

CHEMISTRY

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ABSTRACTS

The Structure of Glucagon. W. W. BROMER, L. G. SINN, A. STAUB and OTTO K. BEHRENS, Lilly Research Laboratories, Indianapolis, Indiana.—Investigations leading to formulation of the amino acid sequence of glucagon were undertaken.

Quantitative amino acid analysis of glucagon provided evidence for the following empirical formula with 29 amino acid residues, and with a minimum molecular weight of 3485:His₅Ser₁Glu₃Gly₁Thr₃Phe₂Asp₄Tyr₁Lys₁Leu₂Arg₂Ala₁Val₁Try₁Met₁(—CONH₂)₄. Histidine was determined as the N-terminal amino acid by means of the dinitrophenylation method; the C-terminal residue is threonine on the basis of data obtained from hydrazinolysis and carboxypeptidase treatment. Carboxypeptidase quantitatively liberated the following amino acids from the C-terminus of glucagon: valine, glutamine, tryptophan, leucine, methionine, asparagine, and threonine. A small amount of alanine and somewhat greater quantities of phenylalanine, aspartic acid, and a second residue of glutamine were also released.

A single lot of carefully purified crystalline glucagon was used throughout the structure studies. The methods employed in the structure determination were as follows: (a) specific enzymatic cleavage with trypsin, chymotrypsin, and subtilisin; (b) resolution of the peptides from these digestions using Dowex 50 chromatography; and (c) characterization of the peptides by quantitative amino acid analysis, dinitrophenylation, and in some cases further degradation with carboxypeptidase and acid.

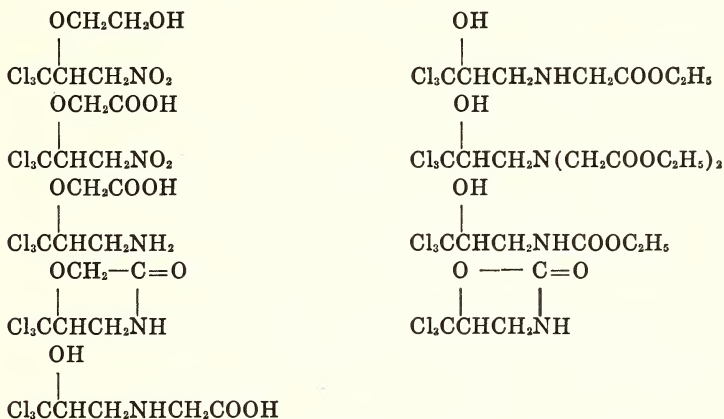
Dowex 50 chromatography of the chymotryptic digest resulted in the separation of six pure peptide fragments. Five fragments were isolated from a 2¼ hour tryptic digestion of glucagon, and seven peptides were obtained from a 50-hour trypsin incubation. Subtilisin digestion followed by chromatographic resolution gave eleven peptides.

In a great majority of cases specific enzymatic splits of greater than 80% were obtained and the resulting peptides were isolated in yields exceeding 50%. With every enzyme used, the sum of the resulting fragments was in complete agreement with the empirical formula of glucagon.

The structure of four peptides was elucidated by partial acid degradation and carboxypeptidase treatment. The locations of the four amide linkages were determined by subjecting selected peptide fragments to chemical analysis.

Further work is being carried out on these reactions and a study of the generality of this method and the possibility of reducing sulfonic acids directly is being made.

Heterocyclic Compounds via 1,1,1-trichloro-3-nitropropanol. HOWARD BURKETT and GUNNER NELSON, DePauw University.—The purpose of this research was to investigate several possible approaches to the synthesis of various types of heterocyclic compounds using 1,1,1-trichloro-3-nitropropanol as the initial starting material. During the course of this study the following new compounds were prepared.



This research is being continued.

An Improved Procedure for the Preparation of Aromatic Thiols. E. CAMPAIGNE and S. W. OSBORN, Chemistry Department, Indiana University.—The preparation of aromatic thiols from the corresponding aryl ethyl xanthates by alkaline hydrolysis has not been satisfactory in many instances. Yields are relatively low due to loss by oxidation, and to incomplete hydrolysis in the case of hindered xanthates. Although Tarbell and Fukushima report yields of 63-75% in the case of *m*-tolyl ethyl xanthate, we have been unable to duplicate these yields with more hindered compounds. In our hands the conventional method gave at best a yield of only 49% in the case of 2,6-dimethylthiophenol, and even poorer yields in the case of *o*-thiocresol (39%) and *o*-phenylthiophenol (21%).

Following the suggestion of Djerassi, reduction of the xanthates by lithium aluminum hydride proved to be a much more effective method for preparing hindered aromatic thiols. Yields of 84-89% were consistently obtained. For example, reduction, of *o*-biphenyl ethyl xanthate with lithium aluminum hydride gave an 84% yield of pure *o*-phenylthiophenol, while even better yields were obtained in the preparation of 2,6-dimethylthiophenol (86%) and *o*-thiocresol (89%) by the reduction of their respective xanthates.

The two new compounds, *o*-phenylthiophenol and 2,6-dimethylthiophenol have been characterized and derivatives prepared.

Studies on the Bromination of Carbostyrils. D. J. COOK, PETER SORTER and R. S. YUNGHANS, DePauw University.—This work involves a study of the products obtained when several methyl substituted carbostyrils were brominated by use of N-bromosuccinimid.

When 1,4-dimethylcarbostyryl is treated with N-bromosuccinimid, the bromine appears to substitute in the 3-position of the heterocyclic ring. A similar study with 4-methylcarbostyryl finds the bromine substituting for a hydrogen in the 4-methyl group. It appears that when a methyl group is attached to the nitrogen of the ring, substitution is preferential in the open 3-position of the ring while bromine will substitute for a hydrogen in the 4-methyl group if no substituent appears on the nitrogen.

When position 3 of the hetero ring is covered with a methyl group, as found in 1,3,4-trimethylcarbostyryl, bromination with N-bromosuccinimid results in bromine entering the 3-methyl position. A bromination of N-methyl- α -methylacetoacetanilide with bromine in chloroform and then ring closure of the intermediate with concentrated sulfuric acid, yielded a product believed to be 1,3-dimethyl-4-bromoethylcarbostyryl. The difference between this compound and the previous mentioned one was used as evidence for the substitution in the 3-methyl group.

In a number of instances the infra-red absorption spectra were helpful in determining the position of bromine substitution.