Available online at www.sciencedirect.com
ScienceDirect
Dyes and Pigments 77 (2008) 111–117
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Synthesis and spectroscopic characterization of 4-carboxyl-2,6-dinitrophenylazohydroxynaphthalenes

Olajire A. Adegoke, Olakunle S. Idowu*, Ajibola A. Olaniyi

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Orita UI, Ibadan, Oyo State, Nigeria

Received 5 December 2006; received in revised form 23 March 2007; accepted 24 March 2007
Available online 27 April 2007

Abstract

A series of azo dyes was prepared by the reaction of 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) with two naphthols and three substituted naphthalene ether derivatives. UV, IR, ^{1}H, ^{13}C and 2D-NMR spectroscopy as well as mass spectral analyses were used to establish the structure of the new azo dyes.

α-Naphthol and its ether gave *para*-substituted azo dyes while β-naphthol and its ethers provided *ortho*-substituted dyes. Dealkylation of the naphthalene ether linkage was found to occur upon coupling with the diazonium ion, to form the corresponding naphthol in the final azo product.

Keywords: CDNBD; Azo dyes; Synthesis; Hydroxynaphthalenes; Dealkylation; Spectroscopic studies

1. Introduction

Azo dyes constitute the largest group of commonly available dyes and pigments [1]. Due to their colour, azo dyes are used as pigments [2], indicators of solvent polarity [3], of molecular environments and of chemical environments [4]; they are also widely used as histological stains [5] and in the colorimetric analysis of pharmaceuticals [6–8]. We have demonstrated that the arenediazonium, 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) is a useful reagent for the instrumental chemical analysis of drugs [9–13]. Its usefulness as an intermediate in the synthesis of a novel series of hydroxynaphthalenes azo dyes is reported in this paper.

2. Results and discussion

The reaction scheme for the synthesis of the five phenylazohydroxynaphthalenes is shown in Fig. 1. CDNBD has been shown to react with naphthols and phenol ethers; the reaction with the latter points to its high reactivity. Phenol ethers are characterized by the presence of ether linkage in addition to the aromatic ring. The alkoxyl group −OR is *ortho*, *para* directing towards electrophilic aromatic substitution and is classified as moderately to weakly activating. Diazo coupling is rare with such ethers except for highly reactive 2,4-dinitrodiazobenzenes which form azo compounds with anisole and phenethole [14]. The *para*-position to the alkoxyl group must be free and coupling is most facile when a methyl group occupies both *meta*-positions to the alkoxyl group i.e. the configuration is that of mesitylene with one methyl group replaced by alkoxyl. The notable characteristic of this coupling is, however, that it proceeds with partial or complete removal of the alkyl group of the phenol ethers, a remarkable occurrence because phenol ethers are by no means easily hydrolyzed. The complete scission of ether linkage has been observed with propranolol, an aryloxy ether giving α-naphthol as a residual that coupled with CDNBD.

Some physicochemical properties and IR vibration bands are presented in Table 1. The ^{1}H and ^{13}C NMR spectra of compounds AZ-01 to AZ-04 were measured and analysed; two-dimensional NMR spectra were used with the aim of assigning proton and carbon chemical shifts unambiguously. $^{1}H-^{1}H$

* Corresponding author. Tel.: +2348058427072.
E-mail address: olakunleid@yahoo.com (O.S. Idowu).

