NEW HYDROPHOBICITY CONSTANTS OF SUBSTITUENTS IN PYRAZINE RINGS DERIVED FROM RP-HPLC STUDY

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Pyrazine derivatives show a wide range of biological activities. 1-Pyrazin-2-ylethan-1-ones have served as food flavourants, and together with pyrazine-2-carbonitriles have been widely used as intermediates in the synthesis of various heterocyclic compounds. In our laboratory, substituted pyrazine-2-carbonitriles and 1-pyrazin-2-ylethan-1-ones have been used as intermediates for the preparation of potential antifungal and antimycobacterial drugs. Using established methods, a library of pyrazine derivatives was synthesized. Homolytic alkylation of commercially available pyrazine-2-carbonitrile yielded a series of 5-alkylpyrazine-2-carbonitriles which were converted into the corresponding 1-(5-alkylpyrazin-2-yl)ethan-1-ones (5-alkyl-2-acetylpyrazines) via the Grignard reaction. Homolytic acetylation of pyrazine-2-carbonitrile yielded 5-acetylpyrazine-2-carbonitrile. Using the same procedure, 3-acetyl-5-*tert*-butylpyrazine-2-carbonitrile was obtained with 5-*tert*-butylpyrazine-2-carbonitrile as a starting material. The hydrophobicity of the compounds was determined both experimentally (RP-HPLC) and by computation (CS ChemOffice Ultra version 9.0, ACD/LogP version 1.0 and ACD/LogP version 9.04), and both the approaches were compared. New hydrophobicity constants π based on experimental results were derived. These constants are markedly different from tabulated constants π valid for benzene rings, and can be widely used in estimating physicochemical properties of new biologically active pyrazines.

Keywords: Lipophilicity; Pyrazinecarbonitrile; Acetylpyrazine; Homolytic alkylation; Homolytic acetylation; Hydrophobicity; Chromatography; Pyrazines.

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The pyrazine moiety can be found in a number of biologically active compounds[1]. Pyrazinamide (**1**) is used as a first-line drug in the treatment of tuberculosis[2], and its analogues have been intensely studied as novel antituberculous agents[3]. Amiloride (**2**) is a potassium-sparing diuretic. It has been widely used clinically, and its structure also serves as a lead structure for the development of sodium channel blockers[4]. Acipimox (**3**), a powerful inhibitor of lipolysis, could play an important role in the treatment of obesity[5]. Some pyrazine-based compounds have also been tested as potential antifungal[6], herbicidal[7] and photodynamic agents[8]. Intense research efforts have also been directed towards synthesis and biological evaluation of various fused heterocycles possessing the pyrazine ring[9]. Among the pyrazine derivatives of natural origin, 2,3,5,6-tetramethylpyrazine (ligustrazine, **4**), a biologically active ingredient originally isolated from *Ligusticum wallichii* Franch (Umbelliferae) is best known. This traditional herbal medicine is widely used in China for the treatment of cardiovascular problems, and the compound is also produced by some bacteria. Its biological effects have been studied and significant therapeutic activities have been discovered[10] (Fig. 1).

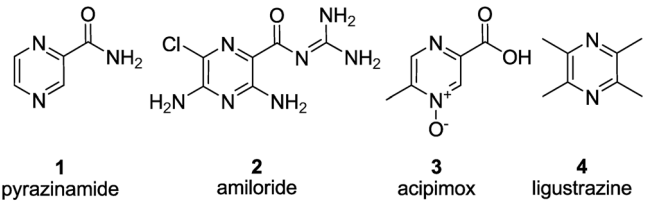


FIG. 1
Structures of clinically used pyrazine derivatives **1**–**4**

Pyrazinecarbonitriles have widely been used as intermediates in the synthesis of various heterocyclic compounds[11], whilst acetylpyrazines (together with alkyl- and alkoxypyrazines) are volatile fragrant compounds which have been employed as food and tobacco flavourants[12]. In our laboratory, various ring-substituted pyrazinecarbonitriles and acetylpyrazines were prepared as a part of studies focused on the synthesis and biological evaluation of pyrazine derivatives[13]. 1-Pyrazin-2-ylethan-1-ones reported here were used for the synthesis of ethyl 3-(5-alkylpyrazin-2-yl)-2,3-epoxybutanoates **5** [14], 3-phenyl-1-pyrazin-2-ylprop-2-en-1-ones **6** [15] and their sulfur derivatives **7** [16], thiosemicarbazones **8** [17], 4,4-dimethylthiosemicarbazones **9** [18], and 5-(1-pyrazin-2-ylethylidene)-2-thioxo-1,3-thiazolidin-4-ones **10** [19] (Fig. 2).

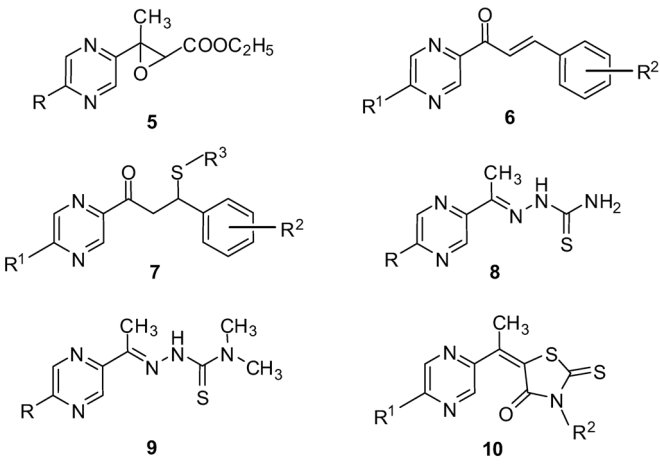


FIG. 2
Potential drugs **5**–**10** obtained by reactions of discussed 1-pyrazin-2-ylethan-1-ones with various reagents

The determination of physicochemical parameters of biologically active compounds has become more important with the advent of rational approaches in drug design[20]. One of the major prerequisites for pharmacological screening and drug development is the prediction of membrane permeability and bioavailability. Most frequently, the drugs cross biological barriers through passive transport, which strongly depends on their lipophilicity. Therefore hydrophobicity is one of the most important physical properties of biologically active compounds. This thermodynamic parameter describes the partitioning of a compound between an aqueous and an organic phase and can be characterized by the partition coefficient (log *P*)[21].

