

Stabilization of Carboxylate Anion with a NH···O Hydrogen Bond: Facilitation of the Deprotonation of Carboxylic Acid by the Neighboring Amide NH Groups

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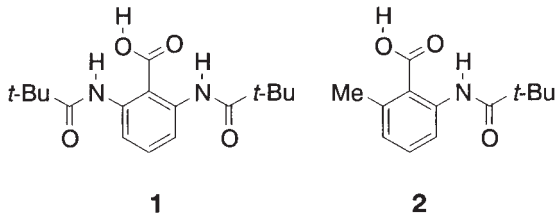
The formation of the NH···O hydrogen bonds of carboxylic acids, 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**) and 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), carboxylate, [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**), and a mixed complex, [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**), were determined by X-ray structure analysis, ^{1}H NMR, and IR spectroscopy, both in the solid state and in solution. The amide NH group forms weak intramolecular hydrogen bond between the NH and O=C group, and no NH···OH hydrogen bond is formed in the carboxylic acid state. Carboxylate anion **3** forms a strong, intramolecular, partially-covalent hydrogen bond between NH···O$^-$ (anion). The strength of the NH···O hydrogen bonds is also maintained in a solution having a low dielectric constant. The pK_a values for **1** and **2**, measured in a micellar solution, indicated that one of the important triggers for deprotonation is a direct interaction toward the oxygen atom of the OH group with the amide NHs.

The change in the pK_a value during an enzymatic reaction is significant for the regulating of the reactivity in the active center of aspartate transcarbamylase (ATcase).[1] In general, the pK_a value of Glu or Asp carboxylic acid in proteins has been considered to depend on the electrostatic interaction around the COOH moiety. Warshel has reported that the enzyme stabilizes ionized acids by combing two contributions: (i) the interaction between the acid charge and the permanent dipoles of the enzyme, ($V_{Q\mu}$), and (ii) the interaction between the acid's charges and the induced dipoles of the enzyme, ($V_{Q\mu}$).[2] It has been theoretically demonstrated that such a model is available to the perturbed pK_a of carboxylic acid inside or outside of biologically important proteins.[2–5] The active center of ATcase shows a different pK_a value between an *N*-(phosphonacetyl)-L-aspartate-binding form or a free one.[6] Thus, we are motivated to experimentally investigate the perturbation to pK_a of carboxylic acid groups by a local electrostatic interaction, such as amide dipoles, which can be recognized as a model of interactions with main chain of proteins, using synthetic the model compounds.

We have synthesized benzoic acid with bulky amide groups at the 2,6-positions for analyses of perturbations from amide groups to the –COOH moiety. The pK_a value of simple carboxylic acids varies with the solvent effect and the electrostatic interaction, including a resonance effect in the case of benzoic acid derivatives.[7] The contribution of the hydrogen bond to lowering the pK_a value has been proposed for *cis*-caronic acid and salicylic acid.[7–10] In these cases, hydroxy groups were thought to direct to the carboxylic acid oxygen. Recently, we reported the presence of a NH···S hydrogen bond between amide NH and thiolate sulfur, which has been established using crystallographic, IR and ^{1}H NMR analyses.[11,12] The relatively large sulfur pπ orbital can interact readily with the amide NH group. A similar NH···O$^-$ hydrogen bond is expected between the carboxylate oxygen and the amide NH, although the pπ of the oxygen atom is smaller than that of the sulfur atom. This paper reports on the properties of NH···O$^-$ hydrogen bonds on the corresponding carboxylic acid oxygen using a series of amidated benzoate compounds (Chart 1).



1 **2**

Chart 1.

Experimental

All solvents were purified by distillation before use. 2,6-Diaminotoluene was synthesized by a method described in our previous report.[13]

2,6-(*t*-BuCONH)$_2$C$_6$H$_3$CH$_3$. To a THF solution (100 mL) of 2,6-diaminotoluene (2.52 g, 2.06×10^{-2} mol) and triethylamine (12.0 mL, 8.24×10^{-2} mol) was added pivaloyl chloride (9.90 mL, 8.24×10^{-2} mol) at 0 °C. After 30 min, volatile materials were removed under reduced pressure. Water was added and white precipitates obtained were collected by filtration. The precipitates were recrystallized by hot methanol. Yield 5.03 g (84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 2H, NH), 8.11 (t, 1H, ArH), 7.00 (d, 2H, ArH), 1.95 (s, 3H, Me), 1.22 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.97, 136.84, 130.79, 124.54, 118.82, 38.57, 27.36, 12.61.

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2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (1). To an aqueous solution (300 mL) of 2,6-bis(pivaloylamino)toluene (2.00 g, 6.89×10^{-3} mol) and magnesium sulfate (0.83 g, 6.90×10^{-2} mmol) was added guranuated potasium permanganate (2.3 g, 1.46×10^{-2} mmol). After vigorous stirring for 6 h, the brown precipitates were removed by filtration and the filtrate was acidified by 12 M (1 M $= 1$ mol dm^{-3}) hydrochloric acid. The obtained white needles were collected by filtration and washed with water. Yield 1.27 g (57.6%). Anal. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74%. Found: C, 63.56; H, 7.62; N, 8.71%. MS (ESI) Calcd (found) *m*/*e*: 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO$^-$, 319.2 (319.2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 2H, NH), 7.92 (d, 2H, ArH), 7.37 (t, 1H, ArH), 1.20 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.79, 169.01, 139.37, 131.22, 116.27, 111.77, 39.41, 27.16.

