Molecular Structure of 2-*p*-Toluidinyl-6-naphthalenesulfonate and Its Relation to Fluorescence Properties

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Abstract: 2-*p*-Toluidinyl-6-naphthalenesulfonate (TNS) is one of a series of compounds which are practically nonfluorescent in water, but fluoresce fairly strongly in organic solvents or when bound to protein molecules. The compound may be crystallized in two forms, one containing water in the lattice and one anhydrous. Fluorescence spectra of the two crystals resemble those of TNS in water and in ethyl alcohol. The molecular structure of anhydrous TNS suggests an extended π-electron system comprised of π electrons of the naphthalene and benzene rings and two p electrons of nitrogen. This extended electron delocalization may be a factor influencing the compound's fluorescent behavior. Anhydrous TNS crystallizes in space group P$\bar{1}$ with $a = 6.158$, $b = 7.379$, $c = 17.454$ Å, $\alpha = 96.84$, $\beta = 95.82$, $\gamma = 92.67°$. The final reliability index, R, is 0.026.

2-*p*-Toluidinyl-6-naphthalenesulfonate (TNS) is one of a series of naphthalenesulfonates employed successfully as extrinsic fluorescent chromophores for the study of protein conformation.[2,3] TNS has been used in the study of antigen–antibody interactions,[4] protein conformation changes produced by the binding of metal ions,[5] and the chymotrypsinogen–chymotrypsin enzyme activation process.[6] The usefulness of TNS and other related fluorescent probes is due to its property of being practically nonfluorescent in water but strongly fluorescent when dissolved in organic solvents or when bound to proteins.[7]

The mechanism by which the fluorescence of these molecules is changed in different media is not fully understood, and although attempts have been made to correlate fluorescence behavior of TNS with solvent properties such as polarity and viscosity,[8] no satisfactory explanation of the large changes in fluorescence quantum yields of these chromophores in different environments has been proposed. We have crystallized TNS in two crystal forms, one containing water in the lattice and the other anhydrous, examined the fluorescence spectra of both crystals, and determined the molecular structure of the chromophore. A preliminary account has been reported earlier.[9]

Experimental Section

TNS potassium salt was dissolved in N,N-dimethylformamide and water was allowed to diffuse slowly into the solution. Two types of crystals were obtained, one containing two molecules of water/TNS molecule and one containing no water. Upon removal from solution the hydrated crystals lose water and change to the anhydrous lattice, cracking the crystals and destroying their usefulness in the process. Good anhydrous crystals can be obtained by careful, very slow evaporation of the dimethylformamide–water solution over a period of time.

A hydrated crystal was sealed in a capillary containing mother liquor, and the unit cell parameters for both types of crystals were determined by least-squares minimization of the difference between calculated and observed θ values for reflections measured at both $+2\theta$ and -2θ using a four-circle diffractometer (λ Mo Kα = 0.71069 Å). Results are shown in Table I.

Table I. TNS Cell Constants

	Hydrated crystal	Anhydrous crystal
a, Å	12.1284 ± 0.0021	6.1583 ± 0.0005
b, Å	7.4774 ± 0.0016	7.3793 ± 0.0007
c, Å	19.9845 ± 0.0047	17.4545 ± 0.0020
α, deg	94.06 ± 0.02	96.838 ± 0.010
β, deg	98.90 ± 0.02	95.820 ± 0.010
γ, deg	93.07 ± 0.01	92.667 ± 0.006
Space group	P$\bar{1}$	P$\bar{1}$
Asymmetric unit	2TNS + $4H_2O$	1TNS

X-Ray intensities of anhydrous TNS were measured on a crystal approximately 0.35 × 0.35 × 0.25 mm to $2\theta = 50°$, corresponding to an interplanar spacing of 0.84 Å. The $\omega/2\theta$ scan technique using Nb-filtered Mo radiation was employed. A total of 2762 independent reflections was measured. Of these 2501 had intensity greater than $2\sigma_c$, where $\sigma_c = (N_{B1} + N_{PK} + N_{B2})^{1/2}$, N_{B1} and N_{B2} are the background counts on each side of the peak, and N_{PK} is the scan count. Frequently measured standard reflections indicated the diffractometer stability to be ±1%; hence the estimated error in the intensity of each reflection is $I = [\sigma_c^2 + (0.01\sigma_c^2)^2]^{1/2}$. Structure factors were obtained from the intensities in the usual fashion; weights were taken as $\sqrt{w} = 1/\sigma_F$. No absorption corrections were applied. The linear absorption coefficient for Mo Kα is 4.8 cm^{-1}.

