As one can see from the Table, all the analogs clearly inhibit amino acid incorporation into protein in the cell-free system. However modification of the various parts of the chloramphenicol molecule affects differently the relation between the antibacterial activity of the antibiotic and its inhibition of protein biosynthesis in ribosomes. In type (I) compounds the difference in antibacterial activity observed between chloramphenicol and its analogs is much less strongly expressed with respect to protein synthesis in a cell-free system. Thus, compounds (Ic) and (If) which display only $^1/_4$ and $^1/_{170}$, respectively, of the antibacterial potency of chloramphenicol, show almost no difference from the antibiotic in the ability to inhibit protein synthesis (see Table). The analog (Ib) approaching chloramphenicol in antibacterial properties is somewhat more active in the cell-free system than compounds (Ia), (Ic) and (If). The analogs (Id) and (Ie), possessing antibacterial activity of the same order of magnitude as (Ic), and much inferior to chloramphenicol in this respect, inhibit protein synthesis somewhat less than the former compounds.

In the series of analogs of type (II) all 3 of the compounds studied are less active in the cell-free system than chloramphenicol. Here, too, as in the type (I) analogs, the differences between the effects on protein synthesis in the ribosomes are much less than the differences in the antibacterial activity. However, the type (II) compounds do fall into the same order with respect to both relative antibacterial activity and protein synthesis inhibition.

These findings lead to the conclusion that the acyl residue of chloramphenicol plays a more important role in the penetration of the antibiotic into the bacterial cell, or in the transformation of the antibiotic on its path towards the ribosomes, than in the actual interaction with the protein synthesizing systems. On the contrary, substitution in the aromatic part of the chloramphenicol molecule affects its interaction with these systems. This is indirectly supported by evidence from the recent paper of VASQUEZ[17], who showed that Ar-substituted D-threo-analogs of chloramphenicol, including (IIa) and (IIb) investigated by us, compete with the antibiotic for the site of attachment to the ribosomes. However, the more equalized effects of type (II) analogs on the cell-free system than the differences in their antibiotic activities indicates that the nitrophenyl part of the molecule also plays a certain role in the penetration of the antibiotic into the microbial cell[18].

Выводы. Сравнительное изучение антибактериальной активности аналогов хлорамфеникола и их влияния на биосинтез белка в бесклеточной системе *E. coli* показало, что ацильный радикал этого антибиотика имеет значение для его проникновения в микробную клетку, а арильный радикал - для взаимодействия с белоксинтезирующей системой.

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[17] D. VASQUEZ, Biochim. biophys. Acta *114*, 277 (1966).
[18] D-threo-1-(*p*-nitrophenyl)-2-aminopropane-1,3-diol, lacking antibacterial activity, does not suppress protein biosynthesis in the cell-free system.
[19] Laboratory of Biological Investigations.
[20] Laboratory for Chemistry of Antibiotics.

Mass Spectrometric Determination of the Amino Acid Sequence in Arginine-Containing Peptides

Earlier[1-3] we showed that the mass spectrometric method can be used for direct determination of the amino acid sequence in peptides containing residues of all the ordinary amino acids except arginine, which causes complications due to the specific behaviour of its guanidine grouping under the mass spectrometric conditions. Because arginine is a component part of many naturally occurring polypeptides and proteins, in order for the method to assume a universal character, its extension to include also the sequential analysis of arginine-containing peptides was highly desirable.

Attempts to overcome the unfavourable effects of the guanidine grouping on the fragmentation of arginine-containing peptides by its diacylation did not lead to positive results, but two other possibilities proved to be quite promising. The first involves conversion of the arginine residue into an ornithine residue in the peptides, since the esters of N^{α}, N^{δ}-diacylated ornithine-containing peptides undergo the normal amino acid type of mass spectrometric fragmentation[1]. For this purpose the peptides could not be incubated with arginase or subjected to alkaline hydrolysis, because this causes not only conversion of the guanidine residue but also intensive cleavage of the amide bonds. On the contrary, refluxing of the arginine-containing peptides with 20% aqueous hydrazine for $^1/_2$–1 h leads to their practically complete transformation into the corresponding ornithine-containing peptides. Although here also one may sometimes encounter partial cleavage of the peptide bonds, the degree of cleavage is usually not very great and can be controlled chromatographically. This is illustrated in Figure 1, lower, by the mass spectrum of N^{α}, N^{δ}-didecanoylornithylleucine methyl ester (1) prepared from arginylleucine by treatment with hydrazine under the above

