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The synthesis and resonance Raman spectra of several new N-acyl glycine ethyl dithioesters  $RC(=O)NH-CH_2-C(=S)SR$  are reported. The resonance Raman spectra of these compounds contain at least two peaks in the "C-S stretching region" between 1050 and 1200 cm<sup>-1</sup>. The solvent, concentration, temperature, and excitation wavelength dependencies of these features have been investigated. The results obtained are consistent with two conformations of the dithioesters co-existing in solution with the conformational differences being controlled by rotational isomerism about one or both of the NH-CH2 and CH2-CS bonds. Strong support for the existence of more than one conformer in solution comes from the Raman spectrum of a single crystal of N-acetyl glycine ethyl dithioester. In the spectrum of the single crystal several Raman bands are absent compared to the spectrum of the dithioester in solution indicating that only one of the conformers found in the solution phase is retained in the crystal. In one of the conformers found in solution there is evidence for an intramolecular interaction between the amide group and the dithioester group. The exact nature of the intramolecular interaction is uncertain, although simple H-bonding and enethiol tautomerism have been eliminated as possibilities. Possible contributors to the interaction are dipole-dipole forces and amide  $\pi$ -electrons interacting through space with sulfur  $d_{\pi}$  orbitals. Both of these putative interactions would be modulated by rotational isomerism. Based on deuterium substitution experiments a resonance Raman band is identified in the 1050-1200 cm<sup>-1</sup> region which contains a contribution from the motion of the amide proton. The unexpected intensity enhancement of an amide mode is attributed to the amide-dithioester intramolecular interaction. The conformer exhibiting the intramolecular interaction is thought to closely resemble the conformation of the substrate in the active site of certain dithioester enzyme-substrate complexes.

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On rapporte la synthèse et les spectres Raman de plusieurs nouveaux dithiosters du N-acyl-glycine d'éthyle, RC(=O)NH-CH<sub>2</sub>—C(=S)SR. Les spectres Raman de ces composés contiennent au moins deux pics dans la région d'élongation de la liaison C= S entre 1050 et 1200 cm<sup>-1</sup>. On a étudié l'effet du solvant, de la concentration, de la température et de la longueur d'onde d'excitation sur ces caractéristiques. Les résultats obtenus sont en accord avec la présence de deux conformations des dithioesters qui co-existent en solution et dont les différences conformationnelles sont controllées par l'isomérie rotationnelle autour d'une ou des deux liaisons NH-CH<sub>2</sub>, CH<sub>2</sub>--CS. Le spectre Raman d'un monocristal de dithioester de N-acyl glycine d'éthyle fournit la meilleure preuve de l'existence de plus d'un conformère en solution. Plusieurs bandes Raman sont absentes dans le spectre du monocristal si on le compare avec le spectre du dithioester en solution, indicant ainsi que seulement un des conformères trouvés dans la solution est retenu dans le cristal. Dans l'un des conformères trouvés en solution, on rencontre des caractéristiques d'une interaction intramoléculaire entre le groupe amide et le groupe dithioester. La nature exacte de l'interaction intramoléculaire n'est pas certaine bien qu'on ait éliminé la liaison hydrogène simple et la tautomérie ènethiol comme d'éventuels responsables. L'interaction des forces dipôle – dipôle et les électrons  $\pi$  de l'amide interagissant à travers l'espace avec les orbitales du  $\pi$  du soufre sont éventuellement responsables de cette interaction. Ces deux interactions putatives peuvent être ajustées par l'isomérie rotationnelle. A partir d'expériences de substitution par du deutérium, on a identifié une bande de résonance Raman dans la région de 1050-1200 cm<sup>-1</sup> qui contient une contribution du mouvement du proton de l'amide. On attribue l'augmentation inattendue de l'intensité d'un mode amide à l'interaction intramoléculaire du dithioester-amide. On pense que le conformère présentant une intramoléculaire ressemble de près à la conformation du substrat dans le site actif de certains complexes enzyme dithioester substrat.

