STUDY OF THE CONFORMATION OF THE ANTIBIOTIC A-128-OP AND ITS DERIVATIVES BY THE METHODS OF OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM

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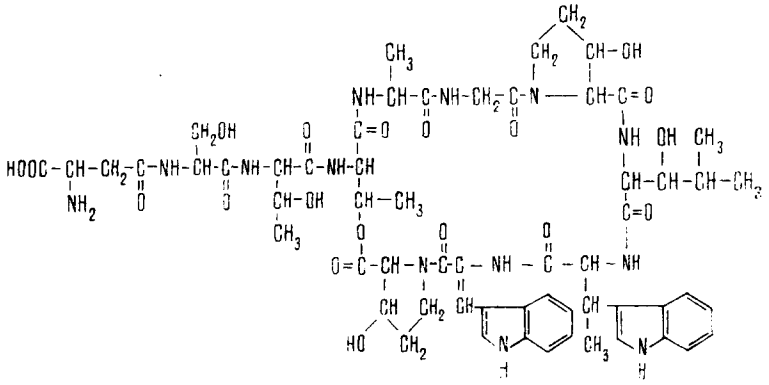
The most important problem of bioorganic chemistry is the study of the interrelationship between the structure and mechanism of the action of biologically active substances. In the final account, the solution of this problem gives real bases for the search for and synthesis of substances with predetermined biological properties and leads to an understanding of the features of the interaction of the molecule of a biologically active substance with a biological receptor.

Recently, great progress has been achieved in the study of the mechanism of the action of polypeptide antibiotics. A definite sequence is observed in the performance of such investigations: the establishment of the primary structure of the antibiotic, the determination of the role of the fractional groups in the manifestation of the biological activity either by their chemical modification or by the synthesis of structural analogs of the natural compound, and the determination by the latest physicochemical methods of the spatial arrangement of all the elements of the structure of the substance under investigation both in solution and in the process of its interaction with the bacterial cell or with artificial membranes [1].

Work on the conformational analysis of known antibiotics – valinomycin [2], gramicidin S [3], gramicidin A [4], stendomycin [5], etc., – is particularly interesting. In the course of the investigations performed it has been shown that in the functioning of these substances a fundamental role is played by the individual functional groups and also by the spatial structure of the molecule as a whole.

The present paper is the first of a series devoted to the conformational state of the polpeptide antibiotic A-128-OP and a number of its derivatives.



Structural formula of the antibiotic A-128-OP

The structural formula of the antibiotic A-128-OP was determined in our laboratory [6]. It is a tripeptide-cyclooctapeptidolactone which includes several amino acids of "nonprotein" nature, and its char-

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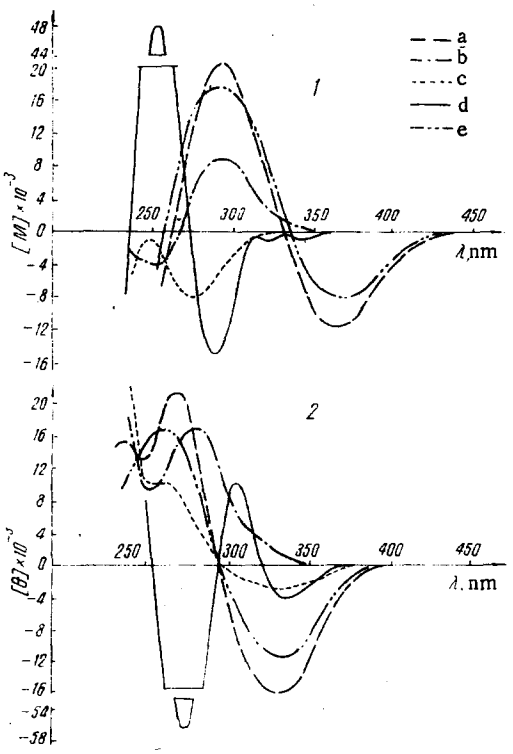

Fig. 1. ORD (1) and CD (2) curves of the antibiotic A-128-OP (a), A-128-OP acid (b) and derivatives of the antibiotic A-128-OP at the β-methyltryptophan and dehydrotryptophan residues – the 2-hydroxy-6-nitrobenzyl derivative (c), 2-nitrophenylsulfenyl derivative (d), and formyl derivative (e) [solvent: methanol–water (2:1)].

acteristic feature is the presence of such hydrophobic amino acids as β-methyltryptophan, dehydrotryptophan, and erythro-β-hydroxyleucine.

