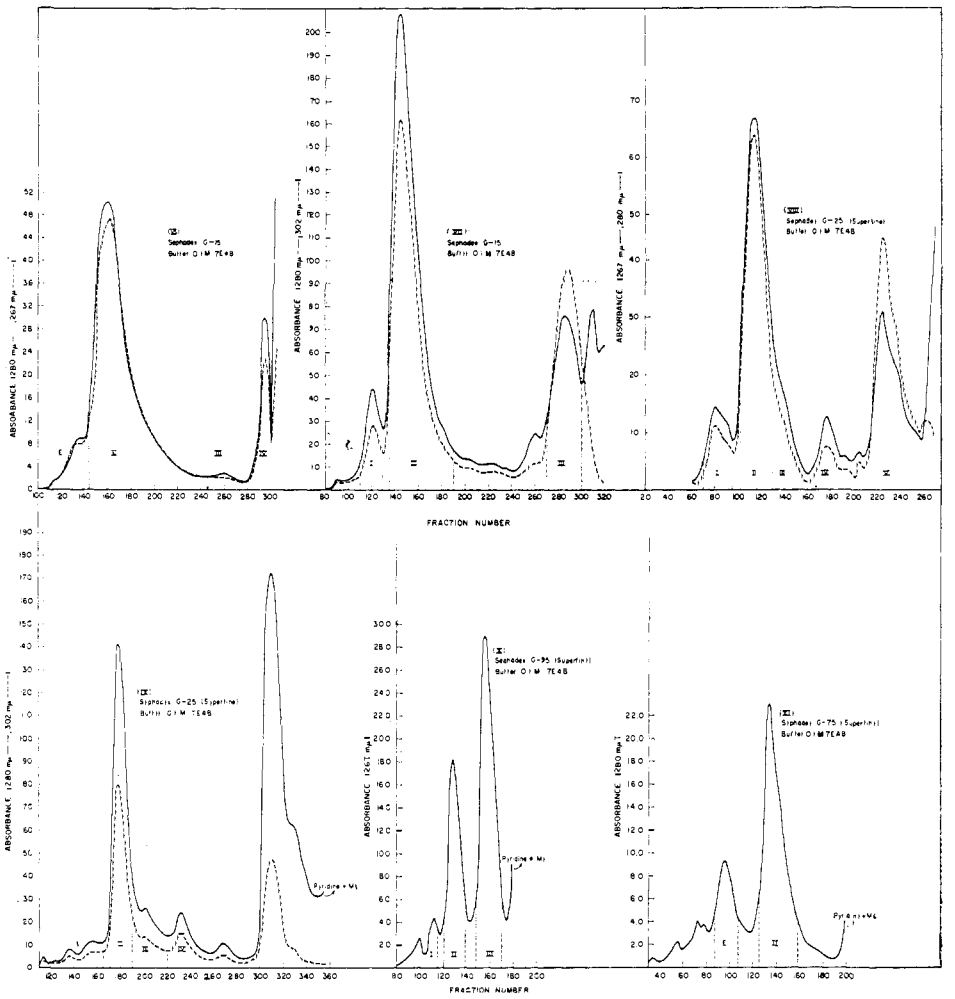

Figure 2. (VI) Chromatography of the reaction mixture on a Sephadex G-15 column (96 × 5 cm) in the preparation of d-pA^{Bz}pT. Fractions of 4 ml were collected every 6 min. (VII) Chromatography of the reaction mixture (one-fourth portion) on a Sephadex G-15 column (96 × 5 cm) in the preparation of d-pA^{Bz}pC^{An}. Fractions of 4 ml were collected every 6 min. (VIII) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-25 (Superfine) column (96 × 5 cm) in the preparation of d-pTpTpA^{Bz}. Fractions of 4 ml were collected every 6 min. (IX) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-25 (Superfine) column (96 × 5 cm) in the preparation of d-pA^{Bz}pC^{An}pA^{Bz}. Fractions of 4 ml were collected every 6 min. (X) Chromatography of the reaction mixture (one-ninth portion) on a Sephadex G-75 (Superfine) column (96 × 2.5 cm) in the preparation of d-pTpTpA^{Bz}pA^{Bz}pTpT. Fractions of 2 ml were collected every 15 min. (XI) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-75 (Superfine) column (96 × 2.5 cm) in the preparation of d-pTpTpA^{Bz}pA^{Bz}pTpTpA^{Bz}pC^{An}pA^{Bz}pA^{Bz}pTpA^{Bz}. Fractions of 2 ml were collected every 15 min.

