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"Antibacterial Activity of 3-Nitrolawsonemonoxime, 3-Aminolawsonemonoxime and their Bivalent Metal-Chelates"

R.B. MHASKE*, MRUDULA KULKARNI, S.B. JOSHI[1], V.G. KSHIRSAGAR[2] AND S.D. PRADHAN[3]
Department of Chemistry, University of Pune.
[1]Department of Chemistry, Wadia College, Pune.
[2]Department of Microbiology, Abasahab Garware College, Pune
[3]National Chemical Laboratory, Pune

3-Nitrolawsonemonoxime, 3-aminolawsonemonoxime and their chelates of Ca(II), Mg(II), Sn(II), Pb(II) and Zn(II) have been synthesized and their antibacterial activity studies were carried out. The results obtained were analysed to find the effect of change in concentration of test compounds, change in the substituents at C-3 position in the quinonoid ring of the ligand and the effect of change of central metal ion. The NO_2 group at C-3 position in the quinonoid ring of the ligand showed greater activity than NH_2 group. The activity of 3-nitrolawsonemonoxime was found to be decreased while that of 3-aminolawsonemonoxime, was observed to be increased due to chelation. In case of the chelates, activity was increased if their concentrations were increased sufficiently (doubled). But in case of Ca(II)-3-aminolawsonemonoximate, generally there has been no change in the activity with concentration. Elemental analysis and TG indicated $ML_2 2H_2O$ molecular composition for the chelates.

3-Nitrolawsonemonoxime [VI] and 3-aminolawsonemonoxime [VII] are the oxime derivatives of lawsone (2-hydroxy-1,4-naphthoquinone). Lawsone [I] is naturally occurring compound in the leaves of *Lawsonia alba* (Mehendi) which is medicinally very important[1]. Lawsone and its oxime derivatives play an important role in coordination chemistry since they act as powerful bidentate ligands forming chelates with most of the metal ions. Some oxime derivatives of lawsone and their chelates showed antimicrobial activities[2]. Similarly antimicrobial studies of transition-metal chelates of lawsone[3] and juglone (5-hydroxy-1, 4-naphoquinone) and their 3-chloro derivatives[4], indicated increase in the activity due to chelate formation as well as chloro-substitution. The antibiotic activity of tetracycline increased due to complexation with Ca(II)[5] but decreases due to complexation with MG(II)[6].

*For correspondence
'Shriyog Society,' Wada Road,
Rajgurunagar, Tal. Khed, Dist: Pune

There are also no reports on the antimicrobial activity of 3-nitrolawsonemonoximates and 3-aminolawsonemonoximates of II and IV group metal ions. All these facts gave us motivation for this study in which the antibacterial properties of the oximes and their chelates were further investigated in order to attempt to correlate antibacterial activity with structural aspects such as hydrogen bonding, chelation and substitution.

MATERIALS AND METHODS

All the chemicals and solvents used were of A.R. grade. Dichlone [II] was the product of Fluka A.G.

Preparation of 3-nitrolawsonemonoxime [VI]

Nitrolawsone [III] (0.01 mol) in 2N NaOH was treated with an aqueous solution of $NH_2OH.HCl$ (0.015 mol) at 50-60° for an hour[7]. The mixture was diluted by ice-cold water and neutralised by HCl (2N). The 3-nitrolawsone was synthesised by reacting dichlone [II] in CH_3OH (0.06 mol) with $NaNo_2$ (0.2 mol) in water at 80° for 3 h. The product was dissolved in warm water and acidified with

HCl to precipitate 3-nitrolawsone.

Preparation of 3-aminolawsone monoxime [VII]:

It was prepared by oximation of 3-aminolawsone, using the same above method[7] used for synthesis of 3-nitrolawsonemonoxime[VI]. The starting material, 3-aminolawsone [IV] was prepared by treating 3-nitrolawsone (0.0025 mol) in 5.5 ml water and 1.8 ml 10% NaOH with 1.6 g of $Na_2S_2O_4$ at 50° for half an hour.

Preparation of metal chelates:

Metal complexes were synthesised by treating alcoholic ligand solution (2 x 10^{-3} mol) with aqueous solutions of corresponding metal nitriate (1 x 10^{-3} mol) and by adjusting pH of the reaction mixture to 5-6 at 60°.

Determination of activity:

The activities of the test compounds have been found by using Agar diffusion disc-assay method suggested by Malekzadeh[8] and Berry[9] with slight modifications and precautions described by Balley[10]. This method has been also reported in some research papers[11,12].

