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〈Research Paper〉

Color Change Redox Behavior of the 1,3-Squaraine Dyes

Kun Jun, Seung-Rim Shin, Jong-Il Shin and Soo-Youl Park*

Korea Research Institute of Chemical Technology, New Chemistry Research Division,
Colorant Chemistry Research Team, Yuseong, Daejeon, Korea

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Abstract— The 1,3-bis(4-aminoaryl)squaraines showed color change behavior, they were found to undergo reduction with sodium borohydride in solution to give colorless leuco compounds, which oxidized readily in air back to the colored squaraine dye. We have shown that initial observations indicated that the derivatives synthesized gave new donor-acceptor chromophores. It is also interesting to note that the oxidation of the leuco squaraines did initially produce a species absorbing about 630-680 wavelengths. The 1,3-squaraines have found many uses as near-infrared absorbers, laser dyes and photoconductive materials. Furthermore their color-change redox behavior has potential in the area of peroxidase-based bioassaysas oxidation sensitive indicator systems were investigated.

Keywords: *Squaraine, Leuco squaraines, Near-infrared absorbing, Redox behavior*

1. Introduction

The redox properties of 1,3-squaraine derivatives have been the subject of several investigations since this class of chromophore was first discovered[1-4]. Electrochemical studies have been directed in most cases towards oxidation of the chromophore. Tong *et al.* examined a range of 1,3-bis(2,3,3-trimethylindolenium-2-ylidene)-squaraines, and noted two reversible oxidation potentials in methanol, the first correlating well with the HOMO energy levels calculated by the AM1-SCF method[5].

Previously, Law *et al.* had also noted that 1,3-bis-arylsquaraines showed two reversible oxidation waves in dichloromethane, and the oxidation potential decreased as the donor strength of the substituted aryl residues increased[6]. Other redox studies have concentrated on the photochemistry of the squaraine dyes, where laser excitation can lead to radical cations and anions derived from the patent chromophore[7-9].

We have investigated this possibility, and have found that reduction can, in most cases, be achieved readily in organic solvents with sodium borohydride. Moreover, the resultant colorless reduced species(*leuco* dyes) are generally chemically stable and can be oxidised back to the colored dye with air or common inorganic oxidants. This meant that the 1,3-squaraines could be used as redox indicators, with possible functional applications in enzyme assays, oxygen detection, or reprographic systems. Thus, the chemistry of the *leuco*-1,3-squaraines was investigated in detail in order to determines their potential value in such areas.

2. Experiments

These symmetrical 1,3-squaraines were prepared from squaric acid[10] and the appropriate *N,N*-dialkylanilines. The now standard technique involves heating squaric acid in a mixture of toluene and n-butanol under azeotropic conditions,

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*Corresponding author. Tel: +82-42-860-7666; Fax: +82-42-860-7669; e-mail: sypark@krict.re.kr

so that the mono-n-butyl squarate ester is formed.

This then undergoes *in situ* nucleophilic substitution by the arylamine, giving the half-condensate. A second condensation reaction then gives the 1,3-bis(4-aminoaryl)squaraine[11].

The efficiency of this reaction is determined by the nucleophilicity of the arylamine and the solubility of the product in the reaction medium.

In those cases where the dye had low solubility in the solvent, the product could be filtered off directly and the purity was generally high. In other cases the product had to be isolated by evaporation of the solvent and purified by column chromatography. The structures of the dyes synthesised are shown in Table 1, together with reaction yields and melting point data.

