New Isomeric 2-*ortho*-(*meta*- and *para*-) Chloro-(bromo and nitro-)benzylthio-6-aminouracils

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Received February 22, 2001

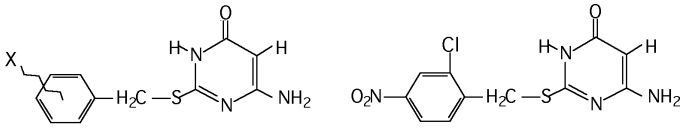
Ten new *ortho*, *meta* and *para* substituted derivatives of 2-benzylthio-6-aminouracils have been prepared. Electron Impact (EI) induced mass spectral fragmentation of these compounds was investigated. Fragmentation pathways are proposed on the basis of accurate mass and metastable transition measurements. The correlation between the intensities of the $M^{+\cdot}$ and the selected fragment ions of these compounds is discussed. The data obtained create the basis for dinstinguishing isomers. The ^{1}H and ^{13}C NMR spectra of these compounds were assigned unambiguously using a combination of heteronuclear (HETCOR) spectra the chemical shifts. The data derived from these spectra can be used to differentiate the isomers.

J. Heterocyclic Chem., **38**, 1425 (2001).

Introduction.

6-Amino-4-chloro-2-benzylthiopyrimidine is a good inhibitor of HIV-1 reverse transcriptase (RT) [1]. 6-Amino-substituted pyrimidine thioethers have been reported to constitute a novel class of non nucleoside HIV-1 reverse transcriptase inhibitors (NNRTI's) with activity against BHAP – resistant HIV [2].

Recently, we have reported on the syntheses and physicochemical properties, as well as mass spectrometric study of 2-*ortho*-(*meta*- and *para*-)chloro-(bromo- and nitro-)benzylthiouracils and 6-methyluracils [3,4] as well as 6-carboxyuracils [5]. However to the best of our knowledge no work has been published about the synthesis and physicochemical properties of S-benzyl substituted derivatives of 2-thio-6-aminouracils. This fact has stimulated us to prepare a series of 2-*ortho*-(*meta*- and *para*-)chloro-(bromo- and nitro-) benzylthio-6-aminouracils (**1** - **9**) as well as 2-[2-chloro-4-nitrobenzylthio]-6-aminouracil (**10**) (Figure 1).



1 - 9 10

1. X = *o*-Cl
2. X = *m*-Cl
3. X = *p*-Cl
4. X = *o*-Br
5. X = *m*-Br
6. X = *p*-Br
7. X = *o*-NO_2
8. X = *m*-NO_2
9. X = *p*-NO_2
10. X = *o*–Cl, *p*–NO_2

Figure 1. The list of structures of compounds.

This paper deals with the synthesis and physicochemical properties of **1**-**10**. The analyses of ^{13}C NMR and EI mass spectra of these compounds have been performed to check the possibility of differentiation of positional isomers. We wished to establish whether it is possible to determine the position of halo- (or nitro-) groups in the phenyl ring on the basis of differences in the values of μ, *i.e*. the ratio of the intensity of selected fragment ion peaks to that of the parent ion peak, and to compare the data with those previously obtained in our laboratory [3,4].

Results and Discussion

A series of ten new *ortho*-(*meta*- and *para*-)chloro-(bromo- and nitro-) substituted 2-benzylthio-6-aminouracils **1-10** has been synthesised in the reaction of 2-thio-6-aminouracil with corresponding benzyl halides. Treatment of 2-thio-6-aminouracil with *o*-(*m*- and *p*-) chloro-(bromo- and nitro-)benzyl bromides (or chlorides), as well as 2-chloro-4-nitrobenzyl chloride in 0. 1 *N* solution of NaOH in methanol at room temperature afforded **1-10** (Figure 1). The structures of all compounds obtained were determined by examining their UV/VIS, IR, ^{1}H and ^{13}C NMR spectra as well as on the basis of elemental analyses (Tables 1- 4).

