Hydrogen bonding in complexes of carboxylic acids with 1-alkylimidazoles: steric and isotopic effects on low barrier hydrogen bonding

Constance S. Cassidy, Laurie A. Reinhardt, W. Wallace Cleland and Perry A. Frey*

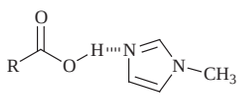
Institute for Enzyme Research, The Graduate School, and Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53705, USA

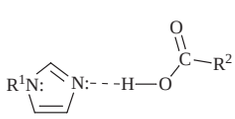
Received (in Gainesville, FL) 11th August 1998, Accepted 13th January 1999

The nature of hydrogen bonding within intermolecular complexes of carboxylic acids and 1-methylimidazole (1-MeIm), 1-*n*-butylimidazole (1-BuIm), and 1-*tert*-butylimidazole (1-*t*-BuIm) in chloroform was characterized by Fourier transform infrared spectroscopy. Earlier spectroscopic studies indicated that carboxylic acid–1-MeIm complexes are of three types: (**I**) neutral complexes with the weaker acids ($pK_a \geqslant 2.2$) in which the antisymmetric carbonyl stretching frequencies are lowered relative to the free acids and the ethyl esters of the acids; (**II**) ionic complexes of stronger acids ($pK_a \leqslant 2.0$) in which the carbonyl stretching frequencies are slightly lower than those for the tetrabutylammonium salts of the acids; (**III**) depolarized partially ionic complexes coexisting with type **II**, in which the carbonyl stretching frequencies are intermediate between those for the tetrabutylammonium salts (bond order 1.5) and the free acids (bond order 2.0).[12] Assignment of the ionic and intermediate carbonyl stretching frequencies was verified by shifts to longer wavelengths in the ^{18}O-labeled 2,2-dichloropropanoic acid–1-MeIm complexes. Type **III** complexes have been postulated to incorporate a low barrier hydrogen bond (LBHB) between the N^3 of the imidazole ring and the carboxylic group. The size of the alkyl group in 1-alkylimidazoles has no significant effect on the carbonyl stretching frequencies in any of the complexes. However, increasing bulkiness in the alkyl group increases the intensity of the type **III** species relative to type **II**, so that the equilibrium is shifted toward low barrier hydrogen bonding in solution. The broad bands at approximately 2500 and 1900 cm^{-1} in the R-COOH–1-alkylimidazole complexes are similar to those classically attributed to strong hydrogen bonds. These bands are absent from the spectra of R-COOD–1-alkylimidazole complexes. Moreover, the antisymmetric carbonyl stretching bands characteristic of the type **III**, or LBHB-bonded complexes, are greatly decreased in intensity in the spectra of R-COOD–1-alkylimidazole complexes, and are shifted to higher wavelengths nearer to those expected for the free R-COOD. The deuteron is more strongly attached to oxygen, whereas the corresponding proton is more free to engage in low-barrier hydrogen bonding. Spectroscopic data indicate that R-COOH–1-alkylimidazole complexes are unexpectedly strong in $CHCl_3$, perhaps because of resonance assisted hydrogen bonding.

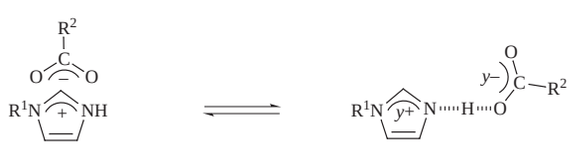
The proton bridging $N^{\delta 1}$ of His 57 and the β-carboxy group of Asp 102 in the active site of chymotrypsin has recently been assigned as a low barrier hydrogen bond (LBHB) in transition state analog complexes.[1–3] Evidence for this assignment includes the low-field chemical shift of the proton (18.3 ppm) at low pH[4,5] and its low fractionation factor of 0.4.[6] The chemical shifts of the protons bridging His 57 and Asp 102 in tetrahedral peptidyl trifluoromethyl ketones range between 18.6 and 19 ppm, and the basicity of $N^{\varepsilon 2}$ in His 57 is dramatically elevated to values of 10.6 to 12.0 in these transition state analog complexes.[2,3,7] The increased basicity of His 57 in these complexes is regarded as evidence of the stabilization of the tetrahedral analogs by low barrier hydrogen bonding.[1–3] LBHBs have been postulated to participate in several enzymatic catalysis systems.[8–11]

The nature of the interaction between His 57 and Asp 102 has been explored by NMR and FTIR spectroscopy in a series of complexes formed between carboxylic acids and 1-methylimidazole (1-MeIm) in aprotic organic solvents.[12] The effect of hydrogen bonding on the antisymmetric carbonyl stretching frequency was examined as a function of the strength of the carboxylic acid, where R = CF_3, $CHCl_2$, $C(CH_3)Cl_2$, CH_2Cl, CH_3OCH_2, $PhCH_2$, and CH_3. The data were compared with the methyl esters of the acids, which are neutral, non-hydrogen bonded species, the tetrabutylammonium salts of the acids, which are purely ion-paired species, and the free acids, which exist as hydrogen-bonded dimers over the concentration ranges studied.[12] Based on the interpretation of the NMR and FTIR data, three types of complexes were proposed to exist in organic aprotic solvents (Scheme 1). For weaker acids ($pK_a \geqslant 2.2$), the





I



II **III**

Scheme 1

carbonyl stretching frequencies are lower relative to the free acids and the ethyl esters of the same acids, indicating neutral, hydrogen-bonding species of type **I**. For stronger acids ($pK_a < 2.0$), the predominant carbonyl stretching frequencies

(type **II**) are very near, but slightly lower than those for the nonhydrogen bonded, ion paired tetrabutylammonium salts. Mixtures of 1-MeIm with acids of pK_a near 2 display a third type of complex with an intermediate carbonyl stretching frequency that is thought to correspond to a complex of type **III** in Scheme 1, in which the ion pair is partially depolarized. These complexes are postulated to incorporate an LBHB and to coexist in equilibrium with the ion-paired complex **II**.[12]

The objective in the present work was to test Scheme 1 critically, with specific reference to characterizing the putative LBHB complexes of type **III.** The effects of ^{2}H substitution for ^{1}H and of ^{18}O for ^{16}O on this species are reported, as well as the effects of steric bulk in the 1-alkylimidazole. The results are consistent with the assignment of the FTIR band at intermediate frequencies to a low barrier hydrogen bonded complex.

