2. A. I. Mukhalev, L. B. Falaleeva, N. I. Schramm, et al., Khim.-farm. Zh. No. 1, 36-39 (1981).
3. A. L. Rusanov, Usp. Khim., 43, No. 9, 1669-1687 (1974).
4. A. Spasov and E. Golovinskii, Zh. Obshch. Khim., 32, No. 10, 3394-3400 (1962).
5. A. D. Toleva, N. V. Gerbeleu, G. P. Syrtsova, et al., Zh. Neorg. Khim., 26, No. 5, 1288-1293 (1981).
6. A. D. Toleva, N. M. Samus', and A. N. Shishkov, Ibid., 28, No. 11, 2848-2851 (1983).
7. A. D. Toleva, N. M. Samus', A. N. Shishkov, et al., Koordinats. Khim., 9, No. 12, 1655-1660 (1983).
8. M. N. Hughes, Inorganic Chemistry of Biological Processes, 2nd Edn., J. Wiley, New York (1981).
9. A. Spassov, E. Golovinsky, and G. Demirov, Chem. Ber., 98, 932-935 (1965).

RELATIONSHIP OF STRUCTURE TO ANTIMICROBIAL ACTIVITY IN ANIONIC SURFACTANTS

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UDC 661.185:547.476:61.281

Many synthetic surfactants are known to display biological activity. Antimicrobial activity is that most commonly found, owing to the ability of these compounds to interfere with the permeability of the cytoplasmic membranes and osmotic equilibria in the cell, and to react with its lipids and proteins. These effects of surfactants result in loss from the bacterial cell of substances important for its vital processes, denaturation of proteins, and interference with enzymic equilibria [7]. More detailed studies are currently in progress on the relationships between antimicrobial activity and the structures and surface activity of cationic surfactants [1]. In the case of anionic surfactants, relationships between structure and antimicrobial activity have been established for the series of n-alkyl sulfates $C_nH_{2n+1}OSO_3Na$ [5]. No such studies have been carried out with surfactants of more complex structure, particularly those containing biologically active groupings.

We have made a systematic study of the synthesis and properties of surfactants derived from sulfosuccinic acid, in the course of which the influence of structure on the antibacterial activity of the surfactants was established for a series of sulfosuccinic acid ester anilides of general formula

$$R^1NHCOCH(SO_3Na)CH_2COOR^2,$$

I — XXIII

where $R^1 = C_6H_5$ (I — IV); $C_6H_4Cl = 4$ (V — VII); $C_6H_3Cl_2 = 2,4$ (VIII—IX); $C_6H_4OH = 2$, (X,XI); $C_6H_4OH = 3$ (XII, XIII); $C_6H_4COONa = 4$ (XIV, XV); $C_6H_4COOC_2H_5 = 4$ (XVI, XVII); $C_6H_4SO_2NH_2 = 4$ (XVIII — XXIII).
$R^2 = C_8H_{17}$ (I, V, VIII, XVIII); $C_{10}H_{21}$ (II, VI, IX, X, XII, XIV, XVI, XIX); $C_{11}H_{23}$ (XX); $C_{12}H_{25}$ (III, VII, XXI); $C_{14}H_{29}$ (IV, XI, XIII, XV, XVII, XXII); $C_{16}H_{33}$ (XXIII).

In this series of compounds, the biological activity of the anilide grouping was varied by changing R^1. The substituted aniline starting materials used were sulfanilamide, chloroanilines, and aminophenols, which are bacteriostats; aniline, which is not bacteriostatic in low concentrations; and p-aminobenzoic acid and its ethyl ester, which are involved in the normal metabolism of bacteria, thereby stimulating their growth [4]. The surface activity of the compounds of this series was varied by changing the size of the radical R^2 from C_8 to C_{16}.

Leningrad Institute of Pharmaceutical Chemistry. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 19, No. 11, pp. 1356-1361, November, 1985. Original article submitted October 17, 1984.

