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MASS SPECTRA OF SULPHUR-CONTAINING AMINO ACIDS AND PEPTIDES

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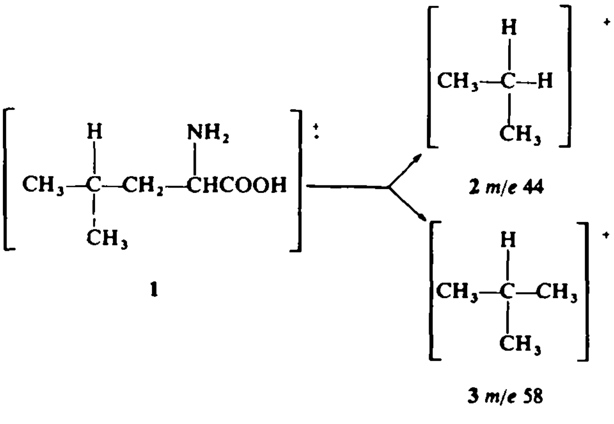
Abstract—More than twenty synthesized S-containing amino acids and peptides with biological interests, have been measured by direct inlet system of the mass spectrometer. Most of these compounds have the large molecular ion abundances and give the reproducible fragmentations. The mass spectral-fragmentation mechanisms of systematically synthesized S-alkyl-L-cysteines, S-alkyl-L-cysteine sulphoxides, S-alkyl-2-methyl-DL-cysteines, glycyl-S-alkyl-DL-cysteines and cyclic sulphur-containing amino acids, etc. are proposed.

INTRODUCTION

THE mass spectra of amino acid and peptide derivatives have been investigated.[1-3] The molecular ion peaks of free amino acids and peptides have relatively the low abundances and it often gives rise to cases in which they are not observed at all, because of the low vapour pressure of these compounds and the instability of the molecular cations. Junk and Svec[4] have succeeded in obtaining a unique and reproducible mass spectrum of each individual amino acid by charging a crucible with the pure acid and then placing the crucible directly into the ionization chamber of the mass spectrometer.

Recently, Martin[5] has speculated the structures of the mass spectral fragments of 22 free amino acids. There is a possibility that the fragment formed by hydrogen rearrangement is one of the thermal decomposition products of an amino acid. For example, the fragment peaks 44 and 58 in the mass spectrum of leucine (**1**) have been formulated as **2** and **3** formed by hydrogen rearrangement,[5] respectively, as shown in scheme 1.



SCHEME 1

But there is no evidence that these fragments are not due to thermal decomposition but mass spectral electron impact. Therefore, the studies on the fragmentation of hydrogen rearrangement should need such consideration as given below.

(1) It is necessary to distinguish the fragments of mass spectra from those of the thermal decomposition products of amino acids and peptides, since these compounds are unstable to heating in the evacuated inlet system.

(2) It is necessary to use amino acids and peptides exchanged by the isotopes: for example, the carboxyl and amino hydrogens of them are deuterated in an excess of 99·9% D_2O.

It is generally known that the molecular ion peaks of S-containing amino acids such as methionine and cysteine have considerably the large relative abundance.[4] However, the mass spectral fragmentation of naturally occurring and related S-containing amino acids and peptides has not been investigated systematically.

We have investigated the photolysis[6] and γ-radiolysis[7-8] of S-containing amino acids, and the precursors of the caucas (*Allium victorialis* L.) flavour.[9] It is interesting to compare the mass spectral fragmentation with the photolysis or the γ-radiolysis of S-containing amino acid, and also, it is important to apply the mass spectrometry to the identification of a trace amount of a naturally occurring S-containing amino acid and peptide, and of the micro-organic metabolite of a naturally occurring and related S-containing amino acid.

This paper deals with the unique mass spectral fragmentation mechanism of more than 20 synthesized S-containing amino acids and peptides of biological interests.

RESULTS AND DISCUSSION

Fragmentations of S-*alkyl*-L-*cysteines* (4)

The reproducible mass spectrum of S-n-propyl-L-cysteine, one of S-alkyl-L-cysteine, is shown in Fig 1.

The base ion peak of S-alkyl-L-cysteine is $(R—S—CH_2)^+$ ion fragment which results from the rupture of the bond β rather than α to the S atom as well as methionine though α-cleavage is preferential in the case of γ-irradiation.[9] The most obvious and

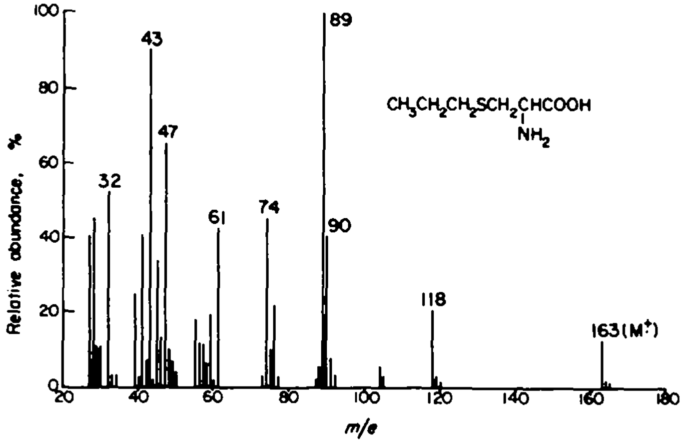
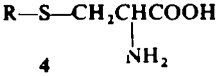


FIG 1.

unique difference between S-alkyl-L-cysteine and methionine is $(R{-}S{-}CH_3)^{+\cdot}$ radical cation peak formed by a hydrogen rearrangement, i.e. the relative abundances in the case of R = methyl, n-propyl, allyl and 1-propenyl were 55·2, 40·3, 55·8 and 72·8 %, respectively.



