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Synthesis, FT-IR, ^{1}H, ^{13}C NMR, ESI MS and PM5 studies of a new Mannich base of polyether antibiotic – Lasalocid acid and its complexes with Li^+, Na^+ and K^+ cations

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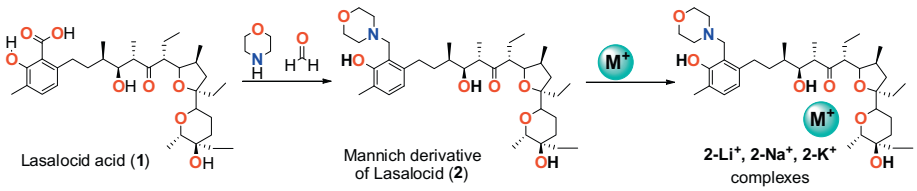
HIGHLIGHTS

- A new *ortho*-phenol Mannich base of Lasalocid was synthesized by chemoselective one-pot reaction.
- The compound obtained is a useful ligand for complexation of monovalent cations.
- Spectroscopic characterization of the ligand and prepared complexes is given.
- Semiempirical calculations of structures of the complexes studied are presented.

GRAPHICAL ABSTRACT



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ABSTRACT

The polyether antibiotic Lasalocid acid has been converted to its Mannich base derivative by a chemoselective one-pot reaction with formaldehyde and morpholine through the decarboxylation process. Spectroscopic studies of the structure of this new derivative have shown that in this *ortho*-phenol Mannich base the O—H···N intarmolecular hydrogen bond is present. The compound forms complexes with Li^+, Na^+ and K^+ cations of exclusively 1:1 stoichiometry. The structures of these complexes have been studied and visualized by semi-empirical calculation based on results of spectrometric and spectroscopic investigation. It is demonstrated that in contrast to Lasalocid acid the novel Mannich type derivative forms preferential complexes with Li^+ cation.

Introduction

Lasalocid acid (Scheme 1) and its derivatives represent a large class of ionophore antibiotics. These compounds show a broad spectrum of bioactivity e.g. antibacterial, antifungal, antiparasitic and antiviral [1–7]. Lasalocid acid sodium salt is used as an antibiotic for poultry and as a growth promoter for ruminants [1,2]. Lasalocid acid isolated from *Streptomyces lasaliensis* is able to form complexes with monovalent and divalent cations and transport them across lipid bilayer. The influx of Na^+ into the cell of Gram-positive bacteria leads to changes in pH and to an increase in the osmotic pressure inside the cell, causing swelling and vacuolization, eventually leading to cell death. The effectiveness of this process strongly depends on the structure of the Lasalocid metal cation complexes [1–4]. In previous studies we have shown that the complex of Lasalocid with allylamine has higher anti-bacterial activity than pure Lasalocid acid [8]. We found also that Lasalocid acid and its complexes are strong cytotoxic agents towards cancer cell lines. The cytostatic activity of Lasalocid and its complexes

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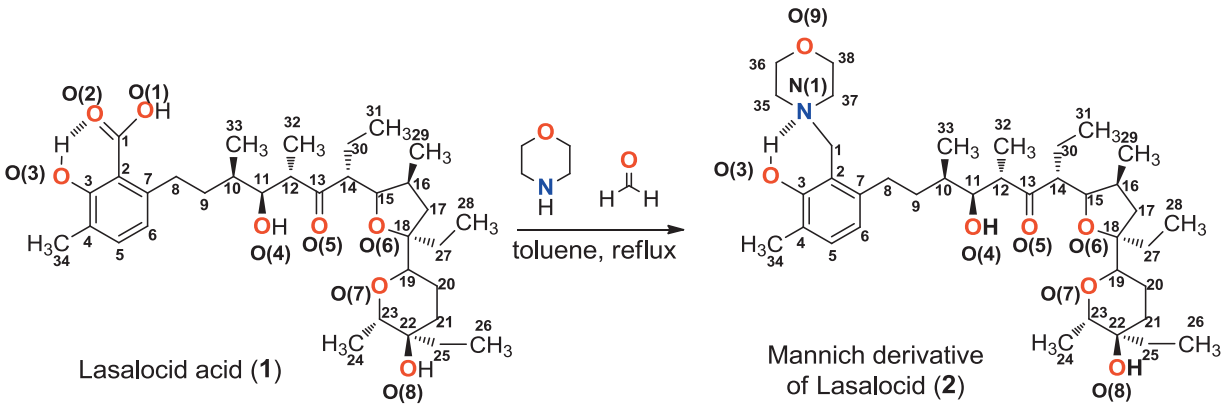

Scheme 1. Mannich reaction conditions.

with amines against human cancer cell lines is higher than that of cisplatin, indicating that Lasalocid and its complexes are promising candidates for new anticancer drugs [9].