Results

Effects of ^{18}O-labeling on the FTIR spectra of 2,2-dichloropropionic acid–1-MeIm complexes in $CHCl_3$

In an earlier study, the FTIR spectra of carboxylic acid–1-MeIm (1 : 1) complexes in $CHCl_3$ were reported.[12] The antisymmetric carbonyl stretching frequencies indicated that 2,2-dichloropropionic acid–1-MeIm consisted of a mixture of complexes, a purely ionic complex ($\nu_{C=O}$ = 1640 cm^{-1}) and an LBHB complex ($\nu_{C=O}$ = 1692 cm^{-1}).[12] To verify these assignments as C–O frequencies, 2,2-dichloro[1-$^{18}O_1$]propionic acid (50% enriched with ^{18}O) was mixed 1 : 1 at 0.25 M with 1-MeIm in $CHCl_3$ and the FTIR spectrum redetermined. The spectrum is compared in Fig. 1 with that of the unlabeled complex. Both the carbonyl bands are shifted, confirming that both correspond to C–O frequencies as originally postulated. The purely ionic carbonyl frequency at 1639 cm^{-1} in the unlabeled compound is shifted to 1624 cm^{-1} by ^{18}O. The putative LBHB species at 1694 cm^{-1} is shifted to 1687 cm^{-1} by ^{18}O. Similar data were obtained at 0.5 M acid and 1-MeIm.

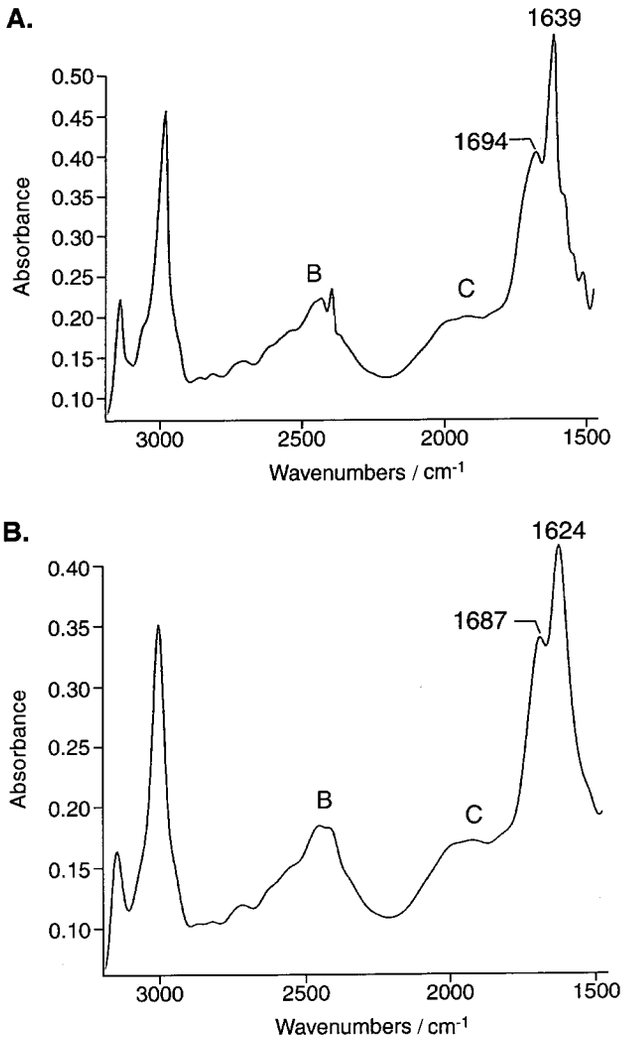


Fig. 1 Effect of ^{18}O on the FTIR spectra of complexes formed between 2,2-dichloropropionic acid and 1-MeIm in $CHCl_3$. A) FTIR spectrum of a 1 : 1 mixture of 2,2-dichloropropionic acid and 1-MeIm. B) FTIR spectrum of a 1 : 1 mixture of ^{18}O-labeled 2,2-dichloropropionic acid and 1-MeIm. The band at 1624 cm^{-1} corresponds to an ion-pair complex that is shifted by 15 cm^{-1} to lower wavenumbers relative to the unlabeled complex. The LBHB species at 1687 cm^{-1} has also been shifted by 7 cm^{-1} to lower wavenumbers.

Dependence of antisymmetric carbonyl stretching frequencies on carboxylic acid strength in 1-alkylimidazole complexes

One-to-one stoichiometric mixtures of 1-MeIm and carboxylic acids were prepared in anhydrous $CHCl_3$ at 0.5 and 0.25 M for FTIR studies. The acids ranged in strength from aqueous pK_a values of 0.2 to 4.8, and the antisymmetric carbonyl stretching frequencies were plotted against the aqueous pK_as of the carboxylic acids in Fig. 2A. The previously reported frequencies for the 1-MeIm complexes were confirmed.[12] The frequencies for the free acids were 20–30 cm^{-1} lower than the corresponding methyl esters because the carboxylic acids existed as strongly hydrogen bonded dimers in $CHCl_3$.[13] The frequencies of the neutral, 1-MeIm complexes of the weakest acids were near those for the free acids but drifted to lower values corresponding to stronger hydrogen bonding as the acid strengths approached pK_a = 2.1. Frequencies of 1-MeIm complexes of the strongest acids were slightly lower (12–16 cm^{-1}) than those of the corresponding non-hydrogen bonded, ion-paired tetrabutylammonium salts reported earlier.[12] As shown in Fig. 2A, varying the alkyl substituent on the 1-alkylimidazole from methyl to *n*-butyl to *tert*-butyl had very little effect on the antisymmetric carbonyl stretching frequencies of carboxylic acid complexes.

