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5-Arylideneaminouracils: II.[1] Synthesis of Sodium and Ammonium Salts

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Abstract—In continuation of studies on the design of new 5-arylideneaminouracil derivatives possessing antimicrobial and antiviral properties, the corresponding ammonium and sodium salts were synthesized. The antimicrobial activity of water-soluble salts of substituted aminouracils *in vitro* was shown to be several times higher than that of parent neutral compounds.

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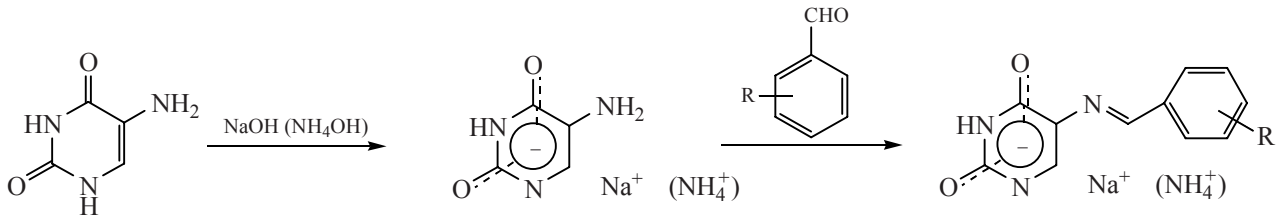
In the previous communication [1] we reported on the synthesis of 5-arylideneaminouracils and showed that their water-soluble derivatives characterized by large lipophilicity parameters log *P* should exhibit enhanced biological activity. In addition, introduction of electron-withdrawing substituents in the aromatic fragment of their molecules is desirable.

We believed that the most appropriate way to obtain such derivatives is the synthesis of 5-arylideneaminouracil sodium and ammonium salts. On the one hand, such salts should be readily soluble in water, and, on the other, the presence of ammonium cations in biological medium should increase antimicrobial activity, taking into account resistance of some mycobacteria to the action of acids [2]. On the basis of the results obtained previously [1], we selected most potent (from the viewpoint of antimicrobial activity) 5-arylideneaminouracils and synthesized their water-soluble sodium and ammonium salts.

The target compounds were prepared in two steps. In the first step, 5-aminouracil was converted into its sodium or ammonium salt, and in the second step the salt thus obtained was brought into condensation with the corresponding aldehyde in alcohol. 5-Arylideneaminouracil sodium and ammonium salts **I** and **II** are brightly colored substances which melt with decomposition at about 300°C (ammonium salts melt above 300°C).