Since various *N*-functionalized morpholines show pharmacological activity, we synthesized a new morpholine Mannich base derivative of Lasalocid acid. Such compounds are reported to exert a number of important physiological activities such as antiemetic or growth stimulant. They are also used in the treatment of inflammatory diseases, pain, migraine, and asthma [10–12]. In this contribution, the nature of complexes formed between Mannich base of Lasalocid acid with morpholine (**2**) and monovalent cations (Li^+, Na^+ and K^+) is studied using ^1H NMR, ^{13}C NMR, FTIR, ESI-MS as well as PM5 semiempirical methods. The structures of these complexes with metal cations are discussed in detail.

Experimental

Materials and methods

Lasalocid sodium salt was isolated from veterinary premix – Avatec® 20 (Alpharma Inc.), which contains about 20% pure Lasalocid sodium salt. Formaldehyde, morpholine and the perchlorates $LiClO_4$, $NaClO_4$ and $KClO_4$ were commercial products of Sigma and used without any further purification. Since the salts were hydrates, it was necessary to dehydrate them at several (6–10 times) evaporation steps from a 1:5 mixture of acetonitrile and absolute ethanol. The dehydration of perchlorates was followed by recording of their FT-IR spectra in acetonitrile.

CD_3CN and CH_3CN spectral-grade solvents were stored over 3 Å molecular sieves for several days. Handling of the compounds was performed in a carefully dried, CO_2-free glove box.

*Isolation of Lasalocid sodium salt (**1**)*

Lasalocid sodium salt was isolated from Avatec® 20 an anticoccidial feed additive distributed by Alpharma Inc. 100 g of Avatec was dissolved in dichloromethane. The solvent was evaporated under reduced pressure and the crude product obtained was purified by dry column vacuum chromatography (gradient solvent mixture hexane/dichloromethane) giving 11 g pure Lasalocid sodium salt.

Preparation of Lasalocid acid

Lasalocid sodium salt (1.5 g) was dissolved in dichloromethane (100 mL) and stirred vigorously with a layer of aqueous sulphuric acid (100 mL, $c = 0.75$ mol/L). The organic layer containing **1** was washed with distilled water and the dichloromethane was evaporated under reduced pressure to dryness to produce the acid.

*Synthesis of Mannich base of Lasalocid acid (**2**)*

A mixture of Lasalocid (1 g, 1.69 mmol), paraformaldehyde (253 mg, 8.45 mmol) and morpholine (736 mg, 8.45 mmol) in toluene (200 mL) was stirred and heated under reflux for 5 h. Water was collected in a Dean–Stark distilling trap during the reflux period. The resulting solution was diluted with petroleum ether and transferred to the separatory funnel and washed once with water and then one with 0.05 M HCl. The organic layer was evaporated under reduced pressure giving 1.6 g of pale yellow resin, which was then purified by dry column flash chromatography, giving 817 mg (75% yield) of final product. Elemental analysis calc. for $C_{38}H_{63}NO_7$: C 70.66%, H 9.83%, N 2.17%, O 17.34% found: C 70.64%, H 9.85%, N 2.14%, O 17.37%. The exemplary spectra are included in the Supplementary material.

*Synthesis of 1:1 complexes of **2** with monovalent cations*

The 0.07 mol/L solutions of 1:1 complexes of **2** with monovalent cations (Li^+, Na^+ and K^+) were obtained by adding equimolar amounts of $MClO_4$ salt (M = Li, Na, K) dissolved in water-free acetonitrile (3.5 mL, $c = 0.05$ mol/L) to acetonitrile solution of **2** (3.5 mL, $c = 0.05$ mol/L). The solvent was evaporated under reduced pressure to dryness and the oily residue was dissolved to the appropriate volume (2.5 mL) using dry CH_3CN or CD_3CN, respectively.

NMR measurements

The ^1H and ^{13}C NMR spectra of **2** and its complexes (0.07 mol/L) with Li^+, Na^+ and K^+ were recorded in CD_3CN solutions using Bruker Avance 600 MHz spectrometer. All spectra were locked to deuterium resonance of CD_3CN. The ^1H NMR measurements were carried out at the operating frequency 600.0018 MHz and the ^{13}C NMR spectra at the operating frequency 150.885 MHz. The temperature 298.0 K and TMS as the internal standard were used in both cases. No window function or zero filling was used. The errors of the ^1H and ^{13}C NMR chemical shift values were 0.01 ppm and 0.1 ppm, respectively. The ^1H and ^{13}C NMR signals were assigned using 2-D (COSY, HETCOR, NOESY and HMBC) whose examples are shown in the Supplementary materials (Figs. S1–S10). 2-D spectra were recorded using standard pulse sequences from Bruker pulse-sequence libraries.

FT-IR measurements

The FT-IR spectra of **2** and its complexes with monovalent cations were recorded in acetonitrile solution (0.07 mol/L). A cell with