

of obligate bicarbonate anions with the formation of pink-colored complexes possessing a characteristic absorption spectrum in the visible region with a maximum at 470 nm also permitted it to be identified as lactoferrin. Since transferrin, which is present in milk in definite amounts, possesses similar properties we made a qualitative analysis of the N-terminal amino acid. In the protein analyzed this was glycine, which is characteristic for lactoferrin, and not valine, which is characteristic for transferrin.

Using this method, it is possible to obtain about 150 mg of lactoferrin from 100 ml of milk.

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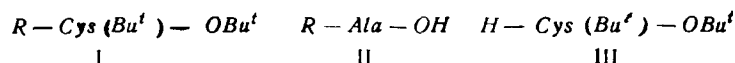
#### PREPARATION AND PROPERTIES OF A CYSTEINE ANALOG OF HUMAN INSULIN

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In connection with a study of the structural and functional organization of insulin, 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<sup>30</sup> position by a protected L-cysteine residue.

The tert-butyl ester of S-tert-butyl-L-cysteine-B<sup>30</sup>-insulin (I, R = de-Thr-B<sup>30</sup>-(human insulin)) was obtained by an enzymatic-chemical method using a scheme involving the tryptic transamidation of porcine insulin (II, R = de-Ala-B<sup>30</sup>-(porcine insulin)).



The trypsin-catalyzed transamidation of porcine insulin (II) takes place on the interaction of the latter with the tert-butyl ester of S-tert-butyl-L-cysteine (III) in an aqueous organic medium (water-dimethylformamide) at 25°C and pH 6.3. Under these conditions, transamidation takes place only at the Lys<sup>B29</sup> residue, and the undesirable side reaction of the Arg<sup>B22</sup> residue does not take place.

The compound (I) formed as the result of transamidation was purified by ion-exchange chromatography on DEAE-Sephadex A-25 followed by gel filtration on Sephadex G-25F. The course and degree of purification were monitored by thin-layer chromatography on silica gel, by electrophoresis in cellulose, and by disc electrophoresis in polyacrylamide gel.

After the lyophilization of the eluate, compound (I) was obtained in analytically pure form.

tert-Butyl Ester of S-tert-Butyl-L-cysteine-B<sup>30</sup>-(human insulin) (I). R<sub>f</sub> 0.48 (C<sub>5</sub>H<sub>5</sub>N-C<sub>4</sub>H<sub>9</sub>OH-H<sub>2</sub>O, 10:15:3:12), 0.78 (iso-C<sub>3</sub>H<sub>7</sub>OH - 25% NH<sub>4</sub>OH, 7:4), 0.8 (C<sub>5</sub>H<sub>5</sub>N-CH<sub>3</sub>COCH<sub>3</sub>-H<sub>2</sub>O, 1:1:2), 0.96 (iso-C<sub>3</sub>H<sub>7</sub>OH - 25% NH<sub>4</sub>OH-H<sub>2</sub>O, 7:4:6) (TLC on Silufol UV-254 plates; Pauly revealing reagent [1]). Electrophoretic mobility: 1.5 (electrophoresis on Whatman No. 1 paper, 450 V, 7 mV, pH 1.9; reference standard: the bis-S-sulfonate of the B-chain of human

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insulin). Amino acid analysis: Asp 2.82 (3), Thr 1.70 (2), Ser 3.00 (3), Glu 7.06 (7), Pro 1.03 (1), Gly 4.00 (4), Ala 1.00 (1), Cys 6.56 (7), Val 3.83 (4), Ile 1.65 (2), Leu 6.03 (6), Tyr 3.70 (4), Phe 3.01 (3), Lys 1.00 (1), His 2.10 (2), Arg 0.85 (1). Results of a determination of the C-terminal amino acids: Asn 0.99 (1); Cys 0.95 (1).

In testing for convulsive effect on mice [2], the biological activity of compound (I) was 100% (in comparison with the activity of an international standard).

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#### HYDROGENOLYSIS OF THE PROTOLIGNIN OF RICE HUSKS

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As a result of a study of the structure of mechanical-grinding lignin (MGL) isolated from the wood of the birch and the oak, and also of the protolignin of *Picea jezoensis* by hydrogenolysis using copper chromite as catalyst [1, 2], a number of monomeric and dimeric hydrogenolysis products have been identified.

We have carried out the hydrogenolysis of rice husks by the methods of Sakakibara et al. [2]. As catalysts we employed copper chromite and a modified nickel catalyst that is used in the deep hydrocracking of petroleum. Hydrogenolysis was carried out for 60 min at an initial hydrogen pressure of 85 atm, a working pressure of 180-190 atm, and a temperature of 200°C; the solvent was dioxane-water (9:1), and the amount of catalyst was 40% on the weight of the raw material.

The combined products obtained were concentrated and were then dissolved in chloroform and extracted with 5% caustic soda. After acidification of the alkaline extract by the addition of hydrochloric acid to pH 8, the hydrogenolysis products were extracted with ether, and then, at pH 2, with ethyl acetate.

The combined ether- and (ethyl acetate)-extracted materials were chromatographed on a column of Sephadex LH-20. The eluogram showed that the combined ether-extracted material consisted of monomeric, dimeric, and oligomeric products, while in the ethyl acetate extract the dimeric fraction predominated.

The components of the monomeric fraction of the combined hydrogenolysis products were identified with the aid of thin-layer chromatography on Silufol (benzene-ethanol (4:1)) with markers, and also by gas-liquid chromatography:

Substance	R <sub>f</sub>		% on the total	
	in the product	of a marker	copper chromite catalyst	catalyst used in the deep hydrocracking of petroleum
p-Hydroxyphenylethane	0.61	0.63	12.3	7.4
p-Hydroxyphenylpropane	0.69	0.70	9.2	68.4
Guaiacylethane	0.81	0.80	1.3	—
1-Guaiacylpropan-1-ol	0.77	0.76	2.5	3.8
3-Guaiacylpropan-1-ol	0.39	0.38	4.5	—
o-Cresol	0.71	0.71	1.3	Сл.
Phenol	—	—	—	12.1
Guaiacol	—	—	—	Сл.
1-Guaiacylethanol	—	—	—	2.4
Guaiacylpropane	—	—	—	5.8

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