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ANTIMICROBIAL ACTIVITY OF ALKYL AMMONIA SALTS OF SPIROARSORANES AND SUBSTITUTED BENZOARSOLES

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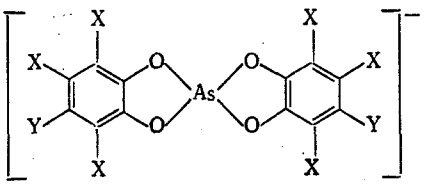
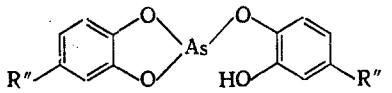
In order to clarify the effect that various structural factors of organoarsenic compounds have on biological activity, we investigated the antimicrobial (fungistatic and bacteriostatic) activity of previously described alkyl ammonia salts of spiroarsoranes (I-VIII) [2], o-oxyphenyleneoxybenzo-1,3,2-dioxaarsole (IX) [3], and newly synthesized substituted benzoarsoles (X, XI).



$\cdot[R_2R'NH]^+$

I—VIII

R = Et (I—III, V, VI, VIII), Pr (VII), — (IV, cation K^+); R′ = H (I—II, V, VII, VIII), Et (III, VI) — (IV); X = Cl (I), Br (II, III), H (IV—VIII); Y = Cl (I), Br (II, III), H (IV—VII), *t*-Bu (VIII).



IX—XI

R″ = H (IX), Cl (X), *t*-Bu (XI).

The latter compounds were obtained by the reesterification of tripropylarsenite with appropriate pyrocatechols. Antimicrobial activity was examined in an aqueous nutrient medium. Obviously, the biological activity of the compounds should depend on their state in solution. Investigation of the compounds by conductometry makes it possible to assay the quantitative parameters which characterize the substances' state in solution. We investigated the electrolytic dissociation of alkyl ammonium salts of spiroarsoranes [4]. Isotherms of electroconductivity were recorded at a temperature of 20°C. Processing of the experimental data by the Bray—Krauss and Shidlovskii methods made it possible to obtain the dissociation constants (K_{dis}) of these salts. We compared antimicrobial activity to the K_{dis} of the alkyl ammonia salts of spiroarsoranes. The data on the compounds' antimicrobial activity are presented in Table 1. An analysis of the data shows that the introduction of electron acceptor substituents into the benzene rings of the spiroarsorane alkyl ammonia salts enhances the antimicrobial properties. Whereas the minimum fungistatic concentration (MFC) for compounds V and

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TABLE 1. Antimicrobial Activity and K_{dis} of Salts of Spiroarsoranes I-VIII and Dioxaarsoles (IX-XI)

Compound	K_{dis} in MeOH	MFC, mole/liter			MBC, mole/liter	
		T. rubrum	T. mentagrophytes	M. canis	St. aureus	E. coli
I	0,3828	$7,8 \cdot 10^{-6}$	$7,8 \cdot 10^{-6}$	$7,8 \cdot 10^{-6}$	$9,68 \cdot 10^{-6}$	$9,68 \cdot 10^{-6}$
II	0,1089	$2,71 \cdot 10^{-5}$	$2,71 \cdot 10^{-5}$	$2,71 \cdot 10^{-5}$	$6,78 \cdot 10^{-6}$	$6,78 \cdot 10^{-6}$
III	0,1948	$1,34 \cdot 10^{-5}$	$1,34 \cdot 10^{-5}$	$1,34 \cdot 10^{-5}$	$6,68 \cdot 10^{-6}$	$6,68 \cdot 10^{-6}$
IV	$1,58 \cdot 10^{-3}$	$3,79 \cdot 10^{-4}$	$3,79 \cdot 10^{-4}$	$3,79 \cdot 10^{-4}$	$3,79 \cdot 10^{-4}$	$1,52 \cdot 10^{-3}$
V	$0,57 \cdot 10^{-3}$	$6,85 \cdot 10^{-4}$	$6,85 \cdot 10^{-4}$	$6,85 \cdot 10^{-4}$	$2,74 \cdot 10^{-4}$	$1,37 \cdot 10^{-3}$
VI	—	$6,36 \cdot 10^{-4}$	$6,36 \cdot 10^{-4}$	$6,36 \cdot 10^{-4}$	$1,27 \cdot 10^{-3}$	$1,28 \cdot 10^{-3}$
VII	$0,48 \cdot 10^{-3}$	$1,27 \cdot 10^{-3}$	$1,27 \cdot 10^{-3}$	$1,27 \cdot 10^{-3}$	$6,36 \cdot 10^{-4}$	$1,27 \cdot 10^{-3}$
VIII	$0,34 \cdot 10^{-3}$	$2,09 \cdot 10^{-4}$	$2,09 \cdot 10^{-4}$	$2,09 \cdot 10^{-4}$	$5,24 \cdot 10^{-5}$	$5,24 \cdot 10^{-5}$
IX	—	$4,28 \cdot 10^{-4}$	$8,56 \cdot 10^{-4}$	$4,28 \cdot 10^{-4}$	$8,56 \cdot 10^{-4}$	$8,56 \cdot 10^{-4}$
X	—	$1,39 \cdot 10^{-4}$	$1,39 \cdot 10^{-4}$	$1,39 \cdot 10^{-4}$	$6,93 \cdot 10^{-5}$	$6,93 \cdot 10^{-5}$
XI	—	$2,47 \cdot 10^{-4}$	$2,47 \cdot 10^{-4}$	$2,47 \cdot 10^{-4}$	$1,24 \cdot 10^{-3}$	$1,24 \cdot 10^{-3}$

