Reaction of Cysteine and Methionine With Malonaldehyde

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Abstract

Malonaldehyde (M), a product of polyunsaturated fatty acid oxidation, reacted with the sulfhydryl as well as with the amino groups of cysteine (cys). The cys-M product had an absorption maximum at 310 mμ, and the extinction coefficient at pH 7.0 was 2.3×10^4. Elementary analysis of the cys-M product agreed with a structure in which 2 moles of cysteine had reacted with 3 moles of malonaldehyde. The molecular weight of cys-M preparations increased on storage and the UV absorption changed from 310 to 315–320 mμ, with a consequent shift to longer wavelength in the visible.

Methionine (meth) reacted with malonaldehyde under the same reaction conditions only at the α-amino group, similar to glycine (gly). The apparent pK_a of the carboxyl group of gly-M increased to 3.36 and that of meth-M to 3.19, representing an increase of about one pK_a unit over the natural amino acids. For gly-M and meth-M the respective absorption maxima were 272 and 282 mμ. The spectral shifts from 267 to 315 mμ of the amino acid-M products with respect to β-oxyacrolein were explained in terms of increasing substitution at the β-carbon of the α,β-unsaturated carbonyl system. When the α-amino-malonaldehyde condensation products of methionine and glycine reacted with semicarbazide the original amino acids and disemicarbazone of malonaldehyde were formed.

Introduction

Malonaldehyde is a product of polyunsaturated fatty acid oxidation (1). It exists primarily in the enolic form, representing an α,β-unsaturated aldehyde; the pK of the enolic hydrogen is 4.6 (11,12). The preferred conformations of different tautomers of the enol have been investigated by NMR spectroscopy (13).

In bovine serum albumin, malonaldehyde reacts with the α-amino groups of lysine side chains and the α-amino group of aspartic acid (6). The preparation of a glycine-malonaldehyde derivative has been reported (5). In a study on the reaction of the structural muscle protein myosin with malonaldehyde, it was observed that among other functional amino acid side chains, cysteine was most reactive (7).

In the present experiments the interaction of malonaldehyde with protein constituents, particularly the sulfur amino acid cysteine which is known to have structural as well as catalytic functions in many proteins, was studied. Derivatives of amino acid-malonaldehyde products, particularly those in which the functional amino acid side chains have reacted, would consequently be of use for the identification of protein-lipid reaction products in hydrolyzates and tissue extracts.

Experimental Procedures

Cys-M

Cysteine solution was prepared by dissolving 5 g (0.028 moles) cys·HCl·H_2O (Calbiochem) in a minimum amount of water. A stream of N_2 was allowed to flow over the solution and by the addition of about 13.5 ml 2 N KOH, the pH was adjusted to 6.5 (a precipitate of cystine will form at this step if an oxidized cysteine preparation is used). The cysteine solution was kept under N_2 until needed. Malonaldehyde was prepared by hydrolyzing 8 ml (0.036 moles) 1,1,3,3-tetraethoxypropane (Eastman Organic Chemicals) with 3 ml N HCl. The solution was kept at 45–50 C, until miscible and clear (20 min). After adding 2 ml water, the hydrolysis mixture was held an additional 20 min at the above temperature. The solution was cooled to room temperature and taken to pH 6.5 with about 10.4 ml 2 N KOH. (If the acetal is not completely hydrolyzed, a cloudy solution was obtained during the neutralization step, such a solution should not be used.)

The cysteine and malonaldehyde solutions were then mixed. The pH of the resulting reaction mixture dropped to 5.4. After 10–15 min under N_2, the pH was raised to 7 with 2 N KOH and kept at this pH for another 15 min. The reaction mixture was then acidified slowly, with N HCl, to pH 3–2.8. The product precipitated as a tan to yellow colored product after the first addition of acid. The flask was cooled in ice and the suspension was filtered with suction. The product was washed with cold water and then with acetone. The resulting tan colored powder was obtained in 48% yield. Spectral evidence indicated that no free malonaldehyde was present in the filtrate. For further purification the cys-M product can be dissolved by adjusting the pH to 7, reprecipitating it with acid, and washing the product with water and then with acetone. Attempts to crystallize the cys-M preparation as the acid or the sodium salt failed. For elemental analysis, the product was dried over NaOH pellets and silica gel at 300–400 μ Hg for 24 hr.

