FLUORESCENT SYMMETRIC PHENAZINES FROM NAPHTHOQUINONES

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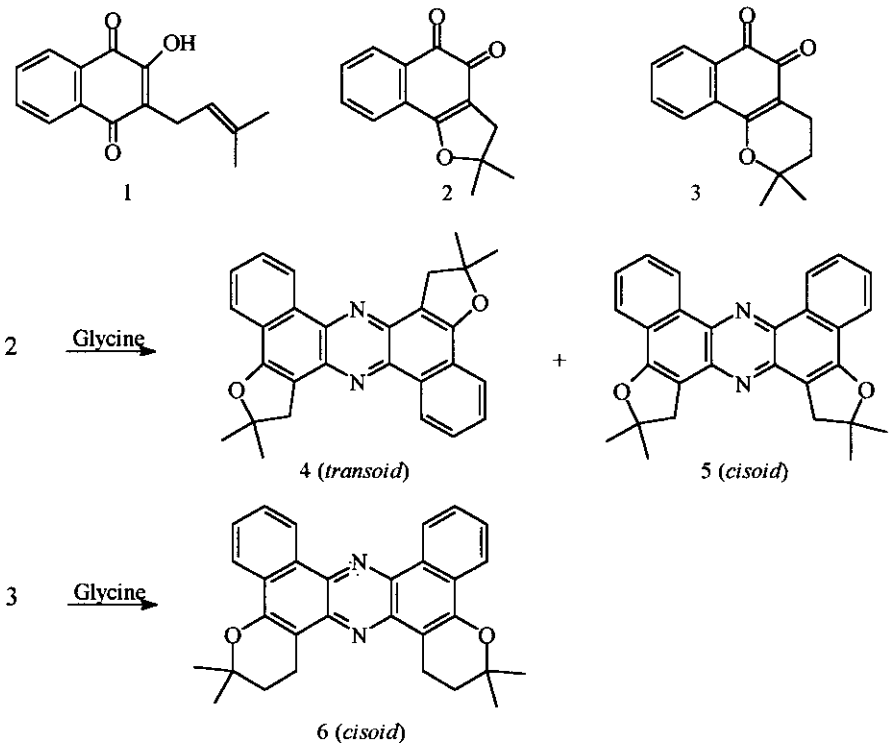
Abstract - Three new symmetric phenazines from the reaction of naphtho-quinones with glycine are characterized spectroscopically. These compounds exhibit high fluorescence emission of potential use to photophysic studies.

Structures containing naphthofurans, naphthopyrans and prenylated naphthalenic quinoidal systems belong to an important class of natural products, occurring mainly in the Bignoneaceae family.[1] There is an interest in studying such quinones because of their wide spectrum of biological activities, such as trypanocidal,[2] virucidal,[3] slight anti-tumor activities[4] and inhibitory activity of DNA topoisomerase I.[5,6] Our interest in these compounds derives both from the point of view of studying their chemical reactivity as well as producing potentially bioactive derivatives for screening studies. In connection with our investigation of the chemistry of biologically active compounds from the Brazilian flora, we have published the reactions of representative compounds belonging to the above described structural systems, such as lapachol (**1**), β-lapachone (**2**) and β-nor-lapachone (**3**), with aminated reagents to lead to new heterocyclic compounds.[7] Of particular interest were the reactions of **2** and **3** with glycine, which produce a time-dependent and very complex mixture of products, from which it was possible to isolate and identify stable naphthyloxazolic type compounds.[6,7] Therefore it was decided to repeat these reactions, focusing on other by-products, which emerge in the low polarity chromatographic fractions in the work-up step.

The reactions of these quinones are run in either aqueous pyridine or aqueous alkaline-ethanol solutions, under water bath conditions, as previously described.[8] Thus, in aqueous alkaline-ethanol, quinone (**2**) yielded the by-products (**4**) (3%) and (**5**) (7%) while **3** gave only **6** (20%). In aqueous pyridine both quinones yielded the above mentioned phenazines only in trace amounts (<0.01%). After removing the solvent the crude products were column-chromatographed. These new by-products are apolar yellow compounds of high R_f value, eluted in the first non-polar fraction on a silica gel column with increasing

solvent polarity (hexane/ethyl acetate). It was noted that these yellow by-products are very fluorescent compounds under ultra-violet light, a fact that has stimulated us to purify and investigate the structure of these compounds, as herein described.

Spectroscopic studies allow us to propose the structures assigned to phenazines (**4, 5** and **6**) as shown in Scheme I. These compounds are easily purified by chromatography, using a silica gel column and eluting with hexane.