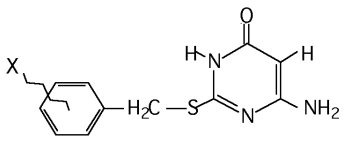
The obtained 2-benzylthio-6-aminouracils **1**–**10** may appear in various tautomeric forms differing by the position of protons [6]. The prototropic tautomerism of thiouracils and their S-substituted derivatives has attracted attention in the last decade [7-13]. This tautomerism is somewhat different than that of uracils, because a substitution of exocyclic oxygen by sulfur atom in thiouracil makes the tautomerization process easier than in uracil. According to literature, 2- methylthiouracil exists only as the 4-oxo form in solution [7], but in the gas phase as nearly equimolar hydroxy-oxo tautomeric equilibrium [9–13].

The UV/VIS spectra of **1**–**10** were measured only as their monoanionic forms in 0.1 *N* NaOH aqueous solutions (pH 13. 0) taking into regard poor solubility of these compounds in the majority of organic solvents. These spectra show absorption maxima at 220 – 223 nm and 264 – 268 nm (Table 3). It ought to be pointed out that UV/VIS spectra of monoanionic forms of **1–10** show blue shifts of the characteristic long wavelength bands as compared to neutral species of 2-*o*-(*m*- and *p*-) chloro- (and bromo-) benzylthio-6-methyluracils [3].

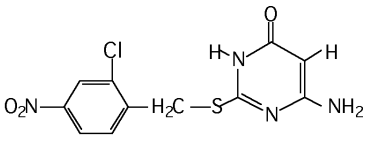
A comparison of these data with those of 1- ethyl-2-thiouracil and 1-methyl-2-ethylthiouracil [14] indicate that **1**–**10** molecules exist in the 0.1 NaOH aqueous solution as 4–oxo tautomers.

The IR spectra of **1**–**10** show absorption bands in the regions 2676 – 2745 and 3459 – 3486 cm^{-1} which have

Table 1
Chemical and Physical Data of Compounds **1**–**10**



1 - 9



10

Comp.	X	Formula (mol. weight)	M. p. [°C]	Yield (%)	R_f TLC	Reaction time (h)	Crystallization solvent
1	*o*–Cl	$C_{11}H_{10}N_3OSCl$ 267. 03	264 - 6	55	0.37	3	EtOH/H_2O 1:3
2	*m*–Cl	$C_{11}H_{10}N_3OSCl$ 267.03	247 - 9	44	0.52	12	EtOH/H_2O 1:3
3	*p*-Cl	$C_{11}H_{10}N_3OSCl$ 267.03	252 - 4	55	0.31	12	EtOH/H_2O 1:3
4	*o*–Br	$C_{11}H_{10}N_3OSBr$ 312.04	270 - 2	74	0.45	2	EtOH/H_2O 1:3
5	*m*–Br	$C_{11}H_{10}N_3OSBr$ 312.04	240 - 1	52	0.51	2	EtOH/H_2O 1:3
6	*p*-Br	$C_{11}H_{10}N_3OSBr$ 312.04	247 - 9	60	0.42	2	EtOH/H_2O 1:3
7	*o*–NO_2	$C_{11}H_{10}N_4O_3S$ 278.03	275 - 6	79	0.43	1	EtOH/H_2O 1:3
8	*m*–NO_2	$C_{11}H_{10}N_4O_3S$ 278.03	272 - 4	79	0.37	1	EtOH/H_2O 1:3
9	*p*–NO_2	$C_{11}H_{10}N_4O_3S$ 278.03	247 - 9	73	0.52	2	EtOH/H_2O 1:3
10	2–Cl–4-NO_2	$C_{11}H_9N_4O_3SCl$ 312.03	241 - 3	76	0.83	1	EtOH/H_2O 1:2

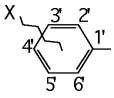
Table 2
Elemental Analyses, UV/VIS, and IR Data of Compounds **1**–**10**

