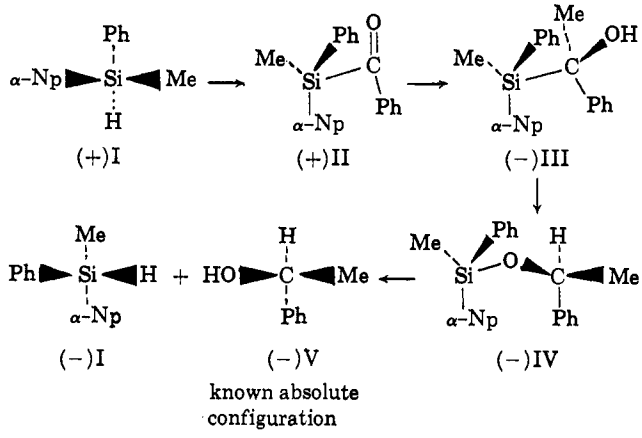
lectivity at the carbon atom since the 1-phenylethanol ultimately produced is a 72:28 mixture of (−) and (+)-enantiomers, based on its observed rotation.

Cram's rule of asymmetric induction[2] should apply in the addition of methyl Grignard reagent to benzoylmethyl-α-naphthylphenylsilane (II) and thus the configuration of the asymmetric carbon atom in the predominant diastereomer formed is defined relative to the configuration of the silicon atom.[3]

Provided that the subsequent reactions of rearrangement or reduction do not alter the configuration at carbon, the isolation of (−)-1-phenylethanol of known absolute configuration[4] can be used to establish the absolute configuration of the predominant carbinol. Reduction of the silyl ether will not affect the configuration since the cleavage occurs at the Si–O bond.[5] The rearrangement of the silylcarbinol by sodium–potassium alloy in diethyl ether is believed to involve an intermediate carbanion or species of considerable carbanionic character.[6] Cram has found that related carbanions under similar conditions retain their configuration,[7] and this, together with the great rapidity with which the rearrangement occurs, leads us to believe that the configuration at the asymmetric carbon atom is retained during rearrangement. Indeed, if the two diastereomeric carbinols were formed in the ratio 72:28, the rearrangement is completely stereospecific and at the very worst had essentially only one carbinol been produced, the rearrangement was significantly stereoselective.

Knowing the absolute configuration of (−)-1-phenylethanol (V) and assuming that the configuration at carbon, established by Grignard addition according to Cram's rule, is retained during the rearrangement of the silylcarbinol to the silyl ether, then the absolute configuration of the key compounds in the Walden cycle are



It is of interest to note that the absolute configuration of (+)-methyl-α-naphthylphenylsilane derived above is in accord with that predicted by Brewster's rules of atomic asymmetry,[8] accepting that α-naphthyl is more polarizable than phenyl. If α-naphthyl is assigned a polarizability value of 4, and Brewster's values are used for other groups, then the directions of rotation of all optically active silanes in the methyl, α-naphthyl, phenyl series for which polarizability data are available are correctly predicted on the basis of the stereochemical transformations of retention or inversion reported by Sommer and Frye.[5,9,10] Thus the chlorosilane known to be formed from (+)-silane (α-Np > Ph > Me > H, clockwise) by retention of configuration[9,10] would be predicted to be levorotatory (Cl > α-Np > Ph > Me, anticlockwise) by Brewster's rules, as is found experimentally. Further work is in progress.

The following are typical experimental data: Treatment of (+)-methyl-α-naphthylphenylsilane, $[\alpha]^{25}D$ +33.2° (cyclohexane, *c*, 5.3), with chlorine gave chlorosilane $[\alpha]^{25}D$ −6.22° (cyclohexane, *c* 9.1). Treatment with benzylsodium in ether–toluene gave 54% benzylsilane, m.p. 69–70° after several recrystallizations from methanol–ethanol, $[\alpha]^{25}D$ −6.68° (cyclohexane, *c* 6.0).

Bromination with two moles of N-bromosuccinimide[11] gave a 94.7% yield of dibromobenzyl compound, m.p. 114–114.5°, $[\alpha]^{25}D$ 12.90° ($CHCl_3$, *c* 6.5) which on hydrolysis with silver acetate in benzene–acetone–water[11] gave, after recrystallization from ethanol, 94% of yellow benzoylsilane, m.p. 68–70°, $[\alpha]^{25}D$ 6.72° (C_6H_6, *c* 9.45).

