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# FTIR studies of hydrogen bonding between $\alpha$ , $\beta$ -unsaturated esters and alcohols<sup>1</sup>

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## Abstract

The enthalpy (and entropy) of hydrogen bond formation has been measured between the ester carbonyl groups of the two  $\alpha$ ,  $\beta$ -unsaturated esters thienylacryloyl (TAOME) and 5-methylthienylacryloyl (5MeTAOME) methyl ester and the hydrogen bond donors ethanol, phenol and 3,5-dichlorophenol in  $\text{CCl}_4$ . For the esters, the hydrogen bonding strengths were measured by quantitating the amount of bound and unbound donor, using the O–H stretching region, as a function of temperature and applying the van't Hoff equation. The decrease in  $\nu_{\text{C=O}}$  of the ester carbonyl group upon hydrogen bond formation ( $\Delta\nu_{\text{C=O}}$ ) has also been measured and correlated with the enthalpy of hydrogen bond formation. A linear correlation is observed between the enthalpy of hydrogen bond formation  $-\Delta H$  and  $\Delta\nu_{\text{C=O}}$ , with  $-\Delta H = 1.36\Delta\nu_{\text{C=O}} - 16.1$ , where  $\Delta H$  is measured in  $\text{kJ mol}^{-1}$  and  $\Delta\nu$  in  $\text{cm}^{-1}$ . Comparison with data for other carbonyl acceptor compounds indicates that the carbonyl group of the above  $\alpha$ ,  $\beta$ -unsaturated esters is more readily polarized than the carbonyl group of saturated esters or ketones. The quantitative relationship between  $-\Delta H$  and  $\Delta\nu_{\text{C=O}}$  derived here has been used to determine the change in the enthalpy of hydrogen bond formation between substrate and enzyme groups in a series of acylserine proteases.

**Keywords:** Hydrogen bonding; Carbonyl; Unsaturated ester; Alcohol

## 1. Introduction

The vibrational spectrum of an enzyme-bound substrate contains detailed information concerning the structure of the bound substrate [1,2].

Potentially, the vibrational data can yield detailed quantitative information, such as the length of individual substrate bonds and the energy of individual enzyme–substrate interactions. In order to translate vibrational frequency changes into interaction energies, detailed studies on simple model compounds are required. The present study sets out to establish a relationship between carbonyl shifts and hydrogen bonding strengths that can be used to measure the strengths of hydrogen bonding interactions in the active sites of enzymes.

Both Raman and FTIR spectroscopies have

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been used to probe the environment of substrate carbonyl groups in enzyme active sites. These techniques have been used to detect polarization of substrate carbonyl groups by the enzyme's active site in a variety of systems, including triose phosphate isomerase [3,4], citrate synthase [5], phospholipase A<sub>2</sub> [6], chloramphenicol acetyltransferase [7], lactate dehydrogenase [8], and ketosteroid isomerase [9]. In addition, several groups have used substrates based on  $\alpha, \beta$ -unsaturated acryloyl esters to probe the active sites of serine and cysteine proteases [1,10–14]. Studies in our laboratory have shown that for a series of acylserine proteases a linear correlation exists between  $\nu_{C=O}$ , the substrate's carbonyl group frequency, and  $\log k_3$ , where  $k_3$  is the deacylation rate constant [2,15].

It is of significant interest to determine the energy of interaction between the substrate's carbonyl group and an enzyme residue. If it is assumed that the substrate polarization results from hydrogen bonding with one or more enzyme donors, then the hydrogen bond enthalpy of this interaction can be determined by performing hydrogen bonding studies on simple model compounds. The model studies are performed using a compound that resembles the enzyme–substrate complex as closely as possible. In addition, it is of particular importance that the model compound is soluble in a non-polar solvent (CCl<sub>4</sub>) so that the solvent does not compete for hydrogen bond formation between the model compound's carbonyl group (acceptor) and the hydrogen bond donor. In this paper we describe hydrogen bonding studies on two esters, 5-methylthienylacryloyl methyl ester and thienylacryloyl methyl ester, shown in Scheme 1. These compounds are models of the acylserine proteases used to generate the linear correlation between  $\nu_{C=O}$  and  $\log k_3$  described above [2,15].

