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Effects of Freezing an Internal Rotation on Intramolecular Catalysis by an Imidazolyl Group. Synthesis and Hydrolysis of 4,5-[1′(4′)-Acetoxymethyltetramethylene]imidazoles and 4,5-[1′(5′)-Acetoxymethylpentamethylene]imidazole

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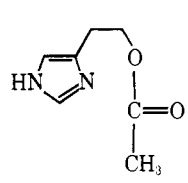
Abstract: As models for acetyl-α-chymotrypsin, 4,5-[1′(4′)-acetoxymethyltetramethylene]imidazole (**2**), its 1′(4′)-methyl-substituted derivative (**3**), and 4,5-[1′(5′)-acetoxymethylpentamethylene]imidazole (**4**) were synthesized via 6-bromo-2-ethoxycarbonylcyclohexanones and 7-bromo-2-ethoxycarbonylcycloheptanone in moderate yields. Their hydrolytic reactivities were determined in water at 50 °C in comparison with their open-chain analogue, 4(5)-(2′-acetoxyethyl)imidazole (**1**). The rate constants (k_1) for intramolecular general base catalysis by an imidazolyl group for **2** and **4** are 2.6 and 11.5 times larger than k_1 for **1**, respectively, but k_1 for **3** is 0.83 times smaller. Thus the 1′(4′)-methyl group in **3** has a retarding effect on k_1, while one methylene unit in the carbocyclic chain of **4** has a large accelerating effect on k_1. The solvent deuterium isotope effects for **2** and **4** are in accord with general base catalysis by the imidazolyl group. The enhancement of 2.6 in k_1 for **2** can be rationalized from the entropy effect of freezing an internal rotation, and the discrepancy from the estimated rate factor of 6 to 11 is mainly attributable to the loose transition state in general base-catalyzed hydrolysis. The large enhancement of 11.5 in k_1 for **4** can be explained in terms of a preferred favorable conformation of the second internal rotation and a basicity correction for the imidazolyl group. ^1H and ^{13}C NMR spectra for those models were discussed for the elucidation of their structures. Comparison of k_1 for **2** and **4** with that of acetyl-α-chymotrypsin suggests that, to mimic the enzymic activity, a rate factor of about 500 must be provided by some modifications other than the freezing of remaining internal rotations in the acetoxymethyl group.

Model studies of the deacylation step of α-chymotrypsin using simple model compounds have been carried out rather extensively.[1-6] The information obtained from them is particularly useful because the simplicity of their structures enables analysis of the results more clearly, and the deacylation step is the microscopic reverse of the acylation.[7]

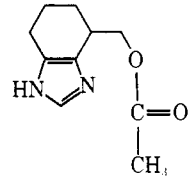
However, most of the models used hitherto are methyl or phenyl esters of carboxylic acid derivatives with an imidazolyl group attached to the α, β, or γ position of the acid carbon skeleton[2,4] or phenyl esters in which an imidazolyl group is bound to the phenyl ring.[1,3,5,6]

Only one model, 4(5)-(2′-acetoxyethyl)imidazole (**1**), with a primary acyloxy group and a 4(5)-imidazolyl group like the active site of acyl-α-chymotrypsin was synthesized, and its hydrolytic reactivity was determined.[2,3] Although the acetate **1** is considered to be hydrolyzed by intramolecular general base catalysis like the enzyme,[5,8] the deacylation rate constant is far below that of the enzyme. This deficiency has been suggested due to the lack of rigidity of the model.[9]

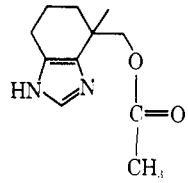
For rate enhancement, ample examples indicate the importance of rigid structures with functional groups at appropriate positions.[7] No model has ever appeared to investigate the rate enhancement caused by freezing an internal rotation in the intramolecular catalysis of ester hydrolysis by an imidazolyl group, although many recent works have concentrated on steric control and steric acceleration in cyclization reactions.[10-13] Therefore, we have synthesized three new models, 4,5-[1′(4′)-acetoxymethyltetramethylene]imidazole (**2**), its 1′(4′)-methyl-substituted derivative (**3**), and 4,5-[1′(5′)-acetoxymethylpentamethylene]imidazole (**4**), and compared their hydrolytic reactivities with the reactivity of **1**. The three new models (**2–4**) apparently have



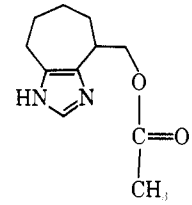
1



2



3



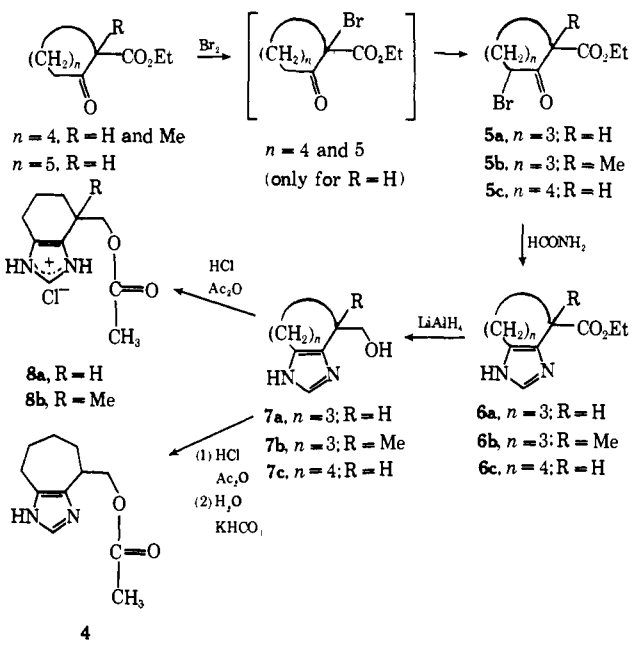
4

one internal rotation frozen in comparison with **1**. The models **3** and **4** are higher homologues of the model **2** with one more carbon atom on or in their carbocyclic chains.

