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Heterocyclic derivatives from natural occurring naphthoquinones: synthesis, characterization and X-ray structure of beta-lapachone hydrazo compounds

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Abstract

β-Lapachone-5-phenylhydrazone and β-lapachone-6-phenyl-hydrazone were synthesised and characterised using ^{1}H and ^{13}C NMR, IR, and UV–visible and X-ray analyses. ^{1}H NMR and UV–visible spectroscopic data indicate that the hydrazo form dominates in solution. Similarly, X-ray crystallographic data indicated that the hydrazo form exists exclusively in the solid state.

Keywords: Hydrazo-derivatives; Naphthoquinone; β-Lapachone; Molecular probes

1. Introduction

It is known that azo dyes are produced in the greatest volume among synthetic dyes available worldwide and their dominance may increase in the future [1]. In addition, it can be seen from the Color Index that over 50% of the listed dyes belong to the azo class [2]. Besides their use as dyes, azo compounds are useful as antiseptics for genitourinary infections and as staining media for tumors and microorganisms [3]. The azo dye Trypan Red was used for many years in veterinary medicine as an antiprotozoal agent [4].

β-Lapachone (2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione; **1**, Fig. 1) is an *ortho*-naphthalenic type quinone isolated from plant extracts of *Tabebuia avellanedae*. Though it was first prepared in 1882 by a sulfuric acid treatment of Lapachol [5] and characterised in 1896 [6], derivatives of β-lapachone have been seldom studied.

Nowadays there is a significant interest in β-lapachone, due to its extremely broad biological activity. The pharmacological activities of this quinone includes antibacterial, antifungal, tumor cell growth inhibition, reduction of HIV-1 replication, suppression of both acute and chronic infections, induction of chromosomal alterations, modification of topoisomerase-I activity, and this

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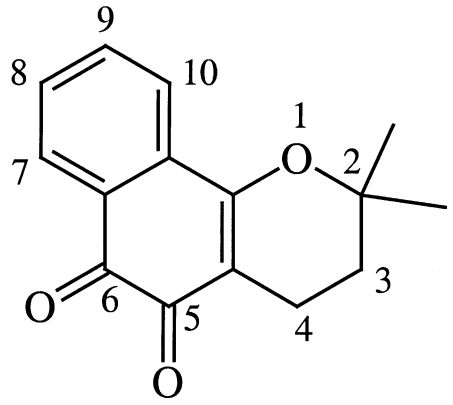

Fig. 1. Structure of β-lapachone.

quinone has potential clinical utility in the treatment of human leukemia and prostate cancer [7–15]. In addition, it inhibits the reverse transcriptase activity of both avian myeloblastosis and Rauscher murine leukemia viruses and induces apoptosis in human prostate cancer cells in vitro [16,17]. In a more recent study, a combination of β-lapachone and taxol combined synergistically to induce cell death in a variety of human carcinoma cells, including ovarian, breast prostate, melanoma, lung, colon and pancreatic cells [18].

Despite the broad spectrum of biological activity observed for β-lapachone, its mechanism(s) of action remain unclear. In this regard, its trypanosomic activity has been studied extensively and is thought to arise from the formation of reactive oxygen species that can damage DNA. Similarly, its antibacterial and cytotoxic activities have also been linked to the formation of reactive oxygen species [19,20]. Like many cytotoxic agents, β-lapachone induces high frequencies of chromosomal aberrations and sister chromatic exchange. Consequently, it has been suggested that there is a critical level of β-lapachone in the G_2 phase of the cell cycle that induces the release of mitochondrial cytochrome C in both apoptosis and necrosis in human carcinoma cells [21,22]. It has been shown that β-lapachone acts as an inhibitor of topoisomerase-I catalytic activity, without inducing a topoisomerase-I cleavable complex [23]. The results of anti-cancer studies showed that β-lapachone activity might involve the mitochondrial cytochrome-*C* oxidase pathway and that cytochrome *C* is involved in the early phase of the cell necrosis [24]. Also, β-lapachone is a potent inhibitor of DNA repair [25].

Chemical studies involving β-lapachone indicate that the chemical reactivity of the *ortho* quinoidal moiety is useful in the synthesis of various heterocyclic structures [26–28]. Due to the wide scope of activity associated with β-lapachone and the need for an approach to cell monitoring of its metabolic pathway, the study of its hydrazones is of interest. Also, dyes of this type can serve as molecular probes for the microenvironment surrounding the associated binding sites.

2. Experimental

2.1. General

Melting points were obtained using a Koffler Apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Unityplus-300 spectrometer. All chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. IR spectra were recorded on a Nicolet FTIR-760 Magna spectrophotometer and mass spectra were recorded on a Varian Micromass MAT-SS-100MS spectrometer.

