

# First solid state alkaline-earth complexes of monensin A (MonH): crystal structure of $[M(\text{Mon})_2(\text{H}_2\text{O})_2]$ ( $M = \text{Mg}$ , $\text{Ca}$ ), spectral properties and cytotoxicity against aerobic Gram-positive bacteria

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**Abstract** Alkaline-earth metal complexes of the monoanionic form of the polyether ionophore monensin A were isolated for the first time in solid state and were structurally characterized using various spectroscopic methods (IR, NMR, FAB-MS). The stoichiometric reaction of monensin acid (MonH) with  $M^{2+}$  ( $M = \text{Mg}$ ,  $\text{Ca}$ ) in the presence of an organic base leads to the formation of mononuclear complexes of composition  $[M(\text{Mon})_2(\text{H}_2\text{O})_2]$ . The structures of magnesium (**1**) and calcium (**2**) monensin complexes in the solid state were established by single crystal X-ray crystallography. The complexes crystallize as  $[\text{Mg}(\text{Mon})_2(\text{H}_2\text{O})_2] \cdot 5\text{MeCN}$  (**1**) and  $[\text{Ca}(\text{Mon})_2(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O} \cdot 5\text{MeCN}$  (**2**) in the monoclinic *P21* space group. The alkaline-earth metal ion

is placed in a distorted octahedral environment, defined by two monensin anions acting as bidentate ligands in the equatorial plane of the complex as well as by two water molecules occupying the axial positions of the inner coordination sphere. The bactericidal activity of **1** and **2** was evaluated against aerobic Gram-positive microorganisms applying the double layer agar hole diffusion method.

**Keywords** Monovalent carboxylic ionophore · Alkaline-earth monensin complexes · Crystal structure · IR · NMR · FAB-MS · Bactericidal activity

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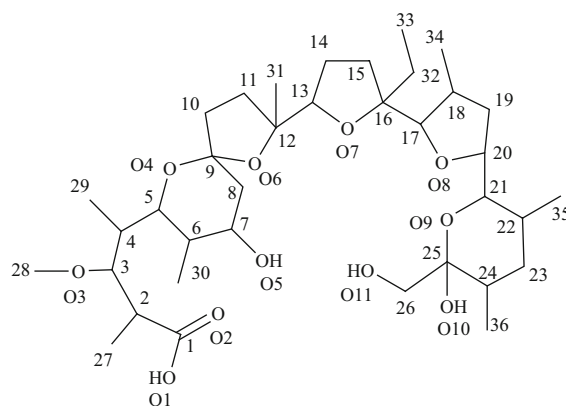
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## Introduction

Recently the most powerful drugs in veterinary medicine effective against coccidiosis and infections induced by Gram-positive organisms represent the group of naturally occurring polyether ionophorous antibiotics (Agtarap et al. 1967; Stern 1977; Berg and Hamill 1978; Liu et al. 1978; Chappe 1979; Long and Jeffers 1982; Westley et al. 1983; Augustine et al. 1987; Koinarski and Sherkov 1987; Folz et al. 1988; Augustine et al. 1992; Varga and Sreter 1996; Wang et al. 2006; Kevin et al. 2009). Their main representative—monensin—is well known and its ability of binding monovalent metal ions is extensively studied.

The neutral ionophore complexes formed are able to penetrate the cell membrane of the microorganisms and parasites and to disturb a series of homeostatic processes after dissociation in the intracellular space causing a death of the corresponding target (Riddell 2002). Due to the high affinity of monensin to complex alkaline metal ions, it is known for a very long time to be a monovalent polyether ionophore (Lutz et al. 1971; Briggs and Hinton 1978; Duax et al. 1980; Garcla-Rosas et al. 1983; Cox et al. 1984; Walba et al. 1986; Pangborn et al. 1987; Rzeszotarska et al. 1994; Wagner-Czauderna et al. 1997; Paz et al. 2003; Huczynski et al. 2007a, b, c, 2008a, b, c, d; Yildirim et al. 2007). From the 1990s up to the present several research groups have drawn attention to the possible complexation of monensin with divalent metal ions (Hebrant et al. 1992; Mimouni et al. 1994; Dassie and Baruzzi 2002; Hamidinia et al. 2002; Huczynski et al. 2006a, b, c, d, 2007a, b, c, 2008a, b, c, d). Although there was only indirect evidence concerning the unusual coordination behaviour of monensin, it was suggested that the complexation chemistry of the ligand (and of other representatives of the monovalent polyether ionophores group) is much broader than we were previously acquainted with (Stiles et al. 1991; Hamidinia et al. 2002, 2004). The hypothesis that monensin possesses previously unknown reactivity prompted our group to start an extensive study on the coordination ability of the ligand towards di- and three-valent metal ions. In our previous publications we demonstrated that monensin could form two types of divalent transition metal complexes depending on the chemical form used in the reactions (Dorkov et al. 2008; Pantcheva et al. 2008, 2009). The compounds obtained possess enhanced antimicrobial activity towards Gram(+) bacteria that is most likely due to the unusual coordination mode of the ligand.

In order to confirm the ability of monensin to bind divalent metal ions, we have now studied its behaviour towards alkaline-earth metals, in particularly magnesium and calcium ions. In the present paper we describe the structure elucidation of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  complexes of the monoanionic form of monensic acid (MonH, Scheme 1) in the solid state by single crystal X-ray crystallography, report on their spectroscopic characterization and on the evaluation of their bactericidal properties against aerobic Gram-positive microorganisms.



**Scheme 1** The chemical structure and numbering sequence of monensic acid

## Materials and methods

### Chemicals

All chemicals were of reagent grade and were used as received. The commercially available sodium monensin was obtained from BIOVET, Ltd. (Bulgaria). Solvents (MeCN, MeOH, DMSO), organic base ( $\text{Et}_4\text{NOH}$ ) and metal salts ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ ) were purchased from Merck.

