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Examination of tryptamine-squaraine complexes as both colorimetric and fluorometric stains in gel electrophoresis

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ABSTRACT

A water-soluble dual pendent squaraine with tryptaminium cations has been mixed with mono-nitroaromatics in solution to create ternary complexes with the aim of creating indole-nitroaromatic charge-transfer pairs. Examination of the X-ray crystal structures of two tryptamine-nitroaromatic complexes, without the squaraine moiety, revealed that neither demonstrated charge-transfer pair or stacking associations between the indole and nitroaromatic rings. Examination of the fluorescence performance upon illumination with 300 nm light of each ternary squaraine complex, particularly in the presence of an equimolar amount of protein, showed that the levels across the series were no more than that observed for the base bis-tryptaminium squaraine, as was observed in a previous study. However, electrophoresis gels stained with the base bis-tryptaminium squaraine, whose X-ray crystal structure is also reported, were found to fluorescence; the only other squaraine dye of this type to do so in addition to the bis-piperidinium squaraine of previous studies.

1. Introduction

Electrophoresis gel documentation systems (or gel docs), those commonly employed to fluorometrically image electrophoresis gels, are fitted with an ultraviolet (UV) light illuminator source emitting UV light at 310 ± 40 nm. For this reason, dyes such as ethidium bromide, which fluoresces a bright orange colour when excited with UV light, is equally commonly employed as a fluorescent tag in detecting nucleic acids in gel electrophoresis techniques [1], where the gels are imaged using a gel doc. In contrast, bis(indolenine)squaraine dyes, studied as fluorescent protein-sensitive molecular probes, fluorescence in the near-infrared region of the visible spectrum and thus require excitation at the wavelength of maximum visible light absorbance (i.e. λ_{max}), which is normally >600 nm [2–4]. It was therefore surprising to discover that the bis-piperidinium salt of a dual pendant sulfonate bis(indolenine)squaraine (**1**) (Fig. 1) fluoresced an intense pink colour when a protein loaded gel, stained with this particular dye, was imaged in fluorescence mode (i.e. illumination with the UV light source) on a gel doc [5]. No other variant in that series of dual pendant sulfonate squaraine dyes, including dyes charge-balanced with alkali metals, could emulate this [6]. Compound **1** does not specifically absorb in the UV region so a synthetic strategy, and subsequent study, was devised that incorporated charge-transfer complexes with the squaraine dye in an attempt to increase the UV absorption of the squaraine-based compound [7]. This was done with the aim of promoting energy transfer from the charge-transfer component to the squaraine moiety to increase the squaraine fluorescence. The synthetic strategy adopted, similar to that illustrated in Fig. 2, involved the use of di-nitro aromatics, which are known to form charge-transfer complexes with the indole ring of the tryptamine cation. However, examination of each of these squaraine-based compounds against that of the squaraine-tryptaminium salt (**2**), both with and without the presence of protein, showed no improvement in fluorescence output upon illumination of UV light (at 300 nm). As a continuation to that study, here we report the use of four mono-nitro aromatics for potential incorporation with compound **2** to create compounds **3**–**6** (Fig. 2). The single crystal structures of **2** and two of the individual complexes employed (**7** and **8**), illustrated in Fig. 3, are reported, as are the results following use of two of the ternary complexes plus compound **2** as both colorimetric and fluorometric stains for gel electrophoresis.

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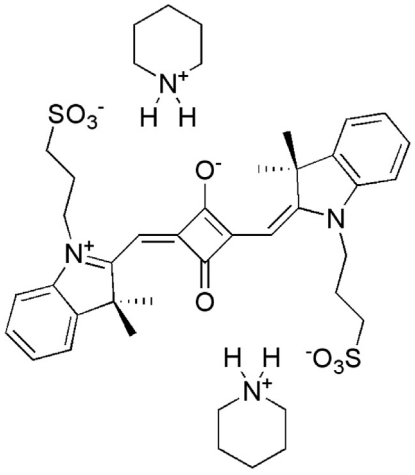

Fig. 1. Chemical diagram for compound **1**.

2. Experimental

2.1. Synthesis

All chemicals were purchased from Sigma-Aldrich and were used as received without further purification. The water used in this study was purified using reverse osmosis techniques. Infrared spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. ^{1}H NMR data were recorded in DMSO-d_6 on a Bruker AVIIIHD 500 MHz FT-NMR spectrometer equipped with a SMART probe. Details of the synthesis of 2,3,3-trimethyl-1-(propan-3-sulphonyl)-indolenine, bis-piperidinium 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl)cyclobutene-1,3-diolate (**1**) [5] and bis-(2-(1*H*-indol-3-yl)ethanaminium) 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl) cyclobutene-1,3-diolate (**2**) [7] have been previously published, with the crystal structure of **1** (as a trihydrate) also previously reported [6].

2.1.1. Preparation of tryptamine-squaraine-nitroaromatic complexes

Bis-(2-(1*H*-indol-3-yl)ethanaminium) 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl)cyclobutene-1,3-diolate hemihydrate (**2**) (2.90 mg, 3.0 μmol) was dissolved with twice molar amounts of 2-nitrophenol (0.83 mg, 6.0 μmol), 4-nitrophenol (0.83 mg, 6.0 μmol), 5-nitroquinoline (1.0 mg, 6.0 μmol) and 5-nitroisoquinoline (1.0 mg, 6.0 μmol), in water (100 mL) with sonication to produce 30 μM stock solutions of **3**,**4**, **5** and **6** respectively.