VI was $6.85 \cdot 10^{-4}$ and $6.36 \cdot 10^{-4}$ mole/liter, the MFC for compounds II and III was $2.71 \cdot 10^{-5}$ and $1.31 \cdot 10^{-5}$ mole/liter respectively. The unsubstituted alkyl ammonium salts of spiroarsoranes exhibit a low level of biological activity. In the series with H, Br, and Cl substituents (compounds, I, II, and V) the fungistatic activity increased approximately 100-fold and was $6.85 \cdot 10^{-4}$, $2.71 \cdot 10^{-5}$, and $7.8 \cdot 10^{-6}$ mole/liter respectively. It is noteworthy that the MFC was not affected by the introduction of electron donor substituents into the anion structure (t-Bu, compound VIII). The MFC values remained the same as for the unsubstituted alkyl ammonia salts of the spiroarsoranes V-VII. The data in Table 1 indicate the symbatic change between the fungistatic properties and their K_{dis}, i.e., the greater the K_{dis} the more active the compound and vice versa. Compound I, which had the largest K_{dis} value (0.3828), also had the highest fungistatic activity ($7.8 \cdot 10^{-6}$ mole/liter) whereas compound V ($K_{dis} = 0.57 \cdot 10^{3}$) was the least active in the series of alkyl ammonia salts ($6.85 \cdot 10^{-4}$ mole/liter) containing a single cation ($Et\overset{+}{N}H_2$) but different substituents (Br, Cl) in the arsoranide anion (compounds I, II, and V). A similar but less pronounced relationship between fungistatic activity and K_{dis} was observed for salts containing a single anion, but different cations (IV-VII). The MFC and K_{dis} values did not significantly change for these compounds (MFC from $3.79 \cdot 10^{-4}$ to $1.27 \cdot 10^{-3}$ mole/liter; K_{dis} from $1.58 \cdot 10^{-3}$ to $0.48 \cdot 10^{-3}$ mole/liter), whereas in the series of compounds I-III and V the K_{dis} decreased by approximately 1000 times but the MFC increased by 100 times. These data indicate that the fungistatic activity of the compounds under study is largely determined by the nature of the arsoranide anion which constitutes a toxophoric group, its status in solution, and the nature and number of substituents in the benzene ring.

The alkyl ammonium salts of spiroarsoranes containing electron acceptor substituents (compounds I-III) dissociate slightly in a methyl alcohol solution (large K_{dis} values), i.e., the ion-ion couple equilibrium is shifted toward the ions:

$$[K^+A^-] \underset{K_{ass}}{\overset{K_{dis}}{\rightleftharpoons}} K^+ + A^-.$$

The absence of substituents in the benzene rings of the salt's anion enhances a shift in equilibrium toward the ion pairs. Evidently, the dissociated ions, i.e., the tetrahalide-containing arsoranide-anions (toxophoric group), have a greater capability of penetrating the lipid membrane of microorganisms, thereby blocking their metabolism.