Synthesis

Imidazole derivatives **2** and **3** were obtained as hydrochlorides **8a,b** as shown in Scheme I. Bromination of 2-

Scheme I



ethoxycarbonylcyclohexanone without solvent gave directly 6-bromo derivative **5a** in 82% yield, which is comparable to that reported by Sheehan and Mumaw,[14] who brominated the starting ketone in chloroform and subsequently rearanged the 2-bromo ketone to **5a** using dry hydrogen bromide. Infrared and NMR spectra definitely indicated that **5a** was a 20:80 mixture of the keto and enol forms, while **5b** was 100% keto form. The reaction of **5a,b** with formamide[15] gave the imidazole derivatives **6a,b** in rather high yields of 65–72% in comparison with only 30% yield for 4,5-tetramethyleneimidazole from 2-bromocyclohexanone and formamide.[16] At present we find no convincing explanation for the improved yield even in the light of the mechanism proposed for the imidazole formation.[15] Use of the Weidenhagen method[17] to obtain **6a,b** from **5a,b** proved unsuccessful, probably owing to vulnerability of the acidic hydrogen and the ester group in the strongly basic solution.

Reduction of **6a,b** with lithium aluminum hydride gave **7a,b** in low yield (40%), accompanied by disruption of the imidazole ring, which was not observed in the reduction of 2-[4′(5′)-imidazolyl]norbornanes.[18] Acetylation of **7a,b** with acetic anhydride gave only *O*-acetylated derivatives **8a,b** in sharp contrast to the cases of 4(5)-(2′-hydroxyethyl)imidazole[2] and 2-[4′(5′)-imidazolyl]norbornanols[18] where *N*- and *O*-diacylated derivatives were isolated. This could be caused by steric hindrance in the 4,5-dialkyl-substituted imidazoles **7a,b**.[19]

The imidazole derivative **4** was prepared by the method used for **8a,b** as shown in Scheme I. A few features of the preparation are worthy of comments. Isomerization of 2-bromo-2-ethoxycarbonylcycloheptanone to 7-bromo derivative **5c** proceeded slowly in the period of 4 days at room temperature, in sharp contrast to the almost instantaneous isomerization in the case of cyclohexanone derivative **5a**. When 7-bromo derivative **5c** reacted with formamide, it gave the crude imidazole derivative **6c** in 30–40% yield. This is only half the yield for **6a,b** and is considered to reflect the strain in the cycloheptene ring system. The imidazole ring of **6c** was found more vulnerable to lithium aluminum hydride than that of **6a** or **6b**. Therefore **6c** was reduced to **7c** under milder conditions. Acetylation of **7c** with acetic anhydride gave *N*- and *O*-diacetylated derivative, which was not found in the reaction of **7a,b**. This was interesting but not pursued further.

Experimental Section

All melting points and boiling points are uncorrected. Elemental analyses were carried out by E. Amano of our laboratory. ^{1}H NMR spectra were recorded at 60 MHz with a Hitachi Perkin-Elmer Model R-24 spectrometer, and tetramethylsilane was used as internal standard unless otherwise noted. ^{13}C NMR spectra were recorded at 20 MHz with a Varian CFT-20 spectrometer equipped with Fourier transform facilities. Mass spectra (70 eV) were obtained using a Hitachi Model RMS-4 mass spectrometer.

6-Bromo-2-ethoxycarbonylcyclohexanone (5a). To 1.0 g (5.9 mmol) of 2-ethoxycarbonylcyclohexanone was added 1.2 g (7.5 mmol) of bromine dropwise with stirring in an ice bath. After the addition was completed, the mixture was stirred further for 15 min in the bath. The mixture was dissolved in ether and washed with water, then with aqueous sodium bicarbonate, and finally with water. After drying ($MgSO_4$), the ether was removed under reduced pressure, and the residue was distilled in vacuo, giving 1.2 g (82%) of a colorless oil: bp 80–92 °C (0.09 mm) [lit.[14] bp 93–95 °C (0.4 mm)]; ir (neat) 1730, 1710 (keto C=O), 1650, 1610 cm^{-1} (enol C=O and C=C); ^{1}H NMR (CCl_4) δ 12.07, 11.92 (0.8 H, ss, enol OH), 4.60 (0.8 H, m, enol CHBr), 4.20 (2.2 H, m, OCH_2 and keto CHBr), 3.2 (0.2 H, m, keto $CHCO_2$), 1.5–2.6 (6 H, m, $(CH_2)_3$), 1.3 (3 H, tt, CH_3); mass spectrum *m/e* 249 (P + 2), 247 (P), 204, 202 (P − OC_2H_5). Anal. ($C_9H_{13}BrO_3$) C, H.

4,5-[1′(4′)-Ethoxycarbonyltetramethylene]imidazole (6a). In a flask fitted with a reflux condenser were placed 15.0 g (0.06 mol) of **5a** and 35 g (0.78 mol) of freshly distilled formamide. The homogeneous solution was heated at 150–160 °C for 1.5 h with stirring. The yellow solution was evaporated in reduced pressure to remove the excess formamide. The residue was dissolved in water, washed with ether, and extracted with chloroform repeatedly after adjusting the pH of the aqueous solution to 8–9 with aqueous sodium carbonate. After drying ($MgSO_4$), the chloroform was removed in vacuo, leaving 8.4 g (72%) of a yellow glassy solid. Crystallization from tetrahydrofuran gave white crystals of **6a**: mp 101.5–102.6 °C; ir (KBr) 3400–2200 (NH), 1725 (C=O), 1610 cm^{-1} (imidazole ring); ^{1}H NMR ($CDCl_3$) δ 7.7 (1 H, s, NH), 7.41 (1 H, s, N-CH=N), 4.10 (2 H, q, OCH_2), 3.68 (1 H, m, $CHCO_2$), 2.56 (2 H, m, =C-CH_2), 2.0 (4 H, m, CH_2CH_2), 1.18 (3 H, t, CH_3); mass spectrum *m/e* 194 (M^+), 149 (M^+ − OC_2H_5). Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