Analysis: Calculated for $C_{15}H_{20}O_7S_2N_2$: C, 44.5; H, 4.9; S, 15.8; N, 6.9. Found: C, 44.5; H, 4.7; S, 15.5; N, 6.7.

The molecular weight of the sodium salt by vapor pressure osmometry was 440 and the absorption maximum at pH 6 was at 310 mμ with an extinction coefficient $E_M = 2.3 \times 10^4$.

Cys-M-Na^+

For solubility reasons, molecular weight determinations by vapor pressure osmometry, were carried out on the sodium salt of cys-M. The sodium salt of cys-M was prepared by adjusting the pH of a cys-M preparation to 7.5 and by eluting it with water from a 95 × 1.5 cm Sephadex G-10 column which was washed extensively with water. The major fraction of cys-M-Na^+ coming off the column was concentrated by freeze drying. For osmometer measurements, water solutions of the cys-M sodium salt were prepared from 0.05 to 0.12 M and compared to standard curves of sodium chloride and sodium acetate. Plotting the resistance readings of the osmometer against the salt concentrations (0.05 to 0.12 M) of the standard salt solutions resulted in overlapping straight lines.

$(Cys\text{-}M)_2 \cdot 3H_2O$

On storage of the cys-M product for about three months, its molecular weight increased. By vapor pressure osmometry, the molecular weight of the

sodium salt of the condensed product was 860. In drying experiments, the acid form of the condensed product took up 6.739% of water from the atmosphere. The hydrated sample was subjected to elemental analysis.

Analysis: Calculated for $C_{30}H_{38}O_{13}S_4N_4 \cdot 3H_2O$: C, 42.6; H, 5.2; S, 15.2; N, 6.6. Found: C, 42.9; H, 5.2; S, 15.6; N, 6.9.

The absorption maximum was at 315–320 mμ with an extinction coefficient $E_M = 4.4 \times 10^4$ at pH 6.

Tests for free sulfhydryl groups were carried out by a nitroprusside procedure (14). Colorimetric tests for amino groups were made with ninhydrin reagents (15). Paper chromatography of the amino acids and their malonaldehyde products was carried out on Whatman 1 paper and 2-butanol-acetic acid-water (450:50:125) was used as solvent.

Meth-M

The methionine-malonaldehyde product was prepared by placing 1 g of L-methionine (0.0067 moles) in a small Erlenmeyer flask with 5 ml water. Malonaldehyde prepared as above, from 1.78 g of tetraethoxypropane and 0.95 ml N HCl, was added to the suspension of the amino acid and the reaction mixture was stirred. After about 10 min the methionine dissolved in the reaction mixture and was shortly followed by the appearance of a granular, yellow precipitate. The suspension was stirred for 10 to 15 min longer and the pH was then adjusted to 2.4. The product was washed on a suction funnel with cold water and then with acetone. It can then be redissolved in water by titrating to pH 7 and be reprecipitated. However, if washed sufficiently, no notable increase in purity was observed by paper chromatography, nor by elemental analysis. Meth-M did not absorb water from the atmosphere, however, drying at reduced pressure over NaOH was carried out for elemental analysis and for titration studies. Attempts to crystallize the product were not successful and the results upon elemental analysis of the amorphous substance are given.

Analysis: Calculated for $C_8H_{13}O_3SN$ (Mol. wt. 203.2): C, 47.3; H, 6.5; S, 15.8. Found: C, 47.5; H, 6.6; S, 15.5.

The molecular weight determined by vapor pressure osmometry was 208 and the equivalent weight was found to be 203.1. Between pH 4 to 9, the compound had an absorption maximum at 282 mμ with an extinction coefficient $E_M = 2.0 \times 10^4$.

Gly-M

Glycine-malonaldehyde was prepared according to Crawford et al. (5) and even though the product was not crystallized, elemental composition and absorption characteristics at 272 mμ could readily be confirmed.

