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2-METHYLPYRIDINIUM 5-(2,4-DINITROPHENYL)-6-OXO-2-THIOXO-1,2,3,6-TETRAHYDROPYRIMIDIN-4-OLATE: SYNTHESIS, CRYSTAL STRUCTURE, AND BIOLOGICAL EVALUATION

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The titled complex is synthesized from the ethanolic solutions of 1-chloro-2,4-dinitrobenzene, 2-thiobarbituric acid, and 2-methylpyridine and characterized by spectral and elemental analyses. The single crystal X-ray diffraction results for the complex are compatible with the spectral observations. The titled complex exhibits anticonvulsant activity and reduces all phases of convulsion even at a low dosage. Acute toxicity studies of the complex are performed to understand the safer dose concentration for clinical trials.

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K e y w o r d s: carbon-bonded anionic sigma complex, 2-thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, single crystal X-ray diffraction studies, anticonvulsant activity, maximal electroshock method.

INTRODUCTION

Electron-deficient nitroaromatic compounds undergo different types of interactions with nucleophiles [1—4]. Strong interactions between them result in the formation of anionic sigma complexes [1]. Generally, ketones, β-diketones, and β-ketoesters form carbon-bonded anionic sigma complexes with nitroaromatics in the presence of tertiary amines [2—4]. A series of carbon-bonded anionic sigma complexes have earlier been synthesized in our laboratories by involving chloronitroaromatic compounds and barbituric acid in the presence of tertiary amines [5—8]. 2-Thiobarbituric acid is an important class of heterocyclic compounds which possesses a bio-active nucleus and displays complex biological activities, such as antibacterial, sedatives, herbicidal, fungicidal and antiviral [9, 10]. Since 2-thiobarbituric acid has an active methylene group, an attempt has been made to synthesize a stable carbon-bonded anionic sigma complex with an electron — deficient compound in the presence of tertiary amine. In 2-thiobarbituric acid, two carbonyl groups and one thiocarbonyl group are present but it also forms a similar type of a carbon-bonded anionic sigma complex like barbituric acid which has three carbonyl groups with 1-chloro-2,4-dinitrobenzene (DNCB) in the presence of 2-methylpyridine. Epilepsy is a chronic neurological disorder in which a person has repeated convulsions over time [11, 12]. Currently, available anticonvulsant agents have notable adverse side effects and insufficient therapeutic applications [13, 14]. Consequently, there is a substantial need to develop new and more effective anticonvulsant agents. This prompted us to synthesize a new type of a carbon-bonded anionic sigma complex of 2-thiobarbituric acid with 1-chloro-2,4-dinitrobenzene (DNCB) and 2-methylpyridine employing the one-pot synthesis guided by the observations that the presence of both pyridine and pyrimidine nuclei in a molecule remarkably increases its biological profile [15, 16].

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EXPERIMENTAL

Materials and methods. Synthesis. Analytical grade 1-chloro-2,4-dinitrobenzene (DNCB), 2-thiobarbituric acid, 2-methylpyridine were used as supplied. 1-Chloro-2,4-dinitrobenzene (2.02 g, 0.01 mol) was dissolved in 40 ml of absolute ethanol and mixed with 2-thiobarbituric acid (1.44 g, 0.01 mol) dissolved in 40 ml of absolute ethanol. After mixing these two solutions, 4 ml of 2-methylpyridine (~0.03 mol) was added, shaken well for 7—8 h and kept as such at 303 K. After a period of two weeks, the excess solvent was removed by distillation under reduced pressure during which a pasty mass was obtained. It was washed with 50 ml of dry ether in 5 aliquots when an amorphous solid was obtained. The dry solid was powdered using an agate mortar and once again washed with 30 mL of dry ether and recrystallized from hot ethanol containing little amount of methanol (yield 90 % ; m.p. 508 K). The IR spectrum was recorded using Perkin-Elmer RXI infrared spectrophotometer as KBr pellets. The UV-visible data were obtained on a Shimadzu UV-VIS 1800 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using Bruker DRX-500 MHz spectrometer with (DMSO-d_6) as solvent and TMS as the internal reference.