4,5-[1′(4′)-Hydroxymethyltetramethylene]imidazole (7a). In 5 ml of dry tetrahydrofuran 0.4 g (9.3 mmol) of lithium aluminum hydride was dissolved under a nitrogen atmosphere at room temperature. To this solution 0.8 g (4.1 mmol) of **6a** in 1.5 ml of dry tetrahydrofuran was added dropwise with stirring at room temperature. After the addition was completed, the reaction mixture was stirred for 30 min, then the excess hydride was destroyed by subsequent additions of 0.5 ml of water, 0.5 ml of 15% sodium hydroxide solution, and finally 1.5 ml of water. The mixture was adjusted to pH 9–10 with hydrochloric acid and filtered. The white precipitate was washed repeatedly with tetrahydrofuran, and, after drying ($MgSO_4$), the combined filtrate and washings were evaporated to dryness in vacuo, giving 0.38 g (60%) of the crude alcohol **7a**. Crystallization from tetrahydrofuran gave white crystals of **7a** in 40% yield: mp 145–147 °C; ir (KBr) 3600–2200 (OH and NH), 1615 (imidazole ring), 1035 cm^{-1} (C-O); ^{1}H NMR (CD_3OD) δ (solvent peak as internal standard, δ 3.35) 7.67 (1 H, s, N-CH=N), 5.30 (s, OH and NH), 3.73 (2 H, AB q, OCH_2), 2.90 (1 H, m, =C-CH), 2.60 (2 H, m, =C-CH_2), 1.87 (4 H, m, CH_2CH_2); mass spectrum *m/e* 152 (M^+), 121 (M^+ − CH_2OH). Anal. ($C_8H_{12}N_2O$) C, H, N.

4,5-[1′(4′)-Acetoxymethyltetramethylene]imidazole Hydrochloride (8a). In a slight excess of hydrochloric acid was dissolved 1.15 g (7.57 mmol) of white crystals of **7a**. After evaporation and

drying in vacuo, the residual imidazole hydrochloride was covered with 7 ml of ethyl acetate and heated at reflux. To this mixture 2.4 g (23.5 mmol) of acetic anhydride was added dropwise. After the crystals were dissolved in the solution, it was refluxed for 5 min, then treated with carbon, and cooled to precipitate 1.17 g (67%) of white crystals of **8a**: mp 155-156.5 °C; ir (KBr) 3200-2200 (NH), 1730 (C=O), 1640 (imidazole ring), 1250 cm^{-1} (C-O); ^1H NMR (Me_2SO-d_6) δ (solvent peak as internal standard, δ 2.50) 8.97 (1 H, s, N=CH=N$^+$), 4.25 (2 H, broad d, OCH_2), 3.14 (1 H, m, =C-CH), 2.57 (2 H, m, =C-CH_2), 2.00 (3 H, s, $COCH_3$), 1.78 (4 H, m, CH_2CH_2). Anal. ($C_{10}H_{15}N_2ClO_2$) C, H, N.

4,5-[1′(4′)-Acetoxymethyltetramethylene]imidazole (2). To obtain the sample for structural studies using NMR spectroscopy, **8a** was dissolved in chloroform and converted into **2** by treatment with aqueous potassium bicarbonate. Recrystallization from a small quantity of chloroform gave colorless crystals: mp 140.5-142 °C; ^1H NMR ($CDCl_3$) δ 11.2 (1 H, s, NH), 7.48 (1 H, s, N-CH=N), 4.23 (1 H, two AB q, OCH_2), 3.08 (1 H, m, =C-CH), 2.58 (2 H, m, =C-CH_2), 2.01 (3 H, s, $COCH_3$), 1.8 (4 H, m, CH_2CH_2). Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

6-Bromo-2-ethoxycarbonyl-2-methylcyclohexanone (5b). 2-Ethoxycarbonyl-2-methylcyclohexanone was brominated with bromine in carbon tetrachloride to give **5b** in 87% yield: bp 122-129 °C (1 mm); ir (neat) 1730 (ester C=O), 1720 cm^{-1} (ketone C=O); ^1H NMR (CCl_4) δ 4.67 (1 H, m, CHBr), 4.15 (2 H, qq, OCH_2), 2.8-1.5 (6 H, m, $(CH_2)_3$), 1.30 (3 H, s, CH_3), 1.26 (3 H, tt, CH_3). Anal. ($C_{10}H_{15}BrO_3$) C, H.

4,5-[1′(4′)-Ethoxycarbonyl-1′(4′)-methyltetramethylene]imidazole (6b). The same procedure as was used for the preparation of **6a** was applied, giving a slightly yellow, viscous oil of crude **6b** in 63% yield. This oil was left at 10 °C for several days to crystallize. White crystals were collected and washed with ether: mp 93.5-94.5 °C; ir (KBr) 3250-2250 (NH), 1720 (ester C=O), 1600 cm^{-1} (imidazole ring); ^1H NMR ($CDCl_3$) δ 11.0 (1 H, s, NH), 7.45 (1 H, s, N-CH=N), 4.10 (2 H, q, OCH_2), 2.75-1.65 (6 H, m, $(CH_2)_3$), 1.51 (3 H, s, CH_3), 1.18 (3 H, t, CH_3). Anal. ($C_{11}H_{16}N_2O_2$) C, H, N.

4,5-[1′(4′)-Hydroxymethyl-1′(4′)-methyltetramethylene]imidazole (7b). The ester **6b** was reduced with lithium aluminum hydride as was applied for **6a**, giving white crystals of **7b** in 41% yield: mp 189-191 °C; ir (KBr) 3500-2100 (OH and NH), 1600 (imidazole ring), 1060 cm^{-1} (C-O); ^1H NMR (Me_2SO-d_6) δ (solvent peak as internal standard, δ 2.50) 7.35 (1 H, s, N-CH=N), 5.3 (broad s, OH and NH), 3.38 (2 H, broad s, OCH_2), 1.14 (3 H, s, CH_3); mass spectrum *m/e* 166 (M^+). Anal. ($C_9H_{14}N_2O$) C, H, N.

4,5-[1′(4′)-Acetoxymethyl-1′(4′)-methyltetramethylene]imidazole Hydrochloride (8b). The same method used for the acetylation of **7a** was adopted: mp 175-178 °C; ir (KBr) 3200-2100 (NH), 1735 (C=O), 1640 (imidazole ring), 1242 cm^{-1} (C-O); ^1H NMR (Me_2SO-d_6) δ (solvent peak as internal standard, δ 2.50), 8.98 (1 H, s, N=CH=N$^+$), 4.11 (2 H, AB q, OCH_2), 2.00 (3 H, s, $COCH_3$), 1.26 (3 H, s, CH_3). Anal. ($C_{11}H_{17}N_2ClO_2$) C, H, N.