### Synthesis of monensic acid

The acidic form of monensin A monohydrate (monensic acid,  $\text{MonH} \cdot \text{H}_2\text{O}$ ) was prepared from sodium monensin as previously reported (Gertenbach and Popov 1975). IR:  $\nu_{\text{COOH}} = 1700 \text{ cm}^{-1}$ .  $^1\text{H-NMR}$  (600 MHz,  $\delta$  (multiplicity, intensity,  $J$ -coupling(s), assignment),  $\text{CDCl}_3$ ): 6.25 (br, 1-OH, 10-OH (sharp), 11-OH,  $\text{H}_2\text{O}$ ), 4.50 (br d, 1H, 7.9, 5-OH), 4.33 (ddd, 1H, 10.8, 5.9, 2.9, 20CH), 4.07 (dd, 1H, 11.6, 2.1, 5CH), 4.03 (d, 1H, 4.0, 17CH), 3.94 (dd, 1H, 10.5, 2.7, 21CH), 3.86 (br s, 1H, 7CH), 3.69 (d, 1H, 11.2, 26 $\text{CH}_2'$ ), 3.50 (d, 1H, 11.2, 26 $\text{CH}_2''$ ), 3.44 (dd, 1H, 10.7, 4.6, 13CH), 3.37 (s, 3H, 28 $\text{OCH}_3$ ), 3.22 (dd, 1H, 10.2, 2.1, 3CH), 2.62 (dq, 1H, 10.2, 6.7, 2CH), 2.25–2.13 (4H, 18CH, 15 $\text{CH}_2'$ , 4CH, 19 $\text{CH}_2'$ ), 2.10 (m, 1H, 6CH), 1.98 (dd, 1H, 14.2, 3.5, 8 $\text{CH}_2'$ ), 1.97 (dd, 1H, 12.3, 8.4, 10 $\text{CH}_2'$ ), 1.89 (dt, 1H, 11.9, 8.7, 11 $\text{CH}_2'$ ), 1.74–1.52 (m, 5H, 8 $\text{CH}_2''$ , 11 $\text{CH}_2''$ , 10 $\text{CH}_2''$ , 14CH<sub>2</sub>), 1.55 (m, 2H, 32 $\text{CH}_2$ ), 1.52–1.33 (m, 5H, 23 $\text{CH}_2'$ , 24CH, 15 $\text{CH}_2''$ , 19 $\text{CH}_2''$ , 23 $\text{CH}_2''$ ), 1.48 (s, 3H, 31 $\text{CH}_3$ ), 1.35 (m, 1H, 22CH), 1.27 (d,

3H, 6.7, 27CH<sub>3</sub>), 1.10 (d, 3H, 6.9, 29CH<sub>3</sub>), 0.95 (t, 3H, 7.4, 33CH<sub>3</sub>), 0.92 (d, 3H, 6.9, 34CH<sub>3</sub>), 0.88 (d, 3H, 7.1, 30CH<sub>3</sub>), 0.87 (d, 3H, 6.4, 36CH<sub>3</sub>), 0.85 (d, 3H, 6.5, 35CH<sub>3</sub>).

#### Synthesis of magnesium complex, [Mg(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], **1**

The consecutive addition of Et<sub>4</sub>NOH (0.5 mmol, 180 µl, 40% in H<sub>2</sub>O) and of freshly prepared solution of MgCl<sub>2</sub>·6H<sub>2</sub>O (0.5 mmol, 100 mg in 5 ml MeCN/MeOH, 10:1) to a solution of MonH (0.5 mmol, 336 mg in 20 ml MeCN/MeOH, 10:1) afforded the precipitation of white solids with a composition of [Mg(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], **1**, which are insoluble in MeCN (228 mg, 65% yield). The complex was filtered off, washed with MeCN and dried over P<sub>2</sub>O<sub>5</sub> at room temperature. Anal. Calcd for MgC<sub>72</sub>H<sub>126</sub>O<sub>24</sub> (MW = 1400.08): H, 9.07, C, 61.77, Mg, 1.74; Found: H, 8.76, C, 60.17, Mg, 2.14%. Slow concentration of diluted reaction mixture affords the formation of colourless crystals of composition [Mg(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].5MeCN. IR:  $\nu_{\text{COO}}^{\text{asym}} = 1550 \text{ cm}^{-1}$ ,  $\nu_{\text{COO}}^{\text{sym}} = 1400 \text{ cm}^{-1}$ . <sup>1</sup>H-NMR (600 MHz,  $\delta$  (assignment), CDCl<sub>3</sub>): 10.98 (11-OH), 6.60 (10-OH), 4.98 (H<sub>2</sub>O), 4.85 (5-OH), 4.30 (20CH), 4.09 (21CH), 4.04 (17CH, 5CH), 3.73 (7CH), 3.78 (26CH<sub>2</sub>'), 3.68 (26CH<sub>2</sub>''), 3.52 (13CH), 3.33 (28OCH<sub>3</sub>), 3.24 (3CH), 2.46 (2CH), 2.33 (6CH), 2.23 (18CH), 2.17–2.04 (15CH<sub>2</sub>', 19CH<sub>2</sub>', 4CH), 2.00–1.94 (8CH<sub>2</sub>', 10CH<sub>2</sub>'), 1.85 (11CH<sub>2</sub>'), 1.75–1.68 (14CH<sub>2</sub>'), 1.70–1.50 (8CH<sub>2</sub>'', 11CH<sub>2</sub>'', 10CH<sub>2</sub>'', 14CH<sub>2</sub>'', 32CH<sub>2</sub>'), 1.54 (31CH<sub>3</sub>), 1.50–1.40 (19CH<sub>2</sub>'', 32CH<sub>2</sub>'', 15CH<sub>2</sub>''), 1.37 (24CH), 1.29 (23CH<sub>2</sub>'', 22CH), 1.18 (27CH<sub>3</sub>), 1.08 (29CH<sub>3</sub>), 0.98–0.82 (33CH<sub>3</sub>, 34CH<sub>3</sub>, 30CH<sub>3</sub>, 36CH<sub>3</sub>, 35CH<sub>3</sub>).

