evaluated from the absence or presence of growth of the microbe after incubation at 37°C for 18-48 h. The last concentration of the preparation at which growth is not observed is its MIC (the method was recommended by order of the Ministry of Public Health of the USSR from March 13, 1975, "Unification of methods for the determination of the sensitivity to chemotherapeutic preparations").

The results obtained are presented in Tables 2 and 3.

An analysis of the data presented in Tables 2 and 3 shows that all of the preparations used for the tests have antimicrobial activity with respect to all of the test microbes used for the investigation. The compounds have the most pronounced action on inducers of intestinal infections (Shigella, Salmonella) and some representatives of quasi-pathogenic bacteria. In addition, they have no effect on Bacillus pyocyaneus and cause a lag in the growth of Sh. stutzeri-shmitzii and Staph. aureus 209. For most of the test microbes the MIC ranges from 50 to 100 μg/ml, while for some it ranges from 6.25 to 12.5 μg/ml (S. paratyphi A, Proteus mirabilis).

Thus it was established that all of the tested preparations have approximately the same antimicrobial properties against most of the test microbes used for the investigations, although S. enterocolitica, Citrobacter, S. paratyphi A and B, and some forms of Shigella proved to be more sensitive to them.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DERIVATIVES OF AMINOBENZOCROWN ETHERS

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We have previously shown [5, 6] that some aliphatic derivatives of crown ethers (CE) display high antimicrobial activity (AMA). Biological activity of a number of other CE is also known [1, 7]. However, the structure–function interrelationship for such compounds has not been adequately studied; the reason for this is the relatively small number of investigated representatives of CE.

The aim of the present research was to study the AMA of derivatives of aminobenzocrown ethers (ABC). We have developed general and convenient methods for the synthesis of some derivatives of ABC and have studied their AMA.

The corresponding N-acyl derivatives III, IV, and Va, b were synthesized in high yields by acylation of 4'-aminobenzo-15-crown-5 (I) and the isomers of diaminodibenzo-18-crown-6 [trans (IIa) and cis (IIb)] with acetic anhydride and benzoyl chloride.

The corresponding formamidinocrown ethers (FCE) VII-XI and acetamidinocrown ether XII were obtained in high yields (up to 95%) by the reaction of I, IIa, b, and diaminodibenzo-24-crown-8 (VI) with dimethylacetals of dimethylformamide, N-formylpiperidine, N-formylmorpholine, and dimethylacetamide.

It was also established that VII, VIIIa, b, and XI are transaminated extremely readily by various amines with the formation of the corresponding FCE. Thus amidines IXa, b and Xa, b were also obtained by transamination of VIIIa, b by, respectively, piperidine and morpholine [2], while amidoxime XIII was obtained by transamination of VIIIa with hydroxylamine hydrochloride at 20°C. Thus the proposed method for the synthesis makes it possible to readily obtain an extensive set of compounds of this type in high yields.

The synthesis and investigation of N,N'-bis(crown ether)-amidines, which have not been previously described in the literature, are of great interest in this respect. We have developed a convenient general method for obtaining bis-CE of this type in yields greater than 75% that does not require column chromatography for their isolation. Thus, for example,

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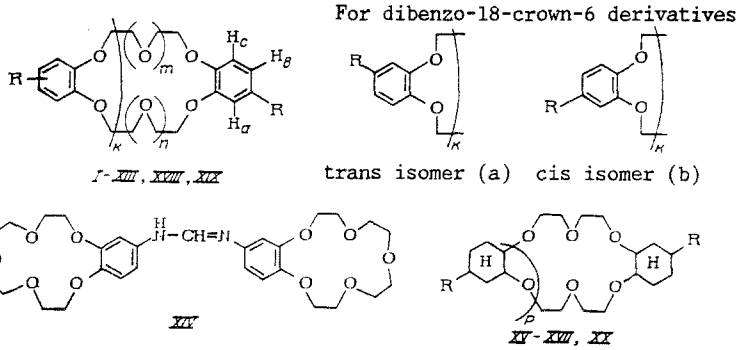

R = H (XVIII, XIX, XX), NH_2 (I, IIa, b, VI, XV, XVI), NHAc (III, Va, b), NHCOPh (IV), N=CH—NMe_2 (VII, VIIIa, b, XI, XVII), N=CH—piperidino (IXa, b), N=CH—morpholino (Xa, b), N=C(Me)—NMe_2 (XII), NH—CH=NOH (XIII); k=0, m=1, n=2 (I, III, IV, VII, XVIII); k=m=n=1 (IIa, b, Va, b; VIIIa, b—Xa, b; XIIa, b; XIII; XIX); k=1, m=n=2 (VI, XI); p=0 (XV), p=1 (XVI, XVII, XX).