[1] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, E. I. VINOGRADOVA, A. I. MIROSHNIKOV, YU. B. ALAKHOV, V. M. LIPKIN, YU. B. SHVETSOV, N. S. WULFSON, B. V. ROSINOV, V. N. BOCHKAREV and V. M. BURIKOV, Nature *211*, 361 (1966).
[2] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV and A. A. KIRYUSHKIN, Report to the 8th European Peptide Symposium, Noordwijk, Holland (1966).
[3] A. A. KIRYUSHKIN, A. I. MIROSHNIKOV, YU. A. OVCHINNIKOV, B. V. ROSINOV and M. M. SHEMYAKIN, Biochem. biophys. Res. Commun. *24*, 943 (1966).

mentioned conditions followed by acylation and esterification of the resultant ornithine-containing peptide according to a procedure described earlier[4]. It can be clearly seen that the mass spectrum of this compound, not subjected to purification, is practically identical to that of an analytically pure sample (see Figure 1, upper).

The second method of transforming arginine-containing peptides into compounds suitable for mass spectrometry consists of blocking the guanidine grouping by its cyclization with β-dicarbonylic compounds. If such peptides are first acylated at the N^{α}-group and then condensed with 1, 1, 3, 3-tetraalkoxypropane in the presence of acid catalysts, the arginine residue is quantitatively transformed into a pyrimidylornithine (Pyr-Orn) residue. Analogously, acetylacetone can be used to form a dimethylpyrimidylornithine (Me_2Pyr-Orn) residue. The first of these reactions is preferable.

[4] A. A. Kiryushkin, Yu. A. Ovchinnikov, M. M. Shemyakin, V. N. Bochkarev, B. V. Rosinov and N. S. Wulfson, Tetrahedron Lett. *1966*, 33.

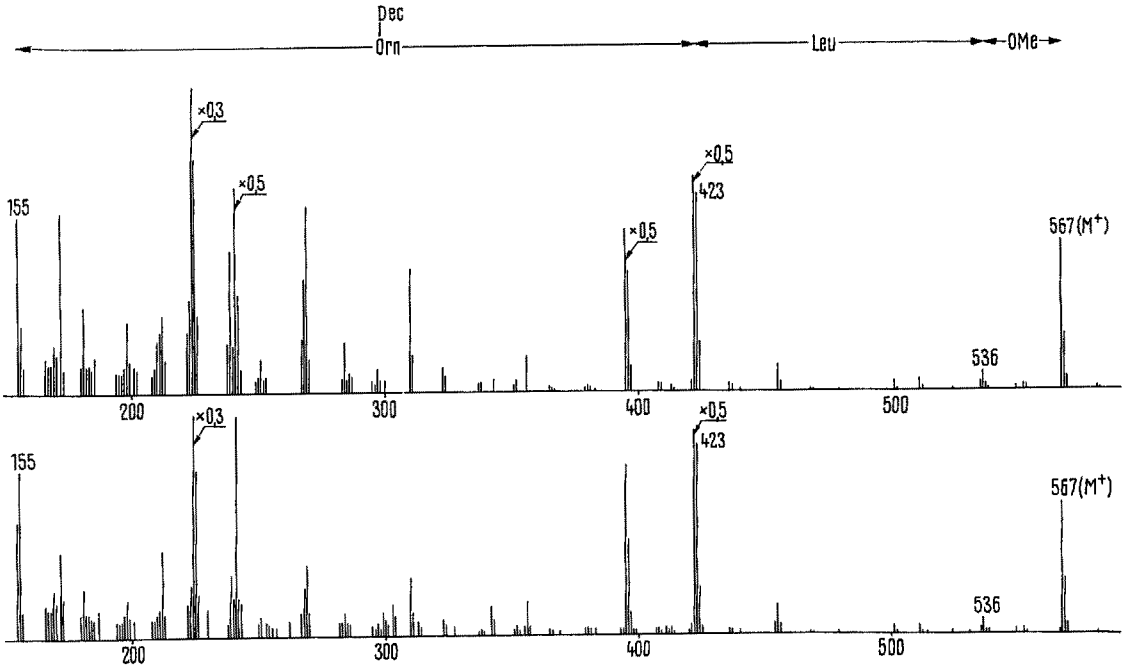

Fig. 1. Upper: Mass spectrum of pure compound (1). Lower: Mass spectrum of raw sample of compound (1) prepared from arginyl-leucine.

