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ARTICLE

The Upper Limit of Luminol's Amphiprotism: The Crystal Structure of 5-Ammonium-2-hydro-1,4-phthalzinediol Sulfate(IV)

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Dedicated to Professor Hans-Jörg Deiseroth on the Occasion of his 75th Birthday

Abstract. Luminol is chemically sufficiently stable to be diprotonated at high proton concentrations as provided by concentrated sulfuric acid. The luminol dication (5-ammonium-2-hydro-1,4-phthalzinediol) sulfate was isolated as macroscopic single crystals and its structure was determined and refined from single-crystal X-ray data collected at 173 K [cell parameters: a = 8.3994(17) Å, b = 6.9985(14) Å, c = 17.486(4) Å, β = 90.85(3)°, V = 1027.8(4) Å^3, space group $P2_1/c$]. The structure is comprised of layers stacked along the b axis. Intralayer interactions are accomplished by strong hydrogen bonds of three luminol dications to one central $[SO_4]^{2-}$ ion. Interlayer interactions are formed by weak hydrogen bonds of one luminol dication to two $[SO_4]^{2-}$ ions in the adjacent layers, respectively, and alternating sandwich and parallel-displaced π-π-stacking of the 1-hydropyridazine-3,6-diol moieties of luminol dications in adjacent layers, respectively.

Introduction

In 1928, German chemist *H. O. Albrecht* reported blood to enhance the luminescence of 5-Amino-2,3-dihydro-1,4-phthalazinedione (luminol) in an alkaline solution of hydrogen peroxide.[1] The chemiluminescence of luminol can also be triggered by a variety of other metals like iron, copper and their complexes. To this very day, luminol is therefore heavily used by crime scene investigators and in environmental and biochemical analytics.[2] More recently, the sodium salt of luminol has regained attention as an active pharmaceutical ingredient in immunomodulating treatment of inflammatory and autoimmune diseases, for instance psoriasis.[3]

Despite its long history and heavy use, the first crystal structure of neutral luminol was reported as late as in 1992 by *Paradies*.[4] The commercially sold luminol powder used for ages in forensics was shown later to be actually a metastable polymorph.[5] The two polymorphs differ in distinct π-stacking motifs. In both crystal structures of neutral luminol the amide-hydroxyimine tautomeric form (Figure 1a, 5-amino-3-hydro-1,4-phthalazine-1-ol-4-one) is present rather than the typically in textbooks assumed amide-amide form (Figure 1b). This indicates that hydrogen bonding motifs contribute significantly to the lattice energy.

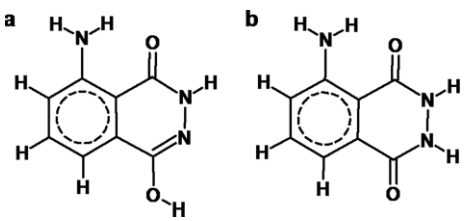

Figure 1. Amide–hydroxyimine (a) and amide–amide (b) tautomeric forms of luminol.

The unique combination of an extended flat π-system and functional groups capable of hydrogen bonding trigger a very rich (pseudo-)polymorphism. Besides a di- and a hexahydrate as many as three polymorphs of the anhydrous Na-luminolate have been structurally characterized.[6]

Luminol, moreover, is amphoteric. Several distinct pK_a values (1.5, 6, and 13–15) are reported in literature while some dispute exists regarding the acidity/basicity ranking of protons in luminol.[7] Based on studies of the oxidation behavior of luminol, it is now generally agreed that the amine moiety is less acidic than the protons of the 1-hydropyridazine-3,6-diol moiety. The amine group at the same time is a weak base (pK_a = 6) and can easily be protonated. It is, however, unclear which atom is the least basic group (pK_a = 1.5). Herein we now report the first crystal structure of the sulfate salt of the luminol dication to answer this question.

Results and Discussion

Crystal Structure of Luminol Dication Sulfate

When attempting to crystallize the dicationic luminol salt from a hot (100 °C) sulfuric acid (98 %, 1 d) the condition turned out to be too harsh even for the very stable luminol and only decomposition products were obtained: *m*-carboxyphen-

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Table 1. Crystallographic data and experimental details of the structure refinement of luminol dication sulfate.

