Influence of structure on antimicrobial activity of some heterocycles
I. Alkylpyrazoles and alkylisoxazoles

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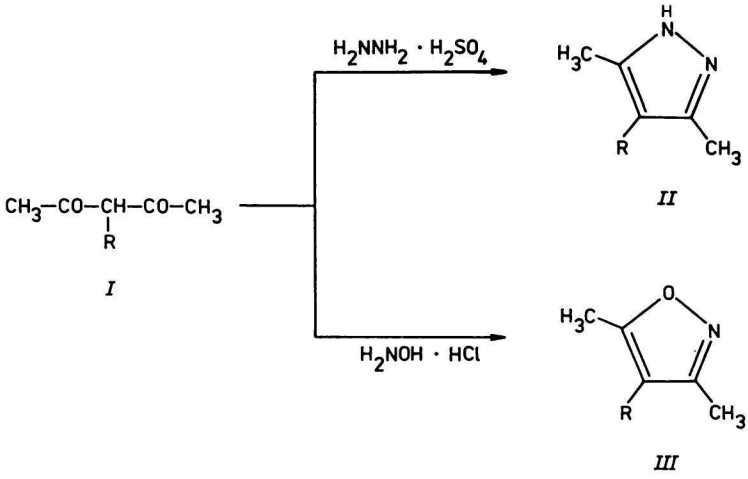
Seven 3,5-dimethyl-4-alkylpyrazoles and seven 3,5-dimethyl-4-alkylisoxazoles were prepared. Their structure was confirmed by spectral data and elemental analysis. Antimicrobial activity of these compounds was also determined and discussed in relation to the structure.

It is generally known that many heterocyclic compounds exhibit antimicrobial effects. Since the preparation of compounds having properties of tenside was our target, we have focused our attention on some alkylpyrazoles and alkylisoxazoles where the mentioned alkyl chain represented hydrophobic component of the molecule. From among pyrazole derivatives, as regards the antimicrobial activity, in most cases more complicated structures are discussed in the literature [1—6]. Regarding more simple structures, mostly various halogen- and aryl-substituted derivatives [7—12] and *N*-acetylated amides [13, 14] are described. Similar situation is found also in the case of isoxazole derivatives [15—18]. In many cases, pyrazole and isoxazole ring is incorporated as a constituent of substituted polycondensed heterocycle [19—24]. However, these compounds do not contain the mentioned hydrophobic alkyl chain, *i.e.* one of the structural elements necessary for surface activity of the final products.

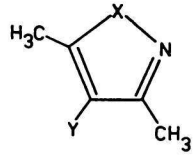
For the preparation of discussed heterocyclic compounds, known synthetic methods [25, 26] have been applied using column chromatography instead of distillation for isolation of products. So, by the cyclization of 3-alkyl-2,4-pentanedione *I* with hydrazine in aqueous-ethanolic medium, corresponding 4-alkyl-3,5-dimethylpyrazoles *II* (Scheme 1) were prepared. Reaction of *I* with hydroxylammonium chloride afforded 4-alkyl-3,5-dimethylisoxazoles *III* (Scheme 1). Alkylation of 2,4-pentanedione sodium salt with corresponding iodoalkanes using ethyl methyl ketone as a solvent proved to be the best method for the preparation of starting 3-alkyl-2,4-pentanediones *I*. The yields were about 50 %. Pentadecyl derivative was obtained only in 27 % yield. By using the general method for alkylation of β-dicarbonyl compounds — addition of bromoalkane and diketone (2,4-pentanedione in our case) into ethanolic solution of sodium ethoxide — required 3-alkyl-2,4-pentanediones were obtained only in



Scheme 1

about 25 % yields. The preparation of the first three derivatives of this group (*Ia—Ic*, Table 1) by using corresponding bromoalkanes by the reaction in autoclave at 180—190 °C yielding about 40 % of products, is described in the literature [27]. 3-Heptylthio-2,4-pentanedione (*Ig*) was prepared by the reaction of 3-chloro-2,4-pentanedione with sodium salt of 1-heptanethiol. As a by-product in this case, heptylthiomethyl methyl ketone resulting by the removal of acetyl group from the main product, was isolated in 22 % yield. Compounds *IIa—IIc* are mentioned in the literature [28] in connection with the study of magnetic spectral properties of Ni^{II} complexes. In addition, compound *IIa* is again published in the work [29] also in the relation to the Ni^{II} and Co^{II} complexes of alkylpyrazoles. However, preparation and characterization of compounds *IIa—IIc* themselves is not given in these papers.