0143-7208/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2007.03.014

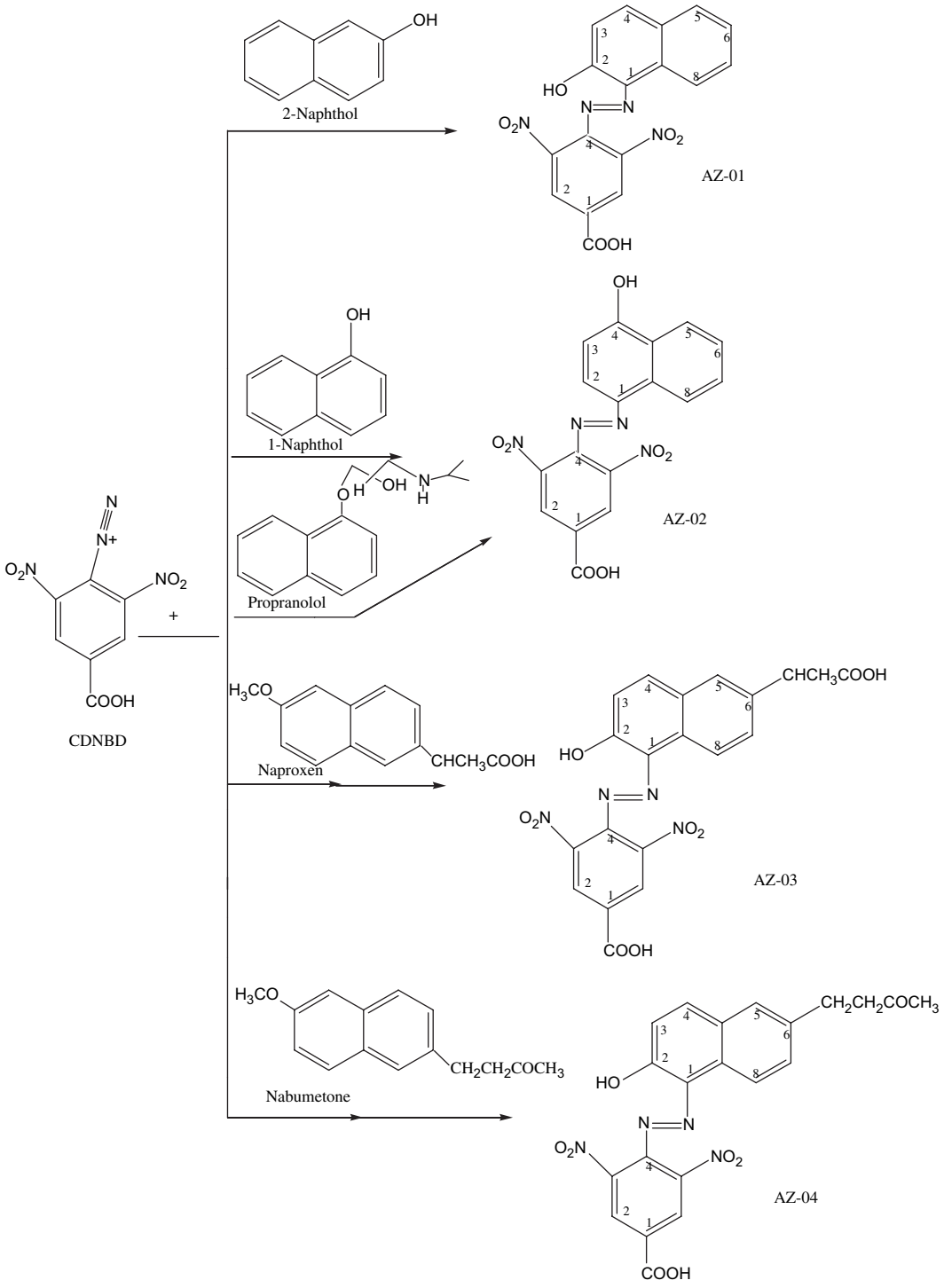

Fig. 1. Synthesis of 4-carboxyl-2,6-dinitrophenylazo hydroxynaphthalenes.

COSY, HMQC and HMBC techniques were applied in the cases of AZ-02 to AZ-04 so as to clearly assign their protons and carbons. The ^{1}H and ^{13}C chemical shifts are collated in Tables 2–5.

As the solubilities of AZ-02 to AZ-04 were very low in common NMR solvents, spectral acquisitions were carried out using deuterated pyridine (d_5). Chemical shift values are referred to the signal of pyridine {$\delta = 8.74$ (^{1}H, H-2) and 150.35 (^{13}C, C-2)}.

The azo dyes obtained from propranolol, naproxen and nabumetone (AZ-02, 03 and 04, respectively) have been used for colorimetric assay [15] while AZ-01 has been evaluated as a pH indicator for weak acid/strong base titration [16].

AZ-01 gave a blood-red azo dye with characteristic absorption maxima at 260 and 470 nm (molar absorptivities in ethylacetate at the two wavelengths were 1.46×10^4 and 1.68×10^4 mol^{-1} dm^3 cm^{-1}, respectively). The compound melted

Table 1
Physicochemical characterization of 4-carboxyl-2,6-dinitrophenylhydroxynaphthalenes

