Conformational Study of *N*-Acyl Amino Acid Esters and Thiol Esters by FT-IR and X-ray Crystallography: Evidence for a N···S Interaction in Thiol Esters[1]

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Abstract: X-ray crystallographic analyses of *N*-benzoylglycine ethyl thiol ester and *N*-(β-phenylpropionyl)glycine ethyl thiol ester demonstrate that both compounds take up a B-type conformation comparable to that found for *N*-acylglycine dithio esters. In CCl_4 solution FT-IR spectra indicate that *N*-acylglycine thiol esters prefer an intramolecularly H-bonded C_5 conformation, while in CH_3CN these compounds assume a mixed population made up of a B and one other non-C_5 conformer. In contrast to their thiol ester and dithio ester analogues *N*-acylglycine dioxygen esters are not generally found in the B conformation in the solid phase. FT-IR data demonstrate that in CCl_4 solution the dioxygen esters are mainly in a C_5 population while in CH_3CN they form a mixed population consisting of rotamers with the N atom trans and gauche to the alkoxy O atom.

The cysteine protease catalyzed hydrolysis of esters, peptides, and polypeptides proceeds via the formation of thiolacyl enzyme intermediates.[1] Due to the transient nature of these intermediates no direct information exists on the conformation of the acyl group and its interactions with the enzyme's active site. Drenth et al.[2] by extrapolating from the X-ray structure of a papain chloromethyl ketone derivative have been able to suggest a possible structure for the covalently bound acyl group. While the X-ray studies serve as the starting point for understanding the chemistry of papain-catalyzed reactions, they are restricted by the resolution of the protein crystallographic data (0.28 nm in the case of the chloromethyl ketone derivative) and by their inability to characterize intermediates under turnover conditions.

In order to obtain detailed information on papain–substrate transients, we have been employing a three-pronged approach involving resonance Raman (RR) spectroscopic and kinetic studies on dithioacyl papains, and X-ray crystallographic studies on suitable model compounds. This work has been described earlier[3,4] and in the two preceding papers in this issue[5,6] and is reviewed elsewhere.[7] Dithioacyl papains are used instead of thiolacyl papains because the dithio ester group provides a suitable chromophoric "handle" by which to obtain the RR and kinetic data. Although the use of C(=S)S instead of C(=O)S represents only a single atom replacement it is of obvious importance to see if the deductions we have made concerning the dithioacyl papains can be transferred to the completely "natural" thiol analogues. To this end in the present paper we compare the conformational preferences of *N*-acylglycine dithio and thiol esters and show that a transfer of information is possible. At the same time we demonstrate that *N*-acylglycine dioxygen esters have quite distinct conformational properties. In particular, the latter esters generally do not form a conformational analogue of the characteristic conformer B, with its N···S(thiol) attraction, seen for thiol and dithioesters.

Experimental Section

The *N*-acylglycine esters were synthesized from glycine methyl ester and the corresponding acid chloride and the *N*-acylglycine thiol esters were synthesized as described by Ingles and Knowles[8] for *N*-acetylglycine ethyl thiol ester. All compounds were purified using silica gel chromatography. The purity of the compounds was checked by NMR, and the results of their elemental analysis agreed, within acceptable limits (±0.02 times calculated percentage), with the theoretical values.

FT-IR spectra were recorded with a Bomem DA 3.02 instrument equipped with a medium-range mercury cadmium telluride detector. Five hundred scans were accumulated by using an optical retardation of 0.5 cm. The resultant interferograms were apodized with a Happ–Genzel function and Fourier transformed to yield a spectral resolution of 2 cm^{-1}. Spectra in the carbonyl region were deconvoluted according to the procedure described by Kauppinen et al.[9a] Demountable liquid cells with CsI windows were used. The path lengths were 1 and 0.1 cm for the NH and C=O region, respectively. For temperature-dependence measurements a thermostated cell mount[9b] was used and the temperature of the solutions was monitored by a thermocouple located in the cell mount.

Crystals of *N*-(β-phenylpropionyl)glycine ethyl thiol ester (PPG) and of *N*-benzoylglycine ethyl thiol ester (BG) were prepared by diffusion of hexane vapor into ether solutions of the respective compounds. Precession photographs indicated the symmetry and approximate cell dimensions, but in each case the accurate measurement of the cell dimensions and of the relative intensity data was made on an Enraf-Nonius CAD-4F diffractometer with Ni-filtered Cu Kα radiation [λ(Cu $K\alpha_1$) = 1.54056 Å]. Intensity data for both compounds were collected up to $\theta = 75°$. The

Table I. Crystal Data

N-(β-Phenylpropionyl)glycine Ethyl Thiol Ester
$C_{13}H_{17}NO_2S$, $M_r = 251.34$
orthorhombic, space group $Pca2_1$
$a = 9.541$ (1) Å, $b = 12.231$ (1) Å, $c = 12.128$ (1) Å
$V = 1415.3$ Å^3, $Z = 4$, $d_x = 1.179$ Mg m^{-3}
$F(000) = 536$, μ(Cu Kα) = 1.92 mm^{-1}
specimen 0.22 × 0.37 × 0.50 mm, prism shaped
$\omega/2\theta$ scans, max scan speed (ω) = 5.0° min^{-1}
1529 independent reflcns measd
1273 obsd ($I_{net} \geq 1.43\ \sigma(I)$)
final $R = 0.037$, $R_w = 0.043$

N-Benzoylglycine Ethyl Thiol Ester
$C_{11}H_{13}NO_2S$, $M_r = 223.28$
monoclinic, space group Pc
$a = 5.675$ (3) Å, $b = 10.439$ (2) Å, $c = 9.944$ (2) Å
$\beta = 93.56$ (1)°, $V = 588.0$ Å^3, $Z = 2$
$d_x = 1.261$ Mg m^{-3}, $F(000) = 236$, μ(Cu Kα) = 2.25 mm^{-1}
specimen 0.15 × 0.50 × 0.50 mm, plate shaped
ω scans, max scan speed = 3.35° min^{-1}
1209 independent reflcns (hkl and $hk\bar{l}$)
1145 obsd ($I_{net} \geq 1.43\sigma(I)$)
final $R = 0.050$, $R_w = 0.066$