0091-150X/85/1911-0797$09.50

TABLE 1. Physicochemical Properties and Bacteriostatic Activity of Ester Anilides of Sulfosuccinic Acid

Compound	Yield, %	Found, %		Empirical formula	Calculated, %		IR spectrum, cm^{-1}				Minimum surface tension ($\sigma_{min} \times 10^3$, J/m^2)	Concentration corresponding to $\sigma_{min} \times 10^3$, mole/liter	Minimum bacteriostatic concentration (C_{bsd}) $\times 10^3$ mole/liter (for Staph. aureus)
		N	S		N	S	$\nu_{C=O}$ ester	$\nu_{C=O}$ amide	ν_{NH}	ν_{SO_2}			
I	81	3,23	7,30	$C_{18}H_{26}NO_6SNa$	3,44	7,85	1720	1618	3250	1043	33	50	2,5
II	72,5	3,69	7,02	$C_{20}H_{30}NO_6SNa$	3,21	7,35	1718	1620	3270	1060	31	10	1,1
III	76,3	2,77	6,85	$C_{22}H_{34}NO_6SNa$	3,02	6,91	1712	1615	3258	1052	31	3,0	0,27
IV	80,2	3,01	6,78	$C_{24}H_{38}NO_6SNa$	2,85	6,52	1720	1619	3250	1060	31	5,0	0,205
V	49	3,38	6,92	$C_{18}H_{25}ClNO_6SNa$*	3,43	7,02	1714	1605	3248	1060	31	10	1,75
VI	82	3,08	6,96	$C_{20}H_{29}ClNO_6SNa$†	2,98	6,82	1728	1624	3250	1052	30	1,0	0,20
VII	79	2,63	6,92	$C_{22}H_{33}ClNO_6SNa$‡	2,81	6,45	1712	1620	3205	1060	30	0,1	0,20
VIII	52,05	—	6,03	$C_{18}H_{24}Cl_2NO_6SNa$••	—	6,72	1720	1614	3242	1040	32	10	>10
IX	53	2,89	6,40	$C_{20}H_{28}Cl_2SO_6SNa$††	2,78	6,34	1720	1610	3205	1060	32	1,0	0,25
X	34	2,66	8,71	$C_{20}H_{30}NO_7SNa$	3,10	7,09	1720	1617	3250	1043	34	1,0	>10
XI	39	2,80	6,97	$C_{24}H_{38}NO_7SNa$	2,74	6,27	1712	1615	3270	1050	34	0,7	>10
XII	43	3,05	7,21	$C_{10}H_{30}NO_7SNa$	3,10	7,09	1717	1619	3280	1066	28	1,0	>10
XIII	57	2,72	6,33	$C_{24}H_{38}NO_7SNa$	2,74	6,27	1714	1619	3260	1060	29	0,5	10
XIV	52	3,03	...	$C_{21}H_{29}NO_8SNa_2$	2,80	...	1720	1607	3205	1066	39	1,0	No bacteriostatic activity
XV	67	2,8	....	$C_{25}H_{37}NO_8SNa_2$	2,51	...	1721	1610	3200	1050	38	0,1	"
XVI	39	2,93	...	$C_{23}H_{34}NO_8SNa$	2,76	...	1724	1605	3210	1050	36	1,0	» »
XVII	42	2,42	...	$C_{27}H_{42}NO_6SNa$	2,61	...	1729	1610	3210	1043	32	1,0	>10
XVIII	54,5	5,84	12,96	$C_{18}H_{27}N_2O_8S_2Na$	5,76	13,15	1720	1605	3210	1050	35	50	2,5
XIX	69,5	5,27	11,68	$C_{20}H_{31}N_2O_8S_2Na$	5,45	12,5	1719	1610	3250	1055	32	10	1,8
XX	76,5	4,80	11,08	$C_{21}H_{33}N_2O_8S_2Na$	5,30	12,1	1720	1610	3210	1052	31	10	1,1
XXI	85	5,45	12,5	$C_{22}H_{35}N_2O_8S_2Na$	5,15	11,80	1720	1619	3220	1050	32	3	0,62
XXII	89,2	4,53	11,42	$C_{24}H_{39}N_2O_8S_2Na$	4,9	11,25	1724	1607	3212	1050	32	0,1	0,31
XXIII	86,8	4,40	10,23	$C_{26}H_{43}N_2O_8S_2Na$	4,68	10,70	1720	1610	3210	1050	31	0,05	0,16