Comp. no.	Colour	Yield (%)	M.P. (°C)	MS data (*m/z*)	Absorption spectra in EtOAc		IR spectrum (KBr) (cm^{-1})
					λ_{max} (nm)	ε_{max} ($mol^{-1}\,cm^2$)	
AZ-01	Blood-red	63.75	275–278	Molecular ion not obtained	260	1.46×10^4	1360–1470 (N=O_{str}) 1600–1700 (Three aromatic rings)
					470	1.68×10^4	1700–1800 (C=O_{str}) 2922 (Carboxylic OH)
AZ-02	Orange	62.84	207–209	382.2	260[a]	8.55×10^6	1161, 1275 (1,4-Substitution) 1701 (C=O_{str})
					440	2.28×10^5	1623 (Aromatic N–O_{str}) 3258 (Bonded OH_{str}) 3430 (OH_{str}, free)
AZ-03	Orange	63.90	187–189	454	260	1.51×10^7	1541 (N····O_{str}) 1724 (C=O_{str})
					470	1.34×10^7	2722 (Phenolic O–H_{str}) 3400 (Carboxylic O–H_{str})
AZ-04	Reddish-orange	72.49	191–193	452.08	260	1.71×10^7	1507 (N=O_{str}) 1614 (C=O_{str}, ketone)
					470	1.20×10^7	1704 (C=O_{str}, carboxylic acid) 3427 (O–H_{str} bonded)

[a] UV data for propranolol-derived AZ-02 are 260 nm and 440 nm (ε_{max} 1.54×10^7 and 2.32×10^7, respectively).

with decomposition at 275–278 °C. Characteristic IR vibration bands were observed at 1600–1700 cm^{-1} (three aromatic rings) with absence of bands at 3000–3500 cm^{-1} (showing the lack of an amino group). The most distinguishing feature of the IR spectrum of AZ-01 was the fingerprint region which showed several sharp peaks at 1300, 1157 and 1136 cm^{-1}, all of which differed from the starting materials. ^1H and ^{13}C chemical shifts for the protons and carbons are shown in Table 2. The mass spectrum gave no molecular ion, probably due to the ready cleavage of the azo linkage in the 70 eV ionization technique. Other daughter ions obtained were at *m/z* 171, 143 and 115.

Table 2
^1H and ^{13}C chemical shifts of 4-[(2-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-01)

C/H no.	δ (^1H)[a]	*J* (^1H–^1H)	δ (^{13}C)
CDNBD residue			
1	–		134.24
2,6	8.85 (2H)		–
3,5	–		139.35
4	–		145.34
*C*OOH	>12 (s)		165
Naphthalene residue			
O*H*	3.50	–	–
2	–	–	181.96
3	6.75 (1H)	d, $^3J_{3,4} = 8.62$ Hz	124.92
4	8.05 (1H)	d, $^3J_{4,3} = 8.62$ Hz	126.47
5	7.91 (1H)	d, $^3J_{5,6} = 7.5$ Hz, $^4J_{5,7} = 2.2$ Hz	129.47
6	7.65 (1H)	m, $^3J_{6,5} = {}^3J_{6,7} = 6.5$ Hz; $^4J_{6,8} = 2.2$ Hz	130.01
7	7.70 (1H)	m, $3J_{7,8} = {}^3J_{7,6} = 6.5$ Hz; $^4J_{7,5} = 2.2$ Hz	130.25
8	7.81 (1H)	d, $^3J_{8,7} = 7.5$ Hz; $^4J_{8,6} = 2.2$ Hz	131.70

[a] Integrals in parentheses.

AZ-02 was obtained as an orange azo dye with absorption maxima at 260 and 440 nm in ethyl acetate ($\varepsilon = 8.5494 \times 10^6$ and 2.2841×10^5 $mol^{-1}\,dm^3\,cm^{-1}$, respectively). Similar UV absorption patterns to the propranolol adduct were obtained ($\varepsilon = 1.5377 \times 10^7$ and 2.3159×10^7 $mol^{-1}\,dm^3\,cm^{-1}$ at 260 and 440 nm, respectively). Both azo dye species melted at 207–209 °C with decomposition. TLC analysis also revealed the presence of spots with similar R_f values. On examination of the ^1H NMR spectra of the azo adduct of propranolol with CDNBD, complete disappearance of peaks in the aliphatic region was observed. The IR spectra of both azo dyes showed principal peaks at 2655, 3065, 3258 (bonded O–H_{str}), and 3430 cm^{-1} (O–H_{str}, free). This superimposability of the IR spectra of both azo dyes suggests similarity of the structures. The presence of sharp vibrational bands at 1161 and 1275 cm^{-1} signifies the likelihood of 1,4-substitution [i.e. substitution of CDNBD at the 4-position of either α-naphthol or propranolol]. The ^1H and ^{13}C chemical shifts for the protons and carbons of AZ-02 are presented in Table 3. The mass spectra for both species of AZ-02 produced molecular ions at 381.2 for the α-naphthol-derived azo dye (negative electrospray) and 383 for the propranolol-derived azo dye (positive electrospray), thereby establishing the M_r of AZ-02 as 382.2.