Crystal data		Data collection		Refinement	ShelXL
Formula	$(C_8H_9N_3O_2)$ (SO_4)	Diffractometer	STOE IPDS II		Refinement of F^2
M_r	275,24	Radiation	Mo-$K\alpha_1$ (λ = 0.71073 Å)	$R[F^2 > 2\sigma(F^2)]$	0.0450
Crystal system	monoclinic	Monochromator	graphite	$wR[F^2 > 2\sigma(F^2)]$	0.1081
Space group	$P2_1/c$	T /K	173	$R(F^2)$ (all data)	0.0560
a /Å	8.3994(17)			$wR(F^2)$ (all data)	0.1155
b /Å	6.9985(14)	Completeness of 2θ	0.987	S	1.074
c /Å	17.486(4)	Collected reflections	7290	Weighting	$1/[\sigma^2(F_o^2)$ $+ (0.0520P)^2 + 1.0857P]$
β /°	90.85(3)	Independent reflections $[I > 2\sigma(I)]$	2758		with $P = (F_o^2 + 2F_c^2)/3$
V /Å^3	1027.8(4)	R_{int}	0.0539	$\Delta\rho_{min}$ /eÅ^3	–0.559
Z / Z′	4 / 1	θ_{min}	2.330	$\Delta\rho_{max}$ /eÅ^3	0.948
ρ /g·cm^{-3}	1.779	θ_{max}	29.263	Refined parameters	199
Crystal description	needle,	h	–11 → 11		
	clear light brown	k	–9 → 9		
Crystal size /mm	0.2 × 0.1 × 0.1	l	–23 → 23		

ylammonium bisulfate[8] and a so far structurally unknown polymorph of hydrazine sulfate[9] were isolated. Reducing the temperature to 80 °C and the reaction time to 3 h of stirring, followed by slowly cooling the solution to room temperature finally yielded single crystals of the luminol dication sulfate. At room temperature the crystals were stable in sulfuric acid. Crystals could be recovered from the mother liquor and mounted on the diffractometer. Diffraction data were collected at 173 K and could be indexed with the following lattice parameters: a = 8.3975 Å, b = 7.0001 Å, c = 17.4897 Å, β = 90.85°. Structure solution and refinement were straightforward. Further details on the data collection and structure refinement are given in Table 1. Figure 2 shows the ORTEP plot of the asymmetric unit of luminol dication sulfate including the numbering scheme. The molecular packing is shown in Figure 3.

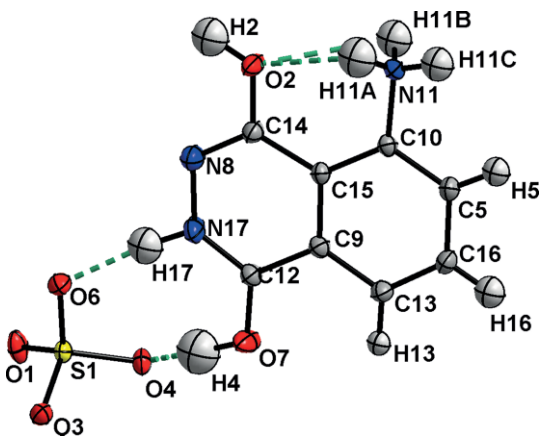

Figure 2. ORTEP plot and crystallographic numbering scheme of the asymmetric unit of the luminol dication sulfate. Displacement ellipsoids are drawn at 50 % probability level. Intra- and intermolecular hydrogen bonds are marked by dashed lines.

The crystal structure indeed proved a twofold protonation of luminol rendering it a dication (Figure 2). Apart from the protonation of the more basic amino-group (N11, H11A, H11B, H11C), additionally the second oxygen of the amid

