mA, deposition standard: the bis-S-sulfonate of the B-chain of human insulin). Amino acid analysis: Asp 3.96 (4), Thr 1.60 (2), Ser 2.62 (3), Glu 7.00 (7), Pro 1.02 (1), Gly 3.84 (4), Ala 1.04 (1), Cys 5.30 (6), Val 3.85 (4), Ile 1.75 (2), Leu 6.10 (6), Tyr 3.54 (4), Phe 2.83 (3), Lys 1.02 (1), His 2.10 (2), Arg 1.02 (1). The results of a determination of C-terminal amino acids: Asn 1.98 (2).

When tested for its convulsive action on mice [3], the biological activity of compound (I) amounted to 85% (in comparison with the activity of an international standard).

LITERATURE CITED

1. Yu. P. Shvachkin, E. N. Voluiskaya, S. P. Krasnoshchekova, and A. M. Nikitina, Khim. Prir. Soedin., 792 (1983).
2. J. M. Stewart and J. D. Young, Solid-Phase Peptide Synthesis, W. H. Freeman, San Francisco (1969).
3. K. L. Smith, Methods Horm. Res., 2, 439 (1962).

PREPARATION AND PROPERTIES OF THE METHYL ESTER OF WILLARDINE^{B30}-(HUMAN INSULIN)

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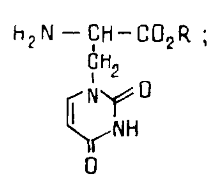
To study the molecular mechanisms of the interaction of human insulin with the receptors, it is necessary to have available structural analogs of this hormone that retain a high biological activity and are readily detectable in complex mixtures by methods of nondestructive analysis. One such method is spectrometry in the near ultraviolet region. However, human insulin and related animal insulins possess extremely limited UV absorption because of the presence in the A and B chains of insulin of residues of the aromatic protein amino acids tyrosine and phenylalanine, which are relatively weak chromophores.

In order to obtain a structural analog of human insulin which is biologically active and possesses intense UV absorption we have investigated the possibility of introducing into the insulin molecule a residue of the natural nonproteinogenic nucleo amino acid willardine [L-β-(uracil-1-yl)-α-alanine (I, R = H)] which is characterized by strong UV absorption due to the presence of the nucleic base uracil in the molecule of this amino acid [1, 2].

After a series of preliminary experiments we succeeded in obtaining a previously unknown structural analog of human insulin satisfying the above requirements and differing from the natural hormone by the replacement of the L-threonine residue in the B30 position by the residue of the methyl ester of L-β-(uracil-1-yl)-α-alanine (Ual–OMe).

The methyl ester of willardine^{B30}-insulin (II, R – de-Thr^{B30}-(human insulin)) was prepared by the trypsin-catalyzed transformation of porcine insulin (III, R – de-Ala^{B30}-(porcine insulin)), which took place when the latter was treated with the methyl ester of L-β-(uracil-1-yl)-α-alanine (I, R = CH_3) [3] in an aqueous organic medium (water–dimethylformamide) at 24°C and pH 6.3. Under these conditions, the tryptic transformation reaction [4] proceeded at the Lys^{B29} residue, and the undesirable side reaction at Arg^{B22} residue did not occur.

I. 

II. R – Ual – OMe

III. R – Ala – OH.

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After purification with the aid of ion-exchange chromatography on DEAE-Sephadex A-25 and gel filtration on Sephadex G-25 (f), the methyl ester of willardine$^{B_{30}}$-(human insulin) was obtained in the analytically pure form.

Methyl Ester of Willardine$^{B_{30}}$-(human insulin) (II). R_f 0.51 (C_5H_5N—C_4H_9OH—CH_3CO_2H—H_2O, (10:15:3:12)), 0.92 (iso-C_3H_7OH—25% NH_4OH, (7:4:6)), 0.56 (iso-C_3H_7OH—25% NH_4OH—H_2O, (7:1:2)), 0.95 (C_5H_5N—CH_3COCH_3—H_2O, (1:1:2)) (TLC on Silufol UV-254 plates, spots revealed with the Pauly reagent [5] and from their UV absorption [3]). Electrophoretic mobility: 1.5 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7 mA, deposition standard: the bis-S-sulfonate of the B chain of human insulin). UV absorption, λ_{max} 267-269 nm, log ε, 4.04 (1% CH_3CO_2H). Amino acid analysis: Asp 3.10 (3), Thr 1.67 (2), Ser 2.70 (3), Glu 7.00 (7), Pro 0.97 (1), Gly 3.86 (4), Ala 1.07 (1), Cys 5.10 (6), Val 3.66 (4), Ile 1.79 (2), Leu 6.04 (6), Tyr 3.28 (4), Phe 2.75 (3), Ual 1.10 (1), His 2.02 (2), Lys 1.86 (1), Arg 1.07 (1). Results of a determination of C-terminal amino acids: Asn 0.98 (1), Ual 0.99 (1).

On testing for its convulsive effect on mice [6], the biological activity of compound (II) was 95% (in comparison with the activity of an international standard).

The preparation of a new active analog of human insulin possessing intense UV absorption expands the possibilities of the use of UV spectrophotometry in investigations of the molecular mechanisms of the action of human insulin and related animal insulins.

LITERATURE CITED

1. Yu. P. Shvachkin, Zh. Obshch. Khim., 49, 1157 (1979).
2. Yu. P. Shvachkin, G. P. Mishin, G. A. Korshunova, Usp. Khim., 51, 311 (1982).
3. V. V. Romanov, N. A. Voskova, G. A. Korshunova, and Yu. P. Shvachkin, Bioorg. Khim., 5, 536 (1979).
4. Yu. P. Shvachkin, E. N. Voluiskaya, S. P. Krasnoshchekova, and A. M. Nikitina, Khim. Prir. Soedin., 792 (1983).
5. J. M. Stewart and J. D. Young, Solid-Phase Peptide Synthesis, W. H. Freeman, San Francisco (1969).
6. K. L. Smith, Methods Horm. Res., 2, 439 (1962).

SCHEME FOR THE SYNTHESIS OF LULIBERIN AND ANALOGS

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In connection with the study of biological features of luliberin [1], a new scheme for the synthesis of this releasing hormone has been developed. The scheme is based on the principle of minimal protection, the use of which considerably decreases the number of stages. In addition, it permits the avoidance of the final deblocking, which is frequently accompanied by side reactions [2]. (Graph, top, following page.)

SCHEME FOR THE SYNTHESIS OF LULIBERIN AND ANALOGS

Luliberin and its analogs with D-phenylalanine in the sixth position has been synthesized by this scheme.

The condensation of benzyloxycarbonylserine with the methyl ester of tyrosine and the 3 + 7 condensation were carried out with the aid of complex F [3]. Hydrogen bromide in acetic acid was used to deblock the methyl ester of benzyloxycarbonylglycylleucine and the amide of benzyloxycarbonylarginylprolylglycine. The heptapeptide 4-10 was deblocked by catalytic hydrogenolysis. The pentafluorophenyl ester of di-tert-butoxycarbonylhistidine was condensed with the tristrimethylsilyl derivative of tryptophan. Silylation was performed with trimethylchlorosilane in the presence of triethylamine in dimethylformamide.

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