TABLE IX

	Mol. wt.	Fluorine, % Calcd.	Fluorine, % Found	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
$CH_3CH_2CO^+SbF_6^-$	293	38.93	38.59	12.30	12.18	1.72	1.79
$(CH_3)_2CHCO^+SbF_6^-$	307	37.15	37.07	15.65	15.78	2.30	2.27
$(CH_3)_3CCO^+SbF_6^-$	321	35.52	35.44	18.71	[a]	2.82	[a]
$CH_3CH_2CH_2CO^+SbF_6^-$	307	37.15	37.03	15.65	15.39	2.30	2.21
$(CH_3CH_2)_2CHCO^+SbF_6^-$	335	34.03	33.87	21.51	21.18	3.31	3.17
$C_6H_5CH_2CO^+SbF_6^-$	355	32.12	31.98	27.07	26.80	1.98	1.78
$(C_6H_5)_2CHCO^+SbF_6^-$	431	26.45	26.13	39.01	38.28	2.57	2.39

[a] Not determined due to relative instability of complex at room temperature.

described previously for $CH_3CO^+SbF_6^-$ and $CD_3CO^+SbF_6^-$,[2] by treating Freon 113 solutions of $CH_3C^{13}OF$ and SbF_5 at -5 to $-10°$ and subsequently isolating the stable, crystalline oxocarbonium salt.

Reaction of Oxocarbonium Salts with Aromatic Compounds. (a) **Without Solvent.**—The appropriate oxocarbonium salt (0.2 mole) was added into 0.5 mole of well-stirred aromatic. The complex salts are generally not soluble in the aromatics. In most cases gentle heating was necessary to start the reaction. The formed ketones give complexes with the by-product Lewis acids and separate from the excess aromatic as a lower layer. After washing the reaction mixtures, they were dried over Na_2SO_4 and the products isolated (fractionation or crystallization).

(b) **In Solution.**—In these experiments the reaction was carried out in nitromethane solutions in which the aromatics and the oxocarbonium salts are both soluble. The reactions are much slower in solvent, and owing to the partial decomposition of the oxocarbonium salts in nitromethane the yields are lower.

Reaction of Oxocarbonium Salts with Alcohols.—Oxocarbonium salt (0.3 mole) was added as nitromethane solution or in small portions as a solid into 0.6 mole of the appropriate stirred and cooled alcohol. A fast reaction takes place. The resulting mixture was washed with water, dried over Na_2SO_4 and fractionated.

Reaction of Oxocarbonium Salts with Mercaptans.—Oxocarbonium salt (0.3 mole) was added in nitromethane solution or in small fractions as a solid to 0.6 mole of well stirred and cooled mercaptan. The reaction is very fast. After completion of the reaction, the mixture was washed with water, dried over Na_2SO_4 and fractionated.

Reaction of Oxocarbonium Salts with Amines.—The solution of 0.3 mole of oxocarbonium salt in nitromethane or SO_2 solution was added to 0.6 mole of the stirred and cooled primary or secondary amine. The products, after water washings, were isolated either by distillation or crystallization.

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[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF CLARK UNIVERSITY, WORCESTER, MASS., AND ILLINOIS INSTITUTE OF TECHNOLOGY, CHICAGO, ILL.]

Pyridoxine and Pyridoxal Analogs. VIII. Synthesis and Infrared Spectra of Metal Chelates[1]

BY DIETRICH HEINERT[2] AND ARTHUR E. MARTELL[2]

RECEIVED NOVEMBER 21, 1962

The preparation and purification of a number of metal chelates derived from the amino acid Schiff bases of pyridoxal analogs 3-hydroxypyridine-4-aldehyde and 3-hydroxypyridine-2-aldehyde are described. Copper(II) chelates containing a 1:1:1 molar ratio of Cu(II):hydroxypyridinealdehyde:amino acid and iron(III) and nickel(II) chelates having a 1:2:2 molar ratio of constituents were synthesized. Glycine, glutamic acid and valine were employed as the amino acids. Probable structures of these compounds were deduced from their stoichiometry, properties and infrared spectra.

