was hydrogenated in the usual manner over palladium in methanol (20 ml.) to yield 0.13 g. (86%) of the dipeptide: $[\alpha]^{25}D$ +13.2° (*c* 2.1, water); lit.[22] $[\alpha]^{20}D$ +12.8 (*c* 2.0, water). *Anal.* Calcd. for $C_{12}H_{16}N_2O_3$: N, 11.9. Found: N, 12.1. Paper chromatography showed one spot, R_f 0.62, with 1-butanol–acetic acid–water, and one spot, R_f 0.80, with phenol–water.

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A Nuclear Magnetic Resonance Study of the Structures of L- and *meso*-Cystine in Aqueous Solutions[1]

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The n.m.r. spectra of L-*cystine, meso-cystine, and their dimethyl esters in acidic and basic solutions are analyzed. The results indicate that the possible configurations for* L-*cystine are stablized by intramolecular interactions between the two moieties. This is in contrast to meso-cystine where no stabilization is observed.*

Introduction

There have been several analyses of the n.m.r. spectra of the L-amino acids, including L-cystine. Taddei and Pratt[2] have observed the chemical shift changes of the various protons as a function of pH. Pachler,[3,4] Fujiwara and Arata,[5,6] and Martin and Mathur[7] have analyzed the high-resolution spectra of these acids in a fashion which can be interpreted to give the relative populations of the three classical rotamers derived from considering the α-amino acids as derivatives of ethane.

The case of the cystines is, however, more complicated than these studies would indicate, and some of the peculiarities of their spectra may throw some light on the interpretation of the spectra of the other amino acids.

The structure of L-cystine in solution has been considered by others[8–10] theoretically, in connection with the very large optical activity exhibited by the molecule. This is a factor of 10 greater than any other amino acid. The disulfide ultraviolet absorption band is optically active,[10a] and studies of this phenomenon in solution indicate that there is only very highly hindered rotation about the disulfide bond. In fact it would appear that the disulfide portion of the structure of the cystines is represented by structures I and II, in which the dihedral angle is 90°.

There are two cases which must be considered in a discussion of the cystines in solution. The first is when R = R′. This is either all L- or all D-cystine. The two structures I and II do not necessarily have the same energy. This is apparent from the use of space filling models and is, of course, theoretically valid. Thus, the possibility of one of the structures (I or II) being predominant in solution obtains.

The second cases arises in a cystine molecule where one moiety is of the L optical isomer, and the other is of the D variety. This is defined as *meso*, or internally compensated, cystine. For this case, by the same argument as above, both structures I and II have the same energy.

There have been speculations[11] concerning the possibility of an endocyclic configuration of L-cystine brought about by binding between the opposing amino and carboxyl groups of the two moieties. The presence of this structure would fit in with the observation that cystine has a strong temperature coefficient of molecular optical rotation, indicating an equilibrium between structural forms.

All previous analyses of the n.m.r. spectra of the simple amino acids in solution are based on the classical rotamer structures which neglect intramolecular interactions other than steric hindrance in the *trans* and *gauche* configurations. Absence of intramolecular interactions leads to the conclusion that both L- and *meso*-cystine should have the same n.m.r. spectra. It was of some interest to determine whether or not this is true.

Experimental Section

The n.m.r. spectra were taken with a DA-60 Varian n.m.r. spectrometer at an in-probe temperature of 26 ± 1° as measured by the ethylene glycol method. An internal standard of TSS (3-trimethylsilyl-1-propanesulfonic acid sodium salt) was used. pH measurements were made with a Beckman one-drop electrode useful in the range 0–11 pH units, and no attempt was

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Table I. Comparison of Computed N.m.r. Constants for L-Cystine, *meso*-Cystine, and L-Cysteine[a]

