SYNTHESIS AND ^1H AND ^{13}C NMR SPECTRA OF SULFUR DERIVATIVES OF PYRAZINE DERIVED FROM AMIDATION PRODUCT OF 2-CHLOROPYRAZINE AND 6-CHLORO-2--PYRAZINECARBONITRILE. TUBERCULOSTATIC ACTIVITY

Karel DLABAL[a], Karel PALÁT[a], Antonín LYČKA[b] and Želmíra ODLEROVÁ[c]

[a] *Faculty of Pharmacy, Charles University, 501 65 Hradec Králové*
[b] *Research Institute of Organic Syntheses, 532 18 Pardubice-Rybitví*
[c] *Research Institute of Preventive Medicine, 833 01 Bratislava*

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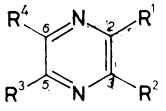
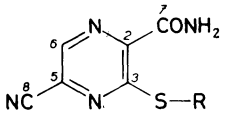
Homolytic amidations of 2-chloropyrazine and 6-chloropyrazine-2-carbonitrile have been carried out to obtain products which have been used to prepare sulfur derivatives of pyrazine as potential tubeculostatic agents. The ^1H and ^{13}C NMR spectra of the products have been measured and interpreted, and the antituberculotic activity has been evaluated.

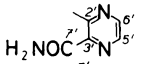
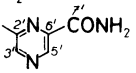
The radical reactions which allow a relatively easy introduction of alkyl, alkoxycarbonyl, amide or acyl groups into molecules of heterocyclic compounds were studied with pyridine and pyrazine compounds[1-6]. It was found[4,6] that the position of attack of the pyrazine ring by a radical is affected by the nature of substituent in monosubstituted pyrazines. The 2-substituents of +M type (Cl, OCH_3, NH_2) favour the 3-substitution to give 2,3-disubstituted pyrazines, whereas those of −M type ($COOC_2H_5$, $CONH_2$, $COCH_3$) favour the 5-substitution to give 2,5-disubstituted pyrazines.



Compound	R^1	R^2	R^3	R^4
Ia	$CONH_2$	Cl	H	H
Ib	$CONH_2$	H	Cl	H
Ic	$CONH_2$	H	H	Cl
Id	$CONH_2$	SH	H	H
Ie	$CONH_2$	H	H	SH
If	Cl	H	H	CN
Ig	$CONH_2$	Cl	CN	H
Ih	$CONH_2$	Cl	H	CN



II a, R = $-CH_2CH_3$
II b, R = $-CH_2CH_2CH_3$
II c, R = $-CH(CH_3)_2$
II d, R = $-CH_2CH_2CH_2CH_3$
II e, R = $-CH_2$- 
II f, R = 

II g, R = 
II h, R = 
II i, R = 

In order to contribute to further elucidation of homolytic amidation of monosubstituted and disubstituted pyrazine compounds, we carried out the amidation of 2-chloropyrazine (i.e. compound with a +M type substituent) and 6-chloro-2-pyrazinecarbonitrile (i.e. compound containing both +M and −M type substituents). The reaction products, i.e. 3-chloro-2-pyrazinecarboxamide (*Ia*) and 3-chloro-5-cyano-2-pyrazinecarboxamide (*Ig*) were used for syntheses of new potential antituberculotics.

EXPERIMENTAL

The melting temperatures were determined with a Kofler apparatus and are not corrected. The samples for elemental analyses were dried above phosphorus pentoxide at 0·1 kPa at room temperature. The purity of compounds was checked by TLC (Silufol UV_{254}, benzene–acetone 1 : 1). The ^1H and ^{13}C NMR spectra were measured with a JNM-FX 100 (JEOL) apparatus at 300 K at 99·602 MHz and 25·047 MHz, respectively. The substances were dissolved in hexadeuteriodimethyl sulfoxide which was used as a lock substance and internal standard. The chemical shifts were transformed to the δ scale after addition of 2·55 ppm (^1H) and 39·60 ppm (^{13}C) to the values measured. The ^1H and ^{13}C NMR spectra of the compounds containing two pyrazine rings were measured with a Bruker AM 400 apparatus at 400·13 MHz and 100·61 MHz, respectively. The twodimensional (2D) NMR spectroscopy H,H-COSY and H-C-COSY was used for assignment of the ^1H and ^{13}C chemical shifts[7,8]. The tuberculostatic activity was tested with the use of *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* PKG 8 on a liquid medium by Šula at pH 5·4 in vitro using pyrazinecarboxamide as the reference.