AZ-03 was obtained as an orange-coloured azo dye with a melting point of 187–189 °C (with decomposition). UV absorption maxima occurred at 260 and 470 nm in ethyl acetate ($\varepsilon = 1.5119 \times 10^7$ and 1.3426×10^7 $mol^{-1}\,dm^3\,cm^{-1}$, respectively). The IR spectra showed a prominent O–H_{str} at 3400 cm^{-1} which appears to be hydrogen bonded. However, the absence of a typical alkyl aryl ether vibrational band around 1604.48 cm^{-1} demonstrates its scission on coupling

Table 3
^{1}H, ^{1}H–^{1}H COSY (400 MHz, pyridine d_5) and HMBC (400 MHz, pyridine d_5) data for 4-[(4-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-02)

C/H no.	δ (^{1}H)[a]	(^{1}H–^{1}H)	δ (^{13}C)	HMBC	
				^2J	^3J
Naphthalene residue					
1	–	–	141.26		
2	8.19 (8.16)	7.02 (7.00)	–	141.26	13.54
3	7.02 (7.00)	8.19 (8.16)	119.20	173.6	128.59, 141.26
4	–	–	173.6	–	–
5	8.54 (8.54)	7.57 (7.58)	125.37	–	135.54
6	7.57 (7.58)	7.74 (7.74), 8.54 (8.54)	127.99	131.55	128.59
7	7.74 (7.74)	8.82 (8.81), 7.57 (7.58)	131.55	–	135.54
8	8.82 (8.81)	7.74 (7.74)	123.45	141.26	135.54
CDNBD residue					
1	–	–	173.00	–	–
2,6	9.05 (9.07)	–	130.02	–	–
3,5	–	–	143.27	–	–
4	–	–	141.62	–	–
*C*OOH	–	–	166.05	–	–

[a] δ Values for propranolol-derived AZ-02 in parentheses.

with the appearance of a phenolic O–H_{str} at 2722.41 cm^{-1} which was not present in naproxen reference. The ^{1}H NMR spectra confirmed the absence of resonance due to the methoxyl protons. Unambiguous assignments of proton and carbon signals were made using ^{1}H–^{1}H COSY, ^{13}C and HMBC spectra (Table 5). The mass spectrum was obtained using

Table 4
^{1}H, ^{1}H–^{1}H COSY (400 MHz, pyridine d_5) and HMBC (400 MHz, pyridine d_5) data for 4-[(7-(1-carboxyethyl)-2-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-03)

C/H no.	δ (^{1}H)	^{1}H–^{1}H	δ (^{13}C)	HMBC	
				^2J	^3J
Aliphatic side chain					
1	4.09	1.73	46.57	–	–
2	1.73	4.09	19.26	–	176.73
3	–	–	176.73	–	–
Naphthalene residue					
1	–	–	135.42	–	8.34, 7.64
2	–	–	182.99	–	6.62
3	6.62	7.64	124.23	182.99	135.80, 135.42
4	7.64	6.62	125.27	135.80	132.01, 182.99
5	7.70	4.09	129.80	46.57	131.06
6	–	–	144.36	–	–
7	7.79	8.34	131.06	–	129.80, 132.01
8	8.34	7.79	127.47	132.01	135.80, 135.42, 144.36
CDNBD residue					
1	–	–	166.33	9.19	
2,6	9.19	–	131.52	140.62	128.79
3,5	–	–	140.62	9.19	–
4	–	–	128.79	–	9.19
*C*OOH	–	–	176.73	–	9.19

Table 5
^{1}H, ^{1}H–^{1}H COSY (400 MHz, pyridine d_5) and HMBC (400 MHz, pyridine d_5) data for 4-[(2-hydroxy-7-(3-oxobutyl)naphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-04)

C/H no.	δ (^{1}H)	^{1}H–^{1}H	δ (^{13}C)	HMBC	
				^2J	^3J
Aliphatic side chain					
1	3.00	–	44.80	–	2.81
2	2.81	–	30.20	–	3.00
3	–	–	207.10	–	–
4	2.13	–	30.08	–	–
Naphthalene residue					
1	–	–	130.70	–	6.61
2	–	–	183.17	–	7.62
3	6.61	7.62	125.23	127.44	130.70
4	7.62	–	127.44	–	–
5	7.34	–	130.30		3.00, 7.62, 7.40
6	–	–	144.06	–	2.81, 8.24
7	7.40	7.34, 8.24	131.63	–	3.00
8	8.24	7.40	145.06	–	7.40, 3.00
CDNBD residue					
1	–	–	136.18	9.16	–
2,6	9.16	–	131.60	9.16	166.15
3,5	–	–	140.56	9.16	–
4	–	–	127.94	–	–
*C*OOH	–	–	166.15	–	9.16

negative electrospray ionization technique and gave a molecular ion at *m/z* of 453; thus the M_r of AZ-03 was 454.