Specimens of both hydrated and anhydrous crystals were sealed in capillaries, surrounded by mother liquor, and their emission spectra were recorded after excitation at 360 mμ.

Structure Determination. A sharpened, origin-removed three-dimensional Patterson map was interpreted to yield the positions of the potassium and sulfur atoms and the positions of the three oxygen and one carbon atom that are arranged tetrahedrally about the sulfur. A Fourier synthesis calculated using phases determined from these six atomic positions clearly showed the positions of the other 17 nonhydrogen atoms. The first postulated structure gave an R of 0.38, which decreased to 0.14 in two cycles of least-squares refinement with individual isotropic thermal parameters and to 0.09 in two cycles with individual anisotropic thermal parameters. A difference electron density map calculated at this stage unambiguously showed the 14 hydrogen atom positions. Further least-squares refinement of all atomic positions with anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the hydrogens reduced R to 0.026.

The final atomic parameters for the nonhydrogen atoms are listed in Table II, in which the B_{ij}'s are coefficients in the expression

$$\exp[-0.25(h^2B_{11}a^{*2} + \ldots + 2klB_{23}b^*c^*)]$$

Final positional and thermal parameters for the hydrogen atoms are

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Table II. Fractional Coordinates and Anisotropic Thermal Parameters (Å^2) for the Nonhydrogen Atoms[a]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
K(1)	0.18012 (6)	0.25071 (5)	0.98248 (2)	3.51	2.62	3.18	0.36	0.39	0.50
S(2)	0.24488 (7)	0.71884 (5)	0.90766 (2)	3.22	2.77	2.42	0.46	0.19	0.15
O(3)	0.18802 (22)	0.88751 (16)	0.95069 (7)	5.82	2.89	3.40	0.18	0.91	−0.43
O(4)	0.10740 (22)	0.56532 (16)	0.92358 (7)	5.89	2.81	3.32	0.11	0.82	0.92
O(5)	0.47440 (21)	0.68685 (22)	0.91677 (8)	3.55	8.52	3.38	1.71	−0.36	0.18
C(6)	0.17609 (26)	0.74085 (21)	0.80838 (9)	2.85	2.13	2.83	−0.01	0.30	0.38
C(7)	0.31356 (27)	0.68861 (21)	0.75451 (10)	2.43	2.15	3.22	0.30	0.07	0.56
C(8)	0.25382 (25)	0.70151 (20)	0.67499 (9)	2.54	1.97	3.12	0.16	0.52	0.55
C(9)	0.04890 (25)	0.77231 (20)	0.65193 (10)	2.47	2.10	3.12	0.10	0.40	0.57
C(10)	−0.08703 (28)	0.82819 (23)	0.71021 (10)	2.38	3.12	3.58	0.69	0.51	0.76
C(11)	−0.02737 (28)	0.81169 (23)	0.78615 (10)	3.01	3.05	3.10	0.60	0.72	0.40
C(12)	−0.00923 (28)	0.78689 (22)	0.57273 (10)	2.51	2.97	3.37	0.60	0.30	0.84
C(13)	0.12616 (26)	0.73483 (20)	0.51726 (9)	3.23	2.51	2.88	0.24	0.42	0.75
C(14)	0.32900 (30)	0.66404 (23)	0.54099 (11)	3.66	3.09	3.26	1.11	1.05	0.79
C(15)	0.38945 (28)	0.64668 (22)	0.61672 (10)	2.80	2.77	3.45	0.83	0.51	0.80
N(16)	0.06091 (27)	0.74340 (22)	0.43916 (8)	2.90	4.75	2.90	0.49	0.34	1.10
C(17)	0.19378 (26)	0.74902 (20)	0.37876 (9)	3.16	2.45	2.83	0.42	0.36	0.74
C(18)	0.11067 (29)	0.67634 (22)	0.30414 (10)	3.27	2.89	3.16	−0.50	−0.01	0.52
C(19)	0.23084 (30)	0.68799 (23)	0.24215 (10)	4.40	3.00	2.76	−0.22	0.19	0.23
C(20)	0.44034 (28)	0.77142 (21)	0.25218 (10)	3.91	2.42	3.55	0.48	1.14	0.73
C(21)	0.52384 (28)	0.84260 (22)	0.32680 (10)	2.83	2.52	4.18	0.05	0.41	0.72
C(22)	0.40431 (27)	0.83342 (22)	0.38956 (10)	3.34	2.52	3.07	0.14	−0.07	0.30
C(23)	0.56828 (43)	0.78645 (36)	0.18337 (14)	5.92	4.43	4.88	0.49	2.54	1.18

[a] Standard deviations are given in parentheses.

