of N^{α},N^{ϵ}-di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysine[10] was coupled with phenylalanylglutamylnitroarginylglutamine benzyloxycarbonylhydrazide monoacetate ($[\alpha]^{24}D$ −19.2° (*c* 2.27, 10% acetic acid); amino acid ratios in the AP-M digest[4] $Phe_{0.99}Glu_{1.04}Narg_{1.00}Gln_{0.98}$. *Anal.* Found: C, 50.3; H, 6.2; N, 18.2; O, 24.7) to give N^{α},N^{ϵ}-di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysylphenylalanylglutamylnitroarginylglutamine benzyloxycarbonylhydrazide. Catalytic hydrogenolysis converted this material into the hydrazide of N^{α},N^{ϵ}-di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysylphenylalanylglutamylarginylglutamine monoacetate dihydrate (*Anal.* Found: C, 52.9; H, 8.0; N, 14.8; O, 24.3); amino acid ratios in acid hydrolysate $Lys_{2.00}Glu_{2.95}Thr_{0.92}Ala_{3.20}Phe_{1.02}Arg_{0.91}$. The azide corresponding to this hydrazide was then coupled with β-(pyrazolyl-3)alanylmethionylaspartic acid *d*-sulfoxide[11] ($[\alpha]^{25}D$ +44.9° (*c* 3.12, water); amino acid ratios in the AP-M digest[4] $Pyr(3)Ala_{0.96}Met_{1.00}Asp_{1.04}$. *Anal.* Found: C, 43.2; H, 5.8; N, 16.2; O, 27.2; S, 7.8), and the ensuing product was deblocked with trifluoroacetic acid to afford the *d*-sulfoxide of II, $[\alpha]^{25}D$ −47.1° (*c* 1.31, 10% acetic acid); amino acid ratios in acid hydrolysate $Lys_{2.04}Glu_{3.11}Thr_{0.96}Ala_{3.05}Phe_{0.98}Arg_{0.97}Pyr(3)Ala_{0.85}Met_{0.52}$[12]$Asp_{1.03}$. For conversion into II the sulfoxide was reduced with aqueous thioglycolic acid;[4] amino acid ratios in acid hydrolysate $Lys_{2.04}Glu_{3.07}Thr_{0.99}Ala_{3.15}Phe_{1.01}Arg_{0.94}Pyr(3)Ala_{0.94}Met_{0.98}Asp_{0.85}ammonia_{0.97}$.[13]

The principles which have led us, by a logical process, to the discovery of a potent antagonist to S-peptide may be useful in development of antagonists to other biologically active polypeptides.[14]

Acknowledgment. The skillful technical assistance of Miss Judy Montibeller, Mrs. Elaine Gleeson, and Mr. Albert Frazier is gratefully acknowledged.

(10) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).

(11) We wish to thank Dr. R. H. Andreatta for supplying us with this compound.

(12) Value not corrected for destruction and homocysteic acid formation.

(13) The ammonia figure is corrected to represent amide ammonia. The methionine sulfoxide content was negligible as determined by AP-M digestion.

(14) In the course of systematic investigations relating structure to function in the oxytocin series, H. Schulz and V. du Vigneaud, *J. Med. Chem.*, **9**, 647 (1966), discovered the antagonistic action of 1-L-penicillamine-oxytocin toward oxytocin; the mechanism of action of this analog is obscure. It should be noted that the replacement of cysteine by the bulkier β,β-dimethylcysteine is not comparable to the isosteric replacement which is described in this communication.