$R{-}S{-}CH_2\underset{NH_2}{\underset{|}{C}}HCOOH$

4

R : CH_3, $CH_3CH_2CH_2$, $CH_2{=}CHCH_2$, $CH_3CH{=}CH$,

However, there is a possibility that $(R{-}S{-}CH_3)^{+\cdot}$ ion fragment is one of the thermal decomposition products. Fig 2 shows the gas chromatograms of head space vapour from thermal decomposed S-allyl-L-cysteine and of authentic methyl allyl sulphide.

The main thermal decomposition products at 200 to 250°, peaks 1 and 2, were identified with allyl mercaptan and diallyl sulphide by using GC-MS,[10] respectively. From the facts that methyl allyl sulphide was not detected in the gas chromatogram and diallyl sulphide ion, *m/e* 74 was not observed in the mass spectral fragments of S-allyl-L-cysteine, $(R{-}S{-}CH_3)^{+\cdot}$ ion fragment is not due to thermal decomposition but mass spectral electron impact.

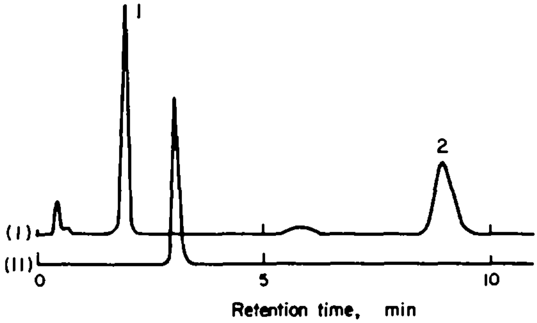
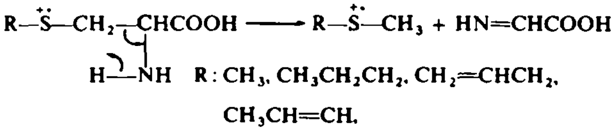


FIG 2. Gas chromatograms of thermal decomposition products of S-allyl-L-cysteine (I) and authentic methyl allyl sulphide (II).
Column: 20% Reoplex 400 coated on C-22 (1 m × 3 mm i.d.). Column temperature: 80°.

Moreover, to elucidate the mechanism of hydrogen rearrangement, the carboxyl and amino hydrogens of S-alkyl-L-cysteines were exchanged stepwise with deuterium. Mass spectral data in the region of mass 88, $(CH_2{=}CHCH_2{-}S{-}CH_3)^{+\cdot}$ ion fragment and the molecular ion of S-allyl-L-cysteine is shown in Fig 3.

At first the carboxyl hydrogen, next one of the amino hydrogens and at last both of the amino hydrogens are exchanged stepwise by using D_2O. The hydrogen rearrangement to $(CH_2{=}CHCH_2{-}S{-}CH_3)^{+\cdot}$ ion fragment resulted from the transfer of the amino hydrogen of S-allyl-L-cysteine as shown in Fig 3. Then, the hydrogen-rearrangement mechanism in proposed in scheme 2.



SCHEME 2

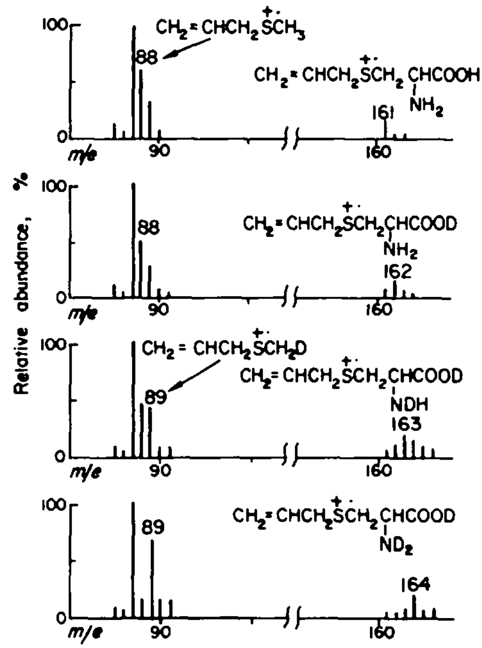


Fig 3. Mass spectra (parts of mass peaks) of S-allyl-L-cysteine deuterated stepwise.