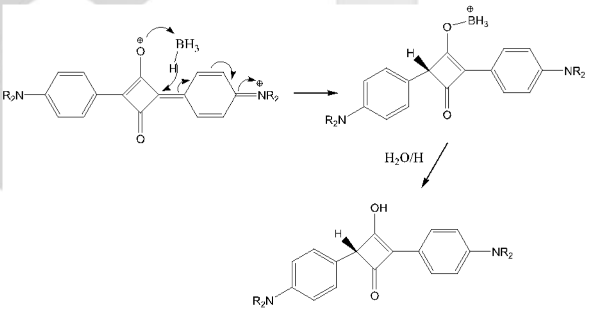
3. Results and Discussion

3.1. Synthesis and characterization of the leuco-1,3-bis(4-aminoaryl)-squaraine dyes

Preliminary experiments showed that the 1,3-bis(4-aminoaryl)squaraines were readily decolorized by a variety of reducing agents. The color could be regenerated by leaving the solutions in air, or by adding oxidizing agents such as ferric chloride. The most convenient method of reduction involved using excess sodium borohydride in a mixture of a dichloromethane and methanol at room temperature. Most of the squaraine dyes being soluble in this medium. A reaction time of 2 hours was sufficient to effect complete reduction. Isolation of the *leuco* dye could be achieved by removal of the solvent on a rotary evaporator, adding water and acetic acid to the residue, and filtering off the precipitate. The white, or lightly colored solid could then be dried in vacuum over calcium chloride. This method was not always successful in producing pure products, as most *leuco* dyes were particularly susceptible to air oxidation. It was also not possible to prevent partial reoxidation once the excess sodium borohydride had been destroyed by acidification. The leuco compounds were, in marked contrast to the parent dyes, generally low melting solids, readily soluble in non-polar solvents. They were also acidic, being insoluble in water but readily soluble in dilute aqueous alkali. The most probable structure for the *leuco* compounds is (1), which would arise by direct transfer of hydride ion from the borohydride anion to the squaraine ring. A probable mechanism is shown in Scheme 1, in which one of the carbonyl groups of the ring becomes attached to boron. Treatment of the resultant complex with aqueous acid would then generate (1).

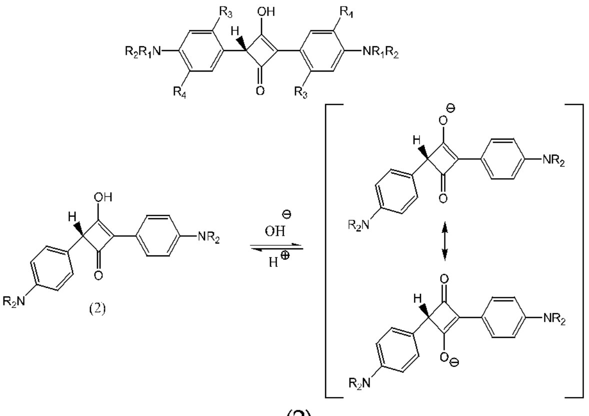
The general structure (1) could be justified on the basis of the following observations:

(a) Mass spectrometry of representative examples shows that the structure corresponds to the parent dye plus two hydrogen atoms (DH_2).



(1)

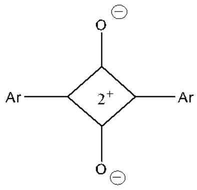
Scheme 1. Formation of leuco 1,3-squaraines.

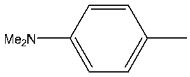
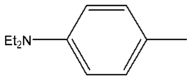
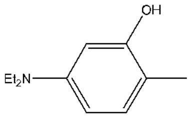
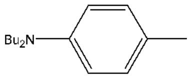
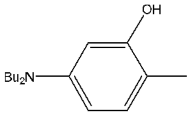
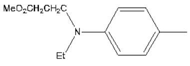
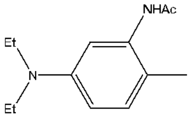
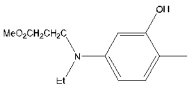


(2)

Scheme 2. Acid-base equilibrium for leuco compounds.