## 2. Experimental methods

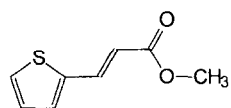
*trans*-5-Methylthienylacrylic acid was synthesized from malonic acid and 5-methyl-2-thiophenecarboxaldehyde (Aldrich) as described previously [16]. The imidazole derivative of the acid was generated using 1,1'-carbonyldiimidazole in dry THF. *trans*-5-Methylthienylacryloyl methyl ester (5MeTAOMe;  $\lambda_{\max}$  317 nm, acetonitrile) was synthesized by incubation of *trans*-5-methylthienylacryloylimidazole in methanol for 6 h followed by HPLC purification on a reverse phase column using a water–acetonitrile gradient [17]. Similarly, 1-<sup>13</sup>C=O *trans*-5-methylthienylacryloyl methyl ester (5MeTA-1-<sup>13</sup>C-OMe) was synthesized using 1,3-<sup>13</sup>C-malonic acid, and *trans*-5-methylthienylacryloyl-2-d methyl ester (–CH=CD–C(=O)–; 5MeTA-2-d-OMe) was synthesized using malonic-d<sub>2</sub> acid-d<sub>2</sub> in the initial condensation with 5-methyl-2-thiophenecarboxaldehyde. *trans*-Thienylacryloyl methyl ester (TAOMe;  $\lambda_{\max}$  306 nm, acetonitrile) was synthesized from 2-thiophenecarboxaldehyde as described above.

Products were analysed by exact mass analysis and NMR spectroscopy. NMR data were obtained in CD<sub>3</sub>CN or CDCl<sub>3</sub>. Data are reported as chemical shift in ppm, multiplicity, integration and coupling in Hz.

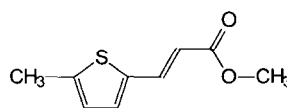
H-NMR: 5MeTAOMe (CDCl<sub>3</sub>),  $\delta$  2.51 (d, 3, 1.0); 3.79 (s, 3); 6.11 (d, 1, 15.4); 6.71 (dd, 1, 1.0, 3.5); 7.06 (d, 1, 3.5); 7.71 (d, 1, 15.4). TAOMe (CD<sub>3</sub>CN),  $\delta$  3.75 (s, 3); 6.28 (d, 1, 15.7); 7.11 (dd, 1, 3.6, 5.1); 7.40 (d, 1, 3.6); 7.54 (d, 1, 5.1); 7.81 (d, 1, 15.7).

For 5MeTA-2-d-OMe, <sup>1</sup>H-NMR indicated that deuterium substitution at C<sub>2</sub> was greater than 85%.

The enthalpy and entropy of hydrogen bond formation were determined in CCl<sub>4</sub> in a manner



Thienylacryloyl methyl ester



5-Methylthienylacryloyl methyl ester

Scheme 1

similar to that described by Thijs and Zeegers-Huyskens [18]. Hydrogen bond donors were ethanol ( $pK$  15.5), phenol ( $pK$  9.99) and 3,5-dichlorophenol ( $pK$  8.18), chosen to represent a large range of  $pK$  values in order to vary the hydrogen bond strength as much as possible.