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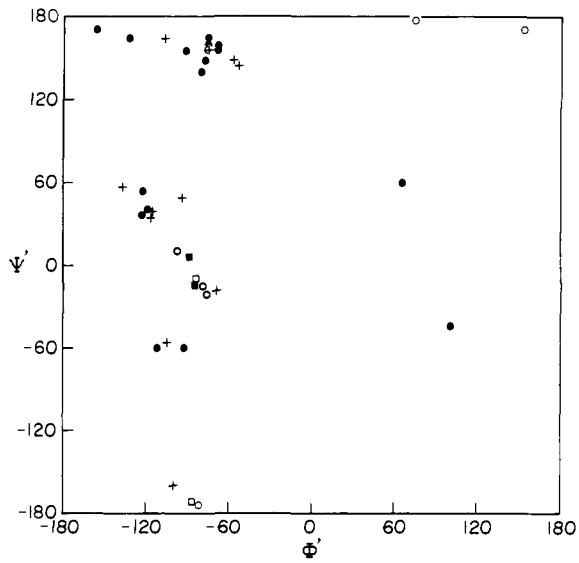


Figure 1. Ramachandran (ϕ',ψ') plot for the X-ray structures of *N*-acyl amino acid esters: where amino acid is glycine (○), an aromatic amino acid (●), or any other (+) and *N*-acylglycine ethyl thiol esters (■) and dithio esters (□).

ω-scan technique was used for compound BG because the crystals showed mosaic spreads up to 0.5°. Table I gives the pertinent crystal data. Intensities of three check reflections were monitored after every 5000 s of exposure time, and these measurements were used to scale the data. The usual Lorentz and polarization corrections were made in both cases, and absorption corrections, using a Gaussian integration method, were applied to the data for compound BG.

Both structures were solved by direct methods, using the *MULTAN* 78 program,[10] and were refined by block-diagonal least squares, minimizing $\sum w(\Delta F)^2$. The non-hydrogen atoms were refined first isotropically, later with anisotropic thermal parameters. Hydrogen atoms were ultimately refined with isotropic temperature factors. The weighting scheme for both structures was of the form $w^{1/2} = 1.0$ if $|F_o| < P_1$, $w^{1/2} = P_1/|F_o|$ if $|F_o| > P_1$. For compound PPG, $P_1 = 15.0$, and for compound BG, $P_1 = 5.0$; these schemes made average values of $w(\Delta F)^2$ essentially independent of $|F_o|$ and $\sin^2\theta$. Unobserved reflections were excluded from both refinements, as were three reflections showing possible extinction effects in the case of compound PPG. Scattering factor values for S, O, N, and C were taken from ref 11, with an anomalous dispersion correction[11] applied to the sulfur scattering curve. Scattering factor values for H were taken from Stewart et al.[12] Difference maps were calculated from the final structure factors [tables of observed and calculated structure factors and of anisotropic thermal parameters for both compounds are available (supplementary material)] and showed maximal residual density of 0.16 and 0.23 e/Å^3 for compounds PPG and BG, respectively. All calculations, unless otherwise noted, were done with the NRC set of crystallographic programs.[13]

Results and Discussion

Survey of Crystallographic Results for Dioxygen Esters. The Cambridge Crystallographic Database was surveyed for *N*-acyl amino acid esters. Of the data sets obtained those involving cyclic peptides and esters formed from either *N*-methyl amino acids or α,α-disubstituted amino acids were discarded, thus leaving the 31 esters listed in Table II (supplementary material). The ϕ' (C–N–C–C) and ψ' (N–C–C–O) angles[14] of these structures are plotted in Figure 1; the majority of the points fall loosely into two

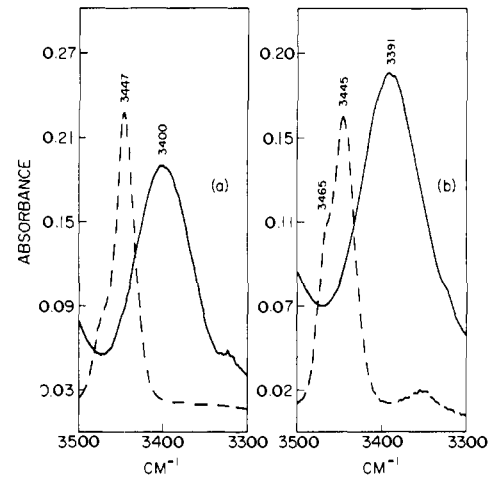
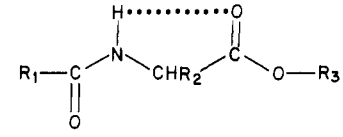


Figure 2. NH stretching region of the FT-IR spectra of *N*-benzoylglycine methyl ester (a) and *N*-benzoylglycine ethyl thiol ester (b) in CH_3CN (—) and CCl_4 (---), 2.3×10^{-3} and 1.3×10^{-3} M, respectively, 1-cm path length.

clusters at $\phi' = -75°,\psi' = 155°$ (12 points) and $\phi' = -120°,\psi' = 45°$ (7 points). The remaining 12 points (2 of which are from glycine esters and 6 from aromatic amino acid esters) are scattered over the whole ϕ',ψ' plot, and one, for *N*-acetylglycyl-L-lysine methyl ester,[15] falls in the area denoting B conformers. The first cluster has the amide nitrogen atom nearly trans with respect to the ester alkoxy oxygen. This conformation is analogous to the conformer A previously described for *N*-acylglycine ethyl dithio esters.[4] The second cluster has a gauche geometry about the C–C_α bond, with respect to the alkoxy oxygen,[16] and as such is unlike any of the conformers found so far for the *N*-acylglycine ethyl dithio esters.