7-Bromo-2-ethoxycarbonylcycloheptanone (5c). To 2.8 g (15.2 mmol) of 2-ethoxycarbonylcycloheptanone in 10 ml of carbon tetrachloride was added dropwise 2.8 g (17.5 mmol) of bromine in 10 ml of carbon tetrachloride with stirring in an ice bath. After the addition was completed, the mixture was stirred further for 20 min in the bath. Then it was allowed to stand at room temperature for 4 days. After washing and drying ($MgSO_4$), the solvent was removed to give 2.8 g (70%) of a colorless oil of **5c**: bp 114-133 °C (0.2 mm); ir (neat) 1740, 1710 (keto C=O), 1640, 1610 cm^{-1} (enol C=O and C=C); ^1H NMR (CCl_4) δ 12.75 (0.5 H, s, enol OH), 4.69 (0.5 H, m, enol CHBr), 4.15, 4.20 (2.5 H, m, OCH_2 and keto CHBr), 3.2-1.5 (8.5 H, m, $(CH_2)_4$ and $CHCO_2$), 1.35, 1.28 (3 H, tt, CH_3).

4,5-[1′(5′)-Ethoxycarbonylpentamethylene]imidazole (6c). The same procedure as was used for the preparation of **6a** was applied, giving a dark brown, viscous oil of crude **6c** in 38% yield: ir (neat) 3500-2300 (NH), 1720 (C=O), 1600 cm^{-1} (imidazole ring); ^1H NMR ($CDCl_3$) δ 10.9 (1 H, s, NH), 7.35 (1 H, s, N-CH=N), 4.10 (2 H, q, OCH_2), 3.88 (1 H, m, $CHCO_2$), 2.72 (2 H, m, =C-CH_2), 1.80 (6 H, m, $(CH_2)_3$), 1.21 (3 H, t, CH_3).

4,5-[1′(5′)-Hydroxymethylpentamethylene]imidazole (7c). The similar procedure as was used for the preparation of **7a** was adopted, except for the addition of hydride at an ice-cold temperature and the destruction of the excess hydride by addition of water. The crude brown product was obtained in 70% yield. The sample for spectrometry was purified by thin-layer chromatography using silica gel and methanol-dichloromethane (1:10): ir (neat) 3500-2200 (OH and NH), 1590 (imidazole ring), 1040 cm^{-1} (C-O); ^1H NMR ($CDCl_3$) δ 7.30 (1 H, s, N-CH=N), 5.11 (2 H, s, NH and OH), 3.65 (2 H, broad d, OCH_2), 2.92 (1 H, m, =C-CH), 2.60 (2 H, m, =C-CH_2), 1.87 (6 H, m, $(CH_2)_3$); mass spectrum *m/e* 166 (M^+), 135 ($M^+ - CH_2OH$).

4,5-[1′(5′)-Acetoxymethylpentamethylene]imidazole (4). In a slight excess of hydrochloric acid was dissolved 1.0 g of crude **7c**. After evaporation and drying in vacuo, the residual solid was covered with 12 ml of ethyl acetate and heated at reflux. To this mixture 4 g of acetic anhydride was added dropwise, and the mixture was heated at reflux for 50 min. Then it was treated with carbon, and the solvent was removed in vacuo to give 1.15 g of a colored oil. This was dissolved in 40 ml of water, and 0.7 g of potassium bicarbonate was added (pH 8-9). After stirring for 20 min, the solution was treated with carbon, and the somewhat decolorized filtrate was evaporated in vacuo. The residual solid was dried in vacuo and extracted with chloroform. Removal of the solvent gave 0.69 g of a brown oil. This was solidified by mixing with a small quantity of anhydrous tetrahydrofuran. The solid was crystallized and washed with a minimum quantity of anhydrous tetrahydrofuran to give 98 mg of white crystals of **4**: mp 135.0-135.5 °C; ir (KBr) 3200-2200 (NH), 1740 (C=O), 1610 (imidazole ring), 1240 cm^{-1} (C-O); ^1H NMR ($CDCl_3$), δ 10.0 (1 H, broad s, NH), 7.37 (1 H, s, N-CH=N), 4.28 (2 H, broad d, OCH_2), 3.18 (1 H, m, =C-CH), 2.70 (2 H, m, =C-CH_2), 2.02 (3 H, s, CH_3), 1.80 (6 H, m, $(CH_2)_3$). Anal. ($C_{11}H_{16}N_2O_2$) C, H, N.

Materials. Potassium chloride and imidazole were reagent grade and used after recrystallization. Hydrochloric acid, sodium hydroxide, and standard buffer solutions were obtained from Nakarai Chemicals (Kyoto). All water used in kinetic runs was deionized. **4,5-Tetramethyleneimidazole** was prepared from 2-chlorocyclohexanone according to the method of Weidenhagen[17] and identified by mp and infrared and NMR spectroscopy. The acetate **1** was prepared by the method of Bruice and Sturtevant[2] and characterized by mp, elemental analysis, and infrared and NMR spectroscopy.

Kinetics. All kinetic measurements for hydrolysis were made by the titration of liberated acid with a titration assembly consisting of a micro buret and a Toa-Dempa Model HM 5A pH meter equipped with a Toa Electronics Type GTC-155 combined glass-calomel electrode. Kinetic solutions (6 ml) in the cell under a nitrogen atmosphere were stirred magnetically and maintained at 50 ± 0.1 °C using a thermoregulated water bath. Kinetic measurements were in water at 50 °C with μ = 0.1 by addition of KCl. The concentration of acetates **1-4** was 0.005 M. The pH of kinetic solution was kept constant within ±0.02-pH meter reading. After each kinetic run, the glass electrode was checked with standard buffer solutions and found to be good to within ±0.03 pH unit. The observed pseudo-first-order rate constant, k_{obsd}, was calculated from a plot of log [acetate] against time using the known initial concentration of acetate. Hydrolyses of the acetates were followed to 3.1 (at pH 6.5)-61% (at pH 9.0) completion for **1**, to 2.3 (at pH 6.0)-61% (at pH 9.0) completion for **2**, to 6.2 (at pH 7.5)-55% (at pH 9.0) for **3**, and to 4.5 (at pH 6.0)-96% (at pH 9.5) for **4**. All runs obeyed good first-order kinetics. Values of k_{obsd} were reproduced within ±5%.

