

No significant biological activity has been reported for the polyhydroxy molecules; however, it has not been determined whether the polyhydroxy moieties might serve as inert carriers of the alkaloid fragment, as active transport agents or as orienting groups for the alkaloid fragment at the active site.

We would like to acknowledge the financial support of the Robert A. Welch Foundation (Grant No. P-074) and the TCU Research Foundation.

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## Fluorescent Probe Conformations: The Crystal and Molecular Structure of Hexa-aquamagnesium Bis(8-anilino-1-naphthalenesulfonate) Hexahydrate

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(Received 22 October 1976; accepted 29 January 1977)

The crystal and molecular structure of hexa-aquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate,  $[\text{Mg}(\text{H}_2\text{O})_6][\text{C}_{16}\text{H}_{12}\text{SO}_3]_2 \cdot 6\text{H}_2\text{O}$ , has been determined.  $P\bar{1}$ ,  $Z = 2$ ,  $a = 11.1303$  (5),  $b = 13.5383$  (6),  $c = 6.9703$  (3) Å,  $\alpha = 102.071$  (5),  $\beta = 97.224$  (6),  $\gamma = 91.009$  (4)°. Structural analysis shows the geometry at the anilino N to be nearly trigonal, a suggested prerequisite for fluorescence. The overall conformation of the molecule is similar to that of one of the two distinct conformers observed in the structure of the ammonium salt of 8-anilino-1-naphthalenesulfonic acid. The torsion angles between the aromatic rings and the C–N–C plane are  $\text{C}(2')\text{--C}(1')\text{--N--C}(1) = 174^\circ$  and  $\text{C}(1')\text{--N--C}(1)\text{--C}(2) = -54^\circ$ . The complex is held together by a strong network of ten intermolecular hydrogen bonds. There is also an N–H...O intramolecular hydrogen bond.

### 1. Introduction

The enhancement of fluorescence properties of a number of polycyclic aromatic compounds as a result of noncovalent interaction with a protein has made them useful as probes of hydrophobic binding sites. They have been used to explore the binding sites of many substrates, enzymes, membranes and proteins (Edelman & McClure, 1968; Brand & Gohlke, 1972; Kolb & Weber, 1975). Generally, the fluorescence of a probe varies with the polarity of the solvent and its fluorescence characteristics are sensitive to its local environment. Probes such as 8-anilino-1-naphthalenesulfonic acid (ANS) (Fig. 1) have been the most widely

utilized in contemporary biochemical research for the assessment of hydrophobicity of binding sites on proteins and as a means of monitoring conformational changes in biological macromolecules.

In addition to its important use as a fluorescent probe, ANS has been shown to act as a competitive inhibitor with thyroxine for the binding sites on the thyroid hormone transport proteins (Green, Marshall, Pensky & Stanbury, 1972; Ferguson, Edelhofer, Saroff, Robbins & Cahnmann, 1975).

Recent X-ray data on the protein crystal structure of horse-liver alcohol dehydrogenase (Eklund, Nordstrom, Zeppenauer, Soderlund, Ohlsson, Boiwe & Branden, 1974) show that there are two binding sites

for ANS, each binding with a different molecular conformation.

Although the mechanism of fluorescence enhancement in proteins is not clearly understood, it is suggested that the relative orientation of the two ring systems in the probes may influence their fluorescence characteristics (Anderson & Weber, 1969; Greene, 1975; Penzer, 1972). The results of an NMR study on ANS (Penzer, 1972) suggest that the aromatic rings are more nearly coplanar in alcohol than in water and that the anilino H participates in a strong interaction with the sulfonate group in alcohol. Anomalous behavior of the proton signals of the H(2), H(2') and H(6') protons in both solvents was reported. Finally, it was suggested that intramolecular hydrogen-bond formation will favor a nearly coplanar conformation.

The crystal and molecular structure of hexa-aquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate was undertaken in order to compare its conformation with those of the thyroid hormones (Cody & Hazel, 1976) and to determine its conformational preference in the light of the current mechanisms proposed for fluorescence.

## 2. Experimental

Crystals of the magnesium salt of 8-anilino-1-naphthalenesulfonic acid, purchased from Eastman

Kodak Chemicals, were grown at room temperature from an aqueous solution. A clear, well formed crystal (0.12 × 0.20 × 0.68 mm) was selected for intensity-data collection. All X-ray measurements were made on a Nonius CAD-4 automated diffractometer using Cu K $\alpha$  radiation. The unit-cell dimensions were obtained from a least-squares calculation based on 2 $\theta$  measurements of 38 *hkl* reflections having 2 $\theta$  > 80° with Cu K $\alpha$  radiation. The crystal data are presented in Table 1.