Comp.	UV/VIS		IR ν [cm^{-1}]			Elemental Analysis (%) Calcd.			Found		
	λ_{max} [nm] (log ε) 0.1 N NaOH (pH 13)		ν S-CH_2	ν NH_2 δ NH_2	ν CO	C	H	N	C	H	N
1	264.0	3.95	2682	3468	1679	49.34	3.77	15.70	48.42	3.51	16.02
	222.0	4.45		1614							
2	264.0	3.94	2745	3467	1699	49.34	3.77	15.70	49.35	3.63	15.14
	222.0	4.45		1611							
3	263.5	3.94	2737	3459	1689	49.34	3.77	15.70	49.60	3.75	15.75
	223.0	4.47		1610							
4	264.0	3.94	2680	3467	1680	42.31	3.23	13.46	41.48	3.01	13.29
	222.0	4.46		1613							
5	264.0	3.96	2681	3486	1694	42.31	3.23	13.46	42.01	3.00	13.52
	222.5	4.47		1619							
6	263.0	3.99	2744	3463	1677	42.31	3.23	13.46	42.20	3.24	13.39
	223.5	4.50		1603							
7	263.5	4.08	2680	3475	1683	47.47	3.63	20.14	47.70	3.63	20.43
	220.5	4.47		1614							
8	265.5	4.16	2683	3488	1692	47.47	3.63	20.14	47.31	3.72	20.09
	221.5	4.4 8		1618							
9	268.5	4.17	2676	3478	1680	47.47	3.63	20.14	47.40	3.60	19.81
	220.0	4.44		1615							
10	267.0	4.18	2682	3469	1678	42.24	2.91	17.92	42.25	2.70	17.87
	220.0	4.45		1613							

been assigned to ν C–S and ν N–H vibrations, respectively. Moreover, the signals assigned to ν C=O and δ N–H appear in the regions 1678 – 1694 and 1611 - 1620 cm^{-1} (Table 3).

Table 3

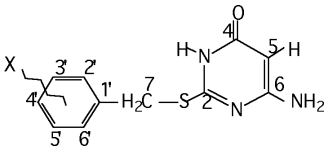
^{1}H-NMR shifts of **1**-**10** [a].

Compound	S-CH_2 (s)	C-6 NH_2 (s)	C-5H (s)	
1	4.41	6.60	4 .97	C-3' H d 7.71 C-5' H t 7.29 C-4' H t 7.47 C-6' H d 7.69
2	4.32	6.60	4.97	C-2' H s 7.53 C-5' H t 7.19 C-4' H d 7.42 C-6' H d 7.34
3	4.31	6.58	4.96	C-2' 6' H d 7.46 C-3' 5' H d 7.36
4	4.42	6.63	4.97	C-3' H d 7.71 C-5' H t 7.29 C-4' H t 7.31 C-6' H d 7.48
5	4.32	6.60	4.97	C-2' H s 7.53 C-5' H t 7.19 C-4' H d 7.42 C-6' H d 7.34
6	4.28	6.50	4.94	C-2' 6' H d 7.49 C-3' 5' H d 7.41
7	4.61	6.58	4.96	C-3' H d 8.00 C-5' H t 7.55 C-4' H t 7.69 C-6' H d 7.89
8	4.45	6.60	4.98	C-2' H s 8.33 C-5' H t 7.60 C-4' H d 8.12 C-6' H d 8.10
9	4.43	6.60	4.98	C-2' 6' H d 8.16 C-3' 5' H d 7.76
10	4.48	6.65	5.02	C-3' H d 8.30 C-6' H d 8.11 C-5' H t 8.14

[a] Spectra determined in dimethyl–d_6 sulfoxide at 25 °C and shifts are reported in ppm (δ) downfield from tetramethylsilane.

Table 4

^{13}C NMR Shifts of **1**-**10** [a]



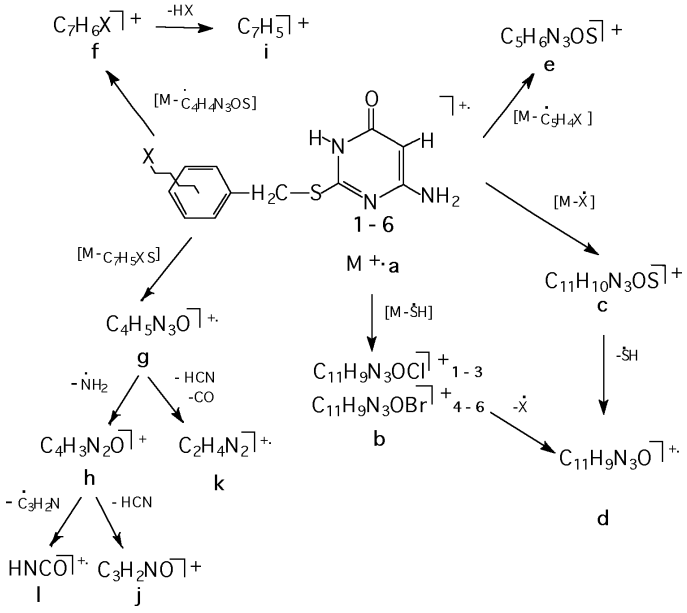
ortho C – 2'
meta C – 3'
para C – 4'