#### Synthesis of calcium complex, [Ca(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], **2**

To a reaction mixture containing MonH (0.5 mmol, 336 mg in 15 ml MeCN/MeOH (10:1)) and Et<sub>4</sub>NOH (0.5 mmol, 180 µl, 40% in H<sub>2</sub>O) the addition of CaCl<sub>2</sub> (0.5 mmol, 56 mg in 5 ml MeCN/MeOH, 10:1) led to the formation of colourless solution that slowly precipitates within 24 h (240 mg, 68% yield). The solid was filtered off, washed with MeCN and dried over P<sub>2</sub>O<sub>5</sub> at room temperature. The elemental analysis data are in agreement with the general composition of the complex, [Ca(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], **2**.

Anal. Calcd. for CaC<sub>72</sub>H<sub>126</sub>O<sub>24</sub> (MW = 1415.86): H, 8.97, C, 61.08, Ca, 2.83; Found: H, 8.56, C, 58.12, Ca, 3.20%. Complex **2** is soluble in MeOH, CHCl<sub>3</sub> and octanol, and possesses very low solubility in MeCN and DMSO. IR:  $\nu_{\text{COO}}^{\text{asym}} = 1560 \text{ cm}^{-1}$ ,  $\nu_{\text{COO}}^{\text{sym}} = 1400 \text{ cm}^{-1}$ . <sup>1</sup>H-NMR (600 MHz,  $\delta$  (assignment), CDCl<sub>3</sub>): 4.95 (5-OH), 4.32 (20CH), 4.23 (17CH), 4.04 (5CH, 21CH), 3.86–3.68 (7CH, 26CH<sub>2</sub>'), 3.56 (26CH<sub>2</sub>''), 3.50 (13CH), 3.37 (28OCH<sub>3</sub>), 3.24 (3CH), 2.48 (2CH), 2.37 (6CH), 2.30 (18CH), 2.22–2.11 (15CH<sub>2</sub>', 19CH<sub>2</sub>'), 2.09 (4CH), 2.00–1.88 (8CH<sub>2</sub>', 10CH<sub>2</sub>', 11CH<sub>2</sub>'), 1.76–1.58 (8CH<sub>2</sub>'', 11CH<sub>2</sub>'', 10CH<sub>2</sub>'', 14CH<sub>2</sub>'), 1.58–1.40 (32CH<sub>2</sub>, 23CH<sub>2</sub>', 24CH, 31CH<sub>3</sub>, 15CH<sub>2</sub>'', 19CH<sub>2</sub>''), 1.40–1.26 (23CH<sub>2</sub>'', 22CH), 1.19 (27CH<sub>3</sub>), 1.12 (29CH<sub>3</sub>), 0.98–0.83 (33CH<sub>3</sub>, 34CH<sub>3</sub>, 30CH<sub>3</sub>, 36CH<sub>3</sub>, 35CH<sub>3</sub>).

Slow concentration of diluted reaction mixture (2–3 days at ambient temperature) affords the precipitation of **2** as colorless crystals. The X-ray analysis showed that **2** crystallizes as [Ca(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O·5MeCN.

#### Physical measurements

Infrared spectra (4000–400 cm<sup>-1</sup>) were recorded on a Specord 75-IR (Carl-Zeiss, Germany) in a nujol mull. FAB-MS spectra were performed using Fisons VG Autospec (Micromass Instruments, UK).

<sup>1</sup>H (600.13 MHz) and <sup>13</sup>C (150.92 MHz) spectra were acquired on an AVANCE AV600 II+ NMR spectrometer (Bruker, Germany). All spectra were recorded in CDCl<sub>3</sub> at room temperature. TMS was used as an internal standard for the <sup>1</sup>H and <sup>13</sup>C spectra. Unambiguous assignment of the signals was made on the basis of the gradient enhanced versions of COSY, TOCSY, HSQC, HMBC and ROESY experiments (Bruker pulse library programs: cosygpmfqq, dipsi2etgpsi, hsqcetdgtpsisp2.2, hmbcgpplndqf, roesyph.2, 2007). The chemical shift values of the individual protons in the compounds have been determined from the HSQC spectra.

Elemental analysis data (C, H, O) were obtained with a VarioEL V5.18.0 Elemental Analyzer (Elementar Analysen Systeme GmbH, Germany). The metal content of complexes was determined by AAS on a Perkin Elmer 1100 B (Waltham, USA) after decomposition of samples with conc. HNO<sub>3</sub> and using a stock standard solution (Merck, 1000 µg/ml); the working reference solutions were prepared after suitable dilution.

## X-ray crystallography

Details concerning data collection, structure solution and refinement of complexes **1** and **2** are given in Table 1. X-ray diffraction measurements were performed on an Oxford Diffraction Xcalibur 2 diffractometer at 112 K, operating with Mo-K $\alpha$  ( $\lambda = 0.71073$  Å) radiation and equipped with a graphite monochromator. The structures were solved by direct methods and were refined by full-matrix least-square procedures on  $F^2$  (Sheldrick 1990; Sheldrick 1997). All non-H atoms were refined isotropically with a riding model.

## Cytotoxicity assay (determination of MIC)

Three aerobic Gram-positive microorganisms were used as test strains to evaluate the cytotoxic properties of the alkaline-earth complexes, MgCl<sub>2</sub>·6H<sub>2</sub>O and CaCl<sub>2</sub>. The bacteria *Bacillus subtilis* (ATCC 6633), *Bacillus mycoides* spp. and *Sarcina lutea* FDA strain PCI 1000 (ATCC 10054) were purchased from the

National Bank for Industrial Microorganisms and Cell Cultures (Bulgaria). The activity of compounds is determined as their minimum inhibitory concentration [MIC, ( $\mu$ M)], which is the lowest concentration causing the visible inhibition of the bacteria growth. Details concerning the experimental procedures using the double layer agar hole diffusion method were carried in accordance with the literature (Andrews 2001) and were similar to those reported previously (Dorkov et al. 2008; Pantcheva et al. 2008, 2009).