As previously described in the 1-MeIm series, 2,2-dichloropropionic acid, and to a lesser degree dichloroacetic acid, form a third type of complex with 1-alkylimidazoles, in which the carbonyl stretching frequency is intermediate between that expected for a hydrogen bonded neutral complex and a purely ionic complex. These unique complexes display frequencies that are expected for low barrier hydrogen bonded complexes, in which the C–O bond order lies between 1.5 and 2.0. The frequencies for these complexes are very similar regardless of whether the alkyl group is methyl, *n*-butyl, or *tert*-butyl.

1-Alkylimidazole complexes of the same acids carrying deuterium in the carboxylic acid group were prepared under identical conditions to obtain further information about the nature of hydrogen bonding. The antisymmetric carbonyl stretching frequencies are plotted in Fig. 2B. The results are generally similar to Fig. 2A for the complexes of weaker and stronger acids; however, they are markedly different for *O*-deuterated 2,2-dichloropropionic acid and *O*-deuterated dichloroacetic acid. Both form two types of complexes, one of which is ionic. The important difference from the protio complexes is the presence of species displaying frequencies that are slightly lower than would be expected for neutral complexes but significantly higher than observed for the putative LBHB complexes of the protio acids. That is, the replacement of hydrogen by deuterium leads to a marked isotope effect on the antisymmetric carbonyl stretching frequency for the LBHB complexes. This is to be expected for LBHBs because of the lower zero point energies of N–D and O–D bonds compared with those for N–H and O–H. The isotope effects support the assignment of LBHBs to complexes with carbonyl stretching frequencies between those for neutral and ionic complexes.

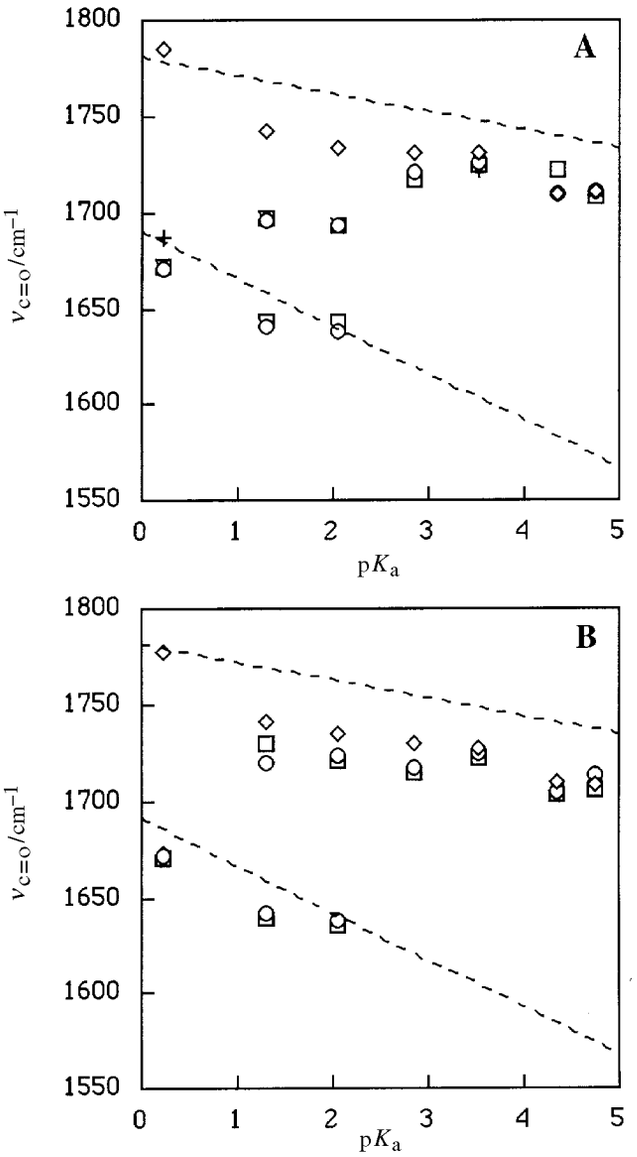


Fig. 2 Effects of acid strength on antisymmetric carbonyl stretching frequencies of complexes formed between carboxylic acids and 1-alkylimidazoles in $CHCl_3$. Part A: Dependence of antisymmetric frequencies for the 1:1 complexes (0.25 M) of carboxylic acids with 1-MeIm, 1-BuIm, and 1-*t*-BuIm on the acidity of the acid. The acids are, in order of increasing pK_a, F_3CCOOH, $Cl_2CHCOOH$, CH_3CCl_2COOH, $ClCH_2COOH$, CH_3OCH_2COOH, $PhCH_2COOH$, and CH_3COOH. The dashed correlation lines represent the previously obtained data[12] with the 0.2 M methyl esters (top line) and the 0.2 M tetrabutylammonium salts of the acids (bottom line). Symbols: ◇, acids; ○, complexes with 1-MeIm; □, complexes with 1-*t*-BuIm; +, complexes with 1-BuIm. The 0.5 M and 0.25 M concentrations gave similar values. NMR data indicated that complexation of these acids is largely complete at 0.25 M. Part B: Dependence of the antisymmetric frequencies for the 1:1 complexes of deuterated carboxylic acids with 1-MeIm, 1-BuIm, and 1-*t*-BuIm. The deuterated acids were run at both 0.2 and 0.5 M. The symbols are identical to those for the protonated acid counterparts. The main difference is in the stretching frequencies for 2,2-dichloropropionic acid and dichloroacetic acid complexes, which are not midway between the free acid and the ion-paired salt, but closer to the free acid.