•Found %: Cl 8,21. Calculated %: Cl 8,24.
†Found %: Cl 7,15. Calculated %: Cl 7,56.
‡Found %: Cl 6,98 Calculated %: Cl 7,15.
••Found %: Cl 14,59 Calculated %: Cl 14,91.
††Found %: Cl 6,97. Calculated %: Cl 7,04.

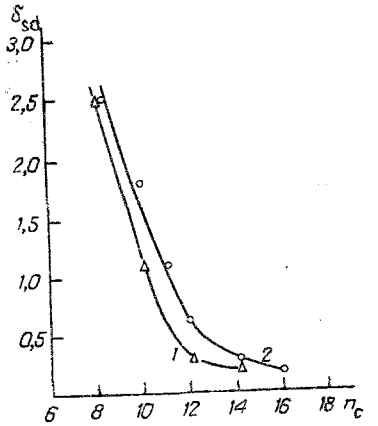
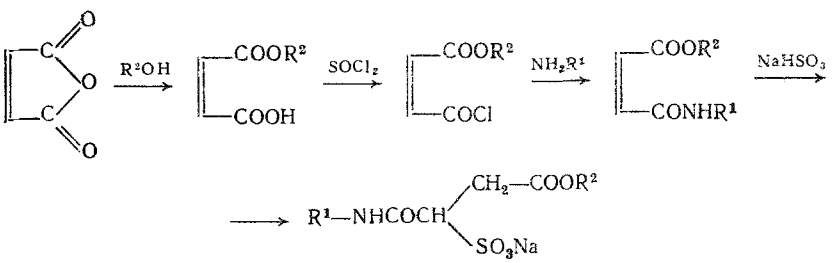


Fig. 1. Plots of bacteriostatic activity versus the length of the hydrophobic grouping in the surfactants. The abscissa represents the number of carbon atoms (n_C) in the hydrophobic moiety of the ester group in the surfactant, equal to $C_{bsd} \cdot 10^3$ (in moles/liter). 1) I-IV; 2) XVII-XXIII.

These sulfosuccinic acid ester anilides were obtained as follows:



The previously described monoesters of maleic acid [3] were obtained by heating solutions of maleic anhydride and the appropriate alcohol in benzene. The acid chlorides of the monoalkyl maleates were obtained in the usual way [2] by boiling the alkyl maleates with thionyl chloride in dry chloroform. The amines were acylated by boiling with the acid chlorides in dry dioxane in the presence of equimolar amounts of sodium carbonate. The ester anilides of sulfosuccinic acid were synthesized by boiling aqueous-alcoholic solutions of the maleate ester anilides with soidum metabisulfite. The desired products were isolated from the reaction mixtures by precipitation with acetone, and twice recrystallized. The physicochemical properties, elemental analyses, and antimicrobial activity of the compounds obtained (I-XXIII) are given in Table 1. Examination of the relationship between structure and surface and biological activity showed that in nearly all these sulfosuccinic ester anilides, increasing the size of R^2 over the range C_8–C_{14} resulted in higher surface activity, σ_{min} for these compounds being reached at reduced surfactant concentrations. In most of the surfactants obtained, σ_{min} fell within a fairly narrow range (30–33 J/m^2), owing to the similarity of their structures. It is noteworthy that the introduction of polar groups into the anilide grouping usually affects adversely the surface active function. For example, in o-aminophenol derivatives, $\sigma_{min} = 34 \cdot 10^{-2}$ J/m^2, and in the case of p-aminobenzoic acid a value of $39 \cdot 10^2$ J/m^2 is reached. This behavior of the surfactants appears to be due to a deterioration in the hydrophiliclipophilic balance (HLB) in the molecule. Derivatives of m-aminophenol are exceptional, since despite the presence of an additional hydrophilic group, the surface activity was very high ($\sigma_{min} = 28$ J/m^2). This appears to be due to the formation of a hydrogen bond between the phenolic hydroxyl and the amide group. This results in a decrease in the hydrophilicity of this grouping, and the HLB of the molecule as a whole is not adversely affected.