When an unsubstituted arsoranide-anion (compounds IV-VII) comes into contact with a counterion (alkyl ammonia cation) in the form of an ion pair, it apparently loses its mobility, and subsequently its penetrability as well. These results are in agreement with contemporary concepts on the penetration of an arsenate-ion through a phosphate transport system into cellular membranes [7]. Existing models of bacterial absorption of phosphate and arsenate through transport systems suggest the cotransfer of H^+ [6]. The presence of a membrane potential (acid–base gradient) where the pH within the cell is maintained at 7.5 while the pH outside the cell is lowered by 1.0 or more, certainly suggests that the input and output pumps are not indifferent to the mobility of the transporting ions.

An analysis of the data on the compounds' bacteriostatic activity shows that it is also primarily determined by the nature of the arsoranide-anion. Compounds containing tetrachloro-

and tetrabromopyrocatechol ligands (compounds I-III) have a higher level of bacteriological activity than the unsubstituted alkyl ammonium salts of spiroarsoranes (compounds V-VII). The introduction of t-Bu and Cl substituents into the structure of the arsoranide-anion (compounds (VII-X) also contribute to lower values for the minimum bacteriostatic concentration (MBC) ($6.24 \cdot 10^{-5}$ and $6.93 \cdot 10^{-5}$ mole/liter). Compound XI is an exception.

Thus, the fungistatic and bacteriostatic activities of the spiroarsorane alkyl ammonium salts primarily depend on the nature of the arsoranide-anion and correlate well with the K_{dis} of these compounds.

EXPERIMENTAL CHEMICAL

The alkyl ammonium salts of spiroarsoranes were obtained by reacting corresponding derivatives of pyrocatechol with trichloroarsenate and amines in a medium of diethyl ether [2]. The 2-oxyphenyleneoxybenzo-1,3,2-dioxaarsoles were obtained by the reesterification of tripropylarsenine with pyrocatechols. The results of the element analysis were in agreement with the calculated values.

2-Oxyphenyleneoxybenzo-1,3,2-dioxaarsole (IX) was obtained from tripropylarsenite (0.1 mole) and 22 g of pyrocatechol (0.2 mole) upon continuous distillation of the resultant propyl alcohol. The crystalline product was recrystallized from benzene. Yield of IX was 90.0%, mp 141-142°C. $C_{12}H_9AsO_4$. The NQR spectrum of compound IX has a ^{75}As frequency of 122.83 MHz. The IR-spectrum (in KBr pellets) has the following absorption bands, cm^{-1}: 650 (As–O), 684, 750, 1510, 1600, 1610 (C_6H_4), 1250 (O–C_{arom}), 3100-3450 (OH).

2-(2-Oxy-4-chloro)phenyleneoxy-7-chlorobenzo-1,3,2-dioxaarsole (X) was obtained from 2.9 g of 4-chloropyrocatechol (0.02 mole) and 2.5 g of tripropylarsenite (0.01 mole). The resultant oily crystals were purified by recrystallization from a benzene–hexane mixture. Yield was 47.1%, mp 108-110°C. $C_{12}H_7AsCl_2O_4$. The product's IR-spectrum had the following absorption bands, cm^{-1}: 630, 670 (As–O); 1230, 1250 (O–C_{arom}), 3200-3400 (OH), and absorption bands of the benzene ring.

2-(2-Oxy-4-tert-butyl)phenyleneoxy-7-tert-butylbenzo-1,3,2-dioxaarsole (XI) was obtained from 3.8 g of 4-tert-butylpyrocatechol (0.023 mole) and 2.9 g of tripropylarsenite (0.0115 mole). The resultant glass-like mass was dissolved in boiling hexane. Upon cooling, white crystals precipitated out of the solution. Yield was 66.7%, mp 130-132°C. $C_{20}H_{25}AsO_4$. The IR spectrum of compound XI (in KBr pellets) has the following absorption bands, cm^{-1}: 636, 645 (As–O), 927, 948 (C–H in tert-butyl), 810, 820, 830, 1486, 1505, 1580 (C_6H_3), 1229, 1258 (O–C_{arom}). The PMR spectrum (in deuteroacetone) δ, ppm: 1.0 (Me, singlet), 6.5 (C_6H_3, multiplet), 7.35 (OH, singlet).