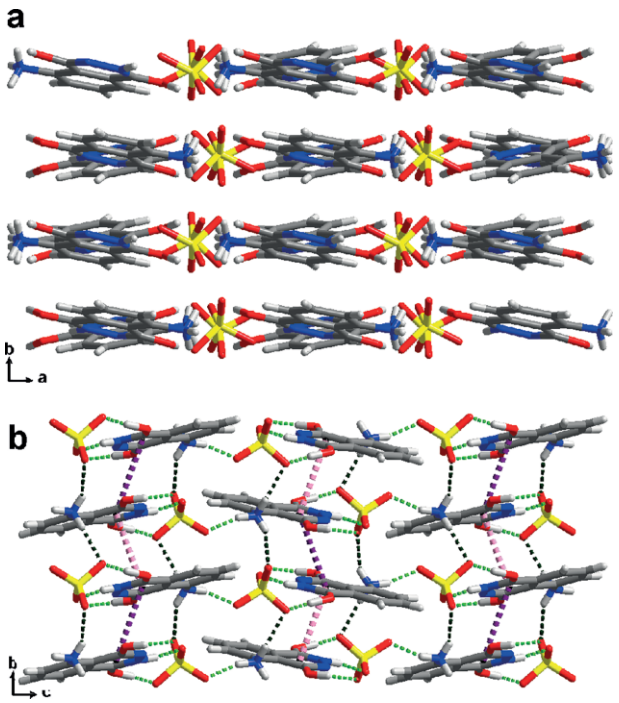

Figure 3. Molecular packing of the luminol dication sulfate with layers stacked along b-axis (a). Packing viewed along a-axis (b). Strong intralayer hydrogen bonds are marked by light green dashed lines. Weak interlayer hydrogen bonds are marked by dark green dashed lines. Light violet dashed lines mark sandwich π–π stacking of 1-hydropyridazine-3,6-diol moieties in adjacent layers. Dark violet dashed lines mark parallel-displaced π–π stacking of 1-hydropyridazine-3,6-diol moieties in adjacent layers.

group in the 5-amino-3-hydro-1,4-phthalazine-1-ol-4-one tautomer[3b,4] (Figure 1a) was protonated at low pH (Figure 2, O2, H2). Refinement yielded a comparatively long O–H bond length for O7–H4 (1.13 Å), while for O2–H2 a value in the usual range was observed (0.95 Å). H4, moreover, is involved in a short hydrogen bond with O4 (1.34 Å, Figure 4 and Table 2). The twofold protonation of luminol paves the way for the formation of a strong hydrogen bond network (H···A

Table 2. Details on two-centered hydrogen bonds in luminol dication sulfate.

D–H···*A*	*D*–H /Å	H···*A* /Å	*D*···*A* /Å	*D*–H···*A* /°	D symm.code
O2–H2···O3	0.95(4)	1.63(3)	2.566(2)	168(3)	–1+x, *y*, *z*
O7–H4···O4	1.13(4)	1.34(4)	2.471(2)	178(3)	x, *y*, *z*
N17–H17···O6	0.98(3)	1.74(3)	2.715(2)	171(3)	x, *y*, *z*
N11–H11A···O3	0.85(4)	2.16(4)	2.902(2)	144(4)	1-x, 2 –*y*, 1 –*z*
N11–H11B···O4	0.93(4)	2.03(3)	2.874(2)	150(3)	1-x, 1 –*y*, 1 –*z*
N11–H11C···O1	0.93(3)	1.83(3)	2.735(2)	164(3)	–1+x, 3/2 –*y*, –1/2 + *z*

interatomic distances significantly below 2 Å) between $[SO_4]^{2-}$ ions and luminol dications in the structure's secondary building motif: Layers with alternating strands of $[SO_4]^{2-}$ ions and luminol dications are stacked along the *b* axis (Figure 3a). Within these strands an interatomic distance [S–S] of 8.74 Å and an angle [S–S–S] of 177.1° is observed along *c*. Nearest luminol dications along *c* within said layers show a slight torsion of the molecular plane of ±11°, respectively. Interlayer interactions (Figure 3b) are governed by a combination of sandwich and parallel-displaced π–π stacking and weak hydrogen bonds. While parallel-displaced π–π stacking of the heteroaromatic moiety is also realized in all other luminol or alkali-luminolate structures, the luminol dication sulfate is the only structure where the complete π system, including the aminobenzyl moiety, is involved in π–π stacking.

