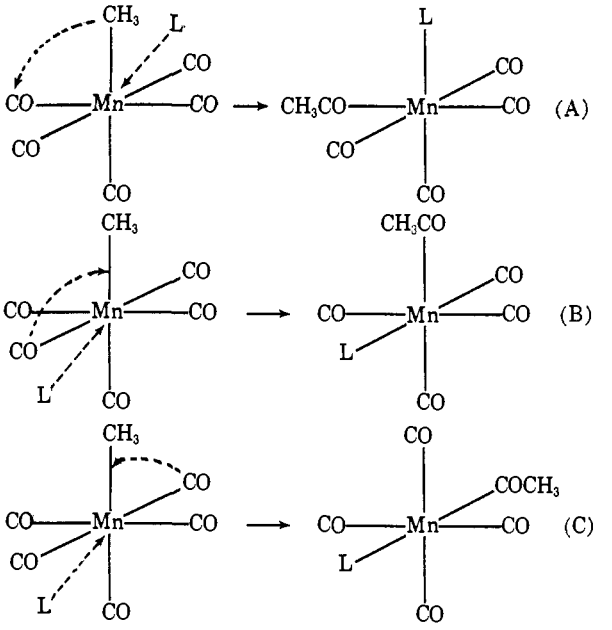
The stereochemistry and hence the mechanism of this type of reaction is, however, rather ill-defined. For example, in the reaction of $CH_3Mn(CO)_5$ with Ph_3P, mixtures of *cis*- and *trans*-$CH_3COMn(CO)_4Ph_3P$ are obtained,[5] and it is not clear whether both isomers are formed by independent reaction paths or whether the reaction is stereospecific and that the other isomer *i.e.*, *cis* or *trans*, is formed in a subsequent thermodynamically controlled step.

The bridgehead phosphites 4-methyl- (or -ethyl-) 2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane react at room temperature with methylmanganese pentacarbonyl in methylene chloride or chloroform solution to give in high yield the complexes *cis*-$CH_3COMn(CO)_4L$, where L is $P(OCH_2)_3CCH_3$ or $P(OCH_2)_3CC_2H_5$. This contrasts with the earlier observation[3] that disubstituted complexes $CH_3COMn(CO)_3L_2$ are formed in the reaction of phosphites with $CH_3Mn(CO)_5$.

When the reaction of $CH_3Mn(CO)_5$ with the ligand $P(OCH_2)_3CCH_3$ is followed by observing the proton magnetic resonance spectrum immediately after mixing the reactants and then at suitable time intervals, the peaks at τ 10.1 (singlet, $CH_3Mn(CO)_5$), 9.31 (singlet, $P(OCH_2)_3CCH_3$), and 6.18 (doublet, J = 2 cps, $P(OCH_2)_3CCH_3$) decrease in intensity and are replaced by peaks corresponding to a single isomer at τ 9.18 (singlet, $MnP(OCH_2)_3CCH_3$), 7.58 (singlet, CH_3COMn),[6] and 5.85 (doublet, J = 5.7 cps, $MnP(OCH_2)_3CCH_3$), with final relative integrated intensities of 1:1:2, respectively. The rate of appearance of the new peaks, which was dependent on phosphite concentration, corresponded exactly to the rate of disappearance of the peaks assigned to the reactants. The infrared spectrum of the carbonyl region of the reaction mixture showed the progressive development of four terminal carbonyl bands at 2082 (m), 2008 (s), 1983 (s), and 1972 (s) cm^{-1}, and an acyl band at 1618 (m) cm^{-1} [7] which corresponds to the formation of the complex *cis*-$CH_3COMn(CO)_4[P(OCH_2)_3CCH_3]$ with C_s symmetry.

These observations are of particular interest in that they demonstrate that in the reaction of $CH_3Mn(CO)_5$ with the sterically compact ligand $P(OCH)_3CCH_3$ a stereospecific reaction occurs. It is suggested that each act of substitution gives *cis*-$CH_3COMn(CO)_4[P(OCH_2)_3CCH_3]$ directly. However, an alternative explanation of these results is that *trans*-$CH_3COMn(CO)_4[P(OCH_2)_3CCH_3]$ is formed in a slow step and then undergoes a very fast irreversible rearrangement to the corresponding *cis* isomer. We regard this as unlikely, because it has been shown[5] that *cis*- and *trans*-$CH_3COMn(CO)_4Ph_3P$ rapidly equilibriate in solution, the *trans* isomer predominating, and it is not clear why in the system *trans*-$CH_3COMn(CO)_4[P(OCH)_3CCH_3] \rightleftarrows$ *cis*-$CH_3COMn(CO)[P(OCH_2)_3CCH_3]$ the equilibrium should now be entirely in favor of the *cis* isomer, as would be required by this alternative explanation.

