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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of a novel barbiturate from 1-chloro-2,4-dinitrobenzene as an anticonvulsant agent

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Abstract A new barbiturate has been synthesized from the ethanolic solution of 1-chloro-2,4-dinitrobenzene, barbituric acid, and triethylamine. The structure of the isolated molecule has been confirmed by ultraviolet (UV)-visible (VIS), infrared (IR), proton magnetic resonance (PMR), ^{13}C nuclear magnetic resonance (NMR), correlation spectroscopy (COSY) and mass spectrometry (MS) spectral studies. Elemental analysis and other qualitative tests have also been carried out to elucidate the proposed structure. The anticonvulsant activity of the synthesized molecule was tested by the maximal electro shock method. Albino rats of either sex weighing 150–200 g were used for the study. The drug was given 1h before the induction of maximal electro shock test. The different stages of convulsions such as tonic flexor, tonic extensor, clonus convulsion, stupor, and recovery/death were examined. Reduction in the extensor phase of convulsion was noted for the synthesized barbiturate.

Keywords Barbiturate · Carbanionic sigma complex · Anticonvulsant · 1-Chloro-2,4-dinitrobenzene

Introduction

Barbiturates are drugs that act as sedative–hypnotic agents. The short-acting barbiturates such as thiopental are used as intravenous anesthetics. The long-acting barbiturates such as phenobarbital are anticonvulsant agents, and are used for the suppression of anxiety, the induction of sleep, and the control of seizures (Nogrady, 1988; Ashutoshkar 1993; Hardman and Limbird, 2001; Yadav 2004; Nadkarni *et al*., 2005; Jain *et al*., 2006). Epilepsy affects approximately 1% of the world's

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population and many of the marketed anticonvulsant drugs have profound side effects (Huseyin *et al*., 1998; Srivastava *et al*., 2000; Dipiro *et al*., 2002; Gitto *et al*., 2006; Rana *et al*., 2007). Hence it is necessary to search for safer and more effective new antiepileptic drugs. A number of stable crystalline adducts have been reported from the electron-deficient nitroaromatics and β-diketones (Terrier 1982; Gnanadoss and Kalaivani 1985; Gnanadoss and Kalaivani 1985). As barbituric acid contains an active methylene group like the β-diketones, an attempt has now been made to prepare a crystalline carbanionic sigma adduct from it.

