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**Short communication**

# Synthesis, reactions and antimicrobial studies of α-aryl-*N*-(2-nitrocyclohexyl)nitrones

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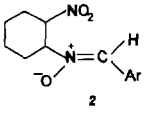
**Summary** — Synthesis of the title compounds from several aryl aldehydes and 2-nitrocyclohexylhydroxylamine has been described and the action of base on these compounds has been investigated. They have all been tested for their antimicrobial activity against the microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Proteus mirabilis*, *Salmonella paratyphi*, *Salmonella typhi* and *Pseudomonas sp* and the results are discussed.

**α-aryl-*N*-(2-nitrocyclohexyl)nitrone / base-catalysed reaction / antimicrobial studies**

Nitrones in general have been shown to be biologically active [1–6]. *N*-Cyclohexyl nitrones in particular have also been shown to be pharmacologically active [7]. It has been found that in general the introduction of nitro group in an organic compound enhances its antimicrobial activity [3] as certain nitro compounds have been shown to possess in vitro growth inhibiting activity against *Staphylococcus aureus* and *S albus* and *Streptobacterium plantarum*. The nitro compounds are also useful in agriculture for the destruction of weeds [8–11]. Hence, one can expect the nitro group containing nitrones to be significantly biologically active compared to simple nitrones. With this view, and in continuation of our work on the chemistry of nitrones [12–20], it was proposed to synthesise α-aryl-*N*-(2-nitrocyclohexyl)nitrones **2** and study their antimicrobial activity. These have been synthesised (scheme 1) by the condensation of various aryl aldehydes with 2-nitrocyclohexylhydroxylamine **1**. The synthesis of **1** has not been reported so far. This was prepared by the nucleophilic addition of free hydroxylamine [21] to 1-nitrocyclohexene [22].

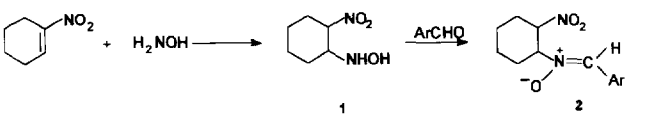
The $^1$H-NMR spectrum of the hydroxylamine **1** shows two multiplets at 3.2–3.7 and 4.5–5.0 ppm accounting for one proton each, viz C1H and C2H. There is a broad peak at 5.8–6.3 ppm accounting for two protons. This peak disappears on $D_2O$ treatment and hence is due to the NH and OH. The multiplet



**2**

**2a** Ar = phenyl; **2b** Ar = 4-chlorophenyl; **2c** Ar = 4-dimethylaminophenyl; **2d** Ar = 2-hydroxyphenyl; **2e** Ar = 4-methoxyphenyl; **2f** Ar = 4-methylphenyl; **2g** Ar = 4-nitrophenyl; **2h** Ar = 2-phenylthiophenyl; **2i** Ar = 2-furyl; **2j** Ar = 2-hydroxy-1-naphthyl.

appearing in the range 1.0–2.6 ppm accounts for all the other alicyclic protons. The yield, melting point and $^1$H-NMR spectral data of all these nitrones are given in table I. In their $^1$H-NMR spectra, the nitrones **2a–i** show a sharp singlet between 7.5 and 8.0 ppm for the azomethine proton, while for the nitrone **2j** it appears at 8.9 ppm [15]. All these nitrones show



**1** **2**

**Scheme 1.**

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**Table I.** Yield, melting point and $^1$H-NMR spectral data of α-aryl-*N*-(2-nitrocyclohexyl)nitrones.

