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The Monoclinic Structure of a Fluorescent Probe: Ammonium 1-Anilino-8-naphthalenesulfonate (ANS) Monohydrate

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Abstract

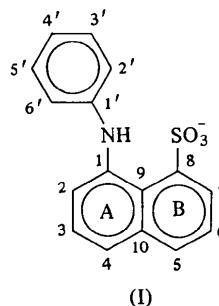
$\text{NH}_4^+ \cdot \text{C}_{16}\text{H}_{12}\text{NO}_3\text{S}^- \cdot \text{H}_2\text{O}$ is monoclinic, $P2_1/c$, with $a = 6.150$ (2), $b = 9.544$ (2), $c = 26.77$ (7) Å, $\beta = 90.35$ (2)°, $Z = 4$, $D_c = 1.413$ Mg m⁻³, $\mu(\text{Mo } K\alpha) = 0.1818$ mm⁻¹. The structure was solved by use of the program *MULTAN* and refined by full-matrix least squares to an R value of 3.5% for 2077 reflections for which $|F| > 3\sigma(|F|)$. The C–N bonds are significantly shorter than the expected single-bonded values. Comparison of independent crystal structures of triclinic ANS and Mg ANS with the present determination revealed that such shortening exists independent of anilino geometry or conformation. The comparison also revealed that ANS probably does not assume a planar conformation, thought possible from its fluorescence properties.

Introduction

N-Arylamino-naphthalenesulfonates have received much attention because of the highly sensitive response of their fluorescence properties to local conditions of chemical environment (Greene, 1975; Kosower, Dodiuk, Tanizawa, Ottolenghi & Orbach, 1975). These compounds have been used as reporter molecules when bound to proteins and other biomacromolecular structures, and they are generally referred to as fluorescent probes (Edelman & McClure, 1968; Brand & Gohlke,

1972). Even though their use has achieved notable popularity, the specific mechanism of their response to environment and their general applicability is still under investigation (De Toma, Easter & Brand, 1976; Dodiuk, Kanety & Kosower, 1979; Johnson, El-Bayoumi, Weber & Tulinsky, 1979; Weber, Tulinsky, Johnson & El-Bayoumi, 1979).

Cody & Hazel (1977*a,b*) have determined the structures of the ammonium and magnesium salts of 1-anilino-8-naphthalenesulfonate (ANS) (I).



Although both salts crystallized in the triclinic system, the former was unusual in that there were two molecules per asymmetric unit. In addition to its fluorescence properties, ANS is also a member of a class of *peri*-substituted naphthalenes which are known to exhibit a distorted naphthyl ring geometry arising from intramolecular steric effects (Balasubramanian, 1966; Robert, Sherfinski, Marsh & Roberts, 1974). Since we were carrying out crystallographic studies of the structure of the ANS- α -chymotrypsin complex

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(Weber, Tulinsky, Johnson & El-Bayoumi, 1979), we naturally became interested in the various conformers of ANS. In recrystallizing ammonium ANS for the protein work, we discovered the crystals to be monoclinic. Since this suggested an additional conformer for ANS, we determined the crystal and molecular structure of this crystal form.

Ammonium ANS was recrystallized by evaporation of an aqueous solution at room temperature. Single-crystal X-ray diffraction photographs fixed the space group as $P2_1/c$. Intensity measurements were performed using a Picker FACS-I computer-controlled diffractometer with Mo $K\alpha$ radiation and a graphite monochromator. The $\theta-2\theta$ scan mode was employed with a 20 s background count at the beginning and end of each range. In this manner, a set of 3073 reflections was collected for $2\theta \leq 50^\circ$, and, of these, 2793 were unique. None of the three intensity standards indicated significant loss in intensity.

The standard deviations of the structure amplitudes were calculated as in Wei & Ward (1976) with application of an instrumental instability factor of 0.02. A weight of $1/\sigma^2$ was applied to structure factors during the least-squares refinement.