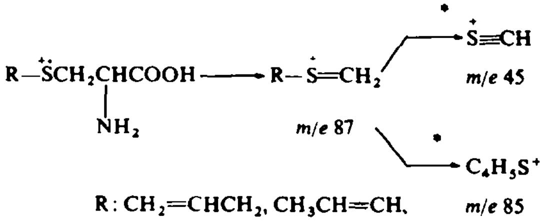
It seems that these fragmentations are very important to distinguish between methionine- and S-alkyl-L-cysteine-containing peptides in *Allium*[11] and *Cruciferous*[12] plants.

In addition, though the fragmentations of S-allyl-L-cysteine and S-(1-propenyl)-L-cysteine indicated the similar pattern to each other, the molecular ion abundance of the latter was larger than that of the former; each molecular ion is 14·2 and 32·1 %, and $(CH_2{=}CHCH_2)^+$ ion was more abundant than $(CH_3CH{=}CH)^+$ ion, i.e. each ion abundance is 87·6 and 40·8 %, respectively.

This information indicates that S-(1-propenyl)-L-cysteine-radical cation is more stable than S-allyl-L-cysteine's since *d*-orbital of the S atom resonates reasonably with 1-propenyl double bond, while the bond of S—C (allyl group moiety) is more easily cleaved than that of S—C (1-propenyl group moiety).

Masses 45 *and* 85 *from unsaturated cysteine derivatives*

The *m/e* 45 fragment was strongly observed by the electron impacts of S-allyl- and S-(1-propenyl)-L-cysteine (4) but not in the cases of S-methyl- and S-n-propyl-L-cysteine.

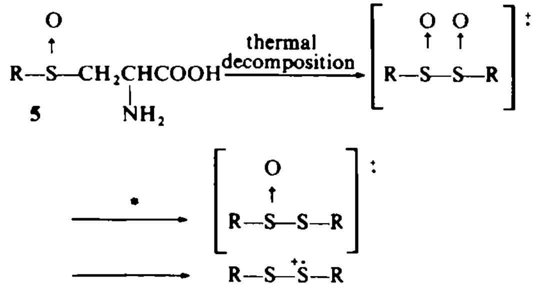


Scheme 3

The metastable ion peak at 23·3 indicates that the ion at m/e 45 is formed from the m/e 87 ion with the concerted loss of two vicinal groups. Similarly, the metastable ion at 83·0 indicates that the ion at m/e 85 is formed from the m/e 87 ion with the concerted loss of two H atoms. These concerted processes are summarized in scheme 3.

Fragmentations of S-alkyl-L-cysteine sulphoxide (**5**)

Sulphoxide amino acids such as S-alkyl-L-cysteine sulphoxide (alkyl: methyl; n-propyl; 1-propenyl; allyl) have been shown to be the characteristic-flavour precursors of onion (*A. cepa* L.),[13] garlic (*A. sativum* L.)[14] and other *Allium* species. The sulphoxides generally exhibit lower vapour pressure than the corresponding sulphides. Though the fragmentations by the electron impact could not be obtained, only the pattern of the thermal decomposition of S-alkyl-L-cysteine sulphoxide was unique and reproducible. Scheme 4 shows the main decomposition process.



R: $CH_3CH_2CH_2$, $CH_2{=}CHCH_2$, $CH_3CH{=}CH$,

SCHEME 4

Fragmentations of S-alkyl-2-methyl-DL-cysteines (**6**)

The introduction of a Me group in the α-position of S-containing amino acid greatly alters the fragmentation mechanisms and further lowers the vapour pressure. The mass spectrum of S-n-propyl-2-methyl-DL-cysteine, one of S-alkyl-2-methyl-DL-cysteines, is shown in Fig 4.

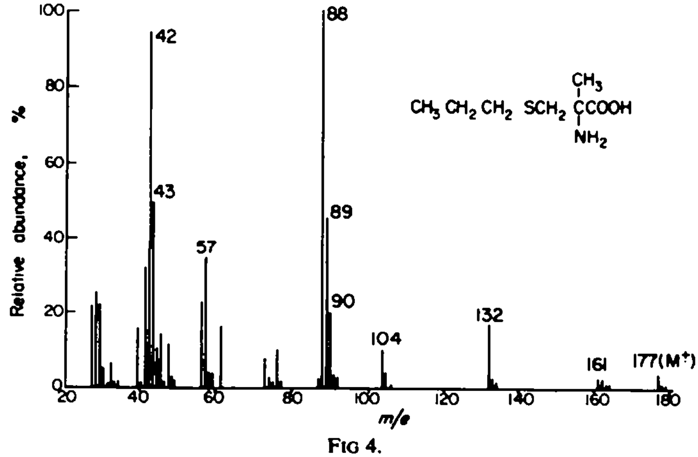


FIG 4.