The intensities of 4175 (2661 observed) independent reflections with  $\theta < 75^\circ$  were measured on the Nonius CAD-4. A reflection was considered unobserved if the net count was less than twice the standard deviation of the background. No significant changes were observed in the intensities of the standard reflections measured during data collection. Intensities were corrected for

Table 1. *Crystal data for hexa-aquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate*

Formula	[Mg(H <sub>2</sub> O) <sub>6</sub> ][C <sub>16</sub> H <sub>12</sub> O <sub>3</sub> S] <sub>2</sub> ·6H <sub>2</sub> O
<i>M<sub>r</sub></i>	418.59
Space group	<i>P</i> 1̄
<i>a</i>	11.1303 (5) Å
<i>b</i>	13.5383 (6)
<i>c</i>	6.9703 (3)
$\alpha$	102.071 (5)°
$\beta$	97.224 (6)
$\gamma$	91.009 (4)
<i>V</i>	1017.91 Å <sup>3</sup>
<i>D<sub>c</sub></i>	1.36 g cm <sup>-3</sup>
<i>Z</i>	2
$\mu$	39.00 cm <sup>-1</sup>
$\lambda$	1.5418 Å
<i>R</i>	6.8% (2661 observed data)
Crystal size	0.12 × 0.20 × 0.68 mm

Table 2. *Positional parameters and hydrogen isotropic thermal parameters for hexa-aquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate*

	<i>x</i> (× 10 <sup>5</sup> )	<i>y</i> (× 10 <sup>5</sup> )	<i>z</i> (× 10 <sup>4</sup> )		<i>x</i> (× 10 <sup>3</sup> )	<i>y</i> (× 10 <sup>3</sup> )	<i>z</i> (× 10 <sup>3</sup> )	<i>B</i> <sub>iso</sub>
Mg	0 (0)	0 (0)	0 (0)	H(1)	734 (5)	336 (4)	455 (8)	6.9 (12)
S	92514 (8)	24963 (6)	5863 (1)	H(2)	375 (3)	449 (3)	345 (5)	3.1 (7)
O(1)	85760 (24)	23364 (18)	3873 (3)	H(3)	273 (4)	310 (4)	185 (8)	7.2 (14)
O(2)	84828 (24)	22946 (17)	7308 (4)	H(4)	57 (4)	288 (3)	106 (7)	4.7 (10)
O(3)	103457 (24)	19084 (18)	5874 (5)	H(5)	882 (5)	381 (4)	123 (8)	7.5 (14)
C(1)	77000 (31)	46465 (24)	6476 (5)	H(6)	763 (3)	503 (3)	193 (6)	3.7 (8)
C(2)	71003 (33)	55445 (27)	6902 (6)	H(7)	153 (3)	339 (3)	668 (5)	3.0 (7)
C(3)	77306 (39)	64506 (27)	7848 (6)	H(2')	562 (5)	230 (5)	339 (9)	8.9 (16)
C(4)	89385 (37)	64898 (25)	8295 (6)	H(3)	398 (5)	170 (4)	424 (8)	7.5 (13)
C(5)	108780 (35)	56870 (26)	8259 (5)	H(4')	302 (5)	220 (4)	711 (8)	8.3 (15)
C(6)	115656 (32)	48859 (29)	7816 (6)	H(5')	411 (5)	365 (4)	925 (8)	7.3 (14)
C(7)	109928 (31)	39337 (26)	7016 (5)	H(6')	612 (5)	423 (4)	874 (8)	7.8 (15)
C(8)	97648 (29)	37929 (22)	6638 (4)	H(5A)	175 (4)	72 (3)	847 (6)	4.6 (10)
C(9)	89876 (28)	46482 (21)	6960 (4)	H(5B)	94 (4)	116 (3)	755 (6)	4.0 (9)
C(10)	96103 (33)	56034 (23)	7838 (5)	H(6A)	157 (3)	48 (3)	304 (5)	3.6 (8)
N(1)	70110 (28)	37758 (23)	5441 (5)	H(6B)	74 (6)	106 (5)	353 (10)	10.4 (18)
C(1')	59646 (32)	33943 (28)	5971 (7)	H(8A)	430 (5)	30 (4)	827 (8)	7.2 (14)
C(2')	53263 (43)	25763 (35)	4660 (9)	H(8B)	644 (5)	41 (4)	112 (8)	7.8 (15)
C(3')	43011 (46)	21578 (41)	5105 (12)	H(4A)	-117 (6)	156 (4)	144 (9)	8.9 (17)
C(4')	38373 (47)	25322 (42)	6819 (13)	H(4B)	-120 (4)	151 (3)	-56 (7)	5.3 (11)
C(5')	44592 (49)	33294 (41)	8124 (11)	H(7A)	666 (5)	26 (4)	503 (9)	8.2 (16)
C(6')	55190 (42)	37659 (35)	7744 (9)	H(7B)	779 (2)	85 (2)	593 (4)	5.5 (5)
O(4)	-10941 (23)	12158 (16)	297 (3)	H(9A)	651 (4)	144 (3)	928 (7)	11.1 (11)
O(7)	70678 (26)	4970 (22)	5908 (5)	H(9B)	535 (4)	199 (3)	914 (6)	9.6 (9)
O(5)	11139 (25)	6721 (23)	-1550 (5)					
O(8)	34910 (28)	737 (27)	8218 (5)					
O(9)	57433 (33)	11392 (31)	9013 (6)					
O(6)	10929 (26)	6318 (21)	2595 (4)					