2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (2). To a THF solution (50 mL) of 2-amino-6-methylbenzoic acid (2.0 g, 13 mmol), triethylamine (2.7 mL, 19 mmol), and pivaloyl chloride (2.0 mL, 16 mmol) were added dropwise at 0 °C. After stirring overnight at room temperature, the reaction mixture was concentrated under reduced pressure to give a brown oil. The oil was dissolved in 200 mL of ethyl acetate and 30 mL of water was added to the solution. The organic layer was successively washed with water, 2% HCl aqueous solution, water, and sat. NaCl aqueous solution, and dried over anhydrous sodium sulfate. The oil, which was obtained by concentration of the solution, was dissolved in hot *n*-hexane and cooled to room temperature. Colorless crystals were obtained and recrystallized from diethyl ether. Yield 1.43 g (46.0%). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95%. Found: C, 66.31; H, 7.14; N, 5.95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H, NH), 9.45 (s, 1H, OH), 8.34 (d, 1H, ArH), 7.39 (t, 1H, ArH), 7.00 (d, 1H, ArH), 2.57 (s, 3H, Me), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.76, 169.29, 137.27, 130.28, 126.13, 124.66, 123.89, 120.04, 39.18, 27.16, 21.42.

4-(*t*-BuCONH)C$_6$H$_4$COOH. To a THF solution (100 mL) of 4-aminobenzoic acid (5.0 g, 36 mmol) was slowly added pivaloyl chloride (4.4 g, 36 mmol) at 0 °C. After stirring for 1 h, 4% $NaHCO_3$ aqueous solution (100 mL) was added and THF was removed under reduced pressure. After being acidified to pH $\sim$ 1 by 10% HCl aqueous solution, products were extracted with ethyl acetate. An oil layer was dried over sodium sulfate, and ethyl acetate was removed under reduced pressure. The residue was recrystallized from hot MeOH. Yield 3.0 g (38%). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33%. Found: C, 64.91; H, 6.84; N, 6.39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1H, COOH), 9.43 (s, 1H, NH), 7.62 (d, 2H, ArH), 7.73 (d, 2H, ArH), 1.23 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6) δ 176.49, 166.55, 143.22, 129.75, 124.70, 119.01, 39.32, 27.02.

[NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (3). Tetraethylammonium acetate tetrahydrate (26.1 mg, 11.0 mmol) and 2,6-bis(pivaloylamide)benzoic acid (32.0 mg, 10.0 mmol) were dissolved in 10 mL of MeOH. After stirring for 30 min, the solvents were removed under reduced pressure. The obtained residue was dissolved in a small amount of ethyl acetate to give colorless needles in 27% yield (12 mg). Anal. Calcd for $C_{25}H_{43}N_3O_4$: C, 66.78; H, 9.64; N, 9.35%. Found: C, 66.33; H, 9.69; N, 9.25%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H, NH), 8.03 (d, 2H, *m*-ArH), 7.26 (t, 1H, *p*-ArH), 3.19 (q, 8H, –CH_2–), 1.23 (s, 9H, *t*-Bu), 1.15 (m, 12H, –CH_3). ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.83, 169.62, 140.21, 130.18, 114.80, 111.57, 51.34, 38.37, 27.28, 7.08.

[N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (4). 2,6-Bis(pivaloylamino)benzoic acid (100 mg, 3.12×10^{-4} mol) and tetrapropylammonium acetate (38.2 mg, 1.56×10^{-4} mol) were dissolved in a mixed solvent (10 mL) of methanol/water (50:50). After evaporation of the solvents, the obtained colorless oil was recrystallized from ethyl acetate. Yield 12 mg (19%). Anal. Calcd for $C_{46}H_{75}N_5O_8 \cdot (H_2O)_{0.5}$: C, 66.16; H, 9.17; N, 8.39%. Found: C, 66.02; H, 9.04; N, 8.41%.

***t*-BuCONHPh.** To a THF solution of aniline (1.0 mL, 1.1×10^{-2} mol) and Et_3N (1.54 mL, 1.10×10^{-2} mol) was slowly added pivaloyl chloride at 0 °C. After stirring for 1 h, an aqueous $NaHCO_3$ solution was added to the solution and THF was evaporated under reduced pressure. The obtained white powder was collected by filtration and recrystallized from hot MeOH. White needles were obtained (500 mg, 26%). Anal. Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90%. Found: C, 74.31; H, 8.56; N, 7.91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NH), 7.62 (d, 2H, *o*-ArH), 7.27 (t, 2H, *m*-ArH), 7.02 (t, 1H, *p*-ArH), 1.23 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.26, 137.89, 128.83, 124.08, 119.84, 39.68, 27.76.

Potentiometric Titration. pH measurements were performed using a Horiba pH Meter M-8s. All pH standard solution, including a 0.1 M NaOH ($f = 1.006$ at 20 °C) solution were purchased from Nacalai tesque. Each pK_a measurement was performed at 21 °C ($\pm$ 1 °C) three times. The concentrations of all solutions were 0.01 M. An aqueous micellar solution was prepared as follows. The samples were dissolved in 0.5% volume (from total volume) of a DMSO solution and a 10% volume of Triton X-100. To the mixed solution was added water, and stirred at a warmed temperature to mix homogeneously. The concentration of Triton X-100 micelle was above a CMC of 2.5×10^{-4} M.

Determination of pK_a Values for Various Carboxylic Acids. The equation below was used to calculate of the pK_a values in aqueous micellar solutions. When arenecarboxylic acid (ArCOOH) is weak acid, [ArCOO$^-$] is equal to [Na$^+$] with [ArCOOH] = [ArCOOH]$_0$ $-$ [ArCOO$^-$]. An equation, K_a = [ArCOO$^-$][H$^+$]/[ArCOOH], leads to pK_a = pH $-$ log[Na$^+$] + log{[ArCOOH]$_0$ $-$ [ArCOO$^-$]}. [ ] and [ ]$_0$ refer to the final concentration and the initial concentration, respectively. A 0.1 M NaOH aquous solution was used to titrate a 0.01 M sample solution at 21 °C.

Physical Measurements. ^{1}H NMR spectra in solutions were taken on a Jeol EX-270 spectrometer. ^{1}H CRAMPS measurements were performed on a CMX 300 spectrometer employing the BR 24 pulse sequence and MAS speed in the range of 1–1.5 kHz. IR spectra were recorded on a Jasco FT-IR/8300 spectrometer. Samples were prepared as KBr pellets or a CH_2Cl_2 solution.