N,N'-bis(m-benzo-15-crown-5)formamidine (XIV) was synthesized from I and ethyl orthoformate with simultaneous removal of the resulting ethanol by distillation.

The high yields under mild reaction conditions and the possibility of obtaining an extensive set of derivatives for each series of compounds, i.e., subjects for systematic medical-biological and other studies, constitute the definite convenience of the above-described methods for the synthesis of derivatives of ABC.

As test compounds for the study of the AMA we also used hydrogenated analogs of ABC – 4'-aminocyclohexano-15-crown-5 (XV)* and trans-diaminodicylcohexano-18-crown-6 (XVI);* from the latter, as in the case of VIIIa, b, we obtained the corresponding formamidine (XVII).

The structures of the synthesized compounds were established by means of PMR, IR, and UV spectroscopy, mass spectrometry, and the results of elementary analysis. The physicochemical characteristics of the synthesized derivatives of ABC are presented in Table 1. The results of the elementary analyses were in agreement with the calculated values.

EXPERIMENTAL (CHEMICAL)

The melting points were determined with a PTP-1 apparatus (USSR). The IR spectra of solutions in $CHCl_3$ were recorded with a Perkin-Elmer 580B spectrometer (Sweden). The PMR spectra of solutions in $CDCl_3$ were recorded with a Tesla BS-467 spectrometer (60 MHz) (Czechoslovakia) with tetramethylsilane (TMS) as the internal standard. The mass spectra were obtained with a Varian MAT-112 spectrometer (West Germany). The UV spectra of solutions in MeOH were recorded with a Specord UV-VIS spectrophotometer (East Germany). Thin-layer chromatography (TLC) on Silufol UV-254 plates (Czechoslovakia) was used to monitor the purity of the substances obtained.

4'-Aminobenzo-15-crown-5 (I) was obtained by the method in [8], the trans (IIa) and cis (IIb) isomers of diaminodibenzo-18-crown-6 were obtained by the method in [9], diaminodibenzo-24-crown-8 (VI, mixture of isomers) was obtained by the method in [3], and 4'-(N,N-dimethylaminomethyleneamino)benzo-15-crown-5 (VII) and the isomers of bis(N,N-dimethylaminomethyleneamino)dibenzo-18-crown-6 (VIIIa, b), bis(N-piperidinomethyleneamino)dibenzo-18-crown-6 (IXa, b), bis(N-morpholinomethyleneamino)dibenzo-18-crown-6 (Xa, b), and bis(N,N-dimethylaminomethyleneamino)dibenzo-24-crown-8 (XI) were obtained by the method in [2].

4'-(N-Acetamido)benzo-15-crown-5 (III). A 1.65-ml (17.5 mmole) sample of Ac_2O was added to a solution of 4.50 g (15.9 mmole) of I in 30 ml of dry benzene, after which the mixture was stirred for 10-15 min, refluxed for 30 min, and cooled to 0°C. The precipitated crystals were removed by filtration and recrystallized from benzene–heptane (2:1). The yield was 4.24 g. UV spectrum, λ_{max}, nm (log ε): 207 (4.61), 251 (3.73), 287 (3.24). PMR spectrum, δ, ppm: 2.15 s (3H, CH_3), 3.7-4.2 m (8H, CH_2O), 6.73 dd (1H, H_b), 6.82 d (1H, H_c), 7.31 d (1H, H_a), 7.87 s (1H, NH). IR spectrum, ν, cm^{-1}: 1635 (C=O), 965, 1115 (C–O–C).

*The authors thank R. N. Gurskii for kindly providing us with samples of crown ethers XV and XVI.