The structure of the prepared compounds was confirmed on the basis of data of elemental analysis, mass and IR spectra. In the mass spectra ($U = 12$ eV) of alkylpyrazoles *IIa—IIf*, the peaks corresponding to the molecular ions $M^{+\bullet}$ (I_r ranged from 12 to 29 %) were observed. The most expressive peaks ($I_r = 100$ %) registered at $m/z = 109$ corresponded to the ions A. Similarly, in the case of



Ion	X	Y	m/z
A	NH	$\overset{+}{C}H_2$	109
B	O	$\overset{+}{C}H_2$	110
C	NH	$SH^{+\bullet}$	128
D	O	$SH^{+\bullet}$	129

alkylisoxazoles *IIIa—IIIf*, the maximum peak registered at $m/z = 110$ corresponded to the ion B. The I_r of molecular ions was within 8 and 30 %. In the case of pyrazole heptylthio derivative *IIg*, maximum peak at $m/z = 128$ corresponding to the ion C was observed. Analogically, isoxazole heptylthio derivative *IIIg* exhibited maximum peak at $m/z = 129$ corresponding to the ion D. In both cases, much more intensive molecular peaks $M^{+\bullet}$ (I_r was 79 resp. 82 %) were observed than in the case of non-sulfur analogues. In the IR spectra of discussed alkylpyrazoles, characteristic expressive bands in the region of $\tilde{\nu} = 1470—1585\ cm^{-1}$ corresponding to the stretching vibration of ring, in the region of $\tilde{\nu} = 955—1060\ cm^{-1}$ corresponding to the planar deformation vibrations of C—H bonds and in the region of $\tilde{\nu} = 820\ cm^{-1}$ corresponding to the nonplanar deformation vibrations of C—H bonds of pyrazole skeleton, were observed. In the case of isoxazole derivatives, the above-mentioned types of vibrations were shown by strong bands in the region of $\tilde{\nu} = 900$, 1040, 1455, and $1640\ cm^{-1}$ The methylene groups of the alkyl chains of discussed compounds exhibited strong absorption in the region of $\tilde{\nu} = 2855$ and $2930\ cm^{-1}$ (ν_{as}(C—H) and ν_s(C—H)), $\tilde{\nu} = 1465\ cm^{-1}$ (δ(C—H)), and $\tilde{\nu} = 1180\ cm^{-1}$ (skeletal vibrations of C—C bonds).

The results of antimicrobial activity testing showed that while pyrazole derivatives *IIa—IIg* demonstrate considerable dependence of minimum inhibitory concentration (MIC) on the length and character of alkyl chain (decrease of efficiency in the case of decyl, dodecyl, and pentadecyl derivatives, more expressively for *Salmonella typhimurium*), effectiveness of alkylisoxazoles *IIIa—IIIg* was relatively low (MIC values about 1000 ppm for all of used microorganisms) regardless of the length of alkyl chain (Table 2). A comparison of MIC values for compounds *IIb*, *IIc*, and *IIg* demonstrates considerable influence of the structure of alkyl chain on the antimicrobial activity. While in the case of gram-negative bacteria (excepting *Salmonella typhimurium*) the efficiency was on the same level for all three compounds, in the case of gram-positive bacteria, heptylthio derivative *IIg* showed ten to hundred times lower value of antimicrobial activity in comparison with non-sulfur analogues *IIb* and *IIc*. As can be seen from Table 2, derivatives *IIa—IIc* exhibit even higher antimicrobial activity than antiseptic agent — [1-(ethoxycarbonyl)pentadecyl]trimethylammonium bromide (Septonex) usually applied in practice, which we used as a standard.