X-ray Structure and Determination. Single crystals of 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**), 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**), and [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**) were sealed in a glass capillary for X-ray measurements. The measurements were performed at 23 °C on a Rigaku AFC7R or AFC5R diffractometer equipped with a rotating anode X-ray generator. The radiation used was Mo Kα monochromatized with graphite (0.71069 Å). The unit cell dimensions were refined by 25 reflections. These standard reflections were chosen and monitored with every 150 reflections, and did not show any significant change. The structures were solved by a direct method and expanded using Fourier techniques using *teXsan* crystallographic software[14] and SHELXL-97.[15] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined only for their positions. Crystal data for **1**: $C_{17}H_{24}N_2O_4$, 320.38, monoclinic, space group $P2_1/a$, with $a = 8.894(2)$, $b = 15.095(3)$, $c = 13.415(2)$ Å, $\alpha = 90.00$, $\beta = 106.267(15)$,

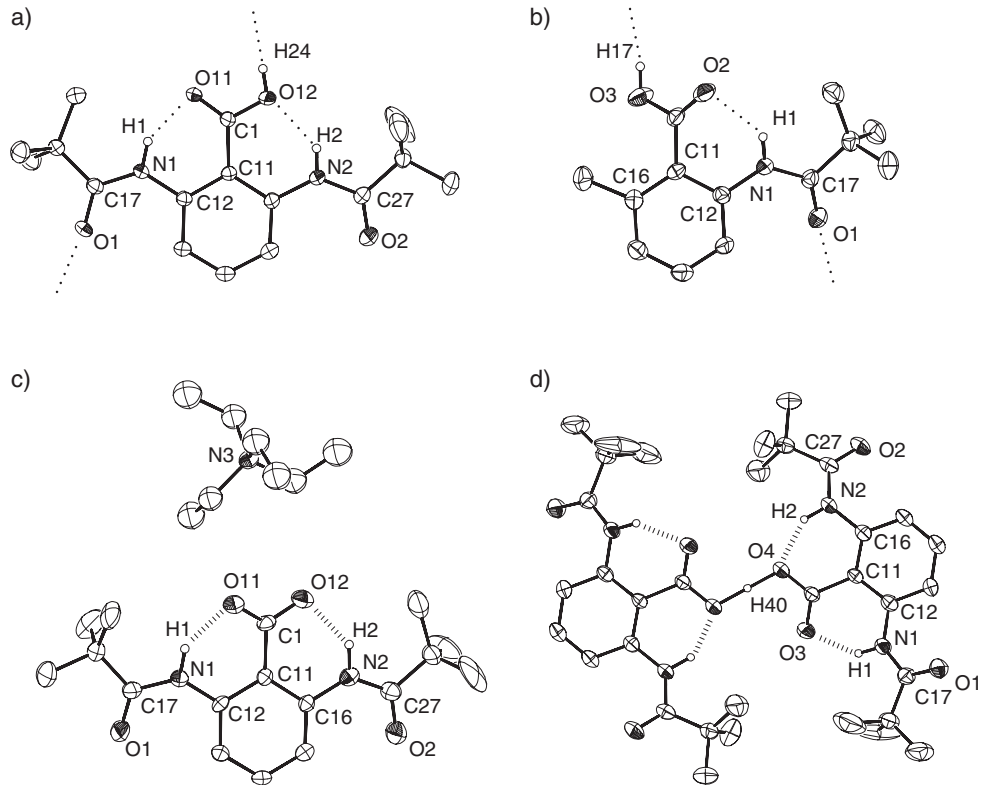

Fig. 1. ORTEP drawings of a) 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**), b) 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), c) anion part of [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**), and d) [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**).

$\gamma = 90.00$, $V = 1728.9(6)$ Å^3, $Z = 4$, $D_{calc} = 1.231$ g/cm^3, no. reflns used = 3970 (all data), $R1 = 0.061$, $wR2 = 0.209$, and GOF = 1.12. Crystal data for **2**: $C_{13}H_{17}NO_3$, 235.28, monoclinic, space group $P2_1/a$, with $a = 10.8443(14)$, $b = 10.4408(14)$, $c = 11.5919(10)$ Å, $\alpha = 90.00$, $\beta = 93.943(9)$, $\gamma = 90.00$, $V = 1309.4(3)$ Å^3, $Z = 4$, $D_{calc} = 1.194$ g/cm^3, no. reflns used = 2569 (all data), $R1 = 0.063$, $wR2 = 0.198$, and GOF = 0.94. Crystal data for **3**: $C_{25}H_{43}N_3O_4$, 449.62, orthorhombic, space group $P2_12_12_1$, with $a = 15.136(3)$, $b = 17.019(4)$, $c = 10.417(3)$ Å, $V = 2683.4(11)$ Å^3, $Z = 4$, $D_{calc} = 1.113$ g/cm^3, no. reflns used = 2973 (all data), $R1 = 0.057$, $wR2 = 0.207$, and GOF = 0.95. Crystal data for **4**: $C_{23}H_{37.50}N_{2.50}O_4$, 413.05, monoclinic, space group $C2/c$, with $a = 30.838(4)$, $b = 12.063(4)$, $c = 13.793(4)$ Å, $\alpha = 90.00$, $\beta = 104.618(19)$, $\gamma = 90.00$, $V = 4965(2)$ Å^3, $Z = 8$, $D_{calc} = 1.105$ g/cm^3, no. reflns used = 3246 (all data), $R1 = 0.070$, $wR2 = 0.208$, and GOF = 1.19.

