SYNTHESIS AND BIOLOGICAL ACTIVITY OF SUBSTITUTED N,N'-ALKYLENEBISPYRIDINIUM SALTS

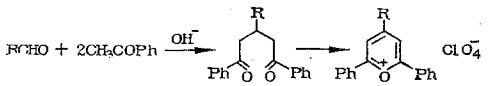
N. A. Morozova, V. A. Sedavkina,
L. K. Kulikova, M. K. Krasheninnikova,
and V. G. Kharchenko

UDC 615.281:547.821.2].012.1

Some pyridinium compounds are known to possess antimicrobial and antiviral activity [1, 2]. A search for new drugs in this series, and the elucidation of structure—activity relationships, are therefore of considerable interest.

We have synthesized some new bisquaternary pyridinium salts by reacting 2,4,6-trisubstituted pyrilium salts with diamines.

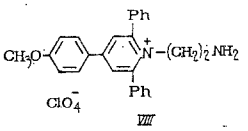
The 1,5-diketones used as starting materials (I-III) were obtained from p-methoxy, 3,4-dimethoxy-, and p-dimethylaminobenzaldehyde and acetophenone [3]. Cyclization of (I-III) in acetic acid in the presence of perchloric acid afforded 2,4',6-trisubstituted pyrilium salts (IV-VI). 2,4,6-Trimethylpyrilium perchlorate (VII) was obtained as described in [4].



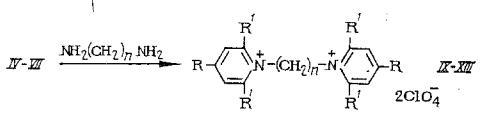
I, IV: R = $C_6H_4OCH_3$-p
III, VI: R = $C_6H_4N(CH_3)_2$-p
II, V: R = $C_6H_3(OCH_3)_2$-m, p

The high reactivity of the oxygen atom in pyrilium salts enabled it to be replaced by nitrogen on reaction with diamines. The 2,4,6-trisubstituted N,N'-alkylenebispyridinium salts were obtained as their perchlorates and iodides (IX-XIII).

The amination was effected with ethylenediamine and hexamethylenediamine in various solvents at 80-90°C, with pyrilium salt—diamine ratio of 2:1. It was found that when nitrobenzene was used as the solvent, it functioned as an oxidizing agent, to give monopyridinium compounds such as (VIII).



The use of ethanol as the solvent led to the formation of the required bispyridinium salts (IX-XIII).



IX: R = $C_6H_4OCH_3$-p, n = 2; X: R = $C_6H_4OCH_3$-p, n = 6; XI: R = $C_6H_3(OCH_3)_2$-m, p, n = 6; XII: R = $C_6H_4N(CH_3)_2$-p, n = 6; XIII: R = R^1 = CH_3, n = 6; IX — XII: R = Ph.

The perchlorates (X) and (XI) were converted by anion exchange into N,N'-hexamethylene-bis-2,6-diphenyl-4-methoxyphenyl- and dimethoxyphenylpyridinium iodides (XIV, XV).

The structures of the bispyridinium compounds were confirmed by their elemental analyses and IR spectra.

The IR spectra of all the salts (IX-XIII) contained valence vibration bands at 1540-1545 cm^{-1}, typical of the pyridinium structure, and stretching vibrations for the aromatic ring at 1610-1600 cm^{-1}. The ClO_4^- anion was identified by the bands at 1110-1090 cm^{-1}. Skeletal vibrations of the polymethylene group were present at 720-750 cm^{-1}.

N. G. Chernyshev Saratov University. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 11, pp. 1312-1315, November, 1983. Original article submitted May 4, 1982.

0091-150X/83/1711-0785$07.50

TABLE 1. Physicochemical Constants of Bispyridinium Salts (IX-XV)

Compound	Yield, %	mp, °C	Found, %				Molecular formula	Calculated, %			
			C	H	N	Cl		C	H	N	Cl
IX	69	139—141	66,93	4,98	3,46	8,14	$C_{50}H_{42}Cl_2N_2O_{10}$	66,59	4,66	3,10	7,88
X	68	245—246	68,10	5,51	3,27	7,76	$C_{54}H_{50}Cl_2N_2O_{10}$	67,71	5,22	2,92	7,42
XI	71	227—228	68,37	5,73	3,15	7,59	$C_{56}H_{54}Cl_2N_2O_{10}$	68,22	5,48	2,84	7,20
XII	69	241—242	68,64	5,97	2,98	7,57	$C_{56}H_{56}Cl_2N_4O_8$	68,36	5,69	2,69	7,22
XIII	63	245—246	50,14	6,23	5,72	—	$C_{22}H_{34}Cl_2N_2O_8$	50,33	6,53	5,34	
XIV	79	190—192	64,38	5,10	2,91	—	$C_{54}H_{50}I_2N_2O_2$	64,03	4,94	2,76	—
XV	77	175—177	62,91	5,32	2,87	—	$C_{56}H_{54}I_2N_2O_4$	62,69	5,04	2,61	—

TABLE 2. Antimicrobial and Antiphage Activity of Substituted N,N'-Alkylenebispyridinium Salts (VIII-XIV)

Compound	Minimum bacteriostatic concentration, μg/ml					% inactivation of phages			
						T_6		MS-2	
	Staph. aureus	E. coli	Pr. vulgaris	Ps. pyocyaneum	Candida albicans	1000 μg/ml	100 μg/ml	1000 μg/ml	100 μg/ml
VIII	50	50	100	50	50	31	29	46	19
IX	50	50	100	100	100	48	34	77	62
X	50	50	100	100	100	53	41	73	29
XI	50	50	100	100	100	48	31	52	42
XII	50	50	100	100	50	45	35	22	12
XIII	100	100	50	100	50	47	21	48	42
XIV	100	100	50	100	50	29	27	40	39
Bleomycin						43	29	91	32
Rubomycin						44	14	30	19

EXPERIMENTAL CHEMISTRY

IR spectra were recorded on a UR-20 spectrometer (East Germany), in vaseline oil and hexachlorobutadiene.