Molecule	pH	Computation scheme	$\delta_{ax(o)}$	δ_{ab}	$J_{bx(o)}$	$J_{ax(o)}$	J_{ab}	Ref.
L-Cystine	<0	ABC	70.62	8.52	4.18	8.17	−15.37	3
	<0	ABC	68.9	5.3	3.7	8.2	−15.1	6
	<0	ABC	70.1	7.1	4.8	7.4	−15.2	*b*
	<0	ABX	70.1	7.3	4.3	7.7	−15.2	*b*
meso-Cystine	<0	ABX	69.2	$1.5 < \delta < 3.6$	$N = 5.9$		−15.2	*b*
meso-Cystine dimethyl ester	<0	ABX	75.2	$1.5 < \delta < 3.6$	$N = 5.9$		−15.2	*b*
L-Cystine dimethyl ester	<0	ABX	73.4	$1.5 < \delta < 3.6$	$N = 5.9$		−15.2	*b*
L-Cystine	>11	ABC	39.74	12.83	4.81	7.66	−13.40	3
	>11	ABC	38.70	12.52	4.59	7.50	−13.40	*b*
meso-Cystine	>11(a)	ABC	37.94	11.64	4.84	7.48	−13.40	*b*
	>11(b)	ABC	37.17	11.22	4.72	7.39	−13.10	*b*
L-Cysteine	<0	AX_2	73.3	0.0	5.06	5.06	...	3
	<0	ABC	74.2	0.0	5.0	5.0	...	6
	<0	A_2B	76.0	0.0	5.2	5.2	...	7
L-Cysteine	>11	ABC	43.29	14.69	4.22	8.28	−13.24	3
	>11	ABC	39.5	13.0	4.5	7.5	−13.2	6
	>11	ABX	40.1	22.3	3.3	9.5	−12.8	7

[a] Constants given in c.p.s. [b] Present work.

made to convert pH to pD. Five per cent w./v. solutions were made of the amino acids and their esters with commercial D_2O. The spectra were obtained immediately after solvation, without degassing, in precision 5-mm. sample tubes. Calibrations of the sweep ranges on the spectrometer were accomplished using the method of Jungnickel.[12] The resolution of the spectrometer was maintained at 0.3 c.p.s.

L-Cystine was obtained from Mann Research Laboratories as homogeneous by paper chromatography. This material, which decomposed at 253–255° (lit. 258–261°), gave the same peak parameters as a known standard of L-cystine on a Beckman amino acid analyzer. In addition, the solid-state infrared spectrum correlated with the literature spectrum.[13] The material was used without further purification.

meso-Cystine was obtained from the same source and was labeled chemically pure. It was analyzed on the amino acid analyzer, and no detectable D,L-cystine was found by comparison of the peak shape with a sample containing L- and *meso*-cystine. The upper limit for the D,L-impurity by this method is estimated at 20%. No optical activity was found in HCl solutions of this preparation. The solid-state infrared spectrum, when compared with the literature spectrum,[13] indicated no more than 10–15% contamination by D,L-cystine. No other materials were detectable. The preparation decomposed at 220–226°. In some experiments described below this material was used without further purification. However, experiments in which D,L-cystine was separated from it by fractional crystallization are also described.

L-Cystine dimethyl ester dihydrochloride was obtained from the same source and was used without further purification.

meso-Cystine dimethyl ester dihydrochloride was obtained from the C.P. *meso*-cystine by a standard synthesis,[14] and was recrystallized once from methanol solution by addition of ether.

Dilutions were made using commercial DCl and NaOD made by dropping D_2O onto sodium oxide.

Spectral analyses for type ABC spectra were performed on an IBM 7090 computer using the Castellano and Waugh method.[15] The computer program was that of Cavanaugh.[16] ABX analyses were carried through manually using the scheme of Pople, Schneider, and Bernstein.[17]

Results

Acidic Solutions. L- and *meso*-cystine display two different types of spectra in acid solution at pH <0 as shown in Figure 1.