2-Chloropyrazine

The compound was prepared by a reaction of 2-hydroxypyrazine with phosphorus oxychloride; b.p. 38–40°C/1·33 kPa (ref.[9] gives b.p. 52°C/2·66 kPa).

6-Chloro-2-pyrazinecarbonitrile (*If*)

The compound was prepared by a reaction of 2-pyrazinecarboxamide-4-oxide with phosphorus oxychloride; b.p. 58–60°C/0·13 kPa (ref.[10] gives b.p. 90–91°C/0·93 kPa; ref.[11] gives b.p. 100–102°C/1·6 kPa).

6-Chloro-2-pyrazinecarboxamide (*Ic*)

The product was prepared by a reaction of 30% hydrogen peroxide and 6-chloro-2-pyrazinecarbonitrile in 2M-NaOH at pH 9; m.p. 170–172·5°C (ref.[12] gives m.p. 172–172·5°C).

Homolytic Amidation of 2-Chloropyrazine

A solution of 19·5 g (0·17 mol) 2-chloropyrazine in 166 g (3·7 mol) formamide was heated at 90°C and treated with 42·8 g (0·18 mol) sodium peroxodisulfate with continuous stirring. The reaction mixture was heated at 90°C for another 60 min, left to stand 24 h, and diluted with 100 ml water. The separated solid was collected by suction, washed with water, and the filtrate was extracted continuously with chloroform for 16 h. The solvent was distilled off and the solid residue was crystallized from acetone to give two products differing in their melting points but giving identical (required) results of elemental analysis. 3-Chloro-2-pyrazinecarboxamide (*Ia*): yield 27%, m.p. 188–190°C (ref.[13] gives m.p. 186–186·5°C). For $C_5H_4ClN_3O$ (157·6) calculated: 38·12% C, 2·56% H, 22·50% Cl, 26·67% N; found: 38·03% C, 2·66% H, 22·73% Cl, 26·88% N. The other substance isolated represents (according to the results of NMR analysis) a mixture of 5-chloro-2-pyrazinecarboxamide (*Ib*) and 6-chloro-2-pyrazinecarboxamide (*Ic*); yield 2·6%.

3-Mercapto- and 6-Mercapto-2-pyrazinecarboxamides (*Id*, *Ie*)

A solution of sodium ethoxide prepared from 0·9 g (0·04 mol) sodium and 20 ml ethanol was mixed with 20 ml dimethylformamide, whereupon one half of the ethanol was distilled off and the residual solution was saturated with dry hydrogen sulfide until it turned dark green. The solution thus prepared was then heated with 3·2 g (0·02 mol) 3-chloro- or 6-chloro-2-pyrazinecarboxamide at 100°C 5 h. Thereafter the solvents were distilled off under reduced pressure, and the evaporation residue was dissolved in a minimum amount of water; the insoluble portions were filtered off, and the filtrate was acidified with conc. acetic acid. The precipitated orange-red crystals of 3-mercapto- or 6-mercapto-2-pyrazinecarboxamide, respectively, were purified by dissolving in an equimolar amount of 2M-NaOH and reacidification. The crystals were collected by suction, washed with water, ethanol, and diethyl ether, and dried in a dessiccator.

3-*Mercapto*-2-*pyrazinecarboxamide* (Id), yield 85%, m.p. 202–204°C (decomp.); for $C_5H_5N_3OS$ (155·2) calculated: 38·70% C, 3·25% H, 27·08% N, 20·66% S; found: 38·52% C, 3·15% H, 26·92% N, 20·40% S.

6-*Mercapto*-2-*pyrazinecarboxamide* (Ie), yield 84%, m.p. 186–189°C (decomp.); for $C_5H_5N_3OS$ (155·2) calculated: 38·70% C, 3·25% H, 27·08% N, 20·66% S; found: 38·60% C, 3·10% H, 26·80% N, 20·51% S.

Homolytic Amidation of 6-Chloro-2-pyrazinecarbonitrile

A solution of 70 g (0·5 mol) 6-chloro-2-pyrazinecarbonitrile (*If*) in 495 g (11 mol) formamide was heated at 90°C and treated with 140 g (0·52 mol) potassium peroxodisulfate added portionwise. The reaction mixture was stirred at 90°C 60 min, left to stand 24 h, and then diluted with 300 ml water. The insoluble portion was removed by suction, washed with a small amount of water, and the filtrate was extracted with chloroform continuously for 48 h. The solvent was removed from the extract by distillation, and the crystalline residue was recrystallized from ethanol to give the product *Ih*. The ethanolic mother liquor was evaporated until dry, and the residue was recrystallized from water to give the product *Ig*. The products *Ih* and *Ig* differ considerably in their melting points but their elemental analyses correspond to the structure presumed.