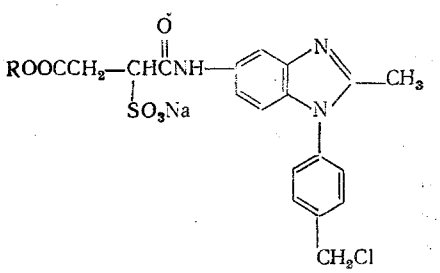
Microbial tests of the surfactants obtained were carried out by the standard serial dilution method against *Staph. aureus*, which is usually sensitive to anionic surfactants, *Escherichia coli*, and the yeast *Candida albicans*. Bacteriostatic activity was shown by nearly all the compounds, except for the p-aminobenzoic acid derivatives, the sensitivity of Gram-positive bacteria (*Staph. aureus*) being usually one to two orders of magnitude greater than that of Gram-negative bacteria (*Escherichia coli*) (C_{bsd} values between $2 \cdot 10^2$—10^4 and $3 \cdot 10$—10^2 mole/liter respectively).

Examination of the minimum bacteriostatic concentrations of the surfactants showed that their antimicrobial activity (against *Staph. aureus*) was not related to that of the anilide grouping. For example, aniline derivatives of sulfosuccinates have C_{bsd} values of the same order as those of chloroaniline and sulfanilamide derivatives, whereas sulfosuccinates obtained from aminophenols are less active. The derivatives of p-aminobenzoic acid and its ester are completely inactive. This could, on the one hand, be the result of their low surface activity, but on the other hand it is possible that a further deactivating factor is the incorporation of cleaved p-aminobenzoic acid in the normal metabolism of the microorganisms.

A closer relationship between surface and biological activity may be discerned in two series of surfactants, namely the aniline derivatives (I-IV) and the streptocide (sulfanilamide) derivatives (XVIII-XXIII). Despite the considerable differences between the structures of the anilide groupings, both series display high biological activity which varies linearly with the length of the hydrophobic radical in the surfactant. Figure 1 shows plots of the minimum bacteriostatic concentration of the surfactants on the length of the radical R^2, from which it will be seen that over the range from C_8 to C_{16} they correspond to the changes in the surface-active properties (it will be seen from Table 1 that as R^2 is increased over this range, the concentrations necessary for the attainment of σ_{min} decrease).

The dependence of bacteriostatic activity on surface-active properties has been studied previously [5], and our findings are in accordance with the established concepts of the effects of surfactants on microorganisms. A novel finding is, however, that as our results show the presence of a bacteriostatic grouping in the combined state in the surfactant molecule does not enhance the antimicrobial acitivity of the surfactant. It is noteworthy that the biological activity of substituted anilines incorporated into the anilide groupings of the surfactants obtained is largely determined by the presence of free amino-groups. They were, however, found to be firmly bound, since the rate of hydrolysis of anilides under neutral conditions (such as are present in the microbiological tests) is extremely slow. For instance, on heating an aqueous solution of (XXI) at 100°C for 30 min, which is equivalent to heating at 37°C for 300 h, no cleavage of sulfanilamide (reaction with β-naphthol [7]) was observed.

