Synthesis of Peptide Analogs of the N-Terminal Eicosapeptide Sequence of Ribonuclease A. XI. Synthesis and Conformational Studies of [Orn^{10}, Nle^{13}]-S-peptide[1,2]

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Abstract: In order to ascertain whether the presence of the thioether function in the side chain of the amino acid in position 13 of the N-terminal sequence of bovine pancreatic ribonuclease is a fundamental prerequisite for such a residue to act as a binding site in the S-peptide–S-protein association, the S-peptide analog was synthesized in which the arginyl residue in position 10 and the methionyl residue in position 13 have been replaced by ornithine and norleucine, respectively. The comparison of the catalytic activity, against RNA, of the [Orn^{10},Nle^{13}]-RNase S′ with that of the [Orn^{10}]-RNase S′ shows that the methionine–norleucine replacement does not significantly affect the ability of the modified synthetic eicosapeptide to activate S-protein. These findings confirm the proposed hydrophobic contribution of the methionine side chain to the S-peptide–S-protein binding and suggest that such an interaction is not highly specific for the thioether function but more generally involves a hydrophobic residue. The ORD and CD spectra of the [Orn^{10}, Nle^{13}]-S-peptide in the far-ultraviolet region are practically identical with those of the S-peptide. The persistence of the flat trough at *ca.* 229 mμ, corresponding to the dichroic band at 227 mμ, in a peptide in which the methionyl residue has been replaced by the norleucyl residue eliminates the possibility that in the upper wavelength range of the spectrum, the observed dispersion arises from optically active transitions involving sulfur.

The importance of methionine-13 as a binding site in the S-peptide–S-protein complex[3] has been already demonstrated. The performic acid oxidation of S-peptide or the reaction with iodoacetic acid and iodoacetamide[4] showed that the conversion of the thioether function of methionine-13 to the sulfone or to the carboxymethyl or carboxyamidomethyl sulfonium salts somewhat lowers but does not destroy the S-protein activating ability of the resulting eicosapeptide.

Determination of the potential catalytic activity of synthetic peptides in which methionine-13 is modified, exchanged, or eliminated[5] rules out such a residue as essential for ribonuclease activity and suggests the role of "binding site" for methionine-13 in the S-peptide–S-protein association process.

The possibility that the methionyl residue in position 13 contributes to the association constant through hydrophobic bonding has already been considered[4,5] and was further supported by the interpretation of the electron density map of the RNase S at 3.5-Å resolution.[6]

Following our own synthetic studies[7] concerning those conformational features responsible for the proper association of S-peptide with S-protein, we carried out the synthesis of the [Orn^{10},Nle^{13}]-S-peptide (Chart I).

In defining the extent to which the importance of a particular group depends, for biological activity, on its functional and polar properties on the one hand and on its steric role on the other hand, a synthetic approach utilizing isosteric replacement can be of great value.

Along this line several synthetic, biologically active analogs of hormones such as ACTH,[8] oxytocin,[9] and gastrin[10] have been constructed by isosteric replacement of a sulfur atom by a methylene group.

We have now used the same approach in examining the role of the thioether group in the methionine-13 side chain in the enzymatically active S-peptide–S-protein system.

The determination of the ability of the partially synthetic [Orn^{10},Nle^{13}]-RNase S′ to catalyze the de-

(1) The peptides and peptide derivatives mentioned have the L configuration. For a simpler description, the customary L designation for individual amino acid residues is omitted. The following abbreviations [IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **241**, 2491 (1966)] are used: Z = benzyloxycarbonyl, Boc = *t*-butyloxycarbonyl, OBu^t = *t*-butyl ester, ONp = *p*-nitrophenyl ester, DMF = dimethylformamide, TFA = trifluoroacetic acid, AP-M = aminopeptidase M, DCCI = N,N′-dicyclohexylcarbodiimide, ORD = optical rotatory dispersion, CD = circular dichroism.

(2) Some of the results recorded in this paper have been presented at the IXth European Peptide Symposium, Orsay, France, April 15, 1968: E. Scoffone, F. Marchiori, L. Moroder, R. Rocchi, and A. Scatturin, Proceedings of the Symposium, in press.

(3) (a) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958); RNase A, the principal chromatographic component of beef pancreatic ribonuclease; RNase S, subtilisin-modified RNase A; S-protein, the protein component obtained from RNase S; S-peptide, the eicosapeptide obtained from RNase S; RNase S′, the reconstituted enzyme obtained by mixing equimolar amounts of S-peptide and S-protein. (b) According to M. S. Dosher and C. H. W. Hirs, *Federation Proc.*, **25**, 527 (1966), natural S-peptide is a mixture of at least [1–20]-S-peptide and [1–21]-S-peptide.