Though the cleavage of β bond to the S atom of S-alkyl-2-methyl-DL-cysteines results mainly, the base ion fragment was amino cation, m/e 88 (8) not containing a S atom. Moreover, relatively the intense and unique peak was mass 42, $(C_2H_4N)^+$ ion fragment. The metastable ion peak at 20·0 indicates that the ion at m/e 42 is formed from the m/e 88 ion as shown in scheme 5. However the metastable ion peak of $(M\text{-}COOH)^+$ ion fragment (7) to m/e 42 ion could not be detected. The ion at m/e 42 can be regarded as either $(CH_2{=}C{=}\overset{+}{N}H_2)$ or $(CH_3{-}C{\equiv}\overset{+}{N}H)$ fragment. Fig 5 shows the mass spectral data in the region of mass 20 of S-ethyl-2-methyl-DL-cysteine exchanged stepwise the carboxyl and amino hydrogens for deuteriums.

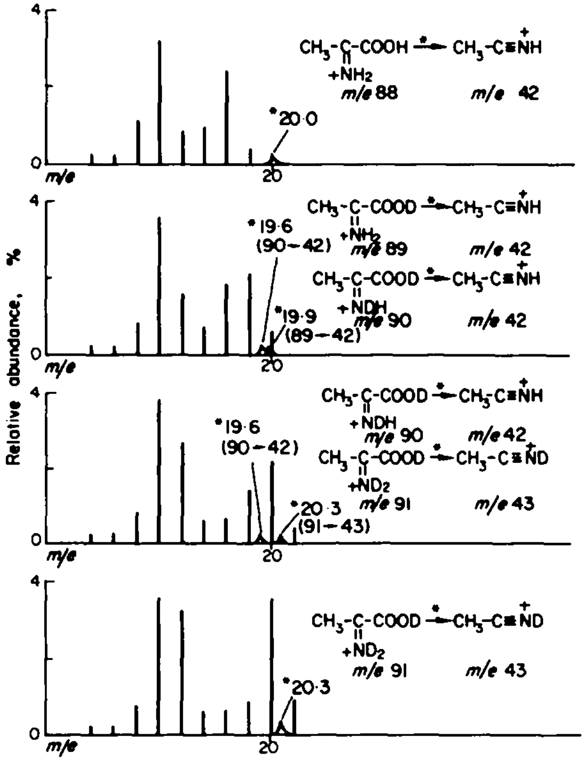
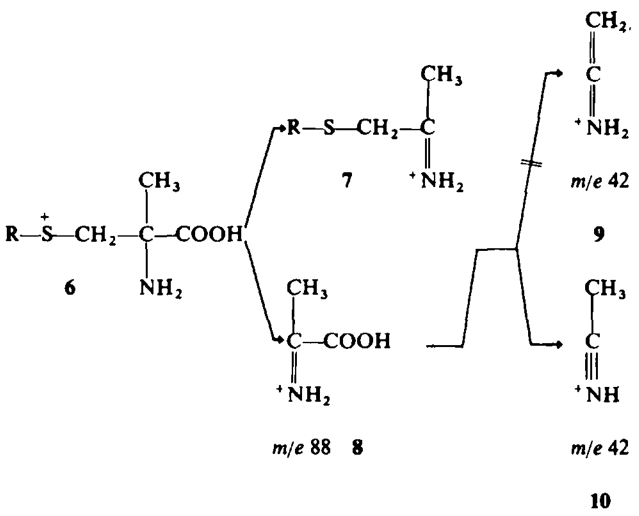


FIG 5. Mass spectra (mass 20 region) of S-ethyl-2-methyl-DL-cysteine deuterated stepwise.

The metastable ion peak was shifted from m/e 20·0 to 20·3 with exchanging the amino hydrogens for deuteriums (Fig 5). From this information, the ion fragment at m/e 42 does not result from the transfer of a Me hydrogen but of an amino hydrogen. The main fragmentation mechanisms of S-alkyl-2-methyl-DL-cysteines are summarized as follows (Scheme 5).

Fragmentations of S-containing peptides

S-containing peptides in *Allium*,[11] *Cruciferous*[12] and *Phaseolus*[15] plants occur in a relatively large amount as γ-glutamyl-S-alkyl-L-cysteine (Alkyl: methyl; n-propyl; allyl; 1-propenyl) and γ-glutamyl-L-methionine.



R : CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $CH_2{=}CHCH_2$, $(CH_3)_2CH$, $CH_3(CH_2)_2CH_2$, $(CH_3)_2CHCH_2$, $CH_3CH_2CHCH_3$, $(CH_3)_3C$, $CH_3(CH_2)_6CH_2$,

SCHEME 5

Glycyl-S-alkyl-DL-cysteines (**11**) and γ-glutamyl-L-methionine (**12**) was dealt with as the proper S-containing peptides. Though it has been generally known that the C—N bond of acetyl peptides[16–17] and N-acyl amino acids[18] is cleaved by electron impact, the C—N bond cleavage of free peptides was not observed. The mass spectrum of Glycyl-S-n-propyl-DL-cysteine, one of S-containing peptides, is shown in Fig 6. In analogy with S-containing amino acids, the molecular abundance of each glycyl-S-alkyl-DL-cysteine was relatively large, i.e. the relative abundances of glycyl-S-n-propyl- (**11a**) and glycyl-S-allyl-DL-cysteine (**11b**) were 4·9 and 6·8 %, respectively. A mass spectrometric survey of dipeptides has shown that the most important ion

