Amino Acid Chemistry in Dipolar Aprotic Solvents: Dissociation Constants and Ambident Reactivity

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The pK_a's of 10 amino acids and their esters were measured spectrophotometrically in dilute Me_2SO solution. The pK_a's of the α-amino acids ranged from 6.3 to 7.5 and the esters from 6.4 to 8.7. The ratio of zwitterion to uncharged form of the amino acids in Me_2SO ranged from 2 to 40, compared to 10^4–10^5 in aqueous solution. The primary reason for this difference is the greater solvation of the carboxylate anion in water compared to Me_2SO, with solvation of the ammonium ion being similar in the two solvents. Although the zwitterion predominates in dipolar aprotic solvents, N-alkylation was competitive with O-alkylation. Reaction of the unprotected amino acids with alkyl halides gave 50–70% yield of esters. Using alkylating agents with "harder" leaving groups increased the ester yield to 75–85%. O-Alkylation yields were improved from 50–70% to 85–90% by addition of 2 equiv of LiBr to the reaction mixture. The improvement in O vs. N selectivity was attributed to a salting in of the zwitterionic form of the amino acid.

Amino acids and peptides constitute one of the most widely studied classes of compounds in chemistry.[1] In synthetic chemistry the amino acids provide a source of enantiomerically pure starting materials for elaboration to more complex molecules. In physical chemistry a vast body of data is available on the physical constants of amino acids in aqueous solution, as crystalline solids and, to some extent, even in the gas phase. Despite this, little is known about the chemistry of amino acids in dipolar aprotic solvents, either as regards thermodynamic constants or in synthetic applications. One reason for this dearth of knowledge is that in vivo reactions take place in aqueous media, so interest in amino acid properties in aqueous solution has predominated. However, in the past two decades it has been shown that water may not be a very good model for in vivo reactions. In enzymes, membranes, and other biologically important media, pK_a's are far different from those in water, as these media tend to be lipophilic rather than hydrophilic.[2] It has been suggested that solvents such as Me_2SO and ethanol provide a better model for in vivo reactions.[3] It is clear that studies in media other than water will be valuable in further understanding amino acid chemistry in living systems. In addition, data in solvents other than water will complement the vast body of data in the solid and gas phases and in aqueous solution, providing insights into solute/solvent interactions. As a start to providing this kind of information on amino acids, we present our studies on (a) the equilibrium acidities of amino acids and their esters in Me_2SO solution and (b) the ambident reactivity of amino acids toward alkylating agents, which culminated in an efficient way to esterify amino acids without resorting to N-protection.

Results and Discussion

I. Equilibrium Acidities and Zwitterionic Content of Amino Acids in Me_2SO. A. pK_a Measurements.

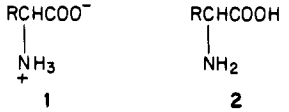
Table I. Dissociation Constants of Amino Acids (Me_2SO Solution at 25 °C)

amino acid	pK_a (app)	pK_a (ester)	pK_{COOH}[a]	pK_{NH}[a]	K_Z
$H_3N^+CH_2COOH$	7.5[b]	8.7[e]	7.5	9.1	40
$MeN^+H_2CH_2COOH$	6.8	7.85[e]	6.8	8.25	27
$Me_2N^+HCH_2COOH$	6.3	6.38[e]	6.45	6.78	2
$Me_3N^+CH_2COOH$	6.4 (6.3)[b]		6.4		
$HOCH_2CH(N^+H_3)$-COOH	7.2	8.3[d]	7.2	8.7	30
$PhCH_2CH(N^+H_3)COOH$	6.8	7.6[d]	6.8	8.0	16
3,4-$(HO)_2PhCH_2C(CH_3)$-$(N^+H_3)COOH$	7.25	7.8[c]	7.3	8.2	9
$H_3N^+(CH_2)_4CH(N^+H_3)$-COOH	6.6	7.75[d]	6.6	8.15	34
$HOOCCH_2CH_2CH$-$(N^+H_3)COOH$	6.7	7.7[d]	6.7	8.1	24
$H_3N^+CH_2CH_2CH_2$-COOH	9.9 (10.3)[b]	10.8[e]	9.9	11.2	19

[a] pK_a's of the amino acids as defined by eq 1a and 1b. [b] Extrapolated from measurements in Me_2SO/water mixtures.[17] [c] Pivaloylethyl ester. [d] Methyl ester. [e] Ethyl ester.

The pK_a's of 10 amino acids and their methyl or ethyl esters were measured in dilute Me_2SO solution (1–2 mM) by the method developed by Matthews, Bordwell, et al.[4] and are listed in Table I. Further details are given in the Experimental Section.

B. Zwitterion vs. Uncharged Form of Amino Acids in Me_2SO Solution. In 1916 Adams[5] recognized, on the basis of dissociation constants, that α-amino acids existed as dipolar ions (1) rather than as uncharged molecules (2) in aqueous solution. Subsequent refined measurements



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in the 1920s and 1930s by Ebert[6] and Edsall and Blanchard[7] confirmed this finding, and these experimentors determined that the ratio of dipolar ion to uncharged molecule for the simple α-amino acids in water was $>10^4$.

(1) Reviews: (a) *Amino Acids, Peptides, and Proteins*; Royal Society of Chemistry: Herts, England, 1969–1983; Vol. 1–14. (b) Cohn, E. J.; Edsall, J. T. *Proteins, Amino Acids, and Peptides*; Reinhold: New York, 1943. (c) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 1–3.

