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ABSOLUTE CONFIGURATION OF THE AMINO ACIDS OF ACTINOIDIN

N. N. Lomakina, V. A. Zenkova, and M. S. Yurina

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As reported previously [1], from the hydrolyzate of the antibiotic actinoidin have been isolated phenylalanine and two derivatives of α-aminophenylacetic acid: p-hydroxyphenylglycine and 3-chloro-4-hydroxyphenylglycine. The structure of the phenylglycine amino acids has been shown by independent synthesis [2]. These amino acids have been obtained for the first time from natural sources, although closely similar amino acids have been found in antibiotics and higher plants: L-phenylglycine [3-5], L-phenylsarcosine [6], D-(m-carboxyphenyl)glycine [7, 8], and D-(3-carboxy-4-hydroxyphenyl)glycine [9].

Table 1

Amino acid	In water		In acid	
	$[\alpha]_D^{20}$, deg	c, %	$[\alpha]_D^{20}$, deg	c, %
Phenylalanine	—35	0.3	—	—
3-Chloro-4-hydroxyphenylglycine	—55.4	1.6 (0.2N NH_4OH)	—75	1.4, 6 N HCl
p-Hydroxyphenylglycine (sample 1)	—29.6	1.85	—	—
p-Hydroxyphenylglycine (sample 2)	—43.5	0.15	—48.1	0.13, 1 N HCl

In the present paper we give the results of a determination of the optical configuration of the p-hydroxyphenylglycine and the 3-chloro-4-hydroxyphenylglycine obtained from actinoidin. The specific rotations of the amino acids of actinoidin are given in Table 1.

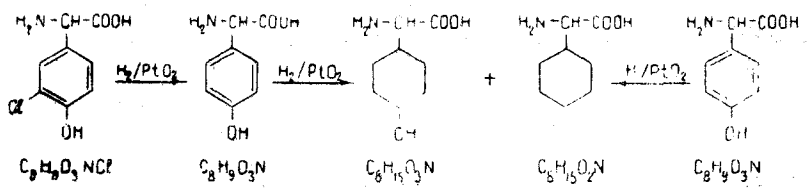
It can be seen from the table that the phenylalanine isolated from actinoidin coincides in the magnitude and sign of the specific rotation with the L-isomer of this amino acid. Phenylglycine amino acids are levorotatory, which is characteristic for phenylglycine derivatives belonging to the D-series [9, 10]. In connection with the absolute magnitude of the specific rotation, it must be borne in mind that phenylglycine and its derivatives readily racemize [11]. As is known from the literature [3-5, 9, 10], when such amino acids were isolated from natural sources the absolute specific rotation was less than for the synthetic isomers. The amino acids of actinoidin could also be partially racemized during their isolation.

Table 2

Substance	$[\alpha]_D^{20}$, deg	c, %
Cyclohexylglycine from 3-chloro-4-hydroxyphenylglycine	—52.0	0.10
Cyclohexylglycine from p-hydroxyphenylglycine	—17.7	0.42
L-cyclohexylglycine [11]	+65.5	—

When the specific rotations of the p-hydroxyphenylglycine and the 3-chloro-4-hydroxyphenylglycine were measured in an acid medium, a negative shift was found in comparison with the magnitude measured in aqueous solution. According to the Clough-Lutz-Jirgensons rule, such a change is characteristic for the D-isomers of the amino acids. However, for p-hydroxyphenylglycine the change in rotation is small.

In view of the unusual structure of the amino acids under consideration, we considered it necessary to confirm their absolute configuration by a direct determination. For this purpose, the amino acids were subjected to catalytic hydrogenation over platinum oxide in aqueous alcoholic solution at room temperature. The aromatic nucleus was hydrogenated and the chlorine was split off [12]. The reduction of the tyrosine by this method was, in addition, accompanied by the partial hydrogenolysis of the phenolic hydroxyl with the formation of cyclohexylalanine [13]. When p-hydroxyphenylglycine and 3-chloro-4-hydroxyphenylglycine were hydrogenated, cyclohexylglycine was found among the reaction products:



The yield of cyclohexylglycine from 3-chloro-4-hydroxyphenylglycine was smaller than from p-hydroxyphenylglycine because of the relative difficulty of the hydrogenolysis of the C—Cl bond.

The mixture of hydrogenation products was separated by ion-exchange chromatography on Dowex 50 × 4 cation-exchange resin in the pyridinium form. The cyclohexylglycine isolated in this way was compared in its chromatographic behavior and its IR spectrum with synthetic cyclohexylglycine. On paper chromatography in various solvent systems, the substances had identical R_f values and mixed samples were not separated. The specific rotations of the cyclohexylglycine preparations (in glacial acetic acid) are given in Table 2.