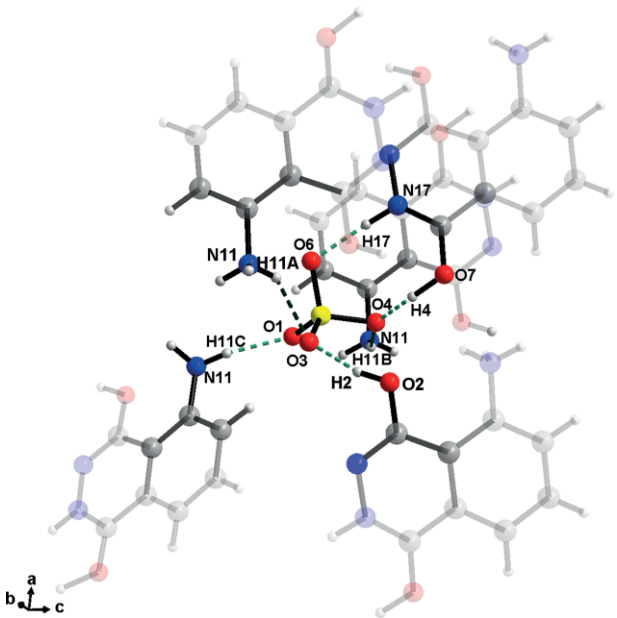

Figure 4. Hydrogen bonds between luminol dications surrounding one $[SO_4]^{2-}$ ion. For details on the respective bonds please see Table 2. In total, five luminol dications form hydrogen bonds to one $[SO_4]^{2-}$ ion. Two weak hydrogen bonds (N11–H11A···O3, N11–H11B···O4) are formed by one luminol dication in an adjacent layer, respectively. Four strong intralayer hydrogen bonds are formed by three surrounding luminol dications.

For the sandwich and parallel-displaced π stacks distances of 3.70 Å (light violet dashed line in Figure 3b) and 3.74 Å (dark violet dashed line in Figure 3b) between the centers of gravity of two adjacent 1-hydropyridazine-3,6-diol moieties are observed, respectively.

For a brief description of the hydrogen bond network, we focus on the shortest two-centered hydrogen bonds and weaker multifurcated hydrogen bonds are neglected for reasons of clarity. Details on the discussed hydrogen bonds are summarized in Table 2. The aforementioned strong intralayer hydrogen bond network is formed by three luminol dications surrounding the central $[SO_4]^{2-}$ ions with one luminol dication addressing the $[SO_4]^{2-}$ ion with two hydrogen bonds (O7–H4···O4 and N17–H17···O6). Weak interlayer hydrogen bonds (N11–H11A···O3 and N11–H11B···O4 with H···A > 2 Å) are formed by one luminol dication in an adjacent layer, respectively.

Conclusions

Luminol is indeed chemically stable enough to become diprotonated at high proton concentration as provided by concentrated sulfuric acid marking the upper limit of luminol's amphiprotism. The O–H group of the amide-hydroxyimine is acidic, the amine group represents a slightly basic group and the oxide of the amid functionality a very weak base allowing to assign the pK_a 13, 6, and 1.5, respectively.

Experimental Section

General: Luminol was purchased from Merck KGaA. Sulphuric acid (p.a) was purchased from Bernd Kraft GmbH.

Synthesis of Luminol Dication Sulfate: Crystals of luminol dication sulfate were obtained from a hot (80 °C) solution of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) (7.4 g) in sulfuric acid (10 mL), that was stirred for 2 h and subsequently allowed to slowly cool to room temperature. Luminol dication sulfate formed clear light brown thin needles.

Single Crystal X-ray Diffraction: Single crystal X-ray data were collected on a STOE IPDS II diffractometer (173 K, graphite monochromated Mo-$K_{\alpha 1}$ radiation (λ = 0.71073 Å)) equipped with an Oxford Cryosystems Cryostream system using a crystal sized 0.2 mm × 0.1 mm × 0.1 mm. Selected crystallographic data are listed in Table 1. The crystal structure was solved and refined using SHELXS[10] and SHELXL[11] as implemented in OLEX^2.[12] Hydrogen atoms were identified in the difference Fourier map and coordinates and isotropic displacement factors were refined freely. The non-symmetrical characteristics of the O7–H4···O4 hydrogen bond are confirmed by the absence of residual electron density in the difference Fourier map. Diamond 4.4[13] was used for the graphical representation of the results.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic

Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository number CCDC-1970866 for luminol dication sulfate (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk)

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Keywords: Luminol; X-ray diffraction; Structure elucidation

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