[Traduit par le journal]

#### Introduction

This paper is the third of a series aimed at elucidating the vibrational spectroscopic properties of dithioesters with particular emphasis on the resonance Raman (RR) spectra of these compounds. The first paper (1) sought to define the normal modes of the simplest dithioesters, methyl and ethyl dithioacetate, while the second paper (2) dealt with the effects on the vibrational spectra of rotational isomerism about bonds in, for example,  $CH_3CH_2 - C(=S)SCH_3$  and  $CH_3C(=S)S- CH_2$ - CH<sub>3</sub>. Both papers (1, 2) precede and accompany the present paper. The overall aim of this work is to aid the understanding of the RR spectra of transient dithioesters (3, 4) which are formed during enzyme substrate interactions (5, 6). The structure of the dithioester intermediates can be written as RC(= O)NHCH<sub>2</sub>C(=S)S—enzyme where the thiol sulfur is from a cysteine in the enzyme's active site. We now report the synthesis and spectroscopic study of a series of six compounds of the type RC(=O)NHCH<sub>2</sub>C(=S)SC<sub>2</sub>H<sub>5</sub> which act, in some respects, as models for the enzyme– substrate intermediates. The dithioesters listed in

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<sup>&</sup>lt;sup>1</sup>NRCC No. 19740.

Table 1 can be considered to be derivatives of N-acyl glycine. They were prepared as described under the Methods section and, to our knowledge, only compound 1 has been reported before (7).

A major finding of the present study is that the *N*-acyl glycine ethyl dithioesters exist in more than one conformation in solution. One of these conformers, in which there appears to be strong interaction between the amide and dithioester groups, is common to all the compounds studied. This conformer assumes special importance because, based on the spectral criteria, it resembles the conformation of the corresponding substrate in the active site of enzymes such as papain.

#### Methods

Absorption spectra were obtained using a Cary 219 spectrophotometer. The apparatus used to obtain the resonance Raman spectra consisted of either a 3000K or a 2000K Coherent Radiation krypton ion laser with a Spex 0.5 m double spectrometer and direct current detection. The near-uv laser lines were separated using two Pellin–Broca prisms and plasma lines were removed from the selected laser line using a tunable grating filter F100 (PTR Optics Corp., Waltham, MA.). Raman spectra obtained with visible excitation were recorded using a Spectra-Physics model 164 Ar ion laser with a Jarell-Ash 25-400 spectrometer.

The sampling systems consisted of a flow cell for the resonance Raman spectra and a rotating cell for the Raman spectra. The variable temperature study was conducted using a small jacketed rotating cell. Under these conditions there was no evidence for photoisomerisation or photodegradation.

Peak frequencies were calibrated using emission lines from Ar and Ne lamps and are accurate to  $\pm 2 \text{ cm}^{-1}$ . Peak heights were taken as reliable measures of band intensities for the variable temperature study.

#### Synthesis

The N-acyl glycine dithioesters and analogues listed in Table 1 were prepared by first treating their respective nitriles with dry HCl gas and an excess of ethanethiol using methylene chloride as solvent (the Pinner reaction) (7-10):



The thioimido ester hydrochlorides produced were treated with  $H_2S$  dissolved in pyridine to give the corresponding dithioesters (7–9):



Nitriles

The nitriles for the synthesis of dithioesters 1, 4, 5, and 6 (Table 1) were synthesised from aminoacetonitrile bisulphate and the corresponding acid chloride (1, 4, and 5) or acid chloroformate (6). The aminoacetonitrile bisulphate (0.1 equiv.)

was dissolved in 50 mL of ice-cold water containing 0.2 equiv. NaOH. The chloride (or chlorformate) (0.1 equiv.) was added dropwise over a period of 30 min while a slightly alkaline pH was maintained by the addition of 5 M NaOH. The modified nitriles separated out as white solids which were collected by filtration, washed with cold water, and dried. They required no further purification. The nitriles used in the synthesis of dithioesters 2 and 3 were produced by the methods of Dinizo *et al.* (11) and Johnson and Gatewood (12), respectively.

#### Thioimido esters

The nitriles were converted to their thioimido ester hydrochloride salts by treatment for 30-60 min with dry HCl gas and excess ethanethiol in ice-cold methylene chloride. The thioimido ester salts produced were precipitated by the addition of anhydrous ether, collected by filtration, and washed with more ether. The thioimido ester salt produced for the synthesis of dithioester **3** is extremely hygroscopic and required careful handling. All the thioimido esters were stored in desiccators and used in the next synthetic step as soon as possible.