Both Mechanisms, A or B, are both consistent with the observed stereochemistry but mechanism C, which involves insertion of a molecule of CO, previously



bonded *trans* to the point of attack of the ligand on manganese between the methyl group and the manganese, can be excluded, because it would afford the *trans* isomer $CH_3COMn(CO)_4L$.

A kinetic investigation of the transformation

$$trans\text{-}CH_3COMn(CO)_4Ph_3P \overset{-CO}{\rightleftharpoons} cis\text{-}CH_3Mn(CO)_4Ph_3P$$

leads to the proposal[8] that mechanism A, *i.e.*, methyl migration, is preferred to mechanism B. However, the fact that *cis*- and *trans*-$CH_3COMn(CO)_4Ph_3P$ rapidly equilibriate in solution makes such a conclusion of doubtful value.

The complexes *cis*-$CH_3COMn(CO)_4[P(OCH_2)_3CR]$ (R = CH_3 or C_2H_5) decarbonylate slowly on heating to give *cis*-$CH_3Mn(CO)_4[P(OCH_2)_3CR]$.

(5) C. S. Kraihanzel and P. K. Maples, *J. Am. Chem. Soc.*, 87, 5267 (1965).

(6) CH_3COMn protons normally have a chemical shift in the range τ 7.4–7.6.

(7) The reaction mixture was evaporated and the spectrum measured in cyclohexane solution. The evaporated solution on redissolving in $CDCl_3$ showed an unchanged pmr spectrum.

(8) R. J. Mawby, F. Basolo, and R. G. Pearson, *J. Am. Chem. Soc.*, 86, 5043 (1964).

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Studies on Polypeptides. XXXV. Synthesis of S-Peptide$_{1-20}$ and Its Ability to Activate S-Protein[1-3]

Sir:

A recent communication by Scoffone, *et al.*,[4] describing a synthesis of 10-ornithine-S-peptide, prompts us to record at this time the synthesis of the eicosapeptide lysylglutamylthreonylalanylalanylalanyllysylphen-

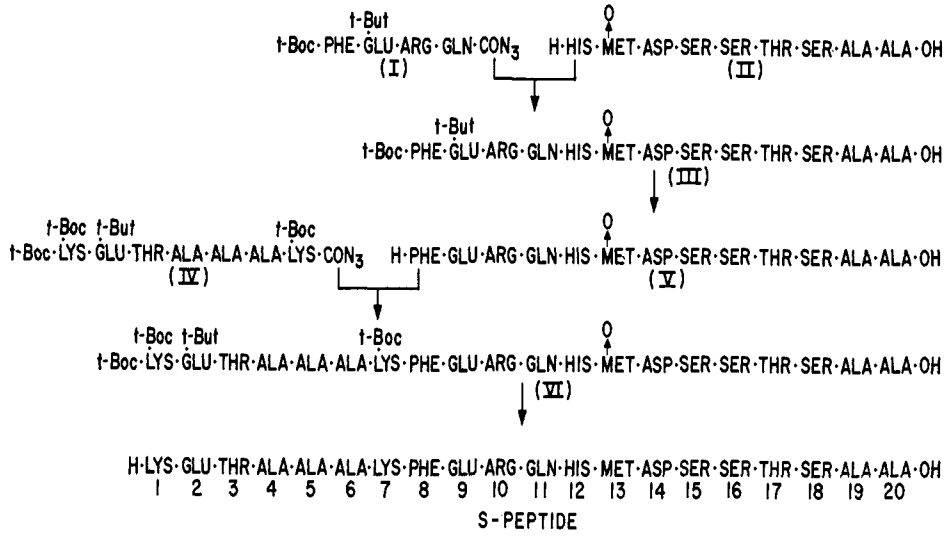
(1) The authors wish to express their appreciation to the U. S. Public Health Service and the American Cancer Society for generous support of this investigation.

(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. General conditions for paper and thin layer chromatography are those given in ref 6; *t*-Boc = *t*-butoxycarbonyl; *t*-But = *t*-butyl ester.

(3) See K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Am. Chem. Soc.*, 88, 3633 (1966), for paper XXXIV in this series.

(4) E. Scoffone, F. Marchiori, R. Rocchi, G. Vidali, A. Tamburro, A. Scatturin, and A. Marzotto, *Tetrahedron Letters*, No. 9, 943 (1966).

