Preparation, characterization, and antimicrobial properties of some 1,3,5-substituted 2-pyrazolines

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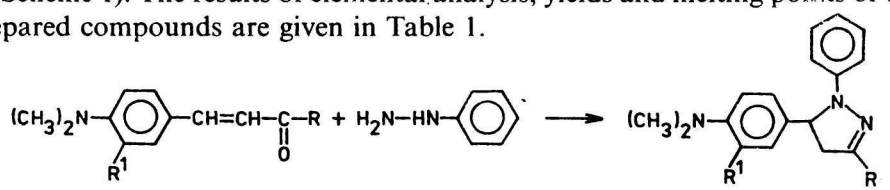
Received 1 December 1988

Eleven new 1,3,5-substituted 2-pyrazolines were prepared by the reaction of phenylhydrazine with some chalcones derived from 4-dimethylaminobenzaldehyde. The structure of these compounds was proved on the basis of mass and IR spectral data and elemental analysis. Antimicrobial effects of the prepared 2-pyrazolines were also studied.

Получено одиннадцать новых 1,3,5-замещенных 2-пиразолинов посредством реакции фенилгидразина с некоторыми халконами, производными 4-диметиламинобензальдегида. Строение полученных соединений было доказано на основании ИК- и масс-спектральных данных, а также элементарного анализа. Изучается также антимикробное действие полученных 2-пиразолинов.

The reaction of hydrazines with α,β-unsaturated aldehydes or ketones is the most utilized method for preparation of 2-pyrazolines. In these cases, required pyrazolines can be obtained either directly or by the subsequent rearrangement of corresponding hydrazones using suitable reaction conditions. Till now, as starting compounds, chalcones and phenylhydrazine were used the most frequently, where corresponding 1,3,5-triphenyl-2-pyrazolines were obtained [1—7].

In the present work, we used as starting compounds some chalcones derived from 4-dimethylaminobenzaldehyde [8] and phenylhydrazine. By the heating of reactants in glacial acetic acid, corresponding 2-pyrazolines were isolated directly (Scheme 1). The results of elemental analysis, yields and melting points of the prepared compounds are given in Table 1.



R = $CH_3(CH_2)_5$, $C_6H_3R^2R^3$

Scheme 1

IR spectra of all the prepared 2-pyrazolines exhibited strong absorption bands in the region of $\tilde{\nu} = 1370$—$1381\ cm^{-1}$, belonging to the deformation vibrations of CH_2 groups of the pyrazoline ring. The absorption bands observed in the region of $\tilde{\nu} = 1591$—$1601\ cm^{-1}$ were assigned to the stretching vibrations of C=N groups. However, in this region, also conjugated C=C double bonds absorb, which can lead to the overlapping and interaction [9]. Likewise, the absorption bands of stretching vibrations of CH—N bonds were observed in the region of $\tilde{\nu} = 1133$—$1152\ cm^{-1}$.

In the mass spectra, the peaks corresponding to the molecular ions $M^{+\bullet}$ were observed as the basic peaks ($I_r = 100\ \%$) in all discussed 2-pyrazolines. Further significant peak at $m/z = 147$ corresponded to the ion $[(CH_3)_2N—C_6H_4—CH=CH_2]^{+\bullet}$ which proves the formation of 2-pyrazoline ring, because this ion could not be formed in the case of the second possible product — noncyclized hydrazone. The peak at $m/z = 121$ corresponded to the ion $[(CH_3)_2N—C_6H_4]^{+\bullet}$. In all spectra, the less significant peak corresponding to the fragmentation $M^{+\bullet} - (CH_3)_2N—C_6H_4^{\bullet}$ was also observed. Derivatives having $R^2 = NO_2$ showed also an intensive peak corresponding to the ion $[M^{+\bullet} - NO]^{+\bullet}$. In the case of compound *XI*, the peak corresponding to the fragmentation $M^{+\bullet} - \dot{C}H_2)_4CH_3$ was also observed.

Since it is known from the literature [10—15] that some pyrazolines exhibit biological activity, antimicrobial efficiency of the prepared 2-pyrazolines against some bacteria and fungi (see Experimental) has been determined. It was found that these compounds exhibit certain antimicrobial activity but only at higher concentrations — minimal inhibitory concentration (MIC) was about $1000\ \mu g\ cm^{-3}$ for majority of tested species of microorganisms (Table 2). In comparison with effects of Septonex — *N*(α-carbethoxypentadecyl)-trimethylammonium bromide used as a standard, we can state that the discussed 2-pyrazolines exhibit weak antimicrobial activity. From this fact we can conclude that those substituents as given on benzene ring in the position 3 of pyrazoline ring, do not influence antimicrobial efficiency expressively. Likewise, introduction of the nitro group into position 3 of benzene ring in the position 5 of pyrazoline ring does not contribute to the substantial increasing of biological activity of the presented 2-pyrazolines. Moreover, either the substitution of the phenyl in the position 3 of pyrazoline ring by the alkyl chain (hexyl in our case) did not show higher efficiency. Hexyl has been chosen on the basis of our knowledge that some heterocyclic compounds having this alkyl chain in the molecule exhibited the best antimicrobial effects in the series of derivatives with different length of the alkyl.