Comp.	C-2	C-4	C-5	C-6	C-7	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
1	162.25	163.69	81.31	164.96	31.33	135.40	133.14	129.34	129.33	127.6	131.76
2	162.34	163.69	81.30	164.94	32.43	141.01	128.80	132.82	127.00	130.13	127.82
3	162.13	163.64	81.32	164.74	32.41	137.41	131.03	128.27	131.69	128.27	131.03
4	162.28	163.71	78.22	164.88	33.91	137.02	124.10	132.57	127.78	129.41	131.78
5	162.23	163.66	81.29	164.84	32.38	141.30	131.72	121.47	129.94	130.49	128.29
6	162.16	163.62	81.28	164.72	32.44	137.86	131.40	131.49	120.20	131.19	131.40
7	163.65	163.70	81.28	164.91	30.46	132.89	147.99	124.90	128.87	133.82	133.74
8	162.43	163.76	81.32	165.10	32.22	141.15	123.59	147.73	121.95	129.69	135.94
9	162.38	163.77	81.33	165.12	32.43	146.46	129.99	123.32	146.82	123.77	130.39
10	162.56	163.70	81.36	165.42	31.03	143.41	134.01	124.09	147.15	121.89	132.56

[a] Spectra determined in dimethyl-d_6 sulfoxide at 25 °C and shifts are reported in ppm (δ)downfield from tetramethylsilane.

The ^{1}H and ^{13}C NMR data of **1** – **10** are given in Tables 3 and 4. Assignments of the ^{1}H NMR and ^{13}C NMR resonances of these compounds were deduced on the basis of signal multiplicities, and by the concerted application of two–dimensional NMR technique HETCOR. The HETCOR results allow unequivocal assignment of the ^{13}C NMR spectra proposed on the basis of chemical shift theory, additivity rules and by comparing the measured and calculated chemical shifts [15,5]. The ^{1}H NMR spectra of **1**-**10** show singlets of C-5H, S-CH_2 as well as NH_2

protons situated at 4.94 – 5.02; 4.28 – 4.48 and 6.58 – 6.65 ppm, respectively. The signals of protons of *ortho*-(*meta*- and *para*-)substituted benzyl groups of **1-10** appear in the range of 7.19 – 8.33 ppm (Table 3). Table 4 gives the ^{13}C NMR data for **1-10**. In order to exemplify the attributions made for each compound on the basis of the analysis of HETCOR spectra the case of **7** is discussed. For this compound the ^{1}H NMR spectrum exhibits two doublets at 7.89 and 8.00 ppm, ascribed to C – 3' H and C – 6'H, respectively. The correlation between the pair of signals at 133.74 ppm and 124.90 ppm with ^{1}H NMR signals at 7.89 and 8.00 ppm allows the assignment of these signals to C-3' and C-6' respectively. Moreover, the triplet at 7. 69 due to C - 4'H in the ^{1}H NMR spectrum correlates with the signal at 128. 87 ppm in ^{13}C NMR spectrum, as well as the triplet at 7.55 ppm due to C-5' H in ^{1}H NMR spectrum correlates with the signal at 133.72 ppm in ^{13}C NMR spectrum. These correlations allow the assignments of these signals to C-4' and C-5', respectively. The remaining two carbons at 30.46 and 81.28 correspond to the singlets of S-CH_2 and C5-H protons at 4.61 and 4.96 ppm, respectively.



Scheme 1. The pathways of the EI–mass fragmentation of the molecular ions of **1-6.**