N,N'-Dimethylenebis-(2,6-diphenyl-4-methoxyphenylpyridinium) Perchlorate (IX). A solution of 2 g (0.004 mole) of (IV) and 0.12 g (0.002 mole) of ethylenediamine in 70 ml of ethanol was heated under reflux at 80-90°C for 4 h. The mixture was kept at room temperature for 10-12 h, and the solution concentrated *in vacuo*, whereupon bright yellow crystals separated. Purification was effected by reprecipitation from chloroform with ether, the yield of (IX) being 2.7 g (69%) (Table 1).

N,N'-Hexamethylenebis-(2,6-diphenyl-4-methoxyphenylpyridinium) Perchlorate (X). A solution of 2 g (0.004 mole) of (IV) and 0.23 g (0.002 mole) of hexamethylenediamine in 100 ml of ethanol was heated for 5 h. The mixture was then poured into 50 g of crushed ice, and the orange crystals which separated were filtered off and washed with a mixture of alcohol and acetone (1:2). Yield of (X), 2.9 g (68%) (Table 1).

Compounds (XI-XIII) were obtained similarly (Table 1).

N,N'-Hexamethylenebis-(2,6-diphenyl-4-methoxyphenylpyridinium) Iodide (XIV). To a solution of 0.0005 mole of (X) in 10 ml of acetone was added 0.0005 mole of potassium iodide in 15 ml of aqueous acetone. The orange crystals which separated were washed with acetone—ether (2:1), mp 190-192°C, yield of (XIV) 79% (Table 1).

Compound (XV) was obtained similarly.

2,6-Diphenyl-4-methoxyphenyl-N-(aminoethyl)pyridinium Perchlorate (VIII). A solution of 1 g (0.002 mole) of (IV) and 0.06 g (0.001 mole) of ethylenediamine in 40 ml of nitrobenzene was heated under reflux at 80-90°C for 4 h. The mixture was kept at room temperature for 10-12 h, and the solution was then concentrated, whereupon yellow crystals separated. Purification was effected by reprecipitation from chloroform and alcohol, mp 152-154°C. Yield of (VIII), 1.2 g (63%). Found, %: C 67.42; H 5.84; Cl 7.87; N 6.37. $C_{26}H_{26}ClN_2O^4$. Calculated, %: C 67.01; H 5.62; Cl 7.61; N 6.01.

EXPERIMENTAL BIOLOGY

The antimicrobial activity of the test compounds was determined by twofold serial dilution in meat-peptone broth (pH 7.2-7.4) against the standard test organisms: *Staph. aureus* 209, *E. coli M*-17, *Pr. vulgaris* 38, *Ps. aeruginosa* 165, and *Candida albicans* 45.

The antiphage activity of the compounds was determined with respect to DNA (T_6)- and RNA (MS-2)-containing phages. The indicator cultures were strains of *E. coli* B and H fr C, respectively.

The numbers of surviving phage particles were determined by the Grazi agar slope method. The antiphage activity was calculated as percentage inactivation, using the formula

$$1-\frac{C_o}{C_c}\cdot 100,$$

where C_o is the number of phage particles surviving in the test, and C_c the number surviving in the controls.

The compounds were dissolved in DMF, followed by dilution with sterile distilled water.

The results of the tests for antimicrobial and antiphage activity are given in Table 2.

All the test compounds displayed moderate antimicrobial activity, inhibiting the growth of the test cultures in concentrations of 50-100 μg/ml.

The tests for antiphage activity were of great interest. When the substituents in the 2- and 4-positions of the pyridinium ring were the same (R = $C_6H_4OCH_3$-p, R' = Ph), increasing the number of methylene groups from 2 to 6 did not result in any change in activity against the RNA phage [compounds (IX, X)].

In the case of the most active compound (IX), antiphage activity was studied in lower concentrations (10 and 1 μg/ml). It was found that (IX) inhibited the reproduction of T_6 phage by 16% in a concentration of 10 μg/ml, and of MS-2 phage by 23% in a concentration as low as 1 μg/ml, whereas antitumor antibiotics (rubomycin and bleomycin) were inactive in these doses.

Replacement of the p-methoxy- and p-dimethoxyphenyl-substituents by p-dimethylaminophenyl resulted in a decrease in activity against the RNA phage (XII). A reduction in activity was also found in this series when the perchlorates were replaced by the iodides (X, XIV).

It is noteworthy that none of the compounds tested were inferior in their antiphage activity to rubomycin and bleomycin, and (IX) was markedly superior.

The compounds were of low toxicity, the LD_{50} by a single intramuscular dose in white mice being 270-280 mg per kg body weight.

These findings therefore indicate the desirability of seeking new, active antiviral compounds in the 2,4,6-trisubstituted pyridinium salt series.

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