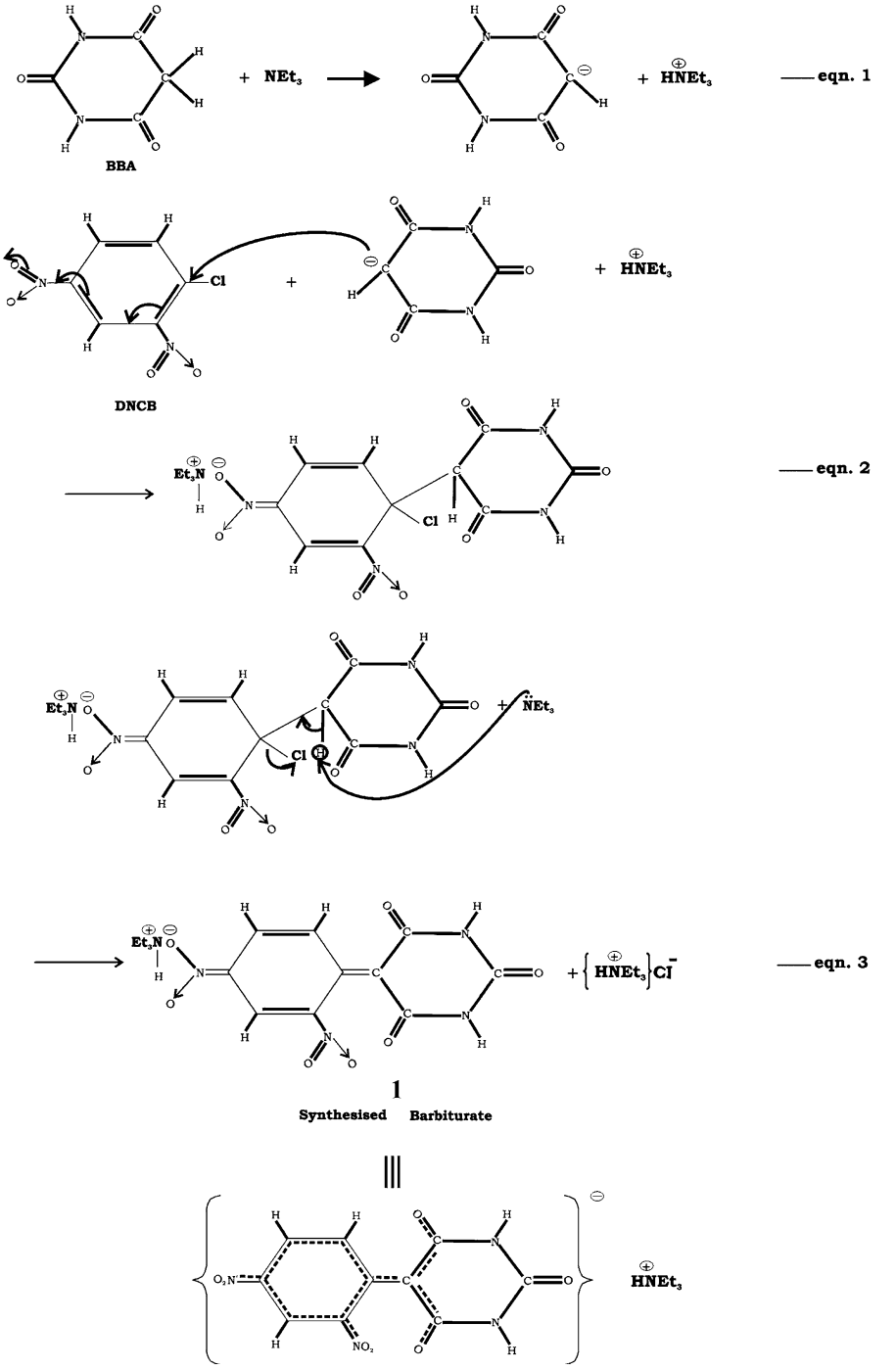
Experimental section

Analytic-grade 1-chloro-2,4-dinitrobenzene (DNCB) and barbituric acid (BBA) were used as supplied. Triethylamine (NEt_3) was refluxed over small quantities of phenyl isocyanate and distilled (Saur, 1963). DNCB (0.01 mol) in absolute ethanol was mixed with 0.01 mol barbituric acid in absolute ethanol. Triethylamine (0.02 mol) was then added and the mixture was shaken well for 5–6 h. The solution was filtered and kept for 48 hours. On standing, maroon red crystals come out of the solution. The crystals were powdered well and washed with copious amount of ethanol and dry ether and recrystallized from absolute alcohol (yield of pure crystals 60%, m.p. 535–537 K, solubility in H_2O 4 g/dm^3 at 298K).

The visible data were obtained on a Perkin–Elmer Lambda 15 UV/VIS spectrometer. The IR spectra were recorded using a Perkin–Elmer spectrum RXI infrared spectrophotometer as KBr pellets. The NMR spectra were obtained from a Bruker DRX–300 spectrometer with deuterated dimethyl-sulphoxide (DMSO) d_6 as the solvent and tetramethyl silane (TMS) as an internal reference. The fast atom bombardment (FAB) mass spectra were recorded on a JEOL SX 102/DA–6000 mass spectrometer. λ_{max} of barbiturate in ethanol −420 nm. IR (DNCB) −1545 cm^{-1} (−NO_2 asym.str.), 1351 cm^{-1} (−NO_2 sym.str.), 732 cm^{-1} (C-Cl str.), IR (BBA) −1716 cm^{-1} (C=O str.), 3479 cm^{-1}, 3529 cm^{-1} (N-H str.). IR (barbiturate) −1516 cm^{-1} (−NO_2 asym.str.) 1343 cm^{-1} (−NO_2 sym. str.), 1712 cm^{-1} (C=O str.), ∼1600 cm^{-1} (C=C str.), 2700–3400 cm^{-1} (N-H str.) $^1H_1$ NMR (DNCB) −δ 8.76 (s, 1H, ring proton of nitro moiety), δ8.46 (d, J = 4Hz , 1H, adjacent ring proton of nitro moiety), δ 7.92 (d, J = 4Hz , 1H, adjacent ring proton of nitro moiety). $^1H_1$ NMR (BBA) −δ 11.09 (s, 2H, N–H protons), δ 3.39 (s, 2H, −CH_2 protons). $^1H_1$ NMR (barbiturate) −δ 9.66 (s, 2H, N–H protons), δ 8.36 (s, 1H, ring proton of nitro moiety), δ 8.20 (m, 2H, adjacent ring protons of nitro moiety), δ 1.50 [t, J = 8Hz , 9H, −CH_3 of $\overset{+}{H}N(CH_2CH_3)_3$], δ 3.08 [q, J = 4Hz, 5Hz, 6H, −CH_2 of $\overset{+}{H}N(CH_2CH_3)_3$]. CHN analysis of barbiturate – found (calculated) C48.83 (48.60), H4.87 (5.31), N 18.08 (17.72).