FT-IR Data for Dioxygen Esters. FT-IR data were obtained for several *N*-acylglycine esters, i.e., *N*-acetyl-, *N*-(β-phenylpropionyl)-, *N*-(*p*-chlorobenzoyl)-, *N*-(*p*-nitrobenzoyl)-, *N*-(*p*-methylbenzoyl)-, *N*-(*p*-methoxybenzoyl)-, and *N*-benzoylglycine methyl ester. However, since the data for each compound are very similar only those obtained for *N*-benzoylglycine methyl ester will be presented.

Figure 2a shows the 3300–3500-cm^{-1} regions of the FT-IR spectra of *N*-benzoylglycine methyl ester dissolved in CCl_4 and CH_3CN. The spectrum taken in CCl_4 solution contains an intense feature at 3447 cm^{-1} with a weak shoulder at $\simeq$3465 cm^{-1}. This result is consistent with that reported by several other workers for a variety of *N*-acyl amino acid esters in CCl_4.[17,18] The intense feature has been assigned to the N–H stretching mode of the weakly hydrogen bonded amide proton in a C_5 conformation (with ψ' and $\phi' \approx 180°$), i.e.,



whereas the shoulder at $\simeq$3465 cm^{-1} has been assigned to the same N–H vibration in a minor population of a non-H-bonded conformer. The frequency difference between the H-bonded (C_5) and non-H-bonded N–H vibrations (3465–3447 cm^{-1}) is designated $\Delta\nu_{NH}$. For a series of *N*-acetylglycine-based compounds (spectra not shown) the $\Delta\nu_{NH}$'s are, for *N*-acetylglycine ethyl ester, 22 cm^{-1}, for *N*-acetylglycine ethyl thiol ester, 20 cm^{-1}, for *N*-

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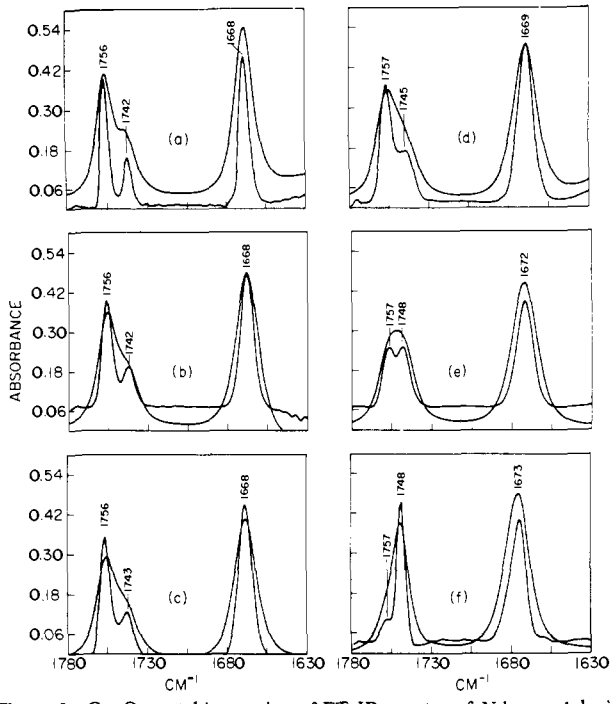

Figure 3. C=O stretching region of FT-IR spectra of *N*-benzoylglycine methyl ester (7.7×10^{-3} M, 0.1-cm cell) in mixed solvents of CH_3CN and CCl_4 (amount of CH_3CN: a, 100%; b, 50%; c, 40%; d, 20%; e, 5%; f 0%). Deconvoluted spectra were obtained using a bandwidth of 12.5 cm^{-1}, a non-Gauss function, a *K* factor of 2.5, and a Bessel function for apodization.[9a]

acetylglycine ethyl thionoester, 59 cm^{-1}, and, for *N*-acetylglycine ethyl dithioester, 61 cm^{-1}. This set of data shows that the interaction in the C_5 conformer involves the C=O or C=S group. It also indicates that a C_5 hydrogen bond involving a carbonyl oxygen atom shifts ν_{NH} by approximately 20 cm^{-1} in esters and thiol esters while the C_5 hydrogen bond to a thiocarbonyl sulfur in thiono and dithio esters shifts ν_{NH} by about 60 cm^{-1}.

Secondary amides tend to form dimers at relatively high concentrations in nonpolar solvents such as CCl_4. For example, *N*-methylbenzamide has its monomeric NH stretching absorption around 3450 cm^{-1} whereas the dimer NH stretching absorption appears in the 3350-cm^{-1} region. To eliminate the possibility of dimer formation influencing the present results, spectra were obtained at low ester concentrations. Moreover, the spectra were shown to be independent of ester concentration in the range 10^{-4}–10^{-2} M.

In CH_3CN solution the amide proton forms a hydrogen bond preferentially with the solvent, as shown by the single feature at 3400 cm^{1} (Figure 2a). In this solvent the competition of the stronger intermolecular H bonds between the NH and the solvent, present at high concentration, prevents the formation of the C_5 conformer.

Figure 3 shows the carbonyl region of the FT-IR spectrum of *N*-benzoylglycine methyl ester dissolved in various CH_3CN/CCl_4 mixed-solvent systems. The peak near 1670 cm^{-1} is an amide feature (it is sensitive to para substitution on the phenyl ring of *N*-benzoylglycine methyl ester, data not shown), and the peaks at $\simeq$1745 cm^{-1} and $\simeq$1755 cm^{-1} are ester carbonyl features. The splitting of the ester carbonyl peak can be explained by the presence in solution of at least two different rotamers. Using the findings from the NH stretching region it is possible to assign, on the basis of its dominant intensity, the feature at 1748 cm^{-1} in the 100% CCl_4 spectrum to the ester carbonyl group of the C_5 conformer and the less intense feature at 1757 cm^{-1} to a non-H-bonded conformer. The difference in the positions of these carbonyl peaks (9 cm^{-1}) is difficult to explain on the basis of weak H bonding alone ($\Delta\nu_{NH}$ is only 18 cm^{-1}) and probably reflects, in addition, the differences in ϕ',ψ' angles between the two conformers. On going to 5% CH_3CN/95% CCl_4 there is a large change in the relative intensities of the two carbonyl peaks. This change appears to be complete on increasing the CH_3CN to 20%.

