Influence of Structure on Antimicrobial Activity of Some Heterocycles
II. Alkylpyrazolones and Alkylcoumarins

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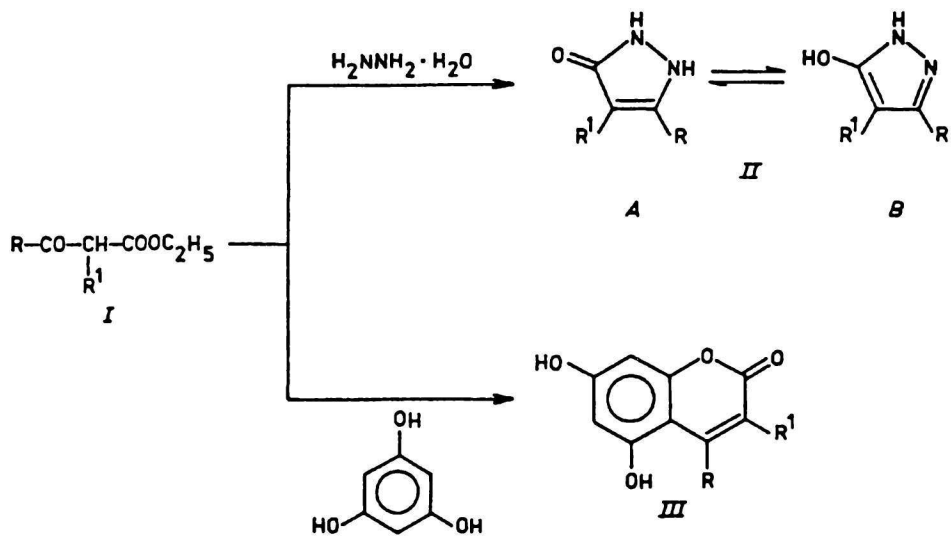
Dedicated to Dr. Ing. Š. Bauer, DrSc., in honour of his 70th birthday

Several substituted 5-pyrazolones and coumarins having long alkyl chain in the molecule were prepared. Their structure was confirmed on the basis of IR, mass, ^{1}H and ^{13}C NMR spectral data and elemental analysis. Antimicrobial activity of these compounds was also determined and discussed in relation to the structure.

Despite of the fact that a lot of 5-pyrazolone derivatives are described in the literature, till now relatively little attention has been paid to the antimicrobial efficiency of these compounds. There are described fungicidal effects of some 4-substituted 5-pyrazolones [1, 2]. Some of them were applied as pesticides [3, 4], other exhibited good analgetic effects [5]. From the point of view of antimicrobial activity, coumarins are published much more. However, derivatives with more complicated substituents on the ring are mostly studied [6—15]. Some derivatives of coumarin are produced industrially as anticoagulant rodenticides, *e.g.* Coumachlor, Coumatetralyl, Warfarin, and Dicumarol [16—18]. The latter two of them were of use also in medicine as drugs with anticoagulant effect [19—21].

With regard to our finding in the previous paper [22] that some alkylpyrazoles exhibit excellent antimicrobial effects, we decided to examine in this respect some alkylpyrazolones. Simultaneously, we also included some alkylcoumarins because their preparation required one common starting compound and the data from literature indicated an assumption to obtain antimicrobially active products. We chose substitution by longer n-alkyl also for the reason that compounds having tenside properties were our target in the next step where the mentioned alkyl chain should represent the hydrophobic part of molecule.