(4) P. J. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343 (1960).

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

(6) H. W. Wyckoff, K. D. Hardmann, N. M. Allewell, T. Inagami, T. Johnson, and F. M. Richards, *J. Biol. Chem.*, **242**, 3984 (1967).

(7) (a) E. Scoffone, R. Rocchi, F. Marchiori, A. Marzotto, A. Scatturin, A. M. Tamburro, and G. Vidali, *J. Chem. Soc., C*, 606 (1967); (b) R. Rocchi, F. Marchiori, L. Moroder, A. Fontana, and E. Scoffone, *Gazz. Chim. Ital.*, **96**, 1537 (1966); (c) F. Marchiori, R. Rocchi, L. Moroder, and E. Scoffone, *ibid.*, **96**, 1549 (1966); (d) E. Scoffone, R. Rocchi, F. Marchiori, L. Moroder, A. Marzotto, and A. M. Tamburro, *J. Am. Chem. Soc.*, **89**, 5450 (1967); (e) R. Rocchi, L. Moroder, F. Marchiori, E. Ferrarese, and E. Scoffone, *ibid.*, **90**, 5885 (1968); (f) F. Marchiori, R. Rocchi, L. Moroder, A. Fontana, and E. Scoffone, *ibid.*, 5889 (1968).

(8) R. A. Boissonnas, St. Guttmann, and J. Pless, *Experientia*, **22**, 526 (1966).

(9) K. Jost and J. Rudinger, *Collection Czech. Chem. Commun.*, **32**, 1229 (1967).

(10) J. S. Morley, *Proc. Roy. Soc.* (London), **B170**, 97 (1968).

Chart I. Amino Acid Sequences of S-Peptide and [Orn[10],Nle[13]]-S-peptide

S-Peptide

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H-Lys-	Glu-	Thr-	Ala-	Ala-	Ala-	Lys-	Phe-	Glu-	Arg-	Gln-	His-	Met-	Asp-	Ser-	Ser-	Thr-	Ser-	Ala-	Ala-OH

[Orn[10],Nle[13]]-S-peptide

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H-Lys-	Glu-	Thr-	Ala-	Ala-	Ala-	Lys-	Phe-	Glu-	Orn-	Gln-	His-	Nle-	Asp-	Ser-	Ser-	Thr-	Ser-	Ala-	Ala-OH

polymerization of RNA permits one to ascertain whether the proposed[4,5] hydrophobic interaction involving the residue in position 13 is specific for methionine or can more generally involve other hydrophobic side-chain residues.

In addition, the study of the [Orn[10],Nle[13]]-S-peptide may be highly interesting in connection with the interpretation of the S-peptide's rotatory properties. It was demonstrated,[11] by ORD and CD measurements, that the S-peptide is essentially randomly coiled in the absence of the partner S-protein.

The ORD spectrum of the S-peptide showed, in addition to those features typical of disordered polypeptides, a trough at *ca.* 230 mμ. Similarly, in the corresponding CD spectrum a minimum appeared at 227 mμ.

Since, on the basis of ORD and CD measurements carried out in 8 *M* urea solution,[11] the observed dispersion in this region can hardly be assigned to the amide n–π* transition of the α helix, one can presume that nonpeptide optically active transitions may have a role in contributing to the optical activity. Recent ORD and CD studies[12] on poly-S-carboxymethyl-L-cysteine in random conformation showed a trough at 236 mμ and a negative dichroic band at 226 mμ which must be ascribed to a possible sulfur lone-pair electron interaction with the peptide.

On the basis of these considerations it was reasonable to assign tentatively the Cotton effect at 230 mμ and the dichroic band at 227 mμ of the S-peptide to an optically active transition involving the sulfur atom of the methionyl residue in position 13.

The determination of the ORD and CD spectra of the [Orn[10],Nle[13]]-S-peptide makes possible the confirmation of this hypothesis.