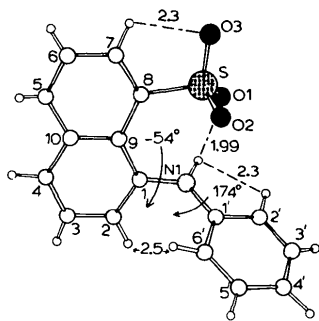


Fig. 1. Numbering scheme for the 8-anilino-1-naphthalene-sulfonate portion of the structure, with the intramolecular hydrogen bond and H...H contact distances.

Lorentz and polarization factors; however, no absorption correction was made.

The structure was solved by direct methods with *MULTAN* (Germain, Main & Woolfson, 1971) and *NQEST* (DeTitta, Edmonds, Langs & Hauptman, 1975). All non-hydrogen atoms for the ANS, Mg and the water molecules were located in the resulting *E* maps. The Mg ion occupies an inversion center and has three pairs of inversion-related waters of hydration associated with it. There are also three water molecules associated with the ANS molecule. All H atoms were located from three-dimensional difference Fourier maps. Both the positional and isotropic thermal parameters for the H atoms were refined. Refinement terminated when there was no further improvement in

the e.s.d.'s. The structure was refined by full-matrix least squares to a final *R* index ( $R = \Sigma |F_o| - |F_c| / \Sigma |F_o|$ ) of 0.068. Only the observed reflections were used throughout the least-squares procedures.

All scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The weighting scheme used in the final refinement was  $w^{-1/2} = \sigma(F_o)$  where  $\sigma(F_o)$  is defined by Stout & Jensen (1968, equation H.14) and the instability correction was 0.06.

The final fractional coordinates for ANS, Mg and water and the positional and isotropic thermal parameters for all H atoms are listed in Table 2.\* Fig. 1 gives the numbering scheme for ANS.

### 3. Results and discussion

The bond lengths and angles calculated from the coordinates in Table 2 are given in Fig. 2. The estimated standard deviations for the nonhydrogen bond lengths of the ANS molecule average 0.005 Å while the corresponding average for the angles is 0.2°. The average e.s.d. for the bond lengths of the water molecules is 0.06 Å and that for the angles is 3.0°. Since the positional parameters of some of these H

\* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32656 (21 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

