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Synthesis, Characterization and Evaluation of Anticonvulsant/Hypnotic Activity and Toxicity Effect: 3–Methylpyridinium 2,6-Dioxo-5-(2,4,6–Trinitrophenyl)–1,2,3,6-Tetrahydropyrimidin–4–Olate

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Abstract: An ethanolic solution of 1-chloro-2,4,6-trinitrobenzene, barbituric acid, and 3-methylpyridine may be used to produce a novel barbiturate (a pyrimidine derivative) in a single reaction vessel. The reported barbiturate is formed by proton abstraction processes and an intermediate sigma complex synthesis. With the use of UV-Vis, FT-IR, 1H NMR, 13C NMR, and elemental analytical data, the structure of the eponymous barbiturate has been determined. Using the Maximal Electro Shock technique, the anticonvulsant activity of the synthesised barbiturate was evaluated in albino rats. Synthesized barbiturates are associated with a shorter ex-tensor phase of convulsion. The LD_{50} values found in the animal model were used to make a first evaluation of toxic manifestation. Class (LD_{50}>1500 mg/kg) describes the substances in question. There were no acute toxicity and behavioural abnormalities seen in the animals. A therapeutic dosage of the medication (100 mg/kg) produces hypnosis in albino mice for 47 min.

Keywords: 1-Chloro-2,4,6-trinitrobenzene, 3-Methylpyridine, Barbiturate, Anticonvulsant, activity, Hypnotic activity

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Introduction

Convulsions are a common neurological disorder, and the aberrant electrical activity in the brain has been linked as a possible cause (Hardman and Limbird, 2001; Fun *et al*., 2011). For the most part, conventional antiepileptic medications such as pyrimidine, phenytoin, carbamazepine, phenobarbital, and valproate are available for the treatment of tonic-clonic seizures. In recent years,

this type of seizure has received a significant amount of attention in the field of pharmacotherapy for convulsions (grandmal type). Despite the fact that these medicines have unwanted side effects, they are nonetheless used in certain clinical contexts (Browne and Holmes, 2001; Bell and Sander, 2002). Because convulsion affects around one per cent of the world's population , it is imperative that research be conducted to identify innovative anticonvulsant medications that have a more selective effect and lower toxicity. In this light, we will discuss the synthesis of a new barbiturate that is composed of 1-chloro-2,4,6-trinitrobenzene (also known as TNCB), barbituric acid (also known as BBA), and 3-methylpyridine. Significant anticonvulsant activity has been shown. It has an LD_{50} value that is much greater than the barbiturates that were previously reported (1,500 mg/kg) (Erickson *et al*., 2009).

Materials and Methods

Solvents and reagents of Analar grade were purchased from a commercial source and utilised unpurified in all synthesis and analysis. With (DMSO-d6) as the solvent and tetramethylsilane serving as the internal standard, ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DRX-500 spectrophotometer. When referring to chemical changes, ppm is the unit of measure. With an FT-IR Perkin-Elmer RXI spectrophotometer, KBr pellets were used to record IR spectra.

Preparation of the Title Molecular Salt (Barbiturate):

In order to produce TNCB, picric acid and phosphorus oxychloride were required (Vogel, 1978). After dissolving barbituric acid (1.3 g, 0.01 M) in 30 ml of absolute ethanol, it was then mixed with TNCB (2.5 g, 0.01 M) in 20 ml of absolute ethanol. The total volume of the reaction was 50 ml of absolute ethanol. After that, we added some additional 3-methylpyridine (3 ml or 0.05 mM) and gave the mixture a thorough shake for another 2 to 3 h. When the solution is allowed to remain, crystals with a colour between rust and orange will begin to form. We screened and ground the crystals with great attention to detail. In order to remove any leftover reactants, the powder that was produced was first washed in 5 ml of 100% ethanol and then washed in 50 ml of dry ether. To re-crystallize the crystals, ethanol was utilised as the solvent (melting point of -114.1 ℃ and 85% pure product output). The results of the micro analysis were C, 44.44, 2.78, and 19.44, whereas the values that were really measured were C, 44.76, 2.18, and 18.91. There are many different types of solvents, including water (16 g/dm^3), ethanol (27 g/dm^3), and dimethyl-sulfoxide (68 g/dm^3). The salt with the same name is the one that is insoluble in ether.