To determine  $-\Delta H$  and  $\Delta S$ , the concentration of donor was maintained at 6 mM for ethanol and 3 mM for phenol and 3,5-dichlorophenol, to avoid self-association. The concentration of acceptor was varied in the range 0–200 mM (TAOMe, ethanol), 0–89 mM (TAOMe, phenol), 0–51 mM (TAOMe, 3,5-dichlorophenol), 0–200 mM (5MeTAOMe, ethanol) and 0–30.3 mM (5MeTAOMe, 3,5-dichlorophenol). FTIR spectra were obtained using a Digilab FTS-60 spectrometer equipped with a DTGS detector. The sample cell had KBr windows and a path length of 0.5 mm. Temperature was controlled with a water bath using cryo-solvent (30% ethylene glycol) circulating through the cell holder in the FTIR sample compartment. The temperature of the cell contents was measured using a thermocouple inserted into the cell through one of the cell's Teflon plugs.

At the beginning of each temperature run, the cell was placed in the cell holder with the temperature bath set at 263 K. At thermal equilibrium, the temperature in the cell was  $275 \pm 1$  K. After acquiring the first FTIR spectrum (64 scans), the bath temperature was changed to 273 K and a second FTIR spectrum was acquired after a 30 min waiting period. This process was repeated in 10 K increments up to a bath temperature of 323 K. Owing to the temperature gradient between the

bath and the cell, the actual cell temperature measured at each point was ( $\pm 1$  K) 275, 280, 287, 295, 303, 310 and 318 K. For each donor–acceptor pair, five concentrations of acceptor were used over the concentration ranges listed above. After subtracting a solvent spectrum, recorded at the appropriate temperature, the concentration of free donor ( $[\text{donor}]_{\text{free}}$ ) was determined by integrating  $\nu_{\text{O}-\text{H}}$  for the free donor ( $\nu_{\text{O}-\text{H}}$  free; ethanol 3632 and 3626  $\text{cm}^{-1}$ ; phenol 3609  $\text{cm}^{-1}$ ; 3,5-dichlorophenol 3599  $\text{cm}^{-1}$ ). Using  $[\text{donor}]_{\text{free}}$ , both  $[\text{donor}]_{\text{bound}}$  and  $[\text{acceptor}]_{\text{free}}$  were calculated and  $K$ , the equilibrium constant for hydrogen bond formation, was determined by plotting  $[\text{donor}]_{\text{bound}}$  against  $[\text{acceptor}]_{\text{free}} \times [\text{donor}]_{\text{free}}$  ( $K = \frac{[\text{donor}]_{\text{bound}}}{([\text{acceptor}]_{\text{free}} \times [\text{donor}]_{\text{free}})}$ ).  $K$  was determined at every temperature and  $-\Delta H$  and  $\Delta S$  were obtained using the Van't Hoff equation,  $\ln K = -\Delta H/RT + \Delta S/R$ , by plotting  $\ln K$  vs.  $1/T$ .

$\Delta\nu_{\text{C}=\text{O}}$ , the decrease in carbonyl frequency upon hydrogen bond formation, was determined either by adding an excess of donor (0.1–0.2 M) such that  $\approx 50\%$  of the acceptor was complexed or by interactively subtracting a FTIR spectrum of free acceptor from that of a solution of acceptor and donor in which only a small fraction of the acceptor was complexed. Using the latter interactive subtraction method, only a small amount of donor needed to be added, therefore ensuring that the carbonyl groups only had a single hydrogen bond donor.

Band fitting was performed with SPECTRACALC (Galactic Industries, NH).

Table 1

The values of  $\Delta\nu_{\text{C}=\text{O}}$ ,  $-\Delta H$  and  $\Delta S$  for each donor–acceptor hydrogen bonding pair

	$\Delta\nu_{\text{C}=\text{O}}/\text{cm}^{-1}$	$-\Delta H/(\text{kJ mol}^{-1})$	$\Delta S/(\text{J K}^{-1}\text{mol}^{-1})$
TAOMe + ethanol	19	9.53	–26.02
5MeTAOMe + ethanol	21	10.34	–26.95
TAOMe + phenol	25	20.52	–50.44
TAOMe + 3,5-diClphenol	29, 26	25.56	–54.12
5MeTAOMe + 3,5-diClphenol	33	26.49	–56.19
5MeTA-2-d-OMe + methanol	18		
5MeTA-2-2-OMe + ethanol	17		
5MeTA-2-d-OMe + phenol	27		
5MeTA-2-d-OMe + 3,5-diClphenol	33		