#### Dithioesters

The thioimido esters were added to pyridine saturated with H<sub>2</sub>S at 0°C and H<sub>2</sub>S was passed through the solution for 20 min after which the solution was allowed to stand 5 min. The deep yellow solution produced was then poured into sufficient ice-cold 4 N HCl to neutralise the pyridine and produce a slightly acidic pH. For some dithioesters crystals of the dithioester formed either immediately or on addition of ice-cold water. The crystals were collected by filtration and washed with water, then recrystallised from an acetone- water mixture. Dithioesters 2 and 4 formed orange or red oils when poured into the HCl solution. These dithioesters were extracted into ether, the ethereal solutions washed with water and dried over anhydrous  $Na_2SO_4$ . The ether was then removed by rotary evaporation. Dithioester 3 is soluble in water and so the HCl solution was extracted several times with ether and the combined ethereal extracts treated as above. The dithioesters were purified by passage down silica gel columns using 90% ether, 10% acetonitrile as solvent.

The results of elemental analysis of the dithioesters all agreed with the theoretical values to within 2%. The purity of all samples was checked by nmr. The yields obtained varied from about 10% for ester 3 to about 25% for ester 5.

## **Results and discussion**

Aliphatic dithioesters normally possess an intense  $\pi \to \pi^*$  absorption band in the range 305–310 nm (13). The  $\lambda_{max}$ 's and extinction coefficients of this transition, for most of the dithioesters discussed in this paper, were determined in a variety of solvents and are given in Table 2. Results obtained for ethyl dithioacetate are included in the table for comparison.

#### (a) Evidence for two forms of dithioester in solution

The RR spectra of solutions of compounds 1-6 (Table 1) are given in Figs. 1–6. Spectra, taken in several solvent systems, were obtained using 324 nm laser light excitation. The RR spectra of the compounds are more complex than those of ethyl and methyl dithioacetates discussed in the preceding paper (1). For these two simple esters in the region between 1050 and 1200 cm<sup>-1</sup> a single feature occurs near 1195 cm<sup>-1</sup>. This band is assigned to a

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TABLE 1. Dithioesters synthesised

TABLE 2.  $\lambda_{max}$  and log  $\varepsilon$  values for various dithioesters

Dithioester	Solvent	$\lambda_{max}(nm)$	Extinction coefficient (log ε)
Ethyl dithioacetate <sup>b</sup>	$\begin{cases} CH_3CN/H_2O^a\\ CH_3CN\\ CCl_4 \end{cases}$	307.5 306 307	4.10 4.07 4.13
1°	$\begin{cases} CH_3CN/H_2O^{\alpha}\\ CH_3CN\\ CCl_4 \end{cases}$	309.5 308 309	4.0 4.0 4.04
2	CH <sub>3</sub> CN/H <sub>2</sub> O <sup>a</sup>	310.5	3.97
3	CH <sub>3</sub> CN/H <sub>2</sub> O <sup>a</sup>	309	4.04
5	$ \left\{ \begin{array}{l} CH_3CN/H_2O^a\\ CH_3CN \end{array} \right. $	310 308	4.06 4.03
6	CH <sub>3</sub> CN/H <sub>2</sub> O <sup>a</sup>	309.5	4.03

<sup>a</sup>20% acetonitrile + 80% water.

<sup>b</sup>Published values for  $\lambda_{max}$  and log  $\epsilon$  for ethyl dithioacetate are 306 nm and 4.10 in ethanol (13), and for dithioester 1 are 309 nm and 4.03 in methanol (7).

mode possessing significant C=S and some C-C stretching character. Each of the spectra of compounds 1-6 (Table 1) is more complex in that it has more than one feature in the 1050 to  $1200 \text{ cm}^{-1}$ region. The dithioester solution spectra (Figs. 1-6) all contain one feature between 1160 and 1185 cm<sup>-1</sup> (which we shall designate band I) and a second feature between 1115 and 1155  $cm^{-1}$  (band II), additionally compounds 3, 4, and 5 have a third feature between 1080 and  $1100 \text{ cm}^{-1}$  (band III). The relative intensities of these three bands exhibit a strong solvent dependency (Figs. 1-6). For all the compounds tested, except dithioester 2, band I is the most intense in CCl<sub>4</sub> solution whereas band II is the most intense in CH<sub>3</sub>CN solution. In the spectra of compound 2 in both CCl<sub>4</sub> and CH<sub>3</sub>CN solution band II is the most intense, however, band I becomes more intense, relative to band II, on changing from  $CCl_4$  to  $CH_3CN$  solution. This intensity change of band I for dithioester 2 is opposite in sign to that of all the other dithioesters tested. Band III, when it appears, exhibits the same solvent dependency as band II.