With new computerized methods of log *P* calculation, the possibility to predict hydrophobicity of large libraries of compounds came into being. However, algorithms that are sensitive to various electronic effects and individual structural aspects are still needed. Reversed phase high performance liquid chromatography (RP-HPLC) methods have become popular and they are widely used for lipophilicity measurement[22]. The general procedure involves the measurement of the directly obtainable retention time under isocratic conditions, with varying amounts of an organic modifier in the mobile phase, and calculating the capacity factor *k*. Log *k* is then used as the lipophilicity index. To compare log *k* values obtained at different concentrations of an organic modifier they are extrapolated to 0% of organic

solvent to get log k_w [23]. However, log k_w approach is not suitable for heteroaromatic compounds as discussed further.

Studies with tetramethylpyrazine and its analogues showed that pyrazine derivatives influence physical properties of biological membranes, such as membrane fluidity, and their effects are dependent on molecular size and hydrophobicity[24]. These facts inspired us to study hydrophobic properties of pyrazine derivatives prepared in our laboratory in greater detail. The aim of this pilot study was to determine lipophilicity (log k) of ring-substituted pyrazinecarbonitriles and acetylpyrazines, to derive the hydrophobic constants π of alkyl substituents in position 5 of the pyrazine ring and to compare them with the hydrophobicity constants π of alkyl substituents in para positions of benzene rings.

RESULTS AND DISCUSSION

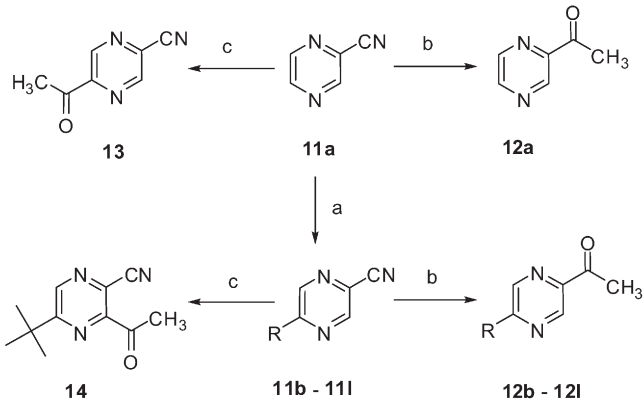
Chemistry

Electron-deficient nitrogen heteroaromatics, including pyrazine, are inert to electrophilic substitution[11b]. Homolytic alkylation and acylation were therefore applied to get the title compounds. Homolytic alkylation of commercially available pyrazine-2-carbonitrile (**11a**) gave 5-alkylpyrazine-2-carbonitriles **11b**–**11l**. The best results were achieved upon alkylation with 2,2-dimethylpropanoic acid. Since the *tert*-butyl radical is bulky, it enters almost exclusively into position 5 of the pyrazine ring. The formation of positional isomers and dialkylated or trialkylated by-products is limited and high yields (80%) of 5-*tert*-butylpyrazine-2-carbonitrile **11f** were obtained. Alkylation with other acids gave a mixture of products, from which the 5-alkyl derivative was separated by column chromatography. The yields (40–50%) were satisfactory up to six carbons in the alkyl chain. Poor solubility of higher fatty acids in water resulted in a dramatic decrease in the yields and only small quantities of nitriles **11i**–**11k** were obtained. Moreover, the unreacted acid could not be completely removed from a mixture with the alkylated nitrile by alkalizing the reaction mixture. Therefore, it was necessary to wash the diethyl ether extract with 10% solution of sodium carbonate to remove the residues of the acid before column chromatography. In order to avoid these problems and to obtain higher yields, the alkylation in water–acetonitrile (1:1) was attempted, but this modification did not lead to better results. Alkylation with phenylacetic acid proceeded without difficulties and gave a good yield (54%) of 5-benzylpyrazine-2-carbonitrile **11l**.

The alkylated pyrazinecarbonitriles were then converted to acetyl derivatives **12a**–**12l** by the Grignard reaction. The yields ranged from 26 to 57% with the exception of compounds **12j** and **12k**. The two compounds could not be separated from the reaction mixture by distillation. A small amount of acetylpyrazine **12k** was finally obtained by column chromatography. Purification and characterization of acetylpyrazine **12j** have failed.

Compounds **13** and **14** were prepared by homolytic acetylation using pyruvic acid as a source of acetyl radicals. In accordance with directing effects[25] of substituents, homolytic acetylation of pyrazine-2-carbonitrile yielded 5-acetylpyrazine-2-carbonitrile as the major product. However, the yields were rather poor (ca. 30%) even if the reaction was performed under nitrogen[13g]. An analogous procedure was then applied to the acetylation of 5-alkylpyrazine-2-carbonitriles. In all cases, a complex mixture of products was obtained, from which individual compounds could not be separated. A pure substance was only obtained from the acetylation of 5-*tert*-butylpyrazine-2-carbonitrile (**12d**). Since the acetyl radical can theoretically attack both position 3 and 6 of the pyrazine ring, a one-dimensional NOE technique was used to discriminate between the two isomers. The isolated compound was found to be 3-acetyl-5-*tert*-butylpyrazine-2-carbonitrile[13h] (**14**).

The general synthetic approach for all the prepared compounds is shown in Scheme 1.