Experimental Section

Materials. RNase A was prepared by the procedure of Crestfield, *et al.*,[13] starting with four times crystallized bovine pancreatic ribonuclease (Fluka AG); RNase S, S-protein, and S-peptide were prepared according to the literature[3,14] by using the proteolytic enzyme designated subtilopeptidase A which was a gift from Novo Industri A/S, Copenhagen, Denmark; AP-M was obtained from Rohm and Haas HmbH. Darmstadt, West Germany; yeast RNA was obtained from Schwarz Laboratories and purified before use by exhaustive dialysis, first against 0.1 *M* sodium chloride and then against water.[15]

Methods. ORD measurements were made on the Cary 60 spectropolarimeter at 24°. Cylindrical fused quartz cells were used with 0.02- and 0.01-cm optical path. The data are expressed in terms of $[m']_\lambda$, the reduced mean residue rotation, defined as $[m']_\lambda = [3/(n^2 + 2)](mrw/100)[\alpha]_\lambda$ deg cm^2 dmol^{-1}, where $[\alpha]_\lambda$ is the specific rotation at wavelength λ, *mrw* is the mean residue weight, and *n* is the refractive index of the solvent. The mean residue molecular weight of 105.3 was employed.

CD measurements were performed on the Cary Model 6001 circular dichroism attachment for the Cary 60 spectropolarimeter.

Mean residue molecular ellipticities $[\theta]_\lambda$ in units of deg cm^2 dmol^{-1} were calculated from CD spectral tracings by the following equation: $[\theta]_\lambda = mrw\theta/lc$, where θ is the observed ellipticity, *mrw* is the mean residue weight, *l* is the optical path length in centimeters, and *c* is the concentration. Solutions were made in doubly distilled water. For the concentrations see the legends of Figures 1 and 2.

Peptide Synthesis.[16] The synthetic route to [Orn[10],Nle[13]]-S-peptide, illustrated in Chart II, is similar to that we used for the preparation of other analogs.[7]

The protected octapeptide *t*-butyloxycarbonylnorleucyl-β-*t*-butylaspartylserylserylthreonylserylalanylalanine *t*-butyl ester (C, 13–20) was prepared by condensing *t*-butyloxycarbonylnorleucine *p*-nitrophenyl ester with B, 14–20.[17,18] The protecting groups were then removed by exposure to TFA and the free octapeptide (D, 13–20) was acylated with the azide prepared from N^α,N^ϵ-*t*-butyloxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^ϵ-*t*-butyloxycarbonyllysylphenylalanyl-γ-*t*-butylglutamyl-N^δ-*t*-butyloxycarbonylornithylglutaminylhistidine hydrazide[7a] (C, 1–12).

The crude S-peptide analog (F, 1–20) (100–200 mg) obtained by treatment of the partially protected eicosapeptide (E, 1–20) with TFA was dissolved in 0.2 *M* sodium phosphate buffer (pH 6.40) and purified by chromatography through an Amberlite CG 50 column (1.8 × 90 cm) with the same phosphate buffer as the eluent. Individual fractions (2.5 ml) were collected (rate *ca.* 15 ml/hr), and the product was detected by the ninhydrin test and the Pauly reaction. The ninhydrin- and Pauly-positive fractions were pooled, concentrated under reduced pressure, lyophilized, and desalted by passing through a Sephadex G-25 column (1.8 × 140 cm) with 5% acetic acid as the eluent (rate *ca.* 20 ml/hr, 5-ml fractions). The peptide

(11) A. Scatturin, A. M. Tamburro, R. Rocchi, and E. Scoffone, *Chem. Commun.*, 1273 (1967).

(12) S. Ikeda and G. D. Fasman, *J. Mol. Biol.*, **30**, 491 (1967).

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

(14) (a) F. M. Richards and P. J. Vithayathil, *ibid.*, **234**, 1459 (1959); (b) G. Gordillo, P. J. Vithayathil, and F. M. Richards, *Yale J. Biol. Med.*, **34**, 582 (1962); (c) A. Marzotto, A. Scatturin, G. Vidali, and E. Scoffone, *Gazz. Chim. Ital.*, **94**, 760 (1964).

(15) D. Wellner, H. J. Silman, and M. Sela, *J. Biol. Chem.*, **238**, 1324 (1963).