Chart I



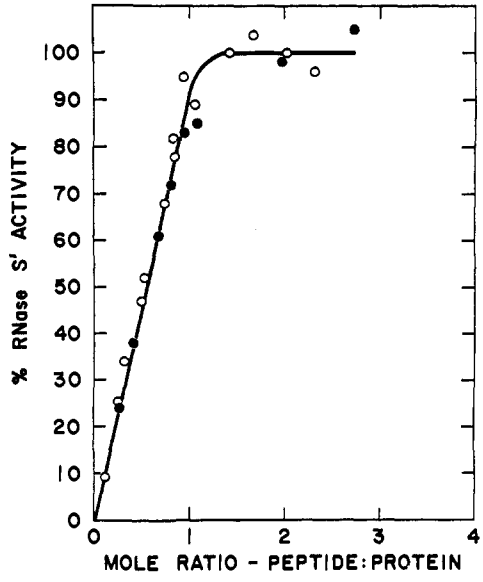


Figure 1. Activity of partially synthetic and natural ribonucleases-S′ using yeast RNA as substrate: ●, partially synthetic enzyme; ○, natural enzyme. Each point is based on triplicate assays. For assay conditions see ref 11.

ylalanylglutamylarginylglutaminylhistidylmethionylaspartylserylserylthreonylserylalanylalanine (S-peptide$_{1-20}$).[5] The highly purified synthetic product brings about activation of S-protein identical with that observed with "S-peptide" (Figure 1). The method of synthesis (Chart I) is based on our previous studies with closely related peptides.[3,6-9] Coupling of the protected tetrapeptide azide I[7] with the nonapeptide II[8] gave the partially protected tridecapeptide III which was isolated in the form of its hexahydrate following countercurrent distribution in the solvent system 10% acetic acid–1-butanol (*Anal.* Found: C, 46.5; H, 7.3; N, 15.6; O, 29.2; S, 2.3); $[\alpha]^{23}D$ −37.4° (*c* 2.07, 10% acetic acid); single ninhydrin-negative, chlorine-, Pauly-, and Sakaguchi-positive spot with R_f^1 0.50, R_f^2 0.30, and R_f^3 2.33 × His; amino acid ratios in acid hydrolysate $Phe_{0.98}Glu_{1.97}Arg_{1.03}His_{1.03}Met_{0.76}Asp_{1.02}Ser_{2.99}Thr_{0.93}Ala_{2.05}$.

Exposure to trifluoroacetic acid converted III into a trifluoroacetate salt of V which was changed to the acetate salt with Amberlite IRA-400. Peptide V was obtained in the form of its acetate hexahydrate (*Anal.* Found: C, 44.4; H, 7.2; N, 15.6; O, 29.8); $[\alpha]^{24}D$ −38.8° (*c* 2.46, 10% acetic acid); single ninhydrin-, Pauly-, Sakaguchi-, and chlorine-positive spot with R_f^3 0.47 × His; single spot on paper electrophoresis at pH 1.9, 3.5, 6.5 and 8.0; amino acid ratios in acid hydrolysate $Phe_{0.96}Glu_{2.24}Arg_{1.06}His_{1.00}Met_{0.78}Asp_{0.98}Ser_{2.89}Thr_{0.90}Ala_{1.98}$; amino acid ratios in aminopeptidase M[10] (AP-M) digest $Phe_{1.15}Glu_{1.20}Arg_{0.91}Gln_{0.84}$-

O
↑

$His_{0.80}Met_{0.86}Asp_{0.82}Ser_{3.26}Thr_{1.07}Ala_{2.09}$.

For preparation of VI the tridecapeptide acetate V was acylated in the presence of triethylamine with the azide IV essentially in the manner described previously.[9] For isolation of VI the crude reaction product was distributed between 2% acetic acid and 1-butanol and the material from the 1-butanol phases was chromatographed on the ion exchanger AG1X2 (acetate cycle) using the solvent systems 2-propanol–methanol–water (1:1:1) and 2-propanol–methanol–0.1 *N* acetic acid (1:1:1) for column development and elution. The partially protected peptide VI (R_f^{VI} 0.2), contaminated with rearrangement products from IV (R_f^{VI} 0.7), was present in the 2-propanol–methanol–0.1 *N* acetic acid eluates. Without further purification this material was dissolved in trifluoroacetic acid and the solution was kept at room temperature for 30 min. Following exchange of the trifluoroacetate ions for acetate ions

(5) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958); RNAase-S, subtilisin modified beef ribonuclease RNAase-A; S-peptide, the peptide obtained from RNAase-S; S-protein, the protein component obtained from RNAase-S; RNAase-S′, the reconstituted enzyme obtained by mixing equimolar proportions of S-protein and S-peptide. According to M. S. Doscher and C. H. W. Hirs, *Federation Proc.*, **25**, 527 (1966), natural S-peptide is a mixture of at least three components, very likely S-peptide$_{1-20}$, S-peptide$_{1-21}$, and S-peptide$_{1-22}$. For clarity natural S-peptide will be designated "S-peptide."