The structure was solved by use of the program *MULTAN* (Germain, Main & Woolfson, 1971), and it was refined using 2077 reflections for which $|F| > 3\sigma(|F|)$. The positions of the 21 skeletal atoms of ANS were located in the first *E* map. After several cycles of full-matrix least-squares refinement with isotropic

thermal parameters ($R = 0.18$, $R = \sum ||F_o| - |F_c|| / \sum |F_o|$), a difference map showed two peaks which could be the counter ion and a water molecule. After additional cycles of isotropic followed by anisotropic refinement ($R = 0.074$), the 12 skeletal H atoms were located in a difference map. These were included in further refinements which reduced R to 0.054. A difference map revealed the locations of the six remaining H atoms. Subsequent cycles of refinement alternately varying all H atom parameters (isotropically) and all non-hydrogen atom parameters (anisotropically) resulted in a final R value of 0.035. The final shifts in non-hydrogen atom parameters averaged 0.05σ .

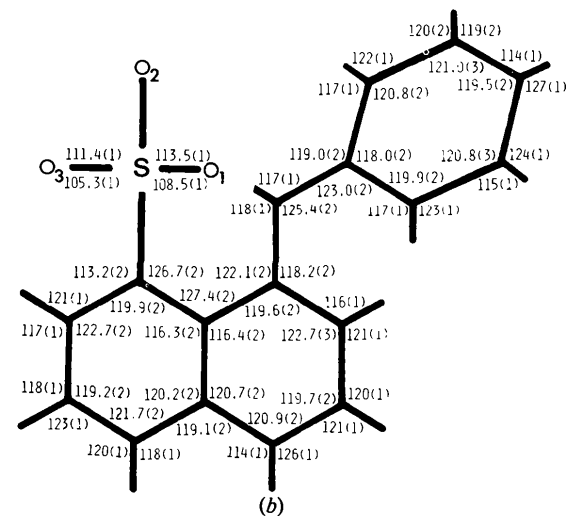
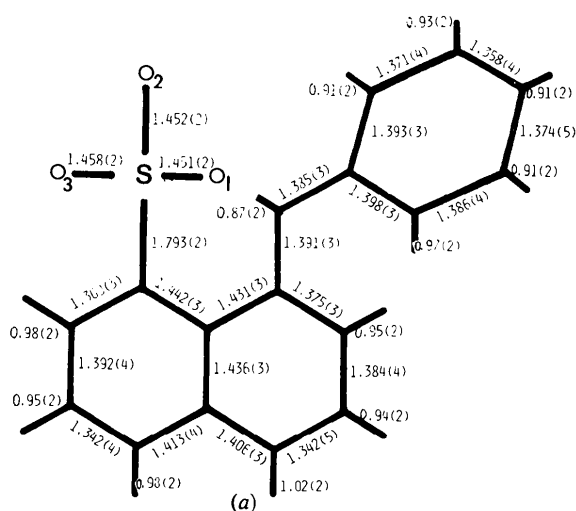
Discussion

The final atomic coordinates of ANS, its ammonium counter ion and water of hydration are listed in Table

Table 1. *Fractional coordinates of ANS non-hydrogen atoms*

N* and O_w correspond to an ammonium ion and a water molecule, respectively.

	x	y	z
S	0.6587 (1)	0.09201 (1)	0.17108 (2)
O(1)	0.8829 (3)	0.0487 (2)	0.16459 (6)
O(2)	0.6367 (3)	0.2318 (2)	0.19186 (6)
O(3)	0.5378 (3)	-0.0107 (2)	0.20017 (6)
C(1)	0.7620 (4)	0.2790 (2)	0.06575 (8)
C(2)	0.7874 (5)	0.3580 (3)	0.0233 (1)
C(3)	0.6420 (6)	0.3531 (3)	-0.0164 (1)
C(4)	0.4710 (5)	0.2659 (3)	-0.01449 (9)
C(5)	0.2605 (4)	0.0848 (3)	0.0268 (1)
C(6)	0.2214 (5)	-0.0011 (3)	0.0654 (1)
C(7)	0.3547 (4)	0.0058 (3)	0.10751 (9)
C(8)	0.5284 (3)	0.0950 (2)	0.11098 (8)
C(9)	0.5817 (3)	0.1849 (2)	0.06950 (8)
C(10)	0.4370 (4)	0.1795 (2)	0.02728 (8)
N	0.9171 (3)	0.2916 (2)	0.10349 (8)
C'(1)	1.0082 (3)	0.4162 (2)	0.11995 (8)
C'(2)	1.2109 (4)	0.4131 (3)	0.14340 (9)
C'(3)	1.3006 (5)	0.5326 (3)	0.1630 (1)
C'(4)	1.1958 (6)	0.6576 (3)	0.1597 (1)
C'(5)	0.9973 (6)	0.6644 (3)	0.1360 (1)
C'(6)	0.9013 (4)	0.5451 (3)	0.11633 (9)
N*	0.8716 (3)	0.4587 (2)	0.24337 (8)
O _w	0.7370 (3)	0.7476 (2)	0.24022 (7)