Product Analysis. The kinetic solutions for **2** and **4** were combined, respectively, adjusted to pH 8-9, and warmed at 50 °C for several hours to complete the hydrolysis. The solution was evaporated below 50 °C in vacuo, and the residual solid was extracted with ethanol. A white solid from the extract was identified as **7a** (or **7c**) using infrared and NMR spectra.

Solvent deuterium isotope effects were determined for the hydrolyses of **2** and **4** at 50° in 99.84% D_2O (Commissariat a l' Energie Atomique de France). By dilution of 20% DCl in D_2O (E. Merck), 0.001 N DCl in D_2O was prepared, and the observed pH meter reading for this solution was compared with that for 0.001 N HCl in H_2O,[21] establishing the equation pD = pH meter readings +0.31 at 50 °C. For preparation of an alkali titrant in D_2O, 40% NaOD in D_2O (E. Merck) was diluted.

pK_a Determinations. pK_a values were determined according to the method of Albert and Serjeant.[22] Imidazole and its derivatives were titrated at 0.005 M using the apparatus for kinetics. The con-

Table I. Hydrolytic Rate Constants of Acetates **1**, **2**, **3**, and **4** and pK_a Values of Their Imidazolyl Groups in Water[a]

Acetate	pK_a[b]	$k_1 \times 10^4$, min^{-1}	Rel rate	$k_{OH} \times 10^{-1}$, $M^{-1}\ min^{-1}$	Rel rate
1	6.50	1.00	1	6.3	1
2	6.90	2.6	2.6	5.4	0.86
3	6.91	0.83	0.83	3.2	0.51
4	7.03	11.5	11.5	7.5	1.19

[a] At 50 ± 0.1 °C, 0.1 M KCl. k_1 and k_{OH}, ±5%; pK_a, ±0.02. [b] For comparison, pK_a values for imidazole and 4,5-tetramethyleneimidazole were determined to be 6.60 and 7.50, respectively.

Table II. Solvent Deuterium Isotope Effects for Acetates **2** and **4**[a]

Acetate	pK_a^D	$k_1^D \times 10^4$, min^{-1}	$k_{OD} \times 10^{-1}$, $M^{-1}\ min^{-1}$	$pK_a^D - pK_a^H$	k_1^H/k_1^D	k_{OH}/k_{OD}
2	7.36	1.0	8.2	0.46	2.6	0.66
4	7.42	4.6	9.8	0.39	2.5	0.77

[a] At 50 ± 0.1 °C, 0.1 M KCl. k_1^D and k_{OD}, ±5%; pK_a^D and pK_a^H, ±0.02.

centration, solvent, temperature, and ionic strength were the same as those for kinetics. The titrant was added in a tenth of an equivalent, and the pK_a was calculated using 7–8 values in the set within a spread of ±0.02.

Results

In Table I are listed the rate constants k_1 (min^{-1}) for intramolecular catalysis of acetate hydrolysis by an imidazolyl group and k_{OH} ($M^{-1}\ min^{-1}$) for bimolecular hydroxide ion attack for **1–4**, which were derived from the observed pseudo-first-order rate constants k_{obsd} at various pH values using the equation $k_{obsd} = \alpha k_1 + k_{OH}[OH^-]$, where α is the mole fraction of the neutral imidazolyl group. Plotted in Figure 1 are values of log k_{obsd} vs. pH (pD) for acetates **1–4**. The solid or dotted curves in the figure were drawn using the above equation and the rate constants and the pK_a values in Tables I and II. The calculated pseudo-first-order rate constants agreed mostly with the corresponding observed rate constants to within ±5%. Since the latter was reproducible to ±5%, the values for k_1 (k_1^D) and k_{OH} (k_{OD}) in Tables I and II can be assumed with an accuracy of ±5%. For the calculation of $[OH^-]$, the ionization constant of water was taken as $10^{-13.2617}$ at 50 °C.[23]

Experiments using D_2O were carried out for **2** and **4** since these were considered adequate for the evaluation of solvent deuterium isotope effects. In Figure 1 are shown the log k_{obsd} vs. pD profiles for **2** and **4**. The results are listed in Table II, employing an ionization constant of $10^{-14.103}$ for D_2O at 50 °C.[23]

Discussion

Solvent Deuterium Isotope Effects.[24] The kinetic solvent isotope effect of k_1^H/k_1^D = 2.5–2.6 obtained for **2** and **4** is in accord with general base catalysis of the attack of water by the imidazolyl group, as anticipated from the pK_a values of the conjugate acids of the imidazolyl and the leaving alkoxide groups and the pK_a of water.[25] Consequently, the imidazolyl group in acetate **1** or **3** is also considered to assist the hydrolysis as a general base catalyst.

Interestingly, the values of 2.5–2.6 are essentially identical with the value of 2.4 for the solvent isotope effect in the deacylation of acetyl-α-chymotrypsin.[26] Similar values were found in the deacylation of various acyl-α-chymotrypsin: 2.5 for *trans*-cinnamoyl-, 2.4 for benzoyl-, and 3.0 for trimethylacetyl-α-chymotrypsin.[27] The effect k_{OH}/k_{OD} = 0.66–0.77 seems to be an expected magnitude for the attack

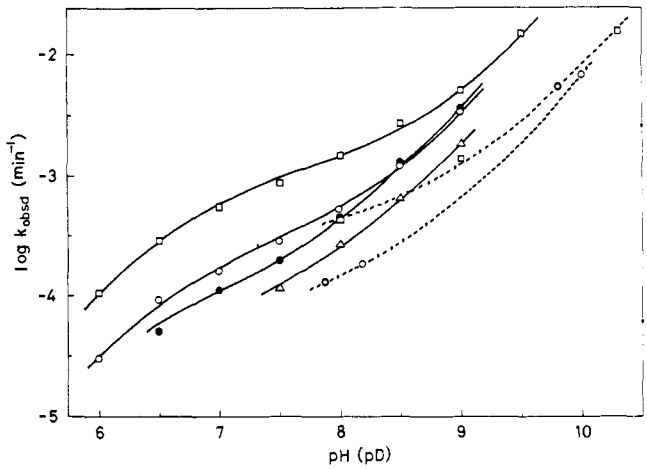


Figure 1. Plots of log k_{obsd} vs. pH (pD) for the hydrolyses of **1** (●), **2** (○), **3** (△), and **4** (□). Solid lines in H_2O and dashed lines in D_2O at 50 °C (0.1 M KCl). The points are experimental and the lines theoretical using $k_{obsd} = \alpha k_1 + k_{OH}[OH^-]$ and the constants listed in Tables I and II.

