ments using a gas buret show that 1 equiv of molecular oxygen is consumed from air (or oxygen) per molar equiv of **1c** reacting;[7] the products isolated are **2a–d**.

The kinetic product distributions obtained on oxidation in aerated methanol have been determined and are reported in Table I. The low and invariant amount of

Table I. Effect of Reaction Conditions on Kinetic Product Distributions in Aerated Methanol at 0°[a]

Substrate	Reagents, equiv			Yields, %[b] 2d[c]	Yields, %[b] (2a + 2b + 2c)[d]	Mole % 2c in acid fraction
	I_2	$NaOCH_3$	NaI			
1a	2.20	5.0		30	54	9.4
1a	1.25	5.0		37	56	8.7
1a	1.25	5.0	2.0	37	57	8.3
1a	1.25	10.0		76	15	9.7
1a	1.25	15.0		81	*e*	
1c[f]			2.0	*e*	66	7.0[g]
1c[f]		10.0	2.0	76	16	8.2
3b		5.1		0	92	8.9

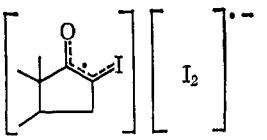
[a] The substrate (3.45×10^{-2} *M*) and reagents were mixed and equilibrated under nitrogen for 20 min prior to saturating with dry air. The products after initial processing were separated into acidic and neutral fractions by extraction with 2.5% NaOH. The extraction caused partial hydrolysis of **2b**, but **2c** and **2d** were not affected. [b] The yields are per cent of theoretical based on substrate. [c] Calculated from weights of neutral fractions, which contained some **1a** (tlc) not exceeding 5% (nmr). [d] Calculated from weights of acid fractions and the mole fractions of **2a–c** as determined from peak heights of the methoxyl and C-13 angular methyl resonances in the nmr (Varian A-60 and C-1024 computer of average transients, 25 scans) [e] Not determined. [f] Initially as suspensions. [g] HA-100 single scan.

2c in the acid fractions suggests that the anhydride **3a** is an intermediate. Basic methanolysis of **3b** (mp 186–189°; $[\alpha]^{24}D$ −148° ($CHCl_3$))[3a] supports this presumption since the percentage of **2c** in the resulting acid mixture is within experimental error of that observed in the acid fractions from oxidation (Table I). Attempts to isolate this intermediate, by aeration of **1c** in methanolic sodium iodide (with or without added methoxide) and quenching immediately after reaction, gives instead the methanolysis products **2b** and **2c**. However, oxidation (I^-, O_2) of **1c** in acetonitrile provides **3a** in 30% yield: mp 209–212° (ether); $[\alpha]^{24}D$ −165° ($CHCl_3$); bands in the infrared at 3609 (hydroxyl) and at 1808 and 1761 (anhydride) cm^{-1}; nmr peaks ($CDCl_3$) at δ 1.23 (3 H, singlet, C-13 angular methyl), 1.78 (1 H, singlet, OH), and 3.55 (1 H, broad multiplet, C-3 α proton).[8] Treatment of **3a** with aqueous pyridine yields **2a** which is identified by comparison of physical properties with an authentic sample.

Partitioning between the anhydride **3a** and the diester **2d** is dependent only on the concentration of base employed. In view of the incorporation of molecular oxygen, an initial hydroperoxide intermediate is presumed (Chart I) which may either rearrange to anhydride or undergo specific nucleophilic attack by methoxide to yield the diester. Under neutral or basic conditions, cleavages like these occur with α-hydroperoxy ketones[9] and in reactions that proceed through α-hydroperoxy ketone intermediates.[10]

The conditions under which oxidation occurs appear to eliminate both base-catalyzed[11] and light- or thermal-initiated free-radical mechanisms. Thus, **1b**, prepared *in situ* with 1 equiv of iodine and excess base, is recovered in 67% yield after aeration for 6 hr. Suspensions of **1c** in aerated methanolic sodium methoxide are stable in the absence of iodide ion (86% recovery of **1c** after 3 hr). Also, the reaction proceeds as rapidly to give the same products in both the presence or absence of light and is not inhibited by added iodoform which is recovered in 70% yield.

The unusual reactivity toward oxygen presently observed may involve a molecular complex (π complex) in equilibrium with both the enolate of **1b** and iodine and with **1c** and iodide ion.[12] Complete electron transfer from the donor to iodine may take place in the ground state to give a charge-transfer complex such as **4**.[13] Such complexes are expected to show free-radical behavior.[14]



4

Acknowledgment. We thank Drs. J. S. Tadanier and H. E. Zaugg of this department and Dr. P. Beak of the University of Illinois for helpful discussions.

(7) Two other enolizable ketones have been briefly studied. They are 5α-androstan-17β-ol-3-one and propiophenone. Quantitative measurements show that in methanol each consumes molecular oxygen (0.75 equiv) under conditions of base-catalyzed iodination (4.5 and 2.5 equiv of iodine, respectively).

