CHEMICAL MODIFICATION OF THE AMINE GROUP OF ANTIBIOTIC A-128-OP

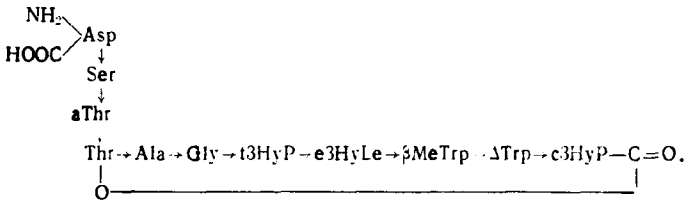
I. G. Smirnova, G. S. Katrukha, and A. B. Silaev

UDC 547.964

The study of the interconnection between the chemical structure and biological properties of antibiotics, and also the investigation of the mechanism of their action at the molecular level, is one of the main directions of the modern chemistry and biochemistry of biologically active substances. A fruitful approach to the solution of these problems is the chemical modification of the functional groups of the molecule of an antibiotic followed by a comparison of the biological properties of the derivatives obtained and of the initial material.

Consequently, we found it necessary to study the interrelationship between the chemical structure and the antibacterial activity of the Soviet polypeptide antibiotic A-128-OP by the method of specific chemical modification.

The antibiotic A-128-OP, the structure of which was established in our laboratory [1], is an octacyclopeptidolactone with a tripeptide side chain which begins with aspartic acid, having a free α-amino group:



The present paper gives the results of the chemical modification of the amine group of the antibiotic A-128-OP with various acylating and alkylating agents. As acylating agents we selected acid chlorides and anhydrides differing in size and hydrophobic properties: benzyl chloroformate, benzoyl chloride, and acetic anhydride. In the modification of the amino group of antibiotic A-128-OP we were attracted by the possibility of introducing an additional carboxy group into its molecule. This would have enabled us to determine how an enhancement of the acidic properties of its molecule would affect the activity of this material. As an N-acyl derivative of such a type we used the N-succinyl derivative of the antibiotic A-128-OP. It is known [2] that under certain conditions succinic anhydride can react not only with amine but also with other functional groups and, in particular, with the OH groups of hydroxy amino acids. In view of this, in the modification of the amino group of antibiotic A-128-OP, which contains several hydroxyamino acid residues, those conditions were selected under which the possibility of O-succinylation was completely excluded.

Another method of changing the basicity of molecules of polypeptides and proteins is the carboxymethylation of the free amino groups with bromoacetic or iodoacetic acid [3]. The results of the analysis of an acid hydrolyzate of the CM* derivative of the antibiotic A-128-OP performed on an amino acid ana-

*Here and below the following abbreviations are used: CM – carboxymethyl; DNS – 1-dimethylaminonaphthalene-5-sulfonyl derivative; DNSP – 3,5-dinitrosulfophenyl derivative; and Cbz – benzyloxycarbonyl derivative.

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 632-636, September-October, 1974. Original article submitted March 14, 1973.

TABLE 1. Physicochemical and Antibacterial Properties of Some Derivatives of the Antibiotic A-128-OP at Its Free α-Amino Group

Derivatives of the antibiotic	mp, °C	$[\alpha]^{20}$, deg. (c 0.5; DMFA)	R_f in systems*		Electrophoretic mobility,† −u	Yield, %	Antibacterial activity‡		
			1	2			St. aureus 209	Bac. megaterium	Bac. subtilis 7241
N-Acetyl-A-128-OP	247—248 (CH_3OH—ether)	−81,0	0,43	0,55	−0,21	55	0,25	0,13	0,17
N-Benzoyl-A-128-OP	250—252 (C_2H_5OH)	83,0	0,67	0,76	0	72	0,50	0,50	0,33
N-Cbz-A-128-OP	260—261 (C_2H_5OH)	−82,5	0,67	0,76	0	78	0,50	0,50	0,33
N-Succinyl-A-128-OP	270—273 (CH_3OH—ether)	−71,0	0,48	0,52	−0,51	53	0,09	0,04	0,08
N-Mono-CM-A-128-OP	230—233 (CH_2Cl_2—ether)	−78,0	0,46	0,55	−0,49	54	0,01	0,09	0,06
N-DNS-A-128-OP	330—332	−86,0	0,49	0,64	0	71	0,50	—	0,20
N-DNSP-A-128-OP	340—342 (C_2H_5OH—ether)	−73,0	0,53	0,55	−1,70	42	0,09	0,06	0,09

* Chromatography in a thin layer of silica gel in 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).

† Electrophoretic mobility $\{u = [cm^2/(sec \cdot V)] \cdot 10^{-5}\}$ in the pyridine−CH_3COOH−H_2O (50 : 2 : 448) system, 500 V, pH 6.5, 2-3 h. (−u) means that the substance migrates towards the anode.

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

lyzer and by paper chromatography showed the presence of mono-CM-aspartic acid in it. The formation of a mono-CM derivative of A-128-OP in place of the possible mixture of mono- and di-CM derivatives is probably due to the influence of the propinquity to the α-amine group of the α-carboxy group of aspartic acid, which prevents the introduction of an additional carboxymethyl residue.

In addition to the derivatives of the antibiotic at the amino group described above, we obtained fluorescing N-DNS and strongly yellow-colored N-DNSP derivatives of the antibiotic A-128-OP. These compounds were used as convenient "tools" in the study of the interaction of the antibiotic and its derivatives with the bacterial cell.

The antibacterial activity of the substances obtained was determined in the Faculty of Soil Biology of Moscow State University by A. N. Polin and Z. M. Petrykina.