SCHEME 1

Synthesis and structures of the studied compounds **11a**–**11l**, **12a**–**12l**, **13** and **14**

R for both **11** and **12**: **b** Pr, **c** i-Pr, **d** Bu, **e** i-Bu, **f** *t*-Bu, **g** Pen, **h** Hex, **i** Hep, **j** Oct, **k** Non, **l** Bn

Conditions: (a) RCOOH, $AgNO_3$, $(NH_4)_2S_2O_8$, H_2O; (b) CH_3MgI, Et_2O; (c) $CH_3COCOOH$, $AgNO_3$, $(NH_4)_2S_2O_8$, H_2O/MeCN or 0.5 M H_2SO_4

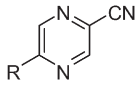
Lipophilicity

Chromatographic behavior and hydrophobicity of diazines have previously been studied[26]. Some research groups used a C_{18}-silica gel column with methanol–water mobile phase to obtain a normalized parameter log k_W, i.e. the retention factor extrapolated to 0% of organic modifier, as an alternative to log P [26d]. According to Yamagami and co-workers[26j,26o,26t], the log k_W approach works well for non-polar solutes but tends to provide overestimated log P values for strong H-acceptors. Systematic studies of their log k obtained under various HPLC conditions led to the conclusion that the use of an eluent containing around 50% methanol is the most convenient and practical procedure for the rapid estimation of log P values for heteroaromatic compounds[26p,26t]. Therefore, we decided to perform our measurements using water–methanol (1:1) as the mobile phase. Log k derived from RP-HPLC retention factors and computational log P values are given in Tables I and II.

As expected, the dependence of log k on the length of the unbranched alkyl substituents in compounds **11a**, **11b**, **11d**, **11g**–**11k** and **12a**, **12b**, **12d**, **12g**–**12k** (H, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}) is linear (for nitrile derivatives **11a**, **11b**, **11d**, **11g**–**11k**: r = 0.9993, n = 8; for acetyl derivatives **12a**, **12b**, **12d**, **12g**–**12k**: r = 0.9983, n = 8). 5-Isopropyl-substituted derivatives **11c** and **12c** should be less lipophilic than the corresponding 5-propyl congeners **11b** and **12b** according to the calculated log P values. Surprisingly, in RP-HPLC they exhibit higher lipophilicity. In the 5-butyl series, lipophilicity increases in the order log $k_{i\text{-Bu}}$ < log k_{Bu} < log $k_{t\text{-Bu}}$. This correlates well with the log P values of ChemOffice, whilst Clog P (ChemOffice) and log P (ACD) rank the three isomers in a different order (log $P_{t\text{-Bu}}$ < log $P_{i\text{-Bu}}$ < log P_{Bu}). Log P is the logarithm of the partition coefficient for octan-1-ol/water. Clog P is the logarithm of octan-1-ol/water partition coefficient based on established chemical interactions. RP-TLC study performed previously with 3-phenyl-1-pyrazin-2-ylpropen-1-ones[27] **6** showed that isobutyl derivatives are clearly the least hydrophobic, whilst *tert*-butyl and butyl derivatives exhibit practically the same lipophilicity. Therefore, it is not surprising that they can easily be interchanged on lipophilicity scales based on approximate computational data.

Relationships among experimentally determined log k, number of carbons (n_C) in alkyl substituent and Taft electronic substituent constants (σ^*) have also been analyzed. Using double linear regression, following dependences have been obtained for pyrazine-2-carbonitriles (*1*) and for 1-pyrazin-2-ylethan-1-ones (*2*):

TABLE I
Comparison of the calculated lipophilicities (log *P*/Clog *P*) with the determined log *k* of the ring-substituted pyrazine-2-carbonitriles **11a**–**11l**

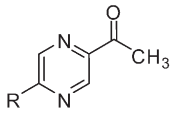


Compound	R	log *k*	log *P*/Clog *P* (ChemOffice)	log *P* (ACD/LogP)
11a	H	0.1061	–0.18/–0.590275	–0.15 ± 0.35[a] –0.153 ± 0.272[b]
11b	*n*-C_3H_7	0.5978	1.42/0.966725	1.37 ± 0.35[a] 1.369 ± 0.274[b]
11c	$(CH_3)_2CH$	0.6840	1.41/0.836725	1.19 ± 0.35[a] 1.186 ± 0.277[b]
11d	*n*-C_4H_9	0.9037	1.84/1.49573	1.90 ± 0.35[a] 1.901 ± 0.274[b]
11e	$(CH_3)_2CHCH_2$	0.8378	1.75/1.36573	1.72 ± 0.35[a] 1.717 ± 0.277[b]
11f	$(CH_3)_3C$	0.9151	1.94/1.23573	1.54 ± 0.36[a] 1.535 ± 0.285[b]
11g	*n*-C_5H_{11}	1.3266	2.26/2.02472	2.43 ± 0.35[a] 2.432 ± 0.274[b]
11h	*n*-C_6H_{13}	1.6876	2.68/2.55373	2.96 ± 0.35[a] 2.964 ± 0.274[b]
11i	*n*-C_7H_{15}	2.0556	3.09/3.08273	3.49 ± 0.35[a] 3.495 ± 0.274[b]
11j	*n*-C_8H_{17}	2.4231	3.51/3.61172	4.03 ± 0.35[a] 4.026 ± 0.274[b]
11k	*n*-C_9H_{19}	2.7878	3.93/4.14072	4.56 ± 0.35[a] 4.558 ± 0.274[b]
11l	benzyl	0.9959	2.20/1.47673	1.84 ± 0.35[a] 1.835 ± 0.278[b]

[a] ACD/LogP version 1.0. [b] ACD/LogP version 9.04.

TABLE II

Comparison of the calculated lipophilicities (log *P*/Clog *P*) with the determined log *k* of the ring-substituted 1-pyrazin-2-ylethan-1-ones **12a**–**12l**, **13**, **14**