Spectral Data:

TNCB has a maximum visible (EtOH) wavelength of 3,600 A; barbiturate has a maximum visible (EtOH) wavelength of 4,600 A.1H NMR [500 MHz, (DMSO-d6)]. TNCB (d) 9.20 (s, 2H, ring protons); BBA (d) 11.09 (s, 2H, two N-H protons); 2-methylpyridine (d) 7.14-8.44 (m, 4H, Ring protons); 2-methylpyridine (d) 2.50 (s, 3H, methyl group); barbiturate (d) 9.75 (s, 2H, -NH); 8.56 (s, 2H, nitro aromatic ring); (s, 3H, -CH3 proton of methylpyridinium cation). TNCB [500 MHz, (DMSO-d6)] d = 149, 146, 127, 123; BBA = 168, 152, 40; 3-methylpyridine = 158, 148, 137, 124, 121, 24.49; barbiturate = 163, 151, 149, 145, 142, 141, 135, 132, 125, 123, 84, 39. The infrared (KBr, cm^{-1}) spectrum for TNCB looks like this: 1,538 [NO (asym. str.)], 1,342 [NO (sym. str.), 718 [C-Cl (str)]; BBA looks like this: 3,479 [N-H (str)], 2,879 [$-CH_2$ (asym. str.), 2,819 [$-CH_2$ (sym. str). The synthesised barbiturate has been tested qualitatively and found to contain nitrogen atoms, nitro groups, and zero chlorine atoms. Thin layer chromatography was used to verify the sample's purity.

Results and Discussion

The methylene group in barbituric acid is active, and in the presence of pyridine (a base), it is able to extract a proton. Following the protocol laid forth in Figure 1, the resultant carbanion easily crystallises into the molecular salt. We have

