

**Synthetic utility of pharmacologically active compound, N-chloroisonicotinamide in the kinetic study of oxidation of *p*-methoxybenzaldehyde di-*n*-butyl acetal**

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**ABSTRACT**

N-chloroisonicotinamide (NCIN), has been found to possess pharmacological activity. It has significant effect on locomotor activity, when compared with the standard drug Chlorpromazine. The results of gross behavioral assessment indicates that the active compound produce a depressant effect on the central nervous system. The compound, NCIN has significant anti-convulsant action against MES induced convulsion, when compared with the standard drug phenytoin. The kinetic study of oxidation of an aromatic diether, *p*-methoxybenzaldehyde di-*n*-butyl acetal by the pharmacologically active N-halo compound, N-chloroisonicotinamide, has been carried out. The reaction follows first order dependence of rate on [substrate] and [oxidant]. The rate increases with the decrease in dielectric constant of the medium. Variation of ionic strength and the addition of isonicotinamide has significant effect on the reaction rate. The stoichiometry and product analysis have been carried out. From the effect of temperature on the reaction rate, the Arrhenius and thermodynamic activation parameters have been calculated.

**Key words:** N-chloroisonicotinamide, locomotor activity, anti convulsant action, acetal, kinetics.

## Introduction

Acetals play a vital role in bio-organic research in exploring biological activities [1] (antimalarial, antiviral etc.). The most stable form of glucose in solution is its cyclic hemiacetal. Acetaldehyde diethyl acetal is an important flavouring compound in distilled beverages. Acetal polymers (polyacetals) are tough and hard plastics used as substitutes for metals. Acetals are sometimes used as protecting group for carbonyl groups in organic synthesis as they are stable with respect to hydrolysis by bases. Acetal molecules are used as intermediates for the production of polymers, vitamins, carotenoid pigments, dyes, pharmaceuticals, pesticides, corrosion inhibitors, fragrances and perfumes. The acetal group plays an important role in glycoside and in the side-chain of steroids. Simple acetals do not seem to occur in nature.

N-chloro compounds are synthesized, characterized and used as versatile reagents in kinetic studies and organic synthesis. The compound N-chloroisonicotinamide (NCIN), prepared by the chlorination [2] of isonicotinamide, offers many advantages like easy method of synthesis, easy handling, low toxicity and mild nature with appreciable stability. N-chloroisonicotinamide, the oxidant in the present study, has been found to possess pharmacological activity. The pharmacological activity of NCIN and the subsequent utility as an oxidant for the organic compounds have not been reported so far. Hence a general review involving isonicotinamide

had been given as a background for the present study.

A comparative rate study on the oxidation [3] of nicotinamide and isonicotinamide by permanganate in acidic medium has been carried out. The oxidation of nicotinamide is reported to be faster than the oxidation of isonicotinamide. It has been reported that the presence of  $-\text{CONH}_2$  group at *meta*- position activates the ring nitrogen more for oxidation than the presence of this group at *para*- position. It has been reported [4,5] that N-cyanomethyl-2-chloroisonicotinamide derivative induces a broad range of disease resistance in rice and tobacco and N-phenylsulphonyl-2-chloroisonicotinamide shows high protective systemic activity against rice blast when applied in granulated form. Crystallization [6] of  $\alpha,\omega$ -alkanedicarboxylic acids with isonicotinamide (INA) in 1:2 and 1:1 stoichiometry has been studied. Supramolecular synthons [7] in phenol-isonicotinamide adducts have also been studied. Experimental thermochemical study [8] of the enthalpies of formation and sublimation of isonicotinamide, picolinamide, nicotinamide, isonicotinamide-N-oxide and nicotinamide-N-oxide have been studied.

The pharmacological activity of NCIN and the kinetic study of oxidation of *p*-methoxybenzaldehyde di-n-butyl acetal by NCIN has been carried out in the present investigation.