Scheme 1

As a starting material, ethyl 2-alkyl-3-oxobutyrate (*I*, R = CH_3, Scheme 1) was used for both types of heterocycles. So, cyclization condensation of *I* with hydrazine hydrate afforded corresponding 4-alkyl-3-methyl-5-pyrazolones (*II*, R = CH_3, Scheme 1). This type of reaction, as it is known from the literature [23, 24], proceeds smoothly with relatively good yields. Analogically, 4-alkyl-3-(2-furyl)-5-pyrazolones (*II*, R = 2-furyl) were prepared from ethyl 2-(2-furoyl)alkanoates (*I*, R = 2-furyl). However, this cyclization required more drastic reaction conditions — heating in butanol for several hours [25], and the yields of products were lower than in the case of compounds *II* where R = CH_3. Starting β-keto esters were prepared by the condensation of ethyl 2-furoate with corresponding ethyl n-alkanoates according to the analogy from the literature [26]. 3-Alkyl-4-methyl-5,7-dihydroxycoumarins (*III*, R = CH_3, Scheme 1) were prepared by cyclization of β-keto esters *I* (R = CH_3) with phloroglucinol under Pechman condensation reaction conditions where trifluoroacetic acid was used as a condensation agent [27]. The yields of coumarins were high — over 85 %. From the second group of starting β-keto esters *I* (R = 2-furyl), we were unable to prepare corresponding coumarins because resinification of reaction mixtures occurred under the mentioned reaction conditions. The survey of prepared compounds and their characteriza-

Table 1. Characterization of the Prepared Compounds

Compound	R	R^1	Formula	M_r	w_i(calc.)/% / w_i(found)/%			Yield	M.p.
					C	H	N	%	°C
If	2-Furyl	Hexyl	$C_{15}H_{22}O_4$	266.34	67.64 67.70	8.33 8.38	–	70	*a*
Ig	2-Furyl	Heptyl	$C_{16}H_{24}O_4$	280.37	68.54 68.50	8.63 8.66	–	72	*b*
Ih	2-Furyl	Octyl	$C_{17}H_{26}O_4$	294.40	69.36 69.40	8.90 8.94	–	69	*c*
Ii	2-Furyl	Decyl	$C_{19}H_{30}O_4$	322.45	70.77 70.71	9.38 9.42	–	66	*d*
Ij	2-Furyl	Dodecyl	$C_{21}H_{34}O_4$	350.50	71.96 71.99	9.78 9.83	–	62	*d*
IIa	Methyl	Hexyl	$C_{10}H_{18}N_2O$	182.27	65.90 65.86	9.95 9.98	15.37 15.41	85	208—209
IIb	Methyl	Heptyl	$C_{11}H_{20}N_2O$	196.30	67.31 67.36	10.27 10.31	14.27 14.29	83	199—200
IIc	Methyl	Octyl	$C_{12}H_{22}N_2O$	210.32	68.53 68.55	10.54 10.58	13.32 13.30	86	186—188
IId	Methyl	Decyl	$C_{14}H_{26}N_2O$	238.38	70.54 70.59	10.99 11.03	11.75 11.77	84	168—170
IIe	Methyl	Dodecyl	$C_{16}H_{30}N_2O$	266.43	72.13 72.10	11.35 11.37	10.51 10.50	81	176—178
IIf	2-Furyl	Hexyl	$C_{13}H_{18}N_2O_2$	234.30	66.64 66.68	7.74 7.76	11.96 11.99	68	128—129
IIg	2-Furyl	Heptyl	$C_{14}H_{20}N_2O_2$	248.33	67.71 67.66	8.12 8.17	11.28 11.30	69	121—122
IIh	2-Furyl	Octyl	$C_{15}H_{22}N_2O_2$	262.36	68.67 68.70	8.45 8.47	10.68 10.69	66	116—117
IIi	2-Furyl	Decyl	$C_{17}H_{26}N_2O_2$	290.41	70.31 70.37	9.02 9.06	9.65 9.64	61	104—106
IIj	2-Furyl	Dodecyl	$C_{19}H_{30}N_2O_2$	318.46	71.66 71.69	9.50 9.56	8.80 8.82	62	92—94
IIIa	Methyl	Hexyl	$C_{16}H_{20}O_4$	276.34	69.54 69.56	7.30 7.33	–	90	200—201
IIIb	Methyl	Heptyl	$C_{17}H_{22}O_4$	290.36	70.32 70.28	7.64 7.66	–	87	203—204
IIIc	Methyl	Octyl	$C_{18}H_{24}O_4$	304.39	71.03 71.04	7.95 7.97	–	89	198—200
IIId	Methyl	Decyl	$C_{20}H_{28}O_4$	332.44	72.26 72.31	8.49 8.52	–	86	185—187
IIIe	Methyl	Dodecyl	$C_{22}H_{32}O_4$	360.50	73.30 73.36	8.95 8.99	–	85	186—188