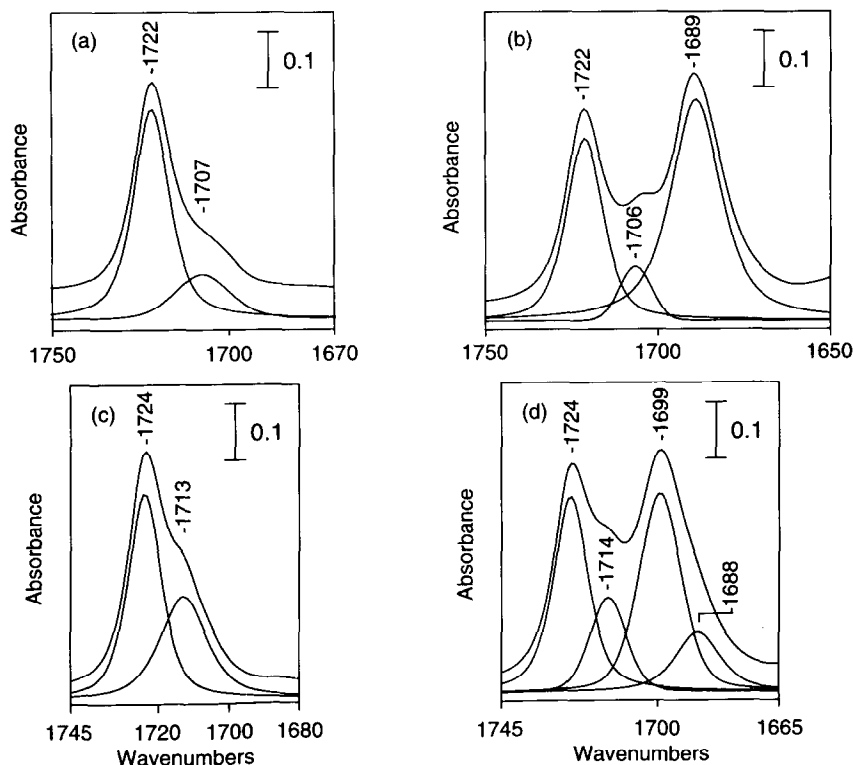


Fig. 1. FTIR spectra of uncomplexed and hydrogen-bonded 5MeTAOMe and TAOMe in  $\text{CCl}_4$ . Spectra were obtained in a 0.15 mm path length cell fitted with KBr windows. The solvent spectrum has been subtracted from each spectrum. (a) 10 mM 5MeTAOMe; (b) 20 mM 5MeTAOMe + 0.11 M 3,5-dichlorophenol; (c) 10.4 mM TAOMe; (d) 22 mM TAOMe + 0.11 M phenol.

### 3. Results and discussion

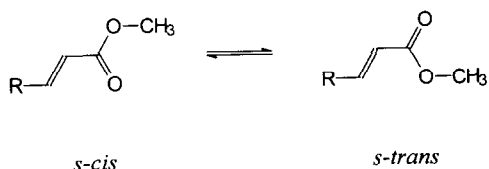
#### 3.1. Determination of $-\Delta H$ and $\Delta S$

The values determined for  $-\Delta H$  and  $\Delta S$  for each donor–acceptor pair are given in Table 1. As expected,  $-\Delta H$  increases as the acidity of the hydrogen bond donor increases. The two esters used, TAOMe and 5MeTAOMe, have similar hydrogen bonding acceptor propensities, as shown by the similarity of  $-\Delta H$  determined for the two esters with ethanol ( $-9.5$  and  $-10.3$   $\text{kJ mol}^{-1}$ , respectively) and 3,5-dichlorophenol ( $-25.6$  and  $-26.5$   $\text{kJ mol}^{-1}$ , respectively).