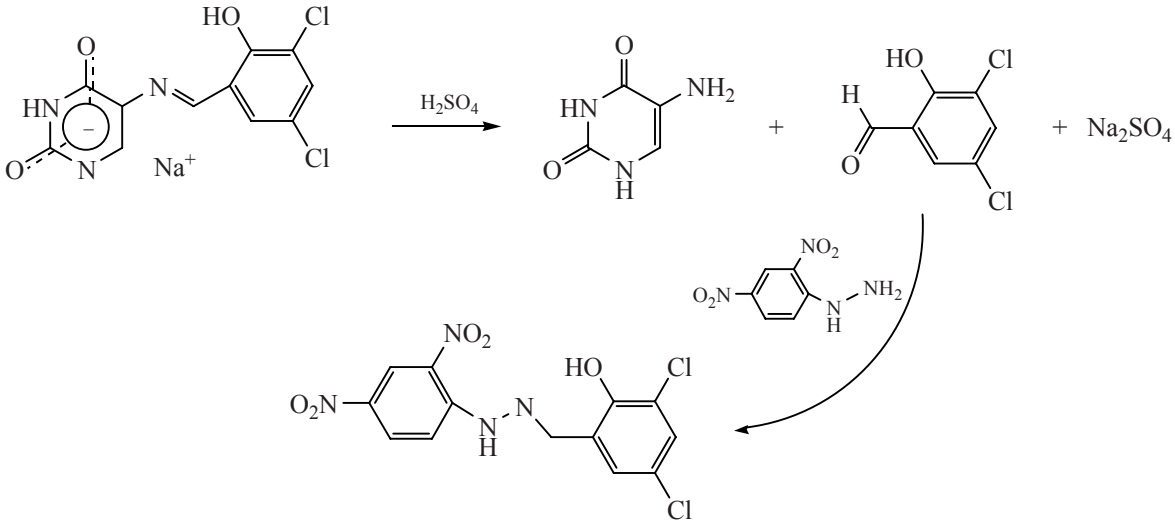
Ia–If, IIb, IId, IIe, IIg

R = 2-HO-5-O_2N (**a**), 2-HO-3,5-I_2 (**b**), 2-OH-3-Br-5-O_2N (**c**), 2,4-Cl_2 (**d**), 2-HO-5,6-benzo (**f**), 2-HO (**g**).

We failed to obtain satisfactory elemental analyses of the isolated sodium salt because of their high ash content. Therefore, we proposed a procedure for their qualitative identification and quantitative determination. No qualitative test for arylideneaminouracils has been reported. For this purpose, we used Brady's reagent (2,4-dinitrophenylhydrazine) which is known to react with carbonyl-containing compounds (aldehyde and ketones) with formation of the corresponding colored crystalline hydrazones. We anticipated that the reaction

[1] For communication I, see [1].

of arylideneaminouracil sodium salts with Brady's reagent may be accompanied by hydrolysis of the azomethine moiety with formation of parent aldehyde. In fact, sodium salt **Ie** in ethanol reacted with a solution of 2,4-dinitrophenylhydrazine with formation of 3,5-dichloro-2-hydroxybenzaldehyde 2,4-dinitrophenylhydrazone which separated from the solution as yellow crystals. This reaction can also be used for quantitative determination of arylideneaminouracil (from the weight of the colored product).



The structure of salts **I** and **II** was confirmed by the ^{1}H NMR spectra. 5-Arylideneaminouracil sodium salts **I** characteristically showed in the ^{1}H NMR spectra a singlet at δ 9.4–9.6 ppm from the N=CH proton, NH signal at δ 11.3–11.5 ppm, and a singlet from 6-H in the pyrimidine ring and signals from aromatic protons in the region δ 7.7–8.1 ppm (Table 1). The ^{1}H NMR spectra of ammonium salts **II** were almost identical to those of sodium salts **I** (Table 2). The only difference was the shape of signals from exchangeable protons: these protons (NH and OH) appeared in the spectra of ammonium salts **II** as broadened signals with strongly reduced intensity.

In the IR spectra of ammonium and sodium salts **I** and **II**, stretching vibrations of the azomethine N=CH bond appeared at 1670 cm^{-1}, a broad band at 3400 cm^{-1} was assigned to OH stretching vibrations, N–H groups in the pyrimidine gave rise to absorption bands at about 3100 cm^{-1}, carbonyl absorption was observed at 1640 cm^{-1}, and C–Cl stretching vibrations had a frequency of 800 to 700 cm^{-1}. The electronic spectra of 5-arylideneaminouracil sodium and ammonium salts contained absorption maxima in the region λ 280–300 nm due to electron transitions in the uracil and aryl fragments, and the entire conjugation system was characterized by absorption band in the visible region (λ_{max} 400 nm; Fig. 1).

Table 1. Yields, melting points, R_f values, and ^{1}H NMR spectra of 5-arylideneaminouracil sodium salts **Ia**–**If**

Compound no.	Yield, %	mp,[a] °C	Formula	R_f[b]	^{1}H NMR spectrum, δ, ppm		
					CH=N	NH	6-H
Ia	92	282	$C_{11}H_6N_3NaO_5$	0.46	9.4	11.4	7.9
Ib	92	268	$C_{11}H_6I_2N_3NaO_3$	0.52	9.5	11.5	7.8
Ic	98	300	$C_{11}H_6BrN_4NaO_5$	0.55	9.6	11.4	8.0
Id	92	295	$C_{11}H_6O_2Cl_2N_3Na$	0.45	9.5	11.3	8.1
Ie	96	290	$C_{11}H_6Cl_2N_3NaO_3$	0.54	9.4	11.4	7.7
If	98	298	$C_{15}H_{10}N_3NaO_3$	0.51	9.4	11.3	8.1

[a] S razlozheniem. [b] Chloroform–ethanol, 7 : 1.