Compound	R	log *k*	log *P*/Clog *P* (ChemOffice)	log *P* (ACD/LogP)
12a	H	0.1697	–0.90/–0.075335	0.16 ± 0.34[a] 0.161 ± 0.266[b]
12b	*n*-C_3H_7	0.7348	0.70/1.48167	1.68 ± 0.34[a] 1.683 ± 0.268[b]
12c	$(CH_3)_2CH$	0.7882	0.68/1.35167	1.50 ± 0.35[a] 1.499 ± 0.271[b]
12d	*n*-C_4H_9	1.0626	1.12/2.01066	2.21 ± 0.34[a] 2.215 ± 0.268[b]
12e	$(CH_3)_2CHCH_2$	0.9901	1.03/1.88066	2.03 ± 0.35[a] 2.031 ± 0.271[b]
12f	$(CH_3)_3C$	1.0818	1.22/1.75066	1.85 ± 0.35[a] 1.849 ± 0.277[b]
12g	*n*-C_5H_{11}	1.4694	1.54/2.53967	2.75 ± 0.34[a] 2.746 ± 0.268[b]
12h	*n*-C_6H_{13}	1.8301	1.96/3.06867	3.28 ± 0.34[a] 3.277 ± 0.268[b]
12i	*n*-C_7H_{15}	2.1987	2.37/3.59767	3.81 ± 0.34[a] 3.809 ± 0.268[b]
12j	*n*-C_8H_{17}	2.5618	2.79/4.12666	4.34 ± 0.34[a] 4.340 ± 0.268[b]
12k	*n*-C_9H_{19}	2.9264	3.21/4.65566	4.87 ± 0.34[a] 4.871 ± 0.286[b]
12l	benzyl	1.1093	1.47/1.99166	2.15 ± 0.35[a] 2.149 ± 0.273[b]
13	CN	0.2742	–0.45/–0.596925	0.41 ± 0.38[a] 0.405 ± 0.383[b]
14[c]	H	1.0207	1.68/1.42908	1.74 ± 0.39[a] 1.745 ± 0.394[b]

[a] ACD/LogP version 1.0. [b] ACD/LogP version 9.04. [c] Compound **14** is 3-acetyl-5-(*tert*-butyl)pyrazin-2-carbonitrile.

$$\log k = -0.336\ (\pm 0.058) + 0.359\ (\pm 0.012) n_C + 0.899\ (\pm 0.147)\sigma^* \qquad (1)$$

$$n = 12,\ s = 0.085,\ r = 0.996$$

$$\log k = -0.208\ (\pm 0.048) + 0.358\ (\pm 0.010) n_C + 0.785\ (\pm 0.121)\sigma^* \qquad (2)$$

$$n = 12,\ s = 0.070,\ r = 0.997.$$

These equations show that the value of log *k* is dependent not only on the number of carbons, but also on the inductive effect of alkyl in position 5. The influence of the inductive effect on log *k* is more pronounced in compounds with more electron-accepting group (nitrile) in position 2. The dependence of log *k* on the number of carbons in aliphatic chain is not influenced by the type of the substituent in position 2.

The relationship between experimental log *k* and calculated log *P* values is approximately linear. Good agreement was observed between log *k* and log *P* values generated with ACD/LogP version 1.0, ACD/LogP version 9.04 and ChemOffice/CLogP software. In pyrazine-2-carbonitrile series, $r = 0.9869$ for both ACD/LogP version 1.0 and version 9.04 and $r = 0.9792$ for ChemOffice/CLogP. In 1-pyrazin-2-ylethan-1-one series, $r = 0.9874$ for both ACD/LogP version 1.0 and version 9.04 and $r = 0.9894$ for ChemOffice/CLogP. The main disadvantage of these three programmes is that they rank 5-butyl isomers in the order $\log P_{t\text{-Bu}} < \log P_{\text{i-Bu}} < \log P_{\text{Bu}}$ which does not correspond to experimental log *k* value, as discussed above. The correlation between log *k* and ChemOffice/LogP values was not so good ($r = 0.9487$ for pyrazine-2-carbonitriles and $r = 0.9236$ for 1-pyrazin-2-ylethan-1-ones), but ChemOffice/LogP is the only software which ranks the 5-butyl substituents in accordance with experimental data. The slopes of the linear regression lines ranged from 0.563 to 0.894 depending on the studied series and software. These data only confirm the well-known fact that each computational algorithm has its advantages and disadvantages. Nonetheless, if the same algorithm is used for a series of structurally related compounds, the obtained values can be used to estimate hydrophobicity without performing time consuming experimental measurements.

Hydrophobicity constants π have been firmly established as the parameter of choice for correlating both binding to biological macromolecules and transport through a biological system[28]. Constant π expresses increments of lipophilicity caused by various groups in the basic skeleton. The π parameters of individual substituents can be calculated using the equation π =

log k_S – log k_U, where log k_S is the determined logarithm of capacity factor for the substituted compound, whereas log k_U denotes the determined logarithm of capacity factor for the unsubstituted compound. The calculated π values of the alkyl substituents in 2,5-disubstituted pyrazines are shown in Table III. The 2,5-substitution pattern in the pyrazine ring corresponds to para arrangement of the substituents in benzene ring. Therefore, hydrophobic constants π for 4-alkyl benzenes[28] are given for comparison.

TABLE III
Comparison of the calculated hydrophobic constants π of the alkyl substituents in the pyrazine derivatives **11a**–**11l** and **12a**–**12l** with those of the alkyl substituents in alkylbenzenes

Alkyl	$\pi_{\mathbf{11a–11l}}$	$\pi_{\mathbf{12a–12l}}$	$\pi_{\text{4-alkylbenzene}}$
H	0	0	0
n-C_3H_7	0.49	0.57	1.60
$(CH_3)_2CH$	0.58	0.62	1.43
n-C_4H_9	0.80	0.89	2.10
$(CH_3)_2CHCH_2$	0.73	0.82	1.93
$(CH_3)_3C$	0.81	0.91	1.88
n-C_5H_{11}	1.22	1.30	2.60[a]
n-C_6H_{13}	1.58	1.66	3.10[a]
n-C_7H_{15}	1.95	2.03	3.60[a]
n-C_8H_{17}	2.32	2.39	4.10[a]
n-C_9H_{19}	2.68	2.76	4.60[a]
benzyl	0.89	0.94	2.01

[a] Extrapolated from other homologues according to the rule: CH_2 = 0.50.

