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

S. V. Nikolaev and V. F. Martinov

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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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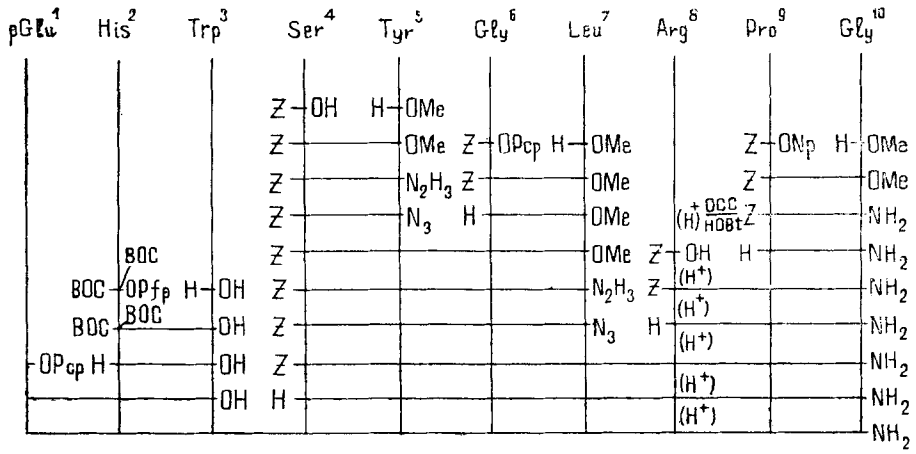

The luliberin and the D-phenylalanyl^6-luliberin were purified on sulfopropyl-Sephadex G-25 in a gradient of pyridine-acetate buffer, 0.05-0.5 M (pH 5.14).

The scheme is suitable for obtaining luliberin in preparative amounts.

LITERATURE CITED

1. H. Matsuo, G. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, Biochem. Biophys. Res. Commun., 43, 1334 (1971).
2. M. Bodanszky, and J. Martinez, Synthesis, 333 (1981).
3. L. Kisfaludy, I. E. Roberts, and R. H. Johnson, J. Org. Chem., 35, 3563 (1970).