This paper describes the synthesis and properties of Fe(III), Cu(II) and Ni(II) chelates of the 3-hydroxy-4-pyridinealdimines (XVI) and 3-hydroxy-2-pyridinealdimines (XVII) containing residues of the natural amino acids glycine, valine, phenylalanine and glutamic acid. Interest in such metal chelate compounds arises from their analogy to pyridoxylideneimine chelates, whose role in vitamin B_6 catalyzed reactions has been the subject of considerable investigation. The role of metal chelates of pyridoxal derivatives of the natural amino acids has been reported by Metzler and Snell[3] and a general mechanism for a wide variety of reactions catalyzed by these compounds was described by Metzler, *et al.*[4] The first crystalline metal chelate of pyridoxal Schiff bases reported was the 1:1 Cu(II) chelate of pyridoxylidenetyrosine described by Baddiley.[5] Christensen isolated and measured spectrophotometrically the formation in solution of the Cu(II), Mn(II), Ni(II), Zn(II), and Mg(II) chelates of pyridoxylideneglycine,[6] containing a 1:1 molar ratio of ligand to metal ion, and the Mn(II), Ni(II), Zn(II), Fe(II) and Fe(III) chelates of a number of other amino acid–pyridoxal Schiff bases[7] containing a 2:1 molar ratio of ligand to metal ion. The formation constants of a number of 1:1 metal pyridoxylideneimine chelates have been reported,[8,9] but little evidence was found for the formation of 2:1 chelates in aqueous solution.[9]

The purpose of the present investigation was to prepare and study metal chelates of amino acid Schiff bases derived from the pyridoxal analogs described in previous communications.[10,11]

Experimental

General Synthesis of Hydroxypyridylmethylene Amino Acid Metal Chelates.—Two mmoles of anhydrous reagent grade amino acid is converted to the potassium salt by the slow addition with stirring of a 0.10 *M* solution of potassium hydroxide in absolute methanol. For the preparation of the Cu(II) and Ni(II) chelates, two equivalents of base was added to one and two equivalents of the amino acid, respectively. For the preparation of the 2:1 Fe(III) chelate, three equivalents of base was added to two equivalents of the amino acid. The methanol solution containing the potassium salt of the amino acid with or without excess base was cooled and combined with an equimolar amount of the hydroxyaldehyde in anhydrous methanol. The resulting yellow Schiff base solution was then slowly combined with the

