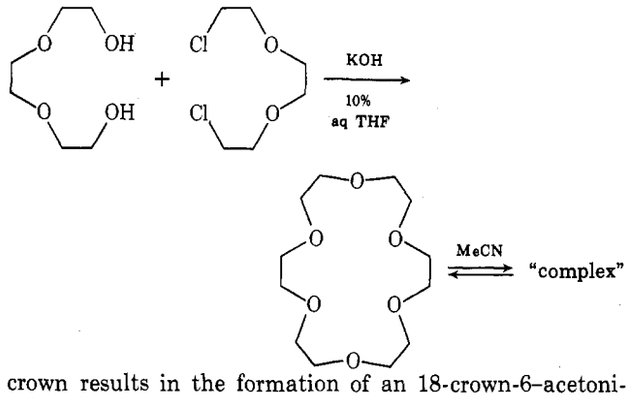

crown results in the formation of an 18-crown-6–acetonitrile adduct (complex)[6] of variable stoichiometry depending on conditions. Evaporation of the acetonitrile leaves crown of high purity. Evidence on the nature of this and other complexes of 18-crown-6 will be published elsewhere.

Experimental Section

A 3-l., three-neck flask equipped with mechanical stirrer, reflux condenser, and addition funnel was charged with triethylene glycol (112.5 g, 0.75 mol) and tetrahydrofuran (600 ml). Stirring was commenced and a 60% KOH solution (109 g of 85% KOH in 70 ml of water) was poured in. The solution warmed but did not boil. After *ca.* 15 min of stirring (the solution darkened) a solution of 3,6-dioxa-1,8-dichlorooctane (140.3 g, 0.75 mol) in THF (100 ml) was added in a stream. After the addition was complete, the solution was heated at reflux and stirred vigorously for 18 hr. The solution was allowed to cool and the bulk of the THF was evaporated under reduced pressure. The resulting thick brown slurry was diluted with 500 ml of dichloromethane and filtered. The salts removed by filtration were washed with more dichloromethane to remove adsorbed crown, and the combined organic solution was dried over $MgSO_4$, evaporated to minimum volume (aspirator vacuum), and then distilled under high vacuum. The distillation should be carried out at the lowest possible pressure; a typical fraction contained 80 g and was collected at 100–160° (0.2 mm).

To 50 g of crude 18-crown-6, bp 125–160° (0.2 mm), in a 250-ml erlenmeyer flask was added 125 ml of acetonitrile. The resulting slurry was heated on a hot plate to effect solution. A magnetic stirring bar was added and the neck was equipped with a $CaSO_4$ drying tube. The solution was stirred vigorously as it was allowed to cool to ambient temperature, and fine white crystals of crown–acetonitrile complex were deposited. The flask was finally cooled in an ice–acetone bath to precipitate as much complex as possible, and the solid was then collected by rapid filtration. The hygroscopic crystals were transferred to a 500-ml round-bottom flask equipped with a magnetic stirring bar and vacuum take-off. The acetonitrile was removed from the complex under high vacuum (0.1–0.5 mm) with gentle heating (≤40°) over 2–3 hr. The pure, colorless crown (20–30 g, 40–60%) crystallized on standing and showed no ions above m/e 265 in the mass spectrum and no significant hydroxyl vibration in the 3500-cm^{-1} region of the infrared. The pure crown had mp 36.5–38.0° (lit.[4] mp 39–40°); nmr (60 MHz, CCl_4) 3.56 ppm (singlet); ir (neat) 2875 (alkane CH), 1450 and 1350 (alkane CH), and 1120 cm^{-1} (ether link); mass spectrum M and M + 1 at m/e 264 and 265, other fragments at m/e 89, 87, 59, 45, 44, 43, and 31.

Registry No.—18-Crown-6, 17455-13-9; triethylene glycol, 112-27-6; 3,6-dioxa-1,8-dichlorooctane, 112-26-5.

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Fluorescence Properties of a Meisenheimer Complex[1]

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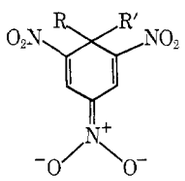
Meisenheimer complexes (below), the σ complexes which are formed as a result of the attack of nucleophilic agents on polynitroaromatic compounds, have been of interest to chemists for over 70 years.[2] Their spectroscopic



R = H, OH, OCH_3; R′ = H, OCH_3, CN, SO_3^-, etc.