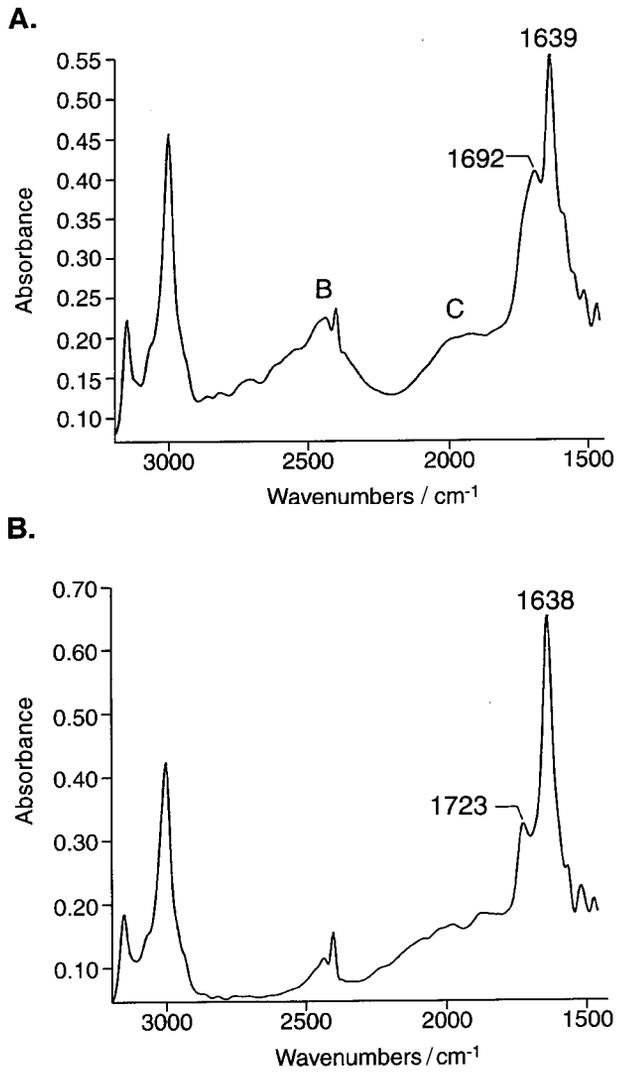


Fig. 3 Effect of deuterium substitution on hydrogen bonding in the complexes of 2,2-dichloropropionic acid and 1-MeIm in $CHCl_3$. A) FTIR spectrum for 2,2-dichloropropionic acid complexed with 1-MeIm. The band at 1639 cm^{-1} represents a conventional ion-pair complex. The band at 1692 cm^{-1} represents an LBHB-bonded complex. B) FTIR spectrum of deuterated 2,2-dichloropropionic acid complexed with 1-MeIm at 0.25 M. The band corresponding to a conventional ion-pair complex remains at 1638 cm^{-1}, however, the band corresponding to an LBHB-bonded complex is decreased in intensity and shifted to higher wavenumbers at 1723 cm^{-1}.

FTIR spectrum of 1-MeIm complexed with *O*-deuterated 2,2-dichloropropionic acid

The FTIR spectrum of the 1-MeIm–*O*-deuterated 2,2-dichloropropionic acid complex between 1400 and 3100 cm^{-1} is compared with that of the undeuterated complex in Fig. 3. The replacement of the band corresponding to the putative LBHB complex at 1692 cm^{-1} of the protio-complex (Fig. 3A) by a much weaker band at 1723 cm^{-1} in the deutero-complex (Fig. 3B) arises from the deuterium isotope effect on the LBHB alluded to in the preceding section. The frequency for the deutero-complex (1723 cm^{-1}) is near that of the free deuterated acid at 1734 cm^{-1} and indicates that the C–O bond order is closer to 2 than in the protio-complex.

The bands marked B and C for the protio-complex in Fig. 3A have been attributed to N–H and O–H stretching modes in strong hydrogen bonds.[14–20] Based on previous interpretations, it is hypothesized that band B represents the antisymmetric stretching frequency and the in-plane stretching frequency (δ_{OH}) in plane summation in Fermi resonance with the ν_{OH} stretching frequency. Band C represents the first overtone out-of-plane bending torsional (γ_{OH}) in Fermi resonance with ν_{OH} and the B-band.[18,21,22] The B and C bands are present in all of the carboxylic acid complexes with 1-MeIm, although conventional (A band) hydrogen stretching in the 3000 cm^{-1} region is also present in the complexes of the weakest and strongest acids. Our spectra in Fig. 3B show that the B and C bands disappear upon substitution of deuterium for protium, in accord with the results of previous authors studying the effect of deuterium substitution on strong hydrogen bonds.[22,23] The