| *Compound* | *Yield (%)* | *Mp (°C)* | *$^1$H-NMR (δ scale)* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *α-H (s)* | *ArH (m)* | *C1H (m)* | *C2H (m)* | *Alicyclic protons (m)* | *Other protons (s)* |
| **2a** | 79 | 123.5 | 7.85 | 7.30–8.70 | 4.45–5.00 | 5.30–5.80 | 1.10–2.90 | – |
| **2b** | 76 | 117.0 | 7.80 | 7.50–8.60 | 4.35–4.90 | 5.10–5.70 | 1.00–2.85 | – |
| **2c** | 75 | 135.0 | 7.55 | 6.75–8.50 | 4.35–4.72 | 5.30–5.70 | 1.25–2.79 | 3.12 (Ar–$NMe_2$) |
| **2d** | 76 | 109.5 | 7.90 | 6.95–7.10 | 4.50–4.95 | 5.10–5.60 | 1.10–2.85 | 12.6 (Ar–OH) |
| **2e** | 80 | Viscous liquid | 7.55 | 6.65–8.40 | 4.10–4.65 | 4.90–5.50 | 0.70–2.55 | 3.69 (Ar–$OCH_3$) |
| **2f** | 77 | 98.5 | 7.75 | 7.42–8.48 | 4.45–4.90 | 5.20–5.75 | 1.20–2.95 | 2.52 (Ar–$CH_3$) |
| **2g** | 75 | 132.5 | 7.95 | 8.30–8.75 | 4.50–5.00 | 5.10–5.60 | 1.10–2.85 | – |
| **2h** | 75 | Viscous liquid | 7.52 | 7.10–8.35 | 4.20–4.85 | 5.20–5.60 | 1.10–2.70 | – |
| **2i** | 71 | 136.0 | 7.69 | 6.55–8.60 | 4.20–4.95 | 5.10–5.65 | 1.00–2.80 | – |
| **2j** | 72 | 165.0 | 8.89 | 7.25–8.25 | 4.80–5.20 | 5.25–5.60 | 1.20–2.95 | 12.5 (Ar–OH) |

s: singlet; m: multiplet.

IR absorptions around 1575 ± 10 $cm^{-1}$ (due to C=N stretching) and 1125 ± 5 $cm^{-1}$ (due to N-O stretching), which are characteristic of the nitrone function. The absorptions around 1540 and 1360 $cm^{-1}$ are due to the nitro group. The UV spectra of these nitrones show characteristic $\lambda_{max}$ bands around 230 and 310 nm.

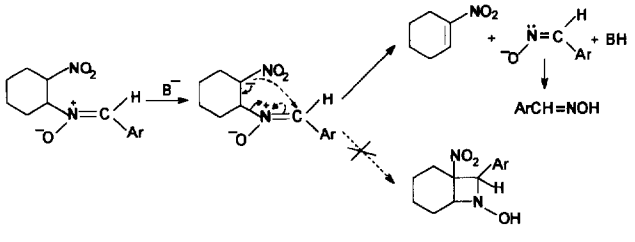
On the basis of the $^1$H-NMR spectra, steric considerations and by anology with the earlier reports [4–6, 12, 13, 15, 16], the configuration of the compound **2** has been assigned to *Z*.

The pattern for C1 and C2 H is very clear. Both are triplet of doublets ($J \approx 12$ Hz, 3 Hz) clearly showing them to be axial. Consequently the substituents are *trans* to each other, ie, diequatorial. Hence the stereochemistry of the compounds is 1*e*-substituted-2*e*-nitrocyclohexane.

With a view to modifying these nitrones as shown in scheme 2, we treated the nitrone **2a** with bases like sodium methoxide. During this treatment, unexpectedly 1-nitrocyclohexene and benzaldoxime were obtained. This interesting result can be visualised as shown in the scheme 2. Other nitrones also gave the corresponding substituted benzaldoximes on similar treatment.

The antimicrobial activity of these nitrones determined by disk diffusion method (filter paper disks) [23, 24] against several microorganisms is listed in the table II. The zone of growth inhibition (in diameter) is expressed in millimeters.

In general, the nitrones **2a–h** show only moderate activity towards *Klebsiella aerogenes* and less activity towards *S aureus* when compared with compound **1**. The nitrones **2a–h** show good activity against *Escherichia coli* while the compound **1** is not active towards the same. The nitrones **2f** and **2h** are very active against *Pseudomonas* sp, the remaining nitrones show less to moderate activity. The effect of **2a** and **2f–h** against *Proteus mirabilis* seems to be maximum and the others show moderate activity. The nitrones **2a–h** show moderate activity against *Salmonella paratyphi*. The nitrones **2a**, **2e**, **2f** and **2h** show good activity towards *S typhi* while the others exhibit moderate activity. It could be seen from the table II that at least in some of the cases, the conversion of the hydroxylamine **1** to nitrones **2**