The results of the biological tests showed (Table 1) that all the derivatives of antibiotic A-128-OP at the α-amino group of the D-aspartic acid possess a lower antibacterial activity than the initial material. Derivatives of the antibiotic in which the NH_2 group was modified by reagents containing an additional carboxy group (N-succinyl-A-128-OP and N-mono-CM-A-128-OP) or a sulfo group (N-DNSP-A-128-OP) had a considerably (5- to 7-fold) lower biological activity than derivatives of the antibiotic with hydrophobic substituents of larger volume – N-DNS-A-128-OP, N-Cbz-A-128-OP, and N-benzoyl-A-128-OP.

Thus, the chemical modification of the amine group of antibiotic A-128-OP with acylating and alkylating agents of different natures leads to a reduction in its antibacterial activity, which shows the important role of the free amino group for the functioning of the antibiotic.

EXPERIMENTAL

Antibiotic A-128-OP was isolated by a procedure described previously [4]. Paper electrophoresis was performed in instruments working on the principle of Durrum's humid chamber [5]. Chromatography was performed in a thin layer of type KSK silica gel on 13 × 18-cm glass plates. The derivatives of the antibiotic were determined with the Cl_2–benzidine reagent [6] and with a 0.005% solution of fluorescein in methanol followed by the examination of the chromatograms in UV light [7]. The analyses of all the compounds corresponded to the calculated figures.

N-Benzyloxycarbonyl-A-128-OP, $C_{67}H_{83}O_{21}N_{13}$, was obtained by a published method [8a] with a 70-fold excess of benzyl chloroformate at pH 9.0 and 0°C.

N-Benzoyl-A-128-OP, $C_{66}H_{81}O_{20}N_{13} \cdot H_2O$, was obtained by a method similar to that given by Greenstein and Winitz [8b] with a 20-fold excess of benzoyl chloride at pH 9.0.

N-Acetyl-A-128-OP was obtained by a modification of a known method [9] using a 50-fold excess of acetic anhydride at –15°C (for the physicochemical constants of N-acetyl-A-128-OP, see Table 1).

Preparation of N-succinyl-A-128-OP, $C_{63}H_{81}O_{22}N_{13} \cdot 4H_2O$. At 0°C, 0.8 mmole of succinic anhydride was added to a solution of 0.1 mmole of the antibiotic A-128-OP in 3 ml of CH_3OH and 3 ml of 3% $NaHCO_3$ solution, and for the next hour, with stirring, the pH was maintained at 8.0 by means of 0.1 N NaOH. After acidification with 1 N HCl to pH 2.0, the precipitate of N-succinyl-A-128-OP was separated off and washed with ether and absolute $CHCl_3$.

N-Carboxymethyl-A-128-OP, $C_{61}H_{79}O_{21}N_{13} \cdot HCl \cdot H_2O$, was obtained by a modification of the method of Korman and Clarke [3], using a 40-fold excess of ICH_2COOH at pH 8.0 followed by the isolation of the N-CM-A-128-OP on a column of Sephadex G-15 using 50% ethanol as the eluent.

N-(1-Dimethylaminonaphthalene-5-sulfonyl)-A-128-OP was obtained as described by Trifonova et al. [10]. The N-DNS derivative of A-128-OP was purified on a column (2 × 5 cm) of Dowex 2 × 8 (Cl^- form) (for the properties of the N-DNS-A-128-OP see Table 1).

N-(3,5-Dinitrosulfophenyl)-A-128-OP, $C_{65}H_{79}O_{26}N_{15}S \cdot 3H_2O$, was obtained by a modification of the method of Katrukha et al. [11] with a 30-fold excess of potassium 4-chloro-3,5-dinitrobenzenesulfonate in 70% aqueous acetone at pH 8.5 and 37°C. The N-DNSP-A-128-OP was purified on a column of Sephadex G-15 using 30% CH_3COOH as eluent.

The physicochemical and antibiotic properties of the derivatives of the antibiotic A-128-OP obtained are given in Table 1.

SUMMARY

1. Seven different derivatives of the polypeptide antibiotic A-128-OP at its α-amino group have been obtained for the first time, and their physicochemical and antibiotic properties have been studied.

2. It has been shown that modification of the amino group lowers the antibacterial activity of the antibiotic. Derivatives with an additional acid grouping have one fifth to one seventh of the biological activity of derivatives of the antibiotic with voluminous hydrophobic substituents.

LITERATURE CITED

1. A. B. Silaev, G. S. Katrukha, Zh. P. Trifonova, R. I. Li, and T. N. Melent'eva, Khim. Prirodn. Soedin., 130 (1971).
2. A. D. Gounnaris and G. E. Perimann, J. Biol. Chem., 242, 2739 (1967).
3. S. Korman and H. T. Clarke, J. Biol. Chem., 221, 113 (1966).
4. A. B. Silaev, G. S. Katrukha, Zh. P. Trifonova, and I. G. Sinyavskaya, Antibiotiki, No. 1, 13 (1968).
5. E. L. Durrum, J. Colloid Sci., 6, 274 (1951).
6. T. Wieland and K. Dose, Angew. Chem., 66, 781 (1954).
7. T. Wieland and L. Bauer, Angew. Chem., 63, 511 (1951).
8. J. Greenstein and M. Winitz, Chemistry of the Amino Acids, Vol. 1, Wiley, New York (1961).
9. H. Fraenkel-Conrat, R. S. Bean, and H. Lineweaver, J. Biol. Chem., 177, 385 (1949).
10. Zh. P. Trifonova, G. S. Katrukha, and A. B. Silaev, Khim. Prirodn. Soedin., 699 (1972).
11. G. S. Katrukha, A. B. Silaev, and S. V. Khartskhaeva, Biokhimiya, 27, 549 (1962).