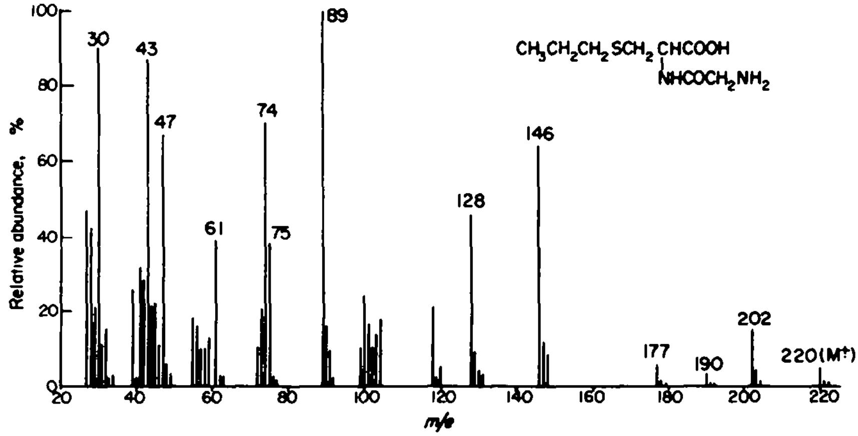
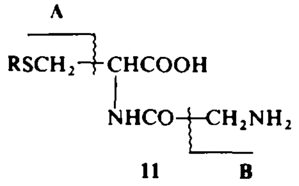


FIG 6.



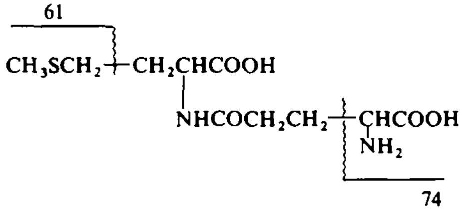
$$\overset{A}{RSCH_2}{-}\underset{\textstyle NHCO{-}\underset{B}{CH_2NH_2}}{CHCOOH}$$

11

R: $CH_2CH_2CH_2$, $CH_2{=}CHCH_2$

corresponds to the amine fragment of the N-terminal amino acid,[19] however the base ion peak of glycyl-S-alkyl-DL-cysteine was not the amine fragment (**B**) but S-containing fragment (**A**) because of d-orbital stabilization.

The base peak of γ-glutamyl-L-methionine was $(CH_3SCH_2)^+$ ion fragment as well as methionine, and the interesting ion peak 74, which was shifted to mass 77 by the treatment of D_2O, is the reasonable fragment to distinguish between γ-glutamyl- and α-glutamyl-L-methionine.



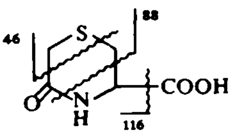
$$\overset{61}{CH_3SCH_2}{-}CH_2\underset{\textstyle NHCOCH_2CH_2{-}\underbrace{\underset{\textstyle NH_2}{CHCOOH}}_{74}}{CHCOOH}$$

12

Fragmentations of cyclic S-containing amino acids

Cyclic amino acids generally give stronger molecular ions than the corresponding acyclic amino acids. L-3-thiomorpholinone-5-carboxylic acid (**13**) and L-thiazolidine-4-carboxylic acid (**14**) as cyclic S-containing amino acids were studied comparing with the corresponding individual-cyclic amino acids.

The relative molecular ion abundances of L-3-thiomorpholinone-5-carboxylic acid (TOCA) and L-thiazolidine-4-carboxylic acid (TCA) were 71·4 and 27·3%, respectively. And, TCA gave much stronger molecular ion than the corresponding individual-cyclic amino acid, proline (< 1 %) because of d-orbital of S. It seems that such a strong molecular ion is attributed to the stabilization of delocalized electrons of S and N in the ring.



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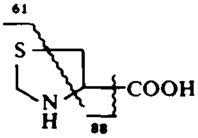

14

EXPERIMENTAL

Instrumentation. To observe a reproducible fragmentation, a Hitachi Model RMS-4 MS was used. All the S-containing amino acids were introduced directly from a heated inlet system into the ionization chamber. The operating parameters were as follows: Inlet temp—130 to 200°; Ion source pressure—7×10^{-7} mm Hg; Ion source temp—200 to 230°; Target current—60 μA; Total emission—80 μA;

Ionization potential—80 eV. With respect to GLC and GC-MS combination, the apparatus and conditions were similar to those previously used.[9]

Deuteration. The carboxyl and amino hydrogens of S-alkyl-L-cysteines were exchanged stepwise by using 99·9% D_2O as shown in the following steps.

(1) Approximately 10 mg of each amino acid was dissolved in 1 ml of D_2O, and immediately each was dried up under a vacuum. (2) 7 mg of deuterated amino acid in the procedure of (1) was dissolved in 5 ml of D_2O and in a sealed tube the soln was heated at 70° for 5 hr. (3) Further, 4 mg of deuterated amino acid in the procedure of (2) was deuterated in 5 ml of D_2O under heating at 80° for 6 hr.

