terms of decreased backbonding from the iridium in passing from an "unsaturated," four-coordinate iridium(I) complex to a "saturated," five-coordinate iridium(I) complex.[10] These absorption bands are similar to the strong band in the 2170–2115-cm^{-1} region found by Allen and Senoff in the spectra of the cationic ruthenium(II)–nitrogen complexes. The Raman band corresponding to the nitrogen triple-bond stretch is found at 2331 cm^{-1}.[2]

A solid sample of III was degraded by heating for 10 min at 165° under argon. The evolved gas was found to be nearly pure N_2 by gas chromatographic analysis. In another experiment a weighed sample of II was heated for 15 min at 200°, and the evolved gases were passed into a vacuum line through a trap cooled with liquid nitrogen. The volume of noncondensable gas was found to be 85% of the value calculated for evolution of 1 mole equiv of N_2. Mass spectral analysis demonstrated that the evolved gas was nitrogen of purity >99%.

The structures assigned to II and III must be considered tentative until X-ray diffraction studies have been completed. It seems possible that other nitrogen complexes can be prepared from organic azides and suitable four-coordinate complexes containing at least one carbonyl group. Experiments in progress are exploring the possibility of reducing coordinated nitrogen and of preparing homogeneous hydrogenation catalysts from II and III.[10a]

Acknowledgment. We are pleased to acknowledge the support of the National Science Foundation. We are also indebted to William Breckenridge, David Harrison, and Edward Weissberger for technical assistance with the gas analyses and to Professor Henry Taube for his encouragement.

(10) Structures I and II depict the nitrogen bound edgewise to the metal. This conjecture stems from our hypothesis that nitrogen–metal π bonds should resemble acetylene–metal bonds. If this supposition is correct, one should seek nitrogen complexes in metal substrates which readily form stable complexes with acetylene or 2-butyne.

(10a) NOTE ADDED IN PROOF. The yellow compound II has been obtained in a crystalline form which is considerably more stable, melting at 151°. Water seems to be required for the formation of II inasmuch as a different product is formed when dry $CHCl_3$ is used as a solvent.

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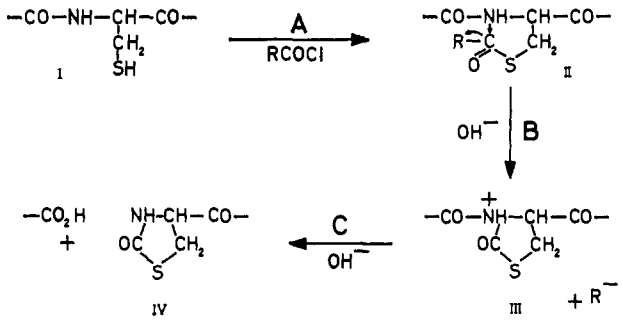
Received May 18, 1966

Specific Cleavage of Peptides at Cysteinyl Residues

Sir:

In studying the sequence of amino acid residues in a peptide chain it is an advantage to cleave the chain quantitatively at specific residues. This can be achieved in some cases by enzymes and by chemical modification of the side chains of amino acid residues.[1] Cysteinyl residues can be converted to S-2-aminoethylcysteinyl residues[2] and to dehydroalanyl residues,[3] and both of these modifications can be used to effect cleavage of the adjacent peptide bond. Recently the use of cyanide ion[4] and of cyanogen bromide[5] is reported to cleave cysteine peptides.

We wish to report a new approach to this problem based on the reaction series outlined below. In step A



the thiol group of I is acylated to form II. Step B involves the elimination of the anion R^- from II and depends on the stability of R^-. In step C the acyl group of the 2-keto-3-acylthiazolidine (III) is hydrolyzed in analogy to the final step in the cyanide cleavage reaction,[4] where a 2-imino-3-acylthiazolidine is involved.

In the preliminary studies reported here we have used as model compounds various N-carbobenzyloxy tripeptides containing cysteine as the middle residue, and the cleavage has been followed quantitatively by converting the N-carbobenzyloxy amino acid into the corresponding free amino acid which is estimated by conventional methods. The acylating agents (RCOCl) used are 4-nitrophenyloxycarbonyl chloride[6] (R = $O_2NC_6H_4O$), phenylthiocarbonyl chloride[7] (R = C_6H_5S), and *n*-butylthiocarbonyl chloride[8] (R = C_4H_9S). The N-carbobenzyloxy tripeptides containing cysteine residues were prepared *in situ* from the corresponding N,S-dicarbobenzyloxy tripeptides[9] by sodium methoxide solution[10] and the thiol groups were then acylated by the appropriate acid chloride in phosphate buffer, pH 7. The liberation of thiol groups and their subsequent acylation were followed quantitatively by iodometric titration. The reaction product was then hydrolyzed in 1.0 *M* potassium hydrogen carbonate (pH 8.1) at 50° for 90 min. After acidification and vacuum evaporation the ether-soluble material was treated with hydrogen bromide in acetic acid[11] to remove quantitatively the N-carbobenzyloxy group; peptide bond cleavage was estimated using the Beckman–Spinco amino acid analyzer.