given a prolonged treatment with aqueous pyridine–triethylamine followed by an alkali treatment to remove the acetyl and cyanoethyl groups. The products were fractionated on columns of an appropriate Sephadex. The separations were rapid and excellent. Figures 1 and 2 show the elution pattern for each step. A summary of the pertinent reaction conditions and separations used in the syntheses of $(pT)_{24}$ and d-pTpTpA^{Bz}pA^{Bz}pTpTpA^{Bz}pC^{An}pA^{Bz}pA^{Bz}pTpA^{Bz} is shown in Table I.

Characterization of all the protected and unprotected intermediate compounds was accomplished by paper chromatography. The final compounds were further characterized by degradation with spleen and venom phosphodiesterase treatment after the enzymic removal of the phosphomonoester group. Enzymic degradation proceeded to completion and the hydrolyzed products (nucleotide and nucleoside) were produced in the expected molar proportions.

This methodology was developed as a necessary preliminary step toward the DNA-directed synthesis of bovine insulin chain A. Further work in this direction will be the subject of forthcoming papers.

(6) To whom all inquiries should be made.
(7) National Research Council of Canada Postdoctorate Fellow, 1967–1968.

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Purification and Structure of Porcine Calcitonin-1

Sir:

We wish to report the isolation in pure form of a porcine calcitonin (PC-1) and the elucidation of its structure.[1]

(1) We also wish to acknowledge the valuable assistance of C. Pidacks for chromatography development work, as well as E. Lindemann and H. Falk for biological assay data.

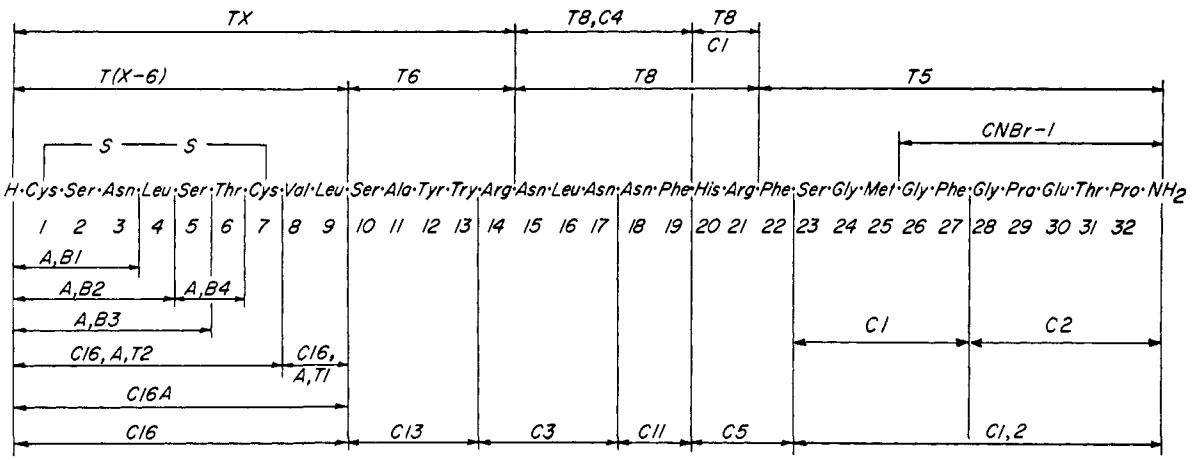

Figure 1.