The azo dye obtained from nabumetone (AZ-04) had a melting point of 191–193 °C; UV absorption maxima occurred at 260 and 470 nm in ethyl acetate ($\varepsilon = 1.7145 \times 10^7$ and 1.2009×10^7 mol^{-1} dm^3 cm^{-1}, respectively).

Principal IR peaks for nabumetone were recorded at 1625 (s), 1550 cm^{-1} (s) as well as 1614.48, 1703.97 (s), 3426.63 (b) and 1507 cm^{-1} (s) for the azo adduct. The IR spectra of both compounds showed the presence of ketonic C=O_{str} at 1625 cm^{-1} (for nabumetone) and 1614.48 cm^{-1} (for the azo adduct). Particularly diagnostic was the absence of a peak at 1550 (s) in the adduct, which is characteristic of alkyl aryl ethers. Both C=O_{str} (carboxylic acid at 1703.97 cm^{-1}) and OH_{str} (3426.63 cm^{-1}) were present in the adduct which can be attributed to the carboxylic acid fragment of CDNBD. Likewise, an N=O_{str} at 1507 (s) indicates the presence of nitro substituents on the nabumetone adduct which is not present in nabumetone. The ^{1}H NMR spectra of nabumetone and its azo dye revealed the complete absence of a signal due to the methoxyl protons in the azo dye.

^{1}H NMR gave the following chemical shift values and integrals (^{1}H, 400 MHz C_5D_5N): δ 2.13 (3H, s, aliphatic H-4), 2.81 (2H, t, J 14.84 Hz, aliphatic H-2), 3.00 (2H, t, J 14.84 Hz, aliphatic H-1). On the naphthalene residue: 6.61 (1H, d, J 9.76 Hz, H-3), 7.34 (1H, s, H-5), 7.40 (1H, dd, J 9.68, 1.48 Hz, H-7), 7.62 (1H, H-4), 8.24 (1H, d, J 8.16 Hz, H-8) and on the CDNBD residual: 9.16 (2H, s, H-2 and H-6). ^{13}C NMR (100 MHz, C_5D_5N): δ 30.08 (aliphatic C-4), 207.1 (C-3), 30.2 (C-2), 44.8 (C-1). On the naphthalene

residue: 131.63 (C-7), 144.06 (C-6), 130.3 (C-5), 127.44 (C-4), 125.23 (C-3), 183.17 (C-2), 130.7 (C-1), 145.06 (C-8) and on the CDNBD residual: 127.94 (C-4), 140.56 (C-3, C-5), 131.6 (C-2, 6), 136.15 (C-1) and 166.15 (COOH).

A notable observation was the absence of a signal due to the methoxyl protons centered at 3.817 ppm which was absent in the proton NMR spectra of the azo dye. Such dealkylation has been observed to occur when phenol ethers couple with highly reactive diazonium ions. The mechanism is not clear but scission appears to occur after coupling and is favored by the use of an acidic medium [17]. It is most likely that stability problems might account for dealkylation as resonance stabilization may be better in the case of −OH rather than −OR. Three sets of signals were therefore observed in the aliphatic region compared to four in the spectra of the nabumetone reference. Five sets of signals were observed in the aromatic region due to the five residual protons on the naphthalene ring imparted by substitution. The sixth aromatic signal was due to protons attached to the CDNBD molecule and occurred as a singlet with an integral of 2 at 9.16 ppm. The $J_{\text{modulated}}$ ^{13}C NMR spectrum showed a total of 18 carbons. The complete assignments of all carbon and proton signals were accomplished using the HMBC and ^{1}H−^{1}H COSY-90 experiments; the resonances assigned are presented in Table 5. An exchangeable proton was found at around 16.4 ppm revealing that the ether linkage changed to an alcohol upon coupling.