Table 1

Characterization of the prepared 2-pyrazolines

Compound	R^1	R^2	R^3	Formula	M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p.
						C	H	N	%	°C
I	H	4-Cl	H	$C_{23}H_{22}ClN_3$	375.68	73.53	5.86	11.18	81	166—167
						73.46	5.93	11.26		
II	H	4-Br	H	$C_{23}H_{22}BrN_3$	420.14	65.75	5.24	10.00	86	186—187
						65.67	5.31	9.96		
III	H	3-NO_2	H	$C_{23}H_{22}N_4O_2$	386.23	71.52	5.70	14.50	78	162—163
						71.43	5.77	14.56		
IV	H	3-Cl	4-Cl	$C_{23}H_{21}Cl_2N_3$	410.13	67.35	5.12	10.24	88	156—157
						67.22	5.20	10.35		
V	H	3-NO_2	4-CH_3	$C_{24}H_{24}N_4O_2$	400.24	72.02	6.00	13.99	74	152—153
						71.93	6.11	13.91		
VI	NO_2	4-Cl	H	$C_{23}H_{21}ClN_4O_2$	420.68	65.66	4.99	13.31	76	145—146
						65.58	5.07	13.36		
VII	NO_2	4-Br	H	$C_{23}H_{21}BrN_4O_2$	465.14	59.39	4.51	12.04	79	141—142
						59.31	4.58	11.99		
VIII	NO_2	3-NO_2	H	$C_{23}H_{21}N_5O_4$	431.23	64.06	4.87	16.23	69	166—167
						64.10	4.93	16.18		
IX	NO_2	3-Cl	4-Cl	$C_{23}H_{20}Cl_2N_4O_2$	455.13	60.69	4.39	12.30	73	165—166
						60.61	4.43	12.21		
X	NO_2	3-NO_2	4-CH_3	$C_{24}H_{23}N_5O_4$	445.24	64.74	5.17	15.72	69	158—159
						64.64	5.21	15.70		
XI	H	R = 1-hexyl		$C_{23}H_{31}N_3$	349.23	79.10	8.88	12.03	71	67—68
						79.14	8.93	12.11		

Table 2

Antimicrobial activity (MIC/($\mu g\,cm^{-3}$)) of the prepared 2-pyrazolines

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Shigella flexneri*	*Salmonella typhimurium*	*Enterobacter aerogenes*
I	1000	1000	1000	1000	1000	1000
II	1000	1000	> 1000	1000	> 1000	1000
III	1000	1000	> 1000	1000	1000	1000
IV	> 100	> 100	1000	> 100	1000	1000
V	1000	1000	> 1000	1000	> 1000	1000
VI	> 100	1000	1000	1000	1000	1000
VII	1000	1000	> 1000	1000	1000	1000
VIII	100	> 100	1000	> 100	1000	1000
IX	100	> 100	1000	> 100	1000	1000
X	> 100	1000	1000	1000	> 1000	1000
XI	100	> 100	1000	1000	1000	1000
Septonex	0.1	0.1	1000	100	1000	1000

Experimental

The starting chalcones were prepared according to the known method [8]. The other used chemicals were commercial products (Lachema, Brno; Fluka, Buchs; Merck, Darmstadt).

The melting points were determined on a Kofler hot-stage. IR spectra (in KBr pellets; 2.0—3.0 mg sample in 400 mg KBr) were measured on a Perkin—Elmer 457 instrument in the region of $\tilde{\nu} = 300$—$4000\,cm^{-1}$. Mass spectra (12 eV) were obtained on a JMS-100 D spectrometer at an emission current of 300 μA, applying direct sample introduction technique. Elemental analyses were performed on a Perkin—Elmer 240 analyzer.

Antimicrobial efficiency was determined by using qualitative suspension method. The used bacteria — *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* were from the Czechoslovak state collection of species cultures. The other microorganisms — *Salmonella typhimurium*, *Shigella flexneri*, and *Enterobacter aerogenes* were from the collection of the Department of Biochemistry and Microbiology, Faculty of Pharmacy, Comenius University, Bratislava. Before using, they were inoculated on a plate with cultivation medium No. 2 Imuna and then were cultivated for 24 h at 37 °C. Inoculum was prepared by the inoculation into 5 cm^3 of cultivation medium Imuna and by the incubation for 18 h at 37 °C. The solutions of tested compounds in 96 % ethanol were used in these experiments. The growth of microorganisms at different concentrations of the tested compound was evaluated and the results were expressed as minimal inhibitory concentration (MIC).

1,3,5-Triphenyl-2-pyrazolines I—X

To the corresponding chalcone (5 mmol) in glacial acetic acid (30 cm^3 for compounds *I—IV*, 50 cm^3 for *V—X*), phenylhydrazine (0.6 cm^3, 20 % excess) was added dropwise. The mixture was left to stand for 1 h under occasional stirring. Then, the mixture was heated — in the case of compounds *I* and *II*, heating just to reflux was sufficient, in the case of compounds *III* and *IV*, refluxing took 1 h and the preparation of compounds *V—X* required at least 2 h of refluxing. Compound *I* crystallized from the solution after 24 h, the other 2-pyrazolines crystallized either immediately from even hot solution or during his cooling. Products *I—III* were recrystallized from dry ethanol, the others from glacial acetic acid.

1-Phenyl-5-(4-dimethylaminophenyl)-3-hexyl-2-pyrazoline (XI)

To the solution of 1-(4-dimethylaminophenyl)-1-nonen-3-one (1.3 g; 5 mmol) in glacial acetic acid (8 cm^3), phenylhydrazine (0.6 cm^3, 20 % excess) was added dropwise. After 48 h of standing at 25 °C, the solution was poured into ice water (60 cm^3) and the product was extracted from the formed emulsion by ether (4 × 50 cm^3). Etheric layer was

washed with water ($3 \times 50\,cm^3$), dried (over Na_2SO_4) and ether was finally distilled off *in vacuo*. The obtained solid compound was crystallized from ethanol ($40\,cm^3$). After recrystallization from ethanol, the product was obtained in the form of cream coloured lustrous scales.

Acknowledgements. The authors thank G. Švihlová and K. Paule (Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, Bratislava) for measurement of mass spectra and for elemental analyses.

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Translated by M. Koóš