At first the carboxyl hydrogen, next one of the amino hydrogens and at last both of the amino hydrogens were exchanged stepwise with deuterium.

S-Alkyl-L-cysteines (**4**) (alkyl: methyl, n-propyl, allyl). The synthetic procedure used is a modification of the method of du Vigneaud *et al.*[20] in preparing S-methyl-L-cysteine from L-cystine. S-Methyl-L-cysteine: m.p. 212–213° (dec); IR (KBr) 3020–2890, 2660, 2170 cm^{-1} (NH_3^+), 1590 cm^{-1} (COO^-). (Found: C, 35·52; H, 6·52; N, 10·28. $C_4H_9NO_2S$ requires: C, 35·55; H, 6·66; N, 10·37%). S-n-Propyl-L-cysteine: m.p. 210–212° (dec); IR 2965–2860, 2580, 2120 cm^{-1} (NH_3^+), 1580 cm^{-1} (COO^-). (Found: C, 43·96; H, 7·88; N, 8·50. $C_6H_{13}NO_2S$ requires: C, 44·15; H, 8·02; N, 8·59%). S-Allyl-L-cysteine: m.p. 208–210° (dec); IR 3020–2870, 2590, 2120 cm^{-1} (NH_3^+), 1580 cm^{-1} (COO^-), 990 and 918 cm^{-1} (allyl double bond). (Found: C, 44·65; H, 6·91; N, 8·64. $C_6H_{11}NO_2S$ requires: C, 44·72; H, 6·88; N, 8·69%).

cis-S-(1-*Propenyl*)-L-*cysteine.* This compound was prepared by the synthetic procedure of Carson and Wong[21] from S-allyl-L-cysteine with t-BuOK in DMSO: m.p. 179–180° (dec); IR 2970–2840, 2580, 2100 cm^{-1} (NH_3^+), 1580 cm^{-1} (COO^-), no absorptions at 990 and 918 cm^{-1} (allyl double bond) and at 967 cm^{-1} (*trans* isomer); NMR (D_2O—NaOD), δ 5·96 (d, 1 H, $J = 9$ Hz, *cis* configuration of the double bond). (Found: C, 44·70; H, 6·81; N, 8·66. $C_6H_{11}NO_2S$ requires: C, 44·72; H, 6·88; N, 8·69%).

(±)*S-Alkyl-L-cysteine sulphoxides* (**5**) (alkyl: n-propyl, allyl, *cis*-1-propenyl). These compounds were derived from corresponding sulphide according to the method of Toennies and Callan[22] in preparing L-methionine sulphoxide from methionine with 30% H_2O_2 in AcOH or water. S-n-Propyl-L-cysteine sulphoxide: m.p. 195–198° (dec); IR 1012 cm^{-1} (sulphoxide). (Found: C, 39·96; H, 7·21; N, 7·79. $C_6H_{13}NO_3S$ requires: C, 40·22; H, 7·26; N, 7·82%). S-Allyl-L-cysteine sulphoxide: m.p. 165° (dec); IR 1020 cm^{-1} (sulphoxide), 990 and 915 cm^{-1} (allyl double bond). (Found: C, 40·51; H, 6·19; N, 7·82. $C_6H_{11}NO_3S$ requires: C, 40·66; H, 6·26; N, 7·91%). *cis*-S-(1-Propenyl)-L-cysteine sulphoxide: m.p. 138° (dec); IR 1009 cm^{-1} (sulphoxide). (Found: C, 40·49; H, 6·20; N, 7·85. $C_6H_{11}NO_3S$ requires: C, 40·66; H, 6·26; N, 7·91%).

S-Alkyl-2-methyl-DL-cysteines (**6**) (alkyl: methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-octyl). The synthetic procedure has been previously reported in detail.[23] Alkylthiopropanones were prepared by the condensation of chloroacetone and sodium mercaptides in EtOH according to the method of Bradsher *et al.*[24] Alkylthiopropanones thus obtained were used for 5-alkylthiomethyl-5-methylhydantoin syntheses by Bucherer's method.[25] Following the directions of Potts,[26] S-alkyl-2-methyl-DL-cysteines were obtained in high yields from 5-alkylthiomethyl-5-methylhydantoins by alkaline hydrolysis.