The spectrum for L-cystine is an ABC type which bears a very strong resemblance to an ABX spectrum. Analyses of this spectrum on the basis of both ABC and ABX schemata were made and the derived constants are given in Table I. The difference between the derived constants, given the line positions, for the two analyses is not great. Comparison of these constants with the other published analyses of the same spectrum show that all differ in varying degrees from each other. This holds true also for the spectrum of L-cysteine which is also included for purposes of comparison. These differences may be due to temperature or pH effects which change the configurational equilibria, or to instrumental differences. It should also be noted that the choice of a negative value for J_{ab} is dictated by *a priori* considerations.[3] The ABX analysis is, of course, invariant to the sign of J_{ab} while the ABC analysis indicates only a negligibly small intensity pattern difference between the two cases.

The *meso*-cystine spectrum at this pH is not adequately fitted by a single species analysis. However, the spec-

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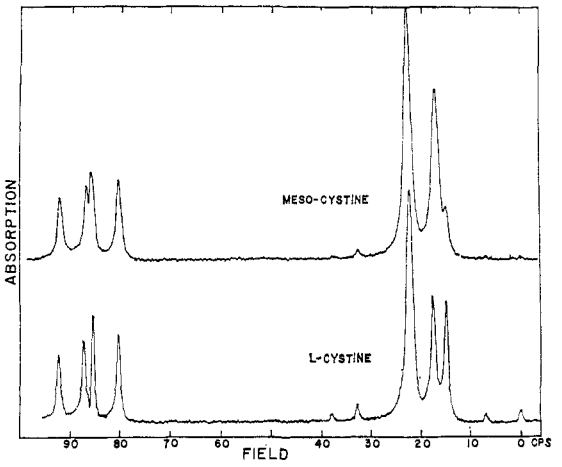

Figure 1. N.m.r. spectra of L- and *meso*-cystine at pH <0; linear absorption scale.

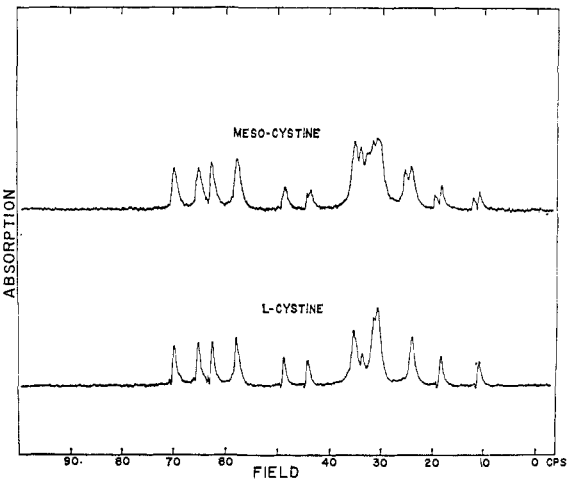

Figure 2. N.m.r. spectra of L- and *meso*-cystine at pH >11; linear absorption scale.

trum fits well into an analysis of a mixture of a large amount of *meso*-cystine (>90%) whose spectrum is discussed below, and a small amount of L-cystine (<10%) whose spectral analysis is discussed above. Thus, the shoulder at about 15 c.p.s. in the spectrum of *meso*-cystine (Figure 1) is due to the D,L-cystine impurity.

This spectrum of *meso*-cystine could result from an impurity of D,L-cystine in the *meso*-cystine preparation, undetected by other methods. In order to examine this point the racemic impurity from the *meso*-cystine was separated from the commercial preparation by fractional crystallization according to the original procedure.[18] After two such crystallizations material was obtained which in acid solution gave a spectrum identical with the L-cystine spectrum. This same solution at alkaline pH (>11) displayed the spectrum discussed below for the *meso*-cystine commercial preparation. However, when the solution was titrated again to pH <0, it displayed a spectrum almost identical with that of the commercial *meso*-cystine at acid pH. The commercial *meso*-cystine carried through the same cycle revealed no change from the original acid solution upon reacidification.

The reason for these observations is easily explained in terms of disulfide exchange[19] which proceeds very rapidly in alkaline solutions in the presence of oxygen. In an alkaline solution of D,L-cystine this effect will cause a mixture of *meso*-cystine and D,L-cystine to result.

