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TECHNICAL NOTE

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Structure Elucidation of Dyes That Are Formed in the Colorimetric Detection of the Improvised Explosive Urea Nitrate

ABSTRACT: Urea nitrate (uronium nitrate, UN) is a powerful, improvised explosive that can be easily made from urea and nitric acid. It is considered the most frequently used, illegal explosive in the Israeli arena, which is responsible for the loss of more than a hundred lives in terrorist incidents. Urea nitrate is a colorless, crystalline substance that looks very much like sugar. A sensitive color test for UN was developed recently. It is based on the formation of a red dye in the reaction between p-dimethylaminocinnamaldehyde and UN under neutral conditions. A similar reaction with p-dimethylaminobezaldehyde produces a yellow dye. The two dyes have been synthesized, and their structures determined by X-ray crystallography. Both dyes are protonated Schiff bases, prevailing in the crystal in a quinoid form. They are identical to the compounds, which are obtained in the colorimetric detection of urea with the same reagents, under strong acidic conditions, whose structures have been postulated in the literature, but never fully proved experimentally.

KEYWORDS: forensic science, urea, urea nitrate, uronium nitrate, p-dimethylaminobenzaldehyde, p-dimethylaminocinnamaldehyde, crystallography, Schiff base, colorimetric detection, nonlinear optics

In a previous article, we described a sensitive and quite specific colorimetric method for the detection of the improvised explosive urea nitrate UN, (Fig. 1). A red dye is formed upon reacting UN with p-dimethylaminocinnamaldehyde (p-DMAC) **2** (Fig. 2) under neutral conditions (1). A similar reaction between UN and the one-vinyl-shorter p-dimethylaminobenzaldehyde (Ehrlich's reagent) **3** (Fig. 2) produces a yellow color under the same conditions. Both reactions can take place without additional acid because of urea nitrate's high acidity. We wish to report the preparation and structure determination of the two dyes that are formed by these reactions. The colored products were characterized by elemental microanalyses, IR, UV, fluorimetric measurements, and X-ray diffraction (XRD).

Our results also provide an unequivocal experimental proof for the structure of the dyes that are formed in the colorimetric detection of urea with reagents 2 and 3 under strong acidic conditions.

Crystals Preparation

Sizeable crystals of both dyes, which are suitable for crystallographic measurements, have been obtained as follows:

*Compound **4**, the Product Between UN and p-dimethylaminobenzaldehyde*

About 97 mg **3** and 40 mg urea nitrate **1** were dissolved in isopropanol (5 mL), in a covered beaker and left overnight at room temperature. The crystals thus formed were washed with cold *n*-propanol and dried in air. Dark red needles with metallic luster (yellow in solution), mp 165.7°C. Anal. Calcd for $C_{10}H_{14}N_3O_4$: C, 47.2; H, 5.6; N, 22.0. Found: C, 47.28; H, 5.53; N, 21.76. IR: 1384, 1558, 1616, 1738, 3411, 3552/cm; UV/Vis: λ_{max} (CH_2Cl_2) 428 nm, (ϵ 52,300/M/cm), 412 nm (shoulder) (Fig. 3); fluorescence: λex 335 nm, λem 372, 671 nm (Fig. 4); λex 385 nm, λem 434, 771 nm.

*Compound **5**, the Product Between UN and p-dimethylaminocinnamaldehyde*

p-DMAC, **2** (30 mg) was dissolved in ethyl alcohol (5 mL). Urea nitrate **1** (21 mg) was added without dissolution and the mixture was maintained at 4°C for 48 h. The dark crystals thus formed were washed with cold ethyl alcohol and dried in air. Dark, nearly black needles with metallic luster (red in solution), mp 167.7°C. Anal. Calcd for $C_{12}H_{16}N_3O_4$: C, 51.4; H, 5.80; N, 20.0. Found: C, 51.37; H, 5.75; N, 19.80. IR: 1384, 1539, 1617, 1746, 2360, 3418, 3551/cm; UV/Vis: λ_{max} (CH_2Cl_2) 520 nm, (ϵ 134,200/M/cm), 411 (weak) (Fig. 5); fluorescence: λex 405 nm, λem 507, 808 nm (Fig. 6); λex 320 nm, λem 355, 640 nm.

Crystallographic Measurements

A single crystal of dye **4** or **5** was attached to a glass fiber, with epoxy glue, and transferred to a Bruker SMART APEX CCD X-ray diffractometer (Bruker Axe GHBH, Karlsrune, Germany) equipped with a graphite-monochromator. The system was controlled by a Pentium-based PC running the SMART software package (2). Data were collected at room temperature using MoKα radiation (λ = 0.71073 Å). Immediately after collection, the raw data frames were transferred to a second PC computer for integration and reduction by the SAINT program package (3). The structure was solved and refined by the SHELXTL software package (4).