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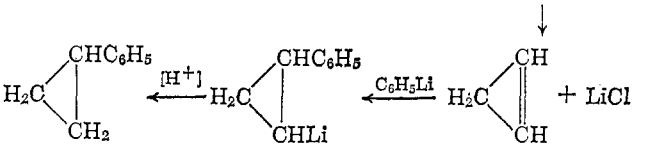
The Stereochemistry of the Addition of Phenyllithum to Cyclopropene

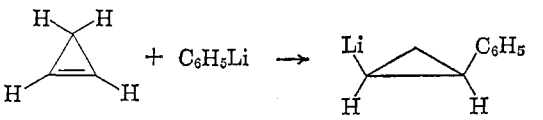
Sir:

Our mechanistic study of the formation of phenylcyclopropane from allyl chloride and phenyllithium led us to postulate a reaction sequence involving the intermediacy of cyclopropene and addition of phenyllithium across the double bond.[1] While hydrocarbons con-

$$CH_2{=}CHCH_2Cl + C_6H_5Li \longrightarrow C_6H_6 + CH_2{=}CHCHLiCl$$



taining conjugated double bonds are known to add organolithium reagents,[2] the reaction with an isolated double bond is quite rare.[3] In no case has the stereochemistry of addition been determined. We now report that the addition of phenyllithium to cyclopropene does, indeed, occur and that the reaction proceeds with greater than 99% stereospecificity to *cis*-2-phenylcyclopropyllithium.[4]



Cyclopropene gas was generated by the tetramethylethylenediamine-promoted reaction of methyllithium with allyl chloride[6,7] and was bubbled *via* a −20° cold trap into a solution of phenyllithium in ether. When the reaction mixture was quenched with water, the only volatile products formed were phenylcyclopropane and allylbenzene in an over-all yield of 2.5% (mole ratio of the two products 20:1, respectively).[9] In a separate experiment, the reaction mixture was poured over freshly crushed Dry Ice; acidification followed by esterification with diazomethane yielded methyl benzoate and *cis*-1-carbomethoxy-2-phenylcyclopropane;[10,11] as little as 0.5% of the *trans* ester would have been detected. The neutral fraction from

(1) R. M. Magid and J. G. Welch, *J. Am. Chem. Soc.*, **88**, 5681 (1966).

(2) E. Grovenstein, Jr., and G. Wentworth, *ibid.*, **89**, 1852 (1967), and references cited therein.

(3) P. D. Bartlett, S. Friedman, and M. Stiles, *ibid.*, **75**, 1771 (1953).

(4) The addition of nucleophiles across the highly strained cyclopropene double bond to yield a relatively stable cyclopropyl anion is an established process with various nitrogen, oxygen, and sulfur bases;[5] we believe that the reaction of phenyllithium with cyclopropene is the first one involving a carbon base.

(5) (a) G. L. Closs, *Advan. Alicyclic Chem.*, **1**, 83 (1966); (b) T. C. Shields, B. A. Shoulders, J. F. Krause, C. L. Osborn, and P. D. Gardner, *J. Am. Chem. Soc.*, **87**, 3026 (1965); (c) T. C. Shields and P. D. Gardner, unpublished results; we thank Professor Gardner for informing us of his work prior to publication.

(6) We thank Professor L. Friedman for suggesting this modification of the procedure of G. L. Closs and K. D. Krantz, *J. Org. Chem.*, **31**, 638 (1966); the three reagents were used in equimolar amounts.

(7) We have been unable to effect better than a 1% conversion in this reaction as judged by the amount of Diels–Alder adduct[8] produced with cyclopentadiene; the reaction, however, appears to be much cleaner using the Friedman modification.

(8) K. B. Wiberg and W. J. Bartley, *J. Am. Chem. Soc.*, **82**, 6375 (1960).

(9) Allylbenzene undoubtedly arises from the reaction[1] of phenyllithium with a small amount of allyl chloride which does not condense in the cold trap; by far the major portion of phenylcyclopropane is *not* formed by the reaction[1] of phenyllithium with allyl chloride since the ratio of phenylcyclopropane to allylbenzene when allyl chloride is added to phenyllithium under the reaction conditions is 0.35:1.