Table III. Fractional Coordinates, Isotropic Thermal Parameters (Å^2), and Bond Lengths (Å) for the Hydrogen Atoms[a]

Atom	x	y	z	B	Bond length, non-H–H
H(7)	0.4448 (28)	0.6453 (22)	0.7687 (9)	2.5	0.905 (17)
H(10)	−0.2250 (29)	0.8758 (23)	0.6954 (10)	3.5	0.958 (18)
H(11)	−0.1301 (29)	0.8551 (24)	0.8261 (11)	4.0	1.022 (19)
H(12)	−0.1413 (28)	0.8293 (22)	0.5564 (10)	2.9	0.916 (17)
H(14)	0.4231 (28)	0.6243 (23)	0.5014 (10)	3.6	0.974 (19)
H(15)	0.5241 (30)	0.5978 (24)	0.6329 (11)	3.6	0.950 (17)
H(16)	−0.0627 (36)	0.7344 (30)	0.4263 (13)	5.0	0.769 (21)
H(18)	−0.0314 (30)	0.6208 (24)	0.2956 (10)	3.5	0.939 (18)
H(19)	0.1700 (29)	0.6387 (24)	0.1921 (11)	3.8	0.939 (18)
H(21)	0.6673 (29)	0.8989 (24)	0.3351 (10)	3.1	0.948 (18)
H(22)	0.4666 (27)	0.8910 (24)	0.4402 (11)	3.3	0.967 (17)
H(23.1)	0.7244 (40)	0.8277 (30)	0.1992 (13)	8.9	0.996 (24)
H(23.2)	0.5879 (36)	0.6729 (33)	0.1576 (14)	9.8	0.922 (23)
H(23.3)	0.5209 (37)	0.8760 (34)	0.1560 (14)	8.5	0.903 (26)

[a] Estimated standard deviations are given in parentheses.

listed in Table III. The table of final observed and calculated structure factors has been deposited with the American Society for Information Science.[10] Programs in the X-ray 63 system[11] were used for most of the calculations.

Results and Discussion

Molecular Structure. Bond lengths and angles for the nonhydrogen atoms are given in Figure 1, and those bond lengths involving hydrogen are listed in Table III. Bond angles involving hydrogen atoms are within two standard deviations of their expected values. No corrections for the effects of thermal vibrations on the bond lengths have been made. Temperature factors for the atoms of the naphthalene and phenyl rings are small and not significantly anisotropic, so thermal corrections to those atomic positions would be small. Comparison of the averaged lengths of chemically equivalent bonds in the naphthalene and phenyl rings of TNS with those found in naphthalene[12] and benzene[13] (Table IV) shows good agreement between corresponding bond lengths even though the rings are substituted in TNS.

Table IV. Bond Lengths in the Naphthalene and Phenyl Rings of TNS Compared to Those in Naphthalene and Benzene[a]

Bond	TNS	Naphthalene	Benzene
C(6)–C(7)	1.363 (2)	1.364 (5)	
C(7)–C(8)	1.414 (2)	1.421 (5)	
C(6)–C(11)	1.417 (2)	1.415 (5)	
C(8)–C(9)	1.427 (2)	1.418 (5)	
Phenyl ring	1.387 (3)		1.392 (10)

[a] Standard deviations are given in parentheses.

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(10) Material supplementary to this article has been deposited as Document No. NAPS-00913 with the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be obtained by citing the document number and remitting $3.00 for photocopies or $1.00 for microfiche. Advance payment is required. Make checks or money orders payable to: CCMIC–NAPS.

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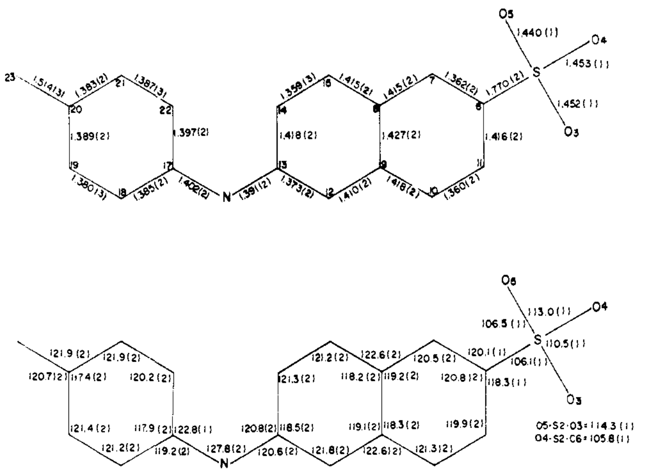

Figure 1. Bond lengths (Å) and angles (deg) in the TNS molecule. Standard deviations are shown in parentheses.