Crystallographic Data

(Excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre

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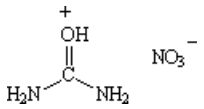

FIG. 1—*Structure of urea nitrate.*

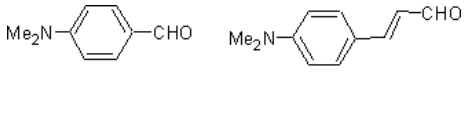

FIG. 2—*Structure of p-DMAC (**2**) and p-DMAB (**3**), the two color-reagents for urea and uronium salts.*

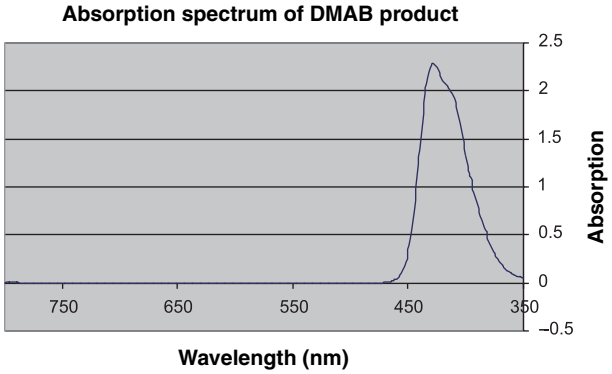

FIG. 3—*Absorption spectrum of dye **4**.*

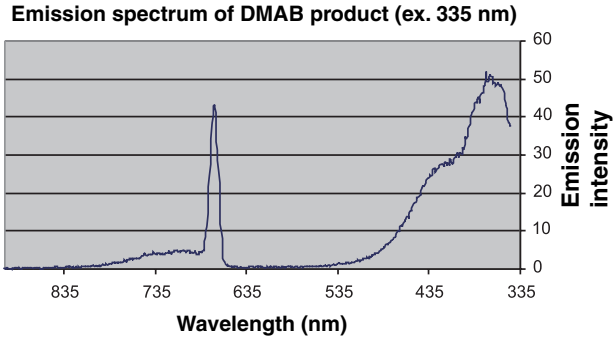

FIG. 4—*Fluorescence emission spectrum of dye **4** (Excitation at 335 nm).*

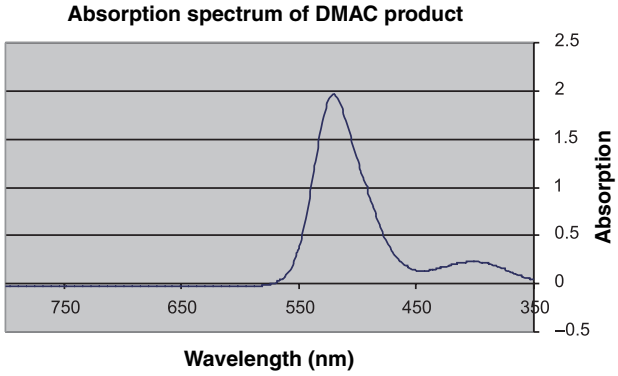

FIG. 5—*Absorption spectrum of dye **5**.*

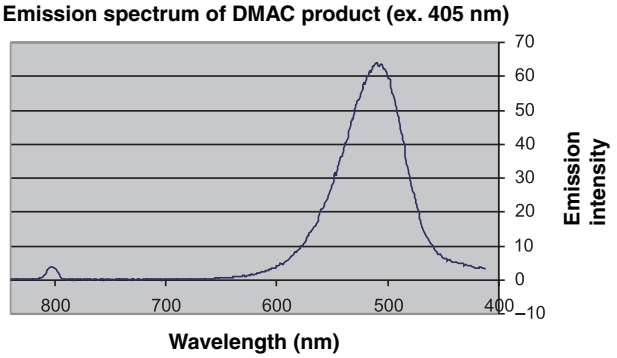

FIG. 6—*Fluorescence emission spectrum of dye **5** (Excitation at 405 nm).*

as supplementary publication numbers CCDC 602720 and 602721. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail deposit@ccdc.cam.uk).