Table III. ^{13}C Chemical Shifts for Acetates 1, 2, and 4[a]

Acetate	Imidazolyl			Acetyl		
	C-2	C-4	C-5	C=O	CH_3	CH_2–O
1	135.0	134.0	117.1	171.1	21.0	63.9
2	133.7	130.8, 129.8		171.2	21.0	67.2
4	131.8	132.9, 132.6		171.1	20.9	66.2

[a] 0.1–0.2 M in $CDCl_3$ at 30 °C and all shifts in ppm from internal Me_4Si with $CDCl_3$ internal lock.

of hydroxide ion on acetates **2** and **4**. Values of 0.75 and 0.58 were reported for the alkaline hydrolyses of ethyl acetate[28] and 1-methyl-5-acetoxymethylimidazole,[29] respectively. The equilibrium solvent isotope effect of $pK_a^D - pK_a^H$ = 0.39–0.46 is also reasonable in comparison with 0.56 and 0.44 for imidazole at 25 °C (μ = 0.11)[30] and 30 °C (μ = 1.0),[31] respectively, and 0.54 for a histidine imidazolyl group at 25 °C (μ = 0.11).[30]

pK_a Values. An alkyl substituent at the 4 or 5 position of an imidazole ring strengthens the basicity by 0.45–0.5 pK_a unit as cited for the pK_a values of imidazole (6.60) and 4,5-tetramethyleneimidazole (7.50) in Table I and as reported for the pK_a values of imidazole (6.95) and 4(5)-methylimidazole (7.45).[20] Therefore, it is apparent from the pK_a value (6.50) for **1** that an acetoxymethyl group bound to the 4(5)-methyl group weakens the basicity by 0.6. This is the same with the case for **2**, which has a pK_a value just 0.6 smaller than that for 4,5-tetramethyleneimidazole. Similar substituent effects can be assumed for **3**. Thus we find no anomalous pK_a value for the cyclic imidazoles **2** and **3** when compared with the open-chain imidazole **1**.

However, the pK_a value (7.03) for **4** seems anomalous. It is larger than the value for **2** by 0.13 and this increment of basicity cannot be attributed to steric effects of the pentamethylene chain, because a methyl group on the tetramethylene chain in **3** hardly affects the pK_a value. As shown in Table III, the ^{13}C chemical shifts (δ values) of the imidazolyl C-2 carbons decrease in the order **1** > **2** > **4**. A similar trend is observed in the ^1H chemical shifts (δ values) of the protons at the imidazolyl C-2 carbons as described in the Experimental Section (**6a** ≃ **6b** > **6c**). Thus, it is probable that the basicity increment for **4** results from a higher electron density at the imidazole C-2 carbon moiety. What brings about the higher electron density, however, is not explicitly explained for the present.

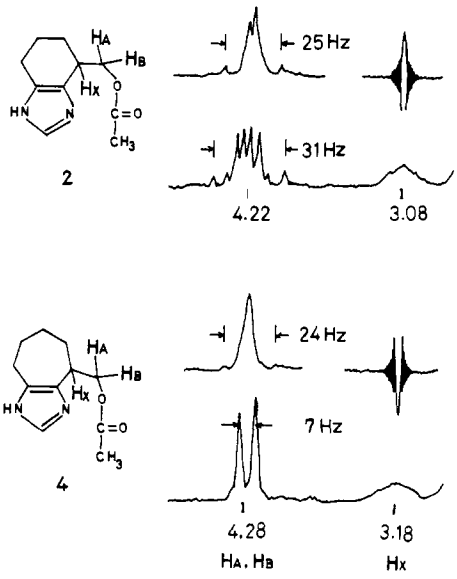


Figure 2. Appearances of ^{1}H NMR spectra of the methylene groups bonded to the acetoxy groups of **2** and **4** in $CDCl_3$ at about 30 °C. The chemical shifts are in ppm from internal Me_4Si.

Effects of Freezing an Internal Rotation. The acetate **2** can be assumed as a typical model for freezing an internal rotation because its six-membered ring is an analogue of nearly strain-free cyclohexene,[32] and its tetramethylene chain is not crowded as compared with the chain for **3** or **4**. Furthermore, rapid ring inversion in cyclohexene was reported by Anet and Haq[33] using ^{1}H NMR spectroscopy, and isomerizations to the pseudoequatorial position under mild conditions were observed in tetralin-1,2-dicarboxylic acids[34] (cis e′,a or a′,e to trans e′,e) and in 1-phenyl-2,3-dicarboxytetralins[35] (cis, trans e′,a,a or a′,e,e to trans, trans e′,e,e). From these results, it is reasonably deduced that the acetate **2** will have its acetoxymethyl group preferably in the more stable pseudoequatorial position, and that this will result in an adequate orientation of the acetoxymethyl group for general base catalysis. Thus the reactivity of **2** will be interpreted in this section in terms of freezing an internal rotation.

On the other hand, the acetate **3** suffers from steric hindrance caused by the 1′(4′)-methyl group, as is evident from this smallest relative rate (0.51) for k_{OH}.[36] A larger retarding effect due to the methyl group found on k_1 rather than on k_{OH}[37] suggests that a steric requirement for the catalysis is rigorous in the vicinity of the methyl group. This situation, in the reverse sense, is seen in the case of **4**, where one carbon increment brings about a large enhancement in k_1. This will be fully discussed in the later section.