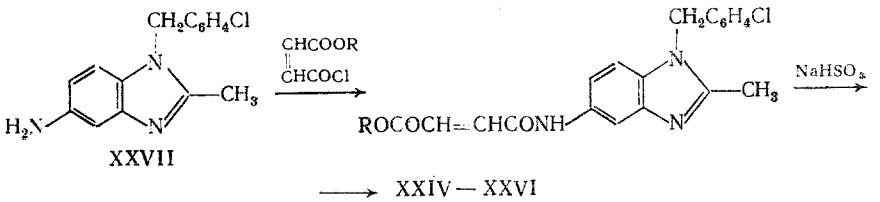
In order to confirm this conclusion in other cases, we synthesized some surfactants of general formula



where R = C_5H_{11} (XXIV), C_8H_{17} (XXV), and $C_{10}H_{21}$, which incorporate as the biologically active grouping the highly active antifungal compound 1-p-chlorobenzyl-2-methyl-5-aminobenzimidazole (XXVII) [8]. The antifungal activity of this compound is virtually independent of the presence of an amino-group or any other substituent in the 5-position of the benzimidazole ring, being fully maintained in the unsubstituted 1-p-chlorobenzyl-2-methyl-benzimidazole. It is noteworthy that the latter compound forms the active constituent of the currently used antifungal preparation chlormidazol, developed by the West German Company Chemie Gruenthal [6].

Hence, in the surfactants (XXIV-XXVI), the incorporation of the surface-active grouping does not block the functional groups responsible for the biological activity of the benzimidazole moiety of the molecule.

Compounds (XXIV-XXVI) were synthesized from the benzimidazole (XXVII) [9] as follows:



Acylation of the benzimidazole amino-group was effected by boiling a solution of the compound in dry dioxane with the acid chloride of the appropriate alkyl maleate in the presence of potassium carbonate. Sulfonation of the maleate ester amides thus obtained was effected by heating with $NaHSO_3$ in aqueous alcoholic solution. It was found that the rate of sulfonation decreased rapidly as the length of the alkyl chain R was increased. As a result of hydrolysis of the ester group on prolonged heating, it was not possible to obtain compounds in which R contained more than ten carbon atoms.

As would be expected, compounds (XXIV-XXVI) displayed surface activity which varied depending on the structure of the hydrophobic protion of the molecule in accordance with the rules mentioned above. For instance, the minimum surface tensions (at a surfactant concentration of $5 \cdot 10^{-2}$ mole/liter) of (XXIV) and (XXVI) were $5.6 \cdot 10^{-2}$ and $3.9 \cdot 10^{-2}$ J/m^2 respectively.

The antimicrobial activity of the original compound (XXVII) and its surface-active derivatives (XXIV-XXVI) were determined by serial dilution against the yeast *Candida albicans*. It was found that the the high biological activity of the original benzimidazole (XXVII), which exerted bacteriostatic effects at a concentration of $4.8 \cdot 10^{-4}$ mole/liter, was considerably reduced when a surface-active groupings was introduced into the molecule. For example, the most active compound (XXVI) had C_{bsd} $1.1 \cdot 10^{-2}$ mole/liter, a value similar to that obtained for anionic surfactants against *Candida albicans*.

It has been reported [8] that the mode of action of antifungal benzimidazoles (chlormidazol) and imidazoles (chlortrimazol) is, like that of anionic surfactants, related to their membranotropic properties. This is also indicated by the synergistic effects of anionic surfactants on the fungistatic activity of these compounds [8]. However, in the case of simple anionic surfactants (alkyl sulfates $C_nH_{2n+1}SO_3Na$), it has been shown that binding to elements of the microbial cell is mediated by the hydrophobic moiety of the molecule [5]. From these observations, in conjunction with our own findings, it may be concluded that in anionic surfactants or more complex structure which incorporate both hydrophobic radicals and biologically active groupings, the latter play no part in interactions with bacterial cell membranes. The structure of the hydrophobic radical plays a dominant part in the antimicrobial activity of the surfactants obtained.