Table 1. Yields and melting point data for 1,3-bis(4-aminoaryl)squaraines



No	Ar	m.p (°C)	Yield (%)	No	Ar	m.p (°C)	Yield (%)
1a		288-290	58	1b		213-215	43
1c		189-191	73	1d		204-206	50
1e		205-208	25	1f		189-191	58
1g		255-257	71	1h		199-201	72

(b) Structures (1) are (2) are the two most stable tautomers that can be drawn for a species DH_2 but the leuco compounds are acidic, and readily soluble in aqueous alkali. This is consistent with the enolic structure (1), but not with the diketone form (2). The acid-base equilibrium that would occur with (1) is shown in Scheme 2, the resultant anion being resonance stabilized as shown.

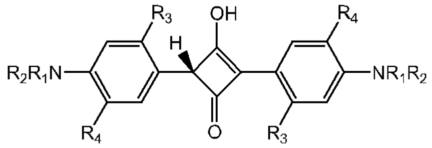
(c) The infrared spectra of the leuco compounds show an intense carbonyl peak at ca. 1710 cm^{-1}. This is consistent with structure (1), in which the frequency of the cyclobutene carbonyl group is lowered by resonance interaction with the double bond and the conjugated hydroxyl group. Structure (2), on the other hand, would be expected to show two carbonyl peaks at ca. 1740 and 1710 cm^{-1} (out-of-phase and in-phase stretching respectively), as observed with tetramethyl cyclobutan-1,3-dione.

(d) Although it was difficult to obtain clean proton nmr spectra for the leuco compounds due to their rapid oxidation back to the 1,3-squaraine dye, it was possible to subtract the peaks due to the latter from the spectra, thus permitting the structure of the leuco compounds to be analysed. The resultant spectra were then fully consistent with general structure (1). Thus, for example, the proton nmr spectrum of leuco dye (2b) shows four groups of aromatic protons, at = 6.90(2H, d, J = 8.5 Hz); 7.10(2H, d, J = 8.5 Hz); 7.20 (2H, d, J = 8.6 Hz); 7.80(2H, d, J = 8.6 Hz), showing that the two para-disubstituted rings in (2b) are not equivalent. In addition (2b) show a 1H singlet at = 4.3, corresponding to the isolated cyclobutane ring proton. The spectrum also shows that there are two slightly different pairs of N-ethyl groups in the com - pound. A range of leuco-1,3-bis(4-aminoaryl) -squaraine dyes was prepared, and these are summarized in Table 2.

3.2 Oxidation characteristics of the leuco-1, 3-bis(4-aminoaryl)-squaraine dyes

As noted previously, the *leuco* squaraine dyes are readily oxidized back to the parent dye, using any conventional oxidizing agent,

Table 2. Yields and physical data for leuco-1,3-squaraine dyes



Leuco dye	Structure				Yield (%)	m.p. (°C)
	R_1	R_2	R_3	R_4		
2a	Me	Me	H	H	80	88-92
2b	Et	Et	H	H	45	64-66
2c	Et	Et	OH	H	67	158-162
2d	n-Bu	n-Bu	H	H	53	128-130
2e	n-Bu	n-Bu	OH	H	80	66-68
2f	Et	$MeO_2C(CH_2)_2$-	OH	H	55	155-158
2g	Et	Et	NHAc	H	50	65-67
2h	Et	$PhCH_2$	NHAc	H	75	82-85

and even air. The type of oxidizing agent used does not affect the nature of the product formed, but does affect the efficiency of the process and the possible formation of by-products. The ability of the *leuco* squaraine dyes to undergo facile air-oxidation places them on a par with the *leuco*- indigoids, *leuco*-thiazinium and oxazinium dyes, and this means that they have many potential applications as color change oxidation-sensitive molecules. They have the additional value of affording chromophores after oxidation with much higher high extinction coefficients and have the capability of exhibiting long wavelength fluorescence. If the *leuco* squaraine system can be stabilized by functionalization (*e.g. esterification or etherification*) then this provides potential source of enzyme substrates or other types of reactive indicator. Removal of the protective group followed by rapid air oxidation would then result in a change from the colorless state to an intense colored state. For such applications, a rapid rate of oxidation in air at room temperature is essential. An indication of the relative ease of oxidation of a range of *leuco* 1,3-squaraine dyes was obtained in the following way. A solution of each squaraine dye in dichloromethane/methanol was reduced fully with a slight excess of sodium borohydride, and after destroying any excess borohydride with a little acetic acid. The rate of coloration of the solution at room temperature in air was then monitored in a spectrophotometer cell by measuring the increase in absorbance at the λ_{max} of the oxidized dye. Oxidations typically took less than 1 hour to go to completion (Fig 1).