The unambiguous assignments of all ^{1}H and ^{13}C resonances of the azo dye were achieved by HMBC in which aliphatic H-2 (δ 2.81) protons produced long range coupling with C-1 (δ 44.8) and vice versa. On the naphthalene ring, H-3 (δ 6.61) produced a ^{3}J with C-1 (δ 183.17) and *ortho* splitting by H-4. Similarly, aliphatic H-1 (δ 44.8) as well as H-4 (δ 7.62) and H-7 (δ 7.40) had long range coupling with C-5 (δ 130.3). ^{3}J coupling was also observed between H-3 (δ 6.61) and C-1 (δ 130.7), while on the CDNBD residue ^{2}J coupling occurred between C-3, 5 (δ 140.56) and H-2, 6 (δ 9.16) as well as between C-2, 6 and their protons. The carboxylic carbon on the CDNBD residue was assigned as 166.15, this being typical of such a group; similarly the ketonic carbonyl carbon occurred at 207.1, which is also typical. The mass spectrum, using negative electrospray technique, gave the molecular ion with an *m*/*z* of 451.08, thereby establishing the M_r of AZ-04 as 452.08. For AZ-01, AZ-03 and AZ-04, the C-2 signals occurred within the range 182−183 ppm indicative of a highly deshielded carbon atom. The chemical shift is consistent with the presence of a hydrazone tautomer, implying that the exchange between the azo and hydrazone configurations was slow enough to fit into the NMR time scale. The hydrazone tautomers are therefore reflected in the structures in Fig. 2.

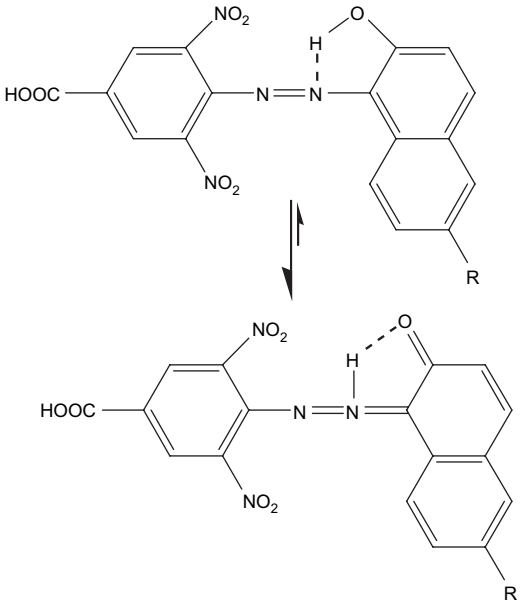

Fig. 2. Proximity effect showing keto−enol tautomerism when the common hydroxyl group is located on the naphthalene ring *ortho* position to the azo group [R = H (AZ-01), R = $CHCH_3COOH$ (AZ-03), R = $CH_2CH_2COCH_3$ (AZ-04)]. ^{13}C NMR data suggest that the keto form is more stable and hence the dominant species.

3. Conclusions

The synthetic intermediate, CDNBD has the ability of producing azo dyes with weakly activated skeletons such as phenol ethers. Unambiguous structure elucidations were carried out using 1D and 2D NMR analyses. Further work will seek to establish the analytical and medicinal utilities of these dyes and also will increase the number of analogs in the azo dye series.

4. Experimental

4.1. General

A solution of CDNBD was prepared using sodium nitrite in sulfuric acid/orthophosphoric acid mixture as previously reported [18]. All compounds were investigated by TLC and purified by recrystallization from organic solvent mixtures. UV spectral analysis was carried out on Unicam Aurora UV spectrophotometer running Helio Scan software (v 1.1) while mass spectra were recorded using a (LCQMS FINNIGAN) mass spectrometer equipped with electrospray technique. IR spectra (KBr pellets) were recorded using a Genesis Series (Ati-Mattson Instruments, USA) FTIR fitted with WINFIRST® software for data acquisition and processing. The 1D- and 2D-NMR spectra were recorded using a Bruker 400 MHz NMR spectrometer.

4.2. Synthesis

4.2.1. Synthesis of 4-[(2-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-01)

A solution of β-naphthol (25 mg) in glacial acetic acid (5 mL) was added to the CDNBD solution, with stirring. The red mixture which formed immediately was stirred gently for 3 h after which time, the reaction mixture was poured on crushed ice and filtered under suction. The crude product

was washed with cold water and dried at 105 °C and the product recrystallized from ethanol/glacial acetic acid (1:1).

4.2.2. Synthesis of 4-[(4-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-02)

A solution of α-naphthol (15 mg) in glacial acetic acid was used for the synthesis of AZ-02 in the place of β-naphthol. Purification was carried out by refluxing the crude dye in ethanol and precipitating the pure dye using ice-cold water.

