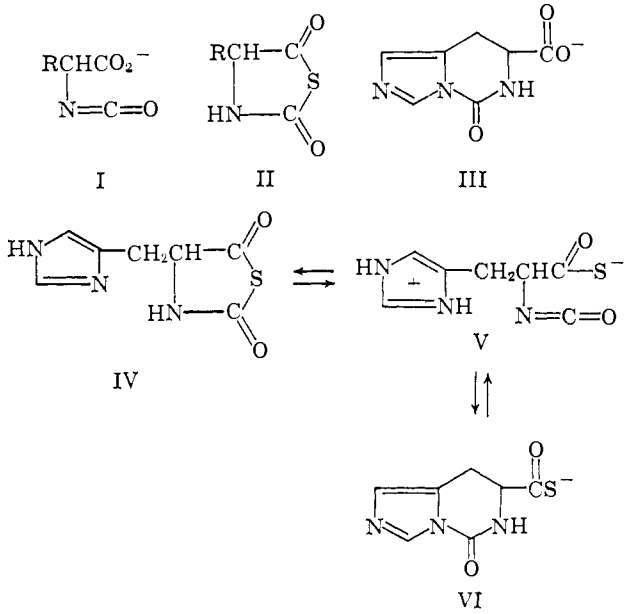
shoulder at 5.69 μ; $[\alpha]^{25}_{589}$ −1.64° (*c* 1.05, CH_2Cl_2). Treatment of a 1% excess of II (R = CH_3) with L-phenylalanine in a Waring Blendor at pH 9.5 and 0° gave the dipeptide in about 90% yield. In this case the optical purity of the dipeptide was determined by comparing the areas of the methyl doublets of the diastereoisomers in the nmr[10] spectrum of the crude lyophilized product in D_2O. We improved the sensitivity of the published analytical procedure[10] about tenfold by use of a ^{13}C–H satellite peak of the predominant L–L isomer as an internal standard. The dipeptide was thus found to contain 98.7 ± 0.3% of the L–L isomer. The ^{13}C–H satellite (abundance of ^{13}C = 1.11%) provides a convenient, reproducible internal reference standard for measurements in this range.

A striking chemical difference was also observed between the NCA and the NTA of imidazyl-unprotected histidine. We have found that the NCA of histidine cannot be used to prepare histidyl peptides because it rearranges to give III, doubtlessly *via* an intramolecular imidazole-catalyzed isocyanate formation. L-Histidine ethylthionourethan, mp 212° dec, $[\alpha]^{24}_{589}$ +24.0° (*c* 2.0, 0.1 *N* NaOH), on treatment with PBr_3 afforded the analytically pure NTA IV as the hydrobromide in 73% yield, $[\alpha]^{25}_{589}$ −7.0° (*c* 2%, methyl Carbitol). This compound, in contrast to the NCA, is useful for the rapid synthesis of imidazyl-unprotected histidyl peptides in aqueous medium possibly because the equilibrium between IV and V lies further to the left than the corresponding equilibrium of the oxygen analogs. Crystalline VI also yielded histidyl peptides, but the NTA IV permitted peptide bond formation at 0° in 3 min whereas higher temperatures and longer reaction times were required with VI. The NTA of histidine (IV) formed as much as 10% of the D-histidyl peptides, but it was often possible to purify the product. The reagent also proved advantageous whenever a simple procedure for the rapid introduction of imidazyl-unprotected histidine in aqueous medium was required, as, for example, in the preparation of reference compounds in connection with sequence determinations.



I II III

IV V

VI

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Thyrocalcitonin. II. Enzymatic and Chemical Sequence Studies

Sir:

The amino acid composition of the dotriacontapeptide thyrocalcitonin was reported from this laboratory in an earlier communication.[1] The assigned composition has been confirmed by two other laboratories.[2,3] From cleavage of the hormone with trypsin we have now obtained three fragments (1–14, 15–21, and 22–32) (Figure 1). Amino acid analyses[4] of these fragments accounted for all of the 32 amino acids (tryptophan was found after enzymatic cleavage) and were in agreement with those just reported by Kahnt, *et al.*[2] On the basis of degradation and synthetic studies summarized below, we report herein the sequence of amino acids 8–32.

Digestion of the hormone with trypsin and chymotrypsin (20 hr) followed by treatment with aminopeptidase M[5,6] (3 days) liberated Asx as asparagines and Glx as glutamic acid. Since the dotriacontapeptide is a monobasic acid,[1] the carboxyl group of the terminal amino acid (proline) is not free. Evidence for the presence of this proline as prolinamide and additional support for the formulation of Glx as glutamic acid was obtained by synthesis[7] of glycylprolylglutamylthreonylprolinamide. This pentapeptide amide was identical with a fragment (Chy-5) obtained by cleavage of the hormone with chymotrypsin. The synthetic[7] and the natural pentapeptides could be distinguished by electrophoresis and tlc from the isomeric pentapeptide glycylprolylglutaminylthreonylproline which we had also synthesized.[7] The presence of a disulfide bridge in thyrocalcitonin is suggested by the absence of free sulfhydryl groups in the hormone as shown by titration with 5,5′-dithiobis(2-nitrobenzoic acid).[8] Performic acid oxidation of fragment 1–14 gave 1.5 cysteic acid residues (theory = 2).