Acetylated derivatives **12a**–**12l** are more lipophilic than the corresponding nitriles **11a**–**11l**. This is in good agreement with the results of Yamagami et al.[26g,26m] who determined the partition coefficients for pyrazine (log *P* = –0.26), pyrazinecarbonitrile (log *P* = –0.01) and acetylpyrazine (log *P* = 0.20). These results enabled us to calculate the π constants for the cyano and acetyl groups:

$$\pi_{CN} = \log P_{pyrCN} - \log P_{pyr} = 0.25 \qquad (3)$$

$$\pi_{acetyl} = \log P_{Acpyr} - \log P_{pyr} = 0.46\ . \qquad (4)$$

Piraprez and co-workers[26d] studied lipophilicity of various flavourants by RP-HPLC, and determined the lipophilicity parameters for pyrazine (log k_{50} = –0.631; log k_W = –0.339) and acetylpyrazine (log k_{50} = –0.282; log k_W = 0.232). Log k_{50} is the logarithm of the capacity factor for water–methanol (1:1) mobile phase, and log k_W is extrapolation to 100% water. Based on these results, the π value for the acetyl

$$\pi_{acetyl} = \log k_{50Acpyr} - \log k_{50pyr} = 0.349 \quad (5)$$

$$\pi_{acetyl} = \log k_{WAcpyr} - \log k_{Wpyr} = 0.571 \; . \quad (6)$$

In this study, the π values for the cyano and acetyl substituents in the 2,5-disubstitution pattern can be generated from the results for compounds **11a**, **12a** and **13**:

$$\pi_{5\text{-CN}} = \log k_{\mathbf{13}} - \log k_{\mathbf{12a}} = 0.1045 \quad (7)$$

$$\pi_{5\text{-Ac}} = \log k_{\mathbf{13}} - \log k_{\mathbf{11a}} = 0.1681 \; . \quad (8)$$

Similarly, the π value for the acetyl substituent bonded in position 3 of 3-acetyl-5-*tert*-butylpyrazine-2-carbonitrile (**14**) can be calculated:

$$\pi_{3\text{-Ac}} = \log k_{\mathbf{14}} - \log k_{\mathbf{11f}} = 0.1056 \; . \quad (9)$$

In the latter case, the π value can naturally be influenced by the presence of *tert*-butyl in position 5 as well. Nonetheless, the results presented here show that, similarly to benzene, the hydrophobicity constants π of individual substituents depend on their mutual position on the pyrazine ring.

Acetylation of the pyrazine ring always results in a significant increase in hydrophobicity, whilst the introduction of a cyano group has a less pronounced effect on the hydrophobic properties. Unsubstituted pyrazine is hydrophilic due to two endocyclic nitrogen lone pairs in the heterocyclic plane, which act as H-acceptors. The introduction of an acetyl substituent (which is considered to be hydrophilic in an aromatic structure) into the heteroaromatic structure of pyrazine results in the masking of one of the nitrogen lone pairs, which in turn leads to a lipophilicity increase[26d]. The lower π value of the cyano group is probably the result of a smaller size and a reduced ability to mask the nitrogen lone pair.

Summarizing, contrary to benzene, the substituent hydrophobicity in pyrazine is influenced by the electronic interactions between the

endocyclic nitrogens and the substituent. The use of benzene-π instead of pyrazine-π would lead to erroneous estimates of hydrophobicity[26p]. The pyrazine-π_{alkyl} parameters listed in Table III and pyrazine-π_{CN} and pyrazine-π_{acetyl} constants based on our experimental work can be used for more precise predictions of hydrophobicity of various pyrazine derivatives, which are of interest as potential drugs.

EXPERIMENTAL

The starting pyrazine-2-carbonitrile (**11a**) was purchased from Aldrich. Alkylation was carried out with isobutyric acid (Aldrich), hexanoic acid (Acros Organics), heptanoic acid (Fluka), octanoic acid (Aldrich), nonanoic acid (Fluka), dekanoic acid (Aldrich) and phenylacetic acid (Lachema Brno). Pyruvic acid (Merck–Schuchardt) served as the source of acetyl radical for homolytic acetylation. Melting points were determined on a Boetius apparatus and are uncorrected. Elemental analyses were performed with EA 1110 CHNS analyzer (Carlo Erba). Silufol 254 plates (Kavalier Votice) were used for thin-layer chromatography. UV spectra of the resulting compounds were determined on a Waters photodiode array detector 2996 (Waters Corp., Milford (MA), U.S.A.) in ca. 9×10^{-4} M methanolic solution and log ε (molar absorption coefficient ε) was calculated for the absolute maximum (λ_{max}, nm) of individual target compounds. IR spectra (ν_{max}, cm^{-1}) were measured on a Nicolet Impact 400 spectrometer in $CHCl_3$, and NMR spectra on a Varian Mercury-Vx BB 300 operating at 300 MHz for ^1H and 75 MHz for ^{13}C. Chemical shifts were recorded as δ values in ppm and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal (7.26 for ^1H, 77.0 for ^{13}C in $CDCl_3$). Coupling constants *J* are given in Hz. Mass spectra were recorded using Finnigan MAT Magnum ion trap GC/MS system with internal electron ionization. The separation was carried out on a ZB5 column (Phenomenex, Inc.), 30 m × 0.25 mm × 0.25 μm, helium 4.5 plus (SIAD Prague) was used as mobile phase. The purity of the obtained compounds was checked by HPLC (see lipophilicity determination). The detection wavelength 210 nm was chosen. Peaks in the chromatogram of the solvent (blank) were subtracted from peaks in the chromatogram of a sample solution. The purity of the individual compounds was determined from the peak area in the chromatogram of sample solution.

Preparation of 5-Alkylpyrazine-2-carbonitriles **11b**–**11l**

Synthesis and characteristics of compounds **11b** and **11d**–**11f** were reported previously[13d]. The following procedure was used to prepare new derivatives. To a solution of pyrazine-2-carbonitrile **11a** (10.5 g, 0.10 mol) in water (300 ml) heated to 80 °C, silver nitrate (1.7 g, 0.01 mol) and corresponding carboxylic acid (0.10 mol) were added. Ammonium peroxydisulfate (25.1 g, 0.11 mol) in water (70 ml) was then added dropwise while stirring and temperature maintained between 75–80 °C. The reaction mixture was stirred for 1 h. After cooling, pH was adjusted to 9–10 with a 10% solution of sodium hydroxide and the mixture continuously extracted with diethyl ether. The organic extract was dried over anhydrous sodium sulfate before evaporating to dryness and then subjected to column chromatography. Chromatographic conditions: silica gel 60 Fluka (0.063–0.2 mm), petroleum ether–ethyl acetate (8:2, for **11j** 9:1).