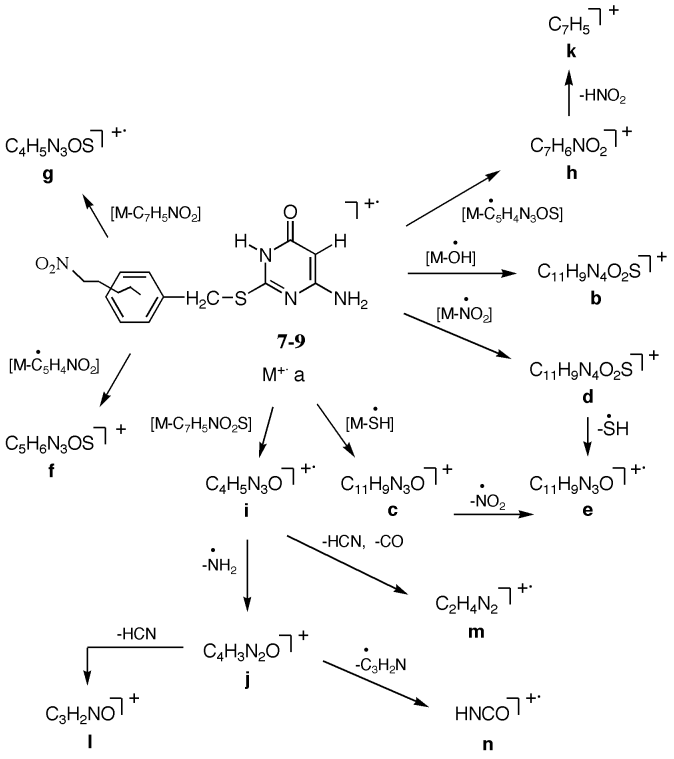
A comparison of the number and positions of the signals of the carbon atoms in the range of 130 – 140 as well as 140 – 150 ppm in ^{13} C NMR spectra of **1 - 9** allows a differentiation between *ortho*-, *meta*- and *para*- substituted in benzylthio group isomers.

This data are given in tabular form below:

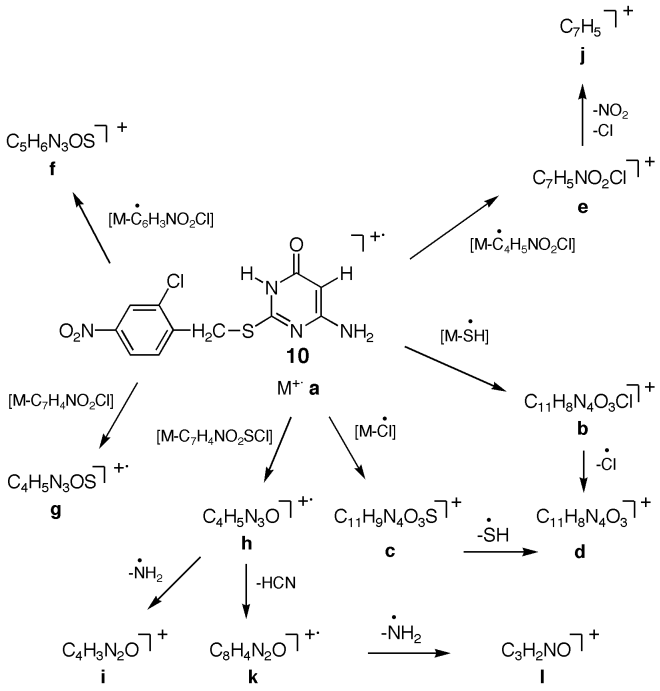
1 – 3 (Cl substituted isomers) 130 – 140 ppm.

ortho	*meta*	*para*
C – 1' 135. 40 ppm	C – 3' 132. 82 ppm	C – 1' 137. 41 ppm
C – 2' 133. 14 ppm	C – 5' 130. 13 ppm	C – 4' 131. 69 ppm
C – 3' 131. 76 ppm		C – 2' 131. 03 ppm
4 – 6 (Br substituted isomers) 130 – 140 ppm		
C – 1' 137. 02 ppm	C – 2' 131. 72 ppm	C – 1' 137. 86 ppm
C – 6' 132. 57 ppm	C – 5' 130. 49 ppm	C – 2',6' 131. 40 ppm
C – 3' 131. 78 ppm		C – 3', 5' 131. 19 ppm
7 – 9 (NO_2 substituted isomers) 140 – 150 ppm		
C – 2' 147. 99 ppm	C – 3' 147. 73 ppm	C – 4' 146. 82 ppm
	C – 1' 141. 15 ppm	C – 1' 146. 46 ppm

On the basis of low and high resolution electron–impact as well as B/E linked scan mass spectra (Tables 5-7), the principal mass spectral fragmentation routes of compounds **1**–**6** are interpreted as shown in Scheme 1, and those of **7 - 9** and **10** in Scheme 2 and 3, respectively. As can be seen from Schemes 1-3 and Tables 5–7 the principal mass fragmentation pathways of 2-benzylthio-6-aminouracils **1-10** are similar to those of 2-benzylthiouracils investigated by us earlier [4]. The common features of the mass spectral fragmentation of the molecular ions of **1-10** are simple cleavages of Csp^2-X and Csp^3-S in the benzylthio substituent *i.e.* elimination of •C_7H_6X and



Scheme 2. The pathways of the EI–mass fragmentation of the molecular ions of **7-9**.