Molecular Orbital Calculations. Ab initio molecular orbital calculations for the carboxylic acid, 2-(*t*-BuCONH)C$_6$H$_4$COOH, and the corresponding carboxylate, 2-(*t*-BuCONH)C$_6$H$_4$COO$^-$, were carried out with the assumption of an idealized coplanar structure between amide and carboxylic acid or carboxylate planes using the Gaussian 98 system of programs.[16] Single point calculations with HF and MP2 levels of theory were performed using the 6-31+G** basis set. DFT calculations using Becke's three-parameter hybrid functional with the correlation functional of Lee, Yang, and Parr (B3LYP)[17–19] were also performed using 6-31+G** basis set. An estimation of the solvation energy in aqueous solution was taken into account by using the PCM method.[20,21]

Results and Discussion

Crystal Structures of Arenecarboxylic Acids and Arenecarboxylates.

In order to investigate the structural difference between carboxylic acid and carboxylate, the crystal structures of 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**) and 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**) as carboxylic acids, and [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**) as a carboxylate and [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**) as a mixed complex of both states, were determined by X-ray analysis. The location of amide groups near carboxylic acid or carboxylate was shown to promote the formation of a NH···O hydrogen bond between an oxygen atom and an amide NH. Figure 1 shows the crystal structures of **1**, **2**, **3**, and **4**. The selected bond distances and bond angles are listed in Table 1.

The crystal structure of a carboxylic acid, **1**, with two amide groups at the *o*,*o*′-positions has one C–O single bond (1.319(3) Å) and one C=O double bond (1.205(3) Å). The two amide NHs direct to each oxygen atom of carboxylic acids with two different N···O distances (2.576(2) and 2.614(3) Å), corresponding to the two different amide C–O bond distances. The long C17=O1 distance (1.227(3) Å) is due to the presence of intramolecular N1–H1···O11=C1 and intermolecular O12′–H24′···O1=C17 hydrogen bonds. The difference between the N1–C17 (1.341(3) Å) and N2–C27 (1.361(3) Å) bond distances is caused by a large bond order of the conjugated N1–C17 bond, involving the inter- and intramolecular hydrogen bonds of the amide group. The results indicate that the hydrogen-bond interaction of N2–H2···O12H is weaker than that of N1–H1···O11=C1 in **1**.

The crystal structure of **2**, with one amide group at the *o*-position of the aromatic ring, indicates the location of the amide group towards the C=O of the carboxyl group. The C–O bond

Table 1. Selected Structural Parameters for **1**, **2**, **3**, and **4**

1		**2**		**3**		**4**	
			Carboxyl group				
C1–O11	1.205(3)	C1–O2	1.205(4)	C1–O11	1.239(7)	C1–O3	1.225(5)
C1–O12	1.319(3)	C1–O3	1.294(4)	C1–O12	1.244(8)	C1–O4	1.289(5)
			Amide group				
C17–O1	1.227(3)	C17–O1	1.224(4)	C17–O1	1.211(8)	C17–O1	1.218(6)
C27–O2	1.209(3)			C27–O2	1.230(8)	C27–O2	1.204(6)
N1–C17	1.341(3)	N1–C17	1.340(4)	N1–C17	1.346(8)	N1–C17	1.355(6)
N2–C27	1.361(3)			N2–C27	1.340(8)	N2–C27	1.350(6)
			Hydrogen bond distances and angles				
N1···O11	2.576(2)	N1···O2	2.648(3)	N1···O11	2.552(7)	N1···O3	2.613(4)
N2···O12	2.614(3)			N2···O12	2.569(7)	N2···O4	2.639(4)
N1H1···O11	140.9	N1···O2	128.1	N1H1···O11	136.8	N1H1···O3	132.5
N2H2···O12	135.2			N2H2···O12	135.6	N2H2···O4	136.3
			Angle between benzene ring and COO plane				
	17.8		29.8		15.7		26.5
			Angle between benzene ring and amide plane				
N1 amide	3.8		33.7		11.8		26.8
N2 amide	21.9				19.3		17.1

distances of carboxylic acid are 1.205(4) Å for C1=O2 and 1.294(4) Å for C1–O3 in **2**. The difference of 0.089 Å in the two C–O bond distances indicates that the carboxylic acid consists of one C=O double bond and one C–OH single bond. The amide NH directs to the C=O oxygen atom to form an NH···O=C hydrogen bond with an appropriate N···O distance of 2.648(3) Å.

The crystal structure of the anion part of **3**, with two amide groups at the *o*,*o*′-positions, indicates that two carboxylate C–O bond distances, C1–O11 and C1–O12, are 1.239(7) and 1.244(8) Å, respectively. This is because conjugation of the two C–O bonds in COO^- anion. The two amide NHs form NH···O–C hydrogen bonds to both oxygen atoms of the carboxylate. Comparing the bond distances of both amide groups, the bond length of C17=O (1.211(8) Å) and C27=O2 (1.230(8) Å) are in a similar range of the N2 amide CO bond of **1**. The N1–C17 bond (1.346(8) Å) and the N2–C27 bond distance (1.340(8) Å) also indicate that both amide groups are involved in NH···O hydrogen bonds. The torsion angles of N1H1···O11C1 and N2H2···O12C1 are 6.1 and −33.3°, respectively, indicating that the N2H2···O12 hydrogen bond gives a more preferable geometry than the N1H1···O11 one.