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(9) A third crystalline modification of N,S-dicarbobenzyloxyglutathione has been obtained; mp 181–182° (uncor), plates from methanol–benzene $[\alpha]^{21}D$ −34° (*c* 1, methanol). *Anal.* Calcd for $C_{26}H_{29}N_3O_{10}S$: C, 54.25; H, 5.08; N, 7.30; equiv wt, 287.8. Found: C, 53.87; H, 5.24; N, 7.52; equiv wt, 286. Two other crystalline modifications, mp 105–107° and 141–143°, $[\alpha]^{25}D$ −32°, have been described previously.[10]

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The results are outlined in Tables I and II. The cleavage reaction is general and, under this particular set of conditions, depends on the nature of the residues adjacent to the cysteinyl residue and on the nature of the S-acyl group. In Table II the glutamic acid formed gives a measure of the specific cleavage and the glycine formed corresponds to the concurrent nonspecific cleavage. In agreement with the intramolecular mechanism we have proposed, it is seen that the extent and specificity of the cleavage depends on the stability of the anion (R^-) formed.

Table I. Cleavage of Model Tripeptides as the S-(4-Nitrophenyloxycarbonyl) Derivatives

Cysteine tripeptides[a]	% cleavage[b]
Cbz-Glu$_\gamma$-Cys(SH)-Gly-OH	30
Cbz-Phe-Cys(SH)-Gly-OH	73
Cbz-Ala-Cys(SH)-Gly-NH_2	53
Cbz-Ala-Cys(SH)-Gly-OH	50

[a] The thiol peptides were acylated *in situ* with 4-nitrophenyloxycarbonyl chloride at pH 7. [b] Yield of cleavage is calculated from the carbobenzyloxy amino acid liberated after 90 min at pH 8.1, 50°, from 5 μmoles of peptide/ml of buffer.

Table II. Effect of Various S-Acyl Groups on the Cleavage of Cbz-Glu$_\gamma$-Cys(SH)-Gly-OH[a]

Thiol derivatives[b]	% glutamic acid formed (specific fission)	% glycine formed (nonspecific fission)
4-$NO_2C_6H_4OCO$	30	2
C_6H_5SCO	47	3
n-C_4H_9SCO	10	3
$(CH_3)_2NCO$	7	5
C_6H_5CO	1	3
H	3	5

[a] The hydrolysis was at pH 8.1, 50°, 90 min, at 5 μmoles of peptide/ml of buffer. The concentrations of glutamic acid and glycine in the reaction mixture were determined after decarbobenzoxylation. [b] Acylations were carried out at pH 7.

To date very little is known of the properties of 2-ketothiazolidinecarboxylic acids[12] or their peptide derivatives. However, 2-imino analogs have been described briefly.[4]

In order to investigate the properties of product IV, in one experiment the pH 8 buffer used for cleavage of the peptide derivatives listed in Table II was 0.2 *M* N-ethylmorpholine acetate[13] (instead of the usual 1.0 *M* potassium hydrogen carbonate); this was subsequently removed by lyophilization to yield a salt-free product. This material after paper chromatography gave new spots which were ninhydrin negative but chlorine–tolidine positive.[14] As previously, treatment of the reaction product with hydrogen bromide in acetic acid gave glutamic acid (identified on paper chromatograms). Oxidation of the reaction product with performic acid gave β-sulfoalanylglycine which was converted to the corresponding DNP derivative and hydrolyzed to DNP-cysteic acid and glycine. These products were all identified with authentic specimens by paper ionophoresis at pH 1.8 (1.0 *M* formic acid) and at pH 2.3 (6% acetic acid). All these observations confirm that the cleavage occurs at the N-acyl bond attached to the cysteine residue.

The elimination step B and the hydrolysis step C must compete with alternative modes of hydrolysis (*e.g.*, of the acylated thiol II to regenerate thiol I and possibly opening of the thiazolidine ring III), and these will decrease the yield of specific fission. So far we have used only one arbitrarily chosen set of hydrolysis conditions (pH 8, 90 min, 50°). Further studies will be required in order to find the optimal conditions for a general cleavage process.

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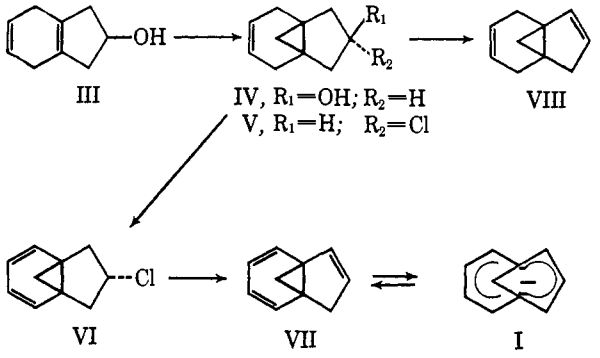
Received May 16, 1966

1,5-Methanocyclononatetraenyl Anion

Sir:

We wish to report the preparation of a new stable 10π electron anion, 1,5-methanocyclononatetraenyl anion (I). I is the methano-bridged derivative of the previously reported aromatic cyclononatetraenyl anion[1] and completes the series of methano-bridged 10π electron aromatic species: neutral compound,[2a] carbonium ion,[2b] and anion.



Synthesis of I proceeded from 2-indanol[3] (II) *via* Birch reduction with sodium in liquid ammonia to give, in 74% yield, 4,7-dihydroindan-2-ol[4] (III), bp 93° (1 mm), in complete analogy to the reaction with indane to give 4,7-dihydroindane.[5] The structure of III was confirmed spectroscopically by its lack of

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