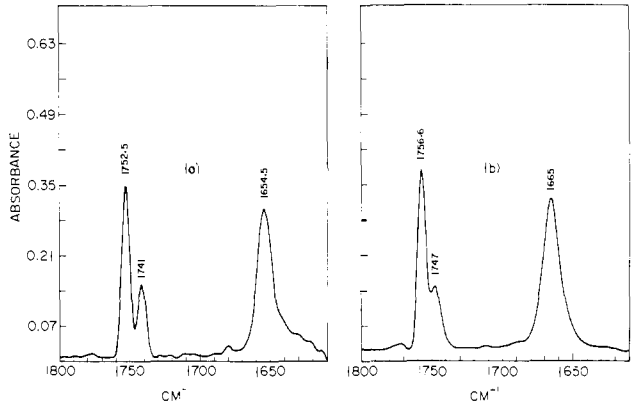

Figure 4. C=O stretching region (deconvoluted) of FT-IR spectra of *N*-methyl-*N*-(β-phenylpropionyl)glycine methyl ester (8.5×10^{-3} M) in CH_3CN (a) and in CCl_4 (b), 0.1-cm cell. The parameters for deconvolution are given in Figure 3.

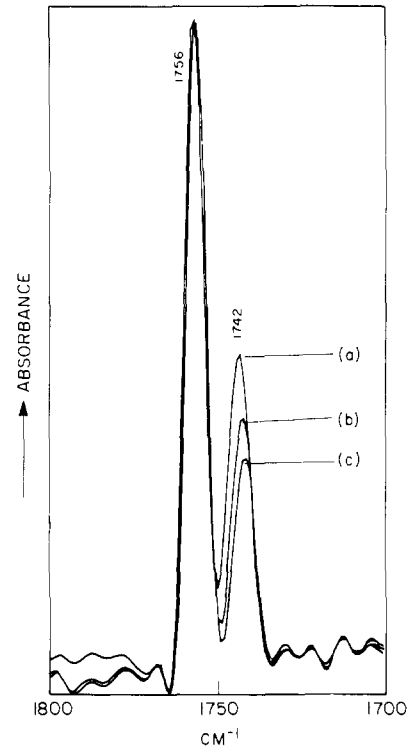

Figure 5. Temperature dependence of carbonyl FT-IR spectra (deconvoluted) of *N*-benzoylglycine methyl ester in CH_3CN (8.0×10^{-3} M, 0.1-cm cell). Temperatures used are the following, from top to bottom: a, +40 °C; b, +27 °C; c, +6 °C. The parameters for deconvolution are given in Figure 3. The peak intensities are normalized with respect to 1756-cm^{-1} band.

Above 20% CH_3CN two features are still present in the spectrum, one at 1756 cm^{-1} and one at 1742 cm^{-1}. Neither feature is due to the C_5 conformer, with its intramolecular H bond retained, since, as mentioned above, CH_3CN competes effectively with the ester carbonyl group for the NH proton and reduces the C_5 population to an undetectable level (Figure 2a). Moreover, the FT-IR spectra of *N*-methyl-*N*-acylglycine esters contain two analogous carbonyl features (Figure 4), both in CH_3CN and CCl_4 solutions, neither of which can be due to an intramolecularly H-bonded C_5 conformer. Therefore, in 100% CH_3CN solution the *N*-acylglycine methyl esters must be present in at least two nonintramolecularly H-bonded conformers that differ in their ϕ',ψ' angles.

The survey of X-ray crystallographic results showed that the major conformational preferences of *N*-acyl amino acid esters consisted of the trans and gauche forms. Therefore we identify the two carbonyl features in CH_3CN solution as each due to one of these forms. Further confirmation that the $\nu_{C=O}$ peaks are

Table III. Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters for the Non-Hydrogen Atoms in *N*-(β-Phenylpropionyl)glycine Ethyl Thiol Ester

$$B_{eq} = \tfrac{8}{3}\pi^2\textstyle\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i\cdot\mathbf{a}_j$$

	X	Y	Z	B_{eq}
S(1)	7088 (1)	2554 (1)	2710 (1)	6.8 (0.0)
O(1)	7840 (3)	1658 (2)	4576 (2)	8.5 (0.1)
O(2)	9508 (2)	4693 (2)	3910 (3)	8.7 (0.1)
N(1)	7220 (2)	4511 (2)	4099 (2)	5.2 (0.1)
C(1)	5875 (6)	510 (3)	2697 (5)	10.4 (0.2)
C(2)	7131 (5)	1115 (3)	2368 (3)	8.5 (0.1)
C(3)	7517 (4)	2491 (2)	4123 (3)	5.8 (0.1)
C(4)	7351 (4)	3539 (2)	4740 (3)	6.2 (0.1)
C(5)	8321 (3)	5047 (2)	3729 (3)	5.7 (0.1)
C(6)	8051 (4)	6071 (2)	3090 (3)	6.1 (0.1)
C(7)	7821 (5)	5836 (3)	1882 (3)	8.1 (0.1)
C(8)	7550 (4)	6865 (2)	1233 (3)	6.2 (0.1)
C(9)	8588 (5)	7394 (3)	684 (4)	8.3 (0.1)
C(10)	8321 (5)	8357 (4)	108 (4)	9.1 (0.1)
C(11)	7030 (5)	8778 (3)	52 (4)	8.3 (0.1)
C(12)	5968 (5)	8262 (3)	594 (4)	8.4 (0.1)
C(13)	6223 (4)	7316 (3)	1186 (4)	7.7 (0.1)