Figure 1d shows the crystal structure of a mixed complex, $[N(n\text{-}Pr)_4][H\{2,6\text{-}(t\text{-}BuCONH)_2C_6H_3(COO)\}_2]$ (**4**), containing both carboxylic acid and carboxylate states. Two carboxyl groups share the same proton with a distance of 1.228(3) Å. Such a short O···O distance of 2.456 Å clearly indicates that the complex forms a low-barrier hydrogen bond consisting of a proton intermolecularly located between the two oxygen atoms of the C–O anion groups in each carboxylate. Actually, the bond distance of C1–O3(H) (1.225(5) Å) and C1–O4 (1.289(5) Å) lies between those of C–O(H) and C–O bond in **1** and **3**. Similar low barrier hydrogen bonds have been found for various carboxylic acids compounds.[22–36]

IR Spectra of Carboxylic Acid and Carboxylate in the Solid State and Solution. Table 2 lists the IR bands in the NH and C=O amide regions for carboxylic acids, 2,6-(*t*-Bu-$CONH)_2C_6H_3COOH$ (**1**) and 2-*t*-BuCONH-6-MeC_6H_3COOH (**2**), and carboxylate, $[NEt_4][2,6\text{-}(t\text{-}BuCONH)_2C_6H_3COO]$ (**3**) in the solid state, compared with those IR bands in a dichloromethane solution. The free amide NH bands are known to appear in the range of 3400–3500 cm^{-1}.[37] **1** shows a free NH band at 3407 cm^{-1} in the solid state. The two amide C=O bands are observed at 1694 and 1636 cm^{-1}. The former band is assigned to the amide whose NH is not hydrogen-bonded with carboxylic acid C=O. The latter band is assigned to the other, which is intramolecularly hydrogen-bonded with carboxylic acid C=O, and also intermolecularly hydrogen-bonded with O–H···O=C interaction. **2** exhibits slightly shifted NH bands at 3330 cm^{-1} assignable to a weak NH···O=C (carboxylic acid) hydrogen bond. The amide ν(CO) bands are observed at 1639 cm^{-1}, due to the amide C=O band participating in the intramolecular NH···O=C (carboxylic acid) hydrogen bond and intermolecularly OH···O=C hydrogen bond. In the carboxylate anion state, the ν(NH) band appears at 3024 cm^{-1}, indicating that the presence of the strong NH···O hydrogen bonds in the solid state. **4** also exhibits a shifted ν(NH) band at 3269 cm^{-1}. Thus, the IR results clearly corroborate that the strength of the NH···O hy-

Table 2. Selected IR Bands (cm^{-1}) in the Amide Region of **1**, **2**, **3**, and **4**

	ν(NH)		ν(CO) (amide)	
	Solid	Solution[a)]	Solid	Solution[a)]
1	3407	3444	1694, 1636	1686
2	3330	3442	1639	1688
3	3024	—[b)]	1664	1662
4	3269	—[c)]	1676	—[c)]

a) In 10 mM dichloromethane solution at room temperature. b) NH signal was broad and unassignable. c) Complex **4** decomposes in the solution.

drogen bonds depends on the basicity of the carboxyl group, even though little geometrical difference has been found in X-ray analyses.

The IR spectra of **1**, **2**, and **3** in 10 mM dichloromethane were obtained at room temperature in order to investigate the intramolecular interaction. A standard amide compound, PhNHCO-*t*-Bu, gives a free amide NH band at 3451 cm^{-1} and a corresponding free CO band at 1682 cm^{-1}, without an inter- or intramolecular interaction in the solution. Carboxylic acid, **2**, indicates an NH band at 3442 cm^{-1} and a CO band at 1688 cm^{-1}, whereas **1** also exhibits two bands at 3444 and 1686 cm^{-1}. These bands of **1** and **2** in dichloromethane indicate that the amide NH and CO groups are not involved in any strong hydrogen bonding. We observed OH (carboxylate) stretching bands for **1** and **2** at ca. 3350 and 3500 cm^{-1}, respectively, as broad bands. Thus, the presence of an amide NH interaction increases the acidity of the carboxyl OH proton. The NH stretching in carboxylate **3** could not be detected in the 2800–3600 cm^{-1} region. The CO band of **3** in the carboxylate anion appears at 1662 cm^{-1}, shifting 20 cm^{-1} from that of the standard PhNHCO-*t*-Bu. The 20 cm^{-1} shift is due to the formation of a strong NH···O=C (carboxylate) hydrogen bond.[38]

^{1}H NMR Spectra (CRAMPS) in the Solid State. Figure 2 shows the ^{1}H NMR spectra of carboxylic acids, 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**) and 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), and carboxylate, [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**) and [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**) in the solid state. **1** shows two amide NH signals at 10.2 and 12.5 ppm assignable to the non-hydrogen bonding amide NH and the intramolecularly NH···O=C hydrogen-bonded NH groups, respectively. A carboxylic acid OH signal was observed at 14.2 ppm. Similarly, an amide NH signal in **2** was observed at 11.6 ppm, which is slightly involved in a weak intramolecular NH···O=C hydrogen bond. A carboxylic acid OH signal appears at 14.1 ppm, that is similar to a common chemical shift for the carboxylic acid proton in the solution. Carboxylate **3** exhibits one broad signal at 14.7 ppm due to the intramolecularly hydrogen-bonded amide NH group in the solid state. Complex **4**, consisting of carboxylic acid and carboxylate, shows a weak hydrogen-bonded amide NH signal at 12.7 ppm and a shifted OH signal at 20.2 ppm. This supports the presence of a low-barrier hydrogen-bonded proton, as reported by the crystallographic analysis of various carboxylate–carboxylic acid complexes. Thus, the low field shift of amide NH signals reflects the acidity of the amide proton forming an NH···X hydrogen bond, as well as the low field shift of OH signal with the increased acidity. The ^{1}H shift of amide NH signal reflects extent of the hydrogen bond of the carboxylic acid and the carboxylate.