Semicarbazones

The semicarbazones were prepared by reacting 1 g of the meth-M or gly-M derivatives or malonaldehyde with 1 g of semicarbazide hydrochloride buffered by 1.5 g of sodium acetate and dissolved in 10 ml of water. The semicarbazones were recrystallized from hot water. Their molecular weights had a range of 181 ± 5 (vapor pressure osmometer). Elemental analysis of the semicarbazones prepared from meth-M and gly-M corresponded to the disemicarbazone of malonaldehyde, mp 208–210 C (decomp).

TABLE I

Titration of Methionine-Malonaldehyde (meth-M)[a]

ml 0.10 M KOH	pH	[HA]	[A⁻]	(H⁺)	$\log \frac{[HA]-(H^+)}{[A^-]+(H^+)}$	pK'a
0.00	2.57	0.010	0.000			
0.05	2.75	0.009	0.001	0.00178	0.43	3.18
0.10	2.88	0.008	0.002	0.00129	0.31	3.19
0.15	3.02	0.007	0.003	0.00095	0.18	3.20
0.20	3.15	0.006	0.004	0.00071	0.05	3.20
0.25	3.25	0.005	0.005	0.00056	−0.10	3.15
0.30	3.40	0.004	0.006	0.00040	−0.25	3.15
0.35	3.58	0.003	0.007	0.00026	−0.42	3.16
0.40	3.81	0.002	0.008	0.00015	−0.64	3.17
0.45	4.25	0.001	0.009	0.00006	−0.98	3.27
0.50	7.10	0.000	0.010			

meth-M pK'a = 3.19 ± (0.04)

[a] Mol. wt. 203.3; 10.16 mg/4.75 ml (0.01 M at half neutralization) were titrated with 0.10 M KOH at 22 C.

$CH_2(CH=N\text{–}NHCO\text{–}NH_2)_2$

Analysis: Calculated for $C_5H_{10}N_6O_2$: C, 32.2; H, 5.4; N, 45.1. M.W. 186.18. Found: M-Semic. C, 32.3; H, 5.3; N, 45.2. Meth-M-Semic. C, 32.3; H, 5.5; N, 45.1. Gly-M-Semic. C, 32.4; H, 5.5; N, 45.3.

Titrations

Titrations to determine ionization constants were carried out with 0.10 M KOH on dilute solutions of amino acids or amino acid-malonaldehyde products. The initial concentrations were adjusted, with carbon dioxide free water, to make the solution 0.01 M at half neutralization, the position of the pK_a (2,3,4).

In Tables I and II, [A⁻] was calculated from the stoicheiometric amount of base added and when subtracted from the original amount of acid the equilibrium amount of [HA] in solution was obtained. Correction for H⁺ already present in solution due to ionization was made by adding or subtracting the hydrogen ion activity (H⁺) to [A⁻] and from [HA] respectively. Since glass electrodes give a measure of hydrogen ion activity rather than of concentration, the respective pH values were converted to hydrogen ion activities.

Titrations of cysteine, glycine and methionine were also carried out in the presence of 1.8% to 12.5% malonaldehyde, similar to the formol titrations of amino acids. The titration of cysteine in the presence of malonaldehyde is given here as an example. A solution containing 0.0878 g of cysteine $HCl \cdot H_2O$ (.0005 moles) in about 4 ml of water was adjusted to pH 7 under a light stream of nitrogen and was then made up to 9.5 ml with neutralized malonaldehyde solution and water, so that its concentration was 5.8% in malonaldehyde. Keeping the solution under nitrogen, it was titrated with 0.05 ml quantities of N KOH. A blank titration of malonaldehyde under the same conditions was then subtracted from the titration curve of the reaction mixture. For the cysteine titration a water blank only was subtracted.