**Scheme 1.**

**Table II.** Antimicrobial activity (zone of growth inhibition, mm) of the nitrones **2a–h**.

| *Microorganism* | *Control* | | *Compound/zone of inhibition* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CHCl_3$ | *Ceftazidime*[a] | ***1*** | ***2a*** | ***2b*** | ***2c*** | ***2d*** | ***2e*** | ***2f*** | ***2g*** | ***2h*** |
| *S aureus* | 0 | 26 | 22 | 13 | 16 | 0 | 12 | 0 | 12 | 0 | 0 |
| *E coli* | 0 | 26 | 0 | 22 | 28 | 30 | 22 | 20 | 26 | 26 | 24 |
| *K aerogenes* | 0 | 22 | 26 | 16 | 22 | 20 | 19 | 13 | 24 | 18 | 20 |
| *P mirabilis* | 0 | 22 | 26 | 28 | 25 | 26 | 24 | 26 | 30 | 28 | 31 |
| *S paratyphi* | 0 | 16 | 18 | 20 | 14 | 18 | 22 | 22 | 20 | 16 | 16 |
| *S typhi* | 0 | 28 | 24 | 24 | 30 | 24 | 24 | 26 | 32 | 22 | 28 |
| *Pseudomonas* sp | 0 | 22 | 22 | 20 | 20 | 20 | 24 | 24 | 28 | 12 | 28 |

[a]Ceftazidime was chosen as the standard because it has an inhibiting effect on all the microorganisms used in this study.

increases its antimicrobial activity and that these nitronitrones are more active than the nitrones namely, α-aryl-*N*-(2-phenylthiophenyl)nitrones, α-(2- and 4-substituted phenyl)-*N*-(4-phenylthiophenyl)nitrones and α-(4-methylthio/methylsulphonylphenyl)-*N*-(2/4-substituted phenyl)nitrones, reported so far from this laboratory [4–6].

## Experimental protocols

Melting points reported are uncorrected. The elemental analyses of the compounds were found to be within ±0.4% of the theoretical values. The UV spectra of nitrones were measured with a Shimadzu UV-Vis 160 spectrophotometer using ethanol as solvent. The IR spectra were recorded on a Perkin Elmer IR 577 spectrophotometer. $^1$H-NMR (90 MHz) spectra were recorded using a R 32 Perkin Elmer spectrometer in $CDCl_3$ solution with TMS as internal reference.

*Preparation of 2-nitrocyclohexylhydroxylamine **1**. Addition of hydroxylamine to 1-nitrocyclohexene*

Hydroxylamine (0.1 mol) in methanol prepared by the addition of methanolic sodium hydroxide with stirring to a methanolic solution of hydroxylammonium chloride till the solution was alkaline to phenolphthalein, was added to the 1-nitrocyclohexene (0.1 mol) in 25 mL methanol. The mixture was refluxed for 30 min and the solvent evaporated under reduced pressure. The resultant hydroxylamine was a viscous liquid, yield 85%.

*α-Aryl-N-(2-nitrocyclohexyl)nitrone **2***

Freshly distilled/crystallised aryl aldehyde (0.01 mol) in ethanol (20 mL) was added to 2-nitrocyclohexylhydroxylamine (0.015 mol) and refluxed for 1 h. Evaporation of the solvent under reduced pressure gave the solid, α-aryl-*N*-(2-nitrocyclohexyl)nitrone, which was then recrystallised with ethanol.

*Reaction with base*

Sodium methoxide, prepared by dissolving 0.005 mol of sodium metal in 15 mL absolute methanol, was added with stirring to a methanolic solution of the nitrone **2a** (0.005 mol). The stirring was continued for further 30 min. Then the mixture was poured into ice, the solid separated was filtered and it was identified as benzaldoxime. The filtrate was extracted with ether, washed with water, dried (anhydrous $Na_2SO_4$) and evaporated to give yellow liquid and was identified as 1-nitrocyclohexene by $^1$H-NMR.

*Antimicrobial activity*

The stock cultures of the selected microorganisms (all these microorganisms are pathogenic in nature and hence these were selected to find out any selection effect) were obtained as a gift from a local diagnosic and research centre with whose help we have already presented some reports regarding the antimicrobial activity [4–6] and the microorganisms were subcultured for identification and for further testing procedures. A single colony (containing approximately 100 000 cells) was taken from the culture by a sterile wire loop. This was introduced into the peptone (1%) water medium tube and the tube was incubated at 37 °C for 30 min. By this time the agar plates were taken from the refrigerator and equilibrated to 37 °C in the incubator [7].

The culture plates were inoculated uniformly with the peptone water culture by flooding the surface. After 2 min, the excess was removed. The open plates were allowed to dry in the inverted position for 10 min.

The filter paper disks (6.25 mm in diameter punched from Whatmann filter paper No 1 and sterilized at 140 °C for 1 h in batches of 50 in screw-capped bottles) were soaked in the chemical substances (1% concentration) in chloroform and were placed on the agar plate suitably spaced apart. The plates were incubated overnight at 37 °C. For each compound, the diameter of the areas of growth inhibition was plotted against the logarithms of the concentration of the chemical compound used for these experiments.

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