(uv-visible, ir, nmr)[3,4] properties have been extensively studied, but the fluorescence behavior of these complexes has been overlooked. In view of the similarity of these complexes to the polynitrophenyl haptens used in many immunochemistry studies,[5] we decided to investigate the possibility of using these complexes as fluorescent biophysical probe molecules.[6] We report here the results of our preliminary investigations of the fluorescence properties of a Meisenheimer complex (where R = R′ = H, tetramethylammonium 1,1′-dihydro-2,4,6-trinitrocyclohexadienate) under differing environmental conditions. We believe that these results represent the first reported observations of Meisenheimer complex fluorescence.

The 1,1′-dihydro-2,4,6-trinitrocyclohexadienate anion fluoresces in acetronitrile with an emission maximum at about 670 nm. The quantum efficiency, which because of instrumental limitations must be considered only an estimate, is about 0.09. The measured lifetime is 1.8 ± 0.4 nsec. These data give a radiative lifetime of about 20 nsec.

In water, the emission maximum is shifted to the red (Figure 1) and the intensity is greatly diminished from that in acetonitrile. However, in the presence of an excess of human serum albumin (HSA) the emission spectrum (Figure 1) is similar to that in acetonitrile, although the quantum efficiency is somewhat less.

It is well known that serum albumins act as nonspecific binding agents for hydrophobic anions[7] and apparently the binding of the 1,1′-dihydro-2,4,6-trinitrocyclohexadienate anion to HSA[8] places it in a sufficiently nonaqueous environment that its emission spectrum more closely resembles that observed in acetonitrile. The lifetimes of

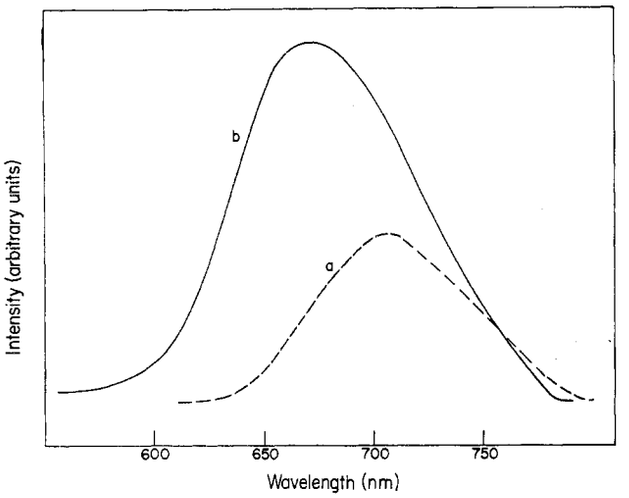


Figure 1. Emission spectra of 10^{-5} *M* tetramethylammonium 1,1′-dihydro-2,4,6-trinitrocyclohexadienate in (a) 0.05 *M* pH 6 phosphate buffer and in (b) the same buffered solution containing 10^{-4} *M* HSA. Excitation wavelength: 478 nm.

this anion in nondeoxygenated aqueous solutions both with and without HSA were less than in acetonitrile, probably near 1 nsec.

The theoretical aspects of the fluorescence of this compound are quite interesting. A general rule in fluorescence spectroscopy states that most electron-withdrawing groups, and nitro groups in particular, rather effectively decrease or eliminate fluorescence in aromatic compounds.[9] We could detect no fluorescence for trinitrobenzene (TNB). Other related compounds such as trinitrotoluene (TNT) likewise exhibit no observable fluorescence.[10] It should be noted, however, that 1,1′-dihydro-2,4,6-trinitrocyclohexadienate is *nonaromatic,* and considerable electron delocalization is possible within the pentadienate anion moiety. This fact, coupled with the observation of low-lying excited states for the molecule,[11] may hold the key to the observed fluorescence. It is likely that these results on 1,1′-dihydro-2,4,6-trinitrocyclohexadienate, the prototype Meisenheimer complex, can be generalized to other related species. The σ complexes formed when amines are added to acetone solutions of TNB[12] exhibited fluorescence properties similar to those we have reported.

We note in passing the resemblance of these systems to the NADH–NAD^+ pair.[13] The nonaromatic NADH molecule has rather intense fluorescence while the aromatic NAD molecule shows little, if any, detectable fluorescence.