(10) The *cis* ester was identified by comparison with a sample prepared by carbonation and esterification of the reaction mixture from butyllithium and *cis*-1-bromo-2-phenylcyclopropane;[12] the *trans* ester was prepared either from the commercially available *trans* acid or from *trans*-1-bromo-2-phenylcyclopropane.[12]

(11) We are confident that 2-phenylcyclopropyllithium, the precursor of the carboxylic acid, *is* formed by addition of phenyllithium to cyclopropene since carbonation of a reaction mixture of phenylcyclopropane and phenyllithium yielded none of the cyclopropanecarboxylic acid.

(12) The *cis*- ahd *trans*-bromocyclopropanes were produced from 1,1-dibromo-2-phenylcyclopropane by the method of D. Seyferth and B. Prokai, *J. Org. Chem.*, **31**, 1702 (1966).

the carbonation contained a small amount of phenylcyclopropane and allylbenzene in the mole ratio 1:2. Since an aliquot removed before carbonation and quenched with water showed phenylcyclopropane and allylbenzene in a ratio of 6.4:1, we can conclude that better than 90% of the cyclopropyllithium reacted with carbon dioxide.

We have, therefore, established that the addition of phenyllithium to cyclopropene does proceed stereospecifically. Were any of the *trans*-lithio derivative formed, it would have been converted into the corresponding carboxylic acid.[13] In view of the well-known tendency of organolithium reagents to form aggregates in solution,[14] there is little that we can say about the composition of the transition state leading to *cis* addition of phenyllithium, although a mechanism similar to that proposed[15] for addition to the carbonyl group would lead to the observed stereochemistry. Further experiments are now in progress in an attempt to increase the yield of cyclopropene and to determine if a generally useful synthesis of alkyl- and arylcyclopropanes might be available by the reaction of cyclopropenes with organolithium reagents.

Acknowledgment. We wish to thank the Robert A. Welch Foundation for partial support of this research.

(13) Both *cis*- and *trans*-1-bromo-2-phenylcyclopropanes are converted stereospecifically by butyllithium into the corresponding lithio derivative and then, upon carbonation, into the carboxylic acid of *retained* configuration; the *trans*-lithio compound is considerably less stable than the *cis*- in ether solution, but carbonation still yields, as the major product, the *trans* acid, along with phenylcyclopropane: R. M. Magid and J. G. Welch, unpublished results.

(14) *Cf.* (a) C. G. Screttas and J. F. Eastham, *J. Am. Chem. Soc.*, **88**, 5668 (1966); (b) P. West and R. Waack, *ibid.*, **89**, 4395 (1967), and references cited therein.

(15) C. G. Swain and L. Kent, *ibid.*, 72, 518 (1950).

(16) National Defense Education Act Fellow.

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The Isolation and Properties of Thyrocalcitonin

Sir:

We wish to report the isolation and preliminary characterization of thyrocalcitonin from porcine thyroid glands. The peptide hormone has a potency of 100 MRC[1] units/mg. The extraction procedure of Tenenhouse, *et al.*,[2] was used with modification to prepare crude trichloroacetic acid precipitate. A further 2000-fold purification was obtained by successive steps of ultrafiltration (two- to threefold increase in activity), 8-stage countercurrent extraction (six- to tenfold increase), 150-stage countercurrent extraction (six- to tenfold increase), 15-stage countercurrent extraction (four- to eightfold increase), and final gel chromatography (four- to eightfold increase).