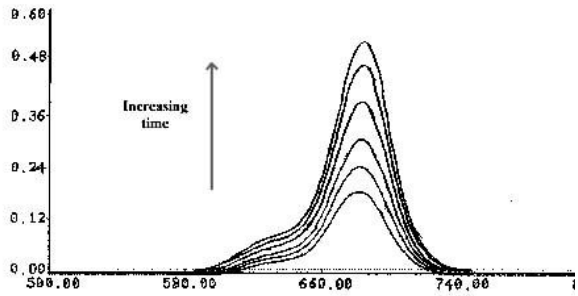


Fig. 1. Air oxidation of a solution of the leuco squaraine dye (2b) in DCM.

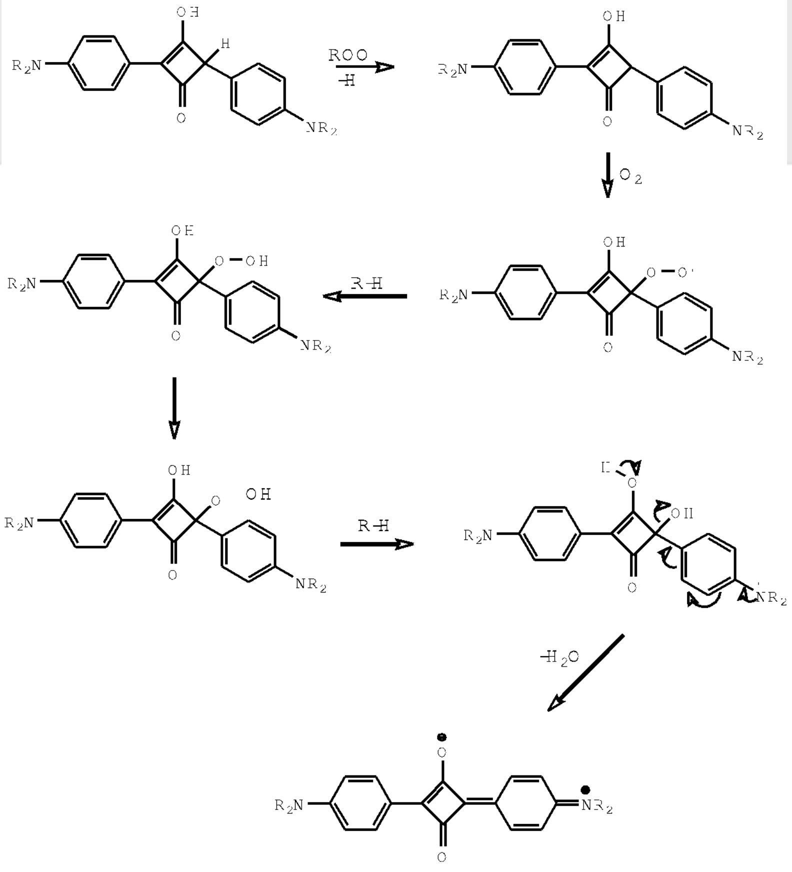
Absorbance/time plots were relatively complex and this method has too many uncertainties to provide meaningful kinetic data(e.g. autoxidation will be a chain reaction and the induction period for initiation and rates of propagation will depend on such factors as solvent and sample impurities, oxygen concentration, incident light etc.). The efficiency of the air-oxidation process could be determined by letting the solutions to oxidize completely, and then comparing final dye absorbance values with the theoretical values for 100 % conversion. The results for several dyes are summarized in Table 3, and oxidation yields are generally high(64-98 %).