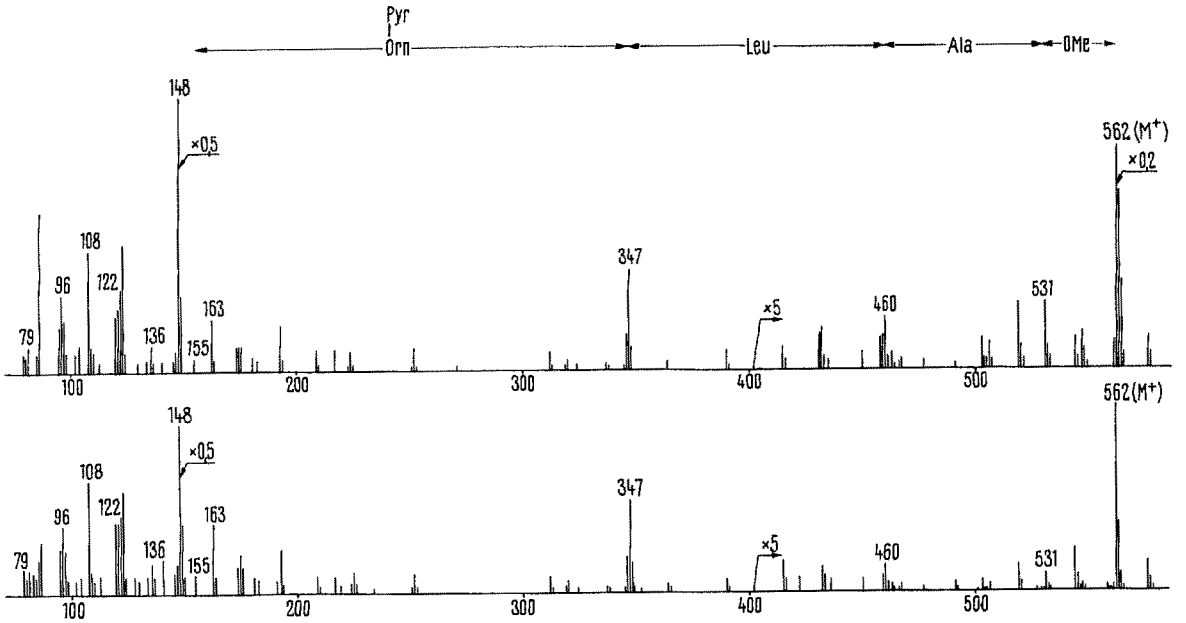

Fig. 2. Upper: Mass spectrum of pure compound (4). Lower: Mass spectrum of raw sample of compound (4).

In order to investigate the mass spectrometric behaviour of peptides containing pyrimidylornithine residue, we synthesized the following compounds:

N^{α}-Dec-N^{δ}-Pyr-Orn-Leu-Ala-OMe (2)

N^{α}-Dec-N^{δ}-Pyr-Orn-Leu-Gly-Leu-Ala-OMe (3)

N^{α}-(Dec-Phe)-N^{δ}-Pyr-Orn-Leu-OMe (4)

N^{α}-Dec-N^{δ}-Me_2Pyr-Orn-Leu-Ala-OMe (5)

N^{α}-(Dec-Phe)-N^{δ}-Me_2Pyr-Orn-Leu-OMe (6)

N^{α}-(Dec-Phe-Phe)-N^{δ}-Me_2Pyr-Orn-OMe (7)

Compounds (2)–(7) were prepared by acylating the free peptides with the N-hydroxysuccinimide ester of decanoic acid in aqueous bicarbonate solution and further condensation of the resultant N^{α}-decanoylpeptides with tetraethoxypropane (acetylacetone) in methanol saturated with dry HCl (10–15 h, 20°). Under such conditions both heterocyclization of the guanidine residue and esterfication of the carboxyl group of the peptide take place. In case the condensation cannot be carried out under such conditions owing to the instability of the arginine-containing peptide in acid medium, it can be achieved with the aid of tetraethoxypropane in the presence of triethylamine, the resultant N^{δ}-pyrimidylornithine containing N^{α}-acylpeptide then being esterified by a suitable reactant (for instance, diazomethane).