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

(7) K. Hofmann, W. Haas, M. J. Smithers, R. D. Wells, Y. Wolman, N. Yanaihara, and G. Zanetti, *ibid.*, **87**, 620 (1965).

(8) K. Hofmann, W. Haas, M. J. Smithers, and G. Zanetti, *ibid.*, **87**, 631 (1965).

(9) K. Hofmann, R. Schmiechen, M. J. Smithers, R. D. Wells, Y. Wolman, and G. Zanetti, *ibid.*, **87**, 640 (1965).

(10) G. Pfleiderer and P. G. Celliers, *Biochem. Z.*, **339**, 186 (1963).

(IRA-400), the product was subjected to chromatography on carboxymethylcellulose (CMC). The free eicosapeptide *d*-sulfoxide was eluted from the column with 0.025 *M* ammonium acetate; ammonium acetate was removed by repeated lyophilization of the peptide; $[\alpha]^{21}D$ −43.1° (*c* 1.04, 10% acetic acid); single Pauly- and ninhydrin-positive spot on paper electrophoresis at pH 1.9, 3.5, 6.5, and 8.0; amino acid ratios in acid hydrolysate $Lys_{2.03}Glu_{3.16}Thr_{1.95}Ala_{5.04}Phe_{0.99}Arg_{0.96}His_{0.96}$-$Met_{0.73}Asp_{0.96}Ser_{2.95}$; amino acid ratios in AP-M digest $Lys_{2.34}Glu_{2.55}Thr_{2.16}Ala_{5.78}Phe_{1.26}Arg_{1.15}Gln_{0.80}His_{0.71}$-

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↑
$Met_{0.74}Asp_{0.80}Ser_{2.65}$.[11]

The S-protein activating potency of a sample of this material has been recorded.[3,12] Reduction of the eicosapeptide *d*-sulfoxide with thioglycolic acid[3] gave, in quantitative yield, the crude eicosapeptide (S-peptide$_{1-20}$). For final purification this material was combined with S-protein and the ensuing RNAase-S′ purified by chromatography on Amberlite CG-50.[13] The highly active, partially synthetic enzyme was then dissociated into S-peptide$_{1-20}$ and S-protein[14] and the peptide separated from protein contaminants by chromatography on CMC. Synthetic S-peptide$_{1-20}$, thus purified, possessed S-protein activating potency identical with that of natural "S-peptide" (Figure 1); single Pauly-, chlorine-, and ninhydrin-positive spot on paper electrophoresis at pH 1.9, 3.5, and 6.5 with mobilities identical with "S-peptide"; amino acid ratios in AP-M digest $Lys_{1.98}Glu_{2.17}Thr_{2.00}Ala_{4.90}Phe_{1.03}Arg_{1.07}Gln_{1.07}$-$His_{0.95}Met_{0.91}Asp_{1.06}Ser_{3.10}$.

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

(11) The low recoveries of glutamine, histidine, methionine, aspartic acid, and serine in the enzymatic digest may be the result of some racemization. This point is under study, particularly since racemization has not been observed in our previous syntheses of similar peptides.[3]

(12) F. M. Finn and K. Hofmann, *J. Am. Chem. Soc.*, **87**, 645 (1965).

(13) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 618 (1963).

(14) F. M. Richards and P. J. Vithayathil, *ibid.*, **234**, 1459 (1959).

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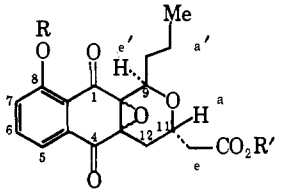
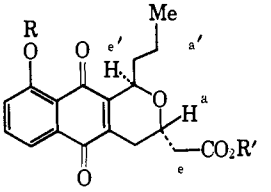
The Structure of Frenolicin

Sir:

In a program of study of microbial metabolites in these laboratories, Van Meter, *et al.*, isolated a pale yellow, crystalline antibiotic named frenolicin from a fermentation of *Streptomyces fradiae*.[1] We now report the characterization of frenolicin as the novel 1,4-naphthoquinone 2,3-epoxide (I)[2] and, in addition, describe the formation of deoxyfrenolicin (V), which exhibits significant inhibitory activity when tested *in vitro* against a variety of fungi and against an experimental ringworm infection in guinea pigs.