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(7) We have found sequential syntheses with N-carboxyanhydrides [R. Hirschmann, R. G. Strachan, H. Schwam, E. F. Schoenewaldt, H. Joshua, H. Barkemeyer, D. F. Veber, W. J. Paleveda, T. A. Jacob, T. E. Beesley, and R. G. Denkewalter, *J. Org. Chem.*, **32**, 3415 (1967), and references cited therein] and N-hydroxysuccinimide esters [G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964)] useful for the rapid synthesis of reference compounds required in support of the structural studies.
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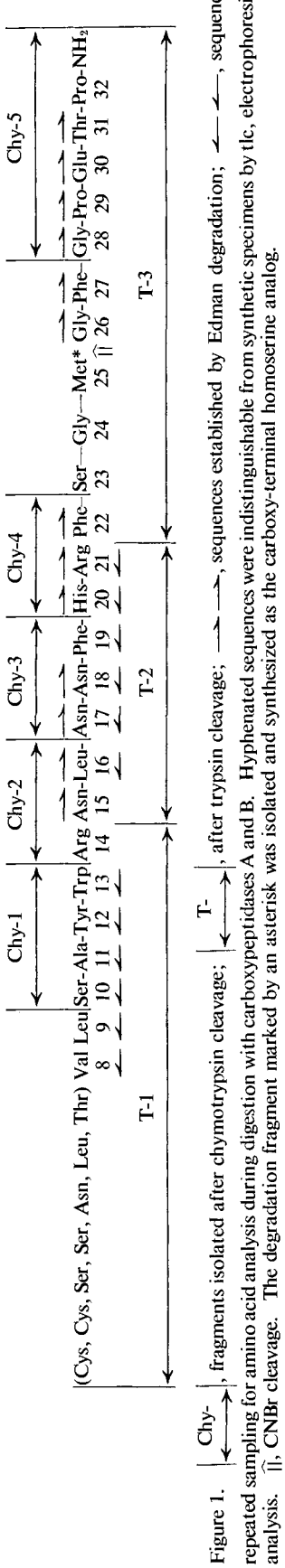

Figure 1. Chy-, fragments isolated after chymotrypsin cleavage; T-, after trypsin cleavage; ⟶, sequences established by Edman degradation; ⟵, sequences determined by repeated sampling for amino acid analysis during digestion with carboxypeptidases A and B. Hyphenated sequences were indistinguishable from synthetic specimens by tlc, electrophoresis, and amino acid analysis. ||, CNBr cleavage. The degradation fragment marked by an asterisk was isolated and synthesized as the carboxy-terminal homoserine analog.

Peptide fragments isolated after cleavage with chymotrypsin are also shown in Figure 1. The important tripeptide 20–22 (Chy-4), which overlaps two tryptic fragments, was identified by Edman degradation and by synthesis. The tyrosyl–tryptophan bond in the chymotryptic fragment Chy-1 was stable to chymotrypsin both in the natural and in the synthetic tetrapeptide.

Treatment of the tryptic fragment 15–21 with thermolysin[9] afforded phenylalanylhistidylarginine as well as fragment 16–18. Degradation of fragment 22–32, obtained from trypsin cleavage, with CNBr gave phenylalanylserylglycylhomoserine as well as fragment 26–32. The presence of an amino-terminal phenylalanine in the tetrapeptide was shown by the dansylation procedure for end-group analysis.[10] It was confirmed by degradation with leucine aminopeptidase which also served to demonstrate the presence of serine in position 23. Moreover, the tetrapeptide was found to be indistinguishable from a synthetic specimen. Sequence 26–31 was established by Edman degradation of fragment 26–32. Leucine aminopeptidase confirmed the presence of the terminal sequence Gly-Phe (26–27).

Hydrolysis of thyrocalcitonin with 0.03 *N* HCl at 100° for 9 hr liberated leucine and aspartic acid as the only detectable amino acids. This result is consistent[11] with the presence of an Asn-Leu-Asn fragment in the hormone. The tryptic fragment 15–21 (T-2) was also found to be identical with a synthetic[7] specimen.

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The Reaction of Derivatives of Tryptophan, Tryptamine, and Other Indoles with 2-Hydroxy-5-nitrobenzyl Bromide (Koshland's Reagent)

Sir:

Although interest in the highly selective reagent 2-hydroxy-5-nitrobenzyl bromide (**1**) for the rapid modification and assay of tryptophan in proteins continues,[1-7] there is no information on the structure of the reaction products.[7a] A recent report[8] on the complex reactions of the reagent with tryptophan, both free and bound in proteins, prompts us to communicate our findings on the reaction of **1** with skatole (**2**), 2,3-dimethylindole (**3**), N-acetyltryptamine (**4**), and N-acetyl-L-tryptophan methyl ester (**5**).

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