The enhancement in k_1 for **2** may be rationalized from the following considerations. (1) Viewing a CPK model of **2** suggests that the acetoxymethyl group is fixed at a favorable conformation with respect to the imidazolyl nitrogen for intramolecular general base catalysis without an internal strain. (2) Conformational restriction in the ground state of **2** in solution is equivalent to a process which involves an entropy change of about 6.5 eu and an enthalpy compensation of about 0.5 kcal/mol, corresponding to a rate factor of about 12 at 25 °C.[38] (3) Since **2** is more basic than **1** by $\Delta pK_a = 0.4$, the efficiency of general base catalysis by the imidazolyl group is expected to increase by a factor of 1.5, if we use the Brönsted exponent $\beta = 0.47$ for general base-catalyzed hydrolysis of ethyl dichloroacetate.[41] There remains a factor of 1.7, which is attributable to freezing an internal rotation. (4) In the imidazolyl group-catalyzed hydrolysis of **1**, two transition states, which are mirror images of each other but identical energetically, may take place during the imidazolyl residue rotation about the methylene–imidazolyl residue bond. But for **2**, since the acetoxymethyl group is expected to take a preferred pseudoequatorial position, only one transition state may be realized (even in an equilibrium with a pseudoaxial position).[42] Therefore, the predicted rate factor ould be divided by 2, yielding a factor in the range of 6 (to 11).[38] (5) If the transition state is loose, **1** is less unfavorable entropically, and the rate acceleration due to freezing an internal rotation will be decreased depending on the looseness.[13]

Quite important is the "looseness" or "tightness" of the transition state in the general base-catalyzed hydrolysis of acetates **1**–**4** because it relates directly not only to the rate factor as considered above but also to the mechanism of proton transfer in the transition state of acetyl-α-chymotrypsin. According to the energetics of neighboring group participation,[13] a measure of the magnitude of anchimeric assistance can be expressed in comparison with the rate of the corresponding intermolecular reaction using "effective concentrations" for the amount of entropy change which is related to the "tightness" or "looseness" of the transition state.

Intermolecular imidazole catalysis in the hydrolysis of ethyl acetate was studied previously by Kirsch and Jencks,[43] who gave a rate constant of 7.5×10^{-6} M^{-1} min^{-1} for the imidazole catalysis at 25° and 1.0 M KCl in water. We have obtained a value of $k_1 = 1.00 \times 10^{-4}$ min^{-1} for the intramolecular imidazolyl group-catalyzed hydrolysis of **1** at 50 °C and 0.1 M KCl in water. Allowing a factor of about 6 ($= 2^{2.5}$) for the temperature difference of 25 °C and neglecting the difference in ionic strength, we obtain 1.7×10^{-5} min^{-1} for the latter at 25 °C, giving an effective concentration of about 2 M.

Noteworthy is the fact that this effective concentration is only several times larger than those for general base-catalyzed enolization and aminolysis reactions (0.5–0.6 M),[13b] but by a factor of 10^3 smaller than that for nucleophilic hydrolysis of phenyl γ-*N*,*N*-dimethylaminobutyrates (1.3×10^3 M).[13b,44] This is in accord with the conclusion that intramolecular general acid- and base-catalyzed reactions generally show small effective concentrations[13b,47] and suggests that the transition state of general base-catalyzed hydrolysis of the acetate **1** is possibly loose and entropically less unfavorable. Thus the observed rate factor of 1.7, which is $1/3.5$ to $1/6.5$ times as small as the predicted factor, could be attributed to the loose transition state.

Enhancement in k_1 for 4. The conformation of cycloheptene has been studied extensively,[48] and experimental results for various benzocycloheptene derivatives[49] are particularly relevant to the present work. Using ^{1}H NMR spectroscopy 4,4,6,6-tetradeuterio-1,2-benzocycloheptene was presumed to take a chair conformation to an extent of 95%. Similar results were obtained for 4,4- and 5,5-dimethyl derivatives of benzocycloheptene. Although these benzocycloheptene derivatives are somewhat different from our imidazocycloheptene derivative **4**, the conclusion may be applied to **4**. Furthermore, the 1′(5′)-acetoxymethyl group of **4** in a preferred chair conformation is expected to be mostly equatorial, where the group is oriented in the proximity of the imidazolyl nitrogen for general base catalysis.

Figure 2 shows the appearances of ^{1}H NMR spectra of the methylene groups bonded to the acetoxy groups of **2** and **4**. Since each of the methylene groups is attached to a tertiary asymmetric carbon on the other side, the spectrum should give two AB quartets in the "rapid rotation" case, three superimposed two AB quartets in the "slow rotation" case, and exchange-broadened signals in the intermediate when the rate of conformer interconversion will become

comparable with the frequency difference between the conformers.[50]

Apparently the form of the spectrum for **2** consists of two AB quartets with eight peaks and that for **4** consists of two exchange-broadened peaks. Thus the acetoxymethyl group in **2** rotates rather rapidly and that in **4** rotates rather slowly. Hence we obtain a valuable conclusion that the former has a rather low-energy barrier for rotation while the latter has a rather high one. This high-energy barrier can be attributed to the one methylene increment in the carbocyclic chain of **4**. Although this does not mean a fixation of the acetoxymethyl group at any conformation, it is quite possible that the group in **4** may take a preferred conformation which is favorable to intramolecular general base catalysis.[51]

Another difference in structure between **2** and **4** is seen in the distances between the carbonyl carbon and the imidazolyl nitrogen. A slightly shorter distance, by only a few tenths of one ångström, can be assumed for **4** using a tentatively static model. This small difference, however, is expected not to affect the catalytic activity because of the "looseness" of the transition state as discussed in the preceding section. The fact that **4** with the shorter distance shows a larger enhancement in k_1 than **2** with the longer distance suggests that steric repulsion between the acetoxy and the imidazolyl groups is not critical to the catalytic action.

The varying ^{13}C chemical shifts of the imidazolyl C-2 carbons in Table III have been discussed earlier in connection with the varying pK_a values of **1, 2,** and **4**. Their acetyl groups, however, have identical chemical shifts, indicating equivalence of their carbonyl bonds. This result, together with the product analysis and the value of $k_1^H/k_1^D = 2.5$, eliminates nucleophilic substitution by the imidazolyl nitrogen with alkyl-oxygen fission for the highly reactive acetate **4**, the mechanism of which was observed in the reaction of strained β-propiolactone with tertiary amines such as 1-methylimidazole to give betaines.[52] The value of k_1^H/k_1^D also rejects nucleophilic catalysis with acyl-oxygen fission.

Thus, the rate factor of 11.5 for k_1 can be assumed as an enhancement in the general base catalysis. The rate factor is reduced to 6.5 by the basicity correction (ΔpK_a 0.53), and the square root of this value gives a factor of 2.5 which may be attributed to freezing an internal rotation. This factor is comparable to a factor of 1.7 found for **2**.