## Materials and methods

Pharmacological evaluation of the N-chloroisonicotinamide (NCIN) was carried out in the Department of Pharmacology, Periyar College of Pharmaceutical Sciences for Girls, Trichy, Tamil Nadu, India. Animal facility of this institute is approved by CPCSEA (Reg. No. 265/CPCSEA). The experimental protocols for the antidiabetic, antiulcer, anti-inflammatory, diuretic, local anaesthetic, sedative and hypnotic, locomotor, antianxiety and antiepileptic activities have been approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Science Academy for the use and care of experimental animals. The animals were maintained at a well ventilated, temperature controlled animal room for seven days prior to the experimental period and provided with food and water. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once. The animals were allowed free access to food and water and were housed at room temperature. All the test compounds were administered via intraperitoneal injection in distilled water with Tween 80 solution.

For the kinetic study of oxidation of *p*-methoxybenzaldehyde di-*n*-butyl acetal by the pharmacologically active oxidant, N-chloroisonicotinamide, acetonitrile was used as a solvent. The oxidant, N-chloroisonicotinamide was prepared by the chlorination of

isonicotinamide. NCIN was characterized by <sup>1</sup>H-NMR spectra.

Acetals are gem-dialkoxy compounds [9,10] and they are formed by the nucleophilic addition of the alcohol to the carbonyl group of an aldehyde producing a hemiacetal which reacts further with another molecule of alcohol to give the acetal in the presence of a catalyst. The substrate *p*-methoxybenzaldehyde di-*n*-butyl acetal was prepared by the method described in the literature [11,12]. The required quantities of the acetal solution, sodium perchlorate and acetonitrile-water mixture were pipetted out in a clean dried reaction bottle, kept in thermostat for about half an hour, set at the desired temperature. The reaction was started by pipetting out the required quantity of NCIN solution, which had also been thermostated for nearly half an hour. The total volume of the reaction mixture was always 25 ml. About 3 ml of reaction mixture was pipetted out into the conical flask and the progress of the reaction was followed iodometrically.

The stoichiometry was found to be 1:1. The product of oxidation, the corresponding ester was confirmed by TLC and <sup>1</sup>H-NMR spectrum.

## Results and Discussion

### *Locomotor activity*

Most of the central nervous system acting drugs influence the locomotor activities in man and animals. The central nervous system depressant drugs such as barbiturates and alcohol reduce the locomotor activity while the stimulants such as caffeine and amphetamine increase the activity. In other words, the locomotor activity can

be an index of wakefulness (alertness) of mental activity. The locomotor activity (horizontal activity) can be easily measured using an actophotometer [13] which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. Actophotometer could have either circular or square area in which the animal moves. Both rats and mice can be used for testing in this equipment. Actophotometer was used to evaluate the locomotor activity. The Swiss albino mice (20-25 g) were divided into eight groups each consisting of six animals. The test compound was injected intraperitoneally (i.p). One group received standard Chlorpromazine 3mg/kg and one group received vehicle (normal saline 5 ml / kg i.p). All the remaining six groups received N-chloroisonicotinamide (30 mg/kg) intraperitoneally. The locomotor activity score of all the animals was noted by placing the animals in the square area of the instrument for ten minutes. Each mouse was retested for activity score after thirty minutes of the test drug administration. The difference in the activity before and after the administration of standard and test compounds was noted (Table 1).

#### **Anti-convulsant activity**

The animals were weighed and numbered. They were divided in to three groups, each consisting of six rats. The animals were held properly, corneal electrode was placed on the cornea and the prescribed current is applied. The different stages of convulsions [14] i.e., tonic flexor,

tonic extensor phase, clonic convulsions, stupor and recovery or death were noted. The same was repeated with other animals of control group. Phenytoin was injected intraperitoneally to the second group and test drug (30 mg/kg) was injected to third group. After thirty minutes, the animals were subjected to electro convulsions as described. The reduction in time or abolition of tonic extensor phase of MES-convulsions was noted (Table 2).

