factors and the diagonal elements of the least-squares normal equation matrix obtained when scattering factors appropriate to carbon atoms were used for both carbon and nitrogen in the least-squares refinement. The location of the hydrogen atoms of rings A–E in a (F_o-F_c) synthesis unequivocally confirmed our assignment of the heteroatoms; the hydrogen atoms of the ethyl and iodoacetate side chains were only diffusedly visible, presumably because of the thermal motion of this part of the molecule.

The results of the X-ray analysis establish that the iodoacetate ester has structure Ic, and it follows, therefore, that camptothecin has structure I. The absolute configuration shown was determined by Bijvoet's method,[16] based on the anomalous dispersion by the iodine atom of the Cu Kα radiation.

We are actively pursuing the synthesis of camptothecin and simpler analogs and the effects of structure modification on biological activity. These and other results will be presented in future communications.

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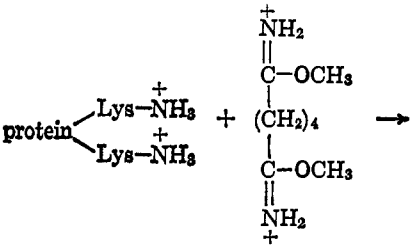
Bifunctional Reagents. Cross-Linking of Pancreatic Ribonuclease with a Diimido Ester[1]

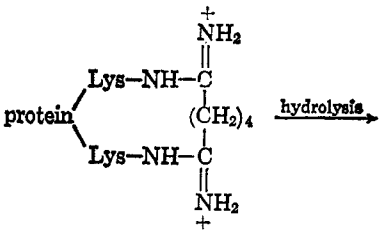
Sir:

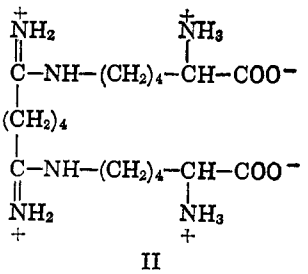
Hunter and Ludwig[2] have shown that water-soluble imido esters react specifically, under mild conditions, with protein amino groups. Since the resulting amidines have pK_a's slightly higher than those of ϵ-amino groups, the amidination should not alter the net charge of the protein in the acid or neutral pH range. Diimido esters might thus provide suitable bifunctional protein reagents, useful in the study of interresidue distances in proteins.[3]

We have investigated the reaction of dimethyladipimidate (I) with bovine pancreatic ribonuclease A (RNAase) according to



I





II

[^{14}C]Adiponitrile, prepared by the reaction of 1,4-dichlorobutane with [^{14}C]NaCN under the conditions of Smiley and Arnold,[4] was converted to [^{14}C]-dimethyl adipimidate (I) as described by McElvain and Schroeder.[5] The diimido ester dihydrochloride, obtained in over-all 85% yields, had a specific activity of 270,000 dpm/μmole and was converted to adipamide, mp 218–220°, during attempted melting point determinations.[6]

N_α,N_ϵ'-Adipamidinobislysine (II) was prepared by the reaction of α-N-formyllysine[7] with I and purified by ion-exchange chromatography on Dowex 50. The purified material moves as a single component, R_f 0.12, during paper chromatography (BuOH–HOAc–H_2O, 7:2:5). It can be measured quantitatively on the automatic amino acid analyzer by elution, as a single peak, from the short column with 0.1 *M* borate buffer (0.35 *M* in acetate), pH 9.7. The observed ninhydrin color yield is equal to that of lysine.

The modification of RNAase was conducted at room temperature in the following manner. [^{14}C]-Dimethyl adipimidate (27 mg, 110 μmoles) was added in 2-mg portions at 5-min intervals to a continuously stirred solution of RNAase (500 mg, 36.7 μmoles) in 50 ml of 0.1 *M* phosphate, pH 10.5. One hour after the addition of reagent was completed, the reaction mixture was subjected to gel filtration on a Sephadex G-75 column previously calibrated with RNAase which had been lyophilized from 50% HOAc to form dimers and higher aggregates.[8] Three fractions corresponding to monomers, dimers, and higher aggregates

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of RNAase were obtained, and analytical data on these fractions are given in Table I. Electrophoresis of the monomeric fraction on cellulose acetate strips demonstrated the absence of native RNAase.