^{1}H NMR Spectra in Solution. In order to examine whether amide NH in carboxylic acids **1** and **2** forms an NH···OH hydrogen bond in carboxylic COOH, the ^{1}H NMR spectra were measured in chloroform-*d*, which is a hydrogen bond-supporting solvent with a relatively low dielectric constant ($\varepsilon = 7$). The chemical shift of the amide NH ^{1}H NMR signal is one of the appropriate monitors for the detection of various hydrogen bonds involving the amide NH. The temperature-dependent gradient for 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**) is estimated in 0.0058 ppm K^{-1} in the range of −30–50 °C. The value in $CDCl_3$ for a linear peptide is known to be 0.0024 ppm K^{-1},

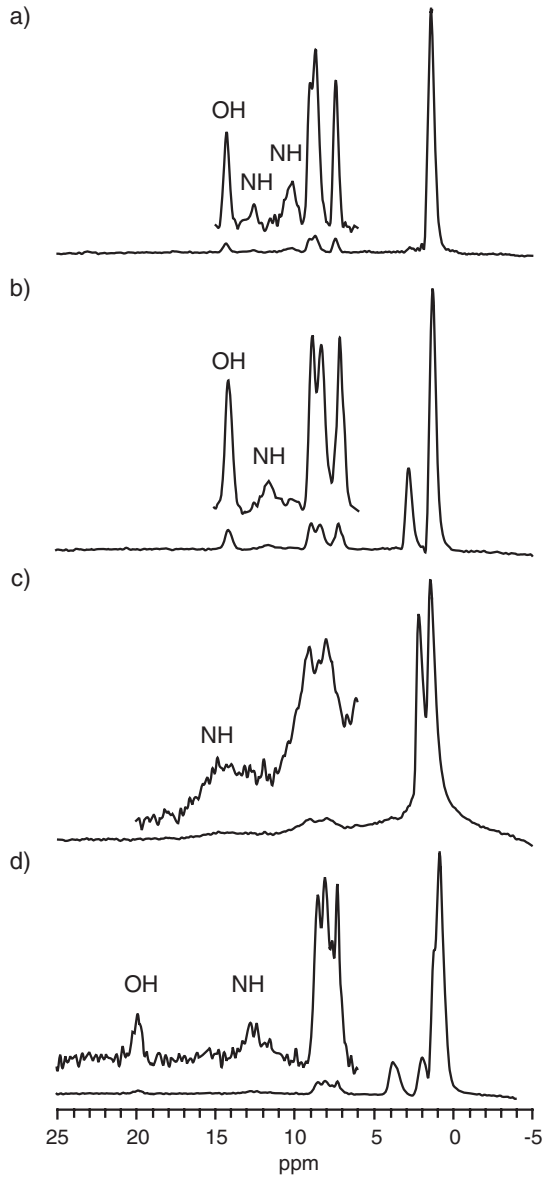

Fig. 2. ^{1}H NMR CRAMPS spectra of a) 2,6-(*t*-BuCONH)$_2$-C$_6$H$_3$COOH (**1**), b) 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), c) [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**), and d) [N(*n*-Pr)$_4$][H{2,6-(*t*-BuCONH)$_2$C$_6$H$_3$(COO)}$_2$] (**4**) in the solid state.

which exhibits no participation of a intramolecular hydrogen bond.[39] 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**) also shows a large value of 0.0038 ppm K^{-1} without a hydrogen bond. No participation of a NH···O (carboxylic acid COOH) hydrogen bond in solution is consistent with the results of IR spectra in the amide region.

On the other hand, carboxylate, [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**), gives a smaller gradient value of 0.00078 ppm K^{-1} than a reported common gradient value for a linear peptide. The amide NH chemical shift of **3**, (13.53 ppm), shifts downfield from that of **1**, (10.19 ppm), as shown in Fig. 3. These results indicate that **3** has a strong NH···O hydrogen bond in solution. Thus, the IR results in the solid state and in solution reveal that the amide NH *in carboxylic acid*

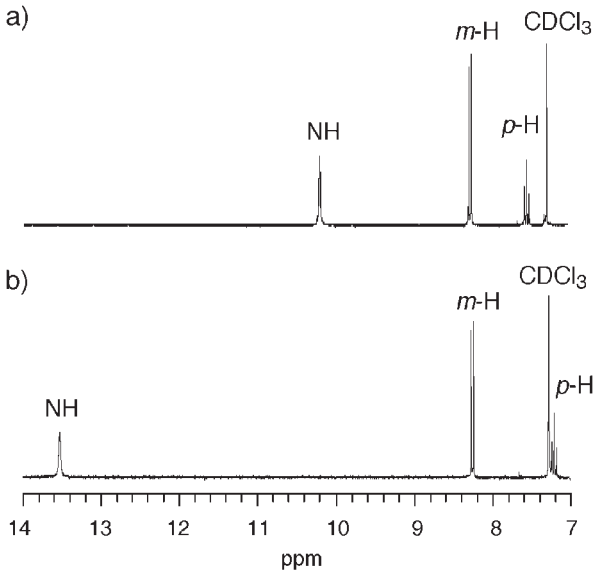

Fig. 3. ^{1}H NMR spectra of a) 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$-COOH (**1**) and b) [NEt$_4$][2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COO] (**3**) in 10 mM $CDCl_3$ solution.

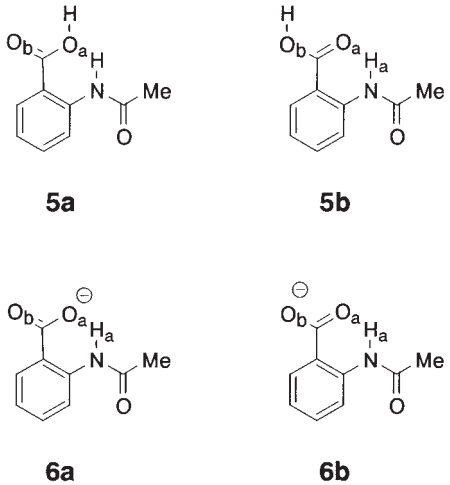

Chart 2.