N-chloroisonicotinamide has significant effect on locomotor activity when compared to the standard drug. The results of gross behavioral assessment indicated that the active compounds produced a depressant effect on the central nervous system. The animals were still responsive and did not exhibit any prominent muscle relaxation. This may be responsible for the modification of mesolimbic system [15].

The given sample produced the loss of extensor phase in MES induced electro convulsion as like standard phenytoin and also time spent by the animals in different phases of convulsion is also reduced when compared to control. Hence, it indicates that the given sample has significant anti-convulsant action against MES induced convulsion [16].

#### **Kinetic study of oxidation of *p*-methoxybenzaldehyde di-*n*-butyl acetal by NCIN**

The kinetic and mechanistic aspects of the oxidation of acetal by N-chloroisonicotinamide have been examined. Alkyl benzoate is the major product of oxidation. Kinetics is followed by

iodometric procedure, i.e. by following the disappearance of NCIN iodometrically in aqueous acetonitrile medium at constant ionic strength. The reactions have been followed under the condition, where the concentration of the acetal is in large excess compared to that of NCIN.

The kinetics of oxidation of acetals by NCIN in aqueous acetonitrile medium at a constant ionic strength corresponding to 0.1 M  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$  is investigated at several initial concentrations of the reactants. Linear plots of  $\log [\text{NCIN}]_t$  vs time shows first order dependence of the rate on  $[\text{NCIN}]$ .

The dependence of rate on acetal concentration has been determined by measuring the first order rate constants for NCIN disappearance for a wide range of acetal concentrations at 50 °C. The pseudo-first order rate constants are found to increase (Table 3) linearly with the increase in  $[\text{acetal}]$ . Plots of  $\log k_1$  vs  $\log [\text{acetal}]$  give straight line with unit slope confirming the first order dependence of rate on  $[\text{acetal}]$ .

The influence of solvent dielectric constant on the rate of NCIN oxidation of acetal has been studied in various solvent mixtures of acetonitrile and water. The rate of oxidation increases with the increase in acetonitrile content of the solvent mixture (Table 3). The plots of  $\log k_1$  vs  $1/D$  are linear.

The influence of variation of ionic strength on the rate of oxidation has been studied by varying the concentrations of  $[\text{NaClO}_4 \cdot \text{H}_2\text{O}]$ . The reaction rate increases (Table 3) with the increase

in ionic strength of the medium. The effect of one of the products of the reaction on the rate of oxidation has been studied by adding various concentrations of isonicotinamide, keeping the concentrations of acetal and NCIN constant. There is a decrease in the reactivity with the increase in the initially added concentration of isonicotinamide.

The oxidation of acetal has been studied at four different temperatures (318 K to 333 K). The temperature dependence on the rates of oxidation is determined by plotting  $\log k_2$  against reciprocal of temperature. The second order rate constants at different temperatures at constant ionic strength for the NCIN oxidation of acetals under investigation are recorded in Table 4. The Arrhenius activation energy, pre-exponential factor, entropy, enthalpy and free energy of activation have been evaluated for all the acetals and the values are listed in Table 4. Addition of the reaction mixture to acrylonitrile do not initiate polymerization, showing the absence of free radical species.

