

PHARMACOLOGY**ANTIBACTERIAL ACTIVITY OF MORIN AND ITS COMPLEXES
WITH La(III), Gd(III) AND Lu(III) IONS**MARIA KOPACZ¹, ELŻBIETA WOŹNICKA¹ and JOLANTA GRUSZECKA²¹Department of Inorganic and Analytical Chemistry, Faculty of Chemistry,
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Abstract: The antibacterial activity of morin, sodium salt of morin-5'-sulfonic acid (NaMSA) and new complexes of La (III), Gd (III) and Lu (III) with morin were tested against three bacterial strains: *Escherichia coli* G (-), *Klebsiella pneumoniae* G (-), *Staphylococcus aureus* G (+) and compared with the activity of penicillin. All of the complexes possess inhibitory action against the tested strains. The activity of the studied compounds depends on their concentration. The complexes at a concentration of 10 µg/cylinder demonstrated higher activity than morin alone, but at a concentration of 100 µg/cylinder morin was the most effective inhibitor against the strains used in this investigation.

Keywords: flavonoids; lanthanides; complexes; antibacterial activity

Flavonoids, 2-phenylbenzo- γ -pyrone derivatives, are a broadly distributed class of naturally occurring pigments present in vascular plants, and are responsible for much of the coloring in nature. Some of them, for example quercetin, rutin and morin (Figure 1) possess antibacterial activity. Recent research shows that not only quercetin but also its complexes with some metal ions show such activity. In (1), the antibacterial activities of the complexes of Mn(II), Co(II), Hg(II) i Cd(II) with quercetin were tested against five bacterial strains: *E. coli*, *S. aureus*, *B. cereus*, *K. pneumoniae* and *P. aeruginosa* and compared with penicillin activity. Penicillin at concentrations of 100 µg/disc exhibit no inhibition towards the strain *B. cereus*, whereas, the mercury (II) complex shows a significant activity against bacterial strains studied in this paper. The other three complexes also had antibacterial activity, though a little weaker than that of penicillin.

The antimicrobial action of quercetin sulfonic derivatives and their complexes with the ions of Zn(II), Fe(II) and Mg(II) against *E. coli*, *S. aureus*, *A. niger*, *C. albicans* was investigated in (2). The results were compared with the activity of nystatin, ampicillin and dirithromycin. Among studied complexes only the complex of Mg(II) with quercetin-5"-sulfonic acid shows no antimicrobial activity, whereas complex of Zn(II) has a wide spectrum of activity and

exhibits inhibition towards the bacteria and fungi studied under test conditions. The most sensitive bacterial strain to the tested compounds was *S. aureus*.

The survey of the literature shows that rare earth elements (lanthanides) have inhibitory activity against bacteria and that they are used as antiseptic medicines (3). They often are applied as complexes with inorganic ligands as well as with organic ones. The antibacterial action depends on the concentration of lanthanide ions. High concentration inhibits growth of bacteria whereas low concentration stimulates it.

In this paper, we report the antibacterial activity of morin, sodium salt of morin-5'-sulfonic acid (NaMSA) and new complexes of La(III), Gd(III) and Lu(III) with morin. The results are compared with the activity of penicillin.

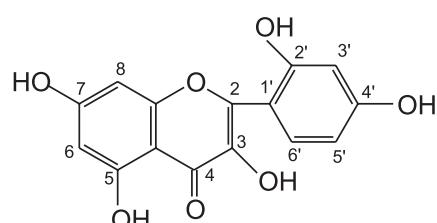


Figure. 1. Morin

EXPERIMENTAL

Synthesis of NaMSA and the complexes

Chemicals: morin ($C_{15}H_{10}O_7 \cdot 2H_2O$) International Enzymes LTD), La_2O_3 , $GdCl_3$ and Dy_2O_3 (Aldrich), methanol, dimethylsulfoxide (POCh). All reagents were analytically pure.

Synthesis of NaMSA ($C_{15}H_9O_{10}SNa \cdot 2H_2O$) and analytical procedures were reported in (4). The complexes La (III), Gd (III) and Lu (III) with morin were obtained as described in (5). The synthesis was done from aqueous – methanol solutions (1: 1) at $c_M : c_L = 1: 3$ (c_M – molar concentration of metal ion, c_L – molar concentration of ligand). The complexes were isolated as water solvates and the molecular formula of the obtained compounds is $Ln (C_{15}H_9O_7)_3 \times nH_2O$ where Ln is the cation of lanthanide, $n = 6$ for La and Lu and $n = 8$ for Gd. The compounds were yellow and amorphous.

Antibacterial activity test

The *in vitro* antibacterial activities of morin and its complexes were tested using two methods: the dilution method and the cylinder-plate diffusion

method. The chosen strains were: *Escherichia coli* G (-), *Klebsiella pneumoniae* G (-) i *Staphylococcus aureus* G (+).