Treatment of benzoylmethyl-α-naphthylphenylsilane in ether at −60° with one mole of methylmagnesium bromide (inverse addition) over 75 min. gave on work-up 86% of a sirupy carbinol mixture, $[\alpha]^{25}D$ −16.0° (C_6H_6, *c* 9.43). Without attempting to separate the diastereomers, the carbinol mixture was treated in ether over 1.7 hours with 2 drops of Na–K alloy. Work-up gave a clear gummy silyl ether in 89% yield, $[\alpha]^{25}D$ −23.7° (cyclohexane, *c* 8.2), which was reduced with lithium aluminum hydride in butyl ether to give by crystallization 98% of crude methyl-α-naphthylphenylsilane, $[\alpha]D$ −25.4°, twice recrystallized from pentane to give 68% of material, m.p. 63–64°, $[\alpha]^{25}D$ −33.5° (cyclohexane, *c* 10.3), and by distillation 38% of 1-phenylethanol, b.p. 47–49° (0.08 mm.), $[\alpha]^{25}D$ −23.2° ($CHCl_3$, *c* 12.79) (reported[12] $[\alpha]^{20}D$ 54.13° ($CHCl_3$, *c* 5.4)), together with additional alcohol which co-distilled with the dibutyl ether, isolated in 10% yield as crude acid phthalate, $[\alpha]^{20}D$ 8.3°. Analyses and infrared spectra were in agreement with the expected structures.

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(2) D. J. Cram and F. A. Abd Elhafez, *J. Am. Chem. Soc.*, **74**, 5828 (1952).

(3) The selectivity of the Grignard addition is markedly temperature sensitive. With addition at room temperature the diastereomeric mixture of carbinols had $[\alpha]D$ +3.03, whereas addition at −60° gave $[\alpha]D$ −16.0° with the specific rotations of the derived 1-phenylethanol being −7.26° and −23.1°, respectively. The actual proportions of diastereomers is not as yet established.

(4) P. A. Levene and S. A. Harris, *J. Biol. Chem.*, **113**, 55 (1936); P. A. Levene and P. G. Stevens, *ibid.*, **89**, 471 (1930).

(5) L. H. Sommers and C. L. Frye, *J. Am. Chem. Soc.*, **82**, 4118 (1960).

(6) A. G. Brook, *ibid.*, **80**, 1886 (1958).

(7) D. J. Cram, A. Langemann and F. Hauck, *ibid.*, **81**, 5750 (1959).

(8) J. H. Brewster, *ibid.*, **81**, 5475 (1959).

(9) L. H. Sommer and C. L. Frye, *ibid.*, **81**, 1013 (1959); **82**, 3796 (1960); **83**, 2210 (1961).

(10) C. L. Frye, Ph.D. Thesis, Pennsylvania State University, 1960.

(11) A. G. Brook, *J. Am. Chem. Soc.*, **79**, 4373 (1957).

(12) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **105**, 1115 (1914).

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STUDIES ON POLYPEPTIDES. XXVI. PARTIAL SYNTHESIS OF AN ENZYME POSSESSING HIGH RNASE ACTIVITY[1-3]

Sir:

We wish to describe experiments which appear to represent the first partial laboratory synthesis of an

(1) The authors wish to express their appreciation to the U. S. Public Health Service, the National Science Foundation and the American Cancer Society for generous support of this investigation.

(2) The peptides and peptide derivatives mentioned are of the L-configuration. In the interest of space conservation the customary L-designation for individual amino acid residues is omitted.

(3) See *J. Am. Chem. Soc.*, **84**, 4481 (1962), for paper XXV in this series.

enzyme. We find that addition to S-protein[4] of synthetic lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminylhistidylmethionine (I) ($[\alpha]^{27}D$ −64.9° in 10% acetic acid; R^2_f 0.4 x his[5]; single ninhydrin, Pauly, Sakaguchi and methionine positive spot on paper electrophoresis at pH 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.09}glu_{3.09}thr_{0.95}ala_{2.95}phe_{1.04}arg_{1.00}his_{1.04}met_{0.95}$) produces a ribonuclease analog which exhibits 68–72% the biological activity of RNase-S′ with yeast RNA as substrate.[6] The reconstructed enzyme attains maximal catalytic activity when approximately 10 moles of peptide are added per mole of S-protein, but an enzyme exhibiting approximately 50% the activity of RNase-S′ results at a molar ratio of 3:1. For maximum activity (62–65% that of RNase-S′) toward uridine-2′:3′-cyclic phosphate[7] approximately 30 moles of I per mole of S-protein are required.

