---|---|---|---|
| | Na–MU | H–MU–Na (A)[a] | H–MU–Na (B) |
| O(1) | 2.35 | – | – |
| O(4) | 2.36 | 2.34 | 2.37 |
| O(6) | 2.28 | 2.42 | 2.32 |
| O(7) | 2.31 | 2.32 | 2.33 |
| O(8) | 2.29 | 2.26 | 2.28 |
| O(9) | – | 2.49 | 2.35 |
| O(12) | – | – | 2.35 |

[a] (A) The structure of H–MU–Na complex in which the C(37)=O group is not involved in coordination of the $Na^+$ cation and (B) the structure of H–MU–Na complex in which the C(37)=O group is involved in coordination of the $Na^+$ cation.

of the metal cation. This is in accordance with the previously published crystallographic data of Monensin A phenylurethane sodium salt [20]. A similar regioselectivity of the dehydration steps was also previously observed for monensin sodium salt and was widely discussed in literature [26–28]. The loss of further $H_2O$ molecule from cation B and C gives rise to a peak at *m*/*z* 776 assigned to ion P. The formation of H fragmentary ion is achieved by abstraction of this part of the molecule that includes the urethane moiety. The cation H which is assigned to the ion of 1:1 complex of monensin acid with $Na^+$ cation shows the same fragmentation pathway, with the formation of the other fragmentary ions (I, J, K, L, M, N, and finally O) like monensin which ESI MS fragmentation was previously described by Lopes et al. [26,27].

The formation of D fragmentary complexes is achieved by cleavage of the urethane group and cation E is form D by the abstraction of water molecules.

It has been demonstrated that the common fragmentation ions were produced *via* a Grob–Wharton type mechanism [28]. The cations F are formed from cations D, by the abstraction of one $C_3H_4$ group. Furthermore, the cation G can be formed by the loss of one water molecule from the cation F.

### *Semiempirical calculation*

On the basis of the spectroscopic results the structures of H–MU, Na–MU and H–MU–Na, were visualised using PM5 semi-empirical method as shown in Figure S3.

**Table 3**
Dimensions of the hydrogen bonds present in the structures of H–MU, Na–MU and H–MU–Na complex and their the heat of formation (HOF) calculated by PM5 method (WinMopac 2003).

| Compound | D–H···A | *d*(D···A) (Å) | <(DHA) (°) | HOF (kcal/mol) |
|---|---|---|---|---|
| H–MU | N(1)—H···O(1) | 2.95 | 152 | 556.6 |
| | O(2)—H···O(4) | 2.90 | 161 | |
| | O(4)—H···O(10) | 2.68 | 105 | |
| | O(10)—H···O(4) | 2.68 | 106 | |
| Na–MU | N(1)—H···O(1) | 2.80 | 112 | −642.3 |
| | O(10)—H···O(2) | 2.65 | 113 | |
| | O(4)—H···O(2) | 2.98 | 165 | |
| H–MU–Na (A)[a] | N(1)—H···O(1) | 2.92 | 137 | −489.5 |
| | O(2)—H···O(4) | 2.87 | 150 | |
| | O(4)—H···O(10) | 2.91 | 127 | |
| | O(10)—H···O(4) | 2.68 | 106 | |
| H–MU–Na (B) | N(1)—H···O(1) | 2.61 | 109 | −446.6 |
| | O(2)—H···O(4) | 2.96 | 124 | |
| | O(4)—H···O(10) | 2.55 | 104 | |
| | O(10)—H···O(4) | 2.55 | 107 | |

[a] (A) the structure of H–MU–Na complex in which the C(37)=O group is not involved in coordination of the $Na^+$ cation and (B) the structure of H–MU–Na complex in which the C(37)=O group is involved in coordination of the $Na^+$ cation.