## Results and discussion

The reaction of monensic acid with MgCl<sub>2</sub> or CaCl<sub>2</sub> in the presence of an organic base leads to the formation of unique alkaline-earth metal complexes of composition [M(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] (M = Mg (**1**), Ca (**2**)). The reaction proceeds in a mixed solvent system (MeCN/MeOH) and at metal-to-ligand-to-base molar ratio of 1:1:1. The addition of Et<sub>4</sub>NOH is essential for the coordination of the ligand to the divalent metal

**Table 1** Crystal data and structure refinement for complexes **1**, [Mg(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]·5MeCN, and **2**, [Ca(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]·H<sub>2</sub>O·5MeCN

Compound	Complex 1	Complex 2
Formula	C <sub>82</sub> H <sub>141</sub> O <sub>24</sub> N <sub>5</sub> Mg	C <sub>82</sub> H <sub>143</sub> O <sub>25</sub> N <sub>5</sub> Ca
<i>M</i>	1605.31	1639.09
Crystal system	Monoclinic	Monoclinic
Space group	P 2 <sub>1</sub>	P 2 <sub>1</sub>
<i>a</i> (Å)	12.3337(5)	12.1855(4)
<i>b</i> (Å)	25.1619(9)	25.0185(11)
<i>c</i> (Å)	14.3112(6)	14.6551(5)
$\beta$ (°)	91.101(4)	90.292(3)
<i>V</i> (Å <sup>3</sup> )	4440.5(3)	4467.7(3)
<i>Z</i>	2	2
<i>D<sub>c</sub></i> (Mg/m <sup>3</sup> )	1.201	1.218
<i>F</i> (000)	1728	1780
$\mu$ (mm <sup>-1</sup> )	0.093	0.145
Crystal size (mm)	0.10 × 0.13 × 0.21	0.38 × 0.35 × 0.17
$\theta_{\min} - \theta_{\max}$ (°)	3.25–27.66	3.22–27.61
Dataset ( <i>h</i> , <i>k</i> , <i>l</i> )	–16/16, –31/32, –18/18	–15/15, –18/31, –18/18
Total Refl./Unique Refl.	57624/20116	33530/14670
Obs. Refl. [ <i>I</i> > 2 $\sigma$ ( <i>I</i> )]	9019	8758
Data/restraints/parameters	20116/8/984	14670/6/1020
R1, wR2 [ <i>I</i> > 2 $\sigma$ ( <i>I</i> )]	0.0605, 0.1398	0.0490, 0.1052
Residuals/eÅ <sup>-3</sup>	1.290/–0.363	0.865/–0.439
GOF	0.807	0.850

ions since it facilitates deprotonation of the carboxylic function of monensic acid during the complexation. In the absence of organic base the reaction does not take place, and the use of inorganic bases such as NaOH or KOH exclusively forms the monovalent metal complexes of monensin, MonNa or MonK, respectively.

#### Crystal structures of $[\text{Mg}(\text{Mon})_2(\text{H}_2\text{O})_2]$ , **1** and $[\text{Ca}(\text{Mon})_2(\text{H}_2\text{O})_2]$ , **2**

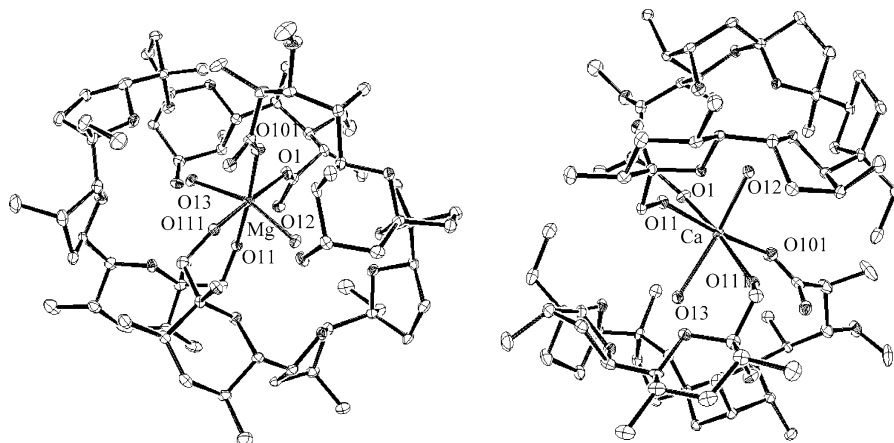
Generally, the magnesium and calcium complexes of monoanionic form of monensic acid consist of a discrete electrically neutral unit  $[\text{M}(\text{Mon})_2(\text{H}_2\text{O})_2]$ . Both compounds crystallize in the monoclinic space group *P21*. The crystals analyzed by X-ray crystallography additionally contain solvents molecules (MeCN and/or  $\text{H}_2\text{O}$ ), which do not affect the coordination mode of the ligand and do not participate in the formation of intramolecular H-bonds with the main unit. Such a solvent insertion was already observed in the cases of  $\text{Mn}^{2+}/\text{Co}^{2+}$  complexes of deprotonated monensic acid (Pantcheva et al. 2008) and of  $\text{Ba}^{2+}$  complex of lasalocid (Johnson et al. 1970a, b).

In compounds **1** and **2**, two monensic anions (Mon) act as bidentate ligands occupying four places in the inner coordination sphere of the alkaline-earth metal ion. Two of the positions in the equatorial plane of the complexes are filled by deprotonated monodentate carboxylic functions of two monensic anions ( $\text{COO}^-$ –M bonds), and other two by methoxy groups of Mon located at the opposite end of the ligand molecule forming two dative  $\text{HO} \rightarrow \text{M}$  bonds, respectively.