Table IV. Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters for the Non-Hydrogen Atoms in *N*-Benzoylglycine Ethyl Thiol Ester

$$B_{eq} = \tfrac{8}{3}\pi^2\textstyle\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i\cdot\mathbf{a}_j$$

	X	Y	Z	B_{eq}
S(1)	2396 (3)	2720 (1)	3182 (2)	5.9 (0.0)
O(1)	6820 (7)	3352 (4)	3200 (7)	9.3 (0.2)
O(2)	4017 (7)	−150 (4)	4935 (3)	6.9 (0.1)
N(1)	4332 (7)	216 (4)	2749 (3)	5.1 (0.1)
C(1)	2924 (20)	4772 (7)	4951 (8)	10.5 (0.3)
C(2)	2342 (10)	4415 (5)	3519 (5)	6.5 (0.1)
C(3)	5393 (8)	2491 (5)	3134 (4)	5.7 (0.1)
C(4)	6216 (8)	1116 (5)	2966 (5)	5.8 (0.1)
C(5)	3316 (8)	−375 (4)	3750 (4)	5.2 (0.1)
C(6)	1328 (8)	−1271 (4)	3418 (4)	5.1 (0.1)
C(7)	601 (11)	−1603 (6)	2107 (5)	6.9 (0.1)
C(8)	−1293 (14)	−2413 (6)	1882 (7)	8.3 (0.2)
C(9)	−2488 (16)	−2888 (6)	2930 (10)	8.7 (0.2)
C(10)	−1760 (15)	−2585 (6)	4215 (8)	8.4 (0.2)
C(11)	116 (11)	−1774 (6)	4461 (5)	6.8 (0.1)

indeed due to different rotamer populations comes from the variable-temperature spectra for *N*-benzoylglycine methyl ester seen in Figure 5. In CH_3CN the relative intensities of the 1756- and 1742-cm^{-1} peaks vary with temperature, with the rotamer giving rise to the 1756-cm^{-1} feature being the most stable. By analogy with α-substituted methyl and ethyl acetates,[19,20] the 1756 cm^{-1} band might be assigned to the trans rotamer, but the increased complexity of the *N*-acylglycine over the simple α-substituted esters renders firm assignment impossible. The important finding remains, however, that there are two rotamers present in CH_3CN solution which may be identified with those found in the crystalline phase; while in non-H-bonding solvents a third form, the C_5 conformer, predominates. The $\nu_{C=O}$ of the C_5 form coincides with $\nu_{C=O}$ from one of the other rotamers. When, as in *N*-methyl-*N*-acylglycine esters, no C_5 form is possible the spectra in CH_3CN and CCl_4 are essentially the same (Figure 4).

Crystallographic Results for Thiol Esters. The final coordinates for *N*-(β-phenylpropionyl)glycine ethyl thiol ester and *N*-benzoylglycine ethyl thiol ester are given in Tables III and IV and their structures are shown in Figure 6. Bond distances, bond angles, pertinent torsional angles, mean plane calculations, and hydrogen-bonding geometries are given in Tables V and VI for the *N*-(β-phenylpropionyl)- and *N*-benzoylglycine thiol esters, respectively.

Both compounds crystallize in the B conformation, i.e., with small N–C–C–S torsional angles leading to an N···S interaction

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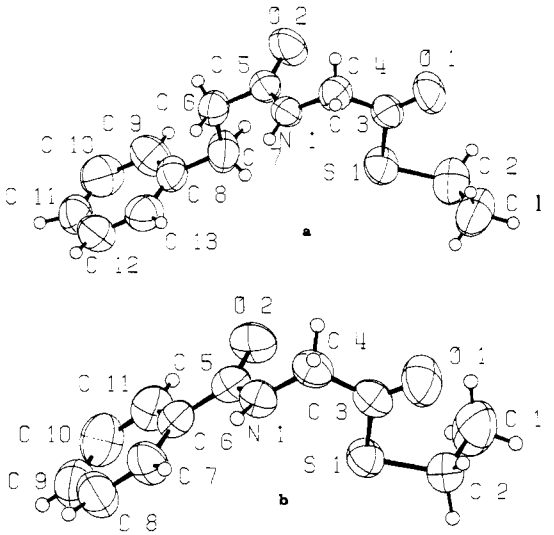

Figure 6. Structures of *N*-(β-phenylpropionyl)glycine ethyl thiol ester (a) and *N*-benzoylglycine ethyl thiol ester (b). Both drawings were made with the ORTEP-II program,[31] and the ellipsoids enclose 50% probability.

Table V. Molecular Geometry for *N*-(β-Phenylpropionyl)glycine Ethyl Thiol Ester

Bond Distances (Å)

S–C(2)	1.809 (4)	C(6)–C(7)	1.509 (5)
S–C(3)	1.764 (4)	C(7)–C(8)	1.507 (5)
O(1)–C(3)	1.197 (4)	C(8)–C(9)	1.357 (6)
O(2)–C(5)	1.232 (4)	C(9)–C(10)	1.392 (6)
N–C(4)	1.427 (4)	C(10)–C(11)	1.337 (7)
N–C(5)	1.317 (4)	C(11)–C(12)	1.362 (6)
C(1)–C(2)	1.463 (7)	C(12)–C(13)	1.384 (6)
C(3)–C(4)	1.492 (4)	C(13)–C(8)	1.382 (6)
C(5)–C(6)	1.495 (4)		

Bond Angles (deg)