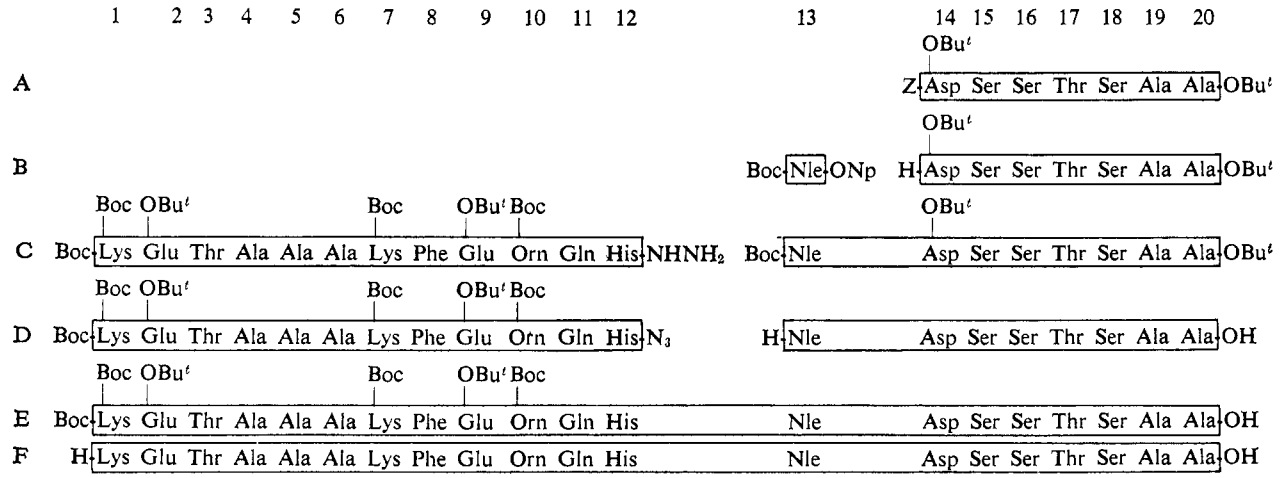
(16) The melting points were determined by the Tottoli capillary melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. The acid hydrolyses were carried out with 6 *N* hydrochloric acid, in sealed evacuated ampoules, for 22 hr at 110°. AP-M digest was prepared as described by K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966). The amino acid composition of acid and enzymic hydrolysates was determined by quantitative analysis using either a Technicon or a Carlo Erba amino acid analyzer. Ascending thin layer chromatography was performed on silica gel (Merck) with the following solvent systems: R_{f_1}, 1-butanol–glacial acetic acid–water (3:1:1); R_{f_2}, ethyl acetate–pyridine–glacial acetic acid–water (60:20:6:14). Electrophoreses were carried out on Whatman No. 1 filter paper, at a gradient of about 20 V/cm, for 3 hr, at pH 1.9 (25% acetic acid), 3.5 and 6.4 [pyridine–acetic acid–water (1:10:89 and 5:0.2:95)], and 9.4 [Na_2CO_3–$NaHCO_3$; G. E. Delory and E. J. King, *Biochem. J.*, **39**, 245 (1945)]. The chlorine [H. N. Rydon and P. Smith, *Nature*, **169**, 922 (1952)] and the Pauly (K. Randerath, "Thin Layer Chromatography," Academic Press, New York, N. Y., 1963, p 176) tests were carried out according to the literature. The hydrazides were also revealed by spraying the chromatograms with 1% picryl chloride solution in 95% ethanol, followed by exposure to ammonia vapors. Unless stated otherwise solvents were evaporated at a bath temperature of 40–50° in a rotatory evaporator. The enzymic activity of the partially synthetic modified ribonuclease was determined with RNA substrate essentially as described by M. Kunitz, *J. Biol. Chem.*, **164**, 563 (1946).

(17) F. Marchiori, R. Rocchi, L. Moroder, G. Vidali, and E. Scoffone, *J. Chem. Soc., C*, 89 (1967).

(18) We reported[17] for this protected heptapeptide the value $[\alpha]^{20}D$ −15.1 ± 0.2° (*c* 1.0, DMF), while Hofmann, *et al.*,[19] found the value $[\alpha]^{26}D$ −4.3° (*c* 1.0, DMF). Such a discrepancy can now be explained as we found that the specific rotation of a 1.0% solution of this compound in DMF changes from *ca.* $[\alpha]^{16}D$ −19° 10 min after solution to *ca.* $[\alpha]^{16}D$ −4° after 5 hr. Such mutarotation is under investigation.

(19) K. Hofmann, W. Haas, M. J. Smithers, and G. Zanetti, *J. Am. Chem. Soc.*, **87**, 631 (1965).

Chart II. Synthesis of the [Orn[10],Nle[13]]-S-peptide



was detected as described above, and the peptide-containing fractions were pooled, concentrated to a syrup, and lyophilized from water to constant weight.