In this paper we give the results of a study of the conformation of the fraction of the molecule of the antibiotic A-128-OP formed by the β-methyltryptophan and dehydrotryptophan residues by the methods of optical rotatory dispersion (ORD) and circular dichroism (CD).

The ORD and CD curves of the antibiotic A-128-OP (Fig. 1) show a positive Cotton effect at 270 nm and a negative Cotton effect at 337 nm. Consequently, the Cotton effects are observed in the regions of the absorption of the aromatic amino acid residues of the antibiotic A-128-OP – β-methyltryptophan and dehydrotryptophan [7]. The appearance of a Cotton effect in the region of absorption of a symmetrical chromophore (dehydrotryptophan) is due to the interaction of the latter with the neighboring amino acid residues. In view of the characteristics of the structure of antibiotic A-128-OP it may be considered that the reason for the induced optical activity of the dehydrotryptophan is the interaction of this chromophore with the β-methyltryptophan residue.* For the effective interaction of β-methyltryptophan and dehydrotryptophan the proximity and a definite orientation of the planes of the indole rings of these amino acid residues relative to one another are necessary. In our opinion, the most probable conformation of the antibiotic in solution is one in which the planes of the indole rings of the β-methyltryptophan and dehydrotryptophan are oriented parallel to one another and are kept in this position by the hydrophobic interaction between them.

Analysis of the molecular structure using Stuart–Briegleb models has shown that the molecule of the antibiotic A-128-OP is compact and extremely rigid. In this structure, the side chains of the β-methyltryptophan and dehydrotryptophan residues are rotated outwards. Free rotation of the indole nucleus of the β-methyltryptophan residue is sterically hindered. The smallest strain in the cyclopeptide part of the molecule of this antibiotic is created with a parallel orientation of the indole rings.

In a comparison of the ORD and CD spectra of the antibiotic A-128-OP and the antibiotically inactive A-128-OP acid [8] (see Fig. 2), it can be seen that the ORD and CD curves of the latter do not have the Cotton effect at 337 nm that appears in the case of the initial antibiotic. The results obtained show that the cleavage of the ester bond in the molecule of the antibiotic is accompanied by a considerable change in the conformation of this compound. On passing from the cyclic structure of the antibiotic to a linear structure, the steric hindrance preventing the free rotation of the β-methyltryptophan residue is probably removed. In view of this, the parallel orientation of the indole rings of the β-methyltryptophan and dehydrotryptophan residues is disturbed and the interaction between them is considerably weakened, which leads to a disappearance of the induced Cotton effect at 337 nm. An analysis of molecular models has shown that on passing from the cyclic structure to a linear structure the molecule of the antibiotic loses its ordered structure and becomes less rigid. Free rotation of the β-methyltryptophan residue is not sterically hindered.

In order to determine the role of the free amino group in the molecule of the antibiotic, we have performed its specific chemical modification by reagents of various chemical natures [9] and have found that all the derivatives possess a smaller antibiotic activity than the initial material.

*In favor of this assertion are the results of investigations of the CD of synthetic peptides of dehydrotryptophan, which will be reported subsequently.

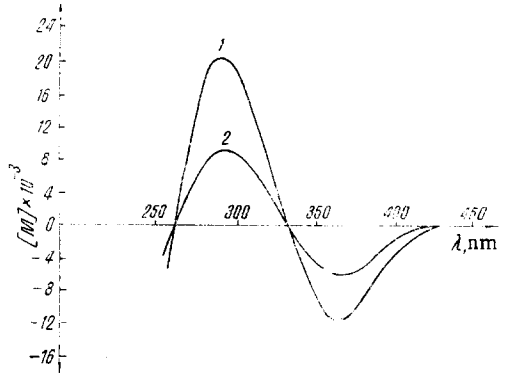

Fig. 2. ORD curves of N-acetyl-A-128-OP (1) and N-DNS-A-128-OP (2) (concentration of the N-DNS-A-128-OP half that of the other compound). The ORD curves of the other N-derivatives of the antibiotic coincide completely with those given above.

The results of a comparison of the ORD curves of the antibiotic A-128-OP and its derivatives at the amino group (N-acetyl-A-128-OP, N-benzyloxycarbonyl-A-128-OP, N-benzoyl-A-128-OP, N-succinyl-A-128-OP, N-monocarboxymethyl-A-128-OP, and N-(1-dimethylaminonaphthalene-5-sulfonyl)-A-128-OP [10]) show that in the wavelength range studied (230-450 nm) the ORD curves considered are identical within the limits of the experimental error (Fig. 2). Consequently, modification of the amide group by acylating and alkylating agents of different natures does not substantially affect the conformation of the "hydrophobic section"* of the molecule of this compound. The decrease in the antibacterial activity of the N-derivatives of the antibiotic shows the important role of the free NH_2 group in its functioning.