Experimental

Iodoalkanes were prepared according to the known method from corresponding bromoalkanes. 3-Chloro-2,4-pentanedione was obtained by the reaction of 2,4-pen-

Table 1

Characterization of the prepared compounds

Compound	R	Formula	M_r	w_i(calc.)/% / w_i(found)/%				Yield[a] %	B.p./*p* °C/Pa
				C	H	N	S		
Ia	Hexyl	$C_{11}H_{20}O_2$	184.11	71.76 71.54	10.86 10.99			54	88—90/266[b]
Ib	Heptyl	$C_{12}H_{22}O_2$	198.12	72.74 72.50	11.10 11.31			52	100—102/266[c]
Ic	Octyl	$C_{13}H_{24}O_2$	212.13	73.60 73.64	11.31 11.40			51	111—113/266[d]
Id	Decyl	$C_{15}H_{28}O_2$	240.15	75.02 75.11	11.66 11.74			48	107—110/13
Ie	Dodecyl	$C_{17}H_{32}O_2$	268.17	76.13 76.04	11.93 12.06			47	123—126/13
If	Pentadecyl	$C_{20}H_{38}O_2$	310.20	77.43 77.25	12.25 12.41			27	*e*
Ig	Heptylthio	$C_{12}H_{22}O_2S$	230.18	62.61 62.69	9.56 9.65		13.93 13.87	60	94—97/13
IIa	Hexyl	$C_{11}H_{20}N_2$	180.11	73.35 73.28	11.10 11.17	15.55 15.68		79	
IIb	Heptyl	$C_{12}H_{22}N_2$	194.12	74.24 74.32	11.33 11.41	14.42 14.53		76	
IIc	Octyl	$C_{13}H_{24}N_2$	208.13	75.02 75.13	11.53 11.63	13.45 13.51		77	*e*
IId	Decyl	$C_{15}H_{28}N_2$	236.15	76.29 76.21	11.86 11.95	11.86 11.98		72	*e*
IIe	Dodecyl	$C_{17}H_{32}N_2$	264.17	77.29 77.18	12.11 12.23	10.60 10.69		69	*e*
IIf	Pentadecyl	$C_{20}H_{38}N_2$	306.20	78.45 78.20	12.41 12.52	9.14 9.07		61	*e*

Table 1 (Continued)

Compound	R	Formula	M_r	w_i(calc.)/% w_i(found)/%				Yield[a] %	B.p./*p* °C/Pa
				C	H	.N	S		
IIg	Heptylthio	$C_{12}H_{22}N_2S$	226.18	63.72	9.73	12.38	14.17	73	*e*
				63.80	9.81	12.48	14.01		
IIIa	Hexyl	$C_{11}H_{19}NO$	181.11	72.94	10.49	7.73		68	*e*
				72.83	10.61	7.81			
IIIb	Heptyl	$C_{12}H_{21}NO$	195.12	73.86	10.76	7.18		65	*e*
				73.94	10.85	7.26			
IIIc	Octyl	$C_{13}H_{23}NO$	209.13	74.66	11.00	6.69		66	*e*
				74.54	11.18	6.78			
IIId	Decyl	$C_{15}H_{27}NO$	237.15	75.96	11.39	5.90		61	*e*
				75.77	11.49	5.99			
IIIe	Dodecyl	$C_{17}H_{31}NO$	265.17	77.00	11.69	5.28		63	*e*
				77.21	11.80	5.36			
IIIf	Pentadecyl	$C_{20}H_{37}NO$	307.20	78.19	12.04	4.56		60	*e*
				78.01	12.16	4.64			
IIIg	Heptylthio	$C_{12}H_{21}NOS$	227.18	63.44	9.24	6.16	14.11	67	
				63.36	9.36	6.23	14.00		

a) After purification (distillation or column chromatography); *b*) 123—124 °C/2.13 kPa in Ref. [27]; *c*) 134—136 °C/2.13 kPa in Ref. [27]; *d*) 145 148 °C/1.47 kPa in Ref. [27]; *e*) viscous oil — b.p. not determined.

