PREPARATION AND PROPERTIES OF DERIVATIVES OF THE ANTIBIOTIC A-128-OP AT THE β-METHYLTRYPTOPHAN AND DEHYDROTRYPTOPHAN RESIDUES

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UDC 547.964

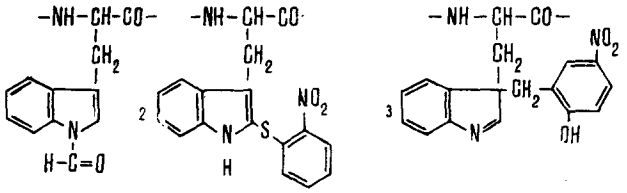
The method of chemical modification is frequently used in bioorganic chemistry for determining the interrelationship between the structure and function of biologically active substances. A comparative study of various derivatives of hormones and antibiotics with the initial materials makes it possible to determine the elements of structure responsible for the biological action of the compound investigated.

In the preceding paper, we gave the results of the chemical modification of the free amino group of the polypeptide antibiotic A-128-OP [1] and we also showed that the amino group is necessary for the manifestation of the activity of the antibiotic and that its modification leads to a fall in its action on a number of microorganisms.

Antibiotic A-128-OP is a tripeptide-cyclooctapeptidolactone [2] in positions 9 and 10 of the cyclopeptide part of which there are residues of dehydrotryptophan and β-methyltryptophan – two amino acids of "nonprotein" nature.

The value of tryptophan residues in the appearance of the biological properties of hormones and enzymes is generally known [3-7]. It is quite likely that in the antibiotic A-128-OP the β-methyltryptophan and dehydrotryptophan residues may play a definite role in the manifestation of antibiotic activity by it.

The present paper gives the results of an investigation of the role of the β-methyltryptophan and dehydrotryptophan residues in the antibiotic A-128-OP by the method of specific chemical modification. For the chemical modification of the antibiotic we selected three reagents highly specific with respect to tryptophan: anhydrous formic acid saturated with gaseous HCl [8], 2-nitrophenylsulfenyl chloride (NPS chloride) [9], and 2-hydroxy-5-nitrobenzyl bromide (HNB bromide) [10]. The first two are capable of replacing the hydrogen atoms in positions 1 and 2, respectively, of the indole rings of the tryptophan residues; HNB bromide leads to substitution in positions 3 of the indole rings:



The corresponding derivatives of the antibiotic were obtained and isolated by the methods described in the literature for each of the reagents with some modifications. These three reagents are interesting by the fact that when they enter the molecule they change the UV spectrum of the initial antibiotic and thereby enable the completeness of the substitution reaction to be followed. It was established by this method that both tryptophan residues in the antibiotic undergo modification.

The results of a determination of antibacterial activity have shown that the HNB and NPS derivatives of the antibiotic A-128-OP are considerably less (15- to 20-fold) active than the initial antibiotic. When

M. V. Lomonosov State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 636-639, September-October, 1974. Original article submitted March 14, 1973.

TABLE 1. Physicochemical Properties and Antibacterial Activities of Derivatives of the Antibiotic A-128-OP at the β-Methyltryptophan and Dehydrotryptophan Residues

Derivatives of the antibiotic	mp, °C	$[\alpha]^{20}$, deg. (c 0.5; DMFA)	R_f in systems*		Yield, %	Antibacterial activity†		
			1	2		St. aureus 209	Bac. megater	Bac. subtilis 7241
1-Formyl-A-128-OP	248–250 (C_2H_5OH)	−76,0	0,42	0,55	88	0,20	0,25	0,20
NPS-A-128-OP	218–220 (C_2H_5OH–ether)	−50,2	0,45	0,60	76	0,17	0,02	0,04
HNB-A-128-OP	260–265° (C_2H_5OH–ether)	−36,4	0,46	0,50	69	0,08	0,02	0,02

* Chromatography in a thin layer of silica gel in the systems: 1) butan-1-ol–CH_3COOH–H_2O (4 : 1 : 1); and 2) butan-1-ol–pyridine–CH_3COOH–H_2O (30 : 20 : 6 : 24).

† Antibacterial activity in relative units in comparison with the activity of the antibiotic A-128-OP arbitrarily taken as 1 for each type of bacterium.

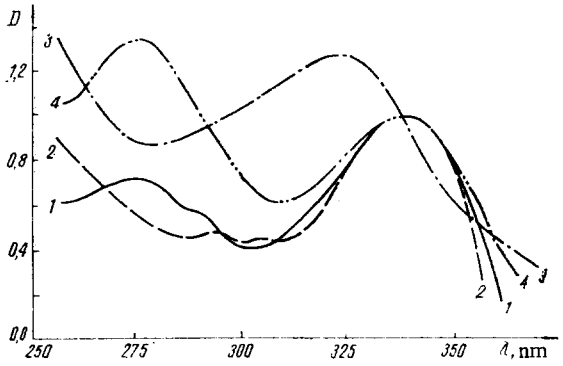


Fig. 1. UV spectra of the antibiotic A-128-OP (1), 1-formyl-A-128-OP (2), HNB-A-128-OP (3), and NPS-A-128-OP (4).

the hydrogen atom in position 1 of the indole ring of tryptophan was substituted (the product of the reaction of the antibiotic with HCOOH/HCl) its activity fell 4- to 5-fold.

It may be assumed that the β-methyltryptophan and dehydrotryptophan residues in the molecule of the antibiotic create a specific "hydrophobic nucleus" which is responsible for the sorption of the antibiotic in the surface of the bacterial cell through a hydrophobic interaction with the elements of the cell coating or the cytoplasmatic membrane. The introduction into the molecule of the large HNB and NPS substituents evidently interferes with the specific conformation of the antibiotic molecule, which leads to the inactivation of the substance. The small formyl radical apparently does not interfere with the overall conformation of the molecule and therefore the level of activity of the antibiotic falls only slightly. In our view, the decrease in the activity of the formyl derivative of the antibiotic shows a definite value for the functioning of this preparation of the hydrogen atoms in position 1 of the indole rings of the β-methyltryptophan and dehydrotryptophan residues.