(8) For comparison **3b** shows carbonyl bands in the infrared ($CHCl_3$) at 1808, 1760 (anhydride), and 1728 (acetate) cm^{-1}, while in the nmr ($CDCl_3$) peaks are observed at δ 1.22 (3 H, singlet, C-13 angular methyl), 2.02 (3 H singlet, acetate), and 4.57 (1 H, broad multiplet, C-3α proton). The C-15 methylene protons of **3a** and **3b** gave superimposable patterns of the ABX type with $|J_{AB}|$ = 18 Hz but only the A proton was completely separated from the resonance envelope of the steroid nucleus.

(9) P. S. Bailey and S. S. Bath, *J. Am. Chem. Soc.* 79, 3120 (1957).

(10) (a) E. Elkik, *Bull. Soc. Chim. France*, 933 (1959); (b) H. Kwart and N. J. Wegemer, *J. Am. Chem. Soc.*, 83, 2746 (1961), and references cited therein.

(11) (a) W. von E. Doering and R. M. Haines, *J. Am. Chem. Soc.*, 76, 482 (1954); (b) G. A. Russell, E. R. Talaty, and R. H. Horrocks, *J. Org. Chem.*, 32, 353 (1967).

(12) The failure of oxidation with 1 equiv of iodine could be the result of one of the following situations. If the rate-determining step precedes the step involving oxygen, then the rate of deiodination of **1b** with iodide ion must be very slow compared to the rate of deiodination of **1c** with iodide ion. Other wiseiodine would accumulate in the presence of **1b** and base, allowing oxidation to take place. However, if the oxygenation step is rate determining, then the equilibrium between **1b** and a mixture of **1a** and **1c** must favor **1b**. Otherwise **1c** would accumulate in the presence of iodide ion and allow oxidation to take place. We thank a referee for suggesting the former possibility.

(13) A referee has pointed out that the donor system is isoelectronic with the stable radicals derived from reduction of 1,2-diones.[11b]

(14) D. N. Stamires and J. Turkevich, *J. Am. Chem. Soc.*, 85, 2557 (1963).

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Studies on Polypeptides. XXXVII. Competitive Inhibition in the S-Peptide–S-Protein System[1,2]

Sir:

Structure–function studies carried out in our laboratories have shown that the six C-terminal amino acid

(1) The authors wish to express their appreciation to the U. S. Public Health Service for generous support of this investigation.

(2) The peptides and peptide derivatives mentioned are of the L configuration.

Chart I

H-Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser-Ser-Thr-Ser-Ala-Ala-OH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
S-peptide$_{1-20}$ (I)

H-Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-Pyr(3)Ala-Met-Asp-OH
12-β-(pyrazolyl-3)alanine S-peptide$_{1-14}$ (II)

residues of S-peptide$_{1-20}$ (I)[3] can be removed without measurably altering the S-protein activating characteristics. S-Peptide$_{1-14}$ is as active as S-peptide$_{1-20}$ as concerns activation of S-protein toward RNA and cytidine 2′,3′-phosphate.[4] S-Peptide$_{1-13}$ is also capable of bringing about essentially full activation of S-protein, but higher molar peptide:protein ratios (approximately 30:1) are required.[5] The *d*-sulfoxides of S-peptide$_{1-13}$, S-peptide$_{1-14}$, and S-peptide$_{1-15}$ are considerably less effective activators of S-protein.[4,5] The amide of S-peptide$_{1-12}$ generates fully active enzyme with S-protein at molar ratios of 188:1,[5] but S-peptide$_{1-11}$ which lacks the histidine residue in position 12 is inactive at molar ratios as high as 8000:1.[5] Removal of lysine-1 exerts little effect on the S-protein activating characteristics of S-peptide$_{1-13}$ but elimination of both lysine-1 and glutamic acid-2 markedly decreases potency.[5]

In 1960 we suggested that amino acid residues in biologically active polypeptides be classified as "binding sites" and "active sites."[6] In order to qualify as an "active site" in the case of S-peptide an amino acid residue must participate directly in the catalytic event and its replacement by other residues must result in totally inactive products. "Binding sites" in S-peptide, according to our definition, are those amino acid residues which are responsible for the firm, noncovalent attachment of the peptide to the protein. Consequently, alterations or replacements at these sites should result in products with a decreased ability to activate S-protein. This effect must be reversible simply by adding excess peptide to shift the equilibrium toward the undissociated complex.

The structure–function studies sumarized above have identified three significant "binding sites" in S-peptide, namely, glutamic acid-2, aspartic acid-14, and methionine-13. Our findings point to histidine-12 as the "active site" of S-peptide and suggest that the rest of the molecule functions as a vehicle for bringing this histidine into the correct conformation in the enzyme's active site. In addition to its role in catalysis,[7] histidine-12 may also contribute to binding, but the available experimental results did not allow conclusions to be drawn regarding this point.