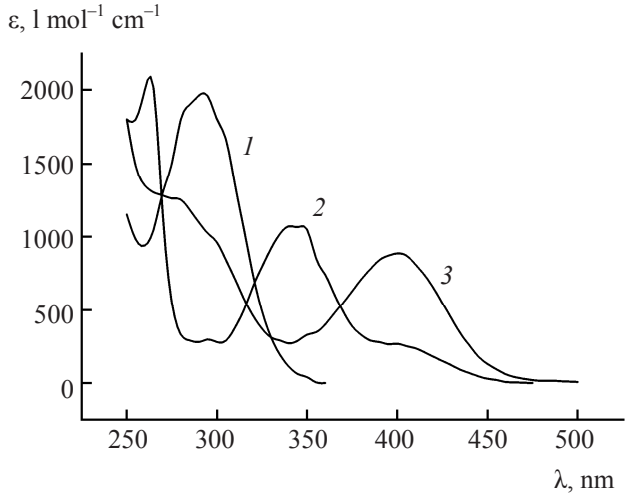


Fig. 1. Electron absorption spectra of (*1*) 5-aminouracil, (*2*) 3,5-dichloro-2-hydroxybenzaldehyde, and (*3*) 5-(3,5-dichloro-2-hydroxybenzylideneamino)uracil sodium salt (**Ie**).

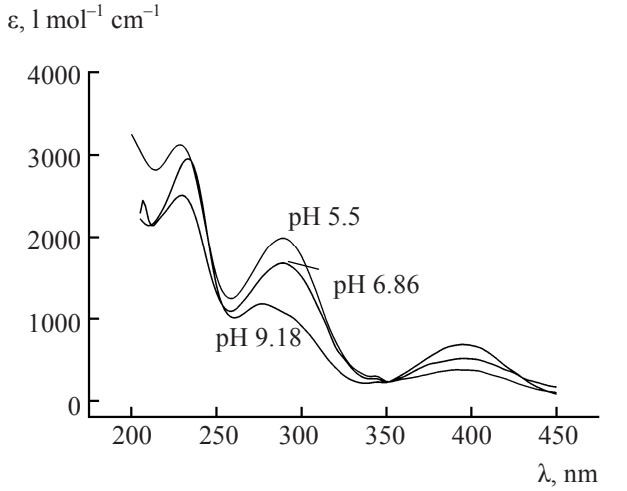


Fig. 2. Electronic absorption spectra of 5-(3,5-dichloro-2-hydroxybenzylideneamino)uracil ammonium salt (**IIe**) at different pH values.

It was important to examine the behavior of 5-arylideneaminouracil salts in aqueous solution at different pH values with a view to estimate their stability in biological media. The UV spectra of salts **I** and **II** were measured in buffer solutions at different pH values (tartrate, pH 3.56; phthalate, pH 5.5; phosphate, pH 6.86; borate $Na_2B_4O_7 \cdot H_2O$, pH 9.18 [4]). We found that salts **I** and **II** decomposed at pH 3.5: long-wave absorption maximum at λ 400 nm disappeared from the UV spectra. Ammonium salts **II** were stable at pH ≥5.5. Figure 2 shows the electron absorption spectra of 5-(3,5-dichloro-2-hydroxybenzylideneamino)uracil ammonium salt (**IIe**) at different pH values. The presence of an isosbestic point and a weak absorption band at λ 340 nm must be noted.

5-Arylideneaminouracil sodium and ammonium salts **I** and **II** were tested for biological activity. It was found that their antimicrobial effect exceeds the effect of the corresponding neutral compounds by a factor of 6 to 10. The results are given in Tables 3 and 4. High and versatile antibacterial activity of 5-(2-hydroxy-3,5-diiodobenzylideneamino)uracil ammonium salt (**IIb**) should be noted, especially with respect to *S. aureus* 29213. The Hantzsch curve in Fig. 3 demonstrates nonlinear relation between biological activity of 5-arylideneaminouracil sodium salts and their lipophilicity. The plot clearly has exponential shape, in keeping with the correlations drawn by us previously [1].