Spectral data. Maroon red crystalline solid; yield: 90 %, m.p. 508 K; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 11.00 (br, s, 2H, N—H protons of the 2-thiobarbiturate moiety), 8.78 (d, 1H, ring proton adjacent to the $\overset{\oplus}{N}$—H group of the 2-methylpyridinium ion), 8.44—8.48 (ring proton *para* to the $\overset{\oplus}{N}$—H group of the 2-methylpyridinium ion, 8.40 (s, 1H, ring proton flanked between two —NO_2 groups), 8.22—8.24 (m, 1H, ring proton adjacent to the —NO_2 group), 8.17—8.19 (m, 1H, ring proton *meta* to the —NO_2 group), 7.88—7.92 (m, 2H, ring proton *meta* to the $\overset{\oplus}{N}$—H group and ring proton adjacent to the carbon-bearing —CH_3 group of the 2-methylpyridinium ion); ^{13}C NMR (500 MHz, DMSO) δ_C (ppm): 174.77, 161.57, 154.32, 147.28, 146.06, 142.17, 142.05, 139.77, 133.60, 127.99, 124.97, 124.63, 119.83, 90.22, 20.07; IR (KBr) ν (cm^{-1}): ~2200—3400 (br, characteristic of amine salt), 1640 (s, sh, C=O str.), 1577 (s, sh, C=S str.), 1539 (s, sh, NO_2 asym. str.), 1336 (s, sh, NO_2 sym. str.), 532 (s, sh, torsional oscillation — cation moiety); UV-Vis λ_{max}: DMSO (490 nm), ethanol (430 nm), water (400 nm); solubility in water (1.14 g / 100 ml). Anal. calcd. for $C_{16}H_{13}N_5O_6S$ (%): C 47.64, H 3.22, N 17.36, S 7.94. Found (%): C 47.28, H 3.01, N 17.99, S 7.63.

Crystal structure determination. The detailed structure information on the isolated carbon-bonded anionic sigma complex has been probed through single crystal X-ray diffraction studies. Good quality crystals of the complex are selected for the single crystal X-ray diffraction analysis and mounted on a Bruker AXS Kappa APEX2 CCD diffractometer with a graphite monochromator. MoK_α radiation is used for the measurements. The structure is solved by direct methods and refined by the full-matrix least squares technique. The non-hydrogen atoms are refined anisotropically. All the hydrogen atoms are placed in their idealized positions and refined as riding on their carrier atoms. The programs used for the crystal structure determination are — Data collection: APEX2 (Bruker, 2004 Cell refinement: APEX2 and SAINT (Bruker, 2004), Data reduction: SAINT and XPREP (Bruker, 2004), structure solving: SIR92 (Altomare et al., 1993); structure refinement: SHELXL 97 (Sheldrick, 2008); molecular graphics: ORTEP (Farrugia, 1997) and Mercury (Macrae et al., 2008) [17—21].

Biological assay. The maximal electroshock (MES) method [22, 23] has been adopted to study the anticonvulsant activity. Albino rats of either sex weighing 150—200 g were divided into groups of six animals each. The positive control used was phenobarbitone (20 mg/kg). The control group was fed with normal saline (5 mL/kg). Another group received the carbon-bonded anionic sigma complex (25 mg/kg). The normal saline, the standard drug, and the synthesized complex are given one hour before the induction of MES (150 mA / 0.2 s). The current was applied to the animals using the corneal electrodes of electro convulsometer (model 100-3, INCO). The different stages of convulsions such as tonic flexion, tonic extensor, clonus convulsion, stupor and recovery /death were noted. The time spent by the animal in each of these phases was noted. The mean value for each group was calculated and compared with the control. The results are expressed as mean ± standard error. The test of significance is analyzed by student′s t-test [24]. Hypnotic activity of the complex has been examined

on albino mice. Acute toxicity studies of the synthesized complex are made as per OECD guidelines (revised draft 423) to understand the safer dose concentration for clinical trials.