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TABLE I

METAL CHELATES OF SCHIFF BASES

Compound[a] Form	R'	No.	Empirical[b] formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Metal, %[c] Calcd.	Metal, %[c] Found	Yield, %
2,3	H	I	$C_8H_6N_2O_3Cu\cdot 2H_2O$	34.60	34.85	3.63	3.15	10.09	9.91	22.88	23.33	60
2,3	H	II	$C_{16}H_{13}N_4O_6Fe\cdot 2H_2O$	42.78	42.71	3.82	3.89	12.47	12.78	12.43	13.07	16
2,3	H	III	$C_{16}H_{14}N_4O_6Ni\cdot H_2O$	44.17	44.06	3.71	4.35	12.88	12.40	13.49	13.10	37
2,3	$CH(CH_3)_2$	IV	$C_{11}H_{12}N_2O_3Cu\cdot {}^1/_2H_2O$	45.12	45.20	4.48	4.54	9.57	9.37	21.70	21.50	66
2,3	$CH(CH_3)_2$	V	$C_{22}H_{25}N_4O_6Fe\cdot 2H_2O$	49.54	49.55	5.48	5.45	10.51	11.11	10.47	10.45	47
2,3	$CH(CH_3)_2$	VI	$C_{22}H_{26}N_4O_6Ni\cdot H_2O$	50.89	50.96	5.44	5.69	10.79	10.81	11.30	11.38	74
2,3	$(CH_2)_2CO_2H$	VII	$C_{11}H_{10}N_2O_5Cu\cdot H_2O$	39.82	39.87	3.65	3.63	8.44	8.39	19.15	19.08	42
2,3	$(CH_2)_2CO_2H$	VIII	$C_{22}H_{21}N_4O_{10}Fe\cdot 2H_2O$	44.53	44.40	4.25	4.18	9.44	9.49	9.41	9.48	62
2,3	$(CH_2)_2CO_2H$	IX	$C_{22}H_{22}N_4O_{10}Ni\cdot 3H_2O$	42.95	42.92	4.59	4.17	9.11	9.02	9.54	10.30	29
3,4	H	X	$C_8H_6N_2O_3Cu\cdot {}^1/_2H_2O$	38.33	38.25	2.82	2.88	11.18	11.05	25.35	25.29	62
3,4	H	XI	$C_{16}H_{13}N_4O_6Fe\cdot 2H_2O$	42.78	42.78	3.82	3.61	12.47	12.66	12.43	12.30	70
3,4	H	XII	$C_{16}H_{14}N_4C_6Ni\cdot H_2O$	44.17	43.86	3.71	3.72	12.88	12.43	13.49	13.90	70
3,4	$CH(CH_3)_2$	XIII	$C_{11}H_{12}N_2O_3Cu\cdot {}^1/_2H_2O$	45.12	45.62	4.48	4.58	9.57	9.72	21.70	21.37	33
3,4	$CH(CH_3)_2$	XIV	$C_{22}H_{25}N_4O_6Fe\cdot 2H_2O$	49.54	49.72	5.48	5.23	10.51	10.81	10.47	11.15	30
3,4	$CH(CH_3)_2$	XV	$C_{22}H_{26}N_4O_6Ni\cdot H_2O$	50.89	50.96	5.44	5.36	10.79	10.87	11.30	11.77	57

[a] Form 2,3 indicates chelates derived from 3-hydroxy-2-pyridinealdimines; form 3,4 such derived from 3-hydroxy-4-pyridinealdimines; R' indicates the various amino acid substituents, indicated by R' in the general formulas A, B and C for Cu, Fe and Ni chelates, respectively. [b] Various crystal water contents (such as 0, 1 or 2 H_2O) may be obtained for most compounds under specific drying conditions. [c] Percentage of the metal, chelated and indicated by the empirical formula.

appropriate amount of 0.25 *M* methanol solution of the metal salt ($CuCl_2\cdot 2H_2O$, $FeCl_3\cdot 6H_2O$, $NiCl_2\cdot 6H_2O$) with rapid stirring. Molar equivalents of the Cu(II) and Schiff base solutions were used, while for the formation of the Fe(III) and Ni(II) chelates the molar ratio of Schiff base to metal salt was 2:1. The Cu(II) and Ni(II) chelate compounds which formed spontaneously under these conditions were crystallized by partial evaporation of the solvent and purified by recrystallization from methanol. The exceptions to this crystallization procedure were the Cu(II) and Ni(II) chelates of the Schiff base formed from glutamic acid and 3-hydroxy-2-pyridinealdehyde, which were insoluble in all solvents investigated and precipitated as amorphous solids.

All of the Fe(III) chelates were too soluble in methanol and other polar solvents for purification by fractional crystallization. These compounds, and the Cu(II) and Ni(II) chelates mentioned above, which were too soluble, were purified by chromatography on Sephadex (Type G-25, Pharmacia Chemical Co., Uppsala, Sweden). A typical reaction mixture containing 2 mmoles of N-(hydroxypyridylmethylene)-amino acid metal chelate was added to a column of 30 g. of Sephadex previously equilibrated with methanol. The metal chelates separated on the column in an intensely-colored relatively narrow band. Elution of the column with absolute methanol gave almost quantitative separation of the metal chelate from potassium chloride, which was found in fractions immediately following the metal chelate. After a second chromatographic separation, final purification was effected by evaporation of the eluate to a small volume or by the addition of a non-polar solvent.