Scheme 3. The pathways of the EI–mass fragmentation of the molecular ion of **10**.

Table 6

Elemental composition and Relative Intensities of the Ion Peaks in the Spectra of **7**-**9** According to High Resolution Data

Ion	M/z	Elemental composition	Relative Intensities (%)		
			7	**8**	**9**
M+· **a**	278	$C_{11}H_{10}N_4O_3S$	7	100	100
b	261	$C_{11}H_9N_4O_2S$	1	38	2
c	245	$C_{11}H_9N_4O_3$	1	6	12
d	232	$C_{11}H_{10}N_3OS$	1	5	2
e	199	$C_{11}H_9N_3O$	1	4	12
f	156	$C_5H_6N_3OS$	1	10	9
g	143	$C_4H_5N_3OS$	100	1	5
h	136	$C_7H_6NO_2$	6	13	7
i	111	$C_4H_5N_3O$	10	75	78
j	95	$C_4H_3N_2O$	5	20	20
k	89	C_7H_5	11	21	23
l	68	C_3H_2NO	30	26	31
m	56	$C_2H_4N_2$	8	8	10
n	43	HNCO	4	12	10

the elimination of •X in the cases of **1** and **4** is strongly connected with the *ortho*-effect. The loss of substituent radical from the *ortho* position of the phenyl ring is favored because it involves the formation of a very stable even–electron tricyclic ion characterized by the quaternization of N (1) (**1**, **4**) of the pyrimidynyl moiety. In the

Table 5

Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **1**-**6** According to High resolution Data

Ion	M/z	Elemental composition	Relative Intensities (%)					
			1	**2**	**3**	**4**	**5**	**6**
M+·	267	$C_{11}H_{10}N_3OSCl$	100	100	100			
a	311/313	$C_{11}H_{10}N_3OSBr$				61/60	100/99	100/99
b	234	$C_{11}H_9N_3OCl$	14	40	34			
	278/280	$C_{11}H_9N_3OBr$				3/2	32/31	32/31
c	232	$C_{11}H_{10}N_3OS$	66	2	1	100	5	3
d	199	$C_{11}H_9N_3O$	43	11	20	74	41	52
e	156	$C_5H_6N_3OS$	13	9	6	9	15	8
f	125	C_7H_6Cl	90	42	78			
	169/171	C_7H_6Br				53/52	38/ 37	81/ 80
g	111	$C_4H_5N_3O$	40	45	38	34	70	62
h	95	$C_4H_3N_2O$	15	12	11	14	21	17
i	89	C_7H_5	38	20	25	38	37	39
j	68	C_3H_2NO	36	22	23	43	40	37
k	56	$C_2H_4N_2$	15	9	10	19	17	17
l	43	HNCO	12	10	8	17	18	12

•X radicals. It ought to be mentioned that in the case of **1**-**6,** during the processes of cleavage of Csp^3-S bonds of the benzylthio substituent the positive charge is stabilized more effectively on the benzyl fragment. It was also found that even–electron fragment ions [M-·X] **c** in the mass spectra of **1** and **4** have 66 and 100 % relative intensity. In the mass spectra of **2**, **3**, **5** and **6** the relative intensity of these ions is in the range 1-2 %. Hence, it is obvious that mass spectra of **7**–**9** the even electron fragment ions [M-·X] **d** have almost the same very low abundances (Table 6). The base peaks in the mass spectra of **8** and **9** are the molecular ions **a**. The odd -electron fragment ion **g** corresponds to the base peak in the mass spectrum of **7**. It was found that ion **g** is formed from the molecular ion by ejection of a neutral fragment C_7H_5X. The cleavages of the Csp^3–S bonds probably follow the Mc Lafferty type of

Table 7

Elemental Composition and Relative Intensities of the Ion Peaks in the Spectrum **10** According to High Resolution Data