***t*-Butyloxycarbonylnorleucine Dicyclohexylammonium Salt.** Norleucine (0.5 g, 3.81 mmol) and MgO (0.307 g, 7.62 mmol) were suspended in 5 ml of water at room temperature. To the stirred suspension a solution of *t*-butyl azidoformate[20] (1.1 g, 7.62 mmol) in dioxane (12 ml) was added. The reaction mixture was kept 20 hr at 45° when water was added. The mixture was extracted twice

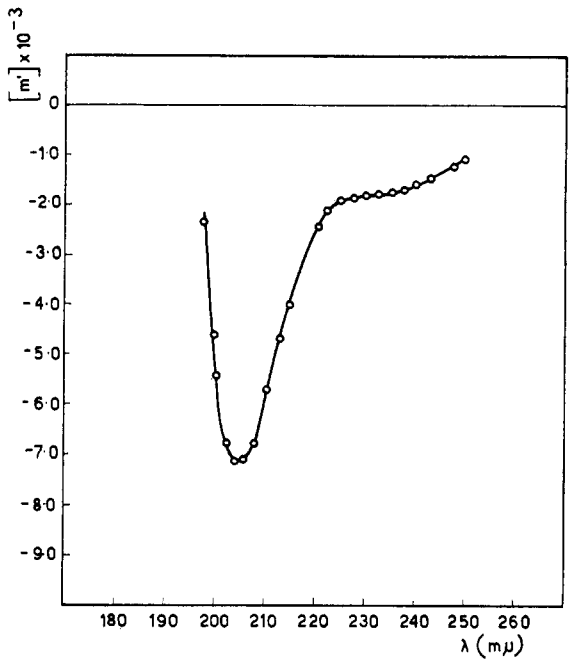


Figure 1. Far-ultraviolet ORD of [Orn[10],Nle[13]]-S-peptide in water. Concentration 0.107% as determined by dry weight.

with ethyl acetate and the combined aqueous layers, cooled to 0°, were acidified by addition of solid citric acid.

The acid solution was extracted twice with ethyl acetate; the organic layers were pooled, washed with water, and dried over sodium sulfate. The drying agent was filtered off, the solvent evaporated under reduced pressure, and the oily residue (0.449 g, 1.94 mmol, 51%) dissolved in ether.

Dicyclohexylamine (0.4 ml, 2 mmol) was added, after 60 min the solution was concentrated under reduced pressure, and precipitation occurred by addition of petroleum ether. The compound was collected and dried: yield 0.585 g (73%); mp 130–132°, $[\alpha]^{20}D$ +8.4 ± 0.2° (*c* 1.0, methanol).

Anal. Calcd for $C_{23}H_{44}N_2O_2$ (412.61): C, 66.9; H, 10.8; N, 6.8. Found: C, 66.6; H, 10.6; N, 6.8.

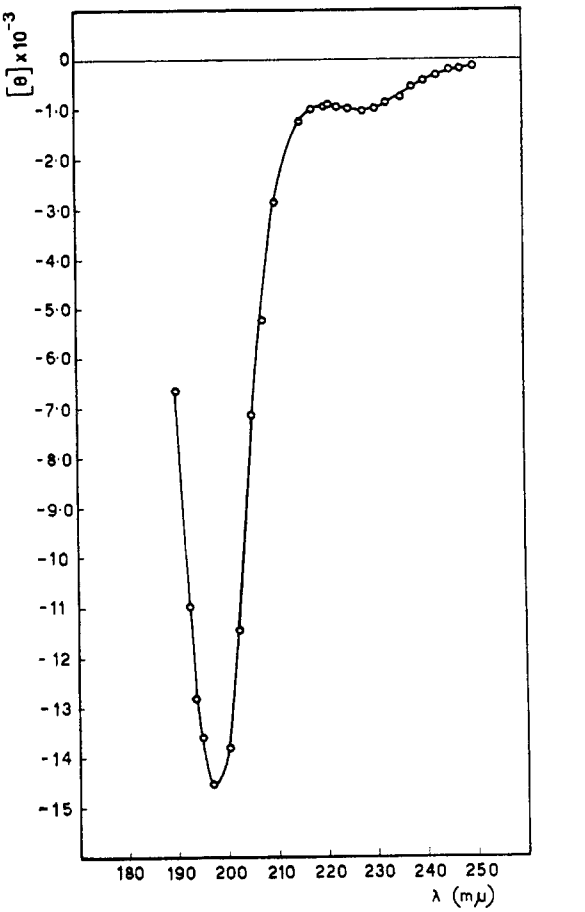


Figure 2. Far-ultraviolet CD of [Orn[10],Nle[13]]-S-peptide in water. The concentration is the same as in Figure 1.