2.1.2. Synthesis of tryptaminium salts for X-ray analyses

Tryptamine (100 mg, 0.62 mmol) was reacted with an equimolar amount of either 2-nitrophenol (86 mg), 4-nitrophenol (86 mg), 5-nitroquinoline (108 mg), or 5-nitroisoquinoline (108 mg) by warming in ethanol (3 mL). Total evaporation of the ethanol from each reaction yielded yellow crystals for **7** and **8**, a yellow-brown gel for **9** (that solidified after several days) and yellow-brown powder for **10**, each identified as organic salt complexes (for **7** and **8**) or a co-crystal adduct (for **9** and **10**) using infrared spectroscopy techniques. Compounds **7** and **8** yielded crystals from which specimens were cleaved for the X-ray analyses.

2.1.3. X-ray crystallographic analysis

Crystallographic data for **2** was collected at 100(1) K on a Rigaku Saturn724+ diffractometer using monochromatized Mo-Kα x-ray radiation (λ = 0.71075 Å) equipped with an Oxford Cryosystem low temperature device, and for **7** and **8** was collected at 200(1) K on an Oxford Diffraction Gemini-S CCD-detector diffractometer using monochromatized Mo-Kα X-ray radiation (λ = 0.71073 Å). All structures were solved by direct methods SHELX97 [8], and refined by full-matrix least-squares calculations. Crystal data for **2**: $C_{52}H_{60}N_6O_8S_2$, Mw = 961.18, monoclinic, $P2_1/n$, Z = 2, a = 14.3879(9), b = 5.8044(3), c = 29.186(2) Å, β = 99.645(7)°, D_{calcd} = 1.328 g cm^{-3}, T = 100(1) K, $F(000)$ = 1020, μ = 0.173 mm^{-1}, 13756 reflections were collected, 4230 unique (R_{int} = 0.0698), 2780 observed ($I > 2\sigma(I)$), 310 parameters, R_1 = 0.0528, wR_2 = 0.1161.Crystal data for **7**:

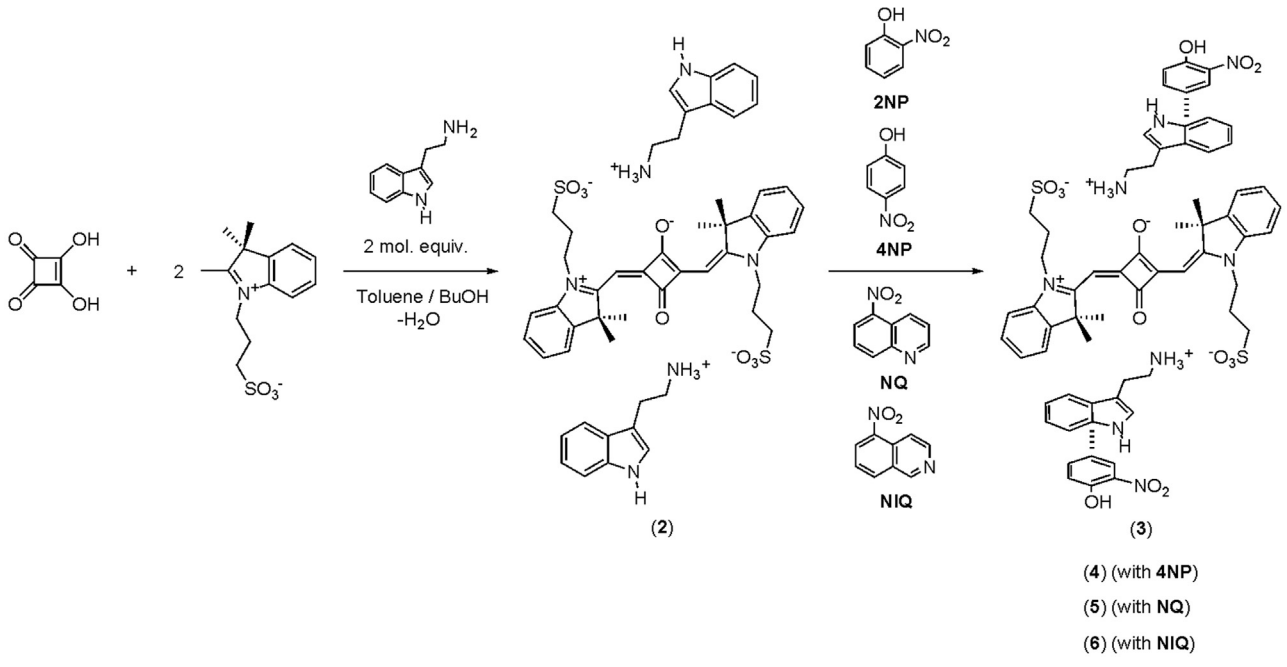

Fig. 2. Schematic showing the synthetic route for compound **2** and complexes **3**–**6**.