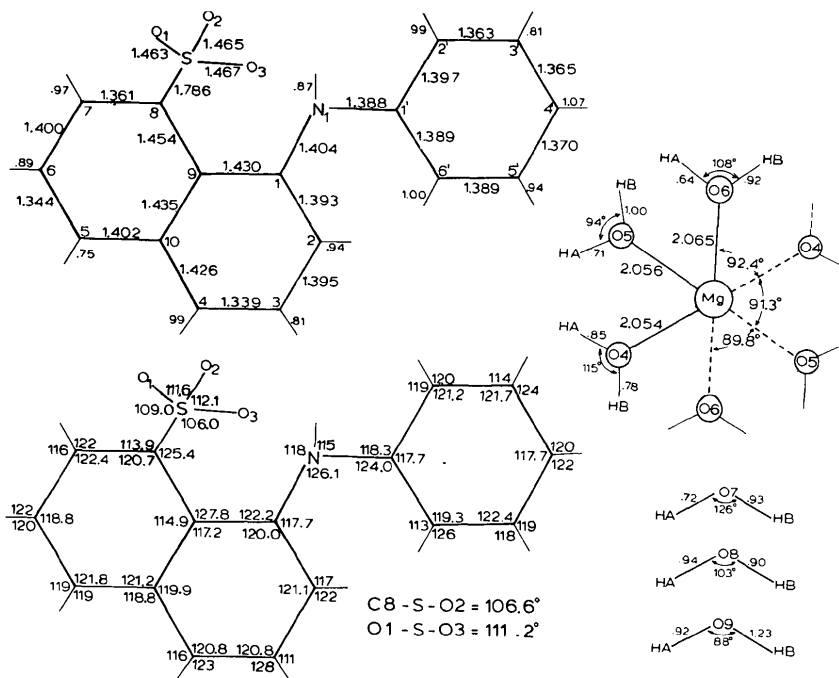


Fig. 2. Bond distances and bond angles for hexaaquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate.

atoms were less well defined than those of other atoms in the Fourier maps, it is most likely that their errors are underestimated.

The coordination about the Mg ions is octahedral with the three O atoms related by inversion through the center of symmetry to the rest of the coordination sphere about Mg (Fig. 2).

#### 4. Naphthalene geometry

As observed in other *peri* or 1,8-disubstituted structures (Balasubramanian, 1966; Einspahr, Robert, Marsh & Roberts, 1973; Bright, Maxwell & de Boer, 1973; Robert, Sherfinski, Marsh & Roberts, 1974) the average lengths of the bonds involving the C(1) and C(8) atoms are longer than the average length of the bonds involving the C(4) and C(5) atoms respectively. In this structure, these values are longer by 0.029 and 0.035 Å, respectively. Overcrowding apparently leads to this redistribution of the bonding electrons within the naphthalene nucleus.

The strain introduced by the 1,8-disubstitution is relieved by angle expansion [ $S-C(8)-C(9) = 125.2^\circ$ ;  $N-C(1)-C(9) = 122.2^\circ$ ] and out-of-plane distortions involving the entire molecule (as described by the angle between the two six-membered rings of the naphthalene structure of  $3.5^\circ$ ) as well as the *peri* substituents [ $N-C(1)-C(9)-C(8)^* = -5.1$ ;  $S-C(8)-C(9)-C(1) = -9.4^\circ$ ]. Despite these deformations the  $S \cdots N$  non-bonded distance (3.08 Å) remains shorter than the normal van der Waals separation of 3.35 Å. Similar effects have been noted in the ammonium-ANS structure (Cody & Hazel, 1976, 1977) and other naphthalene derivatives (Robert, Sherfinski, Marsh & Roberts, 1974).

\* A torsion angle  $\alpha-\beta-\gamma-\delta$  is positive if, when viewed down the  $\beta-\gamma$  bond, the  $\alpha-\beta$  bond will eclipse the  $\gamma-\delta$  bond when rotated less than  $180^\circ$  in a clockwise direction (Klyne & Prelog, 1960).

#### 5. Anilino nitrogen geometry

The ammonium-ANS structure has two molecules in the asymmetric unit. The N atom has pyramidal geometry in one molecule, ANS(1), and nearly planar geometry in the other, ANS(2), and the overall conformations of the two molecules differ radically. In the magnesium complex the N has nearly planar geometry and the overall molecular conformation is nearly identical with that of ANS(2).

In the magnesium complex, just as in ANS(2), the plane of the  $C(1')-N-C(1)$  anilino linkage is nearly coplanar with the phenyl ring and the shortening of the C-N distances would suggest enhanced conjugation between the anilino group and the phenyl ring.

As illustrated in Fig. 1, there is an intramolecular  $N-H \cdots O$  hydrogen bond as well as short intramolecular contacts involving H(1), H(2), H(2') and H(6') which are also observed in the two different conformations of ANS in the structure of the ammonium salt.

The intramolecular hydrogen bond observed in all three conformations of ANS had been predicted only for the coplanar models on the basis of solution NMR data (Penzer, 1972). Since the conformations about the bonds from the naphthalene moiety to the *peri* substituents are essentially the same in the three molecules (Table 3), it would appear that the *peri*-group geometry controls intramolecular hydrogen-bond formation and not the geometry of the anilino N.

The protons which showed an anomalous signal behavior in the NMR study (Penzer, 1972) are the same ones involved in the short intramolecular contacts. These close contacts persist in solution regardless of solvent and produce the anomalous NMR behavior.

