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SPECTROSCOPIC ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SOME 4-PHENYLAZO-PHENOXYACETIC ACIDS

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In this paper we present the results obtained in the study of the structure and the antimicrobial activity of 4-phenylazo-phenoxyacetic acids synthesised by the condensation of sodium azobenzene-4-oxydes with monochloroacetic acid. The structure elucidation of eleven 4-phenylazo-phenoxyacetic acids having general formula Ph=Ph-O-CH_2-COOH is described. The results of this study have demonstrated the structure of all compounds using ^{1}H NMR and mass spectra. The most intense peaks have been used to characterise positive ion mass spectra. The losing of $C_2H_2O_2$ from the parent ion is the dominating reaction. All these compounds were evaluated for antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* by disk diffusion method. The screening data releaved that the title compounds carrying methyle group exhibited good antimicrobial activity.

INTRODUCTION

4-Phenylazo-phenoxyacetic acids are azo dyes of large interest from the point of view of possible applications in medicine because of their antimicrobial activity and in non-linear optics. We have recently reported the synthesis and the physical characterization of some 4-phenylazo-phenoxyacetic acids.[1-3] In continuation of our earlier studies, we report here the ^{1}H NMR spectra, the mass spectra and the antimicrobial activity of eleven acids (Table 1) having the general formula:

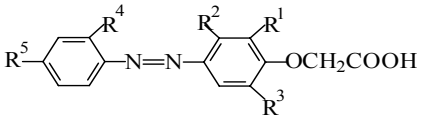

Table 1

4-Phenylazo-phenoxyacetic acids

Compound	*Name*	R^1	R^2	R^3	R^4	R^5
I	2-Methyl-4-phenylazo-phenoxyacetic acid	CH_3	H	H	H	H
II	3-Methyl-4-phenylazo-phenoxyacetic acid	H	CH_3	H	H	H
III	4-(2-Methyl-phenylazo)-phenoxyacetic acid	H	H	H	CH_3	H
IV	2,3-Dimethyl-4-phenylazo-phenoxyacetic acid	CH_3	CH_3	H	H	H
V	2,6-Dimethyl-4-phenylazo-phenoxyacetic acid	CH_3	H	CH_3	H	H
VI	2-Methyl-4-(4-methyl-phenylazo)-phenoxyacetic acid	CH_3	H	H	H	CH_3
VII	4-(4-Chloro-phenylazo)-phenoxyacetic acid	H	H	H	H	Cl
VIII	2-Methyl-4-(4-chloro-phenylazo)-phenoxyacetic acid	CH_3	H	H	H	Cl
IX	3-Methyl-4-(4-chloro-phenylazo)-phenoxyacetic acid	H	CH_3	H	H	Cl
X	2-Chloro-4-(4-chloro-phenylazo)-phenoxyacetic acid	Cl	H	H	H	Cl
XI	2,5-Dichloro-4-(4-chloro-phenylazo)-phenoxyacetic acid	H	Cl	Cl	H	Cl

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The synthesis of organic compounds having biological properties using monochloroacetic acid have received considerable interest in recent years. Treatment of 6-(*p*-chlorophenyl)-1,4,5,6-tetrahydro-s-tetrazine-3(2H)-thione with chloroacetic acid gives 3-(*p*-chlorophenyl)-2,3,6,7-tetrahydro-4H-thiazolo(3,2-b)-s-tetrazin-6-one, evaluated for their antibacterial and antifungal activity.[4] The condensation of 3-arylrhodanines with chloroacetic acid and sodium methoxide affords 3-arylrhodanino(4,5-b)furan-6-(5H)-ones. All the compounds have been tested for their fungicidal activity against *Aspergillus niger* and *Aspergillus flavus*.[5] The reaction of 9-methyl-1,2,4,5-tetraazaspiro[5.5]undecane-3-thione with chloroacetic acid results in the facile synhesis of 4-methyl-6'(7'H)-oxospiro(cyclohexane-1,3'(4H)-(2H)-thiazolo(3,2-b)-s-tetrazine. The antibacterial and antifungal activies have been determined.[6] Several new 5-(4-oxothiazolidin-2-ylidene)-rhodanine were synthesized through the reaction of 5-thiocarbamoylrhodanines with monochloroacetic acid. Some compounds showed promising anticancer activity against particular human cell lines used in the assay.[7] Thiazolo[2,3-b]quinazolines were obtained in one pot synthesis by treating octahydroquinazoline with monochloroacetic acid and aromatic aldehydes. Antifungal activity was shown for some of the synthesized compounds.[8] Treatment of 1',2',4',5',-tetrahydrospiro(adamantane-2,3'-tetrazine)-6-thione with monochloroacetic acid gives spiro(adamantane-2,3'(4'H)-(2H)-thiazolo(3,2-b)-*s*-tetrazin-6'(7')-one. Their antibacterial and antifungal activities have been evaluated.[9]