5-Isopropylpyrazine-2-carbonitrile (**11c**). Yield 8.4 g (63%), white solid, b.p. 100–102 °C/1.60 kPa. $C_8H_9N_3$ (147.2), HPLC purity 99.23%. IR: 3067, 3024, 3020, 3005 (C–H arom.); 2973, 2934, 2875 (C–H alif.); 2239 (CN). UV, λ_{max} (log ε): 227.6 (3.24). ^{1}H NMR: 8.81 d, 1 H, *J*(3,6) = 1.7 (H-3); 8.58 d, 1 H, *J*(3,6) = 1.7 (H-6); 3.26–3.11 m, 1 H (H-1′); 1.34 d, 6 H, *J*(1′,2′) = 6.9 (H-2′). ^{13}C NMR: 166.0, 147.4, 144.1, 128.0, 115.7, 34.4, 21.7. MS, *m/z* (rel.%): 52 (10), 105 (9), 119 (14), 132 (100), 148 (84).

5-Pentylpyrazine-2-carbonitrile (**11g**). Yield 5.8 g (56%), yellow liquid, b.p. 118–125 °C/1.33 kPa. $C_{10}H_{13}N_3$ (175.2), HPLC purity 99.31%. IR: 3067, 3025, 3005 (C–H arom.); 2959, 2931, 2873, 2861 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 221.7 (3.26). ^{1}H NMR: 8.81 d, 1 H, *J*(3,6) = 1.5 (H-3); 8.55 d, 1 H, *J*(3,6) = 1.5 (H-6); 2.89 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 1.84–1.70 m, 2 H (H-2′); 1.42–1.26 m, 4 H (H-3′, H-4′); 0.89 t, 3 H, *J*(4′,5′) = 7.4 (H-5′). ^{13}C NMR: 161.7, 147.4, 145.2, 127.9, 115.7, 35.8, 31.3, 28.6, 22.3, 13.9. MS, *m/z* (rel.%): 119 (100), 132 (19), 146 (7), 176 (72).

5-Hexylpyrazine-2-carbonitrile (**11h**). Yield 6.4 g (44%), yellow liquid, b.p. 125–127 °C/1.33 kPa. $C_{11}H_{15}N_3$ (189.3), HPLC purity 99.63%. IR: 3067, 3024, 3005 (C–H arom.); 2958, 2931, 2872, 2859 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 221.7 (3.01). ^{1}H NMR: 8.81 d, 1 H, *J*(3,6) = 1.5 (H-3); 8.56 d, 1 H, *J*(3,6) = 1.5 (H-6); 2.89 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 1.82–1.69 m, 2 H (H-2′); 1.43–1.21 m, 6 H (H-3′, H-4′, H-5′); 0.87 t, 3 H, *J*(5′,6′) = 7.4 (H-6′). ^{13}C NMR: 161.7, 147.4, 145.2, 127.9, 115.7, 35.9, 31.4, 28.9, 28.7, 22.4, 14.0. MS, *m/z* (rel.%): 119 (100), 132 (17), 146 (6), 160 (5), 190 (52).

5-Heptylpyrazine-2-carbonitrile (**11i**). Yield 5.1 g (30%), yellow liquid, b.p. 135–140 °C/1.33 kPa. $C_{12}H_{17}N_3$ (203.3), HPLC purity 99.48%. IR: 3019, 3011 (C–H arom.); 2958, 2930, 2872, 2858 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 220.5 (2.94). ^{1}H NMR: 8.80 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.55 d, 1 H, *J*(3,6) = 1.4 (H-6); 2.89 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 1.82–1.65 m, 2 H (H-2′); 1.42–1.19 m, 8 H (H-3′, H-4′, H-5′, H-6′); 0.86 t, 3 H, *J*(6′,7′) = 7.4 (H-7′). ^{13}C NMR: 161.7, 147.4, 145.2, 127.9, 115.7, 35.8, 31.6, 29.1, 28.9, 28.9, 22.5, 14.0. MS, *m/z* (rel.%): 119 (19), 204 (100).

5-Octylpyrazine-2-carbonitrile (**11j**). Yield 3.0 g (14%), yellow liquid, b.p. 115–126 °C/1.06 kPa. $C_{13}H_{19}N_3$ (217.3), HPLC purity 99.46%. IR: 2957, 2929, 2857 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 220.5 (2.88). ^{1}H NMR: 8.81 d, 1 H, *J*(3,6) = 1.5 (H-3); 8.55 d, 1 H *J*(3.6) = 1.5 (H-6); 2.89 t, 2 H, *J*(1′,2′) = 7.7 (H-1′); 1.83–1.68 m, 2 H (H-2′); 1.44–1.16 m, 10 H (H-3′, H-4′, H-5′, H-6′, H-7′); 0.86 t, 3 H, *J*(7′,8′) = 6.7 (H-8′). ^{13}C NMR: 161.7, 147.4, 145.2, 127.9, 115.7, 35.8, 31.7, 29.2, 29.1, 28.9, 22.6, 14.0. MS, *m/z* (rel.%): 119 (100), 132 (18), 218 (33).

5-Nonylpyrazine-2-carbonitrile (**11k**). Yield 3.9 g (17%), yellow liquid, b.p. 127–130 °C/1.07 kPa. $C_{14}H_{21}N_3$ (231.3), HPLC purity 99.73%. IR: 2957, 2928, 2857 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 220.5 (2.83). ^{1}H NMR: 8.81 d, 1 H, *J*(3,6) = 1.5 (H-3); 8.55 d, 1 H, *J*(3,6) = 1.5 (H-6); 2.89 t, 2 H, *J*(1′,2′) = 7.8 (H-1′); 1.86–1.67 m, 2 H (H-2′); 1.43–1.17 m, 12 H (H-3′, H-4′, H-5′, H-6′, H-7′, H-8′); 0.87 t, 3 H, *J*(8′,9′) = 6.7 (H-9′). ^{13}C NMR: 161.7, 147.4, 145.2, 127.9, 115.7, 35.9, 31.8, 39.4, 29.3, 29.2, 29.2, 29.0, 22.6, 14.1. MS, *m/z* (rel.%): 119 (100), 132 (14), 160 (8), 232 (100).