C(2)–S–C(3)	100.1 (2)	C(5)–C(6)–C(7)	111.6 (3)
C(4)–N–C(5)	122.0 (3)	C(6)–C(7)–C(8)	111.9 (3)
S–C(2)–C(1)	114.3 (3)	C(7)–C(8)–C(9)	122.0 (3)
S–C(3)–O(1)	122.9 (3)	C(7)–C(8)–C(13)	120.8 (3)
S–C(3)–C(4)	115.2 (2)	C(9)–C(8)–C(13)	117.2 (4)
O(1)–C(3)–C(4)	121.8 (3)	C(8)–C(9)–C(10)	121.0 (4)
N–C(4)–C(3)	116.9 (3)	C(9)–C(10)–C(11)	121.4 (4)
O(2)–C(5)–N	119.9 (3)	C(10)–C(11)–C(12)	118.8 (4)
O(2)–C(5)–C(6)	123.0 (3)	C(11)–C(12)–C(13)	120.5 (4)
N–C(5)–C(6)	117.1 (3)	C(12)–C(13)–C(8)	121.1 (4)

Selected Torsional Angles (deg)

S–C(3)–C(4)–N	−15.4 (4)
O(1)–C(3)–C(4)–N	168.8 (3)
C(1)–C(2)–S–C(3)	79.1 (4)
C(2)–S–C(3)–O(1)	5.5 (4)
C(3)–C(4)–N–C(5)	−84.8 (4)
C(5)–C(6)–C(7)–C(8)	−179.9 (3)
C(6)–C(7)–C(8)–C(9)	95.9 (4)

Deviations (Å) of Atoms from Selected Least-Squares Planes

Plane I: S[a] 0.000 (1), O(1)[a] −0.007 (3), C(3)[a] 0.024 (3), C(4)[a] 0.008 (4)
Plane II: O(2)[a] 0.003 (3), N[a] 0.004 (3), C(4)[a] −0.004 (3), C(5)[a] −0.009 (3)
Plane III: C(8)[a] 0.001 (3), C(9)[a] 0.005 (4), C(10)[a] −0.010 (5), C(11)[a] 0.004 (4), C(12)[a] 0.002 (5), C(13)[a] −0.005 (4), C(7) −0.012 (4)

Hydrogen Bonding Geometry

distances, Å		angles, deg	
N–H(1)	0.85 (3)	N–H(1)···O(2)′	165 (3)
N···O(2)′	2.774 (3)	C(5)–O(2)···H(1)′	139 (1)
H(1)···O(2)′	1.94 (3)		

[a] Atoms used to define plane.

and with the amide and ester planes nearly orthogonal. Here, the N–C(4)–C(3)–S (ψ′) and C(5)–N–C(4)–C(3) (φ′) torsional angles are −15.4° and −84.8° for PPG and 5.4° and −88.7° for BG. In the other four structures (three dithio esters[4,5] and one dioxygen ester[15]) where analogous conformations have been found,

Table VI. Molecular Geometry for *N*-Benzoylglycine Ethyl Thiol Ester

Bond Distances (Å)			
S-C(2)	1.802 (5)	C(5)-C(6)	1.487 (6)
S-C(3)	1.722 (5)	C(6)-C(7)	1.387 (6)
O(1)-C(3)	1.209 (7)	C(7)-C(8)	1.375 (10)
O(2)-C(5)	1.242 (5)	C(8)-C(9)	1.371 (12)
N-C(4)	1.430 (6)	C(9)-C(10)	1.356 (12)
N-C(5)	1.332 (5)	C(10)-C(11)	1.370 (10)
C(1)-C(2)	1.489 (9)	C(11)-C(6)	1.383 (7)
C(3)-C(4)	1.521 (7)		

Bond Angles (deg)			
C(2)-S-C(3)	99.8 (2)	N-C(5)-C(6)	118.9 (4)
C(4)-N-C(5)	123.1 (4)	C(5)-C(6)-C(7)	122.9 (4)
S-C(2)-C(1)	114.7 (5)	C(5)-C(6)-C(11)	118.6 (4)
S-C(3)-O(1)	123.7 (4)	C(7)-C(6)-C(11)	118.5 (5)
S-C(3)-C(4)	116.4 (3)	C(6)-C(7)-C(8)	119.4 (5)
O(1)-C(3)-C(4)	119.9 (5)	C(7)-C(8)-C(9)	121.2 (7)
N-C(4)-C(3)	113.9 (4)	C(8)-C(9)-C(10)	119.7 (8)
O(2)-C(5)-N	119.5 (4)	C(9)-C(10)-C(11)	120.0 (7)
O(2)-C(5)-C(6)	121.6 (4)	C(10)-C(11)-C(6)	121.2 (6)

Selected Torsional Angles (deg)	
S-C(3)-C(4)-N	5.4 (5)
O(1)-C(3)-C(4)-N	-173.3 (5)
C(1)-C(2)-S-C(3)	-79.0 (5)
C(2)-S-C(3)-O(1)	-5.7 (5)
C(3)-C(4)-N-C(5)	-88.7 (5)
N(1)-C(5)-C(6)-C(7)	5.4 (7)

Deviations (Å) of Atoms from Selected Least-Squares Planes

Plane I: S[a] 0.000 (2), O(1)[a] 0.003 (7), C(3)[a] -0.007 (4), C(4)[a] 0.002 (5)

Plane II: O(2) -0.001 (4), N(1)[a] -0.001 (4), C(5)[a] 0.006 (5), C(6)[a] -0.002 (4)

Plane III: C(6)[a] 0.001 (4), C(7)[a] -0.002 (6), C(8)[a] -0.003 (7), C(9)[a] 0.011 (7), C(10)[a] -0.007 (8), C(11)[a] 0.000 (6), O(2) -0.033 (4), C(5) 0.040 (5)

Hydrogen Bond Geometry			
distances, Å		angles, deg	
N-H(1)	0.86 (6)	N-H(1)···O(2)′	149 (6)
N···O(2)′	2.794 (4)	C(5)-O(2)···H(1)′	163 (2)
H(1)···O(2)′	2.03 (6)		

[a] Atoms used to define plane.

the range of ψ' angles is +9.5° to -22.2° and of ϕ' angles is -68.9° to -97.1°. The N···S interaction in PPG, with a nonbonded contact distance of 2.930 (2) Å, must be appreciably weaker than in BG, where the N···S distance is 2.879 (4) Å, or in the dithio esters,[4,5] where the N···S(1) distances are 2.846 to 2.891 Å. This may be a contributing factor to the difference in C(3)-S bond lengths (1.764 (4) in PPG vs. 1.722 (5) Å in BG). The longer bond appears to be more characteristic of thiol esters. In seven other thiol ester structures[21-26] found by a search of the Cambridge Crystallographic Database the corresponding C-S bond lengths ranged from 1.742 to 1.779 Å, with average 1.758 Å (Table VII, supplementary material). The difference in C(3)-S bond lengths in the present structures is accompanied by significant differences in the C(3)-C(4) bond lengths (0.029 Å) and in the C(3)-C(4)-N bond angles (3.0°). More structural data on the *N*-acyl thiol esters are needed to properly assess these differences.