The propranolol–CDNBD azo dye was prepared by weighing propranolol secondary reference (22 mg) into a vial. Glacial acetic acid (10 mL) was added and the propranolol was allowed to dissolve. The ensuing solution was poured into the reagent solution and stirred continuously for 3 h. At the end of this period crushed ice was added to the reaction mixture until cloudiness appeared; the sample was kept overnight in a refrigerator for complete precipitation. The resulting precipitate was filtered and washed several times with distilled water. The orange-coloured adduct obtained was dried in the oven at 70 °C. Several batches of the propranolol adduct were synthesized following this procedure and the crude samples were then combined. The crude propranolol azo adduct was refluxed in ethylacetate on a water bath for 30 min and then filtered under suction and concentrated. Excess *n*-hexane was added to the ethylacetate concentrate in a beaker until just cloudy. The mixture was allowed to stand for 24 h and an orange product was recovered by filtration and washing with excess *n*-hexane. The sample was eventually dried in an oven at 70 °C and stored in sealed vials.

4.2.3. Synthesis of 4-[(6-(1-carboxyethyl)-2-hydroxynaphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-03)

The CDNBD reagent solution was similarly prepared as before. Naproxen (10 mg) was dissolved in glacial acetic acid (5 mL) and this was poured into the reagent solution and reaction was allowed to proceed for 3 h. The red coloured adduct was poured into ice blocks and allowed to separate. The reddish-orange precipitate was filtered and dried at 70 °C; several batches were prepared. The crude reddish-orange dye was dissolved in ethylacetate under reflux and then was filtered while hot and concentrated on a water bath. Excess *n*-hexane was added until the solution became cloudy. The reddish-orange dye was recovered by filtration and stored in sample vials.

4.2.4. Synthesis of 4-[(2-hydroxy-6-(3-oxobutyl)naphthalen-1-yl)diazenyl]-3,5-dinitrobenzoic acid (AZ-04)

For each batch, nabumetone (20 mg) was dissolved in glacial acetic acid (10 mL) and the ensuing solution was poured into the reagent and reaction was allowed to proceed for 4 h. The solution remained purple throughout the reaction period. At the end of reaction, the purple dye was poured into ice and a deep red coloured dye precipitated which was recovered by filtration under gravity. Several batches were prepared. The dried crude extract was dissolved in ethanol under reflux, filtered while hot and concentrated on a water bath. The beaker containing the red dye was placed in an ice-bath and a few ice-flakes were added, upon which, immediate separation of the red azo dye occurred. The sample was allowed to separate and then recovered by filtration. The purified dye was dried at 60 °C and then stored in a vial.

4.3. Spectroscopic characterization

The azo dyes obtained were characterized using UV spectroscopy, NMR, IR and mass spectrometry. AZ-01 was characterized using ^{1}H and ^{13}C NMR spectra; for AZ-02, one dimensional (1D) ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.63 Hz) spectra were recorded with 64 and 320 scans, respectively. Spectral widths were 6410.26 Hz and 25,000 Hz, respectively. All measurements were made in deuterated pyridine and chemical shifts refer to the signal of the solvent {$\delta = 8.74$ (^{1}H, H-2) and 150.35 (^{13}C, C-2)}.

AZ-03 was similarly characterized by weighing 15 mg of the naproxen azo dye and dissolved in 0.7 ml of pyridine (d_5). ^{1}H signals were recorded at 400.14 MHz with a sweep width of 3846.15 Hz and 128 scans. The ^{13}C signals were recorded on $J_{\text{modulated}}$ version with a delay of 31.3 μs and a sweep width of 20,000.00 Hz at a field strength of 100.62 MHz; a total of 306 scans were recorded. All chemical shifts refer to the downfield signals of pyridine. $^{1}H-^{1}H$ COSY (2D) was recorded with a field strength of 400.13 MHz and a sweep width of 9.512 ppm across both axes; a total of 1024 scans were recorded. $^{1}H-^{13}C$ COSY long-range (HMBC) was recorded with a delay of 208.0 μs and 1024 scans. For the $^{1}H-^{1}H$ COSY experiment, data acquisition was done using a sweep frequency of 4310.34 Hz and 64 scans; d_1 (relaxation) was set at 6 s.