TABLE II

Titration of glycine-malonaldehyde (gly-M)[a]

ml 0.10 M KOH	pH	[HA]	[A⁻]	(H⁺)	$\log \frac{[HA]-(H^+)}{[A^-]+(H^+)}$	pK'a
0.00	2.69	0.010	0.000			
0.05	2.88	0.009	0.001	0.00132	0.52	3.40
0.10	3.00	0.008	0.002	0.00100	0.37	3.37
0.15	3.12	0.007	0.003	0.00076	0.22	3.34
0.20	3.26	0.006	0.004	0.00055	0.08	3.34
0.25	3.40	0.005	0.005	0.00040	−0.07	3.33
0.30	3.56	0.004	0.006	0.00027	−0.22	3.34
0.35	3.74	0.003	0.007	0.00018	−0.40	3.34
0.40	3.99	0.002	0.008	0.00010	−0.63	3.36
0.45	4.37	0.001	0.009	0.00004	−0.98	3.39
0.50	8.45	0.000	0.010			

gly-M pK'a = 3.36 ± (0.03)

[a] OHC–CH = CH–NH–CH_2CO_2H (Mol. wt. 130); 6.5 mg gly-M/4.75 ml (0.01 M at half neutralization); were titrated with 0.10 M KOH at 22 C.

The pH measurements were made with an expanded scale pH meter. Spectral measurements were made with a Beckman DB spectrophotometer. Molecular weight studies were carried out with a Hewlett Packard Model 302 vapor pressure osmometer (21).

Discussion

The reaction between free cysteine and aromatic or saturated aliphatic aldehydes generally leads to the formation of 2-substituted thiazolidine-4-carboxylic acids (8) or under more acidic conditions to mercaptals such as djenkolic acid (16). However, reaction of α,β-unsaturated ketones and aldehydes with cysteine failed to give the expected reaction (9,10). In the present experiments malonaldehyde and cysteine were reacted at a molar ratio of 1.29 around neutral pH and room temperature. On lowering the pH a product could be precipitated which in comparison with cysteine gave a ninhydrin color of reddish hue and the development of color required a longer heating period than normally observed for α-amino acids. There was no free malonaldehyde present in the filtrate, as could be judged by the absence of the characteristic absorption peak of this compound in the UV range. During paper chromatography the cys-M product remained close to the origin while cysteine had an R_f value of 0.19. The ninhydrin color of the cys-M spot developed only after heating, indicative of a secondary amine. Determination of free sulfhydryl groups in the cys-M precipitate was carried out by the nitroprusside reaction. While 0.2 μ moles of cysteine per 1.5 ml gave an optical density value of 0.92 at 520 mμ, 0.2 μ moles of cys-M product (M. wt. 404) did not give any nitroprusside color, even after treatment with KCN. However, by examination of more concentrated solutions, cys-M was found to contain 2–3% of cysteine in which the sulfhydryl groups had not reacted. The cys-M product was not soluble in water or in organic solvents such as ethanol, acetone, ether or chloroform. It could be brought back into solution, however, by slowly titrating a water suspension of cys-M to neutrality or by dissolving it in concentrated HCl or H_2SO_4. A graphical interpretation of the titration curve indicated a pK'_a value around 4, while no further buffering regions could be detected in the alkaline pH range. For solubility reasons, a true acid-base equilibrium of cys-M could not be established until after addition of some base, thus preventing the determination of an accurate ionization constant.

Further evidence for the reaction of the sulfhydryl as well as of the amino group was obtained by titrating cysteine in the presence of malonaldehyde. These experiments showed the disappearance of the alkaline buffering region of the sulfhydryl and the amino group.

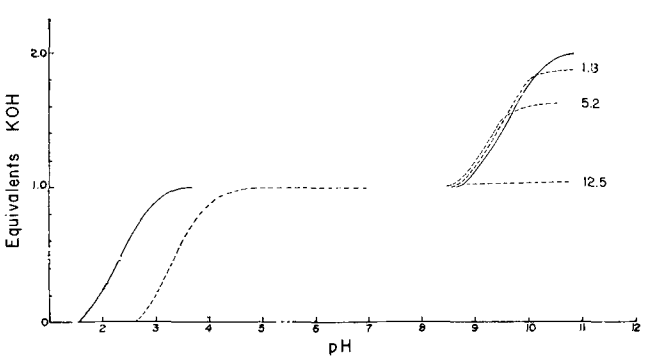
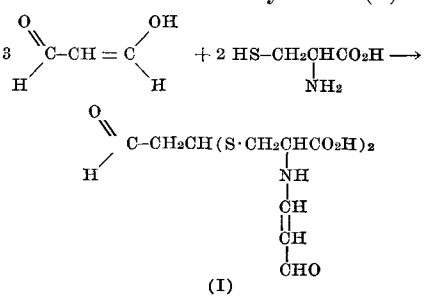


FIG. 1. Titration of glycine (solid line) and glycine in the presence of 1.8%, 5.2% and 12.5% malonaldehyde (dashed lines).