A possible mechanism for the autoxidation of the leuco dyes is shown in Scheme 3. The reaction is presumably initiated by peroxide radical, which could be formed by the solvent or could come from an inefficient reaction between the *leuco* dye and triplet oxygen. Hydrogen abstraction by this radical from the *leuco* dye would then give a resonance stabilized free radical, and this would then be trapped by triplet oxygen as a hydroperoxide radical. Subsequent steps involving hydrogen abstraction, thermolysis of an unstable hydroperoxide, further hydrogen abstraction and finally elimination of water would the give the oxidized squaraine dye.

Table 3. Air oxidation efficiencies for leuco squaraines in dichloromethane solution at room temperature

Leuco compound	Oxidation efficiency (%)	λ_{max} of oxidized form in dichloromethane (nm)
2a	96	630
2b	98	630
2c	88	633
2d	80	642
2e	87	634
2f	96	633
2g	97	682
2h	64	672

General procedure : All the chemicals and solvents used during the reactions were purchased from Aldrich(U.S.A. Chemical Co.), Acros(U.K. Chemical Co), and Junsei (Japan Chemical Co). Before being used, some of them were purified using normal procedures. All reactions were monitored by thin layer chromatography(TLC). Ultraviolet and visible spectra were determined on a Perkin-Elmer Lambda 15 Spectrophotometer. Melting points



Scheme 3. Possible oxidation mechanism for leuco squaraines.

were measured on an electrothermal melting point apparatus. Elemental microanalysis and mass spectrometric analyses were performed by the EPSRC National Mass Spectrometry Service, using either fast atom bombardment sampling in a *m*-nitrobenzyl alcohol matrix, electron impact ionization, chemical ionization or electrospray. Nuclear magnetic resonance spectra(^{1}H NMR) were performed on Bruker 300 spectra at 300.13 MHz in deuteriochloroform.

1,3-Bis(4-aminoaryl)-squaraines (1): Squaric acid(1.14 g, 10 mmole) was refluxed in a mixture of 1-butanol(150 ml) and toluene(75 ml), removing the water formed azeotropically with a Dean-Stark trap. After 1 hour, the appropriate N,N-disubstituted arylamine(20 mmole) was added and the reaction mixture was refluxed for a further 4 hours. The reaction mixture was cooled to room temperature and the solvent removed on a rotary evaporator. The residue was recrystallized from 1-butanol and dried in an oven at 50 °C.

1,3-Bis-(4-NN-dimethylaminophenyl)-squaraine (1a).

Metallic blue crystals(1.86 g, 58 %), m.p. 288-290 °C. Elemental analysis: found C 75.15, H 6.36, N 8.85 % $C_{20}H_{20}N_2O_2$ requires C 74.98, H 6.29, N 8.74 %.

1,3-Bis-(4-NN-diethylaminophenyl)-squaraine (1b).

From NN-diethylaniline: metallic blue crystals, (1.6 g, 43 %), m.p. 213-215 °C. Elemental analysis: found C 76.29, H 7.56, N 7.52 % $C_{24}H_{28}N_2O_2$ requires C 76.56, H 7.50, N 7.44 %. ^{1}H NMR (300 MHz, $CDCl_3$): 1.25 (t, 12H, J=7.0 Hz), 3.46 (quart, 8H, J=7.0 Hz), 6.32 (d, 2H, J=9.0 Hz), 6.75 (d, 2H, J=9.6 Hz), 7.88 (d, 2H, J=8.6 Hz), 8.39 (d, 2H, J=9.2 Hz).

1,3-Bis-(4-NN-diethylamino-2-hydroxyphenyl)-squaraine (1c).