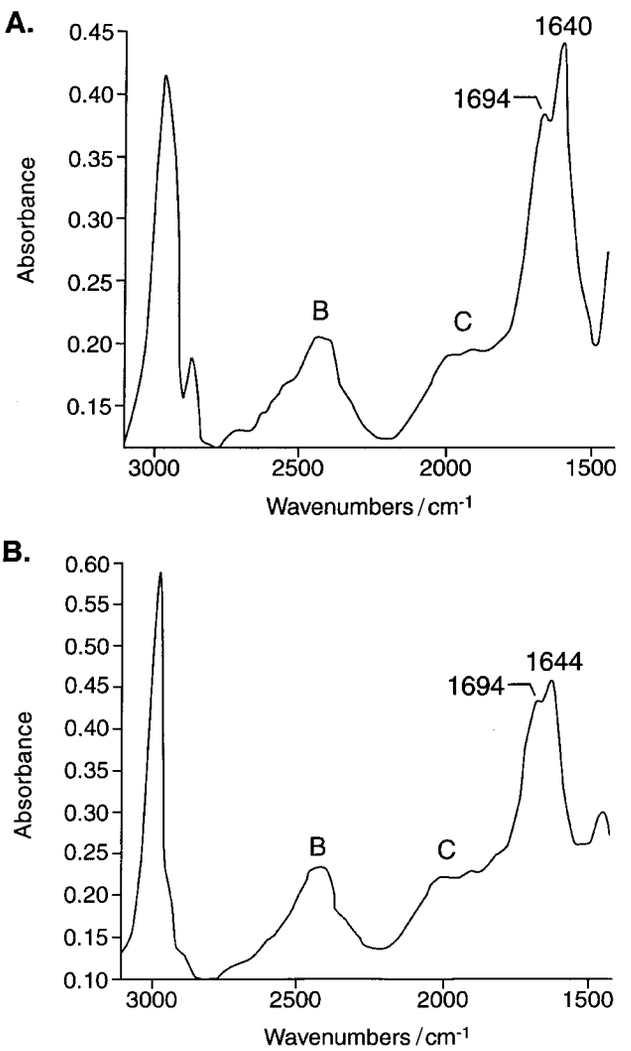


Fig. 4 FTIR spectra for 2,2-dichloropropionic acid complexed with 1-BuIm and 1-*t*-BuIm. A) FTIR spectrum for 2,2-dichloropropionic acid complexed with 1-BuIm. The conventional ion-pair complex remains at 1638 cm^{-1} and the LBHB-bonded complex is at 1694 cm^{-1}. B) FTIR spectrum for 2,2-dichloropropionic acid complexed with 1-*t*-BuIm. The conventional ion-pair complex is at 1645 cm^{-1} and the LBHB-bonded complex is at 1688 cm^{-1} and is increased in intensity relative to the ion-pair complex compared to the ratio seen for the 2,2-dichloropropionic acid complexed with 1-BuIm.

presence of a new band at 2000 cm^{-1} upon deuteration of the acid in the complex is also consistent with strong hydrogen bonding. The B and C bands are absent from the FTIR spectra of the carboxylic acid dimers,[12] in which the hydrogen bonding is weak.

FTIR spectra of 2,2-dichloropropionic acid–1-alkylimidazole complexes

The carbonyl region for the 2,2-dichloropropionic acid–1-MeIm complex is indicative of the strongest hydrogen bonding in the 1-MeIm series. In the free acid, the value of $\nu_{C=O}$ is 1734 cm^{-1}.[12] However, the $\nu_{C=O}$ for 2,2-dichloropropionic acid complexed with 1-MeIm appears as two bands (Fig. 3A). The strong narrow frequency at 1639 cm^{-1} corresponds to the ion-paired complex, and the higher frequency at 1692 cm^{-1} corresponds to the LBHB complex.[12]

FTIR spectra between 1400 and 3100 cm^{-1} for the complexes of 2,2-dichloropropionic acid with 1-*n*-butylimidazole (1-BuIm) and 1-*tert*-butylimidazole (1-*t*-BuIm) at 0.25 M in $CHCl_3$ are shown in Fig. 4. The carbonyl region reveals the effect of increasing the size and branching of the alkyl group in 2,2-dichloropropionic acid–1-alkylimidazole complexes. Comparison of the carbonyl regions for the 1-BuIm and *t*-BuIm complexes in Fig. 4 with that for 1-MeIm in Fig. 3A show that as the bulkiness of the 1-alkyl group is increased, the amount of the LBHB complex increases relative to the purely ionic complex. Taking the ratios of intensities of the bands at ~1640 and ~1690 cm^{-1} as representative of the ratio of ionic to LBHB complexes, the ratio decreases from 1.4 for 1-MeIm to 1.1 for 1-*t*-BuIm in this series. Because the two types of complexes coexist in equilibrium, the change represents a shifting equilibrium toward favoring the LBHB species as the bulkiness of the alkyl group increases.

Table 1 Maximum δ_{obs} for respective carboxylic acids complexed with 1-MeIm or 1-*t*-BuIm. The complexation of the acid with 1-*t*-BuIm results in a chemical shift that is further downfield and supports the hypothesis that the bulkier alkyl group perturbs the equilibrium in favor of the LBHB-bonded complex

Acid	pK_a	δ_{obs} with 1-MeIm/ppm	δ_{obs} with *t*-BuIm/ppm
Trifluoroacetic	0.23	17.20	17.56
Dichloroacetic	1.29	17.88	18.20
2,2-Dichloropropionic	2.06	17.88	18.22
Chloroacetic	2.86	17.66	17.94
Methoxyacetic	3.53	16.70	16.92
Acetic	4.76	15.10	15.38

NMR properties of carboxylic acids complexed with 1-MeIm or *t*-BuIm

One-to-one stoichiometric mixtures of 1-MeIm or 1-*t*-BuIm with carboxylic acids dissolved in $CDCl_3$ exhibit very low-field signals for the acidic proton. The observed chemical shift value (δ_{obs}) varies with the pK_a value for the carboxylic acid and the concentration of the complex.[12]

The interpretation of NMR data is complicated by the presence of several different concentration-dependent equilibria. The situation is analogous to that of mixtures of acetic acid and pyridines of varying basicity, in which mixtures of complexes are observed.[24] The degree of aggregation in carboxylic acid–1-MeIm mixtures appears to depend on the strength of the acid.[12] Taking the maximum observed chemical shift value (δ_{BHA}) for each complex investigated in the present work as a lower limit for the chemical shift of the 1 : 1 complex, a plot of δ_{BHA} *versus* pK_a was biphasic with an intersection point corresponding to a pK_a of 2.1, similar to that previously determined.[12] Significantly, the chemical shift values for the complexes with 1-*t*-BuIm were further downfield by 0.22 to 0.36 ppm for each of the complexes (Table 1). Because the observed ^{1}H NMR chemical shift represents the weighted average of chemical shifts for the various species at equilibrium on the NMR time scale, the lower field values for 1-*t*-BuIm complexes are in agreement with the FTIR results (Fig. 4) showing that the LBHB complexes are more favored than in the 1-MeIm complexes.

Discussion

Previous NMR and FTIR results indicated that 1 : 1 complexes of acids with 1-MeIm in aprotic, organic solvents consisted of the three species in Scheme 1. The LBHB-bonded complex was characterized by the maximum value observed for δ_{BHA} and the intermediate carbonyl stretching frequency (type **III** species) that coexists with that of the ion-pair complex (type **II**).[12]

Because the proton exchange rate is fast within the NMR timescale, other workers have examined complexes of carboxylic acids and pyridine at 109 K, in which the chemical shift values for the species such as BHA and BHAHA can be resolved.[24] The K_d values for complex formation and for the formation of higher order aggregates, which appear to be dependent on the acidity of the carboxylic acid, cannot be

accurately determined at 298 K, but could perhaps be determined at 109 K.