Porcine thyroid acetone-dried powder was extracted at 70° with 2 *N* acetic acid. The crude extract was purified further by adsorption on oxycellulose,[2] at pH 3.0, followed by elution of activity with 0.2 *N* HCl. After removal of chloride ions with Amberlite IRA-400 (acetate) and lyophilization, the material was redissolved in 0.1 *N* acetic acid and subjected to fractionation by gel filtration on Sephadex G-50 columns. The active fraction was further purified by countercurrent distribution (CCD) in the solvent system 1-butanol *vs.* 20% acetic acid. Three active components were resolved: PC-1 (K = 0.66), PC-2 (K = 0.33), and PC-3 (K = 0.14). Preparative amounts of PC-1 and PC-2 were separated from the bulk of the impurities by a room-temperature 20-transfer CCD bottle procedure. The recovered PC-1 and PC-2 were resolved by an extended CCD at 4°. PC-1 and PC-2 were finally obtained in pure form by CM-cellulose chromatography.[3] PC-1 was selected for structural study. The final PC-1 product had a specific activity of about 270 MRC[4] units/mg of protein when assayed in the presence of 0.1% bovine serum albumin.

Amino acid analyses[5,6] of PC-1 gave the following empirical formula:[7] $Cys_2Asp_4Thr_2Ser_4Glu_1Pro_2Gly_3Ala_1Val_1Met_1Leu_3Tyr_1Phe_3His_1Arg_2Try_1$.

The weight-average molecular weight[8] of PC-1 was found to by 3700, which is in excellent agreement with the value required by the above formula (3604 as required by the structure shown in Figure 1).

Failure of PC-1 to react with *p*-mercuribenzoate[9] along with the molecular weight data suggested an intrachain disulfide structure. Dansylation[10] of PC-1, followed by acid hydrolysis, yielded DNS products which on resolution by two-dimensional thin-layer chromatography corresponded to those obtained by hydrolysis of bis-DNS-cystine. Application of the Dansyl–Edman stepwise degradative procedure[11] to PC-1 gave the N-terminal sequence shown for positions 1–9 of Figure 1. Carboxypeptidase studies of PC-1 were inconclusive.

The steps used in the elucidation of the complete structure of PC-1 involved: (a) selective cleavage with trypsin (T peptides), chymotrypsin (C peptides), and cyanogen bromide (CNBr peptides);[12] (b) isolation of peptides in pure form by ion-exchange chromatography (Dowex 50-X2, CM-cellulose) or electrophoresis and chromatography on paper; (c) amino acid analysis of peptides; and (d) specific studies of the isolated peptides by: the Dansyl–Edman sequential method and enzyme digestions with trypsin, chymotrypsin, leucine aminopeptidase (LAP), and carboxypeptidase B (CoB).

All of the peptides shown in Figure 1 except TX, T(X-6), and C16 were isolated and gave satisfactory amino acid ratios. Chymotryptic activity in the trypsin converted TX to T6 and T(X-6) (same as C16). C16 was isolated in pure form after conversion to C16A by β-aminoethylation[13] of the two cysteine residues (AECys).

Dansyl–Edman studies of PC-1, T5, T6, T8, T8,C4, C2, and CNBr-1, along with amino acid data for the remaining peptides, were sufficient to establish the structure sequence for the hormone.

Special experiments were required to place the amide groups in the final structure. (1) Total LAP digestion of T8 released 3 moles of Asn. (2) LAP failed to release

(2) E. B. Astwood, M. S. Raben, R. W. Payne, and A. B. Grady, *J. Am. Chem. Soc.*, 73, 2969 (1951).

(3) M. L. Dedman, T. H. Farmer, and C. J. O. R. Morris, *Biochem. J.*, 78, 348 (1961).

(4) M. A. Kumar, E. Slack, A. Edwards, H. A. Soliman, A. Baghdrantz, G. V. Foster, and I. MacIntyre, *J. Endocrinol.*, 33, 469 (1965).

(5) Tryptophan by the method of J. R. Spies and D. C. Chambers, *Anal. Chem.*, 20, 39 (1948).

(6) Cysteine as cysteic acid after oxidation of PC-1 by the method of S. Moore, *J. Biol. Chem.*, 238, 235 (1963).