Discussion

Colorimetric detection of urea by reaction with Ehrlich's aldehyde, p-dimethylaminobenzaldehyde (p-DMAB) **3**, was first suggested by Barrenscheen >80 years ago (5). The reaction, which requires strong acidic conditions, forms a yellow dye that absorbs at 425 nm (6). The use of its vinilog, p-dimethylaminocinnamaldehyde (p-DMAC) **2**, which produces a deeper color, was introduced by Matsutani for serum urea measurement half a century later. Under similar conditions, it produces with urea a red dye, which absorbs at 520 nm (7). These reagents have been suggested for detection and quantitative determination of urea in biological fluids (8) and in pharmaceutical formulations (9). p-DMAC **2** was proposed for forensic detection of urine stains on fabrics (10) and for the visualization of latent fingerprints on paper (11–13). The assumed structural characteristics of both products—electron donor and acceptor groups connected to a π-conjugated chain—make these compounds potential materials for nonlinear optics (14). To date, however, there has been no unequivocal evidence to confirm the structure of these compounds. Attempts by our group to prepare sizeable crystals, suitable for crystallographic measurements, by adding urea to acidic solution of aldehydes **2** or **3** (*one-stage* synthesis, Fig. 7), failed. Only very small, brittle crystals precipitated from the solution. It was just logical to assume that the same dyes would also be formed by a two-stage process, producing uronium salt first, by reacting urea with a strong acid (nitric acid in this

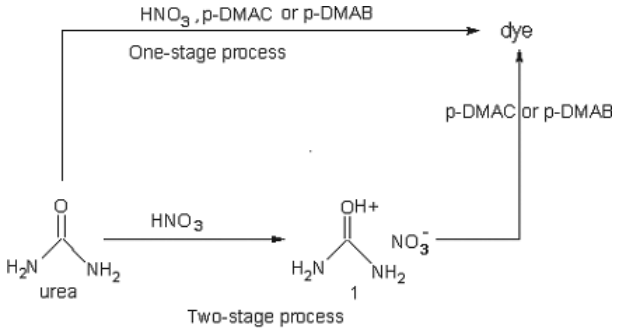

FIG. 7—*One stage and two-stage processes for dye formation in the colorimetric detection of urea.*

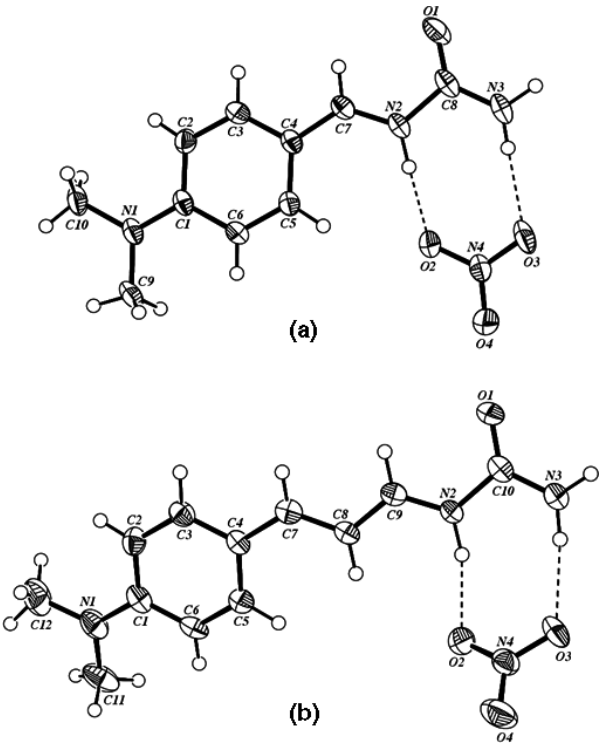

FIG. 8—*Crystal structure of dyes* ***4*** *(a) and* ***5*** *(b). Empty circles represent hydrogen atoms.*