Table 3. Selected Results of Ab initio Calculations

		In gas phase				In aqueous solution			
		Charge			Overlap population	Charge			Overlap population
Models		O^a	O^b	H^a(N)	$O^a \cdots H^a$(N)	O^a	O^b	H^a(N)	$O^a \cdots H^a$(N)
5a	HF	−0.734	−0.536	0.451	−0.0262	−0.764	−0.607	0.486	−0.0250
	MP2	−0.732	−0.535	0.451	−0.0259	−0.764	−0.608	0.486	−0.0250
	B3LYP	−0.590	−0.442	0.371	0.0020	−0.627	−0.508	0.403	0.0036
5b	HF	−0.609	−0.585	0.444	0.0050	−0.666	−0.616	0.465	0.0035
	MP2	−0.609	−0.585	0.444	0.0050	−0.666	−0.616	0.460	0.0035
	B3LYP	−0.514	−0.457	0.365	0.0270	−0.572	−0.486	0.380	0.0258
6a	HF	−0.863	−0.648	0.461	0.0666	−0.999	−0.758	0.472	0.0273
	MP2	−0.860	−0.649	0.461	0.0688	−1.000	−0.758	0.472	0.0274
	B3LYP	−0.684	−0.540	0.364	0.0875	−0.842	−0.661	0.377	0.0549
6b	HF	−0.822	−0.675	0.449	0.0369	−1.017	−0.741	0.466	0.0070
	MP2	−0.824	−0.674	0.450	0.0353	−1.017	−0.741	0.466	0.0070
	B3LYP	−0.679	−0.534	0.353	0.0590	−0.898	−0.612	0.383	0.0300

state does not form a NH···O hydrogen bond, but the amide NH *in carboxylate anion state* can inherently make a hydrogen bond in solution. Furthermore, the carboxylic acid in the solid state gives a weak NH···O=C hydrogen bond, but the bond is not found in solution.

Ab initio Calculations. Ab initio molecular orbital calculations of 2-(*t*-BuCONH)C$_6$H$_4$COOH (**5a**, **5b**) and {2-(*t*-BuCONH)C$_6$H$_4$COO} (**6a**, **6b**) were carried out to investigate the NH···O hydrogen bonding interaction. The calculation was performed using the 6-31+G** base set to obtain the overlap population for the carboxylic acid and the carboxylate as simplified models. Although the crystal structure shows that the amide plane is not coplanar to the aromatic ring, we use model structures fixing the carboxyl and amide plane to a similar plane of the benzene ring (Chart 2). The results are summarized in Table 3. Increasing overlaps due to hydrogen bonding were observed in the carboxylate anion state both in the gas phase and in aqueous solution. The calculated overlap populations of the electron density for **5a** and **5b**, which are in the carboxylic acid state, are 0.0020 and 0.0270, respectively, in DFT methods, whereas those of **6a** and **6b** in the carboxylate anion state are 0.0875 and 0.0590, respectively. Similar tendencies are also observed in HF and MP2 methods. The values of the overlap population of **5a** and **5b** (in carboxylic acid state) in aqueous solution are almost similar to that in the gas phase. The carboxylate anion state (**6a** and **6b**) gives a relatively stronger covalency than that of the carboxylic acid state. The covalency in aqueous solution was, however, decreased compared with a gas phase. In a polar solvent, stabilization of the carboxylate can also work but the random orientation of the dipolar interaction of the polar solvent can not be as effective as the amide groups with a readily formable orientation. The results indicate that the intramolecular NH···O hydrogen bond interaction from amide NH is interfered by solvation, which can

be explained by the previous report.[40] The increased orbital overlaps between (N)H···O indicate the presence of a partial covalency between the amide NH proton and the O atom of the carboxylate anion.

pK_a Shift by NH···O Hydrogen Bond. The pK_a values of various *o*-substituted benzoic acids (ArCOOH), such as 2,4,6-$(CH_3)_3C_6H_2COOH$ without an amide group, 2-*t*-BuCONH-6-MeC_6H_3COOH (**2**) with one amide group, and 2,6-(*t*-$BuCONH)_2C_6H_3COOH$ (**1**) with two amide groups near the carboxylic acid group, were then measured. The pK_a measurements were carried out in a micellar solution in order to examine the effect of amide NH on the pK_a shift of the carboxylic acid. A water-insoluble carboxylic acid allowed us to measure the pK_a value in an aqueous micellar solution. Although the micellar solution consisted of two heterogeneous layers: (hydrophobic and hydrophilic), a titration method was utilized because of rapid proton transfer. Thus, the effect of the NH···O hydrogen bond formed in the hydrophobic layer of the micellar solution can be detected. Figure 4 summarizes the pK_a values of these carboxylic acids in an aqueous solution and in an aqueous micellar solution (10% Triton, 0.5% DMF). The pK_a values of C_6H_5COOH and 2,4,6-$(CH_3)_3C_6H_2COOH$ are 4.6 and 4.8, respectively. *p*-Substituted 4-(*t*-$BuCONH)C_6H_4COOH$ gives the value of 5.4. **1** and **2** which can form single and double hydrogen bond show lower shifted pK_a values of 3.1 and 3.9, respectively. Therefore, the experiment reveals that the *o*-substituted amide groups lower the pK_a values of the carboxylic acid group.

We measured the pK_a values with the corresponding acetylamino derivatives[13] soluble in an aqueous solution. The pK_a

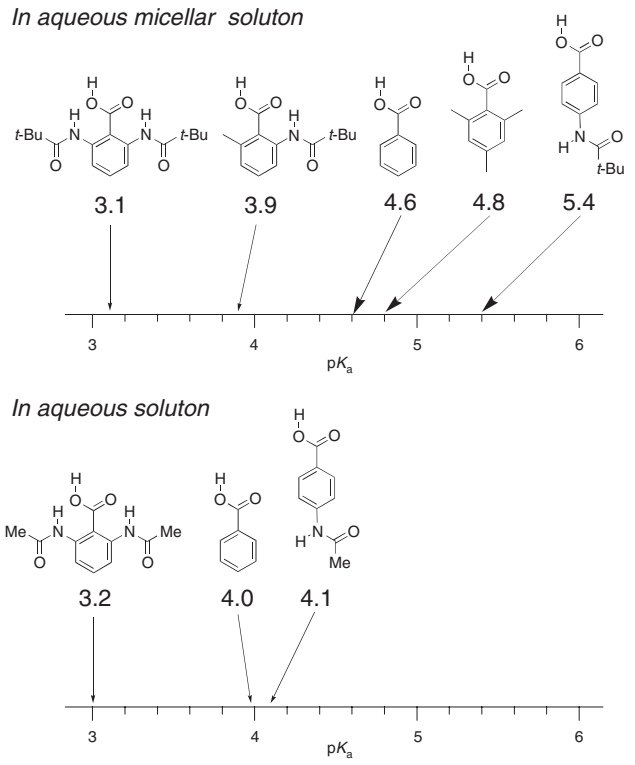

Fig. 4. Summary of pK_a values of series of benzoic acid derivatives.