RESULTS AND DISCUSSION

Spectral interpretation. Qualitative tests on the complex indicate the presence of nitro groups, a nitrogen atom, a sulfur atom and the absence of a chlorine atom [25]. The complex is non-hygroscopic, very stable at room temperature and freely soluble in water. The synthetic route for the formation of the complex is schematically represented in Fig. 1. The complex is maroon red in colour due to the delocalization of negative charge on a large area. The maximum absorption wavelengths are different in solvents of different polarity ($\lambda_{max}(H_2O)$ = 400 nm; λ_{max}(EtOH) = 430 nm; λ_{max}(DMSO) = = 490 nm). In the IR spectrum of the complex, due to the asymmetric and symmetric stretching vibrations of nitro groups, strong sharp bands are noticed at 1539 cm^{-1} and 1336 cm^{-1}. A strong sharp absorption band characteristic of the C—Cl stretching mode is observed at ~732 cm^{-1} in DNCB. This band is absent in the isolated carbon-bonded anionic sigma complex. This clearly specifies that the DNCB chlorine atom is removed during complexation. A broad band at ~2200—3400 cm^{-1} is characteristic of amine salt [26]. Similarly, the band at 532 cm^{-1} reflects the torsional oscillation of amine salt [27]. The band corresponding to C=S appears at 1577 cm^{-1}. In the PMR spectrum of 2-thiobarbituric acid the signal due to N—H protons appears at δ 12.32 ppm, whereas in the complex this signal is shifted to a high field (δ 11.00 ppm) due to the shielding which results from the delocalization of negative charge up to the near vicinity of the N—H group. The ring protons of the nitroaromatic moiety also resonate at a higher field (δ 8.17—8.40) due to shielding by the delocalization of negative charge. The methyl group protons of 2-methylpyridine resonate at 2.49 ppm, which is shifted to a lower field (δ 2.71 ppm) due to protonation at the nitrogen atom during the formation of the carbon-bonded anionic sigma complex. In the ^{13}C NMR spectrum of 2-thiobarbituric acid, the carbon atom attached to the sulfur atom resonates at δ 181 ppm, whereas in the complex it appears at δ 174.77 ppm. This also supports the delocalization of negative charge. Six signals have been observed in the ^{13}C NMR spectrum of DNCB (147.4, 146.1, 133.6, 133.1, 127.3, 120.8). Carbon-bearing Cl appears at δ 133.6 ppm. The synthesized complex exhibits 15 signals due to nine different carbon environments in the anion (174.7, 161.57, 147.28, 142.05, 139.77, 133.60, 124.97, 119.83, 90.22) moiety and six different carbon environments in the cation (154.32, 146.06, 142.17, 127.99, 124.63, 20.07) moiety. The

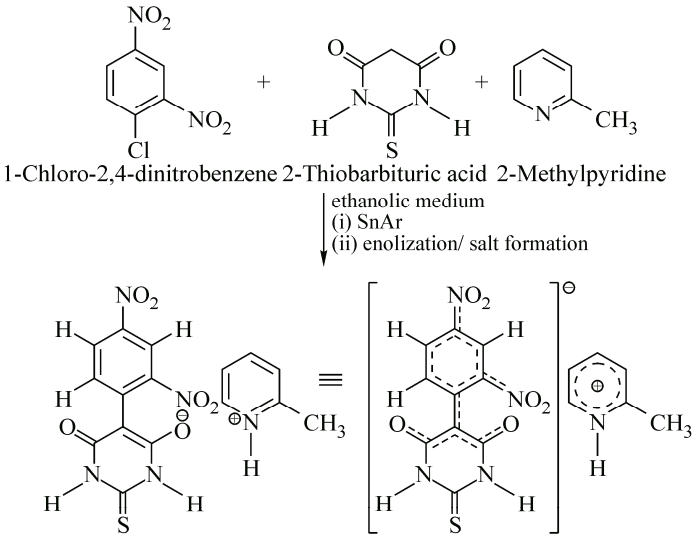

Fig. 1. Schematic representation of the formation of the title compound

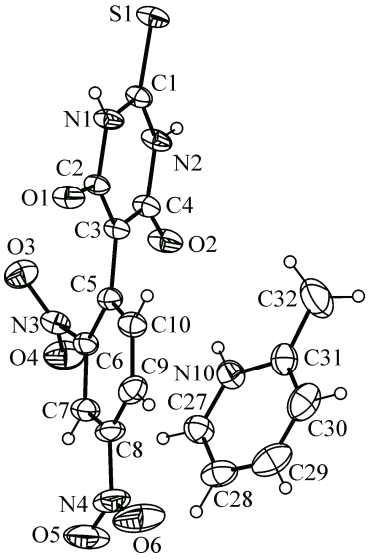

Fig. 2. ORTEP of the title compound

absorption peak at δ 90.22 ppm is noticed in neither DNCB nor 2-thiobarbituric acid but only in the complex due to the newly formed C=C environment [28].