We presumed in [1] that the presence of electron-withdrawing substituents in the aromatic fragment is a necessary condition for high antimicrobial activity of 5-arylideneaminouracil derivatives. This assumption was generally confirmed; nevertheless, the scope of the obtained correlation is fairly limited. It seemed interesting to estimate biological activity of 5-arylideneaminouracils having an *a fortiori* pharmacophoric fragment. For this purpose we synthesized 8-hydroxyquinoline-5-carbaldehyde and its condensation product with 5-aminouracil. It is known that many antimicrobial agents contain a 8-hydroxyquinoline fragment. However, the antimicrobial activity of 5-(8-

Table 2. Yields, R_f values, and ^{1}H NMR spectra of 5-arylideneaminouracil ammonium salts **IIb**, **IId**, **IIe**, and **IIg**

Compound no.	Yield, %	Formula	R_f	^{1}H NMR spectrum, δ, ppm			
				N=CH	NH	6-H, H_{arom}	OH
IIb	14	$C_{11}H_{10}I_2N_4O_3$	0.24[b]	9.9	11.6	7.1–8.1	12.1
IId	76	$C_{11}H_{10}Cl_2N_4O_2$	0.66	9.9	11.2	7–8	–
IIe	61	$C_{11}H_{10}Cl_2N_4O_3$	0.34[a]	9.5	10.9	7–8	13.8
IIg	77	$C_{11}H_{12}N_4O_3$	0.33[c]	9.5	11.2	6.8–7.8	12.8

[a] Chloroform–methanol, 9 : 1. [b] Chloroform–methanol, 15 : 1. [c] CCl_4–propan-2-ol, 7 : 6.

Table 3. Antibacterial activity of 5-arylideneaminouracil ammonium salts **IIb**, **IId**, **IIe**, and **IIg**

Comp. no.	Minimal concentration ensuring 100% inhibition, μg ml^{-1}				
	bacteria		fungi		mycobacteria
	S. aureus 29213	*E. coli* 25922	*Candida Albicans*	*Aspergillus Niger*	*M. Smegmatis*
IIb	6.2	>100	>100	>100	>100
IId	>100	>100	>100	>100	>100
IIe	3.1	100	6.2	12.5	6.2
IIg	50	>100	>100	>100	>100

Table 4. Antibacterial activity of 5-arylideneaminouracil sodium salts **Ib**–**If** and **Ih**

Comp. no.	Minimal concentration ensuring 100% inhibition, μg ml^{-1}				
	bacteria		fungi		mycobacteria
	S. aureus 29213	*E. coli* 25922	*Candida Albicans*	*Aspergillus Niger*	*M. Smegmatis*
Ib	3.1	100	6.2	12.5	6.2
Ic	3.1	>100	>100	>100	100
Id	50	>100	>100	>100	>100
Ie	6.2	>100	>100	>100	>100
If	25	>100	>100	50	50
Ih	>100	50	>100	>100	>100

hydroxyquinolin-5-ylmethylideneamino)uracil sodium salt (**Ih**) turned out to be only moderate, and this compound showed no diverse biological effect: it was active *in vitro* only against *E. coli* 25922.

In fact, taking into account the correlations drawn for antibacterial effect of 5-arylideneaminouracils [1], such parameters of 5-(8-hydroxyquinolin-5-ylmethylideneamino)uracil as log$P$, V, and S imply only medium level of biological activity (log$P$ = 1.47, S = 267 Å^2, V = 424 Å^3). Thus introduction of a pharmacophoric fragment did not enhance antimicrobial activity. These findings confirm structure–activity correlations obtained by us previously for 5-arylideneaminouracils and suggest unique mechanism of their biological action.