#### 3.2. Analysis of ester carbonyl profiles

In order to estimate  $\Delta\nu_{\text{C=O}}$  it is important to understand the carbonyl profiles for both free

and complexed esters. The carbonyl profile for 5MeTAOMe in  $\text{CCl}_4$  and in the absence of donor is characterized by a major band at  $1722\text{ cm}^{-1}$  with a shoulder at  $1707\text{ cm}^{-1}$  (Fig. 1(a)). Similarly, the carbonyl profile for TAOMe is characterized by a band at  $1724\text{ cm}^{-1}$  with a shoulder at  $1713\text{ cm}^{-1}$  (Fig. 1(c)). The basis for interpreting the carbonyl profiles is in the quantum mechanical and vibrational spectroscopic work of Dulce G. Faria et al. on a number of  $\alpha, \beta$ -unsaturated esters, such as methyl cinnamate [19], methyl acrylate [20], and methyl *trans*-crotonate [21]. In the present context, the key finding of Dulce G. Faria et al. is that  $\alpha, \beta$ -unsaturated esters exist in two conformational populations about the  $\text{C}=\text{C}-\text{C}(=\text{O})-$  single bond, namely the *s-cis* and *s-trans* shown in Scheme 2. The *s-cis* is the lower energy form and the carbonyl stretching frequency for that rotamer occurs at  $\approx 10\text{ cm}^{-1}$  lower in frequency compared with the



Scheme 2

*s-trans* form. With this information we assign the peak near  $1720\text{ cm}^{-1}$  in Figs. 1(a) and 1(c) to the *s-trans* rotamer of 5-MeTAOME and the shoulder near  $1707\text{ cm}^{-1}$  to *s-cis*. In keeping with the *s-cis* being the more stable form, the shoulder increases in relative intensity as temperature is lowered (data not shown). The overall low intensity of the *s-cis* band is explained by hypothesizing that the carbonyl of this isomer undergoes a smaller change

in dipole moment with the carbonyl stretching vibration and thus has lower intrinsic infrared absorbance compared with the carbonyl of *s-trans*.

Isotopic substitution was used to confirm the above interpretation of the carbonyl profile and to provide additional data for the hydrogen bonding studies. The analog 5MeTAOME deuterated at the  $\text{C}_2$  position (5MeTA-2-d-OMe) was analysed. For this ester  $\nu_{\text{C}=\text{O}}$  is a broad, apparently single band at  $1717\text{ cm}^{-1}$  (Fig. 2). However, curve fitting of this band required two components to achieve a good fit, with peak maxima at  $1719$  and  $1711\text{ cm}^{-1}$  (Fig. 2). These values are close to those obtained for the unlabelled derivative, the band at  $1719\text{ cm}^{-1}$  being assigned to the *s-trans* conformer and the band at  $1711\text{ cm}^{-1}$  being assigned to the *s-cis* conformer.