EXPERIMENTAL

Reagents

4-Phenylazo-phenoxyacetic acids were obtained according to the literature methods by condensation of monochloroacetic acid with different sodium salts of 4-phenylazo-phenols.[1]

Apparatus

The ^{1}H NMR spectra were registered on Varian EM-360, 60MHz spectrometer, using CCl_4 as solvent and TMS as internal standard.

Mass spectra were run with HPGC-MS 5890 Series II and MSD 5971 Series. We are using an electron ionising energy of 70 eV at 250 ºC (the source temperature).

General procedure

The title compounds were screened for their antibacterial activity using cup plate diffusion method.[10] The bacterial organisms used in the present investigation were isolated from human being with characteristic infections and diseases.

All compounds were tested for antimicrobial activity against 5 microorganisms: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*. Test compounds were dissolved in ethanol. Concentrations 0.2% of the test compounds were obtained. Each well (diameter 6 mm) was loaded with 0.1 mL of test compound solution.

Tests of different isolates of microorganisms used were carried out by pouring 15 mL sterile Mueller Hinton agar in each Petri discs by 9 cm diameter. After solidification, the plates were placed in an incubator at 37 ºC for 30 minutes to remove excessive moisture.

Overnight broth culture was streaked evenly onto medium in three directions using a wooden stick cotton swab. Excess suspension was removed from the swab by rotating it firmly against the side of the tube before seeding the plate surface using sterile forceps. The plates were inoculated aerobically at 37 ^{o}C within 15 minutes. After 24 hours incubation, the diameters of the inhibition zones were measured (including the 6 mm diameter of the disc) with a rule. The results were compared with [1-(ethoxycarbonyl)-pentadecyl]-trimethylammonium bromide (Septonex), a comercial antiseptic agent.

RESULTS AND DISCUSSION

^{1}H NMR spectra

Table 2 gives the chemical shifts, δ (ppm) in the ^{1}H NMR spectra of all compounds.

The 4-phenylazo-phenoxyacetic acids exhibited characteristic ^{1}H NMR spectral data in agreement with earlier reported data.[11-14] The ^{1}H NMR spectra also showed expected signals.

In the ^{1}H NMR spectra of all compounds the presence of CH_2 group is confirmed by the appearance of a signal at δ=4.5-5.5 ppm, an expected singlet. The presence of a singlet at δ=10.1-11.1 ppm may be assigned to COOH protons. The aromatic protons from the two substituted benzene rings came into resonance as a multiplet at δ=6.9-8.7 ppm. Also, it is possible to appear additional signals caused by the substituents. For instance, for 3-methyl-4-phenylazo-phenoxyacetic acid can be observed on additional singlet at δ=2.2 ppm, corresponding to the methyl group protons.