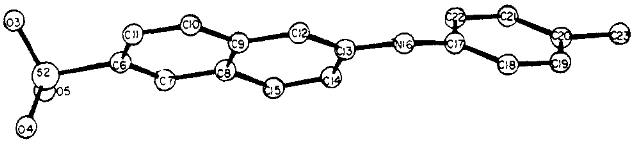

Figure 2. Perspective drawing of the TNS molecule.

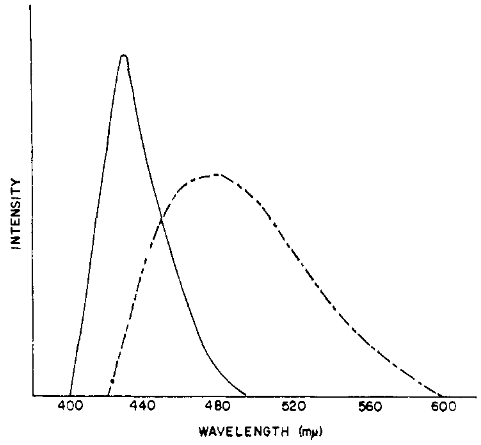

Figure 3. Fluorescence spectra of TNS crystals. Solid line indicates anhydrous crystals, broken line indicates hydrated crystals.

The TNS molecule is made up of a planar naphthalene nucleus joined through N(16) to a planar tolyl group (Figure 2). Least-squares planes through the atoms comprising the naphthalene and phenyl rings were calculated, and deviations of the atoms from these planes are given in Table V.

Table V. Deviations of Atoms From Least-Squares Planes

Plane I		Plane II	
Atom	Δ, Å	Atom	Δ, Å
C(6)	0.0017	C(17)	−0.0017
C(7)	−0.0096	C(18)	0.0039
C(8)	0.0018	C(19)	−0.0022
C(9)	0.0068	C(20)	−0.0017
C(10)	−0.0070	C(21)	0.0039
C(11)	0.0035	C(22)	−0.0022
C(12)	0.0034	N(16)[a]	0.074
C(13)	−0.0051		
C(14)	−0.0049		
C(15)	0.0094		
N(16)[a]	−0.049		
S(2)[a]	−0.040		

[a] These atoms not used in calculating the plane.

There is ample evidence of electronic resonance over the whole TNS molecule. The N atom has a slightly distorted trigonal configuration: the sum of the bond angles about N is 359.7° with the H atom 0.08 Å out of the plane defined by C(13)-N(16)-C(17). Thus the electronic hybridization on N is sp^2 with a p^2 lone pair. The best plane through C(13)-N(16)-C(17)-H(16) makes angles of 154.6 and 152.3° with the naphthalene and tolyl planes, respectively; thus the p^2 orbital of N is only 25.4 and 27.7° out of parallel alignment with the two π-electron systems. The overlap integral between each aromatic π system and the p^2 electrons of N is thus reduced only slightly from the value it would have if all orbitals were parallel. Bond lengths between N and C(13) and C(17) are 1.391 and 1.402 Å, significantly shorter than 1.470 Å, the length of the single bond between trigonal carbon and trigonal nitrogen.[14] These data strongly suggest delocalization of the p electrons of N into an extended π-electron system comprised of N(16) and the naphthalene and phenyl rings. The C–N–C bond angle of 127.8° is larger than normal for trigonal nitrogen (120°); this large angle alleviates steric strain due to C–N bond shortening and at the same time allows a more nearly planar conformation of the molecule than would be possible with the normal trigonal bond angle.

It is worth noting that the naphthalene-N-phenyl system contains 18 p electrons and that such a system may benefit from the Hückel $4n + 2$ rule for aromatic resonance stability. Although that rule was formulated strictly for aromatic compounds, evidence of shortened C–N bonds and widened C–N–C bond angles in many compounds containing two aromatic rings bridged by a trigonal nitrogen,[15–17] and no such bond shortening when two aromatic groups are directly bonded,[18] suggests that it may be applied to systems such as TNS.