Agar diffusion disc-assay method:

Nutrient agar, used as test medium, was sterilised by steam at 15 lb pressure and 12.1° for 15 minutes and was poured in sterilised petri dishes. The agar plates were then incubated with inverted positions for 24 h 37°. The test tube culture of the bacterium was inoculated on the agar plate by pouring and spreading the suspension on the surface. After about 15 min filter paper discs (6.3 mm-diameter) containing the test compounds of desired concentrations prepared by soaking discs in test compound solutions and metal nitrates (used as control compounds) were placed on the agar surface. Petri dishes then were incubated with inverted positions at 37° for 24/48 h and diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Structural analysis: From the elemental analysis, percentage of C, H and metal in the metal complexes were obtained which indicated $ML_2.2\ H_2O$ molecular composition for the chelates synthesized. This molecular composition is also supported by IR and TG studies. Infrared spectra (IR) suggested bonding through phenolic oxygen and nitrogen of oxime group. It also indicated presence of the co-ordinated water molecules which has been also proved by thermogravimetric study. TG studies also helped to find out the order of reaction (n) and energy of activation (E_a) for the decomposition steps of the chelates. Electronic spectral studies described different types of electronic transitions, associated with chelates structure. These are benzenoid electron transfer, quinonoid electron transfer and $n \rightarrow \pi^*$ electronic transitions.

Antibacterial activity:

As compared to dichlone (standard compound) 3-nitrolawsonemonoxime showed greater activity against *E. coli* while 3-aminolawsonemonoxime exhibited nearly the same activity against *B. subtilis* as that of the standard compound. In general, all the chelates except those of 3-nitrolawsonemonoxime with Zn(II), Ca(II) and Mg(II) showed greater activity at 250 µg/disc concentration against *E. coli* and *B. subtilis* (Table-1). Calcium (II) nitrate, Magnesium(II) nitrate, Tin(II) nitrate and Lead(II) nitrate (used as control compounds) did not show activity against bacteria.

Effect of change in substituent at C-3 in quinonoid ring:

3-Nitrolawsonemonoxime and its chelates with Sn(II) and Pb(II) showed greater activities than 3-aminolawsonemonoxime and its chelates with Pb(II) and Sn (II) against *E. coli.* Here it is observed that NO_2 group at C-3 position in quinonoid ring has greater activity than NH_2 group. It is assumed that nitro group prevents the conjungation due to greater electron attracting character but produces greater charge separation as it has strong ability to pull electron cloud. The greater charge separation causing stronger hydrogen bonding might have increased the activity according to the earlier observations and reports[13,14]. The NH_2 group at C-3 in quinonoid ring increases conjugation but shows decreased ability to form hydrogen bond due to its electron donating character and hence antibacterial activities of 3-aminolawsonemonoxime and its chelates with Pb (II) and Sn (II) against *E. coli* are decreased (Table-1).

Effect of change of concentration:

It was observed that chelates of Pb (II) Sn (II) with 3-nitrolawsonemonoxime showed activity against both *E. coli* and *B. subtilis.* This activity was found to increase as the concentrations of these chelates were increased from 125 µg/disc to 250 µg/disc while at lower concentration of 65.5 µg/disc, 125 µg/disc the chelates of Pb

Table 1 - Antibacterial Activity of test compounds

	Test Compound	Inhibition zone diameter (mm)	
		E. coil	*B. subtilis*
1.	Dichlone	8.00	8.00
2.	3-Nitrolawsonemonoxime	17.00	Nil
3.	3-Aminolawsonemonoxime	Nil	8.00
4.	Pb(II)-3-nitrolawsonemonoximate	9.00	9.00
5.	Sn(II)-3-nitrolawsonemonoximate	10.00	10.00
6.	Zn(II)-3-aminolawsonemonoximate	Nil	8.00
7.	Sn(II)-3—aminolawsonemonoximate	8.00	9.00
8.	Ca(II)-3-aminolawsonemonoximate	Nil	10.00
9.	Mg(II)-3-aminolawsonemonoximate	Nil	9.00

Antibacterial activity of test compounds was studied at 250 μg/disc against *E. coli* and *B. Subtilis* using the disc-assay method.