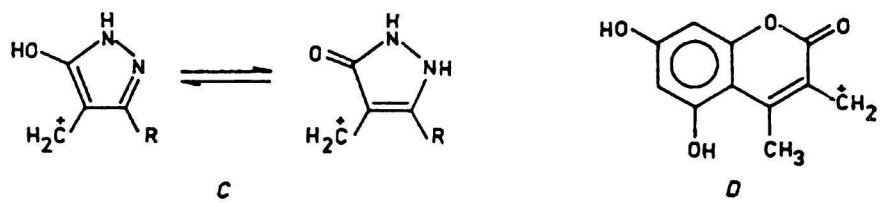
a) B.p. = 115—118 °C (1.3 Pa); *b*) b.p. = 127—130 °C (1.3 Pa); *c*) b.p. = 142—146 °C (1.3 Pa); *d*) undistilled viscous liquid.

Table 2. Antimicrobial Activity (MIC/(μg cm^{-3})) of the Prepared Compounds

Compound	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Streptococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
IIa	0.1	<1	0.1	10	1000	1000	<10
IIb	0.1	0.1	0.1	10	1000	100	<10
IIc	10	10	0.1	1000	1000	1000	10
IId	<100	100	10	1000	1000	1000	100
IIe	1000	1000	1000	1000	1000	1000	1000
IIf	1	1	1	10	1000	1000	100
IIg	1	1	1	10	1000	1000	100
IIh	1	1	10	100	1000	1000	1000
IIi	100	100	100	1000	1000	1000	1000
IIj	1000	1000	1000	1000	1000	1000	1000
IIIa	1	<1	1	10	1000	1000	100
IIIb	1	1	<10	10	1000	1000	100
IIIc	10	10	10	100	1000	1000	1000
IIId	1000	100	1000	1000	1000	1000	1000
IIIe	1000	1000	1000	1000	1000	1000	1000
Septonex	0.1	0.1	0.1	1	1000	100	10

tion is summarized in Table 1. Their structure was confirmed on the basis of elemental analysis and IR, mass, ^{1}H and ^{13}C NMR spectral data.

Depending on the method of measurement of IR spectra, either 3-pyrazolin-5-one (*II*, oxo tautomer *A*) or 5-hydroxypyrazole (*II*, enol tautomer *B*) structure was confirmed. In $CHCl_3$ solution, characteristic bands at $\tilde{\nu}$ = 3465 cm^{-1} (ν(N—H)) and $\tilde{\nu}$ = 1718 cm^{-1} (ν(C=O)) corresponding to the keto form *A* were observed. In the solid state spectroscopic IR technique (KBr pellets), absorption bands at $\tilde{\nu}$ = 3440 and 3290 cm^{-1} (vibrations of associated O—H and N—H groups) and at $\tilde{\nu}$ = 1630 cm^{-1} (ν(C=N)) characteristic of the structure of enol form *B* were registered. Moreover, in both cases expressive bands at $\tilde{\nu}$ = 1530 cm^{-1} and $\tilde{\nu}$ = 1460 cm^{-1} were observed. These bands cannot be unambiguously assigned to one individual type of vibration because skeletal vibrations of pyrazole as well as furan ring may occur in the mentioned region and moreover, the band at $\tilde{\nu}$ = 1530 cm^{-1} may correspond to the second absorption band of secondary amides (δ(N—H)). The presence of the third possible tautomeric form of compounds *II* in solution — 2-pyrazolin-5-one structure — was unambiguously excluded on the basis of ^{1}H and ^{13}C NMR spectral data (see Experimental), where the absence of H-4 proton and the presence of double bond between C-3 and C-4 atoms of pyrazolone skeleton was registered. Moreover, in the IR spectra (measured in solution), characteristic absorption band corresponding to the stretching vibrations of C=N bond was not observed. These spectral data are in accordance with those published for similar 5-pyrazolones which can be used as model compounds [28]. In the case of compounds