1.* The numbering system used is that of formula (I). Bond distances and bond angles of ANS are given in Fig. 1, along with the errors in these quantities based on the standard deviations of the atomic coordinates of the final cycles of refinement.

An inspection of the packing of ANS in the unit cell revealed an extended array of hydrogen-bonded atoms (Fig. 2). Hydrogen bonding takes place between the ammonium ion and water molecule and between these and two of the sulfonyl O atoms of different ANS molecules. This scheme gives rise to a distorted helical arrangement parallel to the *b* axis of the crystal (Fig. 2).

From an examination of the final bond lengths and angles shown in Fig. 1, it is evident that ANS possesses distortions typical of *peri*-substituted naphthalenes and other ANS-like molecules. Notable among these are the in-plane deviations of the bond angles about C(1), C(8), C(9), C(1') and the anilino N atom.

A puckering of the naphthyl ring is accomplished by a twist about the C(9)–C(10) bond, and it is accompanied by a splaying of the *peri* substituents out of the

* Lists of structure factors, anisotropic thermal parameters, H atom coordinates and H isotropic temperature factors have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34836 (14 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

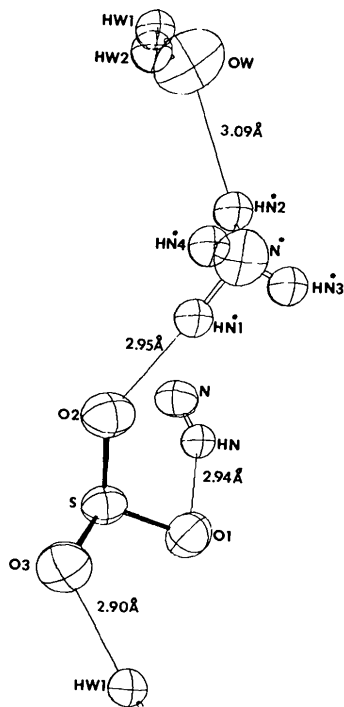


Fig. 2. Perspective ORTEP (Johnson, 1965) drawing of the atoms along *b* involved in the inter- and intramolecular hydrogen-bonding scheme of ANS. Distances between hydrogen-bonded N and O atoms are shown. The crystallographic *c* axis is toward the viewer. The isotropic temperature factor of the H atoms is arbitrary.

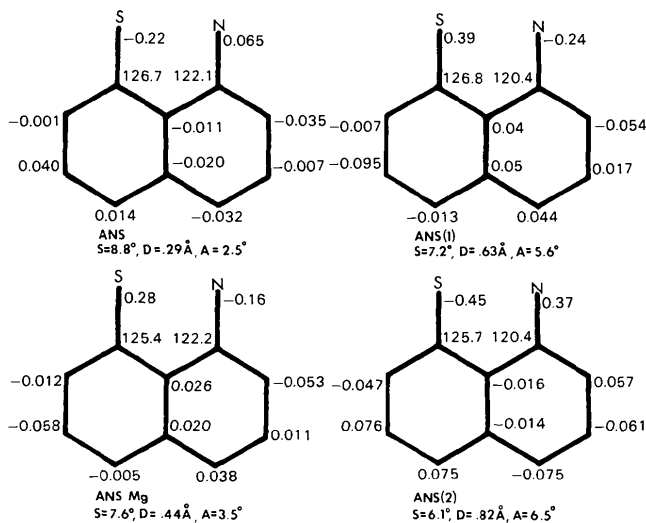


Fig. 3. Deviations (Å) of naphthyl C atoms from the best least-squares plane of several ANS structure determinations: ANS (this work); ANS(1) and ANS(2) (Cody & Hazel, 1977*a*); Mg ANS (Cody & Hazel, 1977*b*). Additional parameters are: $S = \{ \angle[S-C(8)-C(9)] + \angle[N-C(1)-C(9)] - 240^\circ \}$; D = combined distances of S and N atoms from best least-squares plane; A = angle between normals to least-squares planes of six-membered rings (A and D are directly correlated).