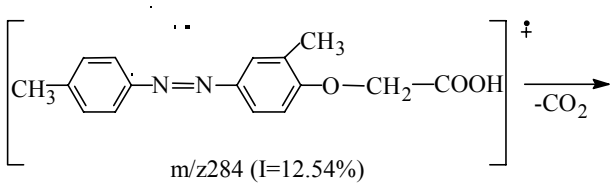
Table 2

Spectral data

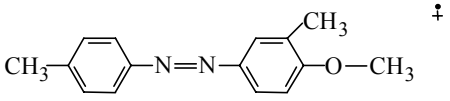
Compound	Phenyl protons (δ ppm)	CH_2 (δ ppm)	COOH (δ ppm)	CH_3 (δ ppm)	Base peak m/z (%)
I	6.9-7.5	4.5	10.4	2.1	77(100%)
II	7.3-7.9	4.8	10.6	2.2	77(100%)
III	7.1-7.7	4.6	10.5	2.2	91(100%)
IV	6.9-7.6	4.5	10.1	2.3	77(100%)
V	7.1-7.6	4.6	10.3	2.6	77(100%)
VI	7.1-7.7	4.5	10.4	2.5	91(100%)
VII	7.6-8.3	5.3	10.7	-	111(100%)
VIII	7.6-8.2	5.2	10.6	3.4	111(100%)
IX	7.5-8.1	5.2	10.5	3.5	111(100%)
X	7.9-8.5	5.5	11.0	-	111(100%)
XI	8.1-8.7	5.2	11.1	-	111(100%)

Mass spectra

Further evidence for the 4-phenylazo-phenoxyacetic acids structure was obtained from mass spectrum. For example, for 2-methyl-4-(4-methyl-phenylazo)-phenoxyacetic acid, Scheme 1 shows the fragmentation process specific to the phenoxyacetic acids by the cleavage of the bond between the C of the methylene group and C atoms of the carboxyl group followed by CO_2 elimination.



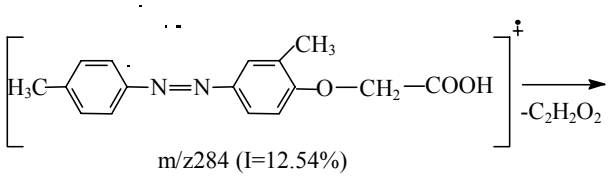
m/z284 (I=12.54%)



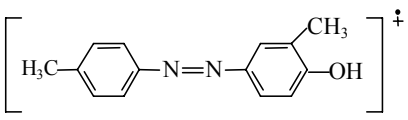
m/z240 (I=21.73%)

Scheme 1

The mass spectrum of 2-methyl-4-(4-methyl-phenylazo)-phenoxyacetic acid, for example, showed the peak at m/z 226, consistent with molecular formula $C_{14}H_{14}N_2O$. The peak at m/z, 226 can be assigned due to the loss of $C_2H_2O_2$ from the parent ion (Scheme 2).



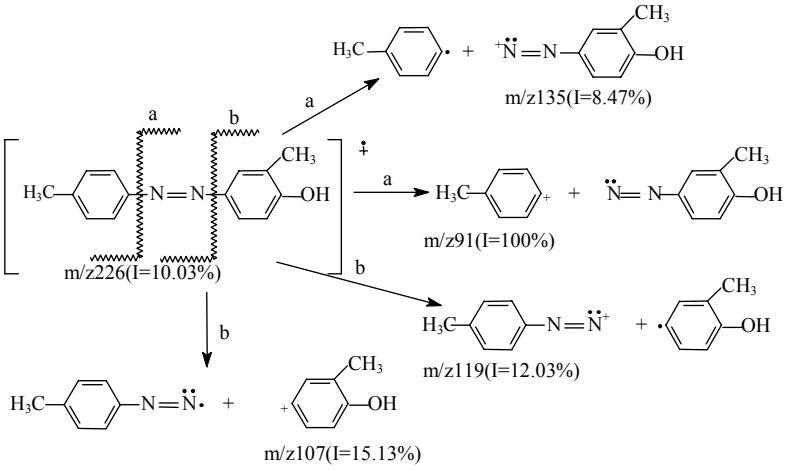
m/z284 (I=12.54%)



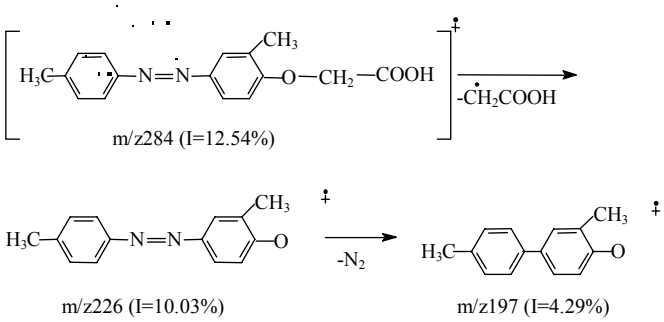
m/z226 (I=10.03%)