These preliminary studies indicate that there is a possibility of using anions such as 1,1′-dihydro-2,4,6-trinitrocyclohexadienate as fluorescent biophysical probe molecules. Compared with its fluorescence in aqueous solution, the relative increase in intensity in the presence of HSA is considerable, but on an absolute basis the yields in these systems are quite low. It may be that immunoglobulins *specific* for the trinitrophenyl moiety will bind this anion in environments which exclude water more efficiently than does HSA. It has been suggested that displacement of bound water from both haptens and immunoglobulins plays a decisive role in the antibody–hapten binding reaction.[14,15] If this is the case it is possible that the fluorescence yield observed under these latter circumstances will be sufficiently enhanced to facilitate their use for physical studies or trinitrophenyl specific antibodies in solution.

There is one other potential application in this work. The fluorescence properties of Meisenheimer complexes might be very useful in analyses for TNT. Though TNT itself is nonfluorescent, it can easily be converted to a Meisenheimer complex by reaction with borohydride[16] or hydropolyborate anions.[17] We have found that sodium cyanoborohydride can be used to prepare the sodium salt of 1,1′-dihydro-2,4,6-trinitrocyclohexadienate from TNB,[16] and we suggest that this milder reducing agent could also be used to convert TNT to a fluorescent species. In this way it should be possible to detect small quantities of TNT (*ca.* 10–100 μg) and related derivatives.

Experimental Section

Tetramethylammonium 1,1′-dihydro-2,4,6-trinitrocyclohexadienate was prepared by previously published methods.[18] Acetonitrile was Aldrich spectrophotometric grade. Human serum albumin was fraction V obtained from Sigma.

The instrument on which the fluorescence spectra were recorded has been described before.[19] It was modified for this study to use a 250-mm Bausch and Lomb emission monochromator (blazed at 750 nm) and an E. M. I. 9558B photomultiplier tube. Spectra were corrected for instrumental response. Quantum yields were estimated using the method of Parker and Rees[20] with fluorescein as the standard.

Fluorescence lifetimes were measured with an instrument based on the single photon timing technique.[21] The excitation beam from a gated deuterium discharge lamp passed through a Jarrell-Ash 0.25-m monochromator. The emission was detected through interference or cutoff filters by an RCA 7265 photomultiplier tube. START and STOP pulses were transmitted to the time to pulse height converter (Ortec 437A) by Ortec NIM modules 417 and 454-453, respectively. The time-correlated signals, after bias amplification (Ortec 444), were analyzed with a Nuclear Data 1100 Series data-handling system. Decay curves were deconvoluted by numerical convolution.[21]

Samples were generally 10^{-5} *M* or less. Samples which were deoxygenated by nitrogen bubbling showed no apparent differences from those which were air saturated. All data were taken at room temperature.

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Registry No.—Tetramethylammonium 1,1′-dihydro-2,4,6-trinitrocyclohexadienate, 27554-58-1.

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Urea Dissociation. A Measure of Steric Hindrance in Secondary Amines

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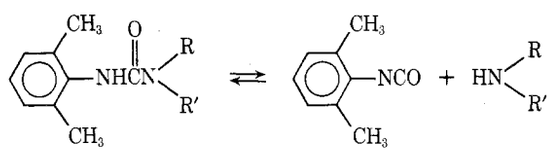
Most ureas are very stable compounds, sometimes used as characterizing derivatives of amines. Thermal dissociation may require high temperatures; *e.g.*, *N,N'*-diphenylurea was 99% dissociated into phenyl isocyanate and aniline at 370° in the gas phase.[1] Sufficient dissociation of some ureas occurs at 175° to give measurable rates of reaction with alcohols to give urethanes.[2] Similarly at 240–280° *N,N*-diphenyl-*N'*-methylurea dissociates enough to allow distillative removal of methyl isocyanate.[3] The possibility that lower temperature dissociation occurs in hindered cases is suggested by the room temperature rearrangement of *N*-*tert*-butyl-*N*-hydroxyureas to urethanes,[4] and the room temperature decomposition of 1,2-di-*tert*-butyl-4-isopropylsemicarbazide.[5]

We have found that ureas 1–8 dissociate appreciably in the range of 20–140° and that the equilibrium constants



1-8 9

1, R = R' = *sec*-butyl
2, R = cyclohexyl; R' = 3-pentyl
3, R = R' = cyclohexyl
4, R = *tert*-butyl; R' = isopropyl
5, R = *tert*-butyl; R' = 3-pentyl
6, R = *tert*-butyl; R' = cyclohexyl
7, R = 2,4,4-trimethyl-2-pentyl; R' = isopropyl
8, NRR' = 2,2,6,6-tetramethylpiperidyl

are readily measured by nmr spectroscopy. The equilibrium constants at two temperatures are given in Table I, and the thermodynamic values for variable-temperature measurements are given in Table II.