The relative intensities of bands I, II, and III were found to be independent of concentration. Spectra were obtained for acetonitrile solutions of dithioester 3 over a concentration range of (0.33 -3.65)  $\times$  10<sup>-4</sup> M and for dithioester 6 over a concentration range of  $(0.37-3.73) \times 10^{-4} M$  and for both compounds the relative intensities of bands I and II (both dithioesters) and band III (dithioester 3) remained constant. The relative intensities of the three bands do, however, exhibit a temperature dependency. Figure 7 shows the changes, in the intensities of bands I and II and where present band III, in the spectra of dithioesters 2, 3, and 4 as a function of temperature. In all cases, as the temperature is lowered, band I decreases in intensity relative to bands II and III. These results along with the solvent dependent changes and lack of any concentration dependency in the RR spectra point to the possibility that two forms of the dithioesters exist through some form of intramolecular rearrangement. Band I would be associated with the higher energy form, which we designate form A, and bands II and III with the lower energy form B. With the assumption that the ratio of the intensities of bands I and II is equal to the ratio of the concentrations of the two conformers, a plot of the logarithm of the intensity ratio against the reciprocal of the absolute temperature yields a  $\Delta H$  value for the two forms. The  $\Delta H$ 's obtained for dithioesters 1, 2, 3, and 4 were 1.0, 0.64, 0.89, and 0.88 kcal/mol, respectively.

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FIG. 1. Resonance Raman spectra of  $4 \times 10^{-4} M$  solutions of ethyl dithiohippurate (1). The solvents used were from top to bottom: carbon tetrachloride, acetonitrile, 20% acetonitrile + 80% H<sub>2</sub>O, and 20% acetonitrile + 80% D<sub>2</sub>O. 10 mW of 324 nm laser excitation were used with a spectral slit width of 10 cm<sup>-1</sup> and a scan speed of 0.75 cm<sup>-1</sup>/s. \* denotes peaks due to solvent and \*\* peaks due to a combination of solute and solvent. The peaks at 637 cm<sup>-1</sup> in carbon tetrachloride and at 1046 cm<sup>-1</sup> in acetonitrile contain very weak contributions from the solvent.

A comparison of the RR spectra obtained for dithioester **3** using three near-uv laser lines (Fig. 8) shows that the intensity ratio of bands II (1145  $cm^{-1}$ ) and III (1096  $cm^{-1}$ ) is constant as a function of excitation wavelength whereas the intensity of band I (1165  $cm^{-1}$ ) changes with respect to bands II and III. There are several possible explanations for these data but they are consistent with the existence of two forms of the dithioester, each form having a slightly different electronic spectrum and a characteristic RR spectrum.

A Raman spectrum obtained from a single crystal of dithioester 3 contains only bands II and III at 1141 cm<sup>-1</sup> and 1088 cm<sup>-1</sup>, respectively (Fig. 9). Band I is completely absent from this spectrum.



FIG. 2. Resonance Raman spectra of  $4 \times 10^{-4} M$  solutions of benzoyl hydroxyacetyl ethyl dithioester (2). The solvents and other conditions were as given in Fig. 1.

Thus, dithioester 3 crystallises entirely in form B. A Raman spectrum of a concentrated acetonitrile solution of dithioester 3 contains all three bands, i.e. bands I, II, and III at 1170, 1143, and 1094 cm<sup>-1</sup>, respectively (Fig. 9). This finding is in agreement with that of the RR study on more dilute solutions of the dithioester (Fig. 3).

The RR spectra of the dithioester solutions (Figs. 1–6) also contain many other features. In the 500–700 cm<sup>-1</sup> region they all contain a peak between 590 and 600 cm<sup>-1</sup>. This peak appears to be associated with form B in that it exhibits the same solvent dependency as band II, i.e. for dithioesters 1, and 3–6 it is more intense in acetonitrile solution than in carbon tetrachloride solution. This 590–600 cm<sup>-1</sup> peak has little or no D<sub>2</sub>O dependency. All the RR spectra also contain features near 690, 1045, and 1340 cm<sup>-1</sup>. Other minor features at approximately 550 and 660 cm<sup>-1</sup> appear in some spectra.