Vacuum ultrafiltration (20–50 mm) through Visking 8/32 cellulose casing at 4° yielded filtrate which usually contained 60% of the thyrocalcitonin and 20–30% of the solids. The lyophilized filtrate was dissolved in 1-butanol–pyridine–0.16 *M* ammonium acetate in 0.16 *M* acetic acid (100:80:235) adjusted to yield a 4% solution containing equal volumes of each phase. A countercurrent extraction was performed using five vessels and passing eight equal volumes of lower phase through each vessel. Each phase was lyophilized and assayed. Solids from the first two upper phases, containing 70–80% of the thyrocalcitonin, were redissolved in the same solvent system at 3–4% concentration and charged to one or two tubes of a 100-stage automatic countercurrent extraction apparatus using 25 ml of each phase per tube. Two hundred upper phase transfers were carried out using a withdrawal technique. Bioassays indicated that transfers 111–130 emerging from the apparatus contained the hormone. These fractions were combined, lyophilized, and further purified by a 15-stage manual countercurrent extraction in the system 1-butanol–acetic acid–water (20:1:20) using a 1.5% concentration. Emulsions encountered were separated by centrifugation. Thyrocalcitonin was recovered from the total contents of tubes 5–10 by lyophilization. The solid was dissolved in 1 ml of 0.4 *M* acetic acid and chromatographed on Biogel P-6 (1.2 × 250 cm) at 28° in the same solvent; 5.1-ml fractions were collected at a flow rate of 1 ml/min. Fractions which contained the thyrocalcitonin were combined and rechromatographed on the same column. Both ultraviolet and refractive index recordings of the column effluent now gave a single Gaussian curve having a band width similar to that obtained with cyanocobalamin on the same column. Based on the retention volumes of insulin and cyanocobalamin, the molecular weight of thyrocalcitonin was estimated to be about 2900. Amino acid analyses of acid hydrolysates carried out before and after oxidation with performic acid gave the following values (μmoles/mg): histidine (0.30, 0.30), arginine (0.59, 0.56), aspartic acid (1.19, 1.22), glutamic acid (0.30, 0.34), threonine (0.59, 0.61), serine (1.17, 1.26), proline (0.62, 0.56), glycine (0.90, 0.94), alanine (0.33, 0.33), half-cystine (0.41, –) cysteic acid (–, 0.65), valine (0.30, 0.31), methionine (0.28, –), leucine (0.90, 0.93), tyrosine (0.30, 0.21), phenylalanine (0.92, 0.93), ammonia (1.67, 5.16). Minor unidentified peaks which may have been tryptophan degradation products were observed. Spectrophotometric studies in 0.01 *N* hydrochloric acid gave maxima at 288 mμ ($E^{1\,cm}_{1\%}$ 13.3) and at 277 mμ ($E^{1\,cm}_{1\%}$ 17.2) indicative of 1 mole of tryptophan.

Thin layer chromatography using silica gel G (Analtech) gave one component at R_f 0.21 using freshly prepared 1-butanol–water–acetic acid (65:25:10) and at R_f 0.71 using 1-butanol–pyridine–acetic acid–water (15:10:3:12) using the nonspecific amide detection reagent of Rydon and Smith.[3] Hypocalcemic activity was recovered from these zones by elution with aqueous acetone. Electrophoresis with paper was unsatisfactory, but essentially single zones were obtained (Rydon–Smith test) at pH 7 and 9 with cellulose acetate strips. At pH 3 and 5 a minor component of lower mobility was observed. Dissolution of the cellulose acetate with acetone and rapid dilution into buffers gave animal activity in the zones indicated.[4] Mobilities are recorded in the table. These data are consistent with a net charge of 3+ at pH 2 and of 1+ at pH 9. Dansylation[5]

(1) T. V. Gudmundsson, I. MacIntyre, and H. A. Soliman, *Proc. Roy. Soc.* (London), **B164**, 460 (1966). We are indebted to the World Health Organization for samples of standard thyrocalcitonin.

(2) A. Tenenhouse, C. Arnaud, and H. Rasmussen, *Proc. Natl. Acad. Sci. U. S.*, **53**, 818 (1965).

(3) H. N. Rydon and P. W. G. Smith, *Nature*, **169**, 922 (1952).

(4) We are indebted to J. Dunn for preliminary studies with this method.

(5) W. R. Gray and B. S. Hartley, *Biochem. J.*, **89**, 60P (1963).