Scheme 1. Structure and reactions

The structures of these phenazines are in agreement with their respective spectral data, such as IR, UV, NMR and MS fragmentation patterns. In support of the structures of the phenazines indicated here are their UV data as well their MS, that indicate a higher m/z value for the molecular ions and an isomeric relationship between compounds (**4** and **5**), synthesized from **2**.

The NMR spectral data do agree with the proposed structures, but do not permit distinguishing between the cisoid (C_{2v}) and transoid (C_{2h}) forms. Although it was expected that IR spectra would be capable of distinguishing the isomers (the centrosymmetric **4** was expected to have fewer peaks because there are more prohibited transitions in the point group C_{2h} than in the point group C_{2v}) this turned out not to be the case. Thus the assignments were only established by studying the electronic absorption spectra of the geometrical isomers. Table I shows the absorption and fluorescence spectral data of phenazines (**4-6**),

which indicate a bathochromic shift with increasing solvent polarity, typical of an π-π^* transition. The absorption spectra of both isomers (**4** and **5**) are shown in Figure 1.

It is well known that geometric isomers with extended π systems, in the absence of steric effects, show some striking differences in their spectral profiles. Thus, the *transoid* form characteristically presents a wide absorption band for the π - π^* transition, sometimes with more vibrational fine structure, compared to the *cisoid* form. The latter usually has a more intense band for the same transition.[9] Using these characteristics as a guide line, it was straightforward to assign the *transoid/cisoid* forms to **4** and **5**, respectively, as indicated in Scheme I. As expected, phenazine (**6**) has absorption and emission spectra that closely resemble those of isomer (**5**).

From a mechanistic point of view, the incorporation of nitrogen from glycine into phenazinic structures *via* quinoidal compounds constitutes a very complex sequence of intermediate reaction steps. Though we have not undertaken any thorough studies in this direction , it was observed that the reaction leading to **4**, **5** and **6** only occurs in the presence of oxygen, an excess of oxygen not having any effect. The presence of excess aqueous ammonia also does not have any influence on phenazine formation from glycine.

Interestingly, in a parallel reaction, phenazine (**6**) can also be produced in 25% yield in a complex reaction of **3** with aqueous ammonia in a closed steel cylinder under water bath conditions. This reaction appears to constitute another new type of reaction, but with no relationship to the formation of phenazine by products from glycine. These questions justify further study.

In conclusion, these biologically active quinones[8,10] are reactive toward nucleophilic nitrogen centers, leading to a wide range of new heterocyclic products. Although the formation of phenazines (**4**, **5** and **6**) is not of preparative utility from a synthetic point of view, to our knowledge it does constitute a new type of reaction for quinones and a new approach of some utility to prepare symmetric phenazines. These reactions also revealed new stable fluorescent compounds whose photophysical properties are under study and will be published elsewhere.

Table 1- Absorbance and fluorescence data for phenazines.

SOLVENT	Phenazine (**4**)			Phenazine (**5**)			Phenazine (**6**)		
	$\lambda_{abs.}$ (nm)	log ε	$\lambda_{fluo.}$ (nm)	$\lambda_{abs.}$ (nm)	log ε	$\lambda_{fluo.}$ (nm)	$\lambda_{abs.}$ (nm)	log ε	$\lambda_{fluo.}$ (nm)
Cyclohexane	487	4.289	489	452	4.798	454	432	4.883	435
Chloroform	488	4.542	511	456	4.588	470	436	4.610	446
Isopropanol	484	3.879	493	451	4.410	469	432	4.454	437
Acetonitrile	487	3.748	510	454	4.398	468	435	4.395	445

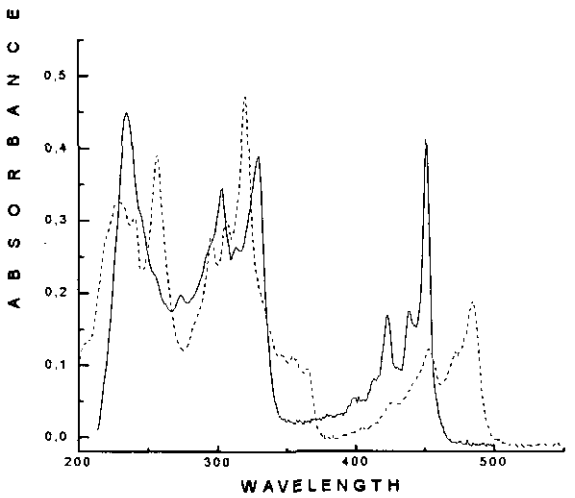


Figure 1- Absorbance spectra of phenazines **(4)** (1.07 x 10^{-5} M) (- - -) and **(5)** (5.95 x 10^{-6} M) (—)