I, R = R′ = H
II, R = Ac, R′ = H
III, R = H, R′ = Me
IV, R = R′ = Me



V, R = R′ = H
VI, R = Ac, R′ = H
VII, R = H, R′ = Me

Frenolicin, $C_{18}H_{18}O_7$ (rather than $C_{13}H_{14}O_5$),[1] *m/e* 346, mp 161–162°, $[\alpha]^{25}D$ −37.7° (*c* 1.5, methanol), is a phenolic carboxylic acid (pK_a' = 10.0 and 5.6 in methanol–water, 1:1), ν_{max}^{KBr} 1710 and 1650 cm^{-1}. It forms a monoacetate (II), $C_{20}H_{20}O_8$, mp 161–163°, ν_{max}^{KBr} 1770 and 1700 cm^{-1}, and is converted with diazomethane to a methyl ester (III), $C_{19}H_{20}O_7$, mp 82–83°, ν_{max}^{KBr} 1735, 1705, and 1665 cm^{-1}. Treatment of frenolicin with methyl iodide in acetone in the presence of potassium carbonate gave O-methylfrenolicin methyl ester (IV), $C_{20}H_{22}O_7$, mp 109–110°, ν_{max}^{KBr} 1740 and 1695 cm^{-1}.

The ultraviolet absorption spectrum of frenolicin, in both neutral and basic media, λ_{max}^{MeOH} 234, 284 (sh), and 362 mμ (ε 18,300, 3460, and 5200), $\lambda_{max}^{0.01\ N\ NaOH}$ (in MeOH) 280 and 425 mμ (ε 6400 and 6150), indicated that it was most likely a derivative of β-hydrojuglone.[3] Alkaline potassium permanganate oxidation of O-methylfrenolicin methyl ester afforded a methoxyphthalonic acid (presumably 3-methoxy) identical with that obtained from a similar oxidation of 1,5-dimethoxynaphthalene.[4]

The 1,4-naphthoquinone oxide structure in frenolicin was suggested by the consumption of 2 moles of hydrogen (10% Pd–C in methanol) to give a colorless compound which was immediately air oxidized to the yellow-orange deoxyfrenolicin (V), $C_{18}H_{18}O_6$, mp 179–181°, ν_{max}^{KBr} 1725, 1665 (sh), 1640, and 1620 cm^{-1}, whose ultraviolet absorption, λ_{max}^{MeOH} 246, 274, and 420 mμ (ε 9070 11,400, and 4290), corresponds to that of eleutherin and isoeleutherin.[5] V was characterized as its monoacetate (VI), $C_{20}H_{20}O_7$, mp 180–182°, ν_{max}^{KBr} 1770, 1715, 1665, and 1590 cm^{-1}, and methyl ester (VII), $C_{19}H_{20}O_6$, mp 120°,

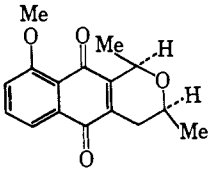
(1) J. C. Van Meter, M. Dann, and N. Bohonos, "Antibacterial Agents Annual–1960," Plenum Press, New York, N. Y., 1961, p 77.

(2) Symbols a′ and e′ denote pseudo-axial and pseudo-equatorial configurations of the bonds in question. All compounds reported here gave satisfactory elemental analyses. Nmr spectra were measured at 60 Mc in deuteriochloroform; shifts are expressed as δ values (parts per million) from tetramethylsilane as internal standard and coupling constants (*J*) are expressed in cycles per second. We thank the Organic Chemical Research Section of these laboratories for the elemental and spectral analyses and Dr. John Lancaster of the Stamford Laboratories for the spin-decoupling experiments.

(3) R. H. Thomson, *J. Chem. Soc.*, 1737 (1950).

(4) C. A. Naylor, Jr., and J. H. Gardner, *J. Am. Chem. Soc.*, **53**, 4109 (1931); W. H. Bentley, R. Robinson, and C. Weismann, *J. Chem. Soc.*, **91**, 104 (1907).

(5) Eleutherin (i) and isoeleutherin (ii) were isolated from the tubers of *Eleutherine bulbosa* by H. Schmid, A. Ebnother, and Th. M. Meijer, *Helv. Chim. Acta.*, **33**, 1751 (1950); H. Schmid and A. Ebnother, *ibid.*, **34**, 1041 (1951). For a detailed nmr analysis of these two compounds see D. W. Cameron, D. G. I. Kingston, N. Sheppard, and Lord Todd, *J. Chem. Soc.*, 98 (1964).



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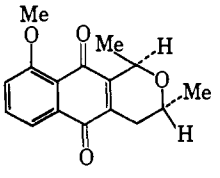

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