The calculated heat of formation (HOF) values (Table 3) prove that the structure of Na–MU complex in which the oxygen carbonyl atom of the urethane group is not involved in the coordination of $Na^+$ cation (type A) is energetically more favourable than that in which the oxygen carbonyl atom of the urethane group is involved in this coordination (type B). This result is in good agreement with the FT-IR data discussed above (Fig. 1b).

The interatomic distances between the oxygen atoms and the sodium cation, for the most energetically favourable structures, are given in Table 2. Analysis of these values for structures of Na–MU salt and both structures of H–MU–Na complex shows that the oxygen atoms O(4), O(6), O(7), O(8) are always involved in the coordination. For the H–MU–Na complex of A type structure in which the C(37)=O group is not involved in the complexation of $Na^+$ cation, the values of the calculated coordination distances are comparable with those in B type structure (in which the C(37)=O group is involved in the complexation of $Na^+$ cation) indicating that both types of complexes are equally probable to be formed.

The calculated lengths and angles of the hydrogen bonds in which the OH and NH groups are engaged are summarised in Table 3. The calculated structures of H–MU acid indicate that the O(2) oxygen atom of the C=O carboxylic group is engaged in the intramolecular hydrogen bonds with O(10)H hydroxyl group. Upon formation of the H–MU–Na complex type A and B this hydrogen bonds is not broken because the oxygen atoms of carboxylic group and O(10)H are not involved in the coordination process of the sodium cation. For this reason the signal of C(1) carbon atom in the $^{13}C$ NMR spectra of H–MU do not shift after complexation of $Na^+$ cation.

In the structures of H–MU and H–MU–Na two O(10)H and O(4)H are bound together by the bifurcated intramolecular hydrogen bonds.

In the calculated structures of Monensin urethane, the N—H···O(1) intramolecular hydrogen bond bound the terminal parts of the molecule and stabilised its pseudo-cyclic conformation. A characteristic feature of such a confirmation of H–MU molecule is the central hole, which incorporates the sodium cation as a guest forming the host–guest H–MU–Na or Na–MU complexes.

The molecular structures of H–MU–Na and Na–MU complexes are compared in Fig. S3. In both complexes the skeleton of Monensin moiety assumes the pseudo-cyclic conformation, which is stabilised by different intramolecular hydrogen bonds whose parameters ale collected in Table 3.

## Conclusion

Previous studies have shown that Monensin phenyl urethanes exhibit high antibacterial activity. Biological activity of this compound has been explained in the chemical literature by two mutually exclusive structures of this compound. To explain molecular reasons behind the antibacterial properties of urethane derivatives of Monensin, the structures of three forms of phenyl urethane of Monensin i.e. acid form (H–MU) and its 1:1 complex with $NaClO_4$ (H–MU–Na) and its sodium salt (Na–MU) have been determined and characterised by FT-IR, $^1H$ and $^{13}C$ NMR spectroscopy, ESI mass spectrometry and PM5 calculation. Within the studied complexes, the sodium cation is coordinated by oxygen atoms in the hydrophilic interior of the Monensin moiety forming a pseudo-cyclic structure wrapped around the $Na^+$ cation. These structures are also stabilized by the "head-to-tail" type of intramolecular hydrogen bonds. The FT-IR and $^{13}C$ NMR spectroscopy and semi-empirical calculations have shown that the oxygen atom of the carbonyl urethane group is involved in the coordination of the $Na^+$ cation only in the H–MU–Na complex structure.

The formation of stable H–MU–Na complex up to $cv = 50$ V is indicated by the electrospray ionisation mass spectra. With increasing cone voltage value, the fragmentation of the complex is detected and is connected primarily with the dehydration process, which is discussed in detail.

For the first time the formation of the complex between urethane derivatives of Monensin in its acidic form and the sodium cation was evidenced and this new discovery certainly contributes to understanding biological properties of these interesting Monensin derivatives.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.03.060.