The shift of one pH unit in the acid direction, upon mixing of the cysteine and malonaldehyde reagents, both of which had previously been adjusted to pH 6.5, can be attributed to the decreasing ratio of RNH_2/RNH_3^+ and RS^-/RSH, as RNH_2 and RS^- are reacting with malonaldehyde.

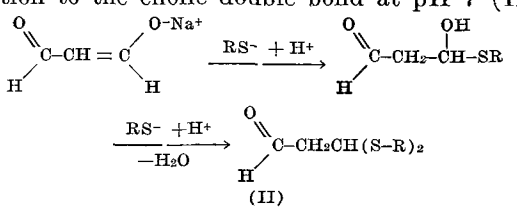
Initial experiments to obtain an approximate molecular weight of cys-M were carried out on a 95 × 1.5 cm Sephadex G-10 column. A V_e/V_o ratio of 1.35 to 1.44 was obtained for the cys-M product while that for cysteine was 2.03 to 2.11 and for malonaldehyde 2.15 to 2.25. In comparison with other standards such as fumaric acid, sucrose, raffinose and some amino acids, this method indicated a molecular weight range of 350 to 450.

The elemental analysis (for cys-M) corresponded to a structure in which 3 moles of malonaldehyde had reacted with 2 moles of cysteine (I).



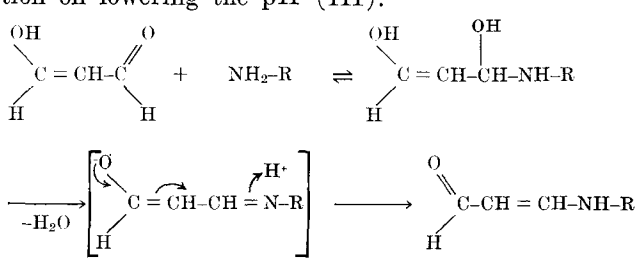
(I)

The reaction of the sulfhydryl groups with malonaldehyde can be visualized as shown below, involving addition to the enolic double bond at pH 7 (II).



(II)

Reactions of similar nature, where the sulfhydryl group of cysteine adds to an α,β-conjugated double bond are known in the case of N-ethylmaleimide, acrylic acid and acrylonitrile, for instance (17,18). Reaction of the amino groups with malonaldehyde may then proceed by an acid catalyzed carbonyl addition on lowering the pH (III).



(III)

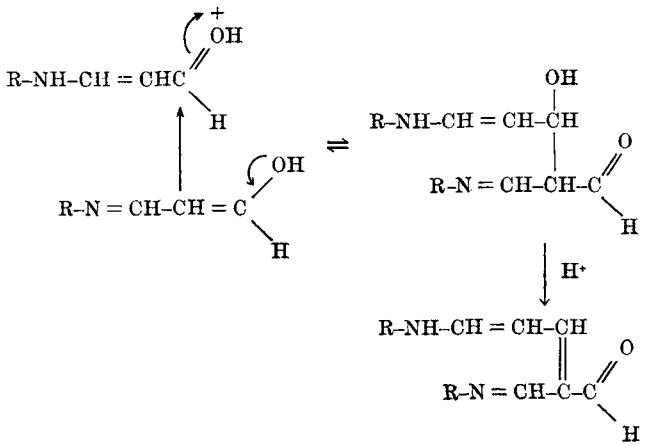
However, amino groups also react readily with α,β-unsaturated compounds at pH 8 (19). A review on the addition of amines to α,β-unsaturated carbonyl systems is available (20). Reaction of the amine by carbonyl addition or by addition to the α,β-double bond of malonaldehyde would lead to the same product, if the double bond addition is to the β-carbon.

Attempts to prepare derivatives of the cys-M product such as semicarbazones, 2,4-dinitrophenylhydrazones, methones, picrates, phenylthioureas, sulfones or acetates failed. Failure, particularly of the aldehyde

groups to react can possibly be explained by the following equilibrium, where the enol is the predominant species (IV).