Crystal structure description. Single crystal X-ray diffraction studies are carried out to ascertain the revealed facts through the spectral analysis. The X-ray analysis also helps to establish various types of the hydrogen bonding which may facilitate the understanding of the mechanism of action of thiobarbiturates on the target center in the future. The asymmetric unit of the complex consists of two anion moieties, two cation moieties, 0.25 methanol molecule (disordered with an occupancy of ~50 %), and 0.25 ethanol molecule (Fig. 2, ORTEP). The important crystal data noticed in the complex are listed in Table 1. The dihedral angles between the dinitrophenyl and thiobarbiturate rings in two asymmetric units are 50.20(7)° and 43.44(6)°. The nitro group which is *ortho* with respect to the carbon atom attached to the thiobarbiturate ring is twisted to an angle of 39.08(10)° in one anionic part and 38.38(11)° in another anionic part from the aromatic ring plane. Also, in one anionic part of the asymmetric unit, the nitro group *para* with respect to the carbon atom attached to the thiobarbiturate ring is deviated to an extend of 6.32(21)°, whereas in another anionic part the deviation observed is 16.13(26)°. Thus, the *para* nitro group is more effectively involved in charge delocalization than the

T a b l e 1

Crystal data of the title compound

Specification	Data
Crystal system; space group	Monoclinic; $P2_1/n$
Unit cell dimensions a, b, c, Å; β, deg.	16.796(6), 10.828(1), 21.715(8); 111.44(1)
Volume, Å^3	3676.40(2)
Z	4
F(000)	1708
Calculated density, g/cm^3	1.493
Radiation	MoK_α
Wavelength, Å	0.71073
Temperature, K	293(2)
Crystal size, mm	0.35×0.30×0.30
θ range for data collection, deg.	1.32 to 26.00
Completeness to θ = 25°, %	100.00
S	1.082
Data / restraints / parameters	7231 / 117 / 604
Scan range	$-20 \le h \le 20$, $-13 \le k \le 13$, $-26 \le l \le 25$
T_{min}, T_{max}	0.902, 0.951
Number of measured / unique reflections	53986 / 7231
$\Delta\rho_{max}$ / $\Delta\rho_{min}$, e/Å^3	0.233 / –0.227
R_1 / wR_2 relative to N1	0.0671 / 0.1192
R_1 / wR_2 relative to N2	0.0405 / 0.0989
CCDC No.	1006268

ortho nitro group. The crystal structure is stabilised by a number of N(5)—H(5)…O(2), N(2)—H(2)…S(2), N(6)—H(6)…O(1) and N(1)—H(1)…O(8) hydrogen bonds.

Biological evaluation. The result of the anticonvulsant activity indicates that the synthesized carbon-bonded anionic sigma complex effectively reduces the various phases of convulsion. The presence of both pyridine and pyrimidine moieties within the same molecule can be responsible for its activity. The presence of the methyl group at the *ortho* position with respect to the nitrogen atom in the 2-methylpyridinium ion can also play a significant role in modulating the ion channel to reduce the various phases of convulsion. The therapeutic dose of the synthesized carbon-bonded anionic sigma complex induces hypnosis in albino mice for a period of 71 min. Acute toxicity studies are required for all biologically active molecules to understand the safer dose concentrations for clinical trials. LD_{50} of the synthesized carbon-bonded anionic sigma complex was made as per OECD guidelines (revised draft 423). The carbon-bonded anionic sigma complex falls under class 4 (LD_{50} greater than 1500 mg/kg); the animal did not show any behavioral changes.

CONCLUSIONS

In the present investigation, a new type of a carbon-bonded anionic sigma complex has been synthesized from DNCB, 2-thiobarbituric acid, and 2-methylpyridine in good yield and high purity employing the one-pot synthesis. New thiobarbiturate possesses admirable characteristic features such as good stability at room temperature, non-hygroscopic nature, and water solubility. The presence of two water molecules of crystallization in the same asymmetric unit with a slightly varying geometry is highly appreciable. Since the complex possesses significant anticonvulsant activity even at a low concentration of 25 mg/kg and a high LD_{50} value (>1500 mg/kg) the molecule can be used as a drug to treat the dreadful disorder epilepsy in forthcoming days.

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Details of the X-ray data collection and final refinement parameters including anisotropic thermal parameters and full list of the bond lengths and angles have been deposited with the Cambridge Crystallographic Data Center in the CIF format as supplementary publication number CCDC 1006268. Copies of these information can be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB21EZ, UK (fax: +44 (0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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