Evaluation of the Effect of Azo Group on the Biological Activity of 1-(4-Methylphenylazo)-2-naphthol

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Abstract: Azo-2-naphthol and 2-naphthol were tested against five microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*. 2-Naphthol was slightly active while azo-2-naphthol was highly active against all the bacteria species tested. The presence of azo group contributed more than 60% of the antibacterial activities exhibited by azo-2-naphthol on all the bacteria tested.

Keywords: Azo compound; 2-Naphthol; Antibacterial activity; *p*-Toluidine.

Introduction

Azo compounds constitute one of the largest classes of industrially synthesized organic compounds. They are important in drugs and cosmetics¹ and show a variety of interesting biological activities including antibacterial²⁻⁵ and pesticidal⁶ activities. Compounds of naphthalene have been reported to show a variety of biological activities including antimicrobial activities^{7,8}, HIV-1 integrase inhibitory effect⁹, anti-inflammatory activity¹⁰, inhibition of protein tyrosine kinases¹¹ and inactivation of enveloped viruses¹². Azo compounds of naphthols have been extensively used as dye¹³⁻¹⁵, but the biological activity is less reported. Since compounds with azo moiety and naphthalene moiety have been reported to exhibit biological activities independently, it is therefore obvious that azo compounds of 2-naphthol will show a higher biological activity.

In this paper, we synthesize azo compound of naphthalene and use it to investigate its inhibitory effect on the biological activities of some bacteria. The contribution of the azo moiety toward the biological activity is also evaluated.

Experimental

General

All chemicals used were of analytical grade. The IR spectra were recorded on a Shimadzu FTIR-IR Prestige (200VCE) as KBr pellets. The C, H, N data were determined using a Perkin-Elmer Instrument (200B).

Synthesis of 1-(4-methylphenylazo)-2-naphthol

The method used for the preparation of azo compounds has been described elsewhere¹³. A solution of hydrochloric acid was prepared by diluting 2 mL of the concentrated acid in 20 mL of distilled water. 10 mmole of *p*-toluidine (1.074 g) was added, stirred and cooled to 0°C. 8 mL of aqueous sodium nitrite (10.88 mmole, 0.7507 g) was added in drops maintaining the temperature between 0-5 °C. The diazonium chloride formed was consecutively coupled with 10 mmole of 2-naphthol (1.449 g) that was dissolved in 15 mL of 10% sodium hydroxide solution. The reaction mixture was stirred at 0°C for 1 hour. The red product that precipitated was recrystallized from ethanol (65%); IR (KBr, cm⁻¹): 3520-3380 (Ar-OH), 3060-3020 (Stretching CH), 2926 (CH₃), 1632 (C=C), 1450 (N=N); Anal. Calcd. for C₁₇H₁₄N₂O: C 77.84, H 5.38, N 10.68; found C 77.78, H 5.21, N 10.58.

Antimicrobial activity

The 2-naphthol and azo-2-naphthol were screened for the presence of antimicrobial constituents against five microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Basillus subtilis* using disk diffusion method⁸. The tested compounds were dissolved in ethanol to give 10 mg/ 2 mL solutions. 20 µL solutions of these were applied to sterile disks and placed on nutrient agar plates with the bacteria. The plates were incubated for 24 hours and the zones of inhibition were measured. Clarithromycin and cerftriflaxon were used as reference antibiotics. Ethanol showed no inhibition zones on all the bacteria tested.

Results and Discussion

The electrophilic substitution reaction of 2-naphthol at 1-position is well documented in the literature^{16,17}. 1-(4-methylphenylazo)-2-naphthol (azo-2-naphthol) **4** was prepared as a red solid from 2-naphthol **3** according to Scheme 1.

Scheme 1

The azo-2-naphthol was obtained in good yield, 65%. The analytical data and IR spectral data support the product obtained.

The azo-2-naphthol and 2-naphthol were examined *in-vitro* against bacteria species. The test results presented in Table 1 suggested that 2-naphthol was slightly active with inhibition zones 6-8 mm. Azo-2-naphthol, combining the activities of azo and naphthalene moieties recorded higher activity against all the bacteria with inhibition zones 17-22 mm. Cerftrioxon and Clarithromycin are well known antibacterial substances and showed inhibition zones 11-13 mm and 5-7 mm respectively for the same pathogenic bacteria. The contribution of the azo moiety toward the antibacterial activity of azo-2-naphthol was evaluated (Table 1). The results showed that azo group contributed more than 60% of the total antibacterial activity of azo-2-naphthol for all the bacteria tested.

Table 1. The effects of azo moiety on the antibacterial activity of azo-2-naphthol

Compound Bacteria	Clarithromycin	Cerftrioxon	2-naphthol	Azo-2- naphthol	% Inhibition* (Azo moiety)
<i>Staphylococcus aureus</i>	+	++	+	+++	a
<i>Eschericia coli</i>	+	+++	+	+++	a
<i>Pseudomonas aeruginosa</i>	+	+++	+	+++	b
<i>Streptococcus faecalis</i>	-	++	+	+++	b
<i>Bacillussubstilis</i>	+	++	+	+++	a

*% Inhibition (azo moiety) = $ZOI(\text{azo-2-naphthol}) - ZOI(2\text{-naphthol})$, $ZOI(\text{azo-2-naphthol})$

Key to symbols

ZOI	=	Zone of inhibition
Highly active	=	+++ (inhibition zone > 12 mm)
Moderately active	=	++ (inhibition zone 9-12 mm)
Slightly active	=	+ (inhibition zone 6-8 mm)
Inactive	=	- (inhibition zone < 5 mm)
% Inhibition	=	a (60 – 64%)
% Inhibition	=	b (> 64%)

Conclusion

Screening of 2-naphthol and azo-2-naphthol independently against bacteria species afforded the evaluation of the contribution of azo moiety to the biological activity. With a high activity (inhibition zones, 17-22 mm) against 2-naphthol (inhibition zones, 6-8 mm), azo-2-naphthol exhibited a combined activities of both azo and naphthalene moieties. The azo moiety contributed more than 60% of the total biological activity exhibited by azo-2-naphthol.

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