The folding of the ligand due to its bidentate coordination mode affords its pseudo-cyclization referred to as “head-to-tail” cyclization, observed even for the free ligand in the absence of metal ions (Lutz et al. 1971). Similarly to the transition metal complexes of Mon (Pantcheva et al. 2008) and barium complex of lasalocid (Johnson et al. 1970a, b), two water molecules are also included in the structures of **1** and **2**, linked by dative  $\text{H}_2\text{O} \rightarrow \text{M}$  bonds in axial positions with respect to the divalent metal ion and completing the octahedral environment of  $\text{Mg}^{2+}/\text{Ca}^{2+}$  (Fig. 1). The data confirm that water molecules in the new monensin complexes play a dual role: first, they occupy the inner coordination sphere of the metal ion, and in the second place they stabilize the strongly folded ligand by various intramolecular H-bonds. Selected bond distances and bond angles of **1** and **2** are presented in Table 2. Intramolecular H-bonds observed are shown in Fig. 2 and Table 3, respectively. No intermolecular H-bonds were detected in the structures. The crystal packing of complexes is depicted in Fig. 3.

The comparison of crystallographic data for the  $\text{Mn}^{2+}/\text{Co}^{2+}$  (Pantcheva et al. 2008) and  $\text{Ca}^{2+}/\text{Mg}^{2+}$  complexes of monoanionic monensic acid shows that the divalent metal center reacts in a similar manner in each case both with monensin ligands and with water molecules. The M–O bond lengths decrease in the order of  $\text{Ca}^{2+} > \text{Mn}^{2+} > \text{Co}^{2+} > \text{Mg}^{2+}$  in accordance with the decrease of the metal ionic radii. The M–O bond angles of metal ion with equatorial monensin ligands forming the *xy*-plane of complexes deviate from the regular ones ( $180^\circ$ ) by ca.  $10^\circ$  and

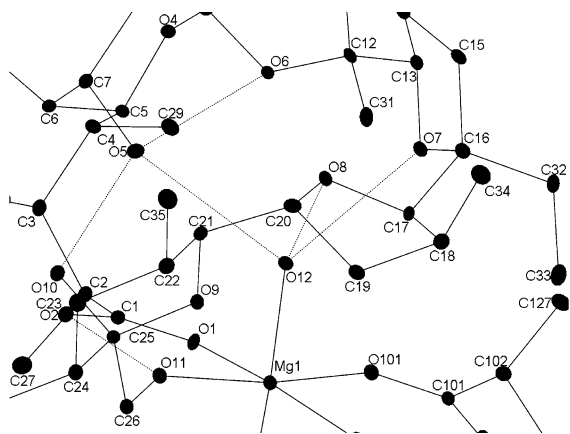
**Fig. 1** ORTEP of  $[\text{Mg}(\text{Mon})_2(\text{H}_2\text{O})_2]$  (**1**) and  $[\text{Ca}(\text{Mon})_2(\text{H}_2\text{O})_2]$  (**2**) at the 30% probability level (protons and solvent molecules are omitted for clarity)



**Table 2** Selected bond distances (Å) and bond angle (°) of complexes **1** and **2**

	Complex <b>1</b>	Complex <b>2</b>
<i>Bond length</i>		
M–O1	2.029(3)	2.266(3)
M–O101	2.029(3)	2.253(3)
M–O11	2.130(3)	2.358(3)
M–O111	2.136(3)	2.370(3)
M–O12	2.107(3)	2.341(2)
M–O13	2.102(3)	2.340(3)
<i>Bond angles</i>		
O1–M–O11	86.17(12)	82.44(9)
O101–M–O1	88.84(12)	94.63(10)
O1–M–O111	171.29(13)	172.17(9)
O101–M–O111	85.89(12)	82.54(10)
O111–M–O11	99.81(12)	101.03(9)
O101–M–O11	172.00(13)	173.94(9)
O12–M–O13	160.41(12)	151.22(9)

Symmetry position:  $-x, y + 1/2, -z$

**Fig. 2** Intramolecular bonds (in dotted line) observed in the structure of alkaline-earth complexes on the basis of complex **1** (the coordination of  $\text{Mg}^{2+}$  with one of the ligands is shown)

no significant difference is observed between them in dependence on the divalent metal center. In contrast, the metal-aqueous oxygen bond angles increase with decreasing metal ionic radii as follows:  $\text{Ca}^{2+}$  ( $151.2^\circ$ ) <  $\text{Mn}^{2+}$  ( $154.4^\circ$ ) <  $\text{Co}^{2+}$  ( $159.1^\circ$ ) <  $\text{Mg}^{2+}$  ( $160.4^\circ$ ). From the data collected up to now it can be concluded that the nature of the divalent metal ion (i.e. its ionic radius) does not affect significantly the equatorial planarity of complexes, but influences the

**Table 3** Intramolecular H-bonds of complexes **1** and **2**

	Complex <b>1</b>	Complex <b>2</b>
O2–O11	2.575	2.612
O102–O111	2.587	2.623
O5–O6	2.763	2.808
O105–O106	2.771	2.794
O5–O10	2.676	2.668
O105–O110	2.689	2.650
O5–O12	3.006	2.930
O105–O13	2.909	2.873
O6–O12	3.081	3.027
O106–O13	3.024	2.974
O7–O12	2.778	2.730
O107–O13	2.772	2.720
O8–O12	2.970	2.885
O108–O13	2.968	2.880