2.2. Synthesis

β-Lapachone was obtained by a sulfuric acid treatment of lapachol (C.I. Natural Yellow 16) [5].

2.3. Phenylhydrazones of β-lapachone

Phenylhydrazine hydrochloride (0.145 g, 1 mmol) was added all at once to a solution of β-lapachone (0.242 g, 1 mmol) in dry methanol (15 ml) and the mixture was stirred at room temperature (~27 °C) for 27 h. The resultant orange-yellow solution was concentrated and the residue was washed with water. The product was purified by column chromatography using silica gel G and CH_2Cl_2. This gave a 3% yield of the 5-phenylhydrazone (dye **2**); mp: 165 °C. IR (KBr): 1608 (w), 1580 (w), 1570 (w), 1495 (w), 1212 (m), 1150 (m) cm^{-1}.

Also obtained from the column was the 6-phenylhydrazone (dye **3**) (orange fraction) in 75% yield; mp: 196–197 °C; JR (KBr): 1609 (w), 1592 (s), 1505 (s), 1390 (s), 1145 (m) cm^{-1}.

2.3.1. Single crystal X-ray diffraction

An orange crystal (~0.08×0.09×0.28 mm) of dye **3** was selected and mounted on a Bruker SMART 1000 CCD area detector diffractometer and analysed (Mo K_α radiation, $\lambda = 0.71073$ Å, $T = 25 \pm 2$ °C). Following routine data collection, the structure model was developed in space group $P2_12_12_1$ (No. 19). H-atoms were positioned geometrically [$d(X\text{-H}) = 1.00$ Å] and refined by attachment to their appropriate N- or C-atoms.

3. Results and discussion

3.1. ^1H and ^{13}C NMR spectra

Tables 1 and 2 provide a summary of the results obtained from the analysis of NMR spectra of dyes **2** and **3** (Fig. 2). We found that the aromatic carbons of dyes **2** and **3** resonate in very narrow ranges of chemical shifts. Consequently, a correlation of

Table 1
^1H and ^{13}C data for dye **2** in $CDCl_3$

H/C	δ (^1H)	δ (^{13}C)	H/C	δ (^1H)	δ (^{13}C)
1	–	143.16	1′	–	142.40
2	–	110.32	2′	7.48	115.77
3	–	130.09	3′	7.38	129.37
4	–	178.17	4′	7.11	124.20
5	8.32	122.00	5′	7.38	129.37
6	7.43	126.58	6′	7.48	115.77
7	7.64	132.74			
8	7.94	128.18			
9	–	133.87			
10	–	132.10			
11	2.87	18.15			
12	1.90	32.62			
13	–	74.55		H(N)	15.67
14	1.43	26.54			
15	1.43	26.54			

Table 2
^1H and ^{13}C data for dye **3** in $CDCl_3$

H/C	δ (^1H)	δ (^{13}C)	H/C	δ (^1H)	δ (^{13}C)
1	–	159.78	1′	–	142.71
2	–	110.92	2′	7.55	115.98
3	–	179.35	3′	7.40	129.37
4	–	124.37	4′	7.14	124.50
5	8.37	121.51	5′	7.40	129.37
6	7.49	125.82	6′	7.55	115.98
7	7.36	128.91			
8	7.94	122.56			
9	–	132.77			
10	–	127.70			
11	2.67	16.14			
12	1.89	31.81			
13	–	77.18			
14	1.45	26.65		H(N)	16.28
15	1.45	26.65			

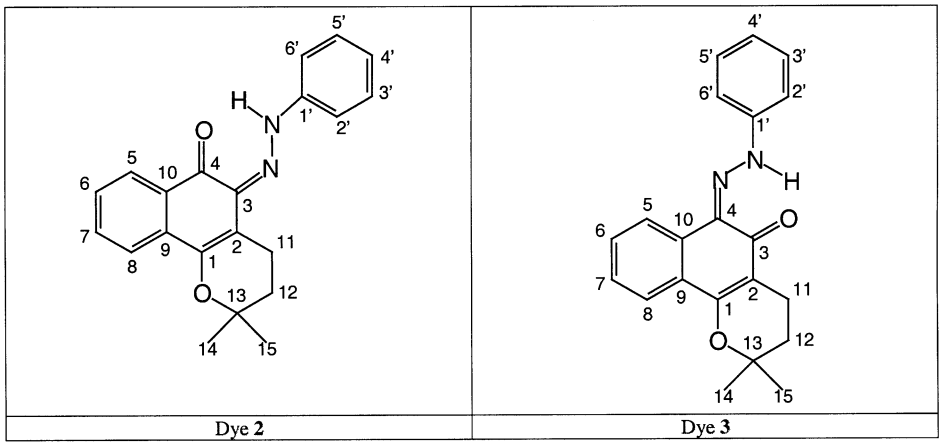

Fig. 2. Structures of hydrazones **2** and **3** with carbon numbering used in NMR assignments.