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FIG. 3. Resonance Raman spectra of  $4 \times 10^{-4} M$  solutions of N-acetyl glycine ethyl dithioester (3). The solvents and other conditions were as given in Fig. 1.

## (b) The RR bands between 1050 and 1200 cm<sup>-1</sup>

For every N-acyl glycine ethyl dithioester, bands I and II appear in the region of the RR spectra associated with C=S stretching motions, and, moreover, derive from different forms of each compound. These facts suggest that bands I and II, respectively, are the corresponding normal modes of forms A and B of the dithioester and that both bands contain some C=S stretching character.

Some insight into the expected positions of C=S stretching bands may be gained by considering the infrared work of Bak *et al.* (14) on dithioesters of type R-CS-SCH<sub>2</sub>COOH. The analysis of Bak *et al.* allows us to estimate the through bond inductive effect of R on the frequency of  $v_{C=S}$ . Assuming that the effect of the carboxymethyl group is constant throughout a series obtained by varying R then any correlation between R and  $v_{C=S}$  should, after



FIG. 4. Resonance Raman spectra of  $4 \times 10^{-4} M$  solutions of *N*-phenylacetyl glycine ethyl dithioester (4). The solvents and other conditions were as given in Fig. 1.

applying a constant correction for the different effects of  $-CH_2COOH$  and  $-CH_2CH_3$  on  $v_{C=S}$ , also be applicable to the dithioesters studied here. A plot of the pK's of the series of 21 acids of the type R-COOH against the  $v_{C=S}$  of the corresponding esters produces a straight line defined by the equation:

[1] 
$$v_{C=S} = 16.8 \text{ pK} + 1143$$

The values in this equation were obtained by a regression analysis which gave a correlation coefficient of 0.79 (significant at P = 0.05). Using a value of 1197 cm<sup>-1</sup> for the  $v_{C=S}$  of ethyl dithioacetate (1) and a pK of 4.75 for acetic acid the equation for a series of ethyl dithioesters becomes:

$$[2] \quad v_{C=S} = 16.8 \text{ pK} + 1116$$

A value of 1180 cm<sup>-1</sup> for the  $v_{C=S}$  of ethyl dithiohippurate, 1, can then be calculated using a





FIG. 5. Resonance Raman spectra of  $4 \times 10^{-4}$  M solutions of N- $\beta$ -phenylpropionyl glycine ethyl dithioester (5). The solvents and other conditions were as given in Fig. 1.

pK of 3.8 for hippuric acid. A similar value (i.e. approx. 1180  $cm^{-1}$ ) can be calculated for the other N-acyl glycine dithioesters studied. The position of band I of the dithioesters falls in the range 1165 cm<sup>-1</sup> to 1185 cm<sup>-1</sup>, thus this band is very close to the predicted value for  $v_{C=S}$ . Therefore, it seems probable that band I of form A of the dithioesters represents a relatively unperturbed C=S stretching vibration, since it is found in the position to be expected for the C=S stretching vibration after taking into consideration only the through bond effects of the substitution of an N-acyl amine onto the acetyl portion of ethyl dithioacetate. By the same token the position of band II is anomalous in that it comes at a lower frequency than that predicted by eq. [2] above. It should be emphasised however that several assumptions, which have not been fully tested, have gone into deriving eq. [2]. For this reason the conclusion we have reached on the expected position for  $v_{C=S}$  is tentative.

All the dithioesters tested, with the exception of **2**, show some band position shifts on going from

FIG. 6. Resonance Raman spectra of  $4 \times 10^{-4}$  M solutions of N-carbobenzoxy glycine ethyl dithioester (6). The solvents and other conditions were as given in Fig. 1.

solution in 80% H<sub>2</sub>O, 20% CH<sub>3</sub>CN, to 80% D<sub>2</sub>O, 20% CH<sub>3</sub>CN (Figs. 1–6). For those compounds that do not possess a band III in their RR spectra, i.e. 1 and 6, band II shifts to higher wavenumbers in the presence of D<sub>2</sub>O, whereas band I remains fixed (Figs. 1 and 6). For compounds that do possess a band III, i.e. 4 and 5, it is this band that shifts to higher wavenumbers, whilst bands I and II remain stationary (Figs. 4 and 5). The behaviour of the *N*-acetyl dithioester 3 is anomalous, since both bands II and III appear to shift and the shifts are also associated with intensity changes, i.e. in D<sub>2</sub>O/CH<sub>3</sub>CN solution band III is more intense than band II, whereas in H<sub>2</sub>O/CH<sub>3</sub>CN band II is the more intense, Fig. 3.

