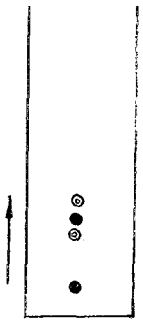

Fig. 3. TLC of the products of the bromosuccinimide cleavage of subunit B.

The hydrolysate was separated on a column (1.1 × 70 cm) of Sephadex G-50 fine equilibrated with 30% CH_3COOH. As in the case of cyanogen bromide cleavage a peptide of low molecular mass was obtained for which the N-terminal amino acid was found to be Val and the amino acid composition: Val_1, Asp_2, Ser_1, Glu_3, Gly_1, Als_1, Leu_1, Phe_1, Arg_1. The yield of peptide was 15%. The compositions of the peptides were determined after hydrolysis in 5.7 N HCl at 110°C for 24 h on a LKB 4101 analyser.

Thus, subunit B contains one Met and one Trp residue. Chemical cleavage at these residues did not lead to the formation of relatively large fragments, since Met and Trp are present in the terminal sections of the polypeptide chain of subunit B.

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PREPARATION AND PROPERTIES OF LEUCINE-B^{30}-INSULIN

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In connection with an investigation of the laws of the structural-functional organization of the insulin molecule, we have performed the synthesis of 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 a L-leucine residue.

Leucine-B^{30}-insulin (I) was obtained by an enzymatic-chemical method using a two-stage scheme involving the use of tryptic transamination [1].

The first stage of the process consisted in the trypsin-catalyzed transamination of porcine insulin (II, R = de-Ala^{B30}-[porcine insulin]) which took place when the latter was treated with L-leucine tert-butyl ester (III) in an aqueous organic medium (water–dimethylformamide) at 24°C and pH 6.3. Under these conditions the enzymatic transamination of the fraction takes place only at the Lys^{B29} residue and the undesirable side reaction at the Arg^{B22} residue does not occur.

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

The second stage of the process consisted in the chemical demasking of the ester derivative of insulin formed (IV) and had the aim of the exhaustive elimination of the C-protective grouping from the Leu^{B30} residue. The ester derivative (IV) was first purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was carried out by treating compound

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(IV) with trifluoroacetic acid at 20°C in the presence of anisole as protector. The leucine-B^{30}-[human insulin] (I, R = de-Thr^{B30}-[human insulin]) formed was isolated from the reaction mixture with the aid of gel filtration on Sephadex G-25 F. The course and degree of purification were monitored by thin-layer chromatography on silica gel, electrophoresis on cellulose, and disc electrophoresis in polyacrylamide gel.

After lyophilization of the eluate, leucine^{B30}-[human insulin] (I) was obtained in the analytically pure state.

Leucine^{B30}-[human insulin] (I). R_f 0.52 (C_5H_5N—C_4H_9OH—CH_3CO_2H—H_2O (10:15:3:12)), 0.65 (iso-C_3H_7OH—25% NH_4OH (7:4)), 0.80 (C_5H_5N—CH_3COCH_3—H_2O (2:1:1)), 0.94 (iso-C_3H_7OH—25% NH_4OH—H_2O (7:4:6)) (TLC on Silufol UV-254, visualization with Pauly's reagent [2]). Electrophoretic mobility: 1.35 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7 mA. Reference standard — the bis-S-sulfonate of the B chain of human insulin).

Amino acid analysis: Asp 2.90; Thr 1.60 (2); Ser 2.70 (3); Glu 7.10 (7); Pro 1.10 (1); Gly 4.00 (4); Ala 1.20 (1); Cys 5.40 (6); Val 3.45 (4); Ile 1.65 (2); Leu 6.85 (7); Tyr 3.25 (4); Phe 2.85 (3); His 1.85 (2); Lys 0.95 (1); Arg 0.95 (1). Results of the determination of C-terminal amino acids: Asn 0.95 (1); Leu 0.98 (1).

When tested for its convulsive effect in mice [3], the biological activity of compound (I) amounted to 90% (in comparison with the activity of the international standard).

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COMPONENTS OF THE LEAVES OF *Phillyrea latifolia*

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We have investigated the leaves of the evergreen tree *Phillyrea latifolia*, family Oleaceae, collected in the Botanical Garden of the Academy of Sciences of the Azerbaidzhan SSR at the beginning of October, 1981. The air-dry raw material was extracted at room temperature successively with chloroform and with ethanol.

Substance (I) was obtained from the chloroform extract by column chromatography (sorbent: silica gel L 60/100 μ; solvents: petroleum ether and dichloroethane).

The ethanolic extract, after concentration, was left for 24 h. The precipitate that deposited was separated off and subjected to recrystallization from aqueous ethanol, giving substance (II). The mother liquor was evaporated to a dry residue, and this was subjected to acid hydrolysis with 5% sulfuric acid for 5 h. The precipitate so formed was separated off and transferred to a column filled with polyamide solvent. Elution was carried out with chloroform—ethanol mixtures containing increasing amounts of the latter. In this way, substances (III), (IV), and (V) were obtained.

Substance (I), $C_{30}H_{48}O_3$, small white acicular crystals soluble in ether, chloroform, acetone, and ethanol and insoluble in petroleum ether and water. mp 302-304°C (ethanol), $[\alpha]_D^{20}$ +78, (c 0.8; chloroform); $\lambda_{max}^{conc\ H_2SO_4}$ 310 nm. The Lieberman—Burchard and Salkowsky reactions were positive.

Substance (II), $C_6H_{14}O_6$, small white acicular crystals. mp 166-168°C (aqueous ethanol). Its hexa-O-acetate had mp 124-126°C. Substance (II) was soluble in water and ethanol, and insoluble in ether and chloroform.

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