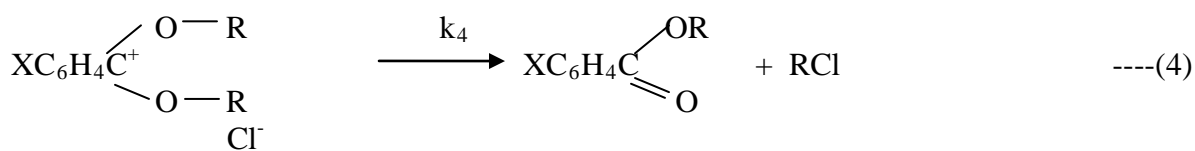
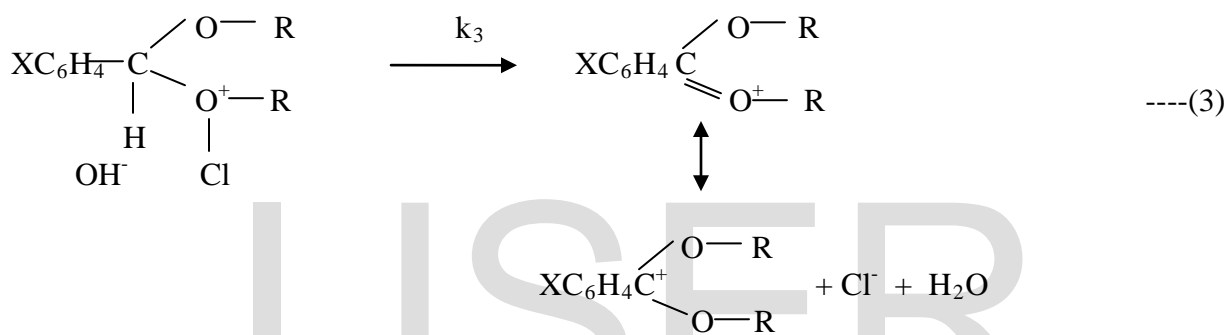
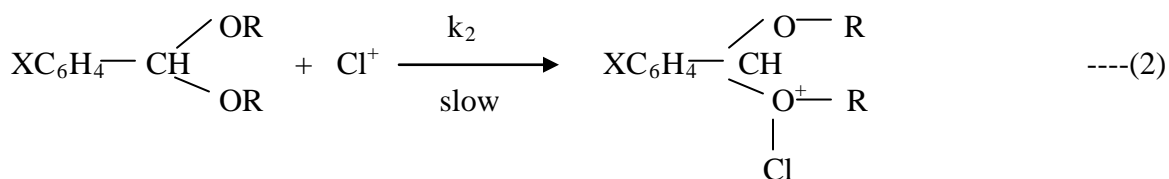
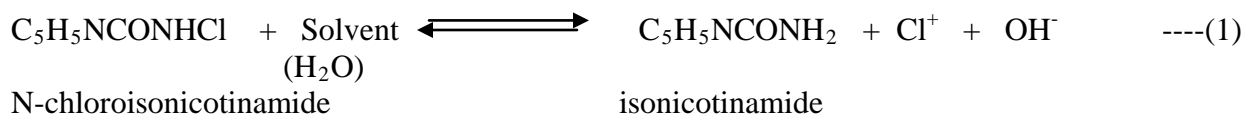
It seems that the results of NCIN oxidation of acetal in the present investigation can be accounted for, by identifying the rate-determining step in the Deno and Potter [17] mechanism for the oxidation of ether by bromine and introducing slight modification to Gopalakrishnan [18] mechanism for the oxidation of alcohols by N-bromosuccinimide. A probable mechanism may involve electrophilic attack of the positive part of oxidising species on the electron – rich oxygen atom of acetal rather than on the

electron – deficient aldehydic hydrogen. The mechanism has been proposed assuming  $\text{Cl}^+$  as the oxidizing species.

Solvent consists of a mixture of acetonitrile and water. The reactive oxidizing species may be  $\text{Cl}^+$  or solvated  $\text{Cl}^+$ . The reactive species formed in the above equilibrium step attacks the acetal molecule in the rate-determining step (2).

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**Figure 1 Mechanism for the kinetic study of oxidation of p-methoxybenzaldehyde-di-n-butyl acetal by NCIN**



R = n-butyl, X = p-OCH<sub>3</sub>

Assuming Cl<sup>+</sup> as the effective oxidizing species, the rate law is given as follows:

$$\frac{-d[\text{NCIN}]}{dt} = \frac{k_1 k_2 [\text{XC}_6\text{H}_4\text{CH}(\text{OR})_2] [\text{NCIN}]}{k_{-1} [\text{INA}]}$$

The above rate law accounts for the first order dependence on [NCIN] and [acetal]. This also explains the retardation in the rate by the addition of isonicotinamide.