S-Methyl-2-methyl-DL-cysteine: m.p. 248–250° (dec). (Found: C, 40·20; H, 7·43; S, 21·26. $C_5H_{11}NO_2S$ requires: C, 40·25; H, 7·43; S, 21·49%). S-Ethyl-2-methyl-DL-cysteine: m.p. 222° (dec). (Found: C, 43·98; H, 7·96; S, 19·65. $C_6H_{13}NO_2S$ requires: C, 44·15; H, 8·02; S, 19·64%). S-n-Propyl-2-methyl-DL-cysteine: m.p. 249° (dec). (Found: C, 47·31; H, 8·38; S, 18·14. $C_7H_{15}NO_2S$ requires: C, 47·43; H, 8·53; S, 18·08%). S-Isopropyl-2-methyl-DL-cysteine: m.p. 195° (dec). (Found: C, 47·35; H, 8·52; S, 17·92. $C_7H_{15}NO_2S$ requires: C, 47·43; H, 8·53; S, 18·08%). S-Allyl-2-methyl-DL-cysteine: m.p. 260° (dec). (Found: C, 47·70; H, 7·44; S, 18·05. $C_7H_{13}NO_2S$ requires: C, 47·98; H, 7·47; S, 18·29%). S-n-Butyl-2-methyl-DL-cysteine: m.p. 225° (dec). (Found: C, 50·38; H, 8·94; S, 16·74. $C_8H_{17}NO_2S$ requires: C, 50·24; H, 8·96; S, 16·76%). S-Isobutyl-2-methyl-DL-cysteine: m.p. 228° (dec). (Found: C, 50·04; H, 8·92; S, 16·84. $C_8H_{17}NO_2S$ requires: C, 50·24; H, 8·96; S, 16·76%). S-sec-Butyl-2-methyl-DL-cysteine: m.p. 248° (dec). (Found: C, 50·19; H, 8·86; S, 16·55. $C_8H_{17}NO_2S$ requires: C, 50·24; H, 8·96; S, 16·76%). S-t-Butyl-2-methyl-DL-cysteine: m.p. 272–275° (dec). (Found: C, 50·22; H, 8·91; S, 16·82. $C_8H_{17}NO_2S$ requires: C, 50·24; H, 8·96; S, 16·76%). S-n-Octyl-2-methyl-DL-cysteine: m.p. 205° (dec). (Found: C, 58·27; H, 10·21; N, 5·53. $C_{12}H_{25}NO_2S$ requires: C, 58·26; H, 10·19; N, 5·66%).

The introduction of a Me group in the α-position of S-containing amino acids showed additionally the IR (KBr pellet) absorption at 1455 cm^{-1}.

DL-2-*Methylmethionine.* Methylthiobutan-3-one was prepared by the condensation of methyl vinyl ketone and methyl mercaptan according to a modification of the method of Catch *et al.*[27] According to the method of Pfister 3rd. *et al.*[28] DL-5-(β-methylthioethyl)-5-methylhydantoin was prepared by the reaction of methylthiobutan-3-one with RCN and ammonium carbonate in aqueous EtOH, and further DL-2-methylmethionine was obtained from DL-5-(β-methylthioethyl-5-methyl hydantoin by alkaline hydrolysis: m.p. 283° (dec), IR 1460 cm^{-1} (methyl group in the α-position). (Found: C, 43·98; H, 7·85; N, 8·46. $C_6H_{13}NO_2S$ requires: C, 44·15; H, 8·02; N, 8·59%).

L-3-*Thiomorpholinone-5-carboxylic acid* (**13**). S-Carbamoylmethyl-L-cysteine was synthesized by the treatment of L-cystine with sodium and α-chloroacetamide in liquid ammonia[29]. S-Carbamoylmethyl-L-cysteine was converted into **13** by heating with EtONa in EtOH or with AcOH under reflux:[30] m.p. 186° (dec); IR 3150 cm^{-1} (NH in lactam), 1710 cm^{-1} (carboxyl), 1660 cm^{-1} (C=O in lactam). (Found: C, 37·03; H, 4·46; N, 8·72. $C_5H_7NO_3S$ requires: C, 37·27; H, 4·36; N, 8·71%).

L-*Thiazolidine-4-carboxylic acid* (**14**). L-Cysteine and formaldehyde were dissolved in 50% EtOH, and the mixture was allowed to stand in a refrigerator over night.[31] Recrystallization was carried out by acidifying the Na salt of **14** in aqueous soln with AcOH: m.p. 194–195° (dec); IR 2600–2150 cm^{-1} ($>\mathrm{NH}_2^+$). (Found: C, 36·24; H, 5·34; N, 10·73. $C_4H_7NO_2S$ requires: C, 36·13; H, 5·27; N, 10·55%).

Synthesis of glycyl-S-n-propyl-DL-cysteine (**11a**).

(a) S-n-*Propyl-DL-cysteine ethyl ester* (**I**). S-n-Propyl-DL-cysteine ethyl ester was prepared from DL-cystine by the method of du Vigneaud *et al.*[29] followed by the method of Fischer.[32]

(b) N-*Carbobenzoxy-glycine* (**II**). N-Carbobenzoxy-glycine was prepared from carbobenzoxy chloride and glycine by the method of Bergmann and Zervas.[33]

(c) N-*Carbobenzoxy-glycyl-S-n-propyl-DL-cysteine ethyl ester* (**III**). A sample of 10·55 g (0·041 mol) of **II**, 8·46 g (0·041 mol) of N, N'-dicyclohexylcarbodiimide (DCC) and 8·0 g (0·041 mol) of **I** were dissolved in 100 ml of THF and allowed to react for 4 hr at room temp. The mixture was shaken occasionally, and after 4 hr, 3 ml of glacial AcOH was added to decompose the excess of DCC. The mixture was stored in a refrigerator overnight. N, N'-Dicyclohexylurea was removed by filtration, the solvent was evaporated under reduced pressure, the residue was dissolved in 80 ml of EtOAc, and insoluble matters were filtered off. The EtOAc soln was washed with 0·5 M $NaHCO_3$ and 0·5 M aqueous soln of citric acid and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the oily residue was obtained in 79% yield (12·3 g).