These equilibrium constants are a good indication of steric hindrance in secondary amines.[6,7] Polar effects should be very slight since we are not comparing relative rates, *i.e.*, stabilities of charged transition states, as in esterifications and hydrolyses.[8] It is interesting to note the very large difference between the disecondary alkyl amines and the secondary tertiary alkyl amines. This dramatic effect is similar to the one found in hydroboration of hindered olefins where the *tert*-butyl group exerts an extraordinary rate-retarding effect.[9] A scale of E^*_s values based on the hydroboration rates[10] was similar to the Taft E_s scale[8] except for the *tert*-butyl group, which showed greater hindrance in hydroboration.

Table I
Urea Dissociation Equilibrium Constants

	40°		127°	
Urea	K[a]	% dissociation[c]	K[a]	% dissociation[c]
1			0.017	12
2			0.032	16
3	2.6×10^{-5} [b]	0.52[b]	0.044	19
4	0.20	36	72.[b]	99[b]
5	0.34	42		
6	1.4	67	117.[b]	99[b]
7	2.1	74		
8	(15.)[d]	(95)[d]		

[a] K = [isocyanate][amine]/[urea]; ±10% of value. [b] Extrapolated values. [c] Calculated for 1 *M* initial urea concentration. [d] At 22°.

Table II
Thermodynamic Values for Urea Dissociation

Urea	$\Delta H°$, kcal/mol	$\Delta S°$, cal/deg mol
3	21.6 ± 0.5	48 ± 1
4	17.2 ± 0.4	51 ± 1
6	12.8 ± 0.2	41 ± 1

Our equilibria showed several inversions of order of steric hindrance in alkyl groups, compared to those indicated by E_s values. For example, we find the 3-pentyl group (E_s = −1.98) less hindering than the cyclohexyl group (E_s = −0.79).[11] In kinetic terms however, we find that urea formation for this pair is qualitatively in line with E_s values. At 40° the reaction of *tert*-butylcyclohexylamine with **9** (0.7 *M*) is at equilibrium (27% associated) in less than 1.5 hr, at which time the reaction of *tert*-butyl-3-pentylamine with **9** is only 14% associated. Even after 2 days, equilibrium is not yet reached (final value after 4 days is 47% associated). Similarly in the reverse direction, **2** required about 20 min to reach equilibrium at 140° by dissociation while **3** required only about 5 min.

Experimental Section

3-Pentylcyclohexylamine was prepared by a modification of the method of Skita and Keil.[12] Platinum oxide (25 mg) and one drop of concentrated HCl were added to 9.9 g (0.10 mol) of cyclohexylamine and 17.2 g (0.20 mol) of 3-pentanone. This was hydrogenated in a Parr shaker at 50 psi initial pressure for 2 days. The solution was decanted, about 200 mg of Na_2CO_3 was added, and then the solution was distilled on a spinning band column to give 11.0 g (65%) of the secondary amine, bp 95° (13 mm) (lit. yield 31%, bp 208–209°).

Di-*sec*-butylamine was prepared similarly from *sec*-butylamine and 2-butanone.

Commercial dicyclohexylamine was purified by distillation.

The secondary alkyl tertiary alkyl amines were prepared as described previously.[13]

All amines were dried over 4A molecular sieves before use.

2,6-Dimethylphenyl isocyanate[14] was prepared by phosgenation of 2,6-dimethylaniline hydrochloride and purified by spinning band distillation, bp 112–113° (35 mm) [lit.[14] bp 90–91° (13 mm)].

Anisole was purified by distillation and dried over 4A molecular sieves. Anisole was chosen as solvent for the higher boiling point (155°). Moreover, the difference in nmr chemical shift between the methyl groups on the isocyanate and those on the ureas (Table IV) were greater than in, *e.g.*, *o*-dichlorobenzene.

Equilibrium Measurements. 2,6-Dimethylphenyl isocyanate (303 mg, 2.06 mmol) was added to a solution of *tert*-butylisopropylamine (217 mg, 0.189 mmol) in 2 ml of pentane under nitrogen. The resulting white solid (**4**) was filtered, washed with pentane, and dried under nitrogen in a glove bag. A sample of the dry urea (97.4 mg) was dissolved in 307.2 mg of anisole under nitrogen in a well-dried nmr tube equipped with a tight cap. The tube was heated in the variable-temperature probe of an A-60 nmr spectrometer at each temperature until the ratio of isocyanate to urea remained constant. The peaks for the benzylic methyl