Table 2 - Antibacterial Activity of test compounds at different concentrations

E. coli	Inhibition zone diameter (mm)			
Compound/Concentration (μg/disc)	65.5	125	250	500
Pb(II)-3-nitrolawsonemonoximate	Nil	Nil	8.00	11.00
Sn(II)-3-nitrolawsonemonoximate	7.00	8.00	10.00	11.00
B. subtilis	**Inhibition zone diameter (mm)**			
Compound/Concentration (μg/disc)	65.5	125.00	250.00	500.00
Ca(II)-3-aminolawsonemonoximate	10.00	12.00	12.00	10.0
Mg(II)-3-aminolawsonemonoximate	Nil	Nil	9.00	11.00
Zn(II)-3-aminolawsonemonoximate	7.00	7.00	8.00	9.00
Sn(II)-3-aminolawsonemonoximate	7.00	9.00	12.00	15.00

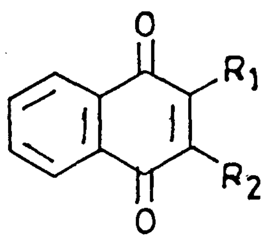
Antibacterial activity of the test compounds was studied at various concentrations ranging from 65.5 to 500 μg/disc against *E. coli* and *B. subtilis* using the disc assay method.

(II) exhibited no activity at all.

The chelates of Ca(II), Mg(II), Zn(II) and Sn(II) with 3-aminolawsonemonoxime were observed to show activity against *B. subtilis*. The chelates of Sn(II) with 3-nitrolawsonemonoxime and 3-aminolawsonemonoxime showed enhanced activity on increase in their concentrations regularly against *E. coli* and *B. subtilis* respectively.

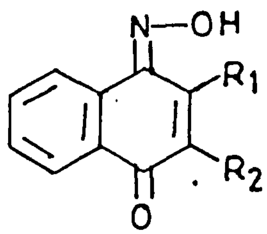
Zn(II)-3-aminolawsonemonoximate showed increased activity as its concentration was increased. Mg(II)-3-aminolawsonemonoximate did not show any activity at the lower concentration (65.5 μg/disc and 125 μg/disc) but its activity was found to be enhanced when its concentration was increased from 250 μg/disc to 500 μg/disc.

In case of Ca(II)-3-aminolawsonemonoximate generally there has been no change in the activity with concentration and hence though in case of other complexes activity was found to be increased with increase in concentration, it may be difficult to have linear and clear dose-response relationship due to less solubilities of chelates in DMSO.



R1 R2	Compound
OH H	Lawsone [1]
C1 C1	Dichlone [II]
OH NO_2	3-nitrolawsone [III]
OH NH_2	3-aminolawsone [IV]

Fig. 1



R1 R2	Compound
OH H	Lawsonemonoxime [V]
OH NO_2	3-nitrolawsonemonoxime [VI]
OH NH_2	3-aminolawsonemonoxime [VII]

Fig. 2

Though experiments were repeated twice and mean zone diameters (mm) were recorded, sample size is not large enough to calculate statistical significance of the observations. Secondly, it will not be appropriate to compare antibacterial activity of these compounds with a known (standard) antibiotic as these compounds are newly synthesised and they are less soluble in DMSO. This study was carried out in order to have supporting evidence of antibacterial study for further structural investigations of chelates synthesized.

This increase in activity may be attributed to increase in concentration of ligand released from the higher concentration of chelate at active or target site of cells of bacteria. Here the chelate may act as carrier of ligand to active site of the cell of concerned bacterium.

Effect of change of metal ions:

Sn(II)-3-nitrolawsonemonoximate exhibited activity against *E. coli*. But when Sn(II) substituted by Pb(II), activity is found to be decreased. Similarly Mg(II)-3-aminolawsonemonoximate showed greater activity against *B. subtilis* than Ca(II)-3-aminolawsonemonoximate, where Mg(II) was substituted by Ca(II). The chelates of Pb(II) with 3-aminolawsonemonoxime did not show activity against *B. subtilis* while Sn(II) chelate with same ligand showed some activity against *B. subtilis*. Thus change in metal ion in chelate of same ligand against same bacterium, changes the activity of chelate.

Effect of chelate formation:

3-Nitrolawsonemonoxime showed greatest activity of all the test compounds as compared to dichlone against *E. coli* while 3-aminolawsonemonoxime exhibited same activity as that of dichlone, (standard) against *B. subtilis*. The chelates of Pb(II) and Sn(II) with 3-nitrolawsonemonoxime showed lower activity against *E. coli* after chelation than the ligand. This may be due to incomplete and ineffective release of ligand from chelate at the site of action in the cells of *E. coli*. All the chelates of 3-aminolawsonemonoxime with Ca(II), Mg(II), Zn(II) Sn(II) metal ions have been reported to have greater activity than the ligand (3-aminolawsonemonoxime) which may be due to easy release of ligand from chelate at the site of action on *B. subtilis* cells.

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