3-*Chloro*-6-*cyano*-2-*pyrazinecarboxamide* (Ih), yield 1·8%, m.p. 246—248°C; for $C_6H_3ClN_4O$ (182·6) calculated: 39·47% C, 1·66% H, 19·42% Cl, 30·69% N; found: 39·45% C, 1·44% H, 19·64% Cl, 30·88% N.

3-*Chloro*-5-*cyano*-2-*pyrazinecarboxamide* (Ig), yield 35%, m.p. 132—134°C; for $C_6H_3ClN_4O$ (182·6) calculated: 39·47% C, 1·66% H, 19·42% Cl, 30·69% N; found: 39·56% C, 1·55% H, 19·29% Cl, 30·46% N.

3-Alkylthio- and 3-Arylthio-5-cyano-2-pyrazinecarboxamides *IIa—IIi*

A mixture of 1·82 g (0·01 mol) 3-chloro-5-cyano-2-pyrazinecarboxamide, 0·01 mol of the respective alkanethiol or arenethiol, 30 ml anhydrous triethylamine, and 30 ml anhydrous diethyl ether (for ethanethiol, 1-propanethiol, 2-propanethiol, and 2-pyrazinethiol) or anhydrous benzene (for benzenethiol, phenylmethanethiol, 3-mercapto-2-pyrazinecarboxamide, 6-mercapto-2-pyrazinecarboxamide, and 1-butanethiol) was refluxed 4—7 h. Then the solvents were distilled off under reduced pressure, and the solid residue was washed thoroughly with water, ethanol, and diethyl ether. The recrystallization was carried out from ethanol or water. Nine sulfides were prepared in this way (Table I).

TABLE I

3-Substituted thio derivatives of 5-cyano-2-pyrazinecarboxamide (*IIa—IIi*)

Compound	Melting point, °C (solvent)	Yield %	Formula (M.w.)	Calculated/Found			
				% C	% H	% N	% S
IIa	165—166·5 (ethanol–water)	43	$C_8H_8N_4OS$ (208·2)	46·14 46·07	3·87 3·69	26·91 27·12	15·40 15·22
IIb	176·5—178 (ethanol–water)	40	$C_9H_{10}N_4OS$ (222·3)	48·63 48·68	4·53 4·57	25·21 25·40	14·40 14·27
IIc	207·5—208·5 (ethanol–water)	45	$C_9H_{10}N_4OS$ (222·3)	48·63 48·41	4·53 4·37	25·21 25·22	14·40 14·31
IId	150—153 (ethanol–water)	38	$C_{10}H_{12}N_4OS$ (236·3)	50·83 50·62	5·12 5·08	23·71 23·52	13·57 13·72
IIe	179—182 (ethanol)	81	$C_{13}H_{10}N_4OS$ (270·3)	57·76 57·85	3·73 3·89	20·73 20·46	11·86 11·68
IIf	230—233·5 (ethanol)	78	$C_{12}H_8N_4OS$ (256·3)	56·24 56·43	3·16 3·00	21·86 21·79	12·51 12·45
IIg	192·5—194 (water)	54	$C_{10}H_6N_6OS$ (258·3)	46·51 46·38	2·34 2·26	32·54 32·65	12·41 12·29
IIh	235—238 dec. (water)	53	$C_{11}H_7N_7O_2S$ (301·3)	43·85 43·95	2·34 2·32	32·54 32·36	10·64 10·52
IIi	278—281·5 dec. (water)	60	$C_{11}H_7N_7O_2S$ (301·3)	43·85 43·84	2·34 2·26	32·54 32·33	10·64 10·73

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of monosubstituted pyrazines were described by Cox and Bothner-By[14]. The authors found the following intervals of the ^nJ(H, H) coupling constants: ^3J(H-5, H-6) = 2·426 to 2·857 Hz, ^4J(H-3, H-5) = −0·112 to −0·462 Hz, and ^5J(H-3, H-6) = 1·331 to 1·451 Hz. Using these values and with application of selective decoupling we assigned the ^{1}H and ^{13}C chemical shifts in the model 2-substituted pyrazines (Table II).