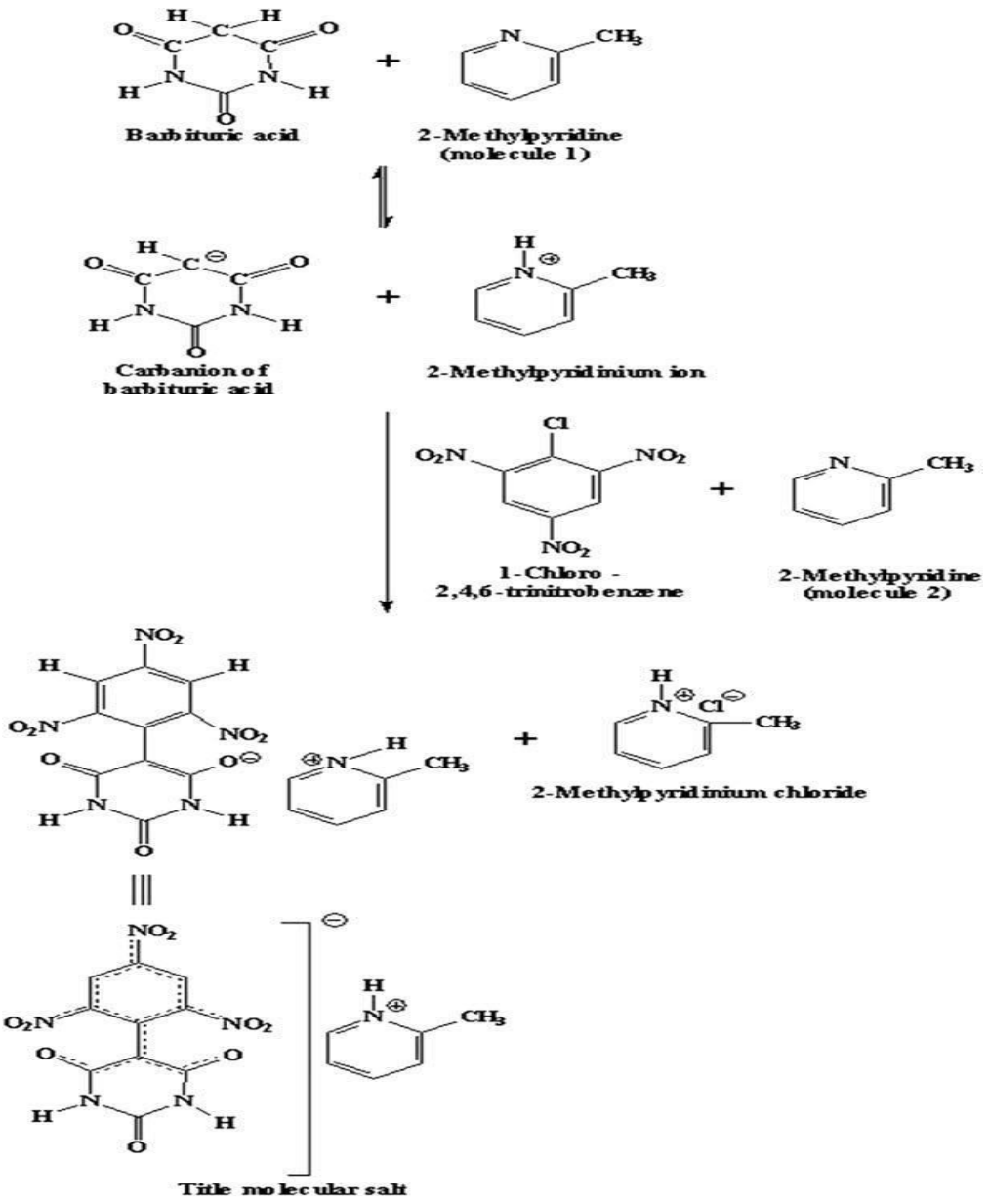

Fig. 1: Synthesis route.

previously documented the existence of molecular salts involving the aliphatic tertiary amines N,N-disubstituted aniline, barbituric acid, and TNCB (Feldmann *et al*., 2009).

Spectral Data:

The study's title refers to barbiturate's poorer absorption than TNCB (3,600 A) (4,600 A). The findings support the idea that the molecular salt anion possesses a wide negative charge (Fig. 1). TNCB and the named molecular salt both lack the conspicuous absorption band at 718 cm^{-1} characteristic of the C-Cl stretching mode, suggesting that one of the chlorine atoms has been removed. The strong band of N-O asymmetric stretching mode, identified in TNCB at 1,538 cm^{-1}, changes frequency due to negative charge delocalization in the molecular salt. This changed the band's frequency (1,530 cm^{-1}). The strong band between 3,500 and 2,500 cm^{-1} may

Table 1: Anticonvulsant activity of title barbiturate against maximal electro shock induced convulsion in Albino rats

S. No.	Treatment	Time (s)				
		Various phases of convulsion				
		Flexor	Extensor	Clonus	Stupor	Recovery/death
1.	Control (saline) (5 ml/kg)	12 ± 1	21 ± 1.5	35 ± 2.5	149 ± 11.0	Recovery
2.	Title barbiturate (25 mg/kg)	10 ± 1.2	20 ± 2.4	33 ± 2.7	186.5 ± 4.6	Recovery
3.	Standard Phenobarbitone (20 mg/kg)	2 ± 0.2	0.0	15 ± 0.8	57 ± 3.9	Recovery

Mean ± SEM, N = 6, P< 0.001 versus standard

Table 2: Hypnotic activity of title arbiturate

S. No.	Treatment	Time of Administration	Time of loss of righting onset of action Recovery			Duration (min)
		(min) (A)	reflex (min) (B)	(min) (B–A)	(min) (C)	(C–B)
1.	Control (normal saline)	0	-	–	-	–
2.	Title barbiturate (100 mg/kg)	0	68 ± 4.8	122 ± 4.8	68 ± 4.8	54.20
3.	Pentobarbitone (20 mg/kg)	0	26.8 ± 1.42	26.8 ± 1.42	168.36 ± 6.4	141.56