The S-C(2) bond lengths in PPG and BG agree satisfactorily with those found in 12 other dithio[4,5,22,27] and thiol[21-26] esters, where the values range from 1.789 to 1.847 Å with average 1.809 Å (Table VII). In four of these cases[22-25] a HOMO···LUMO interaction of the type proposed by Rosenfield, Parthasarathy, and Dunitz[28] and discussed by Varughese et al.[5] is possible between a thiol sulfur (LUMO) and a (nucleophilic) carbonyl oxygen (HOMO), with lone-pair electron density unambiguously directed toward the sulfur atom. In three other cases,[21-23] similar interactions appear possible, two between a thiol sulfur (LUMO) and a thione sulfur (HOMO) and one between a thiol sulfur and an ether oxygen. In the last case the ether substituent makes a C-C-O-C torsional angle of 120°, permitting a lone pair of electrons to point toward the thiol sulfur atom. There appears to be no systematic trend in the S-C(2) bond lengths for this group of compounds as a function of whether or not a HOMO···LUMO interaction is possible.

The inequality of the exocyclic bond angles that is observed in the BG structure, where C(5)-C(6)-C(7) is 4.3° larger than C(5)-C(6)-C(11), is very probably due to repulsion between the amide proton and the hydrogen at C(7). Similar effects occur in *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester[4] and the *p*-chloro and unsubstituted *N*-benzoyl analogues[5] as well as in various cinnamates[29] (where an ethenylic hydrogen is involved instead of the amide proton). In the PPG structure the amide proton is no longer close to the benzene ring, and the exocyclic angles C(7)-C(8)-C(9) and C(7)-C(8)-C(13) do not show such a significant difference.

In the BG structure, both the thiol ester and the amide group are planar within experimental error, but in the PPG crystal structure the thiol ester group is significantly nonplanar (Table V) and the amide group shows small [0.009 (3) Å] deviations from planarity. The dihedral angle between the mean planes of the aromatic ring and the amide group in PPG is 9.4°, with the intervening bridge essentially perpendicular to both. In BG, the aromatic ring is rotated by 5.8° out of the plane of the amide group.

In contrast to the C(3)-S-C(2)-C(1) torsional angles in the other *N*-acyl ethyl thiol and dithio esters, which are either about 180° or -75° to -89°, in PPG the C(3)-S-C(2)-C(1) torsional angle is +79.1°. Thus, the three staggered conformations are all possible. The absolute values of the torsional angles for the two gauche forms are substantially increased from 60°, primarily to avoid too-close contacts of the methyl hydrogens and the carbonyl oxygen (or thione sulfur). The particular conformation adopted is probably governed by packing requirements.

In both present structures, as in the previous dithio esters, the amide proton is involved in an intermolecular hydrogen bond to the amide oxygen. In PPG the strings of hydrogen-bonded molecules extend along *a*, while in BG the chains of molecules are parallel to *c*. Details of the geometry are given in Tables V and VI.

FT-IR Data for Thiol Esters. FT-IR data were obtained for several *N*-acylglycine ethyl thiol esters, namely, *N*-benzoyl-, *N*-(β-phenylpropionyl)-, *N*-acetyl- and *N*-carbobenzoxyglycine thiol esters. For the *N*-acetyl and *N*-(β-phenylpropionyl) thiol esters in the carbonyl region of the spectra, the amide and ester carbonyl features were partially superimposed. However, for the *N*-benzoyl- and *N*-carbobenzoxyglycine ethyl thiol esters the amide and ester carbonyl peaks are well separated and similar results were obtained with both. In the ν_{NH} stretching spectral region all four thiol esters gave similar results. Only the results obtained for *N*-benzoylglycine ethyl thiol ester will be reported and discussed in detail.

Figure 2b shows the 3300-3500-cm^{-1} region of the FT-IR spectra of *N*-benzoylglycine ethyl thiol ester in CCl_4 and CH_3CN solution. In both solvents the peak positions are very similar to those in Figure 2a for *N*-benzoylglycine methyl ester. Thus, in CCl_4, the thiol ester is also found predominantly in a C_5 conformation; however, for the thiol ester the shoulder at 3465 cm^{-1} due to non-C_5 conformer(s) is more intense. This indicates that the C_5 conformer is relatively less stable than in the case of the

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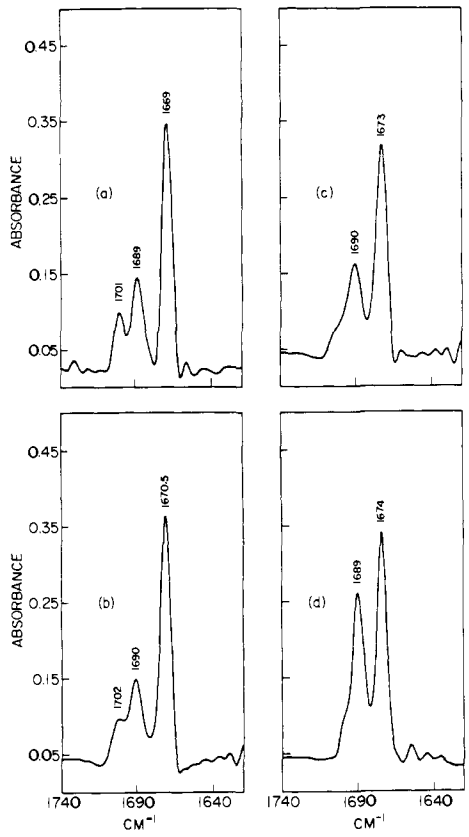

Figure 7. C=O stretching region (deconvoluted) of FT-IR spectra of *N*-benzoylglycine ethyl thiol ester (1.3×10^{-3} M) in mixed solvents of CH_3CN and CCl_4 (amount of CH_3CN: a, 100%; b, 20%; c, 5%; d, 0%). The parameters for deconvolution are given in Figure 3.

methyl dioxygen ester. In CH_3CN solution the shape and position of ν_{NH} indicates that the NH proton is H bonded exclusively to solvent.