In addition to modification of the free amino group, derivatives of the antibiotic at positions 1, 2, and 3 of the indole rings of the tryptophan amino acids have been obtained: formyl, 2-hydroxy-5-nitrobenzyl (HNB), and 2-nitrophenylsulfenyl (NPS) derivatives of the antibiotic A-128-OP [11].

In a study of the optical properties of the HNB and NPS derivatives of this antibiotic, it was found that the ORD and CD curves of these compounds (see Fig. 2) differ substantially from the ORD and CD curves of the initial material. A decrease in the amplitude of the Cotton effect at 337 nm in the HNB and NPS derivatives shows a weakening of the interaction between the β-methyltryptophan and dehydrotryptophan residues in these compounds. A change in the optical activity of the antibiotic A-128-OP with the introduction of the HNB and NPS substituents into positions 2 and 3 of the indole rings of the β-methyltryptophan and dehydrotryptophan is probably connected with a conformational rearrangement of the "hydrophobic section" of the molecule of the antibiotic. It may be assumed that the voluminous HNB and NPS substituents are introduced between the β-methyltryptophan and dehydrotryptophan residues and thereby weaken the interaction between them. The results of a consideration of molecular models confirm the possibility of this hypothesis.

The ORD and CD curves of the antibiotic A-128-OP and its formyl derivative at the tryptophan amino acids (see Fig. 2) have no fundamental differences. The introduction into position 1 of the indole rings of the β-methyltryptophan and dehydrotryptophan of the small formyl substituent apparently has no substantial influence on the spatial arrangement of the indole rings.

In conclusion, it must be noted that the introduction of the HNB and NPS substituents into the molecule is accompanied by a marked decrease in its antibiotic activity. The replacement of the hydrogen atom in position 1 of an indole ring lowers the antibacterial activity of the initial antibiotic by a factor of 4-5.

The results of a study of the conformational state of the antibiotic and its derivatives by the ORD and CD methods in the 230-450-nm region has shown that modification of the amino group of the antibiotic A-128-OP does not affect the conformation of the "hydrophobic section" of the molecule of this compound. Cleavage of the ester bond or modification of the tryptophan amino acids at positions 2 and 3 of the indole ring is accompanied by a considerable change in the conformation of the initial antibiotic.

On the basis of the results obtained it may be assumed that thanks to the hydrophobic nucleus formed by the β-methyltryptophan and dehydrotryptophan residues and to the presence of the cyclic structure in the molecule of the antibiotic A-128-OP a definite "preferred" conformation is created which is most favorable for its functioning. A disturbance of this conformation through the modification of the β-methyltryptophan and dehydrotryptophan residues or the cleavage of the ester bond leads to a considerable decrease in the antibacterial activity of antibiotic A-128-OP.

In our later investigations we shall investigate in detail the conformation of the antibiotic by the ORD and CD methods in the 190-230-nm region.

* By hydrophobic section we mean the β-methyltryptophyl–dehydrotryptophan sequence.

EXPERIMENTAL

The antibiotic A-128-OP was isolated by a procedure described previously [12]. A-128-OP acid and derivatives of the antibiotic at the amino group and the β-methyltryptophan and dehydrotryptophan residues were obtained as described previously [8, 10, 11].

The ORD spectra were taken on a ORD/UV-5 spectropolarimeter (Japan), and the CD spectra on a Jouan II dichrograph (France) in the wavelength range from 230 to 450 nm in cells 1 cm thick. The concentration of the solutions investigated was $(0.5\text{-}1.0) \cdot 10^{-4}$M, and the temperature 20-22°C, the solvent being MeOH–H_2O (2:1). The values of the molecular elipticity (Θ) and the molecular rotation (M) are given without correction for the refractive index of the solvent.

SUMMARY

1. The optical rotatory dispersion and circular dichroism of the antibiotic A-128-OP and derivatives of it have been investigated in the wavelength range from 230 to 450 nm.

2. On the basis of the ORD and CD characteristics and the results of biochemical tests, it has been concluded that the molecule of the antibiotic A-128-OP has a "preferred" conformation which is most favorable for its functioning.

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