***t*-Butyloxycarbonylnorleucine *p*-Nitrophenyl Ester (A, 13).** *t*-Butyloxycarbonylnorleucine dicyclohexylammonium salt (0.581 g, 1.41 mmol) was suspended in 100 ml of ethyl acetate, 1 *N* H_2SO_4 (2 ml) was added, and the suspension was extracted with water. The organic layer was washed with water and dried over sodium sulfate. The drying agent was filtered off, the solution was cooled to 0°, and *p*-nitrophenol (0.196 g, 1.41 mmol) and DCCI (0.29 g, 1.41 mmol) were added. The reaction mixture was stirred for

(20) L. A. Carpino, C. A. Giza, and B. A. Carpino, *J. Am. Chem. Soc.*, **81**, 955 (1959).

2 hr at 0° and then 12 hr at room temperature, the dicyclohexylurea was filtered off, and the solution was extracted with 10% $NaHCO_3$ and water, dried over sodium sulfate, and evaporated to dryness *in vacuo*.

The residue was crystallized from ether–petroleum ether (bp 30–60°); yield 0.312 g (63%), mp 85–86°, $[\alpha]^{20}D$ −45.1 ± 0.2° (*c* 1.0, methanol), R_{f_1} 0.95, R_{f_2} 0.95, single chlorine-positive spot.

Anal. Calcd for $C_{17}H_{24}N_2O_6$ (352.38): C, 58.1; H, 6.9; N, 8.0. Found: C, 57.8; H, 6.8; N, 7.9.

***t*-Butyloxycarbonylnorleucyl-β-*t*-butylaspartylserylserylthreonylserylalanylalanine *t*-Butyl Ester (C, 13–20).** Benzyloxycarbonyl-β-*t*-butylaspartylserylserylthreonylserylalanylalanine *t*-butyl ester[17,18] (A, 14–20) (0.442 g, 0.5 mmol) was hydrogenated for 3 hr over 10% palladized charcoal in a mixture of glacial acetic acid–methanol–water (3:1:1, v/v). The catalyst was filtered off and the solvent evaporated under reduced pressure. The residue (R_{f_1} 0.6, single ninhydrin- and chlorine-positive spot) was dissolved in pyridine (30 ml), and *t*-butyloxycarbonylnorleucine *p*-nitrophenyl ester (0.246 g, 0.7 mmol) and triethylamine (0.15 ml) were added. The solution was kept at room temperature for 24 hr, then the solvent was removed under reduced pressure, the residue was dissolved in DMF, and crystallization occurred when ether was added. Recrystallization from 50% aqueous dioxane gave 0.327 g (68%) of the protected octapeptide ester C, 13–20, $[\alpha]^{20}D$ −16 ± 0.3° (*c* 1.0, DMF), mp 221–223°, R_{f_1} 0.85, R_{f_2} 0.95, single ninhydrin-negative and chlorine-positive spot.

Anal. Calcd for $C_{42}H_{74}N_8O_{17}$ (963.24): C, 52.4; H, 7.7; N, 11.6. Found: C, 52.1; H, 7.6; N, 11.4.

Lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylornithylglutaminylhistidylnorleucylaspartylserylserylthreonylserylalanylalanine (F, 1–20). Sodium nitrite (1 *M*, 0.24 ml) was added to a solution of N^{α},N^{ϵ}-di-*t*-butyloxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butyloxycarbonyllysylphenylalanyl-γ-*t*-butylglutamyl-N^{δ}-*t*-butyloxycarbonylornithylglutaminylhistidine hydrazide[7a] (C, 1–12) (0.228 g, 0.12 mmol) in a mixture of 90% acetic acid (10 ml), 1 *N* hydrochloric acid (0.48 ml), and a 20% solution of sodium chloride (2 ml) at −10°.

After stirring for 15 min at −10°, precooled 20% sodium chloride (80 ml) was added, and the resulting precipitate was collected and washed with ice-cold water. The still-wet material was dissolved in DMF (25 ml) at −10° and dried over sodium sulfate.

Simultaneously *t*-butyloxycarbonylnorleucyl-β-*t*-butylaspartylserylserylthreonylserylalanylalanine *t*-butyl ester (C, 13–20) (0.232 g, 0.24 mmol) was dissolved in TFA (0.5 ml) and kept 1 hr at room temperature.

Ice-cold ether was then added, and the resulting precipitate was collected and dissolved in DMF (45 ml) containing triethylamine (0.07 ml).

Such a solution was added to the azide (D, 1–12) solution prepared above, and the reaction mixture was kept 7 days at 5° and for 1 day at room temperature, concentrated to 10 ml, and diluted with water (150 ml).

The resultant precipitate was centrifuged, washed with water and ether, and dried over phosphorus pentoxide, yielding 0.204 g (65%).