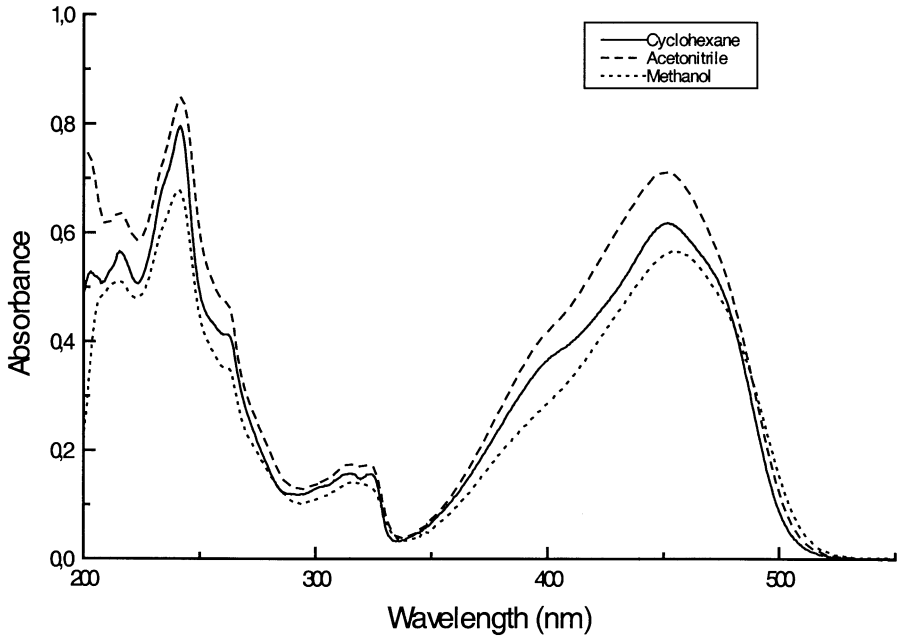

Fig. 3. Absorption spectra of 1.25×10^{-5} M dye **3** in cyclohexane, acetonitrile and methanol.

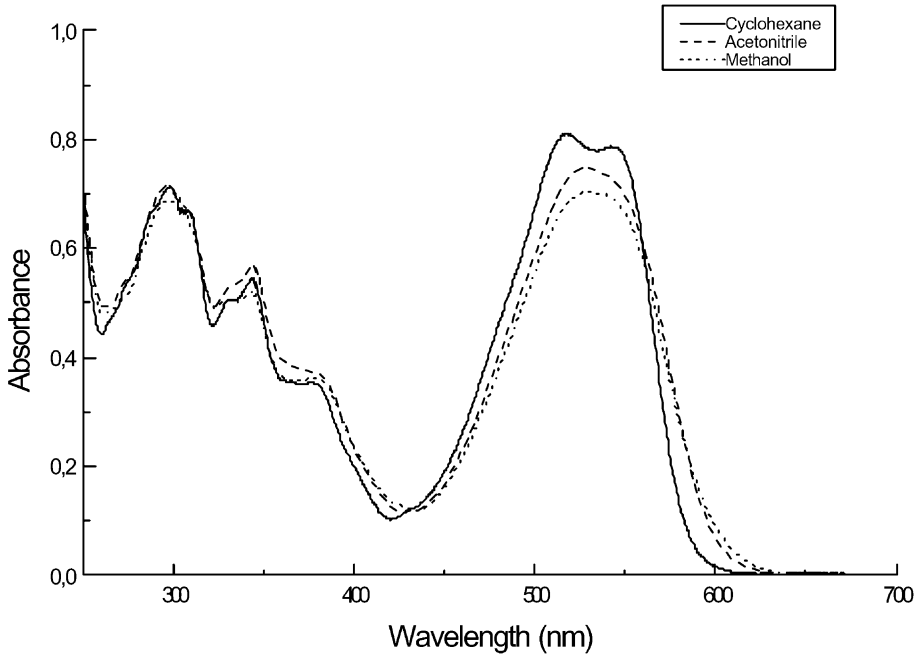

Fig. 4. Absorption spectra of 1.25×10^{-5} M dye **2** in cyclohexane, acetonitrile and methanol.