TABLE 1. Physicochemical Properties of the Synthesized Compounds

Compound	Yield, %	mp, °C	Empirical formula	M^+
III	82	150—01	$C_{16}H_{23}NO_6$	325
IV	85	158—09	$C_{21}H_{25}NO_6$	387
Va	78	273—04	$C_{24}H_{30}N_2O_8$	474
Vb	71	235—06	$C_{24}H_{30}N_2O_8$	474
XIIa	87	135—07	$C_{28}H_{40}N_4O_6$	528
XIIb	85	112—03	$C_{28}H_{40}N_4O_6$	528
XIII	81	165—70 (dec)	$C_{22}H_{28}N_4O_8$	476
XIV	75	147—08	$C_{29}H_{40}N_2O_{10}$	576
XVI	73	Oil	$C_{24}H_{48}N_4O_6$	512

4'-(N-Benzamido)benzo-15-crown-5 (IV). A solution of 3.7 g (13 mmole) of I and 1.75 ml (15 mmole) of PhCOCl in 30 ml of dry benzene was refluxed until HCl evolution ceased (~8 h), after which it was cooled, and the resulting precipitate was removed by filtration and recrystallized from benzene. The yield was 4.30 g. UV spectrum, λ_{max}, nm (log ε): 205 (4.42), 220 (3.89), 289 (3.30). PMR spectrum, δ, ppm: 3.7-4.2 m (8H, CH_2O), 6.83 d (1H, J_{HH} = 2.1 Hz, H_b), 7.05 dd (1H, H_c), 7.52 m (6H, H_a and C_6H_5), 7.91 s (1H, NH). IR spectrum, ν_{max}, cm^{-1}: 1637 (C=O), 965, 1115 (C—O—C).

trans-Bis(N-acetamido)dibenzo-18-crown-6 (Va). A 0.94-ml (10 mmole) sample of Ac_2O was added to a refluxing solution of 1.80 g (4.6 mmole) of IIa in 200 ml of toluene, after which the mixture was stirred for 20 min and cooled, and the precipitate was removed by filtration and washed with benzene. The yield was 1.86 g. UV spectrum, λ_{max}, nm (log ε): 207 (4.72), 251 (3.82), 287 (3.38). PMR spectrum, δ, ppm: 2.15 s (6H, CH_3), 3.7-4.2 m (16H, CH_2O), 6.73 dd (2H, H_b), 6.81 (2H, J_{HH} = 2.1 Hz, H_c), 7.31 d (2H, H_a), 7.87 s (2H, NH). IR spectrum, ν_{max}, cm^{-1}: 1635 (C=O), 965, 1115 (C—O—C).

cis-Bis(N-acetamido)dibenzo-18-crown-6 (Vb). This compound was obtained in the same way as Va. The yield was 1.70 g. With the exception of the melting points, the physicochemical characteristics (see Table 1) were similar to the characteristics of acetamide Va.

trans-Bis[1-(N,N-dimethylamino)ethylideneamino]dibenzo-18-crown-6 (XIIa). A 2-g (15 mmole) sample of dimethylacetamide dimethylacetal was added to a solution of 1.95 g (5 mmole) of IIa in 15 ml of dry benzene, and the mixture was refluxed with stirring for 2 h. It was then cooled, and the solvent was evaporated in vacuo. The residue was crystallized from benzene—hexane (1:2). The yield was 2.30 g. UV spectrum, λ_{max}, nm (log ε): 214 (4.20), 260 (3.58), 294 (3.34). IR spectrum, ν_{max}, cm^{-1}: 1630 (C=N), 960, 1115 (C—O—C). PMR spectrum, δ, ppm: 1.73 s (6H, C—CH_3), 2.90 s (12H, NCH_2), 3.8-4.2 m (16H, OCH_2), 6.13 dd (2H, J_{HH} = 2.2 Hz, H_b), 6.56 d (2H, H_c), 6.70 d (2H, H_a).

cis-Bis[1-(N,N-dimethylamino)ethylideneamino]dibenzo-18-crown-6 (XIIb). This compound was obtained from 1.95 g (5 mmole) of IIb and 2 g of dimethylacetamide dimethylacetal in the same way as XIIa. The yield was 2.24 g. With the exception of the melting points, the physicochemical characteristics (see Table 1) were similar to the characteristics of acetamidine XIIa.