Thus, assigning the small side peaks at 0, 8, 32, and 38 c.p.s. (Figure 1) to *meso*-cystine, a 10-line spectrum remains to be analyzed. In view of the resemblance of the spectrum of L-cystine to an ABX system it is convenient to analyze this spectrum in terms of an ABX spectrum. The conditions under which the normal 12-line ABX spectrum may appear as a 6–10 line spectrum is an intermediate case between the normal and the "deceptively simple," 5-line spectrum.[20] If, $L = {}^1/_2(J_{ax} - J_{bx})$, $N = {}^1/_2(J_{ax} + J_{bx})$, and $\delta_{ab} = \nu_a - \nu_b$ = chemical shift in c.p.s., then in the case where L and $\delta_{ab} << J_{ab}$ it may be shown[20] that the following expressions give the separations of the numbered lines: X portion, $S_{10-11} = 2\delta_{ab}L/J_{ab}$; AB portion, $S_{3-5} = (\delta_{ab} + L)^2/2J_{ab}$, $S_{4-6} = (\delta_{ab} - L)^2/2J_{ab}$.

Applying these expressions to Figure 1 it follows that for *meso*-cystine S_{10-11}, the separation of the inner members of the X quartet, is observable and is, in fact, equal to 0.8 c.p.s. However, $S_{3-5} \simeq S_{4-6} < \Delta\nu_{1/2}$ where $\Delta\nu_{1/2}$ is the full width at half-height for the individual lines making up the observed strong doublet. Assuming that $\Delta\nu_{1/2}$ for *meso*-cystine is the same as for L-cystine, the following relations are obtained from the above expressions when $\Delta\nu_{1/2} = 1$ c.p.s., and $J_{ab} = -15.2$ c.p.s. (measured from the *meso*-cystine spectrum) are inserted: $\delta_{ab,max} = 1.5$ c.p.s., $L_{max} = 4.0$ c.p.s.; $\delta_{ab,min} = 3.6$ c.p.s., $L_{min} = 1.7$ c.p.s. The individual values of J_{ax} and J_{bx} cannot be obtained accurately from this spectrum but their average (N) may be measured. The value obtained, as indicated in Table I, is $N = 5.9$ c.p.s. In contrast to these values the constants for L-cystine under the same conditions are: $\delta_{ab} = 7.1$ c.p.s., $L = 1.3$ c.p.s., $N = 6.1$ c.p.s.

The results are therefore in accord with the conclusion that the spectrum of *meso*-cystine in acid solution is an example of an ABX spectrum where $\delta_{ab} << J_{ab}$, $L << J_{ab}$, in contrast to that of L-cystine where $\delta_{ab} < J_{ab}$, $L << J_{ab}$. The constants which best fit the data indicate that δ_{ab} for *meso*-cystine is about one-half to one-third δ_{ab} for L-cystine.

The spectra of both L-cystine, and *meso*-cystine, dimethyl esters fit into the same analysis, with the same coupling constants as those of *meso*-cystine. As indicated in Table I there are minor differences in δ_{ax} between these compounds.

Basic Solutions. Analysis of these ABC spectra were performed and the derived constants are given in Table I.

The spectrum of L-cystine displays a peak at about 33 c.p.s. (see Figure 2), which is not a member of the L-cystine ABC system. The position of this peak, however, coincides with the position of the sharpest, most intense peak in the spectrum of L-cysteine taken under the same conditions. The assignment of this peak to L-cysteine is in accord with the presence of disulfide exchange. The magnitude of the peak indicates that the cysteine intermediate is present in surprisingly high concentration.