From 3-hydroxy-NN-di-n-butylaniline, metallic blue crystals(2.98 g, 73 %), m.p. 189-191 ℃.

Elemental analysis: found; C 70.23, H 7.04, N 7.01 %; $C_{24}H_{28}N_2O_4$ required for C 70.57, H 6.91, N 6.86 %. ^{1}H NMR (300 MHz, $CDCl_3$): 1.25 (t, 12H, J=7.2 Hz), 3.47 (quart, 8H, J=6.9 Hz), 6.14 (d, 2H, J=2.7 Hz),6.34 (d, 2H, J=2.7 Hz), 7.89 (d, 2H, J=9.0 Hz), 11.4 (s, H), 12.1 (s, H).

1,3-Bis-(4-NN-di-n-butylaminophenyl)-squaraine (1d).

From NN-di-n-butylaniline, gleaming blue crystals(2.45 g, 50 %), m.p. 204-206 ℃.

Elemental analysis: found C 78.40, H 9.20, N 5.60; $C_{32}H_{44}N_2O_2$ requires C 78.65, H 9.07, N 5.73 %. ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (t, 12H, J=7.2 Hz), 1.4 (m, 8H), 1.64 (quint, 8H, J=7.5 Hz), 3.43 (t, 8H, J=7.5 Hz), 6.74 (d, 4H, J=7.5 Hz), 8.34 (d, 4H, J=9.2 Hz).

1,3-Bis-(4-NN-di-n-butylamino-2-hydroxyphenyl)-squaraine (1e).

From3-hydroxy-NN-di-n-butylaniline, metallic blue crystals (1.3 g, 25 %), m.p. 205-208 ℃. $C_{32}H_{44}N_2O_3$ ^{1}H NMR (300 MHz, $CDCl_3$): 0.93 (t, 12H, J=7.0 Hz), 1.40 (m, 8H) 1.63 (quint, 8H, J=6.6 Hz), 3.38 (t, 8H, J=7.2 Hz), 6.12 (s, 2H), 6.34 (d, 2H, J=9.2 Hz) 7.9 (d, 2H, J=9.0 Hz), 11.4 (s, H), 12.1 (s, H).

1,3-Bis-[4-(N-ethyl-N-2-carboxymethylethyl)aminophenyl]squaraine (1f).

From N-ethyl-N-2-carboxymethylethylanilne, blue crystals(3.15 g, 58 %), m.p. 189-191 ℃.

Elemental analysis: found C 68.19, H 6.93, N 5.41 % $C_{28}H_{32}N_2O_6$. requires C 68.0, H 6.90, N 5.68 %.

1,3-Bis-(4-NN-diethylamino-2-acetylamino-phenyl)-squaraine (1g).

From 3-acetylamino-NN-diethylaniline, green crystals(3.5 g, 71 %), m.p. 255-257 °C.

Elemental analysis: found C 68.71, H 7.19, N 11.35 % $C_{28}H_{34}N_4O_4$ requires C 68.55, H 6.99, N 11.42 %. ^{1}H NMR (300 MHz, $CDCl_3$): 1.29 (t, 12H, J=7.0 Hz), 2.36 (s, 6H), 3.54 (quart, 8H, J=7.2 Hz), 6.45 (d, H, J=6.8 Hz), 8.25 (s, 2H), 8.45 (d, 2H, J=7.0 Hz), 8.50 (d, H, J=7.0 Hz), 12.15 (s, 2H).

1,3-Bis-(4-N-benzyl-N-ethylamino-2-acetylaminophenyl)-squaraine (1h).