Fluorescence Spectra. Fluorescence spectra for the hydrated and anhydrous crystals of TNS are shown in Figure 3. The fluorescence intensities of the two spectra are not quantitative relative to each other but peak shapes are accurate. The spectrum of anhydrous TNS crystals resembles very closely the TNS fluorescence spectrum in organic solvents, while that of the hydrated crystals is similar to TNS in water, except that the peak is much broader (see ref 8, p 1913). These similarities indicate that solvent–solute interactions are a likely cause of TNS fluorescence differences in different solvent environments. The two crystal spectra are also consistent with Lippert's theory[19] of fluorescence emission shifts caused by dipole–dipole interactions during the lifetime of the excited state. The broadening of the TNS hydrated crystal fluorescence spectrum is most likely due to the longer time required for solvent

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reorientation in the crystal lattice than in solution. The fact that the water comes out of the crystal so easily when hydrated TNS crystals are left to dry can be taken as evidence that solvent reorientation can indeed occur in the crystal lattice.

Förster[20] has suggested that fluorescence of the anilinonaphthalenesulfonates is observed only when the two rings are planar. Although steric interactions would not allow complete coplanarity, it may be that the resonance delocalization of electrons over the whole molecule suggested for TNS, a property dependent on degree of planarity of the molecule, may be a factor in determining fluorescence behavior. If upon ultraviolet absorption this electronic delocalization stability is lessened, solvents with strong hydrogen bonding properties and appropriate steric size may lower the energy of the excited state by H···N(16) interaction. Also, the geometry of protein binding sites to which TNS is attracted, not only the polarity of such sites, may affect the energy difference between ground and excited states of the probe molecule and influence its emission characteristics. For example, TNS binds to both chymotrypsin and chymotrypsinogen with dissociation constants that differ only by a factor of 2, yet the probe's fluorescence intensity is many times stronger when bound to chymotrypsin than when bound to the zymogen.[21] It is therefore not entirely safe to assume that such probes measure only the polarity of the binding site, as has been suggested.[22] On the other hand, compounds without the extended resonance possible in TNS, for example, 1-dimethylaminonaphthalene-6-sulfonamide,[23] could be used as probes of polarity without fear of the additional complication of altering the structure of the probe.

An elucidation of the arrangement of water molecules about TNS in the hydrated crystals may be extremely significant in more specifically relating structure to fluorescence properties.

Molecular Packing. Short contacts between molecules occur between H(14) of molecules related through a center of symmetry and between O(3) and O(5) and K^+ of neighboring molecules. The H···H distance is 2.1 Å (van der Waals contact is 2.4 Å) and $O(3)\cdots K^+$ $(x, 1 + y, z)$ and $O(5)\cdots K^+ (1 - x, 1 - y, 2 - z)$ distances are 2.68 and 2.60 Å, respectively (van der Waals contact is 2.73 Å).

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Kinetics and Mechanism of the Morpholine–Borane Reduction of Methyl Alkyl Ketones

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Abstract: Deuterium labeling shows that hydrogen is transferred from boron to the carbonyl carbon atom in the reduction of acetone by morpholine–borane *via* both acid-independent (second-order) and acid-catalyzed (third-order) paths. The second-order reaction exhibits a negligible solvent isotope effect and a small (10%) normal B–H substrate isotope effect and is presumed to involve the rate-determining attack of amine–borane on the neutral ketone. The acid-catalyzed reaction exhibits a small substrate isotope effect (20%), but shows a pronounced inverse solvent isotope effect $[k_2(D_2O)/k_2(H_2O) = 2.8]$ which suggests a rate-determining attack of amine–borane on a protonated carbonyl which is formed in a rapid preequilibrium. From data on ketone basicity, it is calculated that protonation renders the carbonyl compound about 10^{11} times more reactive toward reduction by morpholine–borane. For the acid-independent path, a transition state requiring some specific orientation of reactants is proposed consistent with activation parameters of 11.0 kcal/mol and −40 eu. An analogy to certain cycloaddition reactions is suggested. Correlation of rates with Taft σ^* parameters for C-alkyl substituents in the ketone suggests that boron–oxygen bond formation may be important in this transition state. It is speculated that the protonated carbonyl may serve as a general acid promoting decomposition of the amine–borane in a manner analogous to the proposed mechanism for acid-catalyzed amine–borane hydrolysis.

In recent years, the rates of amine–borane reductions of various aldehydes and ketones in aqueous solution have been shown to be enhanced by an increase in acidity, and specific kinetic studies have led to rate expressions which indicate reduction to occur by two pathways, one independent of, and the other first order in, hydrogen ion (eq 1).[2] Also characteristic

$$\frac{-d[\text{amine–borane}]}{dt} = [\text{amine–borane}][\text{RCOR}'][k_1 + k_2(\text{H}_3\text{O}^+)] \qquad (1)$$

of such systems is the fact that, in addition to the

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