

Synthesis and Study of 1,2,4-Oxadiazolines derivatives and their Antifungal activity.

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Abstract: Addition of benzonitrile oxide, generated *in situ* by the action of triethylamine on benzhydroxamoyl chloride, to 2-hydroxybenzaldehyde and its N-phenyl derivatives yielded a mixture of products which was separated into two compounds by fractional crystallization. The major products have been found to be the respective cycloadducts and the minor product was identified as the dimer of the starting nitrile oxide. The cycloadducts have been identified as 1,2,4-oxadiazoline derivatives (Ia-Va) on the basis of elemental analysis and spectral studies. The synthesized 1,2,4-oxadiazolines (Ia-Va) were tested *in vitro* for their antifungal activity against *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum* and *Myrothecium roridum* by employing the standard technique of spore germination inhibition at various concentrations. Some of the compounds have shown promising antifungal activity against the test fungi.

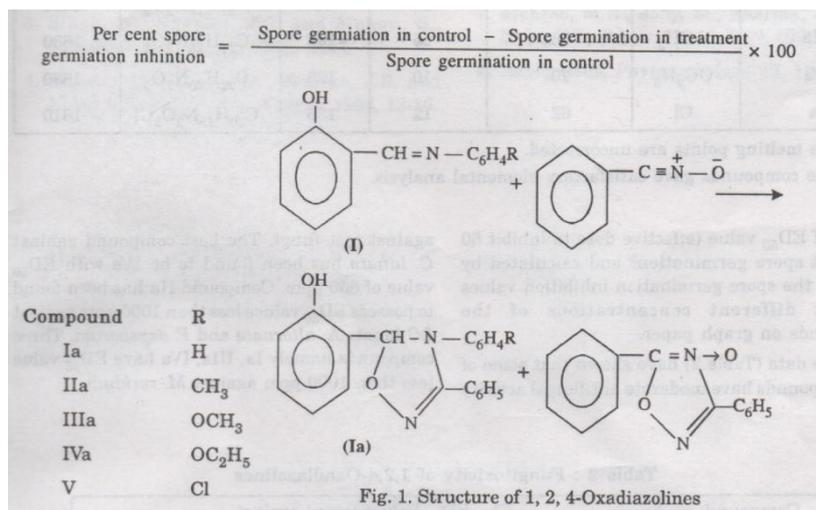
I. Introduction

Addition of benzonitrile oxide to benzaldehydes and cinnamaldehydes results in the formation of 1,2,4-oxadiazolines¹⁻³ which have been reported to possess fungitoxicity. The presence of electron releasing groups such as methoxy⁴, ethoxy⁵, hydroxy⁶ etc. in the phenyl ring increases the activity of parent compound like Schiff bases and thiazolidinones. The present investigation was carried out to study the effect of hydroxyl group in the ortho position of C-phenyl ring on the fungitoxicity of 1,2,4-oxadiazolines because it has been reported that the presence of OH group in ortho position of N-phenyl ring increases the activity of 1,2,4-oxadiazoline⁷ manifold. The results of this study are being communicated in this paper.

II. Experimental

Reaction of benzonitrile oxide, generated *in situ* from benzhydroxamoyl chloride with 2-hydroxybenzaldehyde. 2-Hydroxybenzaldehyde(I) (1.97g, 0.01 mole) was dissolved in ether (10ml) in a beaker (250ml) and was cooled in an ice bath at 0°C. Benzhydroxamoyl chloride (1.1g, 0.01 mole) was then added to the above solution with constant shaking and the contents were kept at 0°C for some time. Triethylamine (1.2ml) in ether (20ml) was then added drop-wise with continuous shaking. After complete addition of triethylamine, the reaction mixture was allowed to stand at 0°C for 2 hours. The ether layer was separated out and the residue was washed thrice with dry ether. The combined ether layer and the washings on evaporation of the solvent yielded a mixture of products. Fractional crystallization yielded two products. The major product was recrystallized from benzene to get 1,2,4-oxadiazoline(Ia), m.p. 190°C yield 66%.