Figure 7 shows the carbonyl region of the FT-IR spectrum of *N*-benzoylglycine ethyl thiol ester dissolved in CH_3CN, CCl_4, and mixtures of the two solvents. As in the case of the methyl dioxygen ester (above) an amide feature is present at approximately 1670 cm^{-1}. However, for the thiol ester the two carbonyl features are found at lower wavenumbers (in the 1690–1705-cm^{-1} region) than they are in the ester spectrum.[30,31] In 100% CCl_4 solution, by comparison with Figure 2b, it is possible to assign the more intense feature at 1689 cm^{-1} to the C_5 conformer and the less intense shoulder at 1701 cm^{-1} to a non-H-bonded conformer. On going through the series of solvents from 100% CCl_4 to 100% CH_3CN there is a large change in the relative intensities of the two carbonyl features. This change with solvent is comparable to that observed for *N*-benzoylglycine methyl dioxygen ester (Figure 3). In 100% CH_3CN the two features are situated at 1689 and 1701 cm^{-1}. The 1689-cm^{-1} feature is not due to residual C_5 conformer, since from the spectra obtained in CH_3CN in the 3300–3500-cm^{-1} region it can be seen that the solvent is interacting with all the N–H protons (Figure 2b). Therefore, as in the case of *N*-acylglycine methyl esters, two major conformational populations of *N*-acylglycine ethyl thiol esters exist in CH_3CN solution and a third conformer, C_5, is also present in CCl_4 solution. Also, the variation in $\nu_{C=O}$ frequencies for the various conformers must again be due to differences in the ϕ',ψ' angles of the various conformers.

(30) Thiol ester carbonyl stretching frequencies normally occur at approximately 1700 cm^{-1} whereas for ester carbonyl the frequencies are normally at approximately 1750 cm^{-1}. See: El-Aasar, A. M. M.; Nash, C. P.; Ingraham, L. L. *Biochemistry* **1982**, *21*, 1972–1976.

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Table VIII. Different Conformations of the Compounds Studied and the Environments in Which They Occur

N-acyl-glycine	conformer			
	trans[a] (A)	gauche[a]	cis[a] (B)	C_5
ester	crystal, CH_3CN (CCl_4)	crystal, CH_3CN (CCl_4)	generally ND	CCl_4
thiol ester	*b*	*b*	crystal, CH_3CN CCl_4	CCl_4
dithio ester	crystal, CH_3CN CCl_4	ND	crystal, CH_3CN CCl_4	CCl_4
dithio-papain	denatured (pH <3)	ND	native (pH >3) denatured (pH <3)	ND

[a] With respect to –O– or –S– atom, ND = not detected. [b] One unknown conformer in solution.

The relative peak intensities in the carbonyl region of *N*-carbobenzoxyglycine ethyl thiol ester in CH_3CN did not vary in the temperature range 12–40 °C (data not shown), indicating that there is little enthalpy difference between the two conformers present or, improbably, that they are separated by a large energy barrier.

One of the $\nu_{C=O}$ peaks is assigned to a B-type conformer, since this is a proven conformational preference. However, it is not known which of the two peaks corresponds to the B rotamer, nor is the nature of the other thiol ester rotamer known with confidence.

Conclusions

The results of this conformational investigation of *N*-acylglycine esters and thiol esters have been combined with the results of previous studies on *N*-acylglycine dithio esters and dithiopapains in Table VIII and Figure 1.

The B conformation is not generally found for esters but is found for thiol esters, dithio esters, and (dithioacyl)papains. Thus this conformation is strongly favored by the presence of the thiol sulfur atom. From the X-ray structures of the thiol esters described here and of dithio esters,[4,5] it can be seen that in the B conformation the amide N and the thiol S are in close contact. The steric repulsion involved in this contact is offset by a favorable interaction which involves the nitrogen lone pair in a HOMO together with a LUMO (and/or a d orbital) based on the sulfur atom.

From the point of view of our work on enzyme–substrate intermediates, an important outcome of the present study is that we may begin to extend the conclusions reached for (dithioacyl)papains to the "natural" (thiolacyl)papains. This follows from the fact that the characteristic B-type conformers found in dithio esters and (dithioacyl)papains[7] have now been identified in thiol esters. Thus, we are able to assume, with some confidence, that the B-type conformation is found in (thiolacyl)papains.

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Registry No. *N*-(β-Phenylpropionyl)glycine ethyl thio ester, 92817-02-2; *N*-benzoylglycine ethyl thio ester, 2979-55-7; nitrogen, 7727-37-9; sulfur, 7704-34-9.

Supplementary Material Available: Fractional coordinates for the hydrogen atoms, thermal vibration parameters in the form of U_{ij} values for non-hydrogen atoms and B values for hydrogen atoms, as well as tables of observed and calculated structure factors for *N*-(β-phenylpropionyl)glycine ethyl thiol ester and *N*-benzoylglycine ethyl thiol ester; in addition, ϕ',ψ' torsional angles are listed for 31 *N*-acyl amino acid esters, and S–C bond lengths are given for 12 other thiol and dithio esters (20 pages). Ordering information is given on any current masthead page.