EXPERIMENTAL (CHEMICAL)

IR spectra were obtained on a UR-20 spectrophotometer with LiF and NaCl prisms, in vaseline oil.

The starting materials for the synthesis of the sulfosuccinic acid ester anilides (maleic acid ester anilides) were obtained by similar methods, as exemplified by the synthesis of tetradecyl maleate m-hydroxyanilide. To a solution of 2.18 g (0.02 mole) of m-aminophenol in 10 ml of dry dioxane was added 2.12 g (0.02 mole) or anhydrous sodium carbonate, and 7 ml (0.02 mole) of tetradecyl maleate acid chloride was then added dropwise over 20 min at a temperature not exceeding 10°C. The mixture was kept for 1 h at 20°C, and heated for 3 h on the water bath. The mixture was cooled, and the product precipitated with 15 ml of water, the solid filtered off, and washed on the filter with water to give 5.5 g (72%) of technical product, mp 142-145°C, which was used in the next step without further purification.

Synthesis of Sulfosuccinic Acid Derivatives (I-XXIII) (general method). To a solution or suspension of 0.02 mole of the technical maleic acid ester anilide in 5 ml of ethanol was added 5 ml of a saturated aqueous solution of sodium metabisulfite, and the mixture heated on the water bath for 4 h. After cooling, the product was precipitated from the mixture with 10 ml of acetone. The product was purified by recrystallization from water or 50% aqueous alcohol to give a colorless powder which did not melt on heating to 280-300°C. The physicochemical properties and elemental analyses of the compounds (I-XXIII) are given in Table 1.

The above method was used to sulfonate maleate ester anilides derived from 1-p-chlorobenzyl-2-methyl-5-aminobenzimidazole, except that the reaction mixture was heated for longer periods of time. In the preparation of (XXIV), the mixture was boiled for 12 h to give a yield of 30%. Found: Cl 6.67; N 2.80; S 6.02. $C_{24}H_{26}ClNO_6SNa$. Calculated: Cl 6.89; N 2.72; S 6.22.

In the preparation of (XXV), the mixture was boiled for 36 h, yield 31%. Found %: Cl 6.50; N 2.63; S 6.01. $C_{27}H_{32}ClNO_6SNa$. Calculated %: Cl 6.34; N 2.50; S 5.75.

In the preparation of (XXVI), the mixture was boiled for 60 h, yield 10%. Found %: Cl 5.93; N 2.28; S 5.62. $C_{29}H_{36}ClNO_6SNa$. Calculated %: Cl 6.07; N 2.40; S 5.47.

LITERATURE CITED

1. V. S. Buryak, V. P. Rudi, G. T. Pis'ko, and N. I. Ganushchak, in: The Physiological Role of Surface-Active Compounds [in Russian], Chernovtsy (1975), pp. 22-23.
2. Weigand-Hilgetag, Experimental Methods in Organic Chemistry [Russian translation from German], Moscow (1969).
3. Yu. B. Gordinskii, V. M. Shimanskii, and R. S. Vishnyakova, Zh. Prikl. Khim., 40, No. 8, 1881-1883 (1967).
4. G. M. Dyson and P. May, The Chemistry of Synthetic Drugs [Russian translation], Moscow (1964).
5. E. F. Panarin and G. E. Afinogenov, Khim.-farm. Zh., No. 1, 79-81 (1978).
6. É. A. Rudzit, Ibid., No. 7, 39-53.
7. A. Schwartz, G. Perry, and G. Birch, Surface-Active Compounds and Wetting Agents [Russian translation], 2nd Edn., Moscow (1960), p. 469.
8. West German Patent No. 1000384 (1957); Chem. Abstr., 51, 15 776 (1957).