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**Table 1 Effect of NCIN on locomotor activity**

S.No	Drug Used	Before Administration	After Administration
1.	Vehicle 5 ml/kg	48.0 ± 1.22	46.28 ± 1.18
2.	Chlorpromazine HCl (3 mg/kg)	44 ± 1.18	7.22 ± 1.02
3.	Test drug (30 mg/kg)	52.2 ± 1.04	22 ± 1.12

**Table 2 Effect of NCIN on anti-convulsant activity**

S.No.	Treatment	Phase of Convulsions				Recovery / death
		Flexor (Sec)	Extensor (Sec)	Clonus (Sec)	Stupor	
1.	Vehicle	6 ± 0.18	15 ± 0.012	22 ± 0.18	211 ± 0.18	Recovered
2.	Phenytoin (25 mg/kg)	2 ± 0.10	--	8 ± 0.08	72 ± 0.12	Recovered
3.	Test compound (30 mg/kg)	4 ± 0.12	2 ± 0.10	18 ± 0.04	81 ± 0.12	Recovered

**Table 3 Effect of variation of [substrate], ionic strength and solvent composition on the rate of the reaction**

X-C <sub>6</sub> H <sub>4</sub> CH(OC <sub>4</sub> H <sub>9</sub> ) <sub>2</sub> x10 <sup>2</sup> (M)	[NCIN] x10 <sup>3</sup> (M)	[NaClO <sub>4</sub> .H <sub>2</sub> O] x10 <sup>3</sup> (M)	CH <sub>3</sub> CN – H <sub>2</sub> O% (v/v)	Temperature (K)	k <sub>1</sub> x10 <sup>4</sup> (s <sup>-1</sup> )
2.0	6.0	1.0	90-10	323	2.02
4.0	6.0	1.0	90-10	323	4.36
6.0	6.0	1.0	90-10	323	7.08
8.0	6.0	1.0	90-10	323	9.09
10.0	6.0	1.0	90-10	323	13.28
8.0	6.0	1.0	88-12	323	5.99
8.0	6.0	1.0	89-11	323	7.81
8.0	6.0	1.0	90-10	323	9.09
8.0	6.0	1.0	91-09	323	11.01
8.0	6.0	1.0	92-08	323	12.50
8.0	6.0	1.0	90-10	323	9.09
8.0	6.0	2.0	90-10	323	11.80
8.0	6.0	3.0	90-10	323	13.41
8.0	6.0	4.0	90-10	323	14.74

**Table 4 Effect of temperature on the rate of oxidation of p-methoxybenzaldehyde-di-n-butyl acetal by NCIN and evaluation of Arrhenius and thermodynamic activation parameters**

[X-C<sub>6</sub>H<sub>4</sub> CH(OC<sub>4</sub>H<sub>9</sub>)<sub>2</sub> = 8.0 x10<sup>-2</sup> M  
[NCIN] = 6.0 x 10<sup>-3</sup> M

[NaClO<sub>4</sub>.H<sub>2</sub>O] = 1.0 x10<sup>-1</sup> M  
Solvent (v/v) = 90% CH<sub>3</sub>CN - 10% H<sub>2</sub>O

X-C <sub>6</sub> H <sub>4</sub> CH(OC <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	k <sub>2</sub> x 10 <sup>3</sup> (s <sup>-1</sup> )				Ea (kJ mol <sup>-1</sup> )	ΔH <sup>‡</sup> (kJ mol <sup>-1</sup> )	ΔS <sup>‡</sup> (J K <sup>-1</sup> mol <sup>-1</sup> )	ΔG <sup>‡</sup> (kJ mol <sup>-1</sup> )	log A
	318 K	323 K	328 K	333 K					
X = p-OCH <sub>3</sub>	6.76	9.86	13.80	19.86	62.80	60.11	-42.51	73.84	8.15

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