On examining the  $H_2O/CH_3CN$  and  $D_2O/CH_3CN$ dithioester solutions by nmr spectroscopy it was found that in the presence of  $D_2O$  the amide proton of the *N*-acyl dithioesters is exchanged for a deuteron. On the time scale of the RR experiments no further proton-deuteron exchanges were obSTORER ET AL.



FIG. 7. Sections of the resonance Raman spectra of acetonitrile solutions of dithioesters  $2(4.2 \times 10^{-3} M)$ ,  $3(6 \times 10^{-3} M)$ , and  $4(3.1 \times 10^{-3} M)$  scanned at three different temperatures. 25 mW of 350.7 nm laser excitation were used with a spectral slit width of 10 cm<sup>-1</sup> and a scan speed of 0.75 cm<sup>-1</sup>/s. The temperature was measured using a copper–constantan thermocouple.



FIG. 8. The variation in the relative intensities of bands I, II, and III in the resonance Raman spectra of dithioester 3 as a function of the excitation wavelength. The concentration of the dithioester was  $9.6 \times 10^{-4}$  M in a 1:1 acetonitrile-water mixture. The power used was 400, 40, and 15 mW for the 350.7, 337.5, and 324 nm excitation lines, respectively. A spectral slit width of 10 cm<sup>-1</sup> was used with a scan speed of 0.75 cm<sup>-1</sup>/s.

served. Thus, the exchange of -NH for -ND must be related to the band position shifts in the resonance Raman spectra. Further evidence that the shifts are due to the exchange of the amide proton for a deuteron is the fact that no shift is observed for compound 2 which does not have an amide proton. The position of band I, for all the dithioesters, is not influenced by the presence of  $D_2O$ , thus the normal mode of form A which gives rise to band I is unaffected by ---NH, ---ND exchange. Form B gives rise to bands II and III in the RR spectra, and one or both of these bands is affected by deuteration of the amide groups. Band II for compounds 1 and 6 probably contains some C=S stretching character and must contain, in some way, a contribution from the motion of the amide proton. For compounds 4 and 5 band II likely contains C=S stretching character but there is no evidence for a contribution from a NH motion. However, band III in compounds 4 and 5 is sensitive to the motion of the amide proton. The situation for compound 3 is more complex and both bands II and III involve amide proton motions with possible C=S stretching contributions to one or both features.

## (c) Nature of the two forms found in solution

Form B, which, according to the temperature dependences of bands I, II, and III, Fig. 7, is of lower energy than form A, must differ from form A by one or more of the following possibilities: (*i*) tautomerism, (*ii*) rotational isomerism, or (*iii*) intramolecular interaction. Intermolecular interac-

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FIG. 9. Raman spectra of N-acetyl glycine ethyl dithioester (3) A, crystals, and B, a concentrated solution, 0.11 M, in a 1:1 acetonitrile-water mixture. B' is a portion of spectrum B recorded at higher sensitivity. The power used was 5 and 360 mW of 5145 nm laser light for spectra A and B, respectively. The spectral slit width was 10 cm<sup>-1</sup> with a scan speed of 0.25 cm<sup>-1</sup>/s.

tions are ruled out by the lack of a concentration dependence in the RR spectra.

# (i) Tautomerism

The enethiolization of thioketones is well known (15, 16). In contrast to such simple thiones dithioesters are generally stable in the thione form (17, 18). The exceptions are special dithioesters (19), for example of the type (20):



There are three pieces of evidence against enethiolization occurring with the N-acyl dithioesters discussed here. Firstly, no band assignable to a C==C stretching vibration can be seen in the spectra of the dithioesters (especially in the Raman spectra of the crystals of dithioester **3** which are entirely in form B, the presumed enethiol). Secondly, if an equilibrium existed between the two tautomers this would provide a mechanism for the exchange of the glycine ---CH<sub>2</sub>---protons, no such exchange is observed in D<sub>2</sub>O (except at high pH). Thirdly, the dithioester chromophore would be destroyed by the formation of the enethiol and thus on going from carbon tetrachloride solution (which favours form A) to acetonitrile solution (which favours form B) a large reduction in the measured extinction coefficients should be observed. A small reduction in the extinction coefficients is seen but it is of the same magnitude as that observed for ethyl dithioacetate (Table 2) on changing solvents. Ethyl dithioacetate is known not to undergo enethiolization (17, 18).