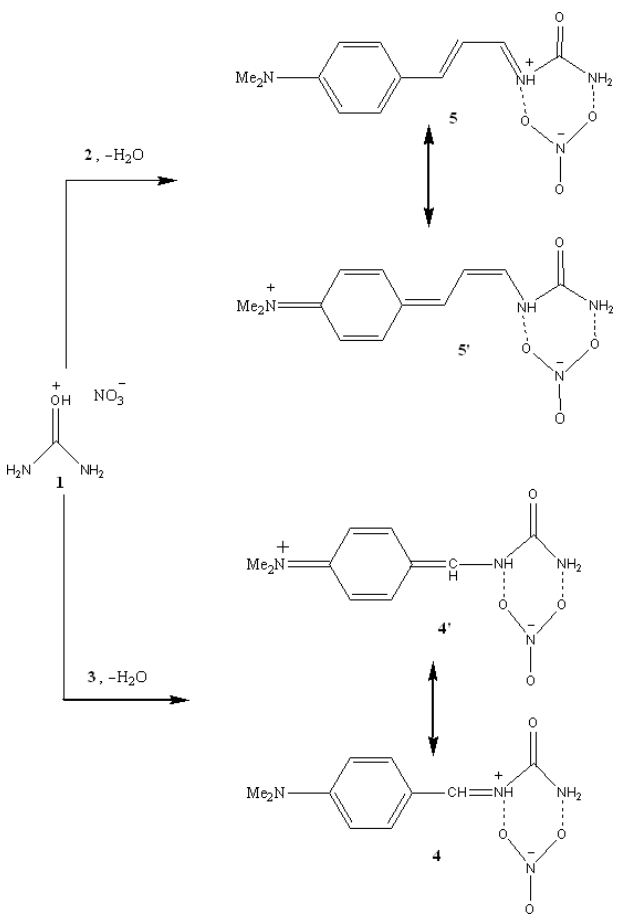

FIG. 9—*The color-producing reactions of p-DMAC (****2****) and p-DMAB (****3****) with urea nitrate.*

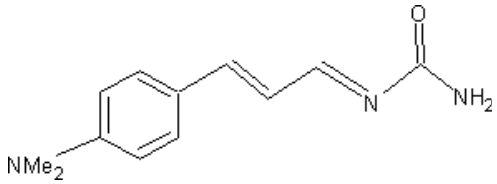

FIG. 10—*The free base structure suggested for the dye that is formed by reaction of urea and p-DMAC under acidic conditions (8).*

TABLE 1—*Bond lengths [Å] for compound* ***4****.*

1.3431 (16)	C(1)-N(1)
1.422 (2)	C(1)-C(2)
1.429 (2)	C(1)-C(6)
1.3584 (19)	C(2)-C(3)
0.878 (16)	C(2)-H(2)
1.4151 (19)	C(3)-C(4)
0.950 (15)	C(3)-H(3)
1.3925 (17)	C(4)-C(7)
1.4205 (19)	C(4)-C(5)
1.3615 (18)	C(5)-C(6)
0.934 (15)	C(5)-H(5)
0.928 (15)	C(6)-H(6)
1.3273 (18)	C(7)-N(2)
0.939 (14)	C(7)-H(7)
1.2143 (18)	C(8)-O(1)

TABLE 2—*Bond lengths [Å] for compound* ***5****.*

1.351 (3)	C(1)-N(1)
1.414 (3)	C(1)-C(6)
1.417 (3)	C(1)-C(2)
1.360 (3)	C(2)-C(3)
0.90 (2)	C(2)-H(2)
1.411 (3)	C(3)-C(4)
0.967 (19)	C(3)-H(3)
1.408 (3)	C(4)-C(7)
1.410 (3)	C(4)-C(5)
1.362 (3)	C(5)-C(6)
0.976 (19)	C(5)-H(5)
0.904 (18)	C(6)-H(6)
1.374 (3)	C(7)-C(8)
0.901 (18)	C(7)-H(7)
1.379 (3)	C(8)-C(9)
0.932 (18)	C(8)-H(8)
1.321 (3)	C(9)-N(2)
0.962 (17)	C(9)-H(9)
1.211 (2)	C(10)-O(1)
1.335 (3)	C(10)-N(3)

case) followed by a reaction with aldehydes **2** or **3**. Indeed, we were able to produce large crystals of the two dyes by the *two-stage* process (Fig. 7). By preparing the dyes from uronium nitrate **1**, it was possible to obtain sufficiently large crystals, determine their molecular structure and provide an experimental proof also for the structure of the dyes that are obtained by the colorimetric detection of urea. Apart from their size, these crystals were identical to the dyes that were prepared from urea, an acid, and the aldehyde by the *one-stage* process. Crystallographic measurements show that both products are mono-ureido nitrate salts (Figs. 8 and 9), which are protonated on one of the urea nitrogen atoms.

In their 1956 article (15), Cline and Fink postulated a protonated Schiff base structure for the product of urea and p-DMAB **3**. Their experimental proof was only partial, resting mostly on microanalysis. In a more recent article (8), Hussain and Shaukat reported

a free-base structure (Fig. 10) for the colored product of urea with p-DMAC **2**. Our crystallographic data (Fig. 8) confirm the protonated Schiff base structure for both products, thus supporting Cline and Fink's assumption (15) and disagreeing with the more recent free-base suggestion (8). Furthermore, examination of the bond lengths in the two dyes (Tables 1 and 2) shows that C(2)-C(3) and C(5)-C(6) are the shortest bonds in both aromatic rings, indicating higher degree of double bond and, hence, that the quinoid form prevails in the crystal lattice (**4**a and **5**a, Fig. 9).

Also, it is unlikely that a compound with the free base structure (Fig. 4), with only two conjugated double bonds, will exhibit light absorption properties such as those exhibited by the two dyes. The intense colors of the two are in accordance with structures **4**a and **5**a, both having quinoid resonating forms.

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