For a stricter interpretation of the results obtained, an additional study of the conformational features of antibiotic A-128-OP and its derivatives is necessary. We shall consider this question subsequently.

EXPERIMENTAL

Antibiotic A-128-OP was isolated by a procedure described previously [11]. Paper electrophoresis was performed in instruments working on the principle of Durrum's humid chamber [12]. Electrophoresis was performed on Filtrak FN-4 chromatographic paper. Chromatography was performed in a thin layer of KSK silica gel on glass plates (13 × 18 cm). The amino acid compositions of the hydrolyzates of the substances under investigation were determined on a Hitachi type KLA-3B amino acid analyzer.

The UV spectrum were taken on a Unicam SP-800 automatic spectrophotometer (England) in the wavelength range from 230 to 450 nm using a cell 1 cm thick. The concentration of the substances under investigation was $4 \cdot 10^{-5}$ M, and the solvent was MeOH–H_2O (2 : 1). The analyses of the compounds agreed with the calculated figures.

Di(1-formyltryptophyl)-A-128-OP (1-Formyl-A-128-OP), $C_{61}H_{77}O_{21}N_{13} \cdot HCl \cdot 2H_2O$, was obtained by a method similar to that of Previero et al. [8]. The completeness of the reaction was checked spectropho-

tometrically from the disappearance of the absorption band at 280 nm and the appearance of a new band at 298 nm characteristic for the 1-formyl derivative of tryptophan.

Di(2-nitrophenylsulfenyl)-A-128-OP (NPS-A-128-OP), $C_{71}H_{83}O_{23}N_{15}S_2 \cdot 3CH_3COOH \cdot 3H_2O$, was obtained [9] with a tenfold excess of 2-nitrophenylsulfenyl chloride. The NPS-A-128-OP was isolated by gel filtration on Sephadex G-15 with 0.2 N CH_3COOH as eluent.

The results of a determination of the number of NPS residues in the molecule of the modified antibiotic performed by the method of Scoffone et al. [9] showed that both tryptophan residues had undergone modification.

Di(2-hydroxy-5-nitrobenzyl)-A-128-OP (HNB-A-128-OP) was obtained by the method of Koshland et al. [10] with a tenfold excess of 2-hydroxy-5-nitrobenzyl bromide. The HNB-A-128-OP was purified on a column of Sephadex G-15, with elution by a 0.2 N solution of ammonia. The quantitative amino acid composition of the HNB-A-128-OP (in residues) was: trans-3-hydroxyproline (1), aspartic acid (1), threonine (2), serine (1), glycine (1), alanine (1), β-hydroxyleucine (1). The number of modified residues was found by the method of Horton and Koshland [13]. The results of the calculation showed that the HNB-A-128-OP molecule contained two HNB residues.

The physicochemical and antibacterial properties of the derivatives of the antibiotic A-128-OP can be judged from the figures given in Table 1.

The authors thank A. N. Polin and Z. M. Petrykina for determining the activity of the antibiotic and its derivatives, and also workers of the Chromatography Division of the Laboratory of Bioorganic Chemistry for performing the analyses on the amino acid analyzer.

SUMMARY

1. Derivatives of the polypeptide antibiotic A-128-OP at positions 1, 2, and 3 of the indole rings of the β-methyltryptophan and dehydrotryptophan residues have been obtained for the first time, and their physicochemical properties have been studied.

2. The introduction into the antibiotic molecule of a 2-hydroxy-5-nitrobenzyl or a 2-nitrophenylsulfenyl substituent lower its antibacterial activity 15- to 20-fold. The replacement of the hydrogen atoms in positions 1 of the indole rings of the β-methyltryptophan and dehydrotryptophan residues leads to a 4- to 5-fold decrease in the activity of antibiotic A-128-OP.

LITERATURE CITED

1. I. G. Smirnova, G. S. Katrukha, and A. B. Silaev, Khim. Prirodn. Soedin., 632 (1974).
2. A. B. Silaev, G. S. Katrukha, Zh. P. Trifonova, R. I. Li, and T. M. Melent'eva, Khim. Prirodn. Soedin., 130 (1971).
3. T. F. Spande and B. Witkop, Methods in Enzymology, 11, 506 (1967).
4. J. Brovetto-Cruz and Choh Hao Li, Biochem., 8, 4695 (1969).
5. Chao Pen Li and E. R. Einstein, J. Biol. Chem., 245, 6397 (1970).
6. A. Holmgren, Eur. J. Biochem., 26, 528 (1972).
7. S. Rambhav and L. K. Ramachandran, Indian J. Biochem. and Biophys., 9, 21 (1972).
8. A. Previero, M. A. Coletti-Previero, and J. C. Cavadore, Biochem. Biophys. Acta, 147 (1967).
9. E. Scoffone, A. Fontana, and R. Rocci, Biochem. Biophys. Res. Commun., 25, 170 (1966).
10. D. E. Koshland, Jr., Y. D. Karlhanis, and H. G. Lathman, J. Amer. Chem. Soc., 86, 1448 (1964).
11. A. B. Silaev, G. G. Katrukha, Zh. P. Trifonova, and I. G. Sinyavskaya, Antibiotiki, No. 1, 13 (1968).
12. E. L. Durrum, J. Colloid Sci., 6, 274 (1951).
13. H. R. Horton and D. E. Koshland, Jr., Methods in Enzymology, 11, 556 (1967).