The results of the analyses of the metal chelate compounds prepared are given in Table I.

Infrared Spectra.—Infrared spectra were measured in the 4000–2000 $cm.^{-1}$ region with a Perkin–Elmer model 21 double beam spectrophotometer equipped with sodium chloride optics. Calibration of the frequency with water vapor, CO_2 gas and polystyrene foil showed the accuracy to be ± 5 $cm.^{-1}$. Potassium bromide pellets containing comparable concentrations of metal chelates (4×10^{-2} mole/l. of IV, and 2×10^{-2} mole/l. of V and VI) were prepared by weighing the exact quantities of metal chelates required to produce pellets of 1 mm. thickness (~400 mg. of KBr) having the desired concentration. To minimize scattering, the pellets were measured, reground and pressed repeatedly until no further increase in intensity of the absorption bands could be obtained.

Results

The metal chelate compounds prepared in this study are listed in Table I. They were all crystalline compounds and, with the exception of those formed from the Schiff base of glutamic acid, are all very soluble in water, and are somewhat soluble in methanol and other polar solvents. Although the Schiff bases from which these metal chelates were formed hydrolyze very rapidly in the presence of water, the metal chelates themselves, once formed, were found to be relatively stable in contact with water, and methanol–water mixtures could be used for recrystallization in some cases. None of the metal chelates was found to be stable in prolonged contact with water.

In the synthesis of these compounds, no advantage was found in using the anhydrous metal salts, but the use of methanol as a solvent for the metal salt led to better analytical results. Sephadex proved to be a superior reagent for the chromatographic purification of metal chelates that could not be purified by recrystallization. The metal chelates were found to be strongly absorbed on all common chromatographic materials and ion exchange resins. The success of the Sephadex is probably due to its very low content of free ion-exchange groups.

Under the conditions employed, it was found that the molar ratio of ligand to metal ion in the metal chelate formed was controlled by the ratios of the constituents used in the synthesis, in marked contrast to the behavior described for aqueous solutions.[9] Thus, there was no disproportionation in alcohol solutions of the 1:1 chelate to a 2:1 compound and metal salt or hydroxide, and the 2:1 chelate did not dissociate appreciably to give the 1:1 chelate compound and free ligand.

Infrared Spectra.—Assignments for the infrared spectra of the Cu(II), Fe(III) and Ni(II) chelates IV,

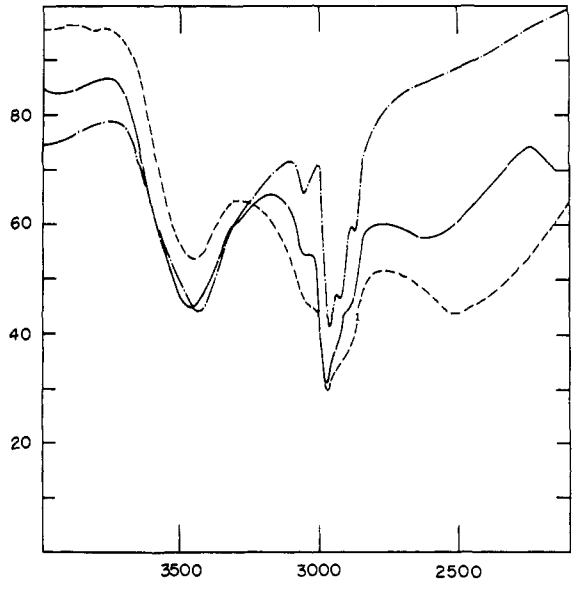
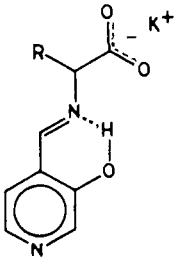


Fig. 1.—Infrared spectra of N-(3-hydroxy-2-pyridylmethylene)-valine chelates in KBr media: - · - · -, 4×10^{-2} *M* Cu(II) chelate; ———, 2×10^{-2} *M* Fe(III) chelate; - - - -, 2×10^{-2} *M* Ni(II) chelate.