(7) Data from our laboratories on the purification and physical properties of PC-1 were presented at a Symposium on Thyrocalcitonin and the C Cells held in London, July 1967 (to be published by William Heinemann Medical Books Ltd., London (S. Taylor, Ed.)). At this meeting Dr. F. J. Wolf described a purified calcitonin giving the same empirical formula as PC-1. In a subsequent publication these workers report their calcitonin does not have a free N terminal, which differs from PC-1 reported here (I. Putter, E. A. Kaczka, R. E. Harman, E. L. Rickes, A. J. Kempf, L. Chaiet, J. W. Rothrock, A. W. Wase, and F. J. Wolf, *J. Am. Chem. Soc.*, 89, 5301 (1967)). It has also recently come to our attention that two other groups have isolated calcitonins with the same empirical formula and have, in addition, purified their trypsin peptides (F. W Kahnt, B. Riniker, I. MacIntyre, and R. Neher, *Helv. Chim. Acta*, 51, 214 (1968); J. Franz, J. Rosenthaler, K. Zehnder, W. Doepfner, R. Huguenin, and St. Guttmann, *ibid.*, 51, 218 (1968)).

(8) D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, 88, 586 (1960).

(9) We wish to thank Dr. E. C. Renzo of our laboratories for this experiment, using the method of Boyer (P. D. Boyer, *J. Am. Chem. Soc.*, 76, 4331 (1954)).

(10) W. R. Gray and B. S. Hartley, *Biochem. J.*, 89, 59P (1963).

(11) P. Nedkov and N. Genov, *Biochim. Biophys. Acta*, 127, 541 (1966).

(12) E. Gross and B. Witkop, *J. Biol. Chem.*, 237, 1856 (1962).

(13) M. A. Raftery and R. D. Cole, *ibid.*, 241, 3457 (1966).

Asp or Asn from PC-1 or any of the peptides containing position 3. This difficulty was overcome by a CoB digestion of C16A which gave the peptides A,B1, A,B2, A,B3, and A,B4 along with Val, AECys, Ser, Thr, and Leu. A,B4 was shown to result from a "chymotryptic-like" split at bond 4–5. Since peptide A,B1 involved positions 1–3 it proved suitable for its Edman study. Two cycles of Edman degradation released Asn from position 3. (3) Extended digestion of CNBr-1 with LAP released Gly, Phe, Glu, traces of Pro, and H-Gly-Pro-OH. A good yield of Glu (0.9 mole) and no release of Gln established Glu at position 30. (4) Prolinamide (Pro-NH_2) at position 32 was established by its release after the sixth Edman degradation cycle on peptide CNBr-1 (identified as DNS-Pro-NH_2 and also as H-Pro-NH_2).

Details of the purification, structure, and enzyme digestion restrictions such as those imposed by the N-terminal disulfide ring of PC-1 will be reported in two publications now in preparation.[14]

(14) Preliminary studies, designed to synthesize PC-1, by G. W. Anderson, F. M. Callahan, A. E. Lanzilotti, and J. E. Zimmerman of these laboratories have given material with hypocalcemic activity. Synthetic work will be reported later.

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Oxidation by Metal Salts. II. The Formation of γ-Lactones by the Reaction of Lead Tetraacetate with Olefins in Acetic Acid[1]

Sir:

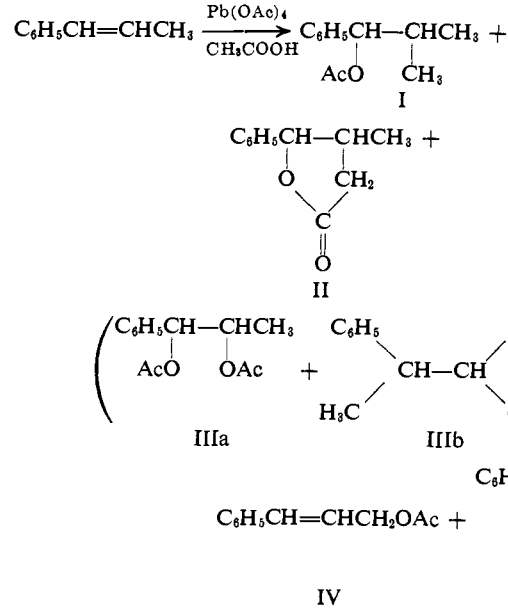
Criegee, in a recent review article, mentioned a peculiar reaction in which a γ-lactone was obtained as a side product from the reaction of lead tetraacetate with octene-4 in acetic acid.[2] The γ-lactone was only observed with octene-4 and the mechanism of the reaction leading to this lactone was unknown.