From a comparison of the figures obtained with those given in the literature, it can be seen that the cyclohexylglycine obtained by the hydrogenation of p-hydroxyphenylglycine and of 3-chloro-4-hydroxyphenylglycine has rotation of the opposite sign to the synthetic L-isomer of the amino acid mentioned. The difference in the absolute magnitudes of the specific rotations of the samples of cyclohexylglycine shows the partial racemization of the amino acids of actinoidin during their isolation. Since on hydrogenation the conversion of the amino acids into cyclohexylglycine takes place without affecting the asymmetric center, it is obvious that p-hydroxyphenylglycine and 3-chloro-4-hydroxyphenylglycine possess the D-configuration. This conclusion concerning the configuration of p-hydroxyphenylglycine agrees with the results of Yu. M. Torchinskii [14], who has established the pyridoxylidene derivative of this amino acid possesses positive circular dichroism. According to the rule established by this author, p-hydroxyphenylglycine belongs to the D-series.

It must be noted that an intermediate in the hydrogenation of 3-chloro-4-hydroxyphenylglycine is p-hydroxyphenylglycine. It was isolated in small amounts from the mixture after hydrogenation and proved to be levorotatory, like the natural D-isomer.

Thus, actinoidin, like many antibiotics of a peptide nature, contains D-amino acids. Their presence may be connected with the appearance of the antibacterial activity of the antibiotics.

Experimental

Hydrogenation of p-hydroxyphenylglycine. A hydrogenation vessel was charged with 50 mg of Adams catalyst and 5 ml of ethanol. The catalyst was activated in an atmosphere of hydrogen while the suspension was stirred with a magnetic stirrer. Then a solution of 51.5 mg of p-hydroxyphenylglycine in 10 ml of water was added, and it was hydrogenated at room temperature for 3 days. After this time, the absorption of hydrogen had practically ceased. The solution was separated from the catalyst and evaporated to dryness in vacuum and the residue was used for the chromatographic isolation of the cyclohexylglycine.

The hydrogenation of 3-chloro-4-hydroxyphenylglycine was carried out similarly.

Isolation of cyclohexylglycine. To separate the hydrogenation products, we used Dowex 50 × 4, 200–400 mesh, resin in the pyridinium form. The solvent was a 0.1 N acetic acid-pyridine buffer solution with pH 4.7 (8 ml of pyridine and 10 ml of glacial acetic acid in 1 *l* of aqueous solution). The resin was charged into a column with a diameter of 1.2 cm; the height of the layer of resin was 150 cm. The mixture of products of the hydrogenation of p-hydroxyphenylglycine obtained from 112 mg of the amino acid, in 2 ml of solvent, was added at the top of the column. The buffer solution was passed into the column at the rate of 6–7 ml/hr. Fractions with a volume of about 3 ml were collected by means of a fraction-collector and were analyzed by paper chromatography. Fractions 6–10 contained the products of the incomplete reduction of p-hydroxyphenylglycine, fractions 39–42 contained cyclohexylglycine, and fractions 57–60 contained p-hydroxyphenylglycine. The fractions containing the cyclohexylglycine were combined and concentrated in vacuum. The cyclohexylglycine deposited in the form of a white crystalline precipitate. This was separated off, washed with water, and dried in a vacuum desiccator, forming 43 mg of chromatographically homogeneous cyclohexylglycine, $[\alpha]_D^{20}$ −17.7° (c 0.4; glacial CH_3COOH). The IR spectrum coincided with that of a synthetic sample.

The mixture obtained from the hydrogenation of 3-chloro-4-hydroxyphenylglycine was separated in precisely the same manner. Cyclohexylglycine, p-hydroxyphenylglycine, and the initial amino acid were isolated.

The paper chromatography was carried out by the descending method on type B paper of the Leningrad no. 2 mill. The following systems of solvents were used: 1-butanol—acetic acid—water (2 : 1 : 1); 1-butanol—pyridine—acetic acid—water (15 : 10 : 3 : 12); n-amyl alcohol—pyridine—water (7 : 7 : 6); and water-saturated phenol. The chromatograms were revealed with a 0.5% solution of ninhydrin in acetone.

Conclusions

It has been established that the p-hydroxyphenylglycine and 3-chloro-4-hydroxyphenylglycine contained in actinoidin possess the D-configuration.

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Institute for the Search for New Antibiotics, AMS USSR