5-Benzylpyrazine-2-carbonitrile (**11l**). Yield 10.5 g (54%), yellow solid, b.p. 91–93 °C/0.40 kPa. $C_{12}H_9N_3$ (195.2), HPLC purity 99.39%. IR: 3088, 3068, 3029, 3010 (C–H arom.); 2966, 2927 (C–H alif.); 2240 (CN). UV, λ_{max} (log ε): 234.7 (3.38). ^{1}H NMR: 8.82 d, 1 H, *J*(3,6) = 1.7 (H-3); 8.56 d, 1 H, *J*(3,6) = 1.7 (H-6); 7.39–7.23 m, 5 H (H-2′, H-3′, H-4′, H-5′, H-6′); 4.26 s, 2 H (CH_2). ^{13}C NMR: 159.9, 147.4, 145.3, 136.4, 129.1, 129.0, 128.2, 127.3, 115.6, 42.3. MS, *m/z* (rel.%): 51 (16), 65 (16), 91 (19), 194 (100).

Preparation of 1-(5-Alkylpyrazin-2-yl)ethan-1-ones **12a**–**12l**

Synthesis and characteristics of compounds **12a**, **12b**, **12d**–**12f** were reported previously[13d]. An analogous procedure was used for the synthesis of new derivatives. A solution of 5-alkylpyrazine-2-carbonitrile (0.128 mol) in absolute diethyl ether (50 ml) was added dropwise to methylmagnesium iodide (49.9 g, 0.300 mol) in absolute diethyl ether (200 ml) while stirring at –10 to +10 °C. The reaction mixture was stirred at the same temperature for 1 h and then poured on cracked ice. Dilute hydrochloric acid (50 ml, 1:1) was then added and the mixture extracted continuously with diethyl ether. The organic portion was dried over anhydrous sodium sulfate, and the solvent removed. Distillation of the respective residues yielded acetyl derivatives.

1-(5-Isopropylpyrazin-2-yl)ethan-1-one (**12c**). Yield 11.1 g (53%), yellow liquid, b.p. 105–107 °C/1.07 kPa. $C_9H_{12}N_2O$ (164.2), HPLC purity 99.12%. IR: 3076, 3027 (C–H arom.); 2972, 2934, 2873 (C–H alif.); 1697 (C=O). UV, λ_{max} (log ε): 273.5 (3.34). ^{1}H NMR: 9.12 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.50 d, 1 H, *J*(3,6) = 1.4 (H-6); 3.27–3.11 m, 1 H (H-1′); 2.68 s, 3 H (CH_3); 1.35 d, 6 H, *J*(1′,2′) = 7.1 (H-2′). ^{13}C NMR: 199.3, 165.9, 145.6, 142.6, 141.7, 34.3, 25.8, 22.0. MS, *m/z* (rel.%): 52 (28), 67 (15), 80 (12), 94 (18), 107 (31), 121 (52), 136 (74), 149 (100), 164 (44).

1-(5-Pentylpyrazin-2-yl)ethan-1-one (**12g**). Yield 7.5 g (30%), yellow liquid, b.p. 120–125 °C/1.33 kPa. $C_{11}H_{16}N_2O$ (196.3), HPLC purity 99.67%. IR: 3075, 3019, 3001 (C–H arom.); 2959, 2931, 2873, 2861 (C–H alif.); 1698 (C=O). UV, λ_{max} (log ε): 278.4 (3.11). ^{1}H NMR: 9.11 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.47 d, 1 H, *J*(3,6) = 1.4 (H-6); 2.87 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 2.68 s, 3 H (CH_3); 1.83–1.69 m, 2 H (H-2′); 1.42–1.27 m, 4 H (H-3′, H-4′); 0.88 t, 3 H, *J*(4′,5′) = 7.4 (H-5′). ^{13}C NMR: 199.4, 161.7, 145.4, 143.0, 142.7, 35.7, 31.4, 28.9, 25.8, 22.4, 13.9. MS, *m/z* (rel.%): 121 (16), 136 (7), 193 (100).

1-(5-Hexylpyrazin-2-yl)ethan-1-one (**12h**). Yield 15.0 g (57%), yellow liquid, b.p. 127–130 °C/1.47 kPa. $C_{12}H_{18}N_2O$ (206.3), HPLC purity 99.55%. IR: 3077, 3019, 3000 (C–H arom.); 2958, 2930, 2872, 2859 (C–H alif.); 1697 (C=O). UV, λ_{max} (log ε): 278.4 (3.20). ^{1}H NMR: 9.12 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.47 d, 1 H, *J*(3,6) = 1.4 (H-6); 2.88 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 2.69 s, 3 H (CH_3); 1.83–1.68 m, 2 H (H-2′); 1.43–1.21 m, 6 H (H-3′, H-4′, H-5′); 0.87 t, 3 H, *J*(5′,6′) = 7.4 (H-6′). ^{13}C NMR: 199.4, 161.6, 145.4, 143.0, 142.7, 35.7, 31.5, 29.2, 28.9, 25.8, 22.5, 14.0. MS, *m/z* (rel.%): 107 (6), 121 (43), 136 (90), 149 (16), 163 (7), 177 (6), 207 (100).

1-(5-Heptylpyrazin-2-yl)ethan-1-one (**12i**). Yield 7.3 g (26%), yellow liquid, b.p. 152–162 °C/1.20 kPa. $C_{13}H_{20}N_2O$ (220.3), HPLC purity 99.83%. IR: 3074, 3019, 3000 (C–H arom.); 2957, 2927, 2871, 2857 (C–H alif.); 1701 (C=O). UV, λ_{max} (log ε): 272.2 (3.16). ^{1}H NMR: 9.11 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.46 d, 1 H, *J*(3,6) = 1.4 (H-6); 2.87 t, 2 H, *J*(1′,2′) = 7.2 (H-1′); 2.68 s, 3 H (CH_3); 1.82–1.67 m, 2 H (H-2′); 1.40–1.17 m, 8 H (H-3′, H-4′, H-5′, H-6′); 0.85 t, 3 H, *J*(6′,7′) = 7.2 (H-7′). ^{13}C NMR: 199.3, 161.6, 145.4, 143.0, 142.7, 35.7, 31.6, 29.3, 29.2, 29.1, 25.8, 22.6, 14.0. MS, *m/z* (rel.%): 53 (7), 121 (30), 136 (100), 149 (20), 221 (26).