## References

[1] D.A. Kevin, D.A.F. Meujo, M.T. Hamann, Exp. Opin. Drug Discov. 4 (2009) 109.
[2] A. Huczyński, Bioorg. Med. Chem. Lett. 22 (2012) 7002.
[3] H.D. Chapman, T.K. Jeffers, R.B. Williams, Poultry Sci. 89 (2010) 1788.
[4] F.G. Riddell, Chilarity 14 (2002) 121.
[5] K. Nakazato, Y. Hatano, Biochim. Biophys. Acta 1064 (1991) 103.
[6] T. Martinek, F.G. Riddell, C. Wilson, C.T. Weller, J. Chem. Soc. Perkin Trans. 2 (2000) 35.
[7] D. Łowicki, A. Huczyński, BioMed Res. Internat. 2013, (2013), Art. ID 742149.
[8] S. Donadio, S. Maffioli, P. Monciardini, M. Sosio, D. Jabes, J. Antibiot. 63 (2010) 423.
[9] A. Huczyński, J. Stefańska, P. Przybylski, B. Brzezinski, F. Bartl, Bioorg. Med. Chem. Lett. 18 (2008) 2585.
[10] A. Huczyński, J. Janczak, B. Brzezinski, J. Mol. Struct. 985 (2011) 70.
[11] D. Łowicki, A. Huczyński, J. Stefańska, B. Brzezinski, Tetrahedron 67 (2011) 1468.
[12] D. Łowicki, A. Huczyński, J. Stefańska, B. Brzezinski, Eur. J. Med. Chem. 45 (2010) 4050.
[13] D. Łowicki, A. Huczyński, B. Brzezinski, F. Bartl, J. Mol. Struct. 990 (2011) 121.
[14] J.W. Westley, C.-M. Liu, R.H. Evans, L.H. Sello, N. Troupe, T. Hermann, J. Antibiot. 36 (1983) 1195.
[15] F. Rabaste, G. Jeminet, G. Dauphin, A.-M. Delort, Biochim. Biophys. Acta 1179 (1993) 166.
[16] M. Rochdi, A.-M. Delort, J. Guyot, M. Sancelme, S. Gibot, J.-G. Gourcy, G. Dauphin, H, Vial, G. Jeminet, J. Med. Chem. 39 (1996) 588.
[17] R. Tanaka, A. Nagatsu, H. Mizukami, Y. Ogihara, J. Sakakibara, Chem. Pharm. Bull. 49 (2001) 711.
[18] E.G. Schildknecht, D. Siegel, R.W. Richle, Chemotherapy 29 (1983) 145.
[19] C.-M. Liu, J. Westley, United States Patent 4,263,427, 1981.
[20] A. Huczyński, M. Ratajczak-Sitarz, J. Stefańska, A. Katrusiak, B. Brzezinski, F. Bartl, J. Antibiot. 64 (2011) 249.
[21] A. Huczyński, J. Janczak, D. Łowicki, B. Brzezinski, Biochim. Biophys. Acta, Biomembranes 1818 (2012) 2108.
[22] Cache Work System Pro Version 7.5.085 UserGuide, Fujitsu, Beaverton, Oregon, USA, 2007.
[23] P. Przybylski, A. Huczyński, B. Brzezinski, J. Mol. Struct. 826 (2007) 156.
[24] G. Zundel, B. Brzezinski, J. Olejnik, J. Mol. Struct. 300 (1993) 573.
[25] B. Brzezinski, G. Schroeder, A. Rabold, G. Zundel, J. Phys. Chem. 99 (1995) 8519.
[26] N.P. Lopes, C.B.W. Stark, P.J. Gates, J. Staunton, Analyst 127 (2002) 503.
[27] N.P. Lopes, C.B.W. Stark, H. Hong, P.J. Gates, J. Staunton, Rapid Commun. Mass Spectrom. 16 (2002) 414.
[28] D.E. Kiehl, R.K. Julian, A.S. Kennington, Rapid Commun. Mass Spectrom. 12 (1998) 903.