(d) N-*Carbobenzoxy-glycyl-S-n-propyl-DL-cysteine* (**IV**). The crude ester **III** (12·3 g) mentioned above was treated with a mixture of 140 ml of dioxane and 100 ml of 1 N NaOH for 6 hr at room temp. After the soln was evaporated under reduced pressure, the residue was dissolved in water (500 ml). After filtration the soln was acidified with 1 N HCl. The oily substance separated was extracted with EtOAc, the EtOAc soln was washed with water, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was dissolved in a small amount of EtOAc and recrystallized by the addition of diethyl ether and light petroleum; yield 3·64 g (31·9%), m.p. 118–119°.

(e) *Glycyl-S-n-propyl-DL-cysteine* (**11a**). A soln of 3·6 g of **IV** in 90% AcOH (50 ml) was hydrogenated over Pd black (0·4 g) as catalyst. The filtrate from the catalyst was evaporated under reduced pressure and the crystalline product was obtained. It was recrystallized from water and EtOH: yield 1·23 g (54·9%); m.p. 191–200° (dec); IR (KBr) 1674, 1564–1546, 1273 cm^{-1} (amide). (Found: C, 43·43; H, 7·28; N, 12·92; S, 14·47. $C_8H_{16}N_2O_3S$ requires: C, 43·62; H, 7·32; N, 12·72; S, 14·55%).

Synthesis of glycyl-S-allyl-DL-cysteine (**11b**).

(a) S-*Allyl-DL-cysteine ethyl ester* (**V**). S-Allyl-DL-cysteine ethyl ester was prepared from DL-cystine and allyl chloride by the same method as mentioned in the preparation of **I**.

(b) N-*Carbobenzoxy-glycyl-S-allyl-DL-cysteine ethyl ester* (**VI**). A sample of 10·6 g (0·04 mol) of **II**, 8·5 g (0·04 mol) of DCC and 7·8 g (0·04 mol) of **V** were dissolved in 100 ml THF and allowed to react for 4 hr at room temp. The mixture was shaken occasionally, and after 4 hr 3 ml of glacial AcOH was added to decompose the excess DCC. The mixture was stored in a refrigerator overnight. The insoluble N,N'-dicyclohexyl urea was removed, the solvent was evaporated under reduced pressure, the residue was dissolved in 60 ml EtOAc, and insoluble matters were filtered off. The EtOAc soln was washed with 0·5 M $NaHCO_3$ and 0·5 M aqueous soln of citric acid, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and oily residue was obtained in 87% yield (13·6 g).

(c) N-*Carbobenzoxy-glycyl-S-allyl-DL-cysteine* (**VII**). The crude ester **VI** (13·6 g) was treated with a mixture of 140 ml dioxane and 100 ml 1N NaOH for 6 hr at room temp. After the soln was evaporated under reduced pressure, the residue was dissolved in water (550 ml). After filtration the soln was acidified with 1N HCl. The oily substance separated was extracted with EtOAc, the EtOAc soln was washed with water, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was dissolved in a small amount of EtOAc and recrystallized by the addition of diethyl ether and light petroleum; yield 10·5 g (74·4%), m.p. 116–121°.

(d) *Glycyl-S-allyl-*DL*-cysteine* (**11b**). To a 200 ml of liquid ammonia in a 500 ml round-bottomed flask equipped with a mechanical stirrer and a soda lime tube, and cooled with dry-ice and EtOH was added 10·5 g of **VII**. Na was then added in small portions until the blue color persisted for 5 min. After the addition of ammonium bromide, 17 ml of allyl bromide was added and the mixture was further stirred for 2 hr. The ammonia was removed and the residual solid was dried *in vacuo*. This material was dissolved in 200 ml water. To this soln 20 ml AcOH was added and the peptide was absorbed by passing the soln through a column of Dowex 50WX-2 (H^+ cycle). The resin was washed with water. The peptide was then eluted from the column with 2N NH_4OH. The soln was then concentrated *in vacuo* to 20 ml, yielding a crystalline mush. After storage in a refrigerator overnight, the crystals were separated from the soln by filtration. It was recrystallized from water and EtOH, yield 1·35 g (20·7%); m.p. 191–196° (dec); IR (KBr) 1682, 1565–1540, 1270 cm^{-1} (amide), 995 and 923 cm^{-1} (allyl double bond). (Found: C, 43·74; H, 6·49; N, 12·84; S, 14·46. $C_8H_{14}N_2O_3S$ requires: C, 44·02; H, 6·46; N, 12·83; S, 14·69%).

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