Under these conditions the retention time of the assumed farnesyl acetate was 27.7 min, and that of a synthetic sample 27.9 min. However, the mass spectrum of this peak differed sharply from that of trans,trans-farnesyl acetate and probably corresponded to the ester of a high-molecular-weight carboxylic acid with methyl alcohol.

On the basis of the results obtained, the component of the sex pheromone of the smooth click beetle has been assigned the structure of geranyl n-butyrate.

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PREPARATION AND PROPERTIES OF A NEW STRUCTURAL ANALOG OF HUMAN INSULIN — ASPARAGINE ^{B30}-INSULIN

Yu. P. Shvachkin, A. M. Nikitina, S. M. Funtova, S. P. Krasnoshchekova, V. P. Fedotov, and A. I. Ivanova

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In connection with the study of the laws of the structural-functional organization of the insulin molecule, we have obtained a previously unknown analog of human insulin differing from the natural hormone by the replacement of the L-threonine residue in the B^{30} position by and L-asparagine residue.

The preparation of the asparagine-^{B30}-insulin (I, R — de-Thr^{B30}-(human insulin)) was performed by an enzymatic-chemical method using a scheme proposed for the enzymatic transamination of porcine insulin (II, R — de-Ala^{B30}-(porcine insulin)) with the chemical splitting out of the protective groups.

The trypsin-catalyzed transamination [1] of porcine insulin (II) takes place on the reaction of the latter with the tert-butyl ester of L-asparagine (III) in an aqueous organic medium (water—dimethylformamide) at 24°C and pH 6.3. Under these conditions, the tryptic transamination reaction proceeded only at the Lys^{B29} residue, and the undesirable side reaction of the Arg^{B22} residue did not occur.

I. R—Asn—OH; III. H—Asn—OBu^t;
II. R—Ala—OH; IV. R—Asn—OBu^t.

The following stage was the chemical demasking of the ester derivative of insulin formed (IV), ensuring the complete elimination of the C-protective groups from Asn^{B30} residues. Compound (IV) was first purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was carried out by treating compound (IV) with trifluoroacetic acid at 20°C in the presence of anisole as protector. The asparagine^{B30}-(human insulin) (I) obtained was isolated from the reaction mixture with the aid of gel filtration on Sephadex G-25f. The course and degree of purification were monitored by means of thin-layer chromatography (TLC) on silica gel, electrophoresis on cellulose, and disk electrophoresis in polyacrylamide gel. After the lyophilization of the eluate, asparagine^{B30}-(human insulin) (I) was obtained in the analytically pure form.

Asparagine^{B30}-(human insulin) (I). R_f 0.46 (C_5H_5N—C_4H_9OH—CH_3CO_2H—H_2O, (10:15:3:12)), 0.40 (iso-C_3H_7OH—25% NH_4OH, (7:4)), 0.88 (C_5H_5N—CH_3COCH_3—H_2O, (1:1:2)), 0.93 (iso-C_3H_7OH—25% NH_4OH—H_2O, (7:4:6)) (TLC on Silufol UV-254 plates, spots revealed with the Pauly reagent [2]). Electrophoretic mobilities: 1.50 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7

Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow.

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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).

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

Yu. P. Shvachkin, A. M. Nikitina,
S. M. Funtova, S. P. Krasnoshchekova,
N. A. Voskova, O. N. Ryabtseva,
V. P. Fedotov, and A. I. Ivanova

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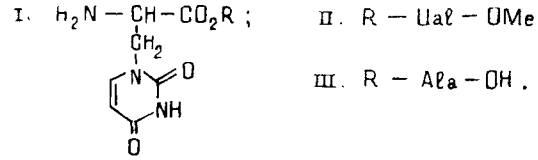
To study the molecular mechanisms of the interaction of human insulin with the receptors, it is necessary to have available structural analogs of this hormone that retain a high biological activity and are readily detectable in complex mixtures by methods of nondestructive analysis. One such method is 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.



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