# (ii) Rotational isomerism

We have previously studied rotational isomers of the two dithioesters:



(2, 21) and determined the effect on the RR spectrum of rotations about the C—C and S—C bonds indicated. By altering the vibrational coupling in the molecules, rotational isomerism does bring about changes in the 500–700 cm<sup>-1</sup> spectral region. However, for ethyl dithioacetate and methyl dithiopropionate rotational isomerism appears to leave the peak near 1195 cm<sup>-1</sup> unchanged. Thus, it is unlikely that a simple rotation is the sole cause of the changes in the 1050–1200 cm<sup>-1</sup> region of the RR spectrum of form B of the present compounds. It does not rule out the possibility that the changes are caused by a rotation coupled with some form of intramolecular interaction.

# (iii) Intramolecular interaction — the difference between forms A and B

Having argued against tautomerism and the effects of rotational isomerism and having dismissed intermolecular interactions as possible causes of the " $v_{C=S}$ " RR peaks of form B we turn now to consider an intramolecular interaction as the source of form B's "perturbed" RR peaks. There are two examples in the literature of intramolecular interactions which have to be taken into account for the systems discussed here. However, we shall argue that neither of these two cases provides an explanation of the observed RR spectrum of form B.

The first example concerns an intramolecular interaction in N-acetyl glycine methylester (22). An intramolecular H-bond was proposed to explain the splitting of the N—H stretching vibration peak in the ir spectra taken in carbon tetrachloride solution, i.e.



This interaction is designated  $C_5$ , since it involves a five-membered ring. Due to the strain in the ring the H-bond must be weak as is reflected by the small change in  $v_{N-H}$  brought about by the interaction i.e.  $24 \text{ cm}^{-1}$  (strong hydrogen bonds can change  $v_{N-H}$  by up to 200 cm<sup>-1</sup>). The lack of any detectable perturbation of the ester  $v_{C=0}$  frequency (22) also supports the supposition that the  $C_5$  interaction must be weak. A dipole–dipole interaction of the amide and ester groups would however help to strengthen the  $C_5$  interaction (23). The  $C_5$  interaction is thought to be important in the determination of protein structure since it is also present in the amides of *N*-acyl amino acids as well as the esters (22–25).

The second example concerns an intramolecular interaction between a sulfur atom and an amide group in *S*-ethylcysteine methylamide (compound *b*). The ir spectrum of this compound has a strong absorption band at 3402 cm<sup>-1</sup> which can be assigned to the N<sub>1</sub>—H<sub>1</sub> stretching vibration (25). However, this frequency is too low to be ascribed to an ordinary C<sub>5</sub> interaction between the N<sub>1</sub>—H<sub>1</sub> and C<sub>2</sub>=O<sub>2</sub> groups. It was concluded, therefore, that the amide proton is involved in a hydrogen bond with the sulfur atom. The structural similarity between the compound and the dithioesters discussed here is shown below:



It is reported that the strength of the N—H....S interaction in S-ethylcysteine methylamide is such that all the molecules present in solution are in the intramolecular interacting form (25). This interaction is unusual since it was shown that the optimal ring size for the interaction is five; the equivalent

methionine derivative, with its potential to form a six-membered ring, did not possess an interaction of this type. This is unexpected since a less strained H-bond could be formed in a six-membered ring. Also such a supposedly strong H-bonded N—H in S-ethylcysteine methylamide would be expected to have a  $v_{N-H}$  at lower wavenumber than that observed. This suggests that the interaction in this compound is not just a simple H-bond.

In both of the literature examples just cited the formation of the hydrogen bond was proposed as the source of the free energy driving the formation of the intramolecular form. However, for the present N-acyl glycine ethyl dithioesters we have good evidence that a hydrogen bond is not energetically important in the intramolecular form. Firstly, does have a form B. Secondly, the solvent dependency of the A and B forms argues against the presence of a strong intramolecular H-bond in B. In consequence of the hydrogen-bond accepting nature of its nitrogen atom acetonitrile is a much stronger H-bond acceptor than carbon tetrachloride. Thus, it would be expected that if form B had a strong intramolecular H-bond this form would predominate in CCl<sub>4</sub>. The opposite is found to be the case; for compounds 1 and 3-6 there is a higher population of form B relative to form A in  $CH_3CN$  compared to  $CCl_4$ .