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The spectrum of *meso*-cystine is clearly that of two species, present in comparable amounts, but with slightly different n.m.r. parameters. The analysis of this spectrum is given in Table I where the two species present are labeled a and b. Unfortunately, under these conditions the spectral lines are rather broad, and resolution is affected. This poor resolution is reflected in the uncertainty of the derived constants. It must be assumed that one species in this spectrum is L-cystine, and the other *meso*-cystine. However, the precision of the values for the constants does not appear to allow an assignment of a or b to *meso*-cystine. The conclusion must still be drawn that even at this pH, *meso*- and L-cystine have different structures. The peak assigned to cysteine in the previous paragraph is also present in this spectrum. The spectra of the methyl esters was not obtained at this pH due to very rapid hydrolysis.

Discussion

In view of the original question these observations indicate that the stabilization of the actual configuration of L-cystine in acid solution is due to intramolecular interaction between the two moieties. This binding appears to be absent in *meso*-cystine as evidenced by the equivalence of the spectrum of *meso*-cystine with that of the esters of both L- and *meso*-cystine. Additional evidence is the very large decrease in δ_{ab} from L-cystine to *meso*-cystine in acid solution, indicating that the β protons have become almost equivalent magnetically in *meso*-cystine. This suggests that the moieties in *meso*-cystine rotate about the disulfide bond much more freely than in L-cystine. This is in accord with the difference in the number of possible stabilizing bonds as discussed in the introduction.

It is interesting that the coupling constants J_{ax} and J_{bx} are relatively invariant to changes in pH for L- and *meso*-cystine and are comparable to those of L-cysteine even though structural changes have clearly taken place. It appears probable, therefore, that the classical rotamer analysis does not apply to the cystines. It should be mentioned here that the spectra of *meso*-cystine and the two methyl esters will equally well fit a six-line A_2B analysis in which the derived constants are $\delta_{aa} \equiv 0$ c.p.s. and $N = 5.9$ c.p.s. Only the presence of the very weak satellites indicate that the system should be more properly regarded as an ABX system with $\delta_{ab} << J_{ab}$.

No conclusion can be drawn as to the stability of either structure I or II so far as the configuration about the disulfide bond is concerned. If the exact nature of the optical rotatory activity of the disulfide transition becomes known it should be possible to combine this knowledge with further n.m.r. measurements to indicate which configuration, left- or right-hand helix, has the lowest energy.

A New Method for the Synthesis of Furanose Derivatives of Aldohexoses[1]

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Disiamylborane was used to reduce tetraacylhexono-γ-lactones to the corresponding tetraacylhexofuranoses. Reduction is almost quantitative and the blocking ester groups are unaffected. The reaction was applied to the newly prepared tetra-O-benzoyl-γ-lactones of L-*gulose,* D-*gulose,* D-*allose,* D-*talose, and* D-*altrose and to* D-*galactono-γ-lactone tetraacetate resulting in the corresponding acylated tetraacylhexofuranoses which were acylated at the anomeric hydroxyl group to form pentaacylhexofuranoses. This method was developed as a general procedure of short reaction route to obtain furanose derivatives of aldoses. It has a potentially important application in biochemistry which lies in its use for the preparation of C-1′ labeled furanosyl nucleosides.*

Hexoses exist primarily in the pyranose ring form, and no general method for obtaining furanose derivatives of hexoses has heretofore been reported. For this purpose special methods have been employed utilizing isopropylidene derivatives to prepare 9-β-D-glucofuranosyladenine[2] and other furanosyl nucleosides of 6-deoxy hexoses.[3] Recently Wolfrom, *et al.*,[4] reported on the use of furanose thioglycosides for the preparation of hexofuranosyl nucleosides of D-glucose and D-galactose. Although these methods may be more generally applicable in many instances, as with the rare hexoses, such intermediates are as yet unknown or the pathway might not be desirable as a practical reaction sequence.

For these reasons, and in view of the advantages of having a single method applicable to a wide variety of carbohydrates, and because of our interest in the prep-

(1) (a) Taken in part from a thesis submitted by L. M. Lerner to the University of Illinois Graduate College in partial fulfillment of the requirements for the Ph.D. degree. (b) Supported in part by Grant P-161 from the American Cancer Society and by Training Grant No. GM-471 from the Division of General Medical Sciences of the U. S. Public Health Service.

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