Mean ± SEM, N = 6, P< 0.001 versus standard

represent an amine salt (Kalaivani and Malarvizhi, 2009). The bright, powerful band at 529 cm^{-1} reflects this amine salt torsional oscillation (Silverstein and Webster, 2004). The barbiturate's PMR spectrum has a greater field at barbituric acid's N-H proton resonance frequency of d 11.09. (d 9.75). Barbiturate has a bigger signal than barbituric acid because the negative charge is spread throughout a broader area, including the keto functionalities close to the N-H groups. The molten salt contains nitro aromatic, barbituric acid, and 3-methylpyri-dine moieties. The nitro aromatic moiety's protons emerge in the molecular salt at a little higher field than TNCB, which helps establish a negative charge. Nitro aromatic ring protons in the molecular salt cause this. A molecular salt weakens the molecule's protons, allowing 3-methylpyridine's nitrogen atom to accumulate a positive charge. The electrostatic force of attraction preserves positive and negative entities within the molecule salt, so changes are minor. The molecular salt's ^{13}C NMR and elemental analyses support the predicted structure. ^{13}C NMR signals arise from different carbon-containing environments.

Biological Activity Studies:

Experiments were conducted as part of the evaluation of biological activities. The Institutional Animal Ethics Committee approved these research, which followed Indian National Science Academy rules for animal use and care (Guidelines, 1992/2000). For seven days before the testing, the animals were fed and watered in a well-ventilated stop animal room at 303 K. The animals were acclimated to the lab before the experiment. Each animal had an ecological purpose.

Evaluation of Anticonvulsant Activity:

Maximum Electro Shock (MES) was used to test for anticonvulsant properties. The albino rats ranged in weight from 150 to 200 g, and each group had six animals of the same gender. Phenobarbitone (20 mg/kg) was employed as a gold standard. The placebo group was given normal saline (5 ml/kg). The artificially produced molecule was given to the second group. In order to induce MES (150 mA/0.2 s), the subjects were given normal saline, the usual medication, and the synthesised barbiturate 1 h beforehand. The electro convulsometer's ocular electrodes were used to apply the electricity to the animals (model 100-3, INCO). Tonic flexion, tonic extensor, clonus convulsion, coma, recovery, and death were identified as distinct phases of convulsions. The duration of each of these states was recorded for each individual animal. The extensor phase of convulsions is thought to be shortened by the use of the drug known as barbiturates (Table 1). The average of each set was compared to the control group. The data was summarised as a mean SE. Student tests were used to investigate the significance test (Kalaivani and Malarvizhi, 2009).

Evaluation of Hypnotic Action:

We used albino mice of both sexes weighing between 25 and 30 g, and randomly assigned them to groups of six. 20 mg/kg of pentobarbital was employed as a reference standard. Standard salt water (1 mL/kg) was administered to the placebo group. The other group was given 100 mg/kg of synthesised molecular salt. The hypnotic activity of the synthesised molecule was determined by tracking its effects throughout time, beginning with injection and continuing through the loss of the righting reflex, the onset of its effects, and the recovery period (Table 2).

Acute Toxicity Study:

Toxicological data on the synthetic barbiturate were analysed to calculate its LD_{50} in accordance with OECD recommendations. Class 4 [LD_{50} (1,500 mg/kg)] is where you'll find molecular salt. There was no evidence of acute toxicity or altered behaviour in the animals.

Conclusion

A simple one-pot synthesis yields very pure and potent (molecular salt) barbiturates for this study. They are very stable and highly soluble in water. Ethanol, a solvent often employed in the preparation process, causes less pollution than other organic solvents. The synthetic approach is cost effective due to the low price of the chemicals employed in the manufacture. The synthesised barbiturates in the current study are very stable, highly soluble in water, and have a moderate therapeutic index, suggesting that they will likely play a key role in the future as anti-convulsion / Hypnotic medicines.

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