AZ-04 (15 mg) was characterized after dissolution in pyridine (d_5). Proton NMR was recorded using a sweep field frequency of 400.13 MHz with a total of 128 scans of 1024. $J_{\text{modulated}}$ ^{13}C spectra were acquired using a relaxation time (d_1) of 6 s. HMBC-spectra were optimized for a long range J_{H-C} of 7 Hz ($d_6 = 0.07$ s). All carbon spectra were acquired with a field strength of 100.61 MHz.

The IR spectra of the azo adducts were also similarly recorded after compression in KBr disk at a pressure of 10 tons using the GENESIS FTIR machine fitted with WINFIRST® software for data acquisition and processing.

Electrospray ionization (ESI) was used for all azo adduct samples, except for AZ-01 (electron impact 70 eV). Positive ESI was adopted for the propranolol azo adduct while negative ESI was adopted for the α-naphthol dyes, AZ-03 and AZ-04.

UV absorption spectra were recorded in ethylacetate.

Acknowledgments

Part of this work was carried out at the School of Pharmacy, Brunswick Square, London through a University of Ibadan/London School of Pharmacy linkage grant to OSI and at the Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow through a University of Ibadan/MacArthur foundation grant awarded to OAA. Both grants are gratefully acknowledged.

References

[1] Nathan SS, Murthy SK. Organic chemistry made simple. London: W.H. Allen; 1968.

[2] Herbst W, Hunger K. Industrial organic pigments: production, properties, applications. Germany: Verlagsgesellschaft mbH; 1997.

[3] Buss V, Eggers L. In: Lindon JC, Tranter GE, Holmes JL, editors. Encyclopedia of spectroscopy and spectrometry. USA: Academic Press; 2000. p. 389–96.

[4] Olaniyi AA, Ogungbamila FO. Experimental pharmaceutical chemistry. Ibadan: Shaneson C.I. Ltd; 1991.

[5] Rageh NM. Electronic spectra, solvatochromic behavior and acid–base properties of some azo cinnoline compounds. Spectrochim Acta Part A 2004;60:103–9.

[6] Bratton AC, Marshall Jr EK. A new coupling component for sulfanilamide determination. J Biol Chem 1939;128(2):537–50.

[7] Nagaraja P, Yathirajan HS, Raju CR, Vasantha RA, Nagendra MS, Hemantha Kumar MS. 3-Aminophenol as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives. IL Farmaco 2003;58:1295–300.

[8] Revanasiddappa HD, Manju B. Spectrophotometric methods for the determination of ritodrine HCl and its application to pharmaceutical preparations. IL Farmaco 2001;56:615–9.

[9] Idowu SO, Tambo SC, Adegoke AO, Olaniyi AA. Novel colorimetric assay of mefenamic acid using 4-amino-3,5-dinitrobenzoic acid (ADBA). Trop J Pharm Res 2002;1(1):15–22.

[10] Idowu SO, Adegoke AO, Olaniyi AA. Colorimetric assay of propranolol by derivatization: novel application of diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA). J AOAC Int 2004;87(3):573–8.

[11] Adegoke AO, Idowu SO, Olaniyi AA. Novel colorimetric determination of indomethacin using 4-carboxyl-2,6-dinitrobenzene diazonium ion. Acta Pharm 2006;56(2):189–202.

[12] Adegoke AO, Idowu SO, Olaniyi AA. A new spectrophotometric method for determination of nadolol. J Iran Chem Soc 2006;3(3): 277–84.

[13] Idowu SO, Adegoke AO, Oderinu BA, Olaniyi AA. Rapid colorimetric assay of diclofenac sodium tablets using 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). Pak J Pharm Sci 2006;19(2):134–41.

[14] Saunders KH. The aromatic diazo-compounds and their technical applications. London: Edward Arnold & Co; 1949.

[15] Adegoke AO. Novel colorimetric assays of some selected pharmaceutical phenol ethers using 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). Ph.D. thesis, University of Ibadan; 2005.

[16] Idowu SO, Olaniyi AA. 1-(4-Carboxyl-2,6-dinitrophenylazo)-2-hydroxynaphthalene as a new pH indicator. J Phytomed Ther 2001; 6(2):108–15.

[17] Bunnet JF, Hoey GB. Dealkylation in connection with diazo coupling of phenol ethers. J Am Chem Soc 1958;80:3142.

[18] Idowu SO, Kolawole AO, Adegoke AO, Kolade YT, Fasanmade AA, Olaniyi AA. Kinetics of thermal decomposition of 4-carboxyl-2,6-dinitrobenzenediazonium ion (CDNBD). J AOAC Int 2005;88(4): 1108–13.