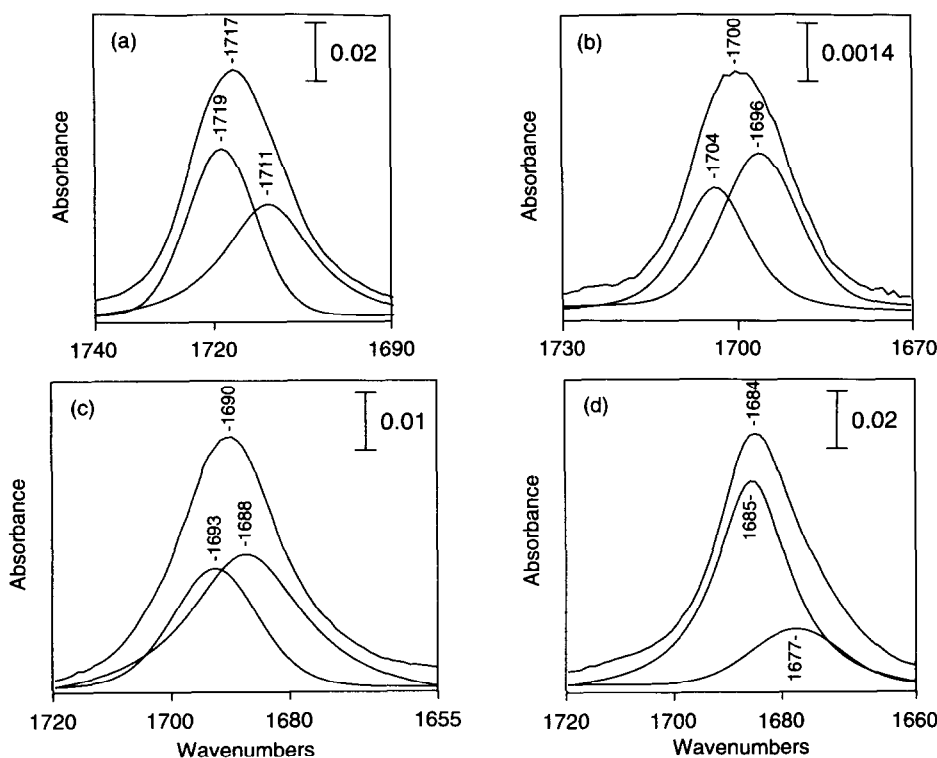


Fig. 2. FTIR spectra of uncomplexed and hydrogen-bonded 5MeTA-2-d-OMe in  $\text{CCl}_4$ . Spectra were obtained in a 0.05 mm path length cell fitted with KBr windows. The solvent spectrum has been subtracted from each spectrum. (a) 10 mM 5MeTA-2-d-OMe; (b) 10 mM 5MeTA-2-d-OMe + 0.1 M ethanol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.827; (c) 10 mM 5MeTA-2-d-OMe + 0.1 M phenol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.467; (d) 10 mM 5MeTA-2-d-OMe + 0.1 M 3,5-dichlorophenol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.182.

Table 2

 $\nu_{C=O}$  values for "free" (non-hydrogen bonded) and hydrogen bonded carbonyls

		Free	Ethanol	Phenol	3,5-Dichlorophenol
TAOMe	<i>s-trans</i>	1724	1705	1699	1695
	<i>s-cis</i>	1713		1688	1687
5MeTAOMe		1722	1701		1689
5MeTA-2-d-OMe		1717	1700	1690	1684
	<i>s-trans</i>	1719	1704	1693	1685
	<i>s-cis</i>	1711	1696	1688	1677

### 3.3. Determination of $\Delta\nu_{C=O}$

$\Delta\nu_{C=O}$  was determined by adding sufficient hydrogen bond donor to complex 50% of the ester (calculated from  $K$ ). Data are shown in Fig. 1 for 5MeTAOMe with 3,5-dichlorophenol and for TAOMe with phenol. Upon addition of 0.11 M 3,5-dichlorophenol to 20 mM 5MeTAOMe, a band is observed at  $1689\text{ cm}^{-1}$ . The interpretation is that the  $1689\text{ cm}^{-1}$  band represents the

hydrogen bonded form of the species giving rise to the band at  $1722\text{ cm}^{-1}$  in the spectrum of the uncomplexed ester, giving  $\Delta\nu_{C=O} = 33\text{ cm}^{-1}$  in this instance. Curve fitting studies showed that the band at  $1689\text{ cm}^{-1}$  required only one component for adequate fitting. Similarly, a single band arising from the hydrogen bonding of 5MeTAOMe with ethanol was observed at  $1701\text{ cm}^{-1}$  (data not shown), giving  $\Delta\nu_{C=O} = 21\text{ cm}^{-1}$ .