If the relative orientation of the two rings has a significant influence upon the mechanism of fluorescence, then the fluorescence properties of the ANS observed here and in the ammonium salt of ANS may be different. The observation of two different

Table 3. *Geometry of the anilino linkage in hexaaquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate*

	Mg-ANS	NH <sub>3</sub> -ANS(2)*	NH <sub>3</sub> -ANS(1)*
C(1')-N	1.388 Å	1.386 Å	1.415 Å
C(1)-N	1.404	1.393	1.409
C(1')-N-C(1)	126.1°	127.3°	123.4°
C(2')-C(1')-N-C(1)	174	171	112
C(1')-N-C(1)-C(2)	-54	-46	22
H-N-C(1')-C(2')	-21	-27	-12
H-N-C(1)-C(9)	-35	-22	-36
Σ valency angles of N	358.7	357.6	335.7
H from C(1')-N-C(1) plane	-0.15 Å	-0.28 Å	-0.75 Å
N from C(1), C(1'), H plane	0.08	0.11	0.35
C(9)-C(8)-S-O(1)	64°	77°	70°

\* Cody & Hazel (1977).

conformations for ANS in these crystals as well as the observation of two conformations (both of which fluoresce) in the protein receptor sites suggests that a coplanar conformation for the two rings may not be essential to fluorescence.

## 6. Crystal packing and hydrogen bonds

The ANS molecule, Mg ion, and six unique water molecules are held together by a network of 10 intermolecular hydrogen bonds which are listed in Table 4 and illustrated in Fig. 3. The only H atoms not involved in the hydrogen-bonding scheme are those of O(9). Their positional parameters were also the least well defined in the difference Fourier maps. The H...O hydrogen-bonded distances are well within the 2.52 Å predicted as the sum of van der Waals radii (Koetzle, 1974) and suggest a strong hydrogen-bonded network. The same is true for the corresponding O...O

Table 4. *Hydrogen bonds in hexaaquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate*

	X—H	H...Y	X...Y	$\angle X-H...Y$
Intermolecular contacts				
O(4)—H(A)...O(1)	0.85 Å	1.85 Å	2.71 Å	177°
O(4)—H(B)...O(2)	0.78	1.99	2.78	178
O(5)—H(A)...O(8)	0.72	2.20	2.80	142
O(5)—H(B)...O(2)	0.93	2.08	2.80	134
O(6)—H(A)...O(8)	0.71	2.15	2.80	152
O(6)—H(B)...O(3)	1.00	1.78	2.77	169
O(7)—H(A)...O(5)	0.64	2.19	2.80	162
O(7)—H(B)...O(3)	0.92	1.89	2.79	166
O(8)—H(A)...O(9)	0.94	1.90	2.80	159
O(8)—H(B)...O(9)	0.88	2.02	2.85	157
Intramolecular contacts				
N—H...O(1)	0.87	1.99	2.89	149

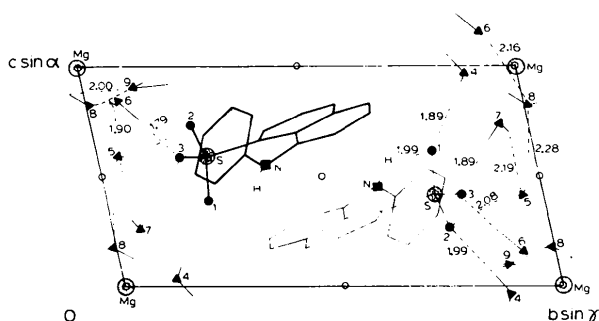


Fig. 3. Packing diagram for hexaaquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate. The large circles are S, the next larger are Mg, the squares are N, the triangles are water and the small circles O. The dark molecules are above the light ones. The dashed lines indicate hydrogen-bonded distances from the H atom to the O atom.

distances when compared with the normal van der Waals separation of 3.04 Å.

The ANS molecules are stacked with their sulfonate groups pointing into the hydrophilic channels formed by the water and Mg ions. Although the *a* and *b* lattice parameters are nearly identical with those in the ammonium-ANS lattice and the waters are in similar sites as the ammonium ions, the ANS molecules are oriented differently.

The authors wish to thank Dr W. L. Duax for helpful discussions, Dr D. C. Rohrer for aid in data collection and Miss G. Del Bel, Mrs C. DeVine and Mrs M. Eрман for their able technical assistance. This research was supported in part by Grant No. AM-15051 from the National Institute of Arthritis, Metabolism and Digestive Diseases, DHEW.

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