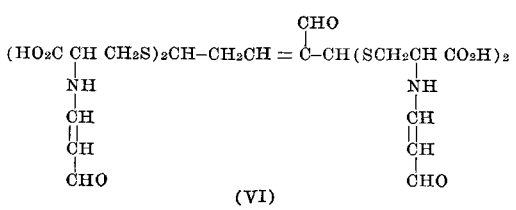
$$\underset{\text{(IV)}}{\text{R–NH–CH = CH–CHO} \rightleftharpoons \text{R–N = CH–CH = CHOH}}$$

On treatment with acid or base or reagents such as acetic anhydride in pyridine, the cys-M product turned dark brown to black, probably due to further inter- and intramolecular polymerizations by aldol condensation type mechanisms (V).



(V)

On rechecking the results on some of the cys-M preparations stored for 3 months, it was found that the elution volume on a Sephadex G-10 column had changed and was now close to the void volume or the values obtained for Blue Dextran 2000. By vapor pressure osmometry the molecular weight of the sodium salt of this cys-M preparation was 860. Elemental analysis was carried out on a sample in the acid form and when equilibrated at atmospheric moisture it contained 6.739% water. The results of the analysis supported a condensation product derived from cys-M. This compound contained 3 molecules of water for a molecular weight of 791; a provisional structure for $(\text{cys-M})_2 \cdot 3H_2O$ is given below (VI).



(VI)

However, the carbonyl groups shown in the above structure were not reactive, suggesting further intramolecular interaction by aldol type condensation, thus increasing the number of conjugated double bonds.

While the freshly prepared cys-M has an absorption maximum at 310 mμ the absorption of the polymerized preparation was at 315 to 320 mμ and the respective extinction coefficients at pH 6 were 2.3×10^4 and 4.4×10^4 respectively (Fig. 3). An infrared spectrum of the condensed product of cys-M as a Nujol mull showed a very pronounced absorption band at 1610 cm^{-1} due to conjugated double bond carbonyl systems and a small band at 1725 due to carbonyl absorption. Compared to the IR spectrum of cysteine, the absorption due to the amino group at 3100 and 3300 cm^{-1} was reduced to a small broad peak in the cys-M product.

Methionine reacted with malonaldehyde, in aqueous solution at room temperature, only at the α-amino

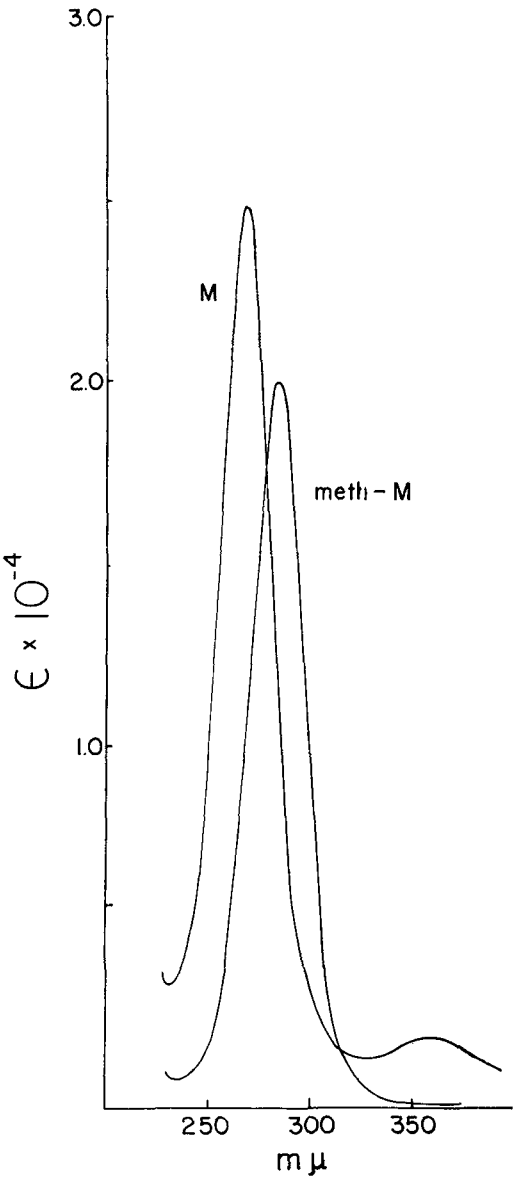
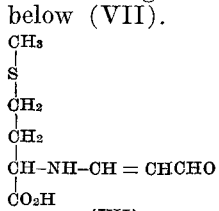


FIG. 2. Absorption curves of malonaldehyde (M) and the methionine-malonaldehyde reaction product (meth-M) at pH 7.5.

group and the structure in agreement with elemental analysis is given below (VII).