Minimal Inhibitory Concentration (MIC) was determined using the method of progressive dilution in liquid media containing 0.15 μ g to 60 μ g/mL of the compound being tested. An appropriate amount of each compound under investigation was dissolved in methanol to make the concentration of 600 μ g/mL. The serial dilutions of all compounds were prepared in phosphate buffer (6). Each solution was inoculated with 0.01 mL of one of the 24 – h bacterial cultures previously prepared. The inoculated solutions were incubated for 24 h at 37°C. After incubation, the bacterial turbidity was estimated. The results are collected in Table 1.

The susceptibility of a bacterium towards morin and its complexes with La (III) and Gd (III) ions was tested by measuring the bacteriostatic diameter and compared to penicillin (7). Nutrient agar (21 cm³) was poured in sterile petridishes and allowed to solidify. About 0.6 cm³ of 24-hour at 36°C broth culture was added to 100 cm³ top agar (kept at ca. 50°C). Then, the top agar (4 cm³) was placed in

Table 1. Antibacterial activity test; the dilution method

Concentration Bacterial strain	0.15 mg/mL	0.3 mg/mL	0.6 mg/mL	0.75 mg/mL	0.9 mg/mL	1.2 mg/mL	6.0 mg/mL	60 mg/mL
Morin								
<i>E. coli</i>	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	+	+	+	+	+	+	+	-
NaMSA								
<i>E. coli</i>	+	+	+	+	+	+	+	-
<i>S. aureus</i>	+	+	+	+	+	-	-	-
<i>K. pneumoniae</i>	+	+	+	+	+	+	-	-
Complex of La(III)								
<i>E. coli</i>	-	-	-	-	-	-	-	-
<i>S. aureus</i>	+	+	+	-	-	-	-	-
<i>K. pneumoniae</i>	+	+	+	+	+	+	-	-
Complex of Gd(III)								
<i>E. coli</i>	+	+	+	+	-	-	-	-
<i>S. aureus</i>	+	+	+	-	-	-	-	-
<i>K. pneumoniae</i>	+	+	-	-	-	-	-	-
Complex of Lu(III)								
<i>E. coli</i>	+	+	+	+	+	+	+	-
<i>S. aureus</i>	+	+	+	+	+	+	+	-
<i>K. pneumoniae</i>	+	+	+	+	+	+	+	-

- – inhibition of bacterial growth

+ – bacterial growth

Table 2. Antibacterial activity test; the cylinder – plate method

Compound	$\mu\text{g}/\text{cylinder}$	Zone of inhibition (mm)		
		<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
Morin	10	0	0	0
Complex of La(III)	10	11	12	0
Complex of Gd(III)	10	12	0	14
Penicillin	10	0	0	23
Morin	100	13	14	13
Complex of La(III)	100	11	12	0
Complex of Gd(III)	100	13	11	12
Penicillin	100	21	10	30

a plate. Four sterile platinum cylinders of 6 mm diameter were put in the agar and completely filled with the test solutions (10 mg and 100 mg/cylinder in DMSO). The plates were incubated for 24 h at 37°C. The mean value obtained for the four cylinders was used to calculate the zone of growth inhibition of each sample. The antibacterial activity of compounds is summarized in Table 2.

RESULTS AND DISCUSSION

From the above investigations it follows that the most effective inhibitor against *E. coli* and *S. aureus* was morin. It has completely inhibited the bacterial growth at whole ranges of concentrations tested in this paper. The same activity against *E. coli* exhibited the complex of La(III). Both the complexes of La(III) and Gd(III) showed a similar activity against *S. aureus*, MIC= 0.75 $\mu\text{g}/\text{mL}$. Moreover, only the complex of Gd(III) at concentrations higher than 0.3 $\mu\text{g}/\text{mL}$ showed an inhibitory effect on *K. pneumoniae*. NaMSA and Lu(III) complexes did not inhibit significantly the growth of tested strains. Thus, these compounds were not used in further investigations.

The susceptibility of certain strains of bacterium towards morin and La(III) and Gd(III) complexes was judged by measuring the inhibition diameter. The results reported in Table 2 show that among the tested compounds at the concentration 10 $\mu\text{g}/\text{cylinder}$ morin exhibits no inhibition of growth of studied strains. On the other hand, morin at the concentration 100 $\mu\text{g}/\text{cylinder}$ shows a higher activity than that of the complexes. In the case of the complexes, the increase of concentration has not any meaning-

ful influence on their antibacterial activity. Both the complexes of La(III) and Gd(III) show a similar activity against *E. coli* under the test conditions. At the concentration 10 $\mu\text{g}/\text{cylinder}$ only the complex of La(III) shows inhibitory effect on *K. pneumoniae*. Finally, the complexes at the concentration 10 $\mu\text{g}/\text{cylinder}$ showed higher activity than morin but at the concentration 100 $\mu\text{g}/\text{cylinder}$ morin was the most effective inhibitor against the strains used in this investigation.

The results of the present paper encourage us to develop further investigations of the related compounds with light lanthanides ions and test them for a wide range of biological activities.

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