Symmetry position:  $-x, y + 1/2, -z$

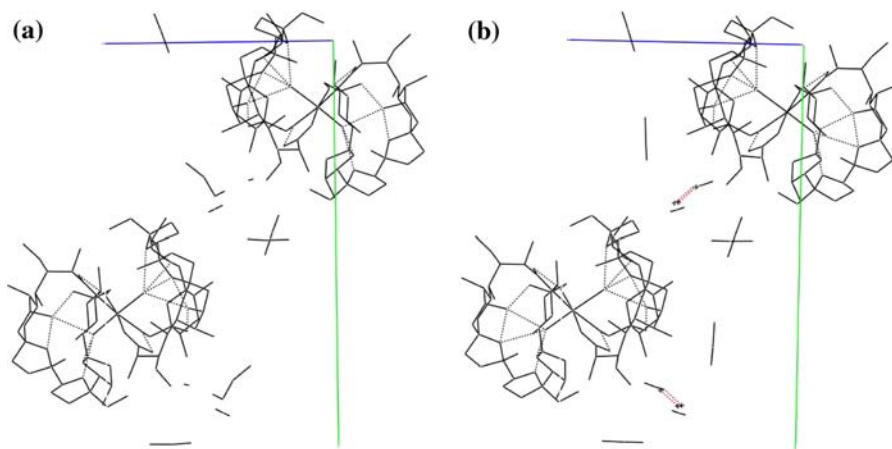
bonding with axial ligands thus leading to different degrees of deformation of the octahedral environment around the divalent cationic center.

The structures of **1** and **2** have clearly been established in the solid state by single crystal X-ray diffraction, nevertheless questions concerning the coordination mode of deprotonated monensic acid especially towards  $\text{Ca}^{2+}$  still remain. These queries arise since calcium and sodium ions are hard acids and their ionic radii are equivalent at coordination number of 6 [ $1.02 \text{ Å}$  ( $\text{Na}^+$ ) vs.  $1.00 \text{ Å}$  ( $\text{Ca}^{2+}$ )], but at the same time  $\text{Ca}^{2+}$  does not “enter” the hydrophilic cavity of ligand at the selected reaction conditions. Although some authors suggest the formation of shell-like alkaline-earth complexes with monovalent ionophores of composition metal-to-ligand ratio = 1:1 (Huczynski et al. 2007a, b, c, 2008a, b, c, d) their isolation as solid phases was not observed in the present research. Despite these hypothesis, the magnesium (**1**) and calcium (**2**) complexes of monoanionic monensic acid of composition of M:Mon = 1:2 clearly represent the first example of alkaline-earth monensin complexes to be isolated and fully characterized in solid state.

### IR spectral properties of complexes **1** and **2**

The IR spectra of **1** and **2** obtained in a nujol mull are very similar to those observed for the transition metal



**Fig. 3** Crystal packing of complexes **1** (a) and **2** (b)

complexes of Mon (Pantcheva et al. 2008) and the results are in agreement with the crystallographic data of new compounds. The main evidence that complexation takes place is found comparing the positions of stretching vibrations of the carboxyl function of MonH and of the carboxylate group of **1** and **2**, respectively. The peak due to  $\nu_{\text{COOH}}$  of MonH ( $1700\text{ cm}^{-1}$ ) disappears in the IR spectra of **1** and **2** thus demonstrating that deprotonation of carboxyl moiety occurs during the complexation. This fact was also confirmed by the appearance of two new peaks in the IR spectra of **1** and **2**, assigned to the stretching vibrations of the deprotonated carboxylic function, absorbing at  $1550\text{ cm}^{-1}$  ( $\nu_{\text{COO}}^{\text{asym}}$ ) and  $1400\text{ cm}^{-1}$  ( $\nu_{\text{COO}}^{\text{sym}}$ ), respectively. The IR spectral data confirming the coordination both of monensin hydroxyl groups and of water ligands to  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  are also in accordance with the data previously reported (Pantcheva et al. 2008). The crystallization water and hydroxyl groups of monensic acid absorb at  $3520$  and  $3320\text{ cm}^{-1}$ , respectively, while in the IR spectra of **1** and **2** the next bands were found and were assigned as follows:  $\nu_{\text{OH}_{\text{water}}}$  ( $3490\text{ cm}^{-1}$ ) and  $\nu_{\text{OH}_{\text{OH-groups}}}$  [ $3360, 3280\text{ cm}^{-1}$  (**1**);  $3280\text{ cm}^{-1}$  (**2**)].

### NMR spectra of MonH, **1** and **2**

To the best of our knowledge, several research teams solved  $^1\text{H}$ - and  $^{13}\text{C}\{^1\text{H}\}$ -NMR spectra of the monovalent metal complexes of Mon (Ajaz et al. 1987; Mimouni et al. 1996; Martinek et al. 2000) or its ester derivatives and their alkali complexes (Huczynski et al. 2006a, b, c, d, 2007a, b, c, 2008a, b, c, d), respectively. The presented study for a first time is

dealing in details with the NMR spectra of monensic acid and its diamagnetic divalent metal derivatives. The  $^1\text{H}$ -NMR data of MonH, **1** and **2** are presented in “Materials and methods” section.  $^{13}\text{C}\{^1\text{H}\}$ -NMR resonances are summarized in Table 4, following the numbering sequence of the ligand, shown in Scheme 1.

Due to the low solubility of metal complexes in MeCN, the NMR spectra were recorded in  $\text{CDCl}_3$  where OH-exchange is taking place thus significantly affecting the position of OH-groups. The 1-OH ( $-\text{COOH}$ ), 10-OH and 11-OH resonances in monensic acid appear as a broad signal at 6.25 ppm and 5-OH is observed at 4.50 ppm. The major evidence that the deprotonation of carboxylic function of MonH occurs during the complexation is the disappearance of 1-OH resonance in  $^1\text{H}$ -NMR spectra of **1** and **2**. At the same time a significant positive deviation in the position of 5-OH is observed whose resonance shifts from 4.50 ppm (MonH) to 4.85 ppm (**1**) and 4.95 ppm (**2**), respectively. The results obtained suggest that in solution this group is more strongly engaged in intramolecular H-bonding in the complexes than in the free acid and this was confirmed by the solid state structure of metal(II) compounds. As can be seen from the crystallographic data (Table 3; Fig. 3), 5-OH participates in three H-bonds by interacting with the aqua ligand ( $\text{H}_2\text{O}-12$ ), the ether oxygen O6 and the hydroxyl group 10-OH.