We wish to report our results, which indicate the generality of this reaction in that γ-lactones have been obtained by the reaction of lead tetraacetate with octene-1, styrene, and β-methylstyrene. We also wish to present a mechanism which explains the formation of these lactones and which predicts the conditions conducive to their formation.

We studied the reaction of lead tetraacetate with *trans*-β-methylstyrene in refluxing acetic acid in some detail. In the presence of added anhydrous potassium acetate (approximately 1 *M* or greater) and low olefin concentrations (0.2 *M* or less) under a nitrogen atmosphere, two major products were obtained. These were identified as the methyl acetate adduct I and the lactone II on the basis of their nmr and infrared spectra and elemental analyses. In addition to these two major products, we isolated a mixture of diacetates, consisting of 65% *threo*- and *erythro*-1,2-diacetates (IIIa) and 35% rearranged 1,1-diacetate (IIIb), together with some allylic acetate IV and traces of the olefin V.

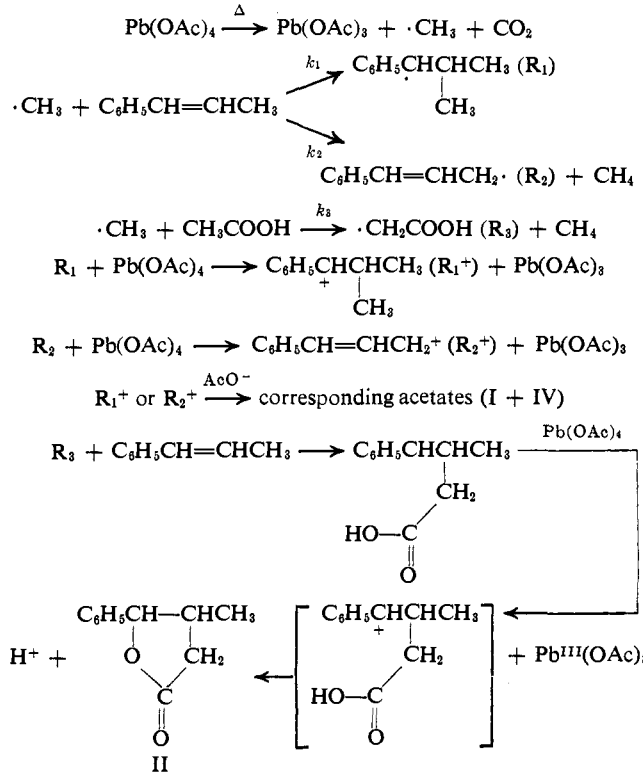
(1) Previous article in this series: E. I. Heiba, R. M. Dessau, and W. J. Koehl, Jr., *J. Am. Chem. Soc.*, 90, 1082 (1968).

(2) R. Criegee in "Oxidation in Organic Chemistry," Part A, K. Wiberg, Ed., Academic Press Inc., New York, N. Y., 1965, p 277.



In a typical experiment, *trans*-β-methylstyrene (35 mmoles) and lead tetraacetate (4.2 mmoles) were refluxed in 100 ml of glacial acetic acid containing 30 g of anhydrous potassium acetate[3] under a nitrogen atmosphere. The following products were obtained: methyl acetate adduct I (1.91 mmoles), lactone II (0.80 mmole), diacetates III[4] (0.36 mmole), allylic acetate IV (0.4 mmole), and the olefin V (0.04 mmole),

Scheme I



(3) The large concentration of potassium acetate was used to increase the temperature of the refluxing solution to 138°.

(4) The formation of diacetates in the oxidation of styrene with lead tetraacetate was attributed to an ionic mechanism by R. O. C. Norman and C. B. Thomas, *J. Chem. Soc.*, *B*, 771 (1967). This explanation is in accord with our results, which indicate that the yield of the diacetate can be suppressed by the addition of potassium acetate and high reaction temperatures.