Formula 1

III, characteristic bands of coumarin skeleton were observed in the region of $\tilde{\nu}$ = 1670 and 1590 cm^{-1} (ν(C=O) and ν(C=C)) as well as the bands at $\tilde{\nu}$ = 3445 cm^{-1} corresponding to the stretching vibrations of hydroxyl groups. The presence of alkyl chain of compounds *II* and *III* was manifested by two strong absorption bands in the region of $\tilde{\nu}$ = 2920 and 2850 cm^{-1} corresponding to the stretching vibrations ν_{as}(C—H) and ν_s(C—H) of methylene groups.

In the mass spectra (U = 70 eV) of all prepared compounds, the peaks of molecular ions $M^{+\bullet}$ were observed. However, their relative intensity I_r considerably differed depending on the type of heterocycle and its substitution. While in the case of compounds *II* where R = CH_3 I_r was from 8 to 12 %, in those compounds *II* where R = 2-furyl, I_r increased to 25—38 % and in the case of compounds *III*, I_r reached up to 75—80 %. Com-

pounds *II* exhibited maximum peaks (I_r = 100 %) at *m/z* = 111 respectively 163 (R = CH_3 or 2-furyl), compounds *III* at *m/z* = 205 corresponding to the ions *C* and *D* (Formula 1). While in the case of compounds *II* no further significant fragmentation was observed, intensive peaks ($I_r \approx$ 60 %) corresponding to the fragmentation $M^{+\bullet} - \dot{C}H_3$ and fragmentation $M^{+\bullet} - \dot{O}H$ ($I_r \approx$ 30 %) were registered in the case of compounds *III*. Starting compounds *I* (where R = 2-furyl) also exhibited peaks of molecular ions $M^{+\bullet}$ ($I_r \approx$ 5 %). Maximum peaks corresponding to the ion $R\dot{C}O$ were registered at *m/z* = 95.

The results of antimicrobial activity testing revealed that some of the prepared compounds *II* and *III* exhibit very good effects against some gram-positive bacteria (Table 2). Similarly, as we found in the case of alkylpyrazoles and alkylisoxazoles [22], in the case of discussed alkylpyrazolones and alkylcoumarins the best efficiency was exhibited by those derivatives where the alkyl chain was represented by hexyl, heptyl, and octyl. Derivatives with longer alkyl chain showed considerably lower efficiency. As can be seen from the results, replacement of methyl group in the position 3 of pyrazolone derivatives by furyl resulted in the decrease of antimicrobial efficiency. As a standard for determination of values of minimum inhibitory concentration (MIC) we used [1-(ethoxycarbonyl)pentadecyl]trimethylammonium bromide (Septonex), antiseptic agent usually applied in practice.

EXPERIMENTAL

Starting ethyl 2-alkyl-3-oxobutyrates were prepared by alkylation of ethyl acetoacetate by corresponding alkyl bromides according to the known method [29]. The other used chemicals were commercially available products (Lachema, Brno; Fluka, Buchs; Merck, Darmstadt).

Melting points were determined on a Kofler hot-stage. IR spectra (in KBr pellets or in $CHCl_3$) were obtained on a Perkin—Elmer G-983 instrument. Mass spectra (70 eV) were measured on a Jeol JMS-100D spectrometer at an emission current of 300 μA, applying direct sample-introduction technique. ^1H and ^{13}C NMR spectra were obtained on a Bruker AM-300 spectrometer operating at 300.13 or 75.46 MHz working frequencies in $CDCl_3$ or DMSO solutions with TMS as an internal standard. For the assignment of signals in ^{13}C NMR spectra, DEPT and semiselective INEPT techniques were used. Elemental analyses were performed on a Perkin—Elmer 240 analyzer.

MIC was determined by using suspension method on solid cultivation media [22].