The reaction of benzonitrile oxide with 2-hydroxybenzal-4-toluidine(II), 2-hydroxybenzal-4-anisidine(III), 2-hydroxybenzal-4-phenitidine(IV) and 2-hydroxybenzal-4-chloroaniline(V) was carried out in the similar way to get 1,2,4-oxadiazolines (IIa-Va) respectively. The stock solution (2000 ppm) was prepared by dissolving the compound (20 mg each) in minimal quantity of ethanol (0.5 ml) and the volume was made up to 10 ml by adding sterilized water. The stock solutions were serially diluted to 1000, 500, 250, 100, 50 and 25 ppm. The cavity slides were used to study the spore germination inhibition by these compounds at different concentrations. The treated spores in cavity slides were kept in petri plates lined with moist filter paper discs and incubated at 24°C for 20 hours. The percent spore germination inhibition was recorded by the following formula:



III. Results And Discussion

Addition of benzonitrile oxide, generated in situ from benzhydroxamoyl chloride by the action of triethylamine, to 2-hydroxybenzalaniline and its N-phenyl derivatives (I-V) after usual work up and solvent removal yields solids which have been separated into two compounds in each case. The major products have been found to be the cycloadducts(Ia-Va) and the minor product has been identified as the dimer of the nitrile oxide. The infra-red spectra of the adducts(Ia-Va) show bands at 1630cm^{-1} which is indicative of the presence of $-\text{CH}=\text{N}-$ linkage. The PMR spectrum of the adduct IIa indicates a three proton singlet at 4.0δ for methoxy protons. Aromatic protons along with the protons of the five membered ring are indicated as a multiplet between 6.7δ to 7.7δ . The PMR spectrum of the compound IIa also contains a three proton singlet at 2.4δ (CH_3 protons) and PMR of compound IVa contains a three three proton triplet at 1.6δ (CH_3 protons of $-\text{OC}_2\text{H}_5$ group) and a two proton quartet at 4.4δ due to OCH_2 protons of OC_2H_5 group. On the basis of spectral studies and elemental analysis, these compounds are assigned 1,2,4-oxadiazoline structures (Ia-Va). The synthesized 1,2,4-oxadiazolines along with their characteristics are recorded in Table 1. The synthesized compounds (Ia-Va) were tested *in vitro* for their antifungal activity against four phytopathogenic fungi viz., *Alternaria alternate*, *Curvularialunata*, *Fusariumoxysporum* and *Myrotheciumroridium* by spore germination inhibition method⁸⁻¹¹.

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Table 1 : Characteristics of 1, 2, 4-Oxadiazolines

Compound	R	Yield (%)		m. p.* (°C)	Molecular Formula**	IR band (cm^{-1})
		Compound	Dimer			
Ia	H	66	12	190	$\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2$	1620
IIa	CH ₃	58	14	92	$\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_2$	1630
IIIa	OCH ₃	55	15	105	$\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3$	1630
IVa	OC ₂ H ₅	70	10	125	$\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_3$	1630
Va	Cl	62	12	135	$\text{C}_{20}\text{H}_{15}\text{N}_2\text{O}_2\text{Cl}$	1610

* All the melting points are uncorrected.
**All the compounds gave satisfactory elemental analysis.

Dithan M-45 and Bavistin were used as check to compare the activity of the compounds. The antifungal activity has been expressed in terms of ED_{50} value (effective dose to inhibit 50 percent spore germination) and calculated by plotting the spore germination inhibition values against different concentrations of the compounds on graph paper.

The data (Table2) have shown that some of the compounds have moderate antifungal activity against test fungi. The best compound against *C. lunata* has been found to be IVa with ED_{50} value of 650ppm. Compound IIa has been found to possess ED_{50} values less than 1000ppm against *LC lunata* A. alternate and *F. oxysporum*. Three compounds namely Ia, IIIa, IVa have ED_{50} value less than 1000 ppm against *M. roridum*.

Table 2 : Fungitoxicity of 1,2,4-Oxadiazolines

Compound	ED ₅₀ Values (ppm) against			
	<i>Curvularia lunata</i>	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Myrothecium roridum</i>
Ia	*	*	*	980
IIa	780	810	750	*
IIIa	790	*	*	880
IVa	650	*	*	990
Va	*	*	*	*
Bavistin**	—	—	9	—
Dithane***	39	35	—	30
M-45				

*More than 1000 ppm

**Standard fungicide for *F. oxysporum*

***Standard fungicide for *A. alternata*, *C. lunata*, *M. roridum*

IV. References

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