(VII)

The apparent pK'_a of the methionine-M product at 22 C was 3.19 and the results for this titration are listed in Table I. The pK'_a of methionine at 22 C was 2.30 in agreement with reported values (2). Details of the titration have been given in the experimental section. Corrections for hydrogen ion concentration at low pH were carried out according to equations derived from the concept of electroneutrality of a solution (4). When titrating a carboxylic acid HA, for instance, with KOH

$$[K^+] + [H^+] = [A^-] + [OH^-] \qquad [1]$$

since all solutions are electrically neutral. The concentration dependent equilibrium constant

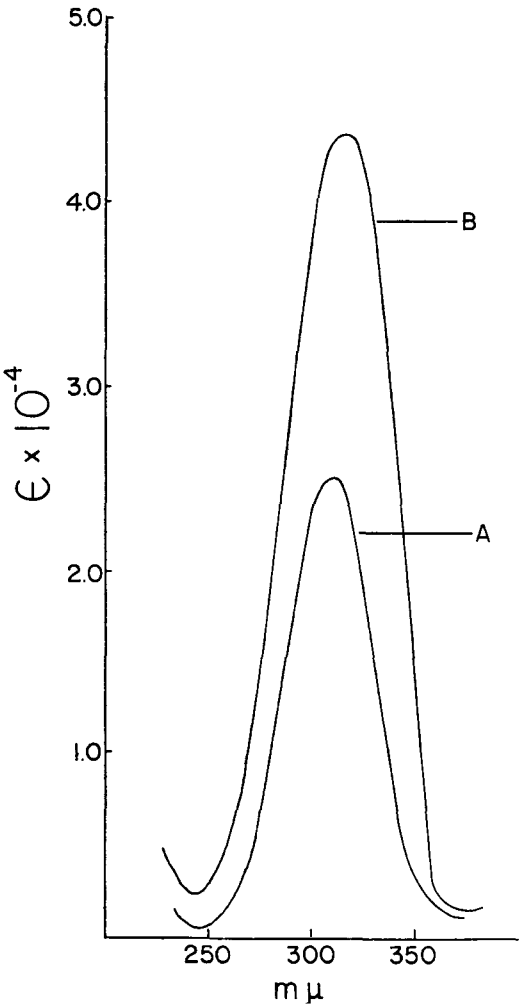


FIG. 3. Ultraviolet spectra of the cysteine-malonaldehyde product at pH 7, after preparation (A) and after prolonged storage (B).

$$K_a = \frac{(H^+)[A^-]}{[HA]} \qquad [2]$$

is related to the thermodynamic ionization constant

$$K_a^\circ = \frac{Ka\,\gamma_{A^-}}{\gamma_{HA}}.$$

In dilute solutions, the activity coefficients (γ) approach unity and $K_a = K_a^\circ$ at zero ionic strength. When the titration is carried out in the acidic region $[OH^-] << [H^+]$ and by substituting equation [1] into [2] the apparent pK_a' becomes

$$pK'_a = pH + \log \frac{[HA]-[H^+]}{[A^-]+[H^+]}$$

and since pH meter readings can readily be converted to hydrogen ion activities the concentration terms $[H^+]$ were substituted by hydrogen ion activities (H^+).

The product of meth-M had an absorption maximum at 282 mμ with an extinction coefficient $E_M = 2 \times 10^4$; the spectral shift relative to the malonaldehyde absorption at pH 7.5 can be seen in Figure 2. While the malonaldehyde spectrum is pH dependent, the spectrum of meth-M did not change between pH 4 and pH 9. When meth-M was reacted with semicarbazide, a crystalline product was obtained mp 208–210 C (once recrystallized). During paper chromatography meth-M migrated at a faster rate than a methionine control and gave a ninhydrin color only upon heating, indicative of a secondary amine. Titrating the alkaline region (pH 7 to 11.5) of methionine in the presence of malonaldehyde, showed the gradual disappearance of the titrable amino groups when the malonaldehyde concentration in different experiments was increased from 1% to 8%.