Ethyl 2-(2-Furoyl)alkanoates *If—Ij*

To the sodium hydride (24.0 g; 1 mol) in hot toluene (500 cm^3) *tert*-butyl alcohol (74.0 g; 1 mol) was added dropwise under stirring. When the reaction was over, a mixture of ethyl 2-furoate (70.1 g; 0.5 mol) and corresponding ethyl alkanoate (1 mol) was added dropwise under continued stirring. Reaction mixture was heated under reflux for additional 2 h, then decomposed by glacial acetic acid under cooling. Toluene solution was washed with water (3 x 100 cm^3) and the solvent removed under diminished pressure. Pure products *If—Ih* were obtained by careful vacuum distillation since distillation mixture foams strongly. In the case of compounds *Ii* and *Ij*, very high boiling points and strong foaming did not enable us to apply vacuum distillation at higher temperatures. These products were purified by careful removing of low-boiling fractions by distillation at diminished pressure and subsequent chromatography of the residue on a column of silica gel L 100/160 using benzene as eluent.

4-Alkyl-3-methyl-5-pyrazolones *IIa—IIe*

To a mixture of ethyl 2-alkyl-3-oxobutyrate (0.05 mol), water (25 cm^3), and ethanol (10 cm^3) hydrazine hydrate (30 % aqueous solution, 8 cm^3) was added gradually under stirring and the mixture was heated under reflux for 0.5 h. Then, further portion of hydrazine hydrate (2 cm^3) was added and heating under reflux continued for another 1 h. After cooling to laboratory temperature, the separated material was filtered off and crystallized from ethanol. The obtained crystalline product was dried in a vacuum desiccator over P_2O_5.

Compound *IIe*: ^1H NMR spectrum (DMSO, 298 K), δ: 3.6 (bs, NH), 2.12 (s, 3H, CH_3 at C-3), 2.26 (t, 2H, the first CH_2 in dodecyl), 1.47 (m, 2H, the second CH_2 in dodecyl), 1.33 (m, 18H, the other CH_2 in dodecyl), 0.95 (t, 3H, CH_3 in dodecyl). ^{13}C NMR spectrum (DMSO, 298 K), δ: 100.9 (C-3), 136.5 (C-4), 159.6 (C-5), 9.9 (CH_3 at C-3), 14.0 (CH_3 in dodecyl), 21.4—31.4 (CH_2 in dodecyl).

4-Alkyl-3-(2-furyl)-5-pyrazolones *IIf—IIj*

A mixture of ethyl 2-(2-furoyl)alkanoate (0.02 mol) and hydrazine hydrate (80 % aqueous solu-

tion, 0.02 mol) in butanol (25 cm^3) was heated under reflux under a nitrogen atmosphere for 20 h. Then, the reaction mixture was slightly acidified by addition of concentrated HBr and left to stand in freezing chamber overnight. Separated crystalline product was filtered off and crystallized from benzene.

Compound *IIi*: ^1H NMR spectrum ($CDCl_3$, 298 K), δ: 6.53 (d, 1H, H-3′), 6.51 (dd, 1H, H-4′), 7.50 (d, 1H, H-5′), 2.52 (t, 2H, the first CH_2 in decyl), 1.53 (m, 2H, the second CH_2 in decyl), 1.06 (m, 14H, the other CH_2 in decyl), 0.98 (t, 3H, CH_3 in decyl). ^{13}C NMR spectrum ($CDCl_3$, 298 K), δ: 102.8 (C-3), 132.5 (C-4), 161.7 (C-5), 144.7 (C-2′), 107.2 (C-3′), 111.6 (C-4′), 142.0 (C-5′), 14.1 (CH_3 in decyl), 22.1—31.9 (CH_2 in decyl). (*Note*: NH groups were not registered in the ^1H NMR spectrum; positions in furan ring are marked with comma.)

3-Alkyl-4-methyl-5,7-dihydroxycoumarins *IIIa—IIIe*

A mixture of phloroglucinol (0.01 mol) and corresponding ethyl 2-alkyl-3-oxobutyrate (0.01 mol) in trifluoroacetic acid (8 cm^3) was heated under reflux for 12 h. After pouring into cold water (30 cm^3), separated product was filtered off and water was removed by refluxing in benzene using Dean—Stark separator. The obtained product was recrystallized from ethanol.

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