Ion	M/z	Elemental composition	Relative Intensities (%)
$M^{+\cdot}$**a**	312	$C_{11}H_9N_4O_3SCl$	100
b	279	$C_{11}H_8N_4O_3Cl$	5
c	277	$C_{11}H_9N_4O_3S$	41
d	244	$C_{11}H_8N_4O_3$	10
e	170	$C_7H_5NO_2Cl$	3
f	156	$C_5H_6N_3OS$	14
g	143	$C_4H_5N_3OS$	5
h	111	$C_4H_5N_3O$	52
i	95	$C_4H_3N_2O$	14
j	89	C_7H_5	17
k	84	$C_8H_4N_2O$	11
l	68	C_3H_2NO	24

rearrangement with hydrogen transfer either to sulfur or the ring annular nitrogen atom. The molecular ions of all the compounds investigated **1**–**10** readily lose ·SH radicals, giving even–electron fragment ions **b** [**1-6**] and **c** [**7-10**] (Tables 5–7). For this loss to occur, a skeletal rearrangement is required with the formation of new carbon–carbon and carbon-nitrogen bonds. The even–electron fragment ions **b** and **c**, which are formed after this rearrangement may have a monocyclic or bicyclic structure. It should be mentioned that loss of a sulphydryl radical is common for aromatic thioethers.

As can be seen from Scheme 3 and the data in Table 7, the principal mass fragmentation pathways of the molecular ion of 2-chloro-4-nitro-benzylthio-6-aminouracil (**10**) are similar to those of **1**– **9**. The molecular ion of **10** readily loses •X (ion **b**), •SH (ion **c**), $C_6H_3NO_2$• (ion **f**) and $C_4H_4NO_3S$• (ion **e**) radicals, as well as $C_7H_3NO_2Cl$ (ion **g**) and $C_7H_4NO_2SCl$ (ion **h**) neutral molecules.

The differences in the fragmentation of isomeric *ortho*- (*meta*- and *para*-) substituted 2-benzylthiouracils (**1**-**9**) have been quantifed by comparing the calculated values of the coefficient μ *i.e*. the abundances of the selected even-electron fragment ions relative to the abundances of the molecular ions. It has been established by us previously [4] that differences in the values of the coefficients μ are useful for differentation of the positional isomers of 2- and 4-substituted alkylthiouracils [16], as well as benzylthiouracils [3,4]. For compounds **1**– **9**, Table 8 presents the ratios of the intensities of the $[M- \bullet X]^+$, $[C_7H_6X]^+$ and $[M- \bullet SH]^+$ ion peaks to those of the parent ions peaks, *i.e.*

$$\mu_1 = \text{int. } [M-\cdot X]^+ / \text{int. } M^{+\cdot}$$
$$\mu_2 = \text{int. } [C_7H_6X]^+ / \text{int. } M^{+\cdot}$$
$$\mu_3 = \text{int. } [M-\cdot SH]^+ / \text{int. } M^{+\cdot}$$

As can be seen from the data in Table 8, the differences between the relative intensities of the peaks of selected fragment ions and $M^{+\cdot}$ ions, *i.e*. the values of μ_1, μ_2, μ_3 for

Table 8

The Values of μ_1- μ_3 Calculated from the EI Mass Spectra of **1–9**

Związek	μ_1	μ_2	μ_3
1	0.66	0.90	0.14
2	0.02	0.42	0.40
3	0.01	0.78	0.34
4	1.66	0.88	0.05
5	0.05	0.38	0.32
6	0.03	0.81	0.32
7	0.14	0.85	0.14
8	0.05	0.13	0.06
9	0.02	0.07	0.12

2-*ortho*- (*meta*- and *para*-)chloro-(bromo- and nitro-)substituted 2- benzylothio-6-aminouracils **1**–**9** may be sufficient to differentiate isomers. *Ortho*- chloro-(bromo- and nitro-) substituted 2- benzylthio-6-aminouracils (**1**, **4**, **7**) may be distinguished from isomeric *meta*- (**2**, **5**, **8**) and *para*- (**3**, **6**, **9**) substituted 2-benzylthio-6-aminouracils on the basis of the highest values of μ_1. *Meta* chloro- and (bromo-) substituted 2-benylthio-6-aminouracils (**2**, **5**) may be distinguished from their *ortho*- and *para*- substituted isomeric counterparts (**1**, **4** and **3**, **6** respectively) on the basis of the lowest values of μ_2. *Para*- chloro- and (bromo-) substituted 2-benzylthio-6-aminouracils (**3**, **6**) may be distinguished from their *ortho*- and *para*- substituted isomers (**1**, **4** and **2**, **5** respectively) on the basis of the lowest values of μ_3.