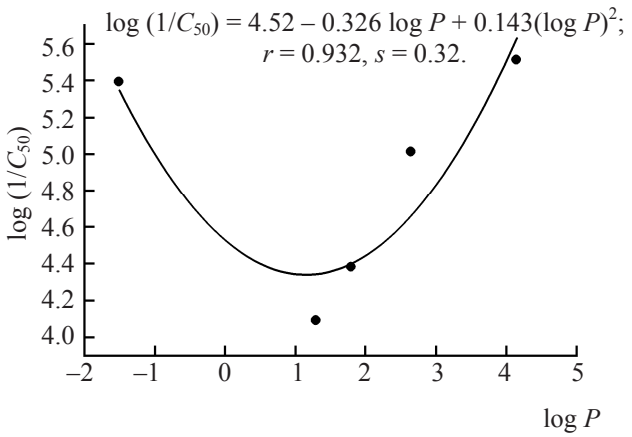


Fig. 3. Plot of log (1/C_{50}) versus log$P$ for antibacterial activity of 5-arylideneaminouracil sodium salts against *S. aureus* 29213.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded from solutions in DMSO-d_6 on a Bruker spectrometer (Bruker-Franzen Analytik GmbH, version 950801.1) at 200 MHz using residual proton signal of the solvent as reference. The electronic absorption spectra were measured on an SF-26 spectrophotometer (MicroCal Origin, version 3.0) from solutions in water with a concentration of 10^{-4} M. The IR spectra were obtained on a Specord M-80 instrument from samples prepared as KBr pellets. The purity of the isolated compounds was checked by thin-layer chromatography on Silufol UV-254 plates.

5-Arylideneaminouracil sodium and ammonium salts **I** and **II** were tested for antibacterial and antiviral activity against standard strains of microorganisms at the Microbiology Department, Pavlov St. Petersburg State Medical University.

5-(3,5-Dichloro-2-hydroxybenzylideneamino)pyrimidine-2,4(1*H*,3*H*)-dione sodium salt (Ie). A solution of 0.113 g of sodium hydroxide in 10 ml of water

was added to 0.356 g of 5-aminouracil, and the mixture was heated on a boiling water bath until complete dissolution of 5-aminouracil. A solution of 0.541 g of 3,5-dichloro-2-hydroxybenzaldehyde in 7 ml of hot ethanol was added in portions over a period of 30 min under stirring on heating on a boiling water bath. An orange–red solid separated, the mixture was stirred for 30 min while heating on a boiling water bath and allowed to cool down to room temperature, and the precipitate was filtered off, washed with 15 ml of ethanol, and dried. Yield 98%. The other 5-arylideneaminouracil sodium salts were synthesized in a similar way.

5-(3,5-Dichloro-2-hydroxybenzylideneamino)pyrimidine-2,4(1*H*,3*H*)-dione ammonium salt (IIe). A mixture of 0.4 g of 5-aminouracil and 30 ml of aqueous ammonia was stirred at room temperature until it became homogeneous. A solution of 0.6 g of 3,5-dichloro-2-hydroxybenzaldehyde in 25 ml of ethanol was added dropwise under stirring. The originally yellow solution turned dark brown. The mixture was heated for 1 h on a water bath, and the brick-red precipitate was filtered off and dried in a desiccator. Yield 61%. The other 5-arylideneaminouracil ammonium salts were synthesized in a similar way.

8-Hydroxyquinoline-5-carbaldehyde. A solution of 40.5 g of quinolin-8-ol in 240 ml of 66% ethanol was added dropwise under stirring to a solution of 50 g of sodium hydroxide in a mixture of 85 ml of water and 180 ml of ethanol. The mixture was heated to the boiling point, 64.8 g of chloroform was added dropwise to the resulting transparent solution, and the mixture was heated for 4 h under reflux. The alcohol and excess chloroform were distilled off under reduced pressure, the residue was diluted on cooling with concentrated hydrochloric acid until a transparent solution was formed, and the solution was made alkaline by adding solid sodium carbonate to pH 8–9. The undissolved material was filtered off and repeatedly washed with a 2 N solution of sodium carbonate. The alkaline solution was carefully acidified with acetic acid, and the precipitate was filtered off, washed with a small amount of water, recrystallized from ethanol, and dried in a vacuum desiccator. Yield 1.5 g (3%), mp 179–180°C; published data [3]: mp 180°C.

Qualitative analysis of 5-arylideneaminouracil salts. 2,4-Dinitrophenylhydrazine, 1 g, was dissolved in 7 ml of concentrated sulfuric acid, and the resulting solution was added under stirring to a mixture of 7 ml of water and 23 ml of 95% ethanol. Mixing of 3 ml of Brady's reagent thus obtained with a solution of 20 mg of sodium salt **I** in 2 ml of 95% ethanol resulted in the formation of a yellow solid.

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