Although it seems unlikely that either structure a or b, with their crucial hydrogen bonds, is equivalent to form B, the --- NH moiety is implicated in form B by the RR results. The sensitivity of the RR spectrum of form B to --- NH, --- ND exchange was removed from the dithioester chromophore. The amide group itself has a  $\pi - \pi^*$  transition at approximately 180 nm ( $\varepsilon_{max} \simeq 10^{-4}$ ) and a  $\pi \to \pi^*$ transition at approximately 220 nm ( $\varepsilon_{max} < 100$ ). However, these electronic absorption bands are too far removed from the Raman excitation wavelength to obtain preresonance enhancement of normal modes of the unperturbed amide chromophore. It is likely, therefore, that the participation of the --- NH motion in the RR spectrum takes place by some form of coupling between the amide and dithioester chromophores. This coupling could include both vibrational and electronic interactions and may take place through the -NH-CH2-C(=S) bonds or "through space", i.e. via nonbonded contacts if the chromophores in form B are placed in close juxtaposition. The nature of the coupling is such that it occurs only in form B. Moreover, since it changes the positions of Raman peak positions (for form B compared to form A) it must perturb the electron distribution in the electronic ground state. (Raman and RR peak positions reflect only electronic ground state properties). However, the coupling has only a modest effect on the electronic spectrum of the dithioester chromophore. Compared to the absorption spectrum of ethyl dithioacetate the spectra of the dithioesters in Table 1 show a red shift in  $\lambda_{max}$  by 2–3 nm (Table 2) and an increase in peak width at half height of approximately 7% in CH<sub>3</sub>CN/H<sub>2</sub>O (8:2) solution and 3% in carbon tetrachloride solution.

The nature of the coupling between the amide and dithioesters in form B is not completely understood although literature evidence (26–31) suggests that a substantial part of the coupling may occur through space. The occurrence of the coupling in form B alone suggests that the amide and dithioester chromophores are in closer contact in form B compared to form A. The preferred means of switching from form A to B would be by rotations about either or both of the NH— $CH_2$ ,  $CH_2$ —C(=S) bonds. Hence, although rotational isomerism by itself does not change the positions of peaks for form B (by altering the vibrational coupling), it is proposed that it brings the amide and dithioester groups into contact. The chromophore-chromophore interaction (which may include electronic and vibrational components) is then thought to change the position of form B's RR features with respect to those of form A. The interaction between the amide and dithioester groups can also include dipole interactions such as the field effects discussed by Bellamy and Williams (32). Field effects have been used to explain the two carbonyl bands in the ir spectra of  $\alpha$ -halogen substituted thiolesters (33). The two bands are due to two conformers, one involving a dipole interaction between the halogen and the carbonyl oxygen (giving rise to the high wavenumber band) and the other involving a dipole interaction between the halogen and the thiol sulfur. The transition between the two forms involves a 180° rotation about a single C-C bond. Another component of the amide-dithioester interaction may be overlap between the sulfur *d*-orbitals and the  $\pi$ -electrons of the amide group. An interaction of this type has been suggested to occur between the  $\pi$  electron cloud and the empty  $d_{\pi}$  orbitals of  $\beta$ , $\gamma$ -unsaturated thiol esters (34).

Although we strongly favor a model in which forms A and B are different rotamers and that a strong intramolecular interaction in form B is absent in A, it is clear that the details of this interaction are speculative. However, X-ray crystallographic studies are under way and by this means we hope to provide detailed information on the geometries of forms A and B.

The dithioesters discussed in this paper are very useful models for the dithioacyl enzymes produced by the action of cysteine proteases on thiono ester substrates (3–6). The similarities between the dithioacyl enzyme resonance Raman spectra and the spectra of the B form of the dithioesters is very striking (3, 4). Thus, the knowledge obtained about the interactions that define the B form of the dithioesters is important to an understanding of the interactions controlling the catalytic properties of dithioacyl enzymes. This point will be discussed at length in a subsequent publication dealing with the resonance Raman spectra of the dithioacyl enzymes.

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