The main amidation product from 2-chloropyrazine exhibits the ^{1}H NMR coupling constant J(H, H) = 2·44 Hz, hence according to ref.[14] the remaining two protons are vicinal, and the main amidation product from 2-chloropyrazine is 3-chloro-2-pyrazinecarboxamide (*Ia*). By means of ^{1}H and ^{13}C NMR spectra it was shown that the separated minor product formed in this amidation is a mixture of two compounds out of which one is 6-chloro-2-pyrazinecarboxamide (*Ic*). The model authetic compound was obtained by the reaction of 6-chloro-2-pyrazinecarbonitrile (*If*) with hydrogen peroxide. The other compound is 5-chloro-2-pyrazinecarboxamide (*Ib*) as the last possible combination of the two substituents in pyrazine ring (with the same elemental composition).

The main amidation product from 6-chloro-2-pyrazinecarbonitrile (*If*) is 3-chloro--5-cyano-2-pyrazinecarboxamide (*Ig*). The structure was assigned on the basis of the fact that the signal of the carbon atom to which the nitrile group is attached (which causes an upfield shift of this signal) is split into a doublet in the proton-coupled ^{13}C NMR spectrum, the coupling constant being ^2J(C, H) = 8·8 Hz. If the amidation took place at the position adjacent to the nitrile group, no such splitting would be observable, since the coupling constants ^4J(C, H) are known to be very small in pyrazine derivatives[15] (usually less than 1·6 Hz). From the amidation product of 6-chloro-2-pyrazinecarbonitrile (*If*) we isolated a minor product (*Ih*) whose ^{1}H and ^{13}C NMR spectra were measured, too. Like in the main product, in the minor product the signal of the carbon atom carrying the nitrile group is split into a doublet with the coupling constant ^2J(C, H) = 12·0 Hz, hence compound *Ih* contains the grouping —N=CH—C(CN)=N— identical with that in the main product. As the elemental analyses of *Ih* and *Ig* are the same, compound *Ih* differs from *Ig* by reversed positions of chlorine and amide group with regard to nitrile group. Hence the side product is 3-chloro-6-cyano-2-pyrazinecarboxamide (*Ih*). This product, however, cannot be formed by amidation of 6-chloro-2-pyrazinecarbonitrile (*If*), and it is formed as an amidation product from 5-chloro-2-pyrazinecarbonitrile (small amounts of the latter compound are always present in the 6-chloroderivative[10,16]). Table III presents the ^{1}H and ^{13}C NMR chemical shifts of compounds *Ia*–*Ih*.

The reactions of 3-chloro-5-cyano-2-pyrazinecarboxamide (*Ig*) with respective alkane- and arenethiols gave the compounds *IIa*–*IIi* whose ^{1}H and ^{13}C NMR spectral characteristics are given in Table IV. The carbon signals were assigned

TABLE II

The ^1H and ^{13}C NMR chemical shifts (δ, ppm) in 2-substituted pyrazines in hexadeuteriodimethyl sulfoxide

Substituent	H-2/C-2	H-3/C-3	H-5/C-5	H-6/C-6
H	8·63	8·63	8·63	8·63
	145·7	145·7	145·7	145·7
Cl	—	8·78	8·60	8·46
	148·6	144·8	143·4	144·5
CN[a]	—	9·23	9·00	8·90
	130·1	148·7	148·3	145·9
$CONH_2$[b]	—	9·25	8·90	8·76
	145·1	143·7	147·5	143·5
$SCH_2CH_2CH_3$[c]	—	8·60	8·34	8·51
	156·4	143·3	139·5	143·9

[a] δ(CN) = 116·0; [b] $\delta(CONH_2)$ = 7·90 and 8·28/165·3; [c] $\delta(SCH_2CH_2CH_3)$ = 3·18/30·8; 1·69/22·3; 1·01/13·3.

TABLE III

The ^1H and ^{13}C NMR chemical shifts (δ, ppm) in compounds *Ia*–*Ig* in hexadeuteriodimethyl sulfoxide

Compound	H-2/C-2	H-3/C-3	H-5/C-5	H-6/C-6	NH_2/CO
Ia	—	—	8·66[a]	8·73[a]	8·26 and 7·99
	144·9	148·4	145·3	142·5	165·6
Ib	—	9·04[b]	—	8·88[b]	8·33 and 7·97
	143·9	143·6	150·8	143·1	164·3
Ic	—	9·16[c]	9·03[c]	—	8·31 and 8·00
	145·0	142·0	147·2	147·0	163·9
If[d]	—	9·10	9·20	—	—
	148·7	147·0	149·0	128·1	
Ig[e]	—	—	—	9·31	8·33 and 8·21
	151·3	145·0	128·6	146·3	164·5
Ih[f]	—	—	9·29	—	8·52 and 8·21
	146·7	149·2	142·4	131·6	162·9

[a] ^3J(H, H) = 2·44 Hz; [b] ^5J(H, H) = 1·40 Hz; [c] ^4J(H, H) < 0·5 Hz; [d] δ(CN) = 114·8; ^4J(H, H) < 0·5 Hz; [e] δ(CN) = 114·8; [f] δ(CN) = 114·5.