Alternatively, we have chosen to further characterize the nature of the hydrogen bonding by expanding the FTIR studies of the molecular complexes of acetic acid derivatives and 1-MeIm, 1-BuIm, or 1-*t*-BuIm. The FTIR timescale is within the order of a molecular vibration so that multiple species may be detected that are not observable on the NMR timescale. If the intermediate species of type **III** complexes is correctly assigned as an LBHB, then perturbing the equilibrium should result in a change of the nature of the stretching frequency corresponding to the LBHB. A substitution of deuterium, which should be more strongly hydrogen bonded to the donor acid than the corresponding hydrogen, should perturb the LBHB-bonded complex.

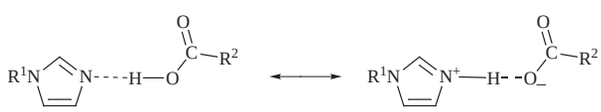
The presence of LBHBs in the carboxylic acids complexed with 1-MeIm is evidenced by the FTIR data of the protonated and deuterated carboxylic acids in Fig. 2. The stretching frequencies represent discrete complexes and not mixtures in chemical exchange. The protonated complexes exhibiting values of C=O stretching frequencies intermediate between those for the neutral and purely ionic complexes correspond to species in which the C=O bond order is between 1.5 and 2.[12] An LBHB species could account for intermediate bond order. The presence of broad bands near 2500 and 1900 cm^{-1} corresponding to B and C bands has been shown to exist in cases of strong hydrogen bonding.[14–20] These B and C bands disappear upon substitution of deuterium in the complex and a new band is present at 2000 cm^{-1}, which is also consistent with the presence of strong hydrogen bonding.[22,23] In the deuterated acid complexes, the band for the LBHB species is also decreased in intensity and shifted to higher wavenumbers, indicating that the deuterium is more strongly bonded to the oxygen than the corresponding hydrogen atom. The intermediate stretching frequency now is closer in value to the deuterated acid, although that for the ion-paired complex is unchanged. This is direct evidence for the double minimum potential energy well of the LBHB, in which the proton is free to vibrate between the two heteroatoms with an intermediate frequency, indicating partial covalent character, while the deuteron is more constrained by the barrier. The shift in the frequency of the intermediate species upon deuteration, the decrease in the relative intensity, and the near disappearance of the B and C bands are positive evidence for low-barrier hydrogen-bonded complexes.

Others have correlated the A, B, and C band changes as a loosening of the proton, indicating a decrease in the barrier in the double minimum proton potential. In the case of a single-well potential, only one band was observed.[25] In our experiments, the B and C bands are observed for all of the complexes, indicating that we are dealing with a double minimum potential well and not a single-well potential.

The relative amounts of the types of complexes in equilibrium cannot be determined without the extinction coefficient of each of the types of complexes in equilibrium. Previous NMR data have indicated that the equilibrium of the intermolecular complexes is concentration dependent[12] and the extinction coefficient cannot be determined by varying the concentration of species in the solution. However, the changes in the relative areas of the carbonyl stretching frequencies upon varying the bulkiness of the substituent on the 1-position of the imidazole group can be compared.

The equilibrium of several types of species in solution is further evidenced by varying the size and bulkiness of the 1-substituent on the imidazole base. The equilibrium between the ion-paired complex **II** of Scheme 1 and the LBHB-bonded **III** was shifted toward the LBHB by bulky substituents on the imidazole ring. An increase in bulkiness in the 1-substituent would interfere with an imidazole face-to carboxy edge interaction in an ion pair by sterically shielding the face of the imidazole ring. Such steric bulk would not interfere with an LBHB interaction in complex **III**. This could account for a decrease in the amount of ion-pair complexation relative to LBHB formation. An alternative explanation could be based on the differences in basicities of the 1-alkylimidazoles. The pK_a values, as measured in this work (see Experimental) are 7.12 (1-MeIm), 7.18 (1-BuIm), and 7.35 (1-*t*-BuIm). Thus, the ratios of dissociation constants are in the same range as the ratios of band intensities.

Several facts indicate that hydrogen bonding between 1-alkylimidazoles and carboxylic acids is strong, even in the neutral complexes of weaker acids. The carbonyl stretching frequencies of the carboxylic acid–1-alkylimidazole complexes are significantly lower than for the carboxylic acid dimers, especially for acids with pK_a values less than 4 (Fig. 2). The $\Delta H°$ for the dimerization of formic acid in $CHCl_3$ was previously determined to be 10.2 ± 0.6 kcal mol^{-1} [13] with each hydrogen bond contributing 5.1 kcal mol^{-1} of stabilization. The formation of 1 : 1, singly hydrogen bonded complexes in the present study between the carboxylic acids and 1-MeIm results in chemical shift values for the complexes that are much further downfield than those for the free acids, showing that the acid–imidazole complexes were more stable than the carboxylic acid dimers in $CHCl_3$. The B and C bands in the FTIR spectra of all of the complexes are indicative of strong hydrogen bonding and are not present in the FTIR spectra of the acids.[12] These results show that the hydrogen bonds between 1-alkylimidazoles and all of the carboxylic acids are favored over the acid dimers in $CHCl_3$, indicating that the hydrogen bonds are likely to be stronger than the two hydrogen bonds linking the acid dimer. Hydrogen bonding in carboxylic acid–imidazole complexes may be augmented by resonance enhancement[26] as illustrated.



In considering the effects of varying acidities in a series of carboxylic acids upon the nature of complexation with bases in aprotic solvents, it is recognized that the acidities are greatly decreased in nonsolvating aprotic solvents, and even in solvents that are hydroxylic but less polar than water.[27] One can consider discussing the effects of varied acid strength in terms of acidity constants that refer to the solvent in which the complexes are generated. However, the effects of solvents on acidities cannot be straightforwardly applied to acid–base complexes because of the unknown microenvironmental effects of the complexing partners. In any case, although solvent effects on acidities can be large, "the relative strengths of acids of the same charge and chemical type are independent of the solvent".[27] Therefore, we shall follow the practice of other investigators[28–31] and quote aqueous acidities, with the understanding that they are intended to refer to the relative acid strengths of the compounds.