Table I. Analytical Data for the Three Gel Filtration Fractions from Amidinated Ribonuclease

Fraction, % of total	Reagent incorpd,[a] moles/mole of protein	Lysine modified,[b] moles/mole of protein	N_ϵ,N_ϵ'-Adipamidinobis-lysine,[c] moles/mole of protein	Enzymic activity,[d] % of native
Monomer, 47	2.3	3.2	1.0	160
Dimer, 29	2.7	4.0	1.2	135
Aggregated, 24	2.8	4.1	1.2	130

[a] Based on radioactivity incorporated. [b] Based on free lysine in acid hydrolysates of dinitrophenylated, deamidinated (with NH_4OH)[11] samples. [c] Amount found in acid hydrolysates, corrected for destruction, using the automatic amino acid analyzer. [d] Activity toward cytidine 2',3'-cyclic phosphate.

The data clearly demonstrate that approximately 60% of the incorporated reagent has reacted monofunctionally. This is perhaps not surprising in view of the known susceptibility of imido esters to hydrolysis.[2,6]

The increased enzymatic activity of the modified RNAase can be attributed either to the chemical modification *per se* or to the stabilization, resulting from the introduction of cross-links, of a superactive conformation of the enzyme. The latter possibility is supported by the fact that modification with methyl hexanoimidate, the corresponding monofunctional imido ester, does not result in increased enzymic activity.

To determine the location of intramolecular cross-links, tryptic digests of performic acid oxidized, amidinated RNAase monomers have been subjected to peptide mapping as described by Anfinsen, *et al.*[9] Autoradiograms of the peptide maps revealed the presence of three major radioactive components, two of which were completely separated from peptides arising from native RNAase.

These two peptides were isolated by preparative paper electrophoresis of 5 mg of trypsin digest, hydrolyzed, and analyzed. The results are given in Table II.

The amino acid composition of peptide 1 is consistent with the presence of a cross-link between lysine residues 31 and 37.[10] A cross-link in this position would result in the release of a peptide composed of residues 11–39 because the peptide bonds involving the amidinated lysine residues are not susceptible to trypsin-catalyzed hydrolysis.[11] Since the maximum distance that the reagent can span is 8.6 A and the minimum distance between the ϵ-amino groups of lysine residues 31 and 37 in a fully extended peptide chain is 12 A, this region of the RNAase molecule must be folded to some extent.

Component 2 appears to be a mixture composed of approximately equimolar quantitites of two peptides, one of which contains residues 1–10 and 34–39 indicating a cross-link between lysine residues 7 and 37, and

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Table II. Amino Acid Analyses of Peptides 1 and 2

Amino acid	No. of residues: Peptide 1 Found[a]	Peptide 1 Calcd[b]	Peptide 2 Found[a]	Peptide 2 Calcd[c]
Di-Lys[d]	0.8	1	0.7	1
Lys	0	0	2.1	2
Arg	1.6	2	2.0	2
Asp	4.6	5	2.1	2
Thr	2.3	2	3.2	3
Glu	2.3	2	3.5	3
Ala	2.3	2	6.0	6
Leu	0.95	1	1.0	1
Phe	....		1.1	1
Tyr	1.0	1	...	
His	0.9	1	...	
Cysteic	0.8	1	...	
Ser	6.3	7	...	
Met SO_2	2.5	3	...	

[a] The following corrections have been made: (1) destruction of Di-Lys, Thr, and Ser during hydrolysis; (2) lysine due to hydrolysis of di-Lys; (3) Gly and Ala found in chromatography paper. [b] Peptide corresponding to residues 11–39 (31–37 bridge). [c] Equimolar amounts of two peptides corresponding to residues 1–10, 34–39 (7–37 bridge), and 1–7 (monofunctional amidination of Lys 1), respectively. [d] N_ϵ,N_ϵ'-Adipamidinobislysine.

the other residues 1–7, whose appearance could be explained by assuming monofunctional modification of the N-terminal lysine. The formation of a 7–37 cross-link is not surprising in view of previous reports[3c,d] of the formation of a 7–41 cross-link with 1,5-difluoro-2,4-dinitrobenzene.

These experiments demonstrate the feasibility of employing diimido esters as bifunctional protein reagents.

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Rate Law for the Oxidation of Methanol and Ethanol by Peroxydisulfate Ion

Sir:

The oxidations of ethanol[1,2] and methanol[1-3] by

$$RCH_2OH + S_2O_8^{2-} \longrightarrow RCHO + 2H^+ + 2SO_4^{2-} \quad (1)$$
$$R = CH_3, H$$

aqueous peroxydisulfate proceed *via* a radical mechanism. The influence of radical traps (allyl acetate[1,3] and diphenylpicrylhydrazyl[4]) on rates is only consistent with both a chain mechanism and initiation by the unimolecular, homolytic scission step

$$S_2O_8^{2-} \longrightarrow 2SO_4\cdot^- \quad (2)$$

However, it is difficult to explain the dependence upon reactant concentrations in the previously proposed rate

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