TABLE IV

^{1}H and ^{13}C NMR chemical shifts (δ, ppm) in compounds *IIa*—*IIi* in hexadeuteriodimethyl sulfoxide

Compound	H-2/C-2	H-3/C-3	H-5/C-5	H-6/C-6	H-7/C-7	H-8/C-8	R[a]				
IIa	—	—	—	8·90	8·35 and 8·02	—	3·09	1·33			
	144·3	158·3	129·5	140·6	164·9	115·6	24·0	13·2			
IIb	—	—	—	8·92	8·35 and 8·02	—	3·05	1·69	1·04		
	144·4	158·5	129·6	140·8	165·2	115·9	31·7	21·3	13·6		
IIc	—	—	—	8·93	8·35 and 8·02	—	3·88	1·38			
	144·4	158·2	129·7	140·8	165·1	115·9	34·3	22·2			
IId	—	—	—	8·92	8·35 and 8·02	—	3·07	1·7	1·5	0·95	
	144·4	158·5	129·6	140·8	165·1	115·9	30·0[b]	29·4[b]	21·7	13·6	
IIe	—	—	—	8·98	8·49 and 8·16	—	4·34	[c]			
	143·7	158·0	129·6	141·3	165·1	115·8	34·5	136·9	129·4	128·5	127·3
IIf	—	—	—	8·59	8·49 and 8·16	—	—	[d]			
	143·2	158·2	129·8	142·0	165·2	115·8	129·1	135·4	129·5	129·6	
IIg	—	—	—	9·05	8·95 and 8·24	—	—	8·91	8·73	8·78	
	143·2	156·4	129·8	142·9	165·0	115·4	150·6	150·8	144·3	145·6	
IIh	—	—	—	9·18	8·41 and 8·08	—	—	—	8·64[c]	8·66[e]	
	145·3	153·6	129·2	144·7	165·3	115·3	152·2	149·6[e]	141·3	146·0	
IIi	—	—	—	9·20	[f]	—	—	[f]			
	[f]	155·9[b]	129·9	142·5	[f]	115·3	152·7[b]	[f]			

[a] The δ(^{1}H) and δ(^{13}C) values in the substituents R are given in the order of their distance from the sulfur atom; [b] the assignment may be reversed; [c] $\delta(^1H)_{arom}$ = 7·25—7·53; [d] a broadened singlet with the centre at 7·54; [e] $\delta(CONH_2)$ = 8·35 and 7·95/166·1, the other signals: 8·64/ /141·3 (C-5′), 8·66/146·0 (C-6); [f] $\delta(CONH_2)$ = 8·63, 8·26, 8·23, 7·95/165·0, 164·5; the other signals: 9·09/149·4 (C-3′); 145·6; 143·3; 9·09/143·1 (C-5′).

on the basis of the appearance of the proton-coupled spectra and results of selective decoupling. The respective C–H pairs were determined after the analysis of heteronuclear shift-correlated spectra (H, C-COSY).

The analysis of ^{1}H and ^{13}C NMR spectra of the compounds prepared shows that our results agree with the conclusions by Houminer et al.[4], i.e. the substituents of +M type favour the radical reactions proceeding predominantly at the *ortho* position (small amount of the *p*- and *m*-isomers being also formed), whereas those of −M type cause the substituent to enter the *para* position.

Table V presents the tuberculostatic activities of some pyrazinecarboxamides. The most active compound of this series is 3-chloro-5-cyano-2-pyrazinecarboxamide (*Ig*) which is twice as active against *M. tuberculosis* and sixteen times as active against *M. kansansii* as compared with pyrazinecarboxamide. If the chlorine atom in *Ig* is replaced by an alkylthio or arylthio group, the activity is substantially decreased.

TABLE V

Tuberculostatic activity of pyrazinecarboxamides *I* and *II*

Compound	MIC, μg/ml	
	M. tuberculosis $H_{37}Rv$	*M. kansasii* PKG 8
Id	>200	>200
Ie	200	>200
Ig	12·5	25
Ih	25	100
IIa	100	400
IIb	100	400
IIc	100	400
IId	50	400
IIe	50	100
IIf	100	400
IIg	400	>400
IIh	200	400
IIi	400	>400
Pyrazinecarboxamide	25	400

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