value of 2,6-$(MeCONH)C_6H_3COOH$ is 3.2, which shifts 0.8 unit lower than that of C_6H_5COOH (4.0). Thus, we have also observed that NH···O hydrogen bonds lower the pK_a values in an aqueous solution. Generally, the pK_a values shift higher in the more hydrophobic environment. Actually, the benzoic acid derivatives without hydrogen bonds in an aqueous micellar solution exhibit around 1 unit higher pK_a than in an aqueous solution. For example, the pK_a value of 4-(*t*-$BuCONH)C_6H_4COOH$ is 5.4 in a micellar solution and that of 4-(MeCONH)-C_6H_4COOH is 4.1 in an aqueous solution. However, the hydrogen-bonded derivative, **1**, shows similar pK_a values to that in an aqueous solution even though in a hydrophobic environment. The results indicate that the pK_a lowering effect of the NH···O hydrogen bond is stronger under hydrophobic circumstances.

Formation of a NH···O Hydrogen Bond between Amide NH and Carboxylate Anion. The crystallographic analysis, IR spectra, and the solid ^{1}H NMR spectra (CRAMPS) of two carboxylic acids, 2,6-(*t*-$BuCONH)_2C_6H_3COOH$ (**1**) and 2-*t*-BuCONH-6-MeC_6H_3COOH (**2**), indicate that the amide NH group forms a very weak intramolecular hydrogen bond between the NH and O=C group and the NH···OH hydrogen bond in the solid state. The presence of a weak NH···OH hydrogen bond of **1** and **2** is supported by the small bond order and a somewhat positively charged OH oxygen obtained from ab initio MO calculations. The large bond order between NH and O^- (anion) atoms was due to charge transfer from the O^- (anion) oxygen, mainly to amide groups as estimated by the ab initio MO calculations. The carboxylate anion **3** has a strong, intramolecular, partially covalent hydrogen bond between the NH···O^- (anion), as demonstrated by crystallographic, IR and solid ^{1}H NMR data. In a solution consisting of a low dielectric constant solvent, the solution structures of **1** and **3** by ^{1}H NMR and IR spectroscopic analyses also indicate the formation of NH···O^- (anion) hydrogen bond in a hydrogen bond-supporting solvent, such as chloroform or dichloromethane.

Lower Shift of pK_a Value by Neighboring Amide NH to Carboxylic OH in Aqueous Micellar Solution. The pK_a values of various carboxylic acids were determined in an aqueous solution to obtain an accurate, reversible, deprotonating point of COOH. However, to evaluate the effect of amide group adjacent to the COOH group in the 2,6-substituted benzoic acids, a low dielectric constant solvent is crucial during deprotonation because of the necessity of maintaining the NH···O hydrogen bond. Therefore, our pK_a measurements were carried out in a 10% Triton X-100 aqueous micellar solution. Such a micellar solution addresses the above two requirements because a proton diffusion rate from micelles to the water part in an aqueous micellar solution occurs quickly enough to record an accurate pK_a value.[41,42] In addition, the inner part in the micelles can support the NH···O^- and NH···O=C hydrogen bonds in hydrophbic environments. The significant pK_a values of **1** and **2** in an aqueous micellar solution are associated with the formation of the NH···O^- (anion) hydrogen bond after deprotonation. In this case, the location of amide NH near the carboxylic acid OH oxygen facilitates deprotonation of the COOH. In our complexes, the amide group at the *o*-position allows an evaluation of the chemical function of the NH···O hydrogen bond. This is because the electronic effect of the amide group is negligible (zero of Hammett σ_p) when the *p*-position is substituted.

One of the important triggers for deprotonation is a direct interaction toward the oxygen atom of the OH group with the amide NH. Of course, the hydrogen bonding or electrostatic interaction of OH with an electron donor, like OH···Donor is also an important factor in facilitating deprotonation.[43,44] Shan and Herschlag have pointed out the importance of a charge rearrangement during deprotonation, in DMSO, of salicylic acid with the hydrogen bond by the adjacent OH.[45] Depronation facilitation of carboxylic acid by neighboring amide NH groups is also thought to be important in understanding the controlled pK_a values of carboxylic acids in proteins.

Conclusion

The formation of the NH···O hydrogen bonds of carboxylic acids, 2,6-(*t*-BuCONH)$_2$C$_6$H$_3$COOH (**1**) and 2-*t*-BuCONH-6-MeC$_6$H$_3$COOH (**2**), carboxylate, [NEt$_4$][2,6-(*t*-BuCONH)$_2$-C$_6$H$_3$COO] (**3**) was determined both in the solid state and in solution. Although an X-ray analysis indicates that the amide NHs intramolecularly interact with O=C of the carboxyl group in the –COOH state, the NH protons are not involved in the strong NH···O hydrogen bonds. The –COO$^-$ state only gives the intramolecular, partially-covalent, strong NH···O$^-$ (anion) hydrogen bond. The strength of the NH···O$^-$ hydrogen bonds is also maintained in a solution with a low dielectric constant. The pK_a values for **1** and **2**, measured in a micellar solution, indicated that a direct interaction toward the oxygen atom of OH group with the amide NH is important for lowering the pK_a values.

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The crystallographic data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

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