Scheme 2

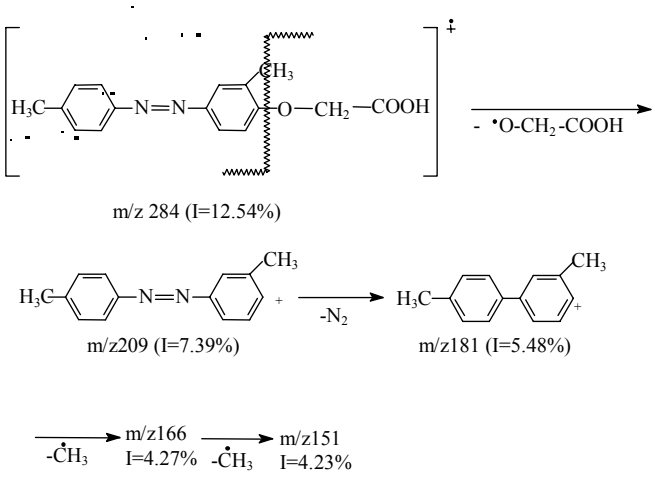
The cleavage of the C-N bonds in this fragmentation produced fragments at m/z 135, m/z 91 (base peak), m/z 119 and m/z 107, respectively (Scheme 3). The cleavage at the O-CH_2 bond and the lost N_2 give another fragment at m/z 197 (Scheme 4). In another fragmentation M^+ lost N_2 and all radicals to produce diphenyl cation at m/z 151 (Scheme 5).



Scheme 3

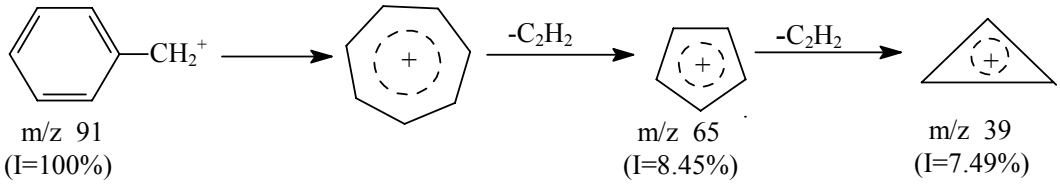


Scheme 4



Scheme 5

The tropilium ion, m/z 91, obtained by skeletal transposition, characteristic of the monoalkyl aromatic compounds, could be eliminate acetylene, and formed the fragment m/z 65 (cyclopentadienilium), which by itself losing an other acetylene molecule generate the ion cyclopropenilium (m/z 39) (Scheme 6).



Scheme 6

All fragmentation process can support the structure formula assigned to the compounds and are in agreement with the literature and with fragmentation of phenoxyacetic acid.[15]

The fragmentation described in Schemes 1-6 for 2-methyl-4-(4-methyl-phenylazo)-phenoxyacetic acid, are characteristic for all compounds mentioned in Table 1.

Antimicrobial activity

The results of screening antimicrobial activity are given in Table 3.

The 4-phenylazo-phenoxyacetic acids thus described were subjected to antibacterial activity screening against two gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and three gram-negative bacteria (*Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*) employing the disk diffusion technique. Test compounds were dissolved in ethanol. Concentrations 0.2% of the test compounds were obtained. Diameter of cup was 6 mm.

The results show that compounds I-VII and XI present antimicrobial activity against most of the tested species. Compounds VIII-X are inactive against all six microorganisms tested. The best efficiency was exhibited by all compounds having methyl substituent.

Table 3

Antimicrobial activities of compounds

Compound	Diameter of zone of inhibition in mm				
	Staphylococcus aureus	Streptococcus pyogenes	Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris
I	15	7	18	15	20
II	16	18	7	13	15
III	18	20	21	10	20
IV	8	10	12	15	8
V	8	7	9	20	20
VI	9	10	7	10	12
VII	18	-	9	20	-
VIII	-	-	-	-	-
IX	-	-	-	-	-
X	-	-	-	-	-
XI	22	20	-	-	-
Septonex	18	17	14	19	16

CONCLUSIONS

The ^{1}H-NMR and mass spectra confirm the structure of all 4-phenylazo-phenoxyacetic acids. Six compounds present antimicrobial activity against all of the tested species. The investigations on the structure-activity relationships confirmed the importance of the nature of substituent in the antimicrobial activity. The best efficiency was exhibited by all compounds having methyl as substituent.

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