From 3-acetylamino-N-benzyl-N-ethylaniline, green crystals(3.7 g, 72 %), m.p. 199-201 ℃. $C_{38}H_{38}N_4O_4$ ^{1}H NMR(300 MHz, $CDCl_3$): 1.32 (t, 6H, J=7.0 Hz), 2.32(s, 6H), 3.62(quart, 4H, J=7.2 Hz) 4.70 (s, 4H), 6.46 (d, H, J=2.5 Hz), 6.49 (d, H, J=2.5 Hz), 7.26 (m, 10H), 8.37 (d, 4H, J=9.4 Hz), 8.45 (d, 2H, J=9.0 Hz), 12.1 (s, 2H).

Leuco squaraine dyes (2) : The squaraine dye(3.7 mmol) was dissolved in a mixture of dichloromethane(30 ml) and methanol(5 ml). Sodium borohydride(0.28 mg, 7.4 mmol) was added in small portions to the stirred solution at room temperature. The stirring was continued for a further 2 hours, or until the color of the squaraine dye had completely disappeared. The solvent was then removed on a rotary evaporator and water(15 ml) was added to dissolve in organics. The suspension was neutralized by dropwise addition of glacial acetic acid and the resultant precipitate was filtered off, and dried in a vacuum desiccator over calcium chloride. The product could be purified by dissolution in dichloromethane and chromatography in the same solvent over a short column of silica gel 60. The resultant colorless solids generally oxidized slowly back to the colored squaraine dye at room temperature and were best stored under nitrogen in a refrigerator.

Leuco squaraine (2a). White solid(0.95 g, 80 %), m.p. 88-92 °C. Mass Spectrum (FAB): found M = 322 required for $C_{20}H_{22}N_2O_2$, M = 322.

Leuco squaraine (2b). White solid(0.62 g, 45 %), m.p. 64-66 °C. Mass Spectrum(FAB): found M = 378 required for $C_{24}H_{30}N_2O_2$, M = 378.

Leuco squaraine (2c). White solid(1.0 g, 67 %), m.p. 158-162 °C. Mass Spectrum(FAB): found M = 410(M/Z) required for $C_{24}H_{30}N_2O_4$, M = 410. Elemental analysis: found C 67.4, H 7.40, N 6.45 % $C_{24}H_{30}N_2O_4.H_2O$ requires C 67.3, H 7.5, N 6.5 %.

Leuco squaraine (2d). White solid(0.96 g, 53 %), m.p. 128-130 °C. Mass Spectrum(FAB): found M = 490; required for $C_{32}H_{46}N_2O_2$, M = 490. Elemental analysis: found: C 75.0, H 9.25, N 5.5 %; $C_{32}H_{46}N_2O_2$. H_2O requires C 74.9, H 9.0, N 5.5 %.

Leuco squaraine (2e). White solid(1.55 g, 80 %), m.p. 66-68 °C. Mass Spectrum(FAB): found M = 522; required for $C_{32}H_{46}N_2O_4$, M = 522.

Leuco squaraine (2f). White solid(1.07 g, 55 %), m.p. 155-158 °C. Mass Spectrum(FAB): found M = 526; required for $C_{28}H_{34}N_2O_8$, M = 526. Elemental analysis (%): found C 61.25, H 6.35, N 5.0 % $C_{28}H_{33}N_2O_8.H_2O$ requires C 61.7, H 6.6, N 5.1 %.

Leuco squaraine (2g). White solid(0.91 g, 50 %), m.p. 65-67 °C. Mass Spectrum(FAB): found M = 492(M/Z) required for $C_{28}H_{36}N_4O_4$, M = 492.

Leuco squaraine (2h). White solid(0.6 g, 75 %), 82-85 °C. Mass spectrum(FAB): found M = 616 required for $C_{38}H_{40}N_4O_4$, M = 616.

4. Conclusions

This color-change redox behavior has potential in the area of peroxidase-based bioassays. The discovery of the facile synthesis of the leuco squaraine system and the demonstration that the leuco compounds can undergo a wide range of chemical inter conversions. This then can be oxidized back to a squaraine system, opens up many possibilities for synthesizing new types of squaraine dye not accessible by existing chemical routes.

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