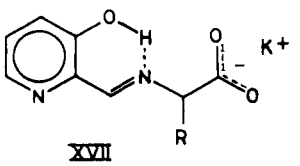
V and VI, shown in Fig. 1, can be made by a comparison with the assignments previously made for the parent Schiff bases.[10] The broad band near 3500 cm.$^{-1}$ is due to the water of hydration indicated in the analytical data given in Table I. The aromatic C–H stretching vibrations appear at 3080 cm.$^{-1}$, and the various C–H stretching modes associated with the valine–isopropyl group appear as a triplet between 2800 and 2980 cm.$^{-1}$. It is noted that for the Cu(II) chelate, there is no absorption band below 2800 cm.$^{-1}$. This is definite evidence for the absence of the 3-pyridol ring in one or more of its protonated tautomeric forms, since it has been shown previously[12] that such compounds have broad absorption bands in this region characteristic of intermolecular hydrogen bonding of the O–H $\cdots$ N or $\overset{+}{\text{N}}$–H $\cdots$ $\overset{-}{\text{O}}$ type. The broad band at 2600 cm.$^{-1}$ for the Fe(III) chelate, and a similar more intense band at 2500 cm.$^{-1}$ for the Ni(II) chelate, are assigned to intermolecular hydrogen bonding of the $\overset{+}{\text{N}}$–H $\cdots$ O$^-$ type, probably involving the positive pyridinium group and a carboxylate oxygen atom.

Discussion

The structures of the metal chelates I–XV listed in Table I, from the ligands XVI and XVII, may be deduced from the analytical data, the infrared spectra



XVI

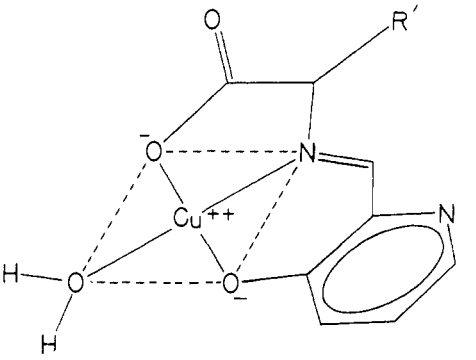


XVII

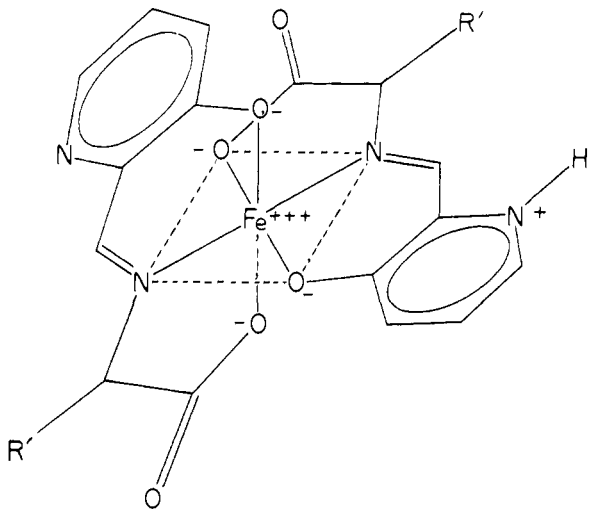
R = H, CH_3, $CH(CH_3)_2$, $CH_2C_6H_5$, CH_2CH_2COOK

and the properties of the ligands described previously.[10] The absence of anions or cations and the stoichiometric ratios established by analysis indicate the compositions of the Cu(II), Fe(III) and Ni(II) chelates illustrated by formulas A, B and C, respectively. Since the ligand must be binegative in the Cu(II) chelate, it must be terdentate. Since the metal ion must have four strong (square-planar) coördination positions, it is apparent that the structure of the compound must be that of formula A, in which the fourth coördination position is occupied by a water molecule. The alternative structure, in which a proton is retained on the aromatic hydroxyl group and a hydroxyl ion is coördinated to the metal, may be excluded on the basis of the infrared spectrum (Fig. 1) and the relatively low pK of the aromatic hydroxyl group previously reported.[13]