trans-Bis(N,N-dimethylaminomethylideneamino)dicyclohexano-18-crown-6 (XVII). This compound was obtained from 1.6 g (4 mmole) of XVI and 1.3 ml (10 mmole) of dimethylformamide dimethylacetal in 20 ml of dry toluene in the same way as XIIa. The yield was 1.49 g. PMR spectrum, δ, ppm: 1.3-1.6 m (12H, cyclohexane CH_2), 2.81 m (14H, cyclohexane NCH_3 and NCH), 3.7-4.2 m (20H, cyclohexane OCH_2 and OCH), 7.33 s (2H, CH). IR spectrum, ν_{max}, cm^{-1}: 1645 (C=N), 950, 1100 (C—O—C).

trans-Bis(N-hydroxyliminomethyleneamino)dibenzo-18-crown-6 (XIII). A solution of 3.80 g (7.6 mmole) of VIIIa and 1.08 g (17 mmole) of $H_2NOH \cdot HCl$ in 25 ml of MeOH was stirred at 20°C for 1 h, after which the resulting precipitate was removed by filtration and washed successively with water and MeOH. The yield was 2.93 g. UV spectrum, ν_{max}, nm (log ε): 216 (4.65), 260 (3.81), 294 (3.42). IR spectrum, ν_{max}, cm^{-1}: 1630 (C=N), 965, 1115 (C—O—C). PMR spectrum, δ, ppm: 3.7-4.2 m (16H, OCH_2), 6.44 dd (2H, J_{HH} = 2.1 Hz, H_b), 6.51 d (2H, H_c), 6.80 d (2H, H_a), 7.01 d (2H, NH), 7.23 d (2H, CH), 9.81 s (2H, NOH).

TABLE 2. Antimicrobial Activity of the Investigated crown Ethers*

Compound	Yield			
	Planococcus citreus	St. aureus p-209	Str. faecalis	Bac. subtilis BKMB-428
I	850	>1000	>1000	>1000
III	>1000	>1000	>1000	>1000
IV	750	750	>1000	>1000
Va	375	>1000	>1000	750
Vb	>1000	>1000	900	>1000
VII	70	>1000	750	750
VIIIa	>1000	>1000	>1000	>1000
IXa	94	375	94	188
Xa	375	850	750	>1000
XI	>1000	>1000	>1000	>1000
XIIa	23	240	750	750
XIII	>1000	>1000	>1000	>1000
XIV	650	900	900	900
XV	>1000	>1000	>1000	>1000
XVI	>1000	>1000	>1000	>1000
XVII	>1000	>1000	>1000	>1000
XVIII	>1000	>1000	>1000	>1000
XIX	>1000	>1000	>1000	>1000
XX	>1000	>1000	>1000	>1000

*The MSC for E. coli K-12 > 1000 μg/ml.

N,N'-Bis(m-benzo-15-crown-5)formamidine (XIV). A mixture of 2.83 g (10 mmole) of I and 8.32 ml (5 mmole) of ethyl orthoformate was heated on an oil bath (bath temperature 150-155°C) for 1 h with simultaneous removal of the resulting ethanol by distillation (distillation was carried out with an efficient column). The mixture was then cooled and treated with 10 ml of benzene, and the resulting precipitate was recrystallized from benzene. The yield was 4.33 g. UV spectrum λ_{max}, nm (log ε): 294 (3.55). IR spectrum, ν_{max}, cm^{-1}: 1640 (C=N), 960, 1115 (C–O–C). PMR spectrum, δ, ppm: 6.58 dd (2H, J_{HH} = 2.2 Hz, H_b), 6.83 d (2H, H_c), 7.12 d (2H, H_a), 7.40 d (1H, CH), 8.06 d (1H, NH).