The following peptides, which correspond to sections of the N-terminal sequence proposed for beef RNase-A, fail to generate enzymic activity[6] upon addition to S-protein in molar ratios as high as 100 to 1: histidylmethionine (II) (*Anal.* Found: C, 46.1; H, 6.4; N, 19.4; $[\alpha]^{28}D$ −15.4° in water; single ninhydrin, methionine and Pauly positive spot; R_f^1 0.31; R_f^2 2.1 x his); phenylalanylglutamylarginylglutaminylhistidylmethionine (III) ($[\alpha]^{26}D$ −35.6° in 10% acetic acid; single ninhydrin, methionine, Pauly and Sakaguchi positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; R_f^2 1.5 x his; amino acid ratios in acid hydrolysate $phe_{1.00}glu_{2.07}arg_{0.98}his_{0.97}met_{1.00}$); aspartylserylserylthreonylserylalanylalanine (IV) ($[\alpha]^{26}D$ −63.5° in water; single ninhydrin positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $asp_{0.94}ser_{3.10}thr_{0.94}ala_{2.03}$); lysylglutamylthreonylalanylalanylalanyllysine (V) ($[\alpha]^{28}D$ −60.8° in 10% acetic acid; single ninhydrin positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.03}glu_{0.97}thr_{0.93}ala_{3.07}$; amino acid ratios in LAP digest $lys_{2.07}glu_{0.97}thr_{0.93}ala_{3.00}$); and lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutamine (VI) ($[\alpha]^{25}D$ −79.2° in 10% acetic acid; single ninhydrin and Sakaguchi positive spot; R_f^2 0.4 x his; single spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.06}arg_{1.02}thr_{0.95}glu_{2.96}ala_{3.05}phe_{0.95}$). Combinations of peptides (V + III), (VI + II) and (II + IV) in the molar ratios 100:100:1 are inactive.

These results support the revised amino acid sequence for positions 1 to 13 in RNase-A[8] and demonstrate that a partially synthetic RNase from which a sizable fragment of the covalent peptide chain (amino acid residues 14 to 20) is eliminated exhibits significant enzymic activity.

Special importance has been attributed to the sequence aspartylserine for catalytic activity of a number of proteolytic and esteratic enzymes.[9] Our experiments unequivocally eliminate aspartylserine (positions 14 to 15) as essential for RNase activity. The ability of peptide I to activate S-protein and the lack of this property in peptide VI emphasizes the significance for catalytic activity of the histidylmethionine portion in the ribonuclease molecule.[10]

We have confirmed the results of Vithayathil and Richards[11] that performic acid-oxidized S-peptide when added to S-protein at a molar ratio of 3:1 regenerates 90% of the activity of RNase-S′ toward RNA. However, oxidation of I with hydrogen peroxide[12] or performic acid[13] results in marked diminution of its ability to activate S-protein against this substrate. Exposure to thioglycolic acid (45–50° for 24 hours) regenerates essentially the full activity of the hydrogen peroxide-treated peptide. These observations implicate the methionine sulfur as the site for the reversible oxidation–reduction behavior and suggest that the thioether sulfur of peptide I is important for maximal activation of S-protein. The ability of the 13α-aminobutyric acid analog of I to regenerate enzymic activity with S-protein is being investigated.

Correlation between ability of S-peptide derivatives to activate S-protein and their capacity to bind to this RNase fragment may contribute significantly toward understanding of protein–peptide interaction on the one hand and topography of the active site on the other. Studies along these lines are in progress.

Current theories pertaining to the active site of RNase may need revision and future ones will have to take into consideration the experimental findings which are presented in this communication.

Acknowledgment.—The skillful technical assistance of Mrs. Chizuko Yanaihara, Mrs. Maria Gunther, Miss Ursula Egli and Mr. John L. Humes is gratefully acknowledged.

(4) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958); the abbreviations used are: RNase-S, subtilisin-modified beef ribonuclease RNase-A; S-peptide, the eicosapeptide obtained from RNase-S; S-protein, the protein component obtained from RNase-S; RNase-S′, the reconstituted enzyme obtained by mixing equimolar proportions of S-peptide plus S-protein; RNA, ribonucleic acid; LAP, leucine aminopeptidase.

(5) R_f^1 values refer to the Partridge system (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948)); R_f^2 values refer to the system 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (S. G. Waley and J. Watson, *ibid.*, **55**, 328 (1953)).

(6) RNase determinations were carried out with a Cary 14 recording spectrophotometer using a scale expander (range 0–0.1 optical density) essentially as described by M. Kunitz, *J. Biol. Chem.*, **164**, 563 (1946).

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RECEIVED JANUARY 31, 1963

AROMATIC SILICON SYSTEMS—A REINVESTIGATION

Sir:

In attempting to extend the investigation we recently described under the heading of Aromatic Silicon Systems,[1] we have discovered major discrepancies in the original work. While it is still too early to be able to state precisely what the difficulties are, it is clear that a significant portion to the published work cannot be duplicated. We deem it advisable to call attention to this fact immediately.

The entire work is under reinvestigation in our laboratory and we hope to report our new findings as soon as possible.

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RECEIVED FEBRUARY 8, 1963

(1) R. A. Benkeser, *et al.*, *J. Am. Chem. Soc.*, **84**, 4723, 4727 (1962). A preliminary announcement of this work appeared in *J. Am. Chem. Soc.*, **83**, 3716, 5029 (1961).