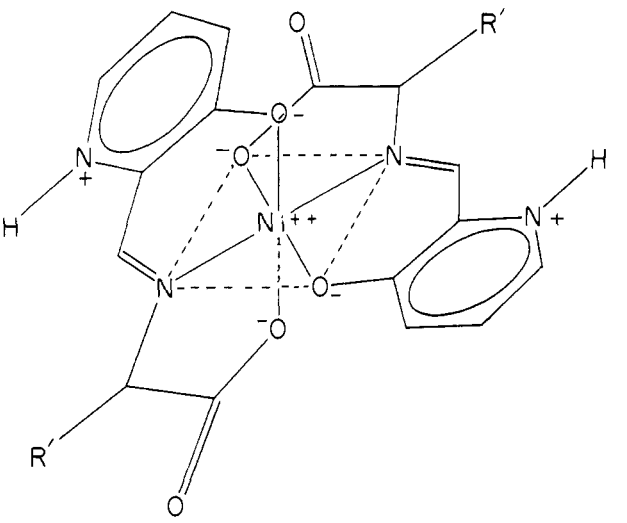
For the Fe(III) chelate compounds, such as B, electroneutrality can be achieved by dissociation of two protons from one of the Schiff base ligands, and one proton from the other. In the case of the Ni(II) chelate, only one proton need be displaced from each ligand. On the other hand, the formation of a terdentate ligand arrangement in these structures can occur readily by transfer of the proton from the phenolic group to the pyridine nitrogen atom. In this way electroneutrality would be maintained, and the metal chelate would assume a more favorable steric arrangement in which the two terdentate ligands would completely satisfy the octahedral coördination requirements of the ligand. The tautomerism of compounds containing the 3-pyridol group has been studied extensively,[14,15] and it is known that conversion of the neutral to the dipolar form of 3-pyridol takes place very easily, so that both forms are in equilibrium in polar solvents. Thus the metal chelate structures shown could stabilize the dipolar form as indicated in B and C. The use of



A



B



C

(12) D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, **81**, 3933 (1959).
(13) D. Heinert and A. E. Martell, *ibid.*, **85**, 188 (1963).
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molecular models reveals very low, if any, strain in the planar arrangements shown for the three donor groups of the ligand. Since resonance between the imine and aromatic ring would tend to keep the bonds adjacent to the imine nitrogens in the same plane, the planar arrangement for the ligands shown in B and C is favored over other possible structures.

Additional evidence for the proposed chelate structures may be obtained by a comparison of the infrared spectra in Fig. 1. The absence or presence of broad bands near 2500–2600 cm.$^{-1}$ indicates no intermolecular hydrogen bonding for the Cu(II) chelate, and increasing amounts of such bonding for the Fe(III) and Ni(II) chelates. The intensities of these bands correlate exactly with what would be expected for the presence of none, one, and two pyridinium protons capable of forming intermolecular hydrogens bonds as indicated in structures A, B and C, respectively. The alternative structures in which water is coördinated to the metal and the 3-pyridol group is engaged in intermolecular hydrogen bonding cannot be excluded for Fe(III) and Ni(II) on the basis of infrared evidence. On the other hand, such alternative structures are considered improbable on the basis of what is known about the affinities between metal ions and various types of donor groups in organic ligands.