The crude material (E, 1–20) was dissolved in anhydrous TFA (1.5 ml), and the solution was kept for 150 min at room temperature.

Ice-cold ether (50 ml) was added and after 30 min at 0° the peptide was collected by centrifugation, washed with ether, and dried.

The residue dissolved in 0.2 *M* sodium phosphate buffer was purified by passing through an Amberlite CG 50 column, desalted by gel filtration on a Sephadex G-25 column, and lyophilized as described previously.

The product (F, 1–20) (0.046 g, 28%) had $[\alpha]^{20}D$ −80.6 ± 2° (*c* 0.11, water), single ninhydrin- and Pauly-positive component on paper electrophoresis at pH 1.9, 3.5, 6.4, and 9.5; amino acid ratios in acid hydrolysate: $Lys_{2.10}Glu_{3.10}Thr_{1.85}Ala_{5.00}Phe_{0.90}Orn_{1.00}His_{0.90}Nle_{1.00}Asp_{1.00}Ser_{2.90}$; amino acid ratios in AP-M digest: $Lys_{2.05}Glu_{2.00}Thr_{1.95}Ala_{4.95}Phe_{0.95}Orn_{1.05}(Gln + Ser)_{3.90}His_{1.00}Nle_{1.05}Asp_{1.00}$.

Results

The ORD spectrum of [Orn^{10}Nle^{13}]-S-peptide in the 190–250-mμ region recorded as a plot of $[m']_\lambda$ values against wavelength is shown in Figure 1. The curve is characterized by two troughs at about 204 and 229 mμ.

The reduced mean residue rotation value at the low-wavelength trough is $[m']_{204}$ −7.100° and at the longer wavelength is $[m']_{229}$ −1.900°.

In the corresponding CD curve (Figure 2) two negative dichroic bands appear centered at *ca.* 198 and 227 mμ. The mean residue molecular ellipticities are respectively $[\theta]_{198}$ −14.600° and $[\theta]_{227}$ −1.050°.

The trough at 204 mμ as well as the large negative dichroic band at 198 mμ are unambiguously associated with the amide π–π* transition of the polypeptide in a disordered form.[21] The assignment of the trough at 229 mμ, corresponding to the minimum at longer wavelength in the CD curve, is still under investigation.

The capacity of the [Orn^{10},Nle^{13}]-S-peptide to catalyze the depolymerization of RNA after recombination with S-protein in different molar ratios, expressed as percentage of RNase S′ as well as [Orn^{10}]-RNase S′[7a] activity, is reported in Table I. The activity data show

Table I. S-Protein Activating Capacity of [Orn^{10},Nle^{13}]-S-peptide[a]

Molar ratio of peptide–protein	RNase S′ activity, %	[Orn^{10}]-RNase S′[7a] activity, %
1:1	30	48
10:1	55	63
100:1	60	68
200:1	70	80

[a] Substrate RNA.

that the catalytic potency of the [Orn^{10},Nle^{13}]-S-peptide–S-protein complex (1:1 molar ratio) is about 50% of the [Orn^{10}]-RNase S′ activity and that this potency goes up to 80% when the peptide–protein ratio is increased. The gradual enhancement of the ribonuclease activity which accompanies the increased value of the ratio between [Orn^{10},Nle^{13}]-S-peptide and S-protein indicates that the methionine–norleucine replacement is accompanied by a decrease in the peptide–protein association.

Discussion

Both the ORD and CD spectra that we obtained for the [Orn^{10},Nle^{13}]-S-peptide show that such a synthetic eicosapeptide possesses those conformational features that we previously found for the S-peptide.[11] Indeed critical wavelengths and rotational strengths of the optically active transitions appear to be similar in the synthetic peptide and the natural one.

A noteworthy feature is the presence of the flat trough at *ca.* 229 mμ corresponding to the dichroic band at 227 mμ. The persistence of such rotatory bands in a peptide in which the methionyl residue has been replaced by the norleucyl residue eliminates the possibility that, in the upper wavelength range of the spectrum, the observed dispersion arises from optically active transitions involving sulfur.

In principle such rotatory bands could be assigned either to the amide n–π* transition of a very short α-helical segment or to the existence of a state of helix ⇌ coil equilibrium in which only a very small proportion of molecules are in a rigid conformation.

The main objections to such an interpretation are the persistence of the trough at 230 mμ and the minimum at 227 mμ for the S-peptide in 8 *M* urea solution[11]

(21) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, 87, 218 (1965).

and the noncoincidence of the wavelength values with the standard values for an α helix (233 and 222 mμ, respectively).