We reasoned that if these interpretations were correct and if histidine-12 did not contribute significantly to binding, it should be possible to construct competitive inhibitors to S-peptide$_{1-20}$ *via* histidine substitutions.

To test this prediction experimentally we synthesized 12-β-(pyrazolyl-3)alanine S-peptide$_{1-14}$ (II) (see Chart I) and explored its ability to compete with S-peptide for S-protein with RNA as the substrate. This analog was selected because the molecular dimensions of β-(pyrazolyl-3)alanine [Pyr(3)Ala] are very similar to, if not identical with, those of histidine; the two amino acids differ markedly as concerns the acid–base properties of the ring portions of their molecules.[8]

At molar ratios as high as 1000:1 peptide II fails to activate S-protein, but, as is apparent from the results presented in Figure 1, this peptide is a potent S-peptide inhibitor. At a 1:1 molar ratio of peptides II:I approximately 50% inhibition is observed. Complete inhibition ensues at a molar ratio of approximately 20:1. The inhibition can be completely reversed by adding S-peptide to the inactive complex. The inhibition curve follows closely the one which can be calculated for equal binding of the two peptides, a result which demonstrates that in the presence of RNA peptide II associates with S-protein almost as firmly as does S-peptide$_{1-20}$. It follows that the binding contribution of β-(pyrazolyl-3)alanine and histidine-12 must be of the same order of magnitude and that histidine-12 is the "active site" of S-peptide.

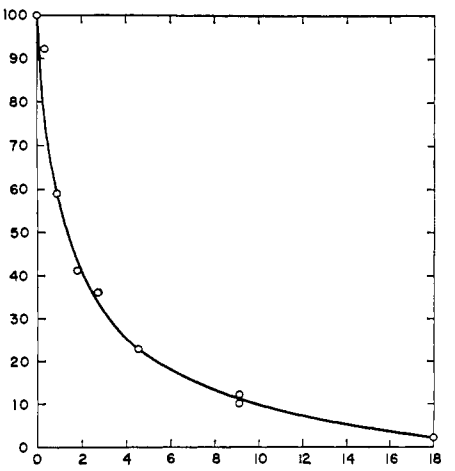

Figure 1. Inhibition of RNase S′ by 12-Pyr(3)Ala S-peptide$_{1-14}$ with yeast RNA as the substrate. Molar ratio S-peptide:S-protein 1:1. Abscissa: molar ratio of inhibitor to S-peptide. Ordinate: per cent activity remaining.

The importance of the reduced methionine sulfur for binding, previously observed in activation studies with S-peptide$_{1-13}$, S-peptide$_{1-14}$, and S-peptide$_{1-15}$,[4] receives full confirmation from inhibition studies with the *d*-sulfoxide of peptide II. In our system this molecule is a weak S-peptide antagonist, exhibiting 50% inhibition at a molar ratio of approximately 500:1. The observation that 12-β-(pyrazolyl-3)alanine S-peptide$_{1-12}$ amide[8] fails to compete with 13-α-aminobutyric acid S-peptide$_{1-13}$[5] at molar ratios as high as 1000:1 provides independent support for the importance of methionine-13 as a binding site in S-peptide$_{1-20}$.

The method of synthesis of II is based on our previous studies with closely related peptides.[9] The azide

(3) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958).
(4) K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Am. Chem. Soc.*, **88**, 631 (1966).
(5) F. M. Finn and K. Hofmann, *ibid.*, **87**, 645 (1965).
(6) K. Hofmann, *Brookhaven Symp. Biol.*, **13**, 184 (1960).
(7) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2413 (1963).
(8) K. Hofmann and H. Bohn, *J. Am. Chem. Soc.*, **88**, 5914 (1966).
(9) K. Hofmann, R. Schmiechen, M. J. Smithers, R. D. Wells, Y. Wolman, and G. Zanetti, *ibid.*, **87**, 640 (1965).

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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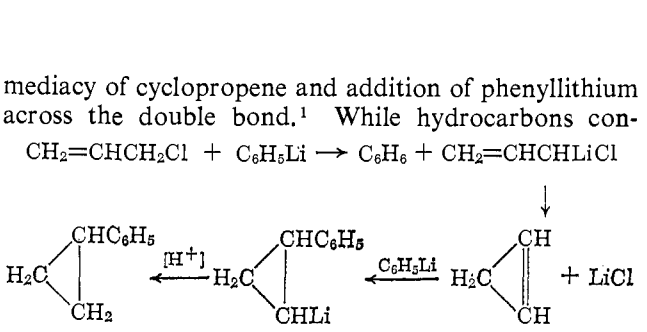
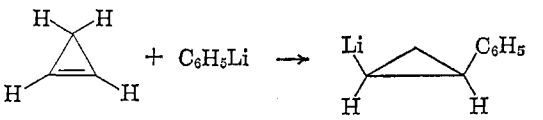
The Stereochemistry of the Addition of Phenyllithum to Cyclopropene

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-



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).