EXPERIMENTAL

Procedures for **4-6**: To 1 mmol of **2** (or **3**) in a mixture of ethanol (20 mL)/$NaHCO_3$ (15 mL, 1%), or 25 mL of aqueous pyridine (1:9) was added glycine (10 mmol), followed by reflux for 24 h (or 16 h), respectively. After cooling, the solvents were vacuum evaporated and the residues were chromatographed in a silica gel column starting with hexane as eluent. In preparing **6,** the compound is found in the hexane fraction. In the case of **4** and **5**, these isomers are found in the hexane/ethyl acetate gradient, corresponding to 0.5 % and 1.0 %, respectively. Phenazine **(6)** can also be obtained from the reaction of **3** (1 mmol) and 15 mL of concentrated aqueous ammonia in a sealed glass ampoule in a water bath for 10 h. After this the ampoule was opened and the reaction mixture was diluted with 20 mL of ethanol and air continuously bubbled for 2 h, followed by vacuum distillation. The residue was chromatographed on a silica gel column, which permits the isolation of **6** from the hexane fraction.

Physical and spectral data to 4, 5 and 6:

1,1,9,9-Tetrahydro-2,2,10,10-tetramethyldibenzo[*e,l*]difuran[*b',c-b',j*]phenazine (4).

mp 307°C (decomp); IR (KBr): 3060; 2960; 2910; 2840; 1120; 1590; 1520; 1490; 1460; 1450; 1420; 775; 750 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.55 (1H, m); 8.13 (1H, m); 7.78 (2H, m); 3.60 (2H, s); 1.72 (6H, s) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 157.693(s); 136.401(s); 133.320(s); 128.954(d); 128.389(d); 126.015(d); 124.416(s); 122.984(d); 114.351(s); 90.543(s); 42.695(t); 29.736(q) ppm. MS (70eV, m/z (%)):420 (79); 405 (100). HRMS calcd for $C_{28}H_{24}N_2O_2(M^+)$: 420.183778. Found: 420.183431. *Anal.* Calcd for $C_{28}H_{24}N_2O_2$: C, 79.98 ; H, 5.75 ; N, 6.67. Found : C, 80.10; H, 5.88; N, 6.81 .

1,1,14,14-Tetrahydro-3,3,13,13-tetramethyldibenzo[*e,j*]difuran[*b',c-b',l*]phenazine (5).

mp 247°C (decomp). IR (KBr): 3060; 2960; 2910; 2850; 1630; 1600; 1525; 1455; 1415 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.40 (1H, m); 8.10 (1H, m); 7.78 (2H, m); 3.70 (2H, s); 1.72 (6H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 155.496(s); 139.092(s); 131.885(s); 129.120(d); 127.475(d); 125.798(d); 124.745(s); 122.370(d); 114.404(s); 89.752(s); 42.281(t); 29.187(q) ppm. MS (70eV, m/z (%)):405(71); 420(100). HRMS calcd for $C_{28}H_{24}N_2O_2(M^+)$: 420.183778. Found: 420.183850. *Anal.* Calcd for $C_{28}H_{24}N_2O_2$: C, 79.98; H, 5.75; N, 6.67. Found: C, 80.08: H, 5.85; N, 6.78.

1,1,2,2,10,10,11,11-Octahydro-3,3,12,12-tetramethyl-3*H*,12*H*-dibenzo[*e,j*]dipyran[*b',c-b',l*]phenazine (6).

mp 236°C (decomp). IR (KBr): 3070; 2980; 2930; 1615; 1595; 1565; 1525; 1480; 1460; 1445; 1415; 775; 750 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.50 (1H, m); 8.35 (1H, m); 7.75 (2H, m); 3.38 (2H, t, J=1 Hz); 2.08 (2H, t, J=1 Hz); 1.55 (6H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 150.581(s); 142.698(s); 134.680(s); 130.895(s); 127.881(d); 126.947(d); 124.236(d); 121.777(d); 110.196(s); 75.533(s); 32.520(t); 26.772(q) ; 18.222(t); ppm. MS (70eV, m/z (%)): 448 (100); 433 (10); 405 (45); 393 (46); 392 (40); 378 (10); 377 (36); 350 (9); 349 (30); 337 (35); 336 (22). HRMS calcd for $C_{30}H_{28}N_2O_2(M^+)$: 448.215078. Found: 448.215084. *Anal.* Calcd for $C_{30}H_{28}N_2O_2$: C ,80.33; H, 6.29; N, 6.24. Found: C, 80.09; H, 6.47: N, 6.06.

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