The titrable amino groups of glycine as well could be seen to decrease gradually when titrated in the presence of increasing malonaldehyde concentrations (Fig. 1). Alkylation of the amino group of glycine with malonaldehyde was found to shift the pK'_a from 2.36 to 3.36. Data for the titration of gly-M are tabulated in Table II. The positively charged amino group in glycine has an electron withdrawing or acid strengthening effect in comparison with acetic acid. In gly-M, the double bond in the vicinity of the amino group reduces the electron withdrawing effect on the carboxyl group and thus decreases the acid strength.

Paper chromatography of gly-M showed similar results as those obtained with meth-M. The secondary amine migrates faster than the original amino acid and the ninhydrin color is only fully developed during heating at 100 C. When reacted with semicarbazide a crystalline product was obtained mp 208–210 C (once recrystallized).

At pH 7 the absorption spectrum of gly-M had a maximum at 272 mμ compared to 267 mμ for malonaldehyde. This shift to longer wavelength could be anticipated since the maximum absorption for a carbon-carbon double bond conjugated with a carbonyl group (malonaldehyde as the β-oxyacrolein) is shifted to longer wavelength with increasing substitution at the α- or β-carbon. In case of meth-M, the size of the substituent to the β-carbon has been increased over gly-M and the spectral shift relative to the original β-oxyacrolein absorption has also increased. Since the shift is however greater than anticipated, it may possibly be assumed that the sulfur atom in the substituent has an intensifying effect. In cys-M the substituent effects of glycine and methionine on the β-oxyacrolein spectrum are further compounded and the absorption maximum is shifted from 267 to 310 mμ. While the ionizable hydroxyl group of β-oxyacrolein has a strong pH dependent effect on the spectrum, when replaced by the amino acid substituents, no pH dependency was noted between pH 4 and 9.

Spectra of the acrolein compounds in which the β-carbon has been substituted by the α-amino groups of glycine and methionine are in general agreement with the spectra of β-aminoacrylate compounds reported elsewhere (22). There is an increasing bathochromic shift of the acrylate spectra when the substitution at the β-carbon changes from ethoxy to amino to methylamino. However, spectra of acrolein compounds with sulfur containing β-substituents do not seem to be available in the literature.

Aldol type condensations and an increase in double bond conjugation, if present in $(cys\text{-}M)_2$ for instance, would provide a further reason for long wavelength shifts.

Reaction of gly-M with 2-thiobarbituric acid (TBA) showed the formation of the characteristic blue-violet TBA-malonaldehyde precipitate. A solution of this reaction product, after washing with water, alcohol and ether, also showed the characteristic absorption of the TBA-malonaldehyde complex at 530 mμ (1).

Similar results were obtained for meth-M. However, the reaction product of cys-M-TBA showed some characteristic differences. The precipitate which formed was red, instead of blue-violet and a spectrum at pH 2 contained an additional peak at 442 mμ in addition to the TBA-malonaldehyde peak at 530 mμ.

The semicarbazones of meth-M and gly-M had identical appearance and melting points and were, therefore, subjected to further analysis. Their molecular weights were 181 $\pm$ 5/mole and the elemental analysis of both semicarbazones corresponded to the disemicarbazone of malonaldehyde. Semicarbazide, therefore, seems to affect hydrolysis of the propenal substituent from the α-amino group. A probable mechanism would involve first addition of semicarbazide to the β-carbon of the α,β-double bond of propenal, followed by carbonyl addition of a second molecule of semicarbazide to the aldehyde group. Acid catalyzed hydrolysis and addition of water would reform the original amino acid and a hydroxydisemicarbazone of malonaldehyde which on dehydration forms the disemicarbazone of malonaldehyde. Cys-M did not give a derivative with semicarbazide hydrochloride which seems to indicate a more stable bond and possibly further intramolecular reactions of the N-propenal groups.

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