The main point of emphasis when discussing the  $^{13}\text{C}\{^1\text{H}\}$ -NMR spectra of MonH, **1** and **2** is focused on the positions of those carbon atoms, that are closely attached to the coordination sites of the ligand and whose resonances deviate significantly in the spectra on moving from MonH to complexes **1** and **2**. As can

**Table 4**  $^{13}\text{C}\{^1\text{H}\}$ -NMR (150 MHz,  $\text{CDCl}_3$ ) resonances of MonH and complexes **1–2**

C-atom	$\delta$ (ppm)			$\Delta$ (ppm)	
	MonH	<b>1</b>	<b>2</b>	$\Delta_1$	$\Delta_2$
1	177.09	181.94	181.81	4.85	4.72
2	41.94	43.70	44.29	1.76	2.35
3	81.56	82.28	82.49	0.72	0.93
4	36.70	37.08	37.40	0.38	0.70
5	67.01	65.66	68.21	−1.35	1.20
6	34.54	34.31	34.23	−0.23	−0.31
7	70.74	70.61	70.71	−0.13	−0.03
8	34.03	34.36	34.33	0.33	0.30
9	107.79	107.65	107.55	−0.14	−0.24
10	38.36	38.51	38.75	0.15	0.39
11	33.73	34.12	33.83	0.39	0.10
12	85.14	85.11	85.29	−0.03	0.15
13	83.42	85.06	83.91	1.64	0.49
14	27.88	27.60	27.78	−0.28	−0.10
15	31.49	29.26	32.83	−2.23	1.34
16	86.18	87.91	87.12	1.73	0.94
17	85.11	85.44	84.35	0.33	−0.76
18	34.45	34.31	34.23	−0.14	−0.22
19	32.58	32.75	31.25	0.17	−1.33
20	77.01	77.58	77.46	0.57	0.45
21	73.83	72.94	73.56	−0.89	−0.27
22	32.80	33.40	33.34	0.60	0.54
23	36.59	36.50	36.73	−0.09	0.14
24	35.68	36.30	35.90	0.62	0.22
25	96.99	98.38	98.63	1.39	1.64
26	67.98	67.85	66.35	−0.13	−1.63
27	15.66	16.94	16.81	1.28	1.15
28	58.03	57.75	57.94	−0.28	−0.09
29	10.37	11.23	11.36	0.86	0.99
30	10.75	10.67	10.76	−0.08	0.01
31	27.69	27.93	27.43	0.24	−0.26
32	31.19	30.66	30.79	−0.53	−0.40
33	8.61	8.84	8.73	0.23	0.12
34	15.75	15.84	15.74	0.09	−0.01
35	17.54	17.39	17.63	−0.15	0.09
36	16.32	16.88	16.97	0.56	0.65

$$\Delta_1 = \delta_1 - \delta_{\text{MonH}}; \Delta_2 = \delta_2 - \delta_{\text{MonH}}$$

be seen (Table 4), the position of 1C shows a very significant downfield shift in the spectra of **1** and **2** comparing to MonH ( $\Delta = 4.85$  ppm (**1**),  $\Delta = 4.72$  ppm (**2**),  $\Delta = \delta_{\text{complex}} - \delta_{\text{MonH}}$ ). Such behaviour is in agreement with the presence of monensin

deprotonated carboxylic function and reveals that both complexes retain their coordination in solution. A downfield shift in the spectra of **1** and **2** is also observed for the carbons 2, 3, 25, 27 and 29 ( $\Delta = 0.7$ – $2.5$  ppm), which are in close vicinity of atoms O1 and O11 directly coordinated to the divalent metal ions. The formation of H-bonds between the water ligands ( $\text{H}_2\text{O}$ -12 and  $\text{H}_2\text{O}$ -13) and ether oxygens O5–O8 also shifts some of the carbon-13 resonances of **1** and **2** significantly. The experimental data obtained show that positive as well as negative shifts are observed depending on the extent of deformation, that the ligand endures through the complexation. The NMR data obtained for Mg- and Ca-complexes of Mon correspond closely and confirm that (i) the coordination mode of monensin is analogous in **1** and **2**; (ii) the metal ions retain their linkage with bidentate monensinic anions both in solid state and in solution.

#### FAB-MS spectra of complexes **1** and **2**

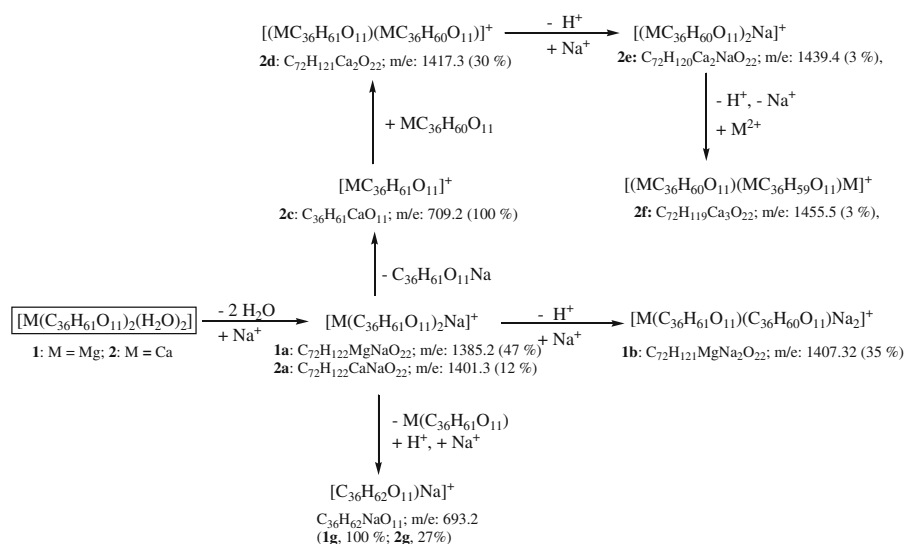
The major species found in the FAB-MS spectra of complexes **1** and **2** are presented in Scheme 2. The spectra consist of a great number of signals some of which are assigned to the molecular ions of complexes after loss of water ligands and association with sodium ions,  $[\text{M}(\text{C}_{36}\text{H}_{61}\text{O}_{11})\text{Na}]^+$  (**1a**:  $\text{M} = \text{Mg}$ ; **2a**:  $\text{M} = \text{Ca}$ ), as well as to the ions generated from association/dissociation processes occurring with the complex species in the gas phase (**1b**, **2c–f**). It should be mentioned that FAB-MS spectra of monensin complexes contain peaks of  $[\text{MonH} + \text{Na}]^+$  due to sodium traces from the matrix used (3-nitrobenzylalcohol), an observation that is in accordance with the high affinity of monensin to bind sodium ions and with data reported previously (Chamberlin and Agtarap 1970; Volmer and Lock 1998).