Ortho-(*meta*- and *para*-)nitro- substituted isomers of 2-benzylthiouracils may be differentiated on the basis of the following sequence of the value of μ_2.

$$\mu_2 \textit{ ortho} > \mu_2 \textit{ meta} > \mu_2 \textit{ para}$$

Moreover, 2-*meta*-nitrobenzylthio-6-aminouracil **8** may be distinguished from its *ortho*- and *para*- substituted isomers (**7** and **9** respectively) on the basis of the lowest value of μ_3.

Conclusions.

The basic mass fragmentation of **1**-**10** is due to cleavages of the Csp^3–S and Csp^2–S bonds of the S-benzyl group, as well as Csp^2–X bonds of X- substituted phenyl group. The values μ_1, μ_2 and μ_3 (*i.e*. the ratio of the intensities of the selected fragment ion peaks to those of the molecular ion peaks $M^{+\cdot}$) depend on the structures of *ortho*-(*meta*- and *para*-) substituted chloro-(bromo- and nitro-)benzylthio-6-aminouracils (**1** - **9**). The differences in the values of μ_1 - μ_3 in the series of **1**-**9** are useful for differentiation between *ortho*- (*meta*- and *para*-) substituted isomers of 2-benzylthio-6-aminouracils **1**–**9**. The differences in the ^{13}C NMR spectra of **1**-**9** in the number and positions of the signals of the carbon atoms in the range of 130 – 140 ppm (**1**–**6**) and 140 – 150 ppm (**7**–**9**) allow a differentiation between *ortho*-, *meta*- and *para*- substituted in benzylthio groups isomers.

EXPERIMENTAL

Purity of all described compounds was checked by m.p.'s, TLC and elemental analysis. Melting points (uncorrected) were determined on a Böetius microscope hot stage. R_f values refer to TLC silica gel F_{254} TLC plates (Merck) developed with $CHCl_3$ - MeOH 5:1 and observed under UV light (λ=254 and 366 nm). UV/VIS spectra were recorded with a Specord UV/VIS spectrophotometer in methanol. IR spectra were recorded with a FT-IR Bruker IFS – 113 v Spectrophotometer in KBr pellets. ^1H NMR spectra were determined with a Varian Gemini 300 (300 MHz) spectrophotometer in DMSO-d_6 solution with TMS as internal standard. Elemental analyses were performed with a Perkin-Elmer 240 C-CHN analyzer. Low- and high-resolution mass spectra were recorded on an AMD - Intectra GmbH-Harpstedt D-27243 Model 402 two - sector mass spectrometer (ionizing voltage 70 eV, accelerating voltage 8 kV, resolution 10 000). Samples were introduced by a direct insertion probe at the source temperature of ~150 °C. The elemental compositions of the ions were determined by a peak matching method relative to perfluorokerosene and using the same instrument. All masses measured agreed with those of the composition listed in column 3 of the Tables 5 - 8 to within ± 2 ppm. The B/E linked scan spectra in the first field-free region were investigated using helium as the collision gas at a pressure of 1,73 x 10^{-5} with the ion source temperature of 180 °C, ionization energy of 70 eV and an accelerating voltage of 8 kV. The values of μ_1, μ_2, μ_3, were calculated as averages of three measurements. 2-Thio-6-aminouracil was obtained according to literature [17,18].

The Synthesis of 2-*ortho*-(*meta*- and *para*-)Chloro-(bromo- and nitro-)benzylthio-6-aminouracils (1–10).

General Procedure.

A methanol solution consisting of 4 mmoles of 2-thio-6-aminouracil in 40 ml of 0.1 *N* NaOH was stirred at room temperature while 10 mmoles of corresponding *ortho*- (*meta*- and *para*-) chloro- (bromo- and nitro-)benzyl halide, or 2-chloro-4-nitrobenzyl bromide were added dropwise. After stirring for 1–12 hours (Table 1) the precipitated solid was isolated by filtration, washed with ethanol, dried at room temperature and recrystalized from a selected solvent (Table 1).

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