In the mass spectra of the N^{δ}-pyrimidylornithine-containing peptides (2)–(7), the amino acid type of fragmentation is usually predominant (see Figure 2, upper). The fact that herein the molecular peak is often among the most prominent bears evidence of the high thermal stability of these peptide derivatives. All the earlier described specificities introduced into the mass spectra by the other amino acid residues are retained in the N^{δ}-pyrimidylornithine-containing peptides. A characteristic feature of all the mass spectra investigated is the presence of a group of peaks which correspond to the fragments formed in the stepwise breakdown of the N^{δ}-pyrimidylornithine residue (m/e 79, 96, 108, 122, 136, 148, 163). Because of the presence in the N^{δ}-pyrimidylornithine residue of a secondary amino group which under the mass spectrometric conditions can undergo intermolecular methylation, the molecular peaks are ordinarily accompanied by +14 m.u. satellites, similar to the behaviour of histidine- and tryptophane-containing peptides[1–3]. All such specificities in the mass spectrometric behaviour of N^{δ}-pyrimidylornithine-containing peptides are manifested also in the respective derivatives of N^{δ}-dimethylpyrimidylornithine.

From the viewpoint of application, it is of particular importance that the mass spectra of the methyl esters of N^{α}-decanoylpeptides containing N^{δ}-pyrimidylornithine residues prepared from microquantities of free arginine peptides without purification at any stage of the transformation give mass spectra that in no way differ from those of the analytically pure samples (Figure 2).

Выводы. Показано, что аргинин-содержащие пептиды перед их масс-спектрометрированием следует превращать в соответствующие орнитиновые или пиримидилорнитиновые пептиды путем гидразинолиза или гетероциклизации с β-дикарбонильными соединениями.

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Étude biochimique des poissons: 1. Variations semestrielles de la teneur en eau et en protéines du tissu musculaire de la truite Arc-en-ciel (*Salmo gairdnerii* Rich)

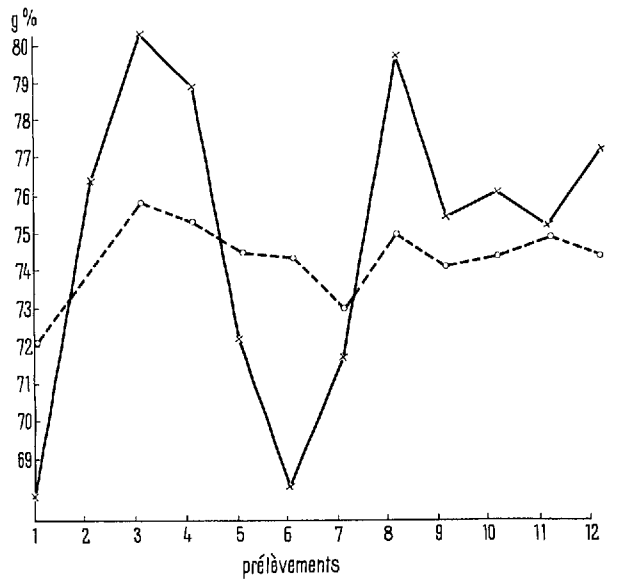
Les conditions écologiques générales ainsi que le phénomène de sexualité jouent un rôle considérable dans la biologie des vertébrés inférieurs. De nombreux auteurs ont signalé l'influence de ces facteurs chez la truite. Ces divers paramètres provoquent des modifications de la composition des tissus de l'animal et en particulier de la masse musculaire.

Nous présentons dans cette note les variations de la teneur en eau et en protéines du muscle dorsal de la truite au cours de la période comprise entre les mois de septembre et de mars, c'est-à-dire, pendant que s'accomplissent les phénomènes de sexualité active.

Nous avons utilisé des truites adultes appartenant à la même souche de reproducteurs et élevées dans des conditions absolument identiques.

La teneur en eau a été évaluée par passage à l'étuve à 50 °C jusqu'à poids constant. Les protéines totales ont été dosées par la technique de Parnas et Wagner modifiée par DUMAZERT[1].

La courbe reproduit les variations enregistrées pour les 6 mois considérés (un prélèvement tous les 15 jours).



Variations de la teneur en eau et en protéines totales du muscle dorsal de la truite Arc-en-ciel (*Salmo gairdnerii* Rich.). x——x protéines totales, o——o eau.