Results and discussion

Although a number of trinitro aromatics gave a pasty mass with barbituric acid, the ethanolic solution of 1-chloro-2,4-dinitrobenzene and barbituric acid in the presence of excess triethylamine yielded a crystalline product. The isolated barbiturate is colored because of charge delocalization. Qualitative tests (Vogel, 1978) on the



Scheme 1 Synthetic route for the formation of the barbiturate

Table 1 Anticonvulsant activity of barbiturate against maximal electro-shock-induced convulsion in albino rats

S. no.	Treatment	Time (s) in various phases of convulsion				
		Flexor	Extensor	Clonus	Stupor	Recovery/ death
1.	Control (saline)	3.24 ± 0.09	13.3 ± 0.25	9.24 ± 0.21	137 ± 8.24	Recovered
2.	Barbiturate (25 mg/kg)	4.20 ± 0.23	13.2 ± 0.51	7.20 ± 0.31	105.5 ± 6.8	Recovered
3.	Barbiturate (50 mg/kg)	3.10 ± 0.05	6.5 ± 0.65	5.10 ± 0.61	82.2 ± 6.1	Recovered
4.	Barbiturate (100 mg/kg)	2.90 ± 0.05	2.0 ± 0.0	5.60 ± 0.46	85.7 ± 6.9	Recovered
5.	Phenytoin (25 mg/kg)	2.66 ± 0.55	0.0 ± 0.12	4.60 ± 0.38	62.1 ± 3.3	Recovered

synthesized barbiturate revealed the presence of the nitrogen atom, nitro groups, and the absence of chlorine. In DNCB, a strong sharp absorption band characteristic of C–Cl stretching mode was observed at 732 cm^{-1}, which is absent in the isolated barbiturate, clearly shows that chlorine is removed from DNCB during the formation of barbiturate. Since, upon the formation of the barbiturate, the aromatic nitro group is converted to one bearing partial negative charge, the asymmetric and symmetric absorption frequencies are expected to decrease (Terrier, 1982; Gnanadoss and Kalaivani, 1985; Kalaivani *et al*., 2005). This was observed in the present study also. The broad band observed at 2700–3400 cm^{-1} in barbiturate is characteristic of the triethyl ammonium cation (Silverstein and Webster, 2004). In the synthesized barbiturate a C=C stretching band appears at $\sim$1600 cm^{-1} as a strong sharp band instead of at $\sim$1640 cm^{-1}. This may be due to stretching of the C=C bond during delocalization. In barbituric acid the N–H proton signal appears at δ 11.09. During the formation of the barbiturate, the negative charge is delocalized over a large area up to the keto functions nearer the N–H groups. Hence the N–H protons are shielded to some extent and show a shift towards high field. The adjacent ring protons of the nitro moiety appear as double doublet in DNCB. whereas in barbiturate it became a multiplet centered at δ 2.33, supporting the delocalization of the negative charge. The quartet at δ 3.08 and triplet at δ 1.50 correspond to the $-CH_2$ and $-CH_3$ of the cation moiety, respectively. In COSY, $^1H-^1H$ coupling was noticed between the ring protons of the nitro moiety and ethyl group protons in the cation moiety. The ^{13}C spectrum of barbituric acid exhibits two signals at δ 151.7 and δ 167.8 due to the keto group carbon atoms. Six signals were observed in the ^{13}C spectrum of DNCB. The carbon atom bearing the Cl appears at δ 133.6. The synthesized barbiturate exhibits 11 signals in the ^{13}C spectrum. The absorption peaks at δ 8.6 and δ 45.8 are due to the carbon environments of the $-CH_3$ and $-CH_2$ groups, respectively, of the triethylammonium ion. The absorption peak at δ 86.6 is not observed in DNCB and barbituric acid but only in the barbiturate, possibly due to the newly formed carbon environment (C=C) (Kemp, 1991). In the FABMS spectrum, the base peak corresponds to triethylammonium cation (m/z = 102). Based on the spectral observations, the barbiturate may have structure 1 shown in Scheme 1.

The synthesized barbiturate was screened for anticonvulsant activity (Kulkarini, 1999). The barbiturate was given 1 h before the induction of the maximal electro shock test (150 mA, 0.2 s). The current was applied to the animals using corneal electrodes. The substance showed activity even at a low concentration of 50 mg/kg (Table 1).

The LD_{50} value of the synthesized barbiturate was calculated as per OECD guidelines (revised draft 423). The barbiturate falls under class 3 (LD_{50} >1000 mg/kg). The animals did not show any signs of acute toxicity or behavioral changes. The synthesized barbiturate may be a potent drug in future due to its extraordinary stability, low toxicity, high solubility in water, and easy method of preparation.

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