#### Cytotoxicity assay

The aerobic Gram-positive microorganisms *B. subtilis*, *S. lutea* and *B. mycoides* were used as test strains to evaluate the cytotoxicity of **1**, **2**,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  and  $\text{CaCl}_2$ . The bactericidal properties of MonH were also confirmed in the present study and its determined activity is in agreement with the data previously reported (Pantcheva et al. 2008).

The alkaline-earth chlorides, similarly to  $\text{MnCl}_2$  and  $\text{CoCl}_2$  (Dorkov et al. 2008; Pantcheva et al. 2008),





**Scheme 2** Major ions observed in the FAB-MS spectra of monensin alkaline-earth complexes **1** and **2**

cannot be defined as bactericidal agents because their minimum inhibitory concentrations fall in the millimolar concentration range (Table 5). Thus the effect of metal salts is uninfluential on the visible growth of bacteria strains and can be disregarded in comparison with the corresponding metal complexes studied.

The alkaline-earth complexes of monoanionic form of monensic acid possess strongly enhanced cytotoxicity against selected strains in comparison to the free ligand (Table 5) and furthermore, the antimicrobial activity of **1** and **2** cannot be assigned to the simple additive effect of partners in the complexes. Instead, it may probably be attributed to increased penetration of the new compounds through the bacteria's cell membrane. Previously we suggested that the increased activity of the  $\text{Co}^{2+}$  complex of

Mon (Pantcheva et al. 2008) could be explained in the terms of an unusual coordination mode of the monovalent polyether ionophorous antibiotic. The current results manifest that the role of divalent metal ion in mononuclear complexes of deprotonated monensic acid should be reconsidered, since the coordination mode of the antibiotic remains unchanged both in transition and in alkaline-earth metal complexes, respectively, but a difference (2–7 multiple) is observed in their cytotoxic properties.

It is still early to predict the intimate antibacterial mechanism of monensin divalent metal complexes, although it seems to be closely linked to the ability of the ligand to bind metal(II) ions and to transfer them across the cell membrane as neutral complexes, followed by further dissociation of the compounds

**Table 5** Bactericidal activity of MonH and complexes **1–2** expressed as MIC ( $\mu\text{M}$ )

Compound	<i>B. mycoides</i>	<i>B. subtilis</i>	<i>S. lutea</i>	References
MonH	11.9	23.9	23.9	Pantcheva et al. (2008)
[Ca(Mon) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ], <b>1</b>	0.7	1.4	1.4	This work
[Mg(Mon) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ], <b>2</b>	0.7	1.4	1.4	This work
[Co(Mon) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	1.4	2.8	2.8	Pantcheva et al. (2008)
[Mn(Mon) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	5.1	10.3	10.3	Pantcheva et al. (2008)
CaCl <sub>2</sub>	$1 \times 10^3$	$5 \times 10^3$	$5 \times 10^3$	This work
MgCl <sub>2</sub> ·6H <sub>2</sub> O	$1 \times 10^3$	$5 \times 10^3$	$5 \times 10^3$	This work
MnCl <sub>2</sub> ·4H <sub>2</sub> O	$5 \times 10^3$	$5 \times 10^3$	$5 \times 10^3$	Dorkov et al. (2008) and Pantcheva et al. (2008)
CoCl <sub>2</sub> ·6H <sub>2</sub> O	$2 \times 10^3$	$2 \times 10^3$	$2 \times 10^3$	Dorkov et al. (2008) and Pantcheva et al. (2008)

and by subsequent disturbance of homeostatic processes thus causing inhibition of bacteria growth.

## Conclusion

Alkaline-earth complexes of monoanionic form of monensin acid with  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were isolated for the first time in solid state. The complexes are of general composition  $[\text{M}(\text{Mon})_2(\text{H}_2\text{O})_2]$  ( $\text{M} = \text{Mg}, \text{Ca}$ ) and consist of an alkaline-earth metal ion placed in distorted octahedral environment. Monensin ligands act in a bidentate coordination mode via their monodentately bound carboxylate- and methoxy functions positioned at the both opposite sites of monensin anions. Additionally, two water molecules form two dative bonds with the alkaline-earth metal ion completing the sixfold geometry around the metal center and stabilizing the “head-to-tail” cyclization of the ligand molecule. The cytotoxicity assay against aerobic Gram-positive bacteria shows strongly enhanced activity of alkaline-earth monensin complexes in comparison to monensin acid that is most likely due to the unusual coordination mode of the ligand although the influence of the divalent metal ion should be also taken into account.

## Supplementary data

CCDC 734913 (**1**) and 734912 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk> (or an application from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 01223 336033; or e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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