An enhanced k_{OH} value for **4** should be noted here although the enhancement is only 1.2 times the value of k_{OH} for **1**. Considering that values of k_{OH} are in the order **1** > **2** > **3** and this is an expected order of the rate constants for alkaline hydrolysis, the result for **4** is contrary to the expectation. However, the rate factor of 1.2 is very small compared with that of 11.5 for k_1, and the discussion given above may be valid in principle.[53]

Comparison with Acetyl-α-chymotrypsin. Comparison of $k_1 = 1.7 \times 10^{-5}$ min^{-1} at 25 °C for **1** with a deacylation rate constant of 4.08×10^{-1} min^{-1} at 25 °C for acetyl-α-chymotrypsin[55] suggests the presence of other factors for the enzymic rate acceleration. Even if the three remaining internal rotations in the acetoxymethyl group were frozen in **2**, the total enhancement would be a factor of about 50 (2.6^4) at the highest. Then, to mimic the enzymic activity, a factor of about 500 must be provided by such modifications as adjustment of the spatial alignment of the imidazolyl and the acetoxy groups, or the presence of a carboxylate group which affords the so-called "charge-relay" system[56] like the enzyme, or by both of them.

If the looseness in the transition state is to function, the spatial alignment will be less important and the "charge-relay" system will make a major contirbution. A quantum-mechanical calculation indicated the importance of the "charge-relay" system,[57] but, experimentally, Rogers and Bruice[6] were able to realize a rate factor of but three in the presence of an intramolecular carboxylate group in the hydrolysis of substituted 2-(2′-acetoxyphenyl)imidazoles. Thus the circumstances still remain veiled.

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(37) The retarding effect of the methyl group is 0.83/2.6 = 1/3.1 for k_1 and 0.51/0.86 = 1/1.7 for k_{OH}.
(38) Page and Jencks[13] gave a rate factor of 5 at 25 °C, corresponding to 0.99–0.76 kcal/mol for the loss of each internal rotation in the formation of 4-, 5-, and 6-membered rings. They estimated 4.5 eu as a representative value for the entropy that may be lost upon freezing an internal rotation around the methylene–methylene bond. Using their data about ring-closure reactions of unsaturated hydrocarbons, we estimated

6.5 eu for the entropy which is lost upon freezing an internal rotation adjacent to the imidazolyl double bond. We also took account of a partial compensation by a favorable enthalpy function change of 0.5 kcal/mol as they did, and obtained 1.44 kcal/mol, corresponding to a rate factor of about 12 at 25 °C. This free energy is comparable to 1.8 kcal/mol (corresponding to a rate factor of 21) which was given by Page and Jencks[13a] for the free rotation about the methylene–carboxyl residue bond. They estimated the free energy change using data of gas-phase unimolecular ester cleavages, but we have no related data about imidazoles. The reasons that the imidazolyl group can be approximated by the carboxyl group are as follows: (1) the reduced barrier to rotation for the former may be comparable to that for the latter (0 kcal/mol for carboxyl and probably ~1 kcal/mol for imidazolyl);[39] (2) the free-rotor partition function for the former must be somewhat larger than that for the latter but it will not cause a significant change in entropy;[13a,39] (3) solvation effects in solution take place for both groups,[13] but the effects must be minor as far as the internal rotation is concerned, although the effects are expected to raise the barrier to rotation through solute–solvent interactions. The thermodynamic quantities cited above were calculated at 25 °C while our experiments were carried out at 50 °C. The temperature dependence of the entropy of internal rotation is negligible for the above temperature difference[40] but the corresponding free energy $T\Delta S$ is 1.08 times larger than $T\Delta S$ for 25 °C. Thus the rate factor at 50 °C should be 14. Considering the uncertainties involved, we prefer the rate factor in the range of 12 to 21.

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Laser-Excited Raman Spectroscopy of Biomolecules. VIII. Conformational Study of Bovine Serum Albumin

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Abstract: From the Raman spectrum of 4% aqueous bovine serum albumin the polypeptide backbone is found to be predominantly α-helical (~60%) as revealed by the distribution of intensity in the amide III region and by the relative sharpness of the amide I line. The remainder of the backbone is random-coil and no evidence for β-pleated-sheet conformation can be detected. The intensity ratio (10:7) of the tyrosine doublet at 852 and 827 cm^{-1} indicates that most of the phenolic hydroxyl groups of the 19 tyrosyl residues are weakly hydrogen bonded. The disulfide line is rather broad and low in frequency (503 cm^{-1}), showing that there is some variation in the local geometries of the disulfide groups. Their conformations, however, appear to be limited to the gauche, gauche, gauche form.

Although the amino acid composition of bovine serum albumin (BSA), a major protein constituent of plasma with a molecular weight of about 67000 daltons, has been known[1] since 1949, the complete sequence has apparently not yet been determined.[2,3] Previous investigations of its conformation include reports on its optical rotatory dispersion,[4,5] infrared absorption,[6] and an earlier study of its Raman spectrum in aqueous solution from this Laboratory.[7] These investigations agree in assigning a large amount of α-helical conformation to the native protein. In the present paper much improved spectra of the protein are reported and since much progress has been made in the interpretation of the Raman spectra of proteins in the past five years, more detailed conclusions about the conformation of BSA can now be drawn, especially about the polypeptide backbone conformation and the state of the tyrosyl residues in the protein.

Experimental Section

BSA of high purity was kindly provided by Dr. E. R. Simons and Professor E. R. Blout of the Harvard Medical School. It was used without further treatment at a concentration of 4% by weight in 0.1 *M* NaCl, pH 6. The solution (3 μl) was placed in a "Kimax" melting point capillary (1 mm i.d.) used as a Raman cell.

Raman spectra were recorded on a Spex Ramalog 4 double-monochromator spectrometer equipped with photon counting electronics, an RCA C31034 photomultiplier, and a Coherent Radiation 52G argon-ion laser tuned to 4880 Å as the exciting line. The scattered radiation was observed at 90° to the incident beam.

Results and Discussion

A representative Raman spectrum of aqueous BSA is shown in Figure 1 as recorded, and the frequencies and relative peak intensities of the Raman lines are listed in Table I. Interpretation of the spectrum begins with those lines that are characteristic of the peptide backbone.

Amide III Region. It has been recognized for some time that the region between 1225 and 1300 cm^{-1} in the Raman spectra of proteins is sensitive to the geometry of the peptide groups making up the backbone of the protein. The amide III vibration has been shown[8-10] to be a mixture of C–N bond stretching and in-plane N–H bond bending, which gives rise to a frequency near 1230–1235 cm^{-1} for