The carbonyl profile for uncomplexed TAOMe

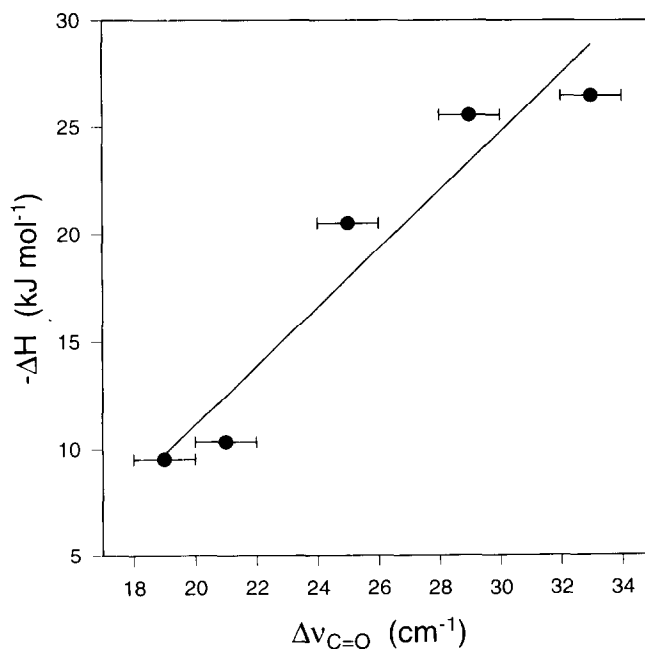


Fig. 3. Plot of  $-\Delta H$  vs.  $\Delta\nu_{C=O}$  using the data presented in Table 1. The data have been fitted by linear regression to the equation  $-\Delta H = 1.36\Delta\nu_{C=O} - 16.10$ .

Table 3

Collated data relating  $\Delta H$  and  $\Delta\nu_{C=O}$ :  $-\Delta H$  (kJ mol<sup>-1</sup>) = slope  $\Delta\nu_{C=O}$  (cm<sup>-1</sup>) + constant

Acceptor	Slope	Constant	<i>r</i>	$-\Delta H$ /(kJ mol <sup>-1</sup> ) required to shift $\nu_{C=O}$ by 20 cm <sup>-1</sup>
TAOMe and 5MeTAOMe <sup>a</sup>	1.36	-16.10	0.92	11.10
Methyl acrylate, methyl <i>trans</i> -crotonate, methyl <i>trans</i> -cinnamate <sup>b</sup>	0.46	4.53		13.17
<i>N,N</i> -Dimethylacetamide <sup>c</sup>	2.63	-42.63	0.833	9.97
Methyl acetate <sup>c</sup>	1.67	-22.95	0.999	10.45
Acetophenone <sup>c</sup>	1.28	-2.11	0.967	23.49
Benzophenone <sup>c</sup>	1.49	-4.40	0.992	25.4
Acetone <sup>c</sup>	1.96	-0.47	0.969	38.73

<sup>a</sup> This work.<sup>b</sup> Ref. [22].<sup>c</sup> Ref. [18].

is characterized by a band at 1724 cm<sup>-1</sup> with a shoulder at 1713 cm<sup>-1</sup> (Fig. 1(c)). Upon addition of 0.1 M phenol (Fig. 1(d)) a new band is observed at 1699 cm<sup>-1</sup>. Curve fitting of this band required two components to achieve a good fit, with peak maxima at 1699 and 1688 cm<sup>-1</sup>. The interpretation, consonant with the observations on methyl cinnamate [22], is that the bands at 1699 and 1688 cm<sup>-1</sup> represent the hydrogen bonded forms of the species giving rise to the carbonyl bands at 1724 and 1713 cm<sup>-1</sup>, respectively, in uncomplexed TAOMe. This gives  $\Delta\nu_{C=O}$  values of 25 and 25 cm<sup>-1</sup>, respectively. Similarly, for TAOMe with 3,5-dichlorophenol, the hydrogen bonded carbonyl profile is composed of two components, at 1695 and 1687 cm<sup>-1</sup> (data not shown), giving  $\Delta\nu_{C=O}$  of 29 and 26 cm<sup>-1</sup>, respectively. When ethanol was used as hydrogen bond donor, only a single component was required to fit the hydrogen bonded carbonyl band, with a maximum at 1705 cm<sup>-1</sup>. Assuming that the hydrogen bonded carbonyl arises from the uncomplexed species with  $\nu_{C=O}$  1724 cm<sup>-1</sup>, this gives a  $\Delta\nu_{C=O}$  of 20 cm<sup>-1</sup>.