The present results are analogous to those obtained in complexes formed between trifluoroacetic acid and pyridines of varying basicities.[28–31] The present work differs in that the basicities of the 1-alkylimidazoles are similar, and the acidities of the carboxylic acids are varied. In the study of pyridines with trifluoroacetate, the strongest hydrogen bonding was found between trifluoracetic acid (pK_a, 0.2) and pyridine (pK_a, 5.2), corresponding to a difference in aqueous pK_a of 5, in parallel with the difference in pK_a between 1-MeIm and 2,2-dichloropropionic acid, the strongest hydrogen bonding pair under our experimental conditions.

The results presented here support the assignment of LBHBs in complexes of carboxylic acids with 1-substituted imidazole when acidities are similar. Recently completed calorimetric studies of the same complexes indicate that the enthalpies of the LBHBs correspond to −12 to −15 kcal mol^{-1}.[32] Complexes

are presented as a model for the interaction of His 57 and Asp 102 in chymotrypsin, and the low field ^{1}H NMR chemical shifts and the antisymmetric carbonyl stretching bands in the FTIR spectra are provided as additional evidence for the existence of low-barrier hydrogen bonding in the association of histidine with carboxylic acids.

Experimental

Materials

The carboxylic acids, dichloroacetic anhydride, 1-MeIm, and 1-BuIm were purchased from Aldrich Chemical Company. Methoxyacetyl chloride and phenylacetyl chloride were obtained from Fisher Chemical Company. These compounds were repurified by fractional distillation or recrystallization in water before use. Chloroform was purchased from Aldrich Chemical Company as an anhydrous solvent in a sealed vial and the FTIR spectrum of this chloroform was identical to that of chloroform dried over P_2O_5 followed by distillation. [^{18}O]-Water, 95–98% ^{18}O, was purchased from Cambridge Isotope Laboratories.

Preparation of 1-*t*-BuIm

The method used was a slight modification of a literature procedure.[33] *tert*-Butyl isothiocyanate (100 g, 0.88 mol) and aminoacetaldehyde diethyl acetal (115.6 g, 0.916 mol) in EtOH (anhydrous) (900 mL) were refluxed for 7 h under N_2 gas. The reaction was cooled and the solvent removed *in vacuo*. ^{1}H NMR: 6.24 (br s, 1H), 5.95 (br s, 1H), 4.58 (t, 1H), 3.73–3.52 (series of m, 6H), 1.36 (s, 9H), 1.17 (t, 6H). The residue (219 g, 0.88 mol), a clear thick liquid, was then refluxed with 10% H_2SO_4 (aq). As the temperature increased, the acetal dissolved. After 10 h, the reaction was cooled and placed on ice. 1-*tert*-Butylmercaptoimidazole precipitated as a brownish white solid (130 g, 95%) and was collected by Buchner filtration. ^{1}H NMR: 6.80 (d, 1H), 6.63 (d, 1H), 1.77 (s, 9H). Thiol (30 g, 0.19 mol) was added to 10% HNO_3 (aq) in a 4 L flask and carefully heated to reflux. When the solid was dissolved, a few drops of concentrated HNO_3 (aq) were added to initiate the reaction, and NO_2 (g) evolved. After neutralization with solid Na_2CO_3, the reaction mixture was extracted with copious amounts of ether. The extraction was repeated several times and the organic layers were combined and dried over anhydrous $MgSO_4$. Filtration followed by short path vacuum distillation yielded 1-*tert*-butylimidazole (9.2 g, 38%). ^{1}H NMR ($CDCl_3$): 7.61 (s, 1H), 7.01 (m, 2H), 1.51 (s, 9H).

Titration of 1-MeIm, 1-BuIm, and 1-*t*-BuIm for pK_a determination

The three imidazole bases were titrated with HCl (aq) at 25 °C to determine their respective pK_a values. The data of the plot of volume of HCl (aq) (mL) *versus* the pH were fitted to $Y = A + B/(1 + H/K_a)$ by the least squares method. The pK_a values for 1-MeIm, 1-BuIm, and 1-*t*-BuIm were 7.12 ± 0.01, 7.18 ± 0.003, and 7.35 ± 0.003, respectively. The aqueous pK_a value for 1-MeIm of 7.12 is similar to the literature value of 7.06.[34]

Carboxylic acid complexes with 1-MeIm, 1-*t*-BuIm, and 1-BuIm

The compounds were dried by use of a double manifold. The deuterated acids were prepared by reacting either the acyl chloride or anhydride with 100% D_2O in anhydrous ether followed by distillation or pumping under vacuum. 2,2-Dichloropropionyl chloride was not commercially available and was synthesized by reaction of the carboxylic acid with thionyl chloride in benzene.[35] All of the acids were distilled under vacuum or nitrogen and immediately transferred to a dry box containing P_2O_5 and protected from moisture throughout the experiments.

One-to-one complexes of protonated or 1-deuterated acids with 1-alkylimidazoles were prepared from stock solutions of 1 M acid and base and diluted to concentrations of 0.5 and 0.25 M in anhydrous $CHCl_3$. FTIR spectra were acquired with a Nicolet 5PC FTIR spectrometer in an inert atmosphere using a sealed cell with CaF_2 plates.

Synthesis of 2,2-dichloro[^{18}O]propionic acid

2,2-Dichloro[^{18}O]propionic acid was synthesized by the hydrolysis of the acyl chloride with ^{18}O-labeled water in anhydrous ether. The solvent was removed by rotary evaporation *in vacuo*, and the acid was distilled and immediately transferred to the dry box.

200 MHz NMR of carboxylic acids

It was necessary to verify that the carboxylic acids exist as dimers or higher order aggregates between concentrations of 0.5 and 0.25 M. Stock solutions containing 1 M of acid were prepared in chloroform and diluted to concentrations ranging from 0.8 M to 25 mM in the dry box. The samples were stored in a desiccator and run on a 200 MHz Bruker NMR spectrometer. The chemical shift of the acid proton was plotted as a function of the concentration of the acid. The data were

$$\delta_{obs} = \delta_{dimer} + \delta_{monomer}\,(K_d/[\text{monomer}])/(K_d/[\text{monomer}] + 1) \quad (1)$$

fitted to eqn. (1), which represents a simple equilibrium of the monomeric and dimeric acid forms, as described by Lazaar and Bauer.[13] The results (data not shown) indicated that the acids existed primarily as dimers between 0.25 and 0.5 M.