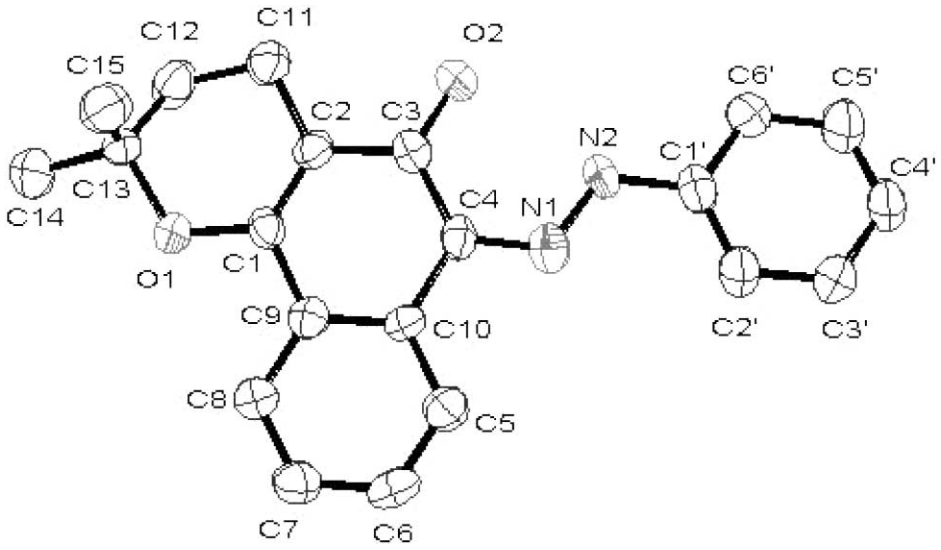

Fig. 5. Crystal structure of dye **3** (50% thermal ellipsoids).

their chemical shifts with those assigned to the C-atoms in β-lapachone **1** spectra and the use of 2-D NMR techniques (H–H and H–C COSY) were necessary for spectral assignments. Despite the fact that azo-hydrazone tautomerism may in principle occur in solution, no evidence for the existence of hydroxyl protons was produced in $CDCl_3$. Not only was a very low concentration of the azo form evident in solution it also appeared that the relaxation time of acidic proton was very short. Therefore, the associated very broad signal was below the background noise level. The chemical shifts for the C-atoms of the phenyl group have been used for semi-quantitative characterization of azo-hydrazone equilibria. In this regard, it was reported [29,30] that compounds existing in the hydrazone form also exhibit relatively constant chemical shift values for C-1′ to C-4′, which is in agreement with the results obtained in this work.

3.2. UV–visible spectra

It is clear from the results of previous studies involving azo-hydrazone tautomerism that the nature of the environment employed determines whether one form is favored over another [31–34]. The compounds studied in this work, exhibit differences in the distribution of tautomeric forms upon solvent changes, as would be anticipated. Dye **3** gave absorption bands that are typical of the azo (400 nm, shoulder) and hydrazone (454 nm) forms. Changes in the intensities of the two bands in solvents of different polarities can be seen in Fig. 3. The sensitivity of dye **2** to solvent polarity was much more evident. In cyclohexane, absorption maxima attributable to the azo and hydrazone forms were observed at 501 nm and 552 nm, respectively. When solvent polarity was increased for (e.g. CH_3CN and CH_3OH) the azo-hydrazone equilibrium shifted to the hydrazo form (cf. Fig. 4).

3.3. X-ray crystal structure

To firmly establish the position of the hydrazino moiety in the β-lapachone derivative, X-ray analysis was performed on the major product of the condensation reaction between **1** and phenylhydrazine. The results in Fig. 5 indicate dye **3** was the major reaction product and that the hydrazone tautomer was the only form present in the solid state. Tables 3 and 4 provide crystal cell data and selected bond distances and angles for this dye.

Table 3
X-ray crystal cell data for dye **3**

Composition	$C_{21}H_{21}N_2O_2$
M_r	332.39
Crystal system	Orthorhombic
Space group	$P2_12_12_1$ (No. 19)
a, Å	5.8302(6)[a]
b, Å	11.9175(12)[a]
c, Å	24.724(3)[a]
α, °	90
β, °	90
γ, °	90
V, Å^3	1717.9
Z	4
T °	25±2
R(F)	0.037
wR(F)	0.037

Table 4
Selected bond distances and bond angles for dye **3**

Atoms	Distance (Å)	Atoms	Angle (degree)
C3–O2	1.257 (6)	C4-N1-N2	118.3 (5)
C3–C4	1.456 (7)	C3-C4-N1	125.3 (5)
C4–N1	1.334 (6)	O2-C3-C4	120.0 (5)
N1–N2	1.322 (6)	N1-N2-C1'	117.8 (5)
N2–C1′	1.408 (6)		

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