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On Cysteine and Cystine Peptides. II. S-Acylcysteines in Peptide Synthesis[1,2]

BY LEONIDAS ZERVAS, IPHIGENIA PHOTAKI AND NICOLAOS GHELIS

RECEIVED OCTOBER 31, 1962

An approach to the synthesis of unsymmetrical cystine peptides containing at least two –S–S– bridges which entails the use of selectively removable S-protecting groups is discussed. In addition to S-trityl- and S-diphenylmethyl-, S-benzoyl-, S-acetyl- and S-carbobenzoxycysteines are shown to be very useful intermediates for the incorporation of cysteine residues into a peptide chain. The removal of these S-acyl groups which are, in a certain sense, "active esters" can easily be achieved by methanolysis in the presence of sodium methoxide. Furthermore, the S-benzoyl group is not attacked by the N-decarbobenzoxylating agents trifluoroacetic acid and hydrogen bromide in acetic acid. The S-acetyl group is resistant to trifluoroacetic acid but is removed by hydrogen bromide in acetic acid and the S-carbobenzoxy group is split off by trifluoroacetic acid but survives the treatment with 2 *N* hydrogen bromide in acetic acid to a very great extent. Consequently, these S-acylcysteine residues can be used for peptide synthesis by means of their N-carbobenzoxy derivatives. Using S-acylcysteine residues, several cysteine-containing peptides have been synthesized and converted to the corresponding cystine peptides. The use of the above-mentioned S-protecting groups for overcoming the unique difficulties inherent in establishing a disulfide bridge specifically between two of three cysteine residues of a peptide chain, as in fragment IX of insulin, has been explored.

Introduction

Many methods for the incorporation of amino acid residues, including cysteine residues, into a peptide chain are now well established.[3a] In particular, the synthesis of symmetrical cystine peptides[3a,b] and cyclic peptides of the oxytocin type[3b] is, in principle, no longer a problem in peptide chemistry. On the other hand, the synthesis of unsymmetrical cystine peptides with two or more –S–S– bridges is an extremely difficult task.[3c] A random solution to this problem may be that of synthesizing the proper sequences of amino acids, including cysteine residues, as they are represented in a natural product, *e.g.*, insulin, in the hope that the oxidation of these synthetic cysteine peptides would lead to the formation of the natural product and its characteristic –S–S– bridge system. Indeed, it has recently been reported that when insulin is reduced and the sulfhydryl chains so obtained are reoxidized, some insulin activity is regenerated.[4] Many fragments of the insulin molecule, some of which contain S-benzylcysteine residues, have already been synthesized in different laboratories.[5]

Another, much more controlled approach to the synthesis of unsymmetrical cystine peptides containing at least two –S–S– bridges requires that the following conditions be fulfilled: (a) cysteine residues bearing different S-protecting groups which may be removed selectively must be available; and (b) procedures must be developed for preventing the rearrangement of cystine chains during synthesis, so that the desired multimembered ring system may be formed.[3c]

Concerning the first of the above requirements S-trityl-(Tr) and S-diphenylmethyl-(DPM) L-cysteine have recently been proposed as intermediates for the incorporation of cysteine residues into a peptide chain.[3c] It has been found that S-acylcysteines are also suitable for the above purposes since the S-acyl groups, like the S-trityl and S-DPM groups, can be very easily removed without affecting peptide bonds or other sensitive parts of the molecule. Very useful, in our opinion, are the S-benzoyl- (Ia) and S-acetyl-L-cysteine(Ib) and especially their N-carbobenzoxy derivatives (Ic,Id) which can be easily prepared by reduction of N,N′-dicarbobenzoxy-L-cystine with zinc–hydrochloric acid followed by acylation of the N-carbobenzoxy-L-cysteine

(1) (a) A summary of a part of this paper was presented at the 4th European Peptide Symposium, Moscow, August, 1961; L. Zervas, *Collection Czechoslov. Chem. Communs.*, **27**, 2229 (1962); (b) a summary of this paper was presented at the 5th European Peptide Symposium, Oxford, England, September, 1962; L. Zervas, I. Photaki, A. Cosmatos and N. Ghelis, Proceedings of the 5th European Peptide Symposium, Pergamon Press, London, in press.

(2) This investigation was supported by the Royal Hellenic Research Foundation, to which we are greatly indebted.

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