Table 2

Antimicrobial activity (MIC/(μg cm^{-3})) of the prepared compounds *II* and *III*

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*	*Pseudomonas aeruginosa*
IIa	< 0.1	< 0.1	1000	10	1000
IIb	< 0.1	< 0.1	< 1000	< 10	1000
IIc	< 0.1	< 0.1	1000	10	1000
IId	< 1	< 1	1000	1000	1000
IIe	< 1	< 1	1000	1000	1000
IIf	100	100	> 1000	1000	1000
IIg	1	10	1000	1000	1000
IIIa	< 1000	< 1000	1000	1000	1000
IIIb	< 1000	< 1000	1000	1000	1000
IIIc	< 1000	< 1000	1000	1000	1000
IIId	1000	1000	> 1000	1000	> 1000
IIIe	1000	1000	> 1000	1000	> 1000
IIIf	1000	1000	> 1000	1000	> 1000
IIIg	1000	1000	> 1000	1000	1000
Septonex	0.1	0.1	1000	1000	1000

tanedione with sulfuryl chloride [30]. 2,4-Pentanedione, 1-heptanethiol and the other used chemicals were commercially available products (Lachema, Brno; Fluka, Buchs).

Mass spectra ($U = 12$ eV) were measured on a Jeol JMS-100D mass spectrometer at an emission current of 300 μA, applying direct sample-introduction technique. IR spectra (liquid samples as liquid films of indefinite thickness) were obtained on a Perkin—Elmer 457 instrument.

MIC was determined by using suspension method on solid cultivation media. Before using, bacteria were inoculated on a plate with cultivation agar and then cultivated for 24 h at 37 °C. Inoculum was prepared by inoculation into cultivation medium No. 2 Imuna (5 cm^3) and by incubation for 2 h at 37 °C. For testing, solutions of the tested compounds in 96 % ethanol were used. The growth of microorganisms at different concentrations of the tested compound was evaluated. The used microorganisms originated from the Czechoslovak state collection of culture species.

3-Alkyl-2,4-pentanediones Ia—If

A mixture of 1-iodoalkane (0.05 mol) and 2,4-pentanedione sodium salt (0.04 mol) in ethyl methyl ketone (100 cm^3) was stirred and heated under reflux for 72 h. Then, solvent was distilled off and water (50 cm^3) was added to the residue followed by the extraction with ether (4 × 100 cm^3) and drying of etheric layer (Na_2SO_4). After evaporation of ether, product was obtained by distillation under diminished pressure. The yields were about 40 %.

3-Heptylthio-2,4-pentanedione (Ig)

Small pieces of sodium (1.15 g; 0.05 mol) were added in portions into dry ethanol (50 cm^3). After cooling to room temperature, 1-heptanethiol (6.6 g; 0.05 mol) was added dropwise and the mixture was stirred additional 0.5 h. Then, the mixture was cooled in ice-water, 3-chloro-2,4-pentanedione (6.68 g; 0.05 mol) was added dropwise and the mixture was stirred additional 2 h at room temperature. Separated NaCl was filtered off, solvent removed by distillation and product distilled under diminished pressure. On the basis of comparison of spectral data with those of the standard sample, the first fraction collected at 55—60 °C and $p = 0.13$ kPa was characterized as heptylthiomethyl methyl ketone.

4-Alkyl-3,5-dimethylpyrazoles IIa—IIg

To the stirred solution of hydrazinium(2+) sulfate (0.025 mol) in 10 % NaOH (20 cm^3), 3-alkyl-2,4-pentanedione (0.025 mol) in ethanol (5 cm^3) was added dropwise in the course of 0.5 h at 15 °C. The mixture was stirred additional 1 h at this temperature, then water (20 cm^3) was added and the product was extracted by ether (4×25 cm^3). Combined ethereal extracts were washed with saturated solution of NaCl (20 cm^3), dried (K_2CO_3) and finally, solvent was evaporated *in vacuo*. The obtained product was purified on a column of silica gel L 100/160 using a mixture ethyl acetate—n-hexane ($\varphi_r = 3:2$) as an eluent.

4-Alkyl-3,5-dimethylisoxazoles IIIa—IIIg

To the solution of hydroxylammonium chloride (13 mmol) and K_2CO_3 (6 mmol) in water (5 cm^3), 3-alkyl-2,4-pentanedione (10 mmol) in ethanol (5 cm^3) was added. The reaction mixture was heated under reflux for 2 h, then cooled and poured into water (15 cm^3). The product was extracted by ether (4×25 cm^3). After drying of ethereal extracts over Na_2SO_4, the solvent was evaporated and remaining dark oil was purified on a column of silica gel L 100/160 using a mixture ethyl acetate—n-hexane ($\varphi_r = 3:2$) as an eluent.

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