The problem of the unambiguous assignment of the optically active transition in the 225–235-mμ range is so far unsolved, and the possibility that chromophores other than the amide group or the thioether function are involved must not be neglected.

The comparison of the enzymic activity of the [Orn^{10},-Nle^{13}]-RNase S′, against RNA, with that of the [Orn^{10}]-RNase S′[7a] shows that the replacement of methionine by norleucine does not significantly affect the ability of the modified synthetic eicosapeptide to give an enzymatically active complex with S-protein.

These findings provide independent support for the proposed[4,5] hydrophobic contribution of methionine side chain to the S-peptide–S-protein binding and suggest that such an interaction is not highly specific for the thioether function but may more generally involve other hydrophobic residues.

Moreover, while one should be cautious about interpreting results obtained from studies with enzymes in the same light as those obtained with hormones one may suppose that the methionyl residue plays a similar role in the S-peptide and in the above-mentioned polypeptide hormones.

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Synthesis of Sugar-Like Phosphates by the Oxyphosphorane Condensation. Reaction of Glyoxal with Trialkyl Phosphites and Preparation of Phosphate Esters of Glycolaldehyde, α-Hydroxy β-Keto Aldehydes, and Hydroxymalonaldehyde Chloride

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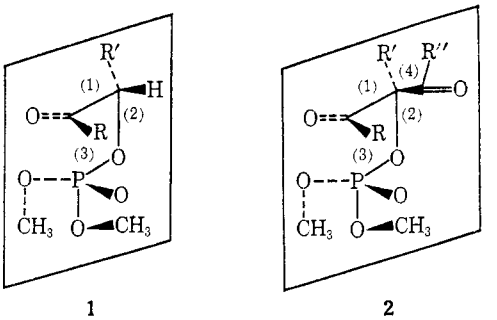
Abstract: Commercially available "glyoxal trimer dihydrate" was converted into monomeric anhydrous glyoxal which was allowed to react with trimethyl phosphite to form 2,2,2-trimethoxy-2,2-dihydro-1,3,2-dioxaphospholene. The phospholene was transformed into dimethyl 2-oxoethyl phosphate ("diose phosphate") by anhydrous HCl, and into a series of α-hydroxy β-keto aldehyde phosphates by carboxylic acid chlorides. The reaction of the phospholene with phosgene, $COCl_2$, gave dimethyl phosphohydroxymalonaldehyde chloride. The ^{31}P and ^{1}H nmr and infrared spectra were studied.

This research was undertaken to provide general procedures for the synthesis of polyfunctional carbonyl-containing phosphate esters of biological interest.[2] The phosphates can be represented schematically[3] by formulas **1** and **2**. These are arbitrarily chosen conformations which can serve as a basis for discussion of the possible interactions between the polar groups, P=O and C=O, and the effects of other groups, R, R′, and R″, on these interactions.



1 **2**

R and R′ = H, alkyl, or aryl; R″ = H, Cl, alkyl, aryl

The availability of pure anhydrous samples[4,5] of these low molecular weight highly reactive phosphates is

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(2) (a) T. C. Bruice and S. J. Benkovic, "Biorganic Mechanisms," Vol. 2, W. A. Benjamin, Inc., New York, N. Y., 1966; (b) H. Khorana, "Some Recent Developments on the Chemistry of Phosphate Esters of Biological Interest," John Wiley & Sons, Inc., New York, N. Y., 1961; (c) M. Calvin and J. A. Bassham, "The Photosynthesis of Carbon Compounds," W. A. Benjamin, Inc., New York, N. Y., 1962; (d) D. M. Brown, *Advan. Org. Chem.*, **2**, 141 (1963); (e) F. Cramer, *Angew. Chem.*, **72**, 236 (1960); (f) J. R. Cox, Jr., and B. Ramsay, *Chem. Rev.*, **64**, 317 (1964).

(3) Four atoms are placed on the plane indicated, *i.e.*, aldehyde C, alcohol C and O, and phosphoryl P. Wedges and dashes denote group in front and back of plane, respectively.

(4) The preparation of aqueous solutions of the dicyclohexylammonium salt of glycolaldehyde dihydrogen phosphate has been thoroughly described by Ballou and MacDonald.[5] The "hydrate" of the diethyl acetal derivative of this material is offered commercially at $6 per 25 mg (Calbiochem, Los Angeles, Calif.).

(5) C. E. Ballou and D. L. MacDonald in "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler, M. L. Wolfrom, and J. N. BeMiller, Ed., Academic Press, New York, N. Y., 1963, p 272.