In order to confirm the  $\Delta\nu_{C=O}$  values calculated for unlabelled 5MeTAOMe,  $\Delta\nu_{C=O}$  was also determined for 5MeTA-2-d-OMe with ethanol, phenol and 3,5-dichlorophenol. The FTIR carbonyl profiles are shown in Fig. 2. For the spectra obtained in the presence of a hydrogen bond donor (Fig. 2(b,c,d)), the spectrum of uncomplexed 5MeTA-2-d-OMe has been subtracted, leaving  $\nu_{C=O}$  arising from only the hydrogen bonded

ester carbonyls. Curve fitting has been performed, each carbonyl band requiring two components for an adequate fit. For the deuterated analog,  $\Delta\nu_{C=O}$  values calculated using peak maxima from the raw data are in good agreement with values calculated for the individual deconvoluted *s-trans* or *s-cis* features. The assignments for  $\nu_{C=O}$  "free" and hydrogen bonded are given in Table 2. It can also be seen from Table 2 that there is good agreement between the  $\Delta\nu_{C=O}$  values obtained with 5MeTA-2-d-OMe compared with those obtained with unlabelled 5MeTAOMe.

#### 3.4. Relationship between $-\Delta H$ and $\Delta\nu_{C=O}$

Fig. 3 shows a plot of  $-\Delta H$  against  $\Delta\nu_{C=O}$ . The plot is linear and fits to the equation  $-\Delta H = 1.36\Delta\nu_{C=O} - 16.10$  ( $r = 0.92$ ), where the units are kJ mol<sup>-1</sup> for  $\Delta H$  and cm<sup>-1</sup> for  $\Delta\nu_{C=O}$ . These data have been used to quantitate hydrogen bonding strengths in the active sites of chymotrypsin and subtilisin [15].

Table 3 is a compilation of some of the quantitative data that exist relating  $-\Delta H$  and  $\Delta\nu_{C=O}$ . It can be seen that significantly less energy is required to bring about a unit shift in the carbonyl frequency of  $\alpha,\beta$ -unsaturated esters compared with ketones (by a factor of 2–7), but that the values for saturated and unsaturated esters are similar. The data presented here are similar to the values reported for other  $\alpha,\beta$ -unsaturated esters by Dulce G. Faria et al. [22], demonstrating that the

substitution on the acryloyl moiety does not markedly change the inherent “stiffness” of the  $\alpha, \beta$ -unsaturated ester carbonyl.

Using the relationship we have established between  $-\Delta H$  and  $\Delta\nu_{C=O}$ , we have quantitated the change in  $\nu_{C=O}$  observed through a series of acylserine proteases in which the acyl donor was thienylacryloyl or 5-methylthienylacryloyl. This allowed us to estimate that the decrease in  $\nu_{C=O}$  of  $54\text{ cm}^{-1}$  through the acyl enzyme series could be accounted for by an increase in hydrogen bond enthalpy to the carbonyl oxygen of  $57\text{ kJ mol}^{-1}$  [2,15]. It should be noted that the values of  $\Delta H$  given in Refs. [2] and [15] are low by a factor of 2.3, due to an arithmetic error.

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