500 MHz NMR studies of carboxylic acids complexed with 1-MeIm and 1-*t*-BuIm

The carboxylic acids (trifluoroacetic, dichloroacetic, 2,2-dichloropropionic, chloroacetic, methoxyacetic, and acetic acid) were mixed in 1:1 stoichiometry with 1-MeIm or 1-*t*-BuIm and dissolved in $CDCl_3$ at concentrations of 25 mM to 0.8 M. The solutions were prepared in a dry box and protected from moisture throughout the NMR studies.

Acknowledgements

Supported by Grant No. GM 51806 from the National Institute of General Medical Sciences. C. S. C. was supported by the predoctoral training grant GM 08505 from the National Institute of General Medical Sciences. This study made use of the National Magnetic Resonance Facility at Madison, which is supported by NIH grant RR02301 from the Biomedical Research Technology Program, National Center for Research Resources. Equipment in the facility was purchased with funds from the University of Wisconsin, the NSF Biological Instrumentation Program (grant DMB-8415048), the NIH Biomedical Research Technology Program (grant RR02301), the NIH Shared Instrumentation Program (grant RR02781), and the US Department of Agriculture.

References

1 P. A. Frey, S. A. Whitt and J. B. Tobin, *Science*, 1994, **264**, 1927.
2 C. S. Cassidy, J. Lin and P. A. Frey, *Biochemistry*, 1997, **36**, 4576.
3 J. Lin, C. S. Cassidy and P. A. Frey, *Biochemistry*, 1998, **37**, in the press.
4 G. Robillard and R. G. Shulman, *J. Mol. Biol.*, 1972, **71**, 507.
5 J. L. Markley, *Biochemistry*, 1978, **17**, 4648.
6 J. L. Markley and W. M. Westler, *Biochemistry*, 1996, **35**, 11092.
7 T. C. Liang and R. H. Abeles, *Biochemistry*, 1987, **26**, 7603.
8 W. W. Cleland, *Biochemistry*, 1992, **31**, 317.
9 J. A. Gerlt and P. G. Gassman, *Biochemistry*, 1993, **32**, 11943.
10 W. W. Cleland and M. M. Kreevoy, *Science*, 1994, **264**, 1887.

11 J. A. Gerlt, M. M. Kreevoy, W. W. Cleland and P. A. Frey, *Chem. Biol.*, 1997, **4**, 259.
12 J. B. Tobin, S. A. Whitt, C. S. Cassidy and P. A. Frey, *Biochemistry*, 1995, **34**, 6919.
13 K. I. Lazaar and S. H. Bauer, *J. Am. Chem. Soc.*, 1985, **107**, 3769.
14 W. A. Bueno, N. A. Blaz and M. J. M. Santos, *Spectrochim. Acta*, *Part A*, 1981, **37**, 935.
15 M. Szafran and Z. Dega-Szafran, *J. Mol. Struct.*, 1994, **321**, 57.
16 Z. Dega-Szafran, E. Dulewicz and M. Szafran, *J. Chem. Soc.*, *Perkin Trans. 2*, 1984, 1997.
17 G. M. Barrow, *J. Am. Chem. Soc.*, 1956, **78**, 5802.
18 P. Hadzi, *Pure Appl. Chem.*, 1965, **11**, 435.
19 S. L. Johnson and K. A. Rumon, *J. Phys. Chem.*, 1965, **69**, 74.
20 R. Lindeman and G. Zundel, *J. Chem. Soc.*, *Faraday Trans. 2*, 1972, **68**, 979.
21 S. Bratoz, D. Hadzi and N. Sheppard, *Spectrochim. Acta*, 1956, **8**, 249.
22 D. Hadzi and N. Klibarov, *J. Chem. Soc. A*, 1966, 439.
23 F. E. G. Henn, G. M. Cassanas, J. C. Giuntini and J. V. Zanchetta, *Spectrochim. Acta*, *Part A*, 1994, **50**, 841.
24 N. S. Golubev, S. N. Smirnov, V. A. Gindin, G. S. Denisov, H. Benedict and H.-H. Limbach, *J. Am. Chem. Soc.*, 1994, **116**, 12055.
25 G. Zundel and U. Bohner, *J. Phys. Chem.*, 1986, **90**, 964.
26 G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, Oxford University Press, New York, 1997.
27 R. P. Bell, *The Proton in Chemistry*, Cornell University Press, Ithaca, New York, 1959, p. 58.
28 Z. Dega-Szafran and J. Kuzendorf, *Pol. J. Chem.*, 1979, **53**, 623.
29 Z. Dega-Szafran and E. Dulewicz, *J. Chem. Soc.*, *Perkin Trans. 2*, 1983, 345.
30 P. Barczynski, Z. Dega-Szafran and M. Szafran, *J. Chem. Soc.*, *Perkin Trans. 2*, 1985, 765.
31 Z. Dega-Szafran and M. Szafran, *Heterocycles*, 1994, **37**, 627.
32 L. A. Reinhardt, K. Sackseder and W. W. Cleland, *J. Am. Chem. Soc.*, 1999, in the press.
33 C. D. Bedford, R. N. Harris, R. A. Howd, D. A. Goff, G. A. Koolpe, M. Petesch, I. Koplovitz, W. E. Sultan and H. A. Musallan, *J. Med. Chem.*, 1989, **32**, 504.
34 Y. Nozaki, F. R. N. Gurd, R. F. Chen and J. T. Edsall, *J. Am. Chem. Soc.*, 1957, **79**, 2123.
35 P. E. Bratchanskii and G. G. Komissarova, *Zh. Prikl. Khim.*, 1974, **47**, 242.

Paper 8/06387G