EXPERIMENTAL BIOLOGICAL

Fungistatic activity was tested with reference to fungal dermatophylic etiological agents such as Trichophyton rubrum 695, Trichophyton mentagrophytes var. gypseum 1773, and Microsporum canis 84, employing a generally accepted standard method for testing all antifungal compounds [5]. Observations were made over a period of 14 days at a temperature of 26°C. Fungal sensitivity to a preparation was determined by the minimum dose at which no fungal growth was observed. Bacteriostatic properties were tested by the series dilution method in accordance with method [1] for E. coli BKMB-129 and St. aureus 209-P. Experimental bacterial load was 300,000 microbial bodies per 1 ml. The experimental results were tabulated every 24 h for a period of 5 days during which the presence of growth (turbidity) or inhibition of culture growth in the medium was checked.

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF BISQUATERNARY SALTS OF 3,12-BIS(3'-HALOACYL)-3,12-DIAZA-6,9-DIAZONIADISPIRO[5.2.5.2]HEXADECANE

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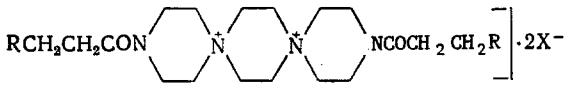
Spirobromine, which is the dichloride of 3,12-bis(3'-bromo-1'-oxopropyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane (IIa), is a new compound with an original chemical structure used for the treatment of acute leucosis, cancer of the cervix, malignant lymphomas, and cancer of the larynx [2, 3].

In the present report we describe a method of synthesis and the physicochemical properties of bisquaternary salts of 3,12-bis(3'-haloacyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]-hexadecane (IIc, d) and also the results of a comparative biological study of spirobromine (IIa) and bisquaternary salts (IIb-d) in relation to the effect on the reproduction of herpes viruses of simple Types 1 and 2 in cell cultures and in animals.

Spirobromine and bisquaternary salt IIb were obtained by us according to the method in [3].

Acylation of the dichloride (Ia) and dibromide (Ib) of 3,12-diaza-6,9-diazoniadispiro-[5.2.5.2]hexadecanes with β-chloropropionyl chloride in an aqueous medium in the presence of AcOLi, which is used as an acceptor for combining with HCl generated during the reaction, gives bisquaternary salts IIc and IId.



IIa-d

R = Br(IIa, b), Cl (IIc, d); X = Cl (IIa, d), Br (II b, c).

Compounds IIc and IId are white crystalline products which do not have a characteristic melting point. Compound IId is readily soluble in water, compound IIc is moderately soluble in water; compounds IIc and IId are virtually insoluble in organic solvents.

The structure of compounds IIc and IId has been confirmed by the elemental analysis data and by IR, UV, and PMR spectroscopy. In the IR spectra of compounds IIc and IId there are absorption bands in the region of 1660 cm^{-1} which can be assigned to the CO group of amides. The UV spectra of compounds IIa and IIc have λ_{max} at 199 and 202 nm (ε 22,760 and 23,467 respectively). In the PMR spectrum of compound IIc there are characteristic signals from protons of the $COCH_2$ groups at 3 ppm (triplet) and the CH_2Cl groups at 3.64 ppm (triplet) and also signals from protons in the spiro system at 4.20, 4.03, and 3.91 ppm.

EXPERIMENTAL CHEMICAL

IR spectra of the synthesized compounds were recorded in petrolatum oil on a Perkin-Elmer 457 (USA) instrument; UV spectra were recorded in aqueous solution on a Perkin-Elmer 575 (USA) instrument; PMR spectra were recorded in D_2O on a JEOL (Japan) instrument with operating frequency of 100 MHz and tetramethylsilane as internal standard. The proton signals are recorded on the δ scale. The elemental analysis figures found corresponded to those calculated.

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