EXPERIMENTAL (BIOLOGICAL)

The bacteriostatic activity of the CE was evaluated from their minimal concentrations that suppress the growth of microorganisms. The minimal suppressing concentrations (MSC) were determined by the method of twofold serial dilutions [4]. In our research we used periodic cultures of the following microorganisms: Planococcus citreus, Staphylococcus aureus P-209, Streptococcus faecalis, Bacillus subtilis BKMB-428, and Escherichia coli K-12. The microorganisms were grown in standard meat-peptone broth (digestion by the Hottinger method) at 37°C. For the determination of the MSC the investigated CE were dissolved in ethanol, or aqueous alcohol solutions were prepared. Data on the determination of the AMA of the compounds that we synthesized, as well as benzo-15-crown-5 (XVIII), dibenzo-18-crown-6 (XIX), and dicyclohexano-18-crown-6 (XX), which were used for comparison are presented in Table 2.

In the analysis of the experimental data presented in Table 2 one must note the different susceptibilities of the test cultures of microorganisms used to the active CE (IV, Va, VII, IXA, XIIa, and XIV). The indicated compounds suppress the growth of only the Gram-positive microorganisms used in the research. The MSC differ markedly (23-900 μg/ml) both for most of the Gram-positive test cultures and in the series of active compounds for each of the cultures. The AMA of investigated CE is manifested most strongly for typical representatives of coccus microflora (Planococcus citreus and Staphlococcus aureus). The Gram-negative bacteria Escherichia coli K-12 are resistant to the action of I-XX under the conditions of our experiments.

It is apparent from Table 2 that AMA is absent in CE XVIII-XX, which do not contain substituents, and aminocrown ethers I, XV, and XVI. Only functional derivatives of ABC with respect to the amino group suppress the growth of Gram-positive microorganisms. In the case of Va, b, which are trans and cis isomers of bis(N-acetamido)dibenzo-18-crown-6, it was noted that only trans isomer Va displays high AMA. To a first approximation, the differences in the AMA of these compound are in agreement with their different physicochemical properties and, primarily, with their own solubility and the solubility of the starting IIa, b in organic solvents. These facts enabled us to subsequently restrict our examination only to the trans isomers.

In the indicated series one should note the low activity of benzamide IV with respect to Planococcus citreus and Staphylococcus aureus and the absence of AMA for acetamide III. The amidinocrown ethers are most interesting from the point of view of the structure-function interrelationship. The data on the AMA of the synthesized formamidines indicate the existence of a relationship between the size of the cavity of the macrocycle and its AMA. Thus VIIIa, XI, and XVII, which are, respectively, derivatives of dibenzo-18-crown-6, dibenzo-24-crown-8, and dicyclohexano-18-crown-6, are inactive. At the same time, formamidinobenzo-15-crown-5 (VII) has a relatively high AMA. In analogy with VIIIa, XI, and XVII, formamidines IXa and Xa should not display AMA; however, these compounds are highly active (see Table 2); the activity of IXa is higher than the activity of Xa. This fact, in our opinion, constitutes evidence that the antimicrobial activity in this case is determined by the introduction of a piperidine substituent in the case of IXa and a morpholine substituent in the case of Xa; the higher activity of IXa correlates with the known higher lipophilicity of the piperidine fragment as compared with the morpholine fragment. The presence of a methyl group at the C atom in amidine XIIa (as compared with VIIIa) leads to the development of high AMA. Acetamidinocrown ether XIIa, obtained in this research, is the most active of all of the compounds that we investigated. It might be assumed that acetamidinocrown ethers will prove to be a promising class of effective antimicrobial agents.

The investigation of AMA has not been carried out for bis-CE of any type. For the first time we have investigated a compound of this type, viz., XIV, which displays an activity evaluated as medium (see Table 2); however, the method for the synthesis of amidine XIV is universal and makes it possible to obtain a sufficiently complete set of compounds of this type that contain macrocycles of any size and thus makes it possible to make a detailed investigation of the interrelationship between the structure of such CE and their physiological activity. On the basis of the data obtained one may draw certain conclusions regarding the structure–function relationship for a new class of derivatives of CE. A difference in the AMA for trans and cis isomers Va, b was established for the first time. A difference in the AMA for the crown forms and acetamidines and the existence of an effect of some substituents, as well as the macrocycle factor, were observed. The observed relationships between the structure and the AMA in the series of investigated CE and the MSC for the active compounds make it possible, in our opinion, to regard this class of CE as promising for the search for and incorporation in the practice of public health of new highly effective antimicrobial preparations.

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