1-(5-Octylpyrazin-2-yl)ethan-1-one (**12j**). Obtained as brown oil. HPLC purity 62.10%. UV, λ_{max} (log ε): 272.2 (3.12).

1-(5-Nonylpyrazin-2-yl)ethan-1-one (**12k**). The distilled product was subjected to column chromatography. Chromatographic conditions: silica gel 60 Merck (0.040–0.063 mm), petroleum ether–ethyl acetate (9:1). Yield 1.0 g (3%), yellow liquid, b.p. 77–81 °C/0.30 kPa. $C_{15}H_{24}N_2O$ (248.4), HPLC purity 99.47%. IR: 2957, 2927, 2856 (C–H alif.); 1699 (C=O). UV, λ_{max} (log ε): 272.2 (3.01). ^{1}H NMR: 9.12 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.47 d, 1 H, *J*(3,6) = 1.4 (H-6); 2.88 t, 2 H, *J*(1′,2′) = 7.7 (H-2′); 2.69 s, 3 H (CH_3); 1.83–1.68 m, 2 H (H-2′); 1.41–1.17 m,

12 H (H-3′, H-4′, H-5′, H-6′, H-7′, H-8′); 0.86 t, 3 H, *J*(8′,9′) = 6.7 (H-9′). ^{13}C NMR: 199.4, 161.7, 145.4, 143.0, 142.8, 35.8, 31.8, 29.4, 29.3, 29.3, 29.3, 29.2, 25.8, 22.7, 14.1. MS, *m/z* (rel.%): 121 (25), 107 (5), 136 (100), 149 (16), 163 (5), 177 (5), 248 (5).

1-(5-Benzylpyrazin-2-yl)ethan-1-one (**12l**). Yield 7.3 g (27%), yellow liquid, b.p. 130–133 °C/1.20 kPa. $C_{13}H_{12}N_2O$ (212.3), HPLC purity 99.09%. IR: 3087, 3066, 3031, 3008 (C–H arom.); 2927, 2855 (C–H alif.); 1701 (C=O). UV, λ_{max} (log ε): 280.8 (3.37). ^{1}H NMR: 9.15 d, 1 H, *J*(3,6) = 1.4 (H-3); 8.48 d, 1 H, *J*(3,6) = 1.4 (H-6); 7.38–7.21 m, 5 H (H-2′, H-3′, H-4′, H-5′, H-6′); 4.25 s, 2 H (CH_2); 2.68 s, 3 H (CH_3). ^{13}C NMR: 199.2, 159.9, 145.5, 143.2, 142.8, 129.1, 129.0, 128.9, 127.0, 42.2, 25.8. MS, *m/z* (rel.%): 51 (7), 65 (8), 91 (8), 115 (22), 142 (5), 169 (20), 211 (100).

Preparation of Compounds **13** and **14**

Synthesis and characteristics of compound **13** were reported in our previous paper[13g]. Compound **14** was prepared in an analogous manner using the following method: A stirred mixture of 5-*tert*-butylpyrazine-2-carbonitrile **11b** (2.00 g, 0.0125 mol), ammonium peroxydisulfate (14.25 g, 0.0625 mol) and silver nitrate (0.325 g, 0.0019 mol) in 225 ml of 0.5 M sulfuric acid was heated to 40 °C before adding pyruvic acid (3.3 g, 0.0375 mol). The resulting mixture was stirred at 40 °C for 2 h. After cooling to room temperature, the pH was adjusted to 9 with 10% solution of sodium hydroxide, and the mixture continuously extracted with diethyl ether. The organic extract was dried over anhydrous sodium sulfate and subjected to column chromatography. Chromatographic conditions: silica gel 60 Merck (0.040–0.063 mm), petroleum ether–ethyl acetate (8:2). The product was then crystallized from anhydrous ethanol.

3-Acetyl-5-tert-butylpyrazine-2-carbonitrile (**14**). Yield 0.53 g (21%), white solid, m.p. 82–83.5 °C (ethanol). For $C_{11}H_{13}N_3O$ (203.2) calculated: 65.01% C, 6.45% H, 20.68% N; found: 65.33% C, 6.63% H, 20.52% N. HPLC purity 98.73%. IR: 3023, 3015 (C–H arom.); 2937, 2911, 2871 (C–H alif.); 2239 (CN); 1712 (C=O). UV, λ_{max} (log ε): 248.8 (2.73). ^{1}H NMR: 8.91 s, 1 H (H-6); 1.47 s, 9 H (H-2′); 2.75 s, 3 H (CH_3). ^{13}C NMR: 196.8, 166.3, 148.0, 144.3, 124.6, 115.2, 37.5, 29.5, 26.2.

HPLC Determination of Lipophilicity (Capacity Factor *k*/Calculated log *k*)

A HPLC separation module Waters Alliance 2695 XE, Waters photodiode array detector 2996 (Waters Corp., Milford (MA), U.S.A.) and a chromatographic column Symmetry® C_{18} 5 μm, 4.6 × 250 mm, Part No. WAT054275 (Waters Corp., Milford (MA), U.S.A.) were used. The HPLC separation was monitored with Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford (MA), U.S.A.). A mixture of MeOH p.a. and H_2O-HPLC–Milli-Q Grade (1:1) was used as a mobile phase. Total flow in the column was 0.9 ml/min, injection 30 μl, column temperature 22 °C and sample temperature 10 °C. The detection wavelength 210 nm was chosen. A KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes.

The capacity factors *k* were calculated using the Millennium32® Chromatography Manager Software using the formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained using an unretained analyte. The log *k* values of the individual compounds, calculated from the capacity factor *k*, are shown in Tables I and II.

Lipophilicity Calculations

Log *P* and Clog *P* values (ChemOffice) were calculated using the program CS ChemOffice Ultra version 9.0 software (CambridgeSoft, Cambridge (MA), U.S.A.). Log *P* values were also generated with ACD/LogP version 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Log *P* values calculated using ACD/Labs software version 9.04 were found in the SciFinder Scholar™ database[29]. The results are shown in Tables I and II.

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