mean plane of the ring system. Another consequence of this deformation is that the normals to the planes formed by six-membered portions of the naphthalene fragment tilt by about 2.5° from their parallel orientations. Similar effects have been noted in previous studies (Robert, Sherfinski, Marsh & Roberts, 1974; Cody & Hazel, 1977*a,b*). Distortions of the naphthyl portions of ANS are summarized in Fig. 3 for several different ANS structural determinations. In contrast, Cruickshank & Sparks (1960) found naphthalene to be planar to within 0.01 \AA . From Fig. 3 it can be seen that for each conformation the sums of the in-plane expansions of the S–C(8)–C(9) and N–C(1)–C(9) angles bear an inverse relationship to the amount of splaying, the latter measured by the sum of the N and S distances normal to the least-squares plane.

Penzer (1972) measured chemical shifts of ANS ring protons by NMR experiments in deuteromethanol and D_2O . He found ring current deshielding to be selectively operative in the former medium, wherein ANS is highly fluorescent. It was concluded that this implied a more planar conformation in deuteromethanol. A conformation-linked fluorescence dependence was suggested which was to be governed by solvent-enhanced favorability of intramolecular hydrogen bonding in non-polar media. However, Cody & Hazel (1977*a,b*) pointed out that such hydrogen bonding was observed in all known crystal structures. This is true of the present study as well. In addition, further comparison (Table 2) reveals that, contrary to the earlier NMR

Table 2. *Conformational parameters of ANS observed in several X-ray crystallographic structure determinations*

	ANS*	ANS (1)†	ANS (2)†	Mg ANS‡
C(1')-N (Å)	1.385	1.415	1.386	1.388
C(1)-N (Å)	1.391	1.409	1.393	1.404
C(8)-S (Å)	1.793	1.788	1.796	1.786
∠C(2')-C(1')-N-C(1) (°)	-156	112	171	174
∠C(1')-N-C(1)-C(2) (°)	42.5	21.8	-46.0	-54
Σ bond angles of N (°)	360.0	335.7	357.6	358.7

* This work.

† Cody & Hazel (1977a).

‡ Cody & Hazel (1977b).

interpretation, as the coplanarity measured by the torsion angle C(1')-N-C(1)-C(2) increases, that measured by C(2')-C(1')-N-C(1) actually decreases.

Based on a solution spectroscopic study of 2-anilino-6-naphthalenesulfonate (2,6-ANS), Kosower *et al.* (1975) suggested conformation dependence which results from variable excited-state intramolecular charge transfer. Two key requirements for the formation of an intramolecular charge-transfer complex are: the ability of the phenyl ring to communicate electronically with the naphthyl, for which a planar conformation in the excited state has been presumed necessary, and the ability of the environment to stabilize the charge separation. Quenching in polar media was proposed to occur *via* a second excited-state charge-transfer pathway which involves the solvent reaction field and which leads to ground-state ANS (Kosower, Dodiuk & Kanety, 1978). The conformational considerations oppose those of Penzer (1972), but the solution-NMR-based arguments apply only to the ground state.

However, in all four molecular structures (Table 2) the C(1')-N and C(1)-N bond lengths are significantly shortened with respect to single-bonded values, which would be 1.470 ± 0.005 Å for a pure C(sp²)-N(sp²) case (Camerman, 1970) and would be slightly longer for a pure C(sp²)-N(sp³) case (due to the greater *p*-orbital character of the N hybrid atomic orbitals). It is thus evident from all the crystallographic

results that coplanarity is not a requirement for significant anilino-naphthyl π -orbital overlap in ANS, and this suggests a less sensitive dependence of the spectral properties on conformation than has been supposed.

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