(2) (a) Murdock, A. L.; Grist, L. K.; Hirs, C. H. W. *Arch. Biochem. Biophys.* **1966**, *114*, 375. (b) Hill, R. J.; Davis, R. W. *J. Biol. Chem.* **1967**, *242*, 2005. (c) Timasheef, S. N.; Gorbunoff, M. J. *Annu. Rev. Biochem.* **1967**, *36*, 40. (d) Carty, R. P.; Hirs, C. H. W. *J. Biol. Chem.* **1968**, *243*, 5254. (e) Schmidt, D. E., Jr.; Westheimer, F. H. *Biochemistry* **1971**, *10*, 1249.

(3) Crosby, J.; Stone, R.; Lienhard, G. E. *J. Am. Chem. Soc.* **1970**, *92*, 2891–2900.

(4) Matthews, W. S.; Bares, J. E.; Bartmess, J. E.; Bordwell, F. G.; Cornforth, F. J. Drucker, G. E.; Margolin, Z.; McCallum, R. J.; McCollum, G. J.; Vanier, N. R. *J. Am. Chem. Soc.* **1975**, *97*, 7006–7114.

(5) Adams, E. Q. *J. Am. Chem. Soc.* **1916**, *38*, 1503–1510.

(6) Ebert, L. *Z. Phys. Chem. (Leipzig)* **1926**, *121*, 385.

(7) Edsall, J. T.; Blanchard, M. H. *J. Am. Chem. Soc.* **1933**, *55*, 2337–2353.

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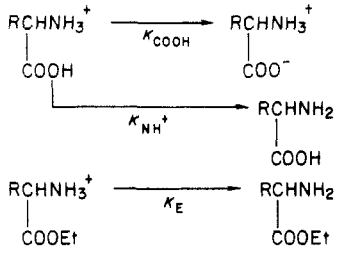
As the interest in amino acid chemistry grew and as modern instrumentation became available, investigations of amino acid structures in media other than water began. In the 1950s IR studies showed that α-amino acids in the solid phase also existed as the dipolar ion (1), stabilized by intermolecular hydrogen bonding.[8] More recent X-ray and neutron diffraction studies on amino acids have given more precise details of bond lengths, bond angles, and packing arrangements of the dipolar ion structure in crystals.[9] In contrast to the data in the solid phase and in aqueous solution, theoretical studies have suggested[10] that the uncharged form is more stable than the dipolar ion form in the gas phase by 29 to 100 kcal/mol.[11] The microwave spectrum of glycine vapor[12] is in accord with these predictions, as are the pK_a data of amino acids in the gas phase reported by McIver and Locke.[13]

Data for assessing the zwitterionic vs. the uncharged form in solvents other than water are scarce. In 90% EtOH/water the zwitterionic forms for leucine and glycine predominate over the molecular forms by 500/1 and 1000/1, respectively.[7] In benzene solution Nash and Tam found that α-, β-, and δ-*N*,*N*-di-*n*-butyl amino acids exist as the uncharged form, while the zwitterionic form predominates in acetonitrile.[14]

In solutions other than water, it is not clear which form should predominate. With no solvation (gas phase) the molecular form is by far the more stable. In water strong hydrogen bonding stabilizes the zwitterionic form. In polar solvents that cannot hydrogen-bond donate, solvation will stabilize the polar zwitterion but further stabilization by hydrogen bonding will not be possible. It is therefore difficult to predict a priori which form should predominate in dipolar aprotic solvents.

Method of Determining Zwitterion/Uncharged Form Equilibrium in Me_2SO: Acidities of Dimethylglycine and Its Ester. To determine the zwitterion/uncharged form equilibrium in Me_2SO solution, we have used the method of Edsall and Ebert[6,7] in which the pK_a of the ester NH_3^+ group (eq 2, K_E) is assumed to be



equal to the pK_a of the NH_3^+ group (eq 1b, K_{NH^+}) in the amino acid. The amount of zwitterion (K_Z) is then calculated from eq 3 from the overall pK_a of the amino acid, pK_a (app), containing contributions from both eq 1a and 1b, and the pK_a of the ester (eq 2).

$$K_Z = K_{COOH}/K_{NH^+} = K_{COOH}/K_E = (K_{app} - K_E)/K_E \quad (3)$$

Table II. Chemical Shift of Methyl Group of *N*,*N*-Dimethylglycine and Derivatives

compound	δ_{Me}	$\Delta\delta_{Me}$
$Me_2N^+HCH_2COOEt$ p-TsO^-	2.84	0.61
Me_2NCH_2COOEt	2.23	
$Me_3N^+CH_2COOH$ Cl^-	3.24	0.09
$Me_3N^+CH_2COO^-$	3.15	
$Me_2N^+HCH_2COOH$ Cl^-	2.84	
$Me_2N^+HCH_2COO^- \rightleftharpoons Me_2NCH_2COOH$	2.59	

Using eq 3 and the data in Table I, K_Z values of about 10 result for most amino acids. However, this method of determining zwitterion content has been criticized by Sargeant and co-workers,[15] who found that for phenylglycine in water, $pK_E = pK_{NH^+} - 0.2$. In other words, the ammonium group in the amino acids is 0.2 unit less acidic than that of the amino acid ester. To determine whether this is also true in Me_2SO solution, ^1H NMR was used to determine the amount of zwitterion present in solution for *N*,*N*-dimethylglycine. The pK_a's in Table I and eq 3 suggest that only about 20% of the zwitterion is present, the only amino acid measured in which the uncharged form predominates. The chemical shifts of the methyl groups of *N*,*N*-dimethylglycine and the ethyl ester are given in Table II. Inspection of this table shows that an upfield shift of 0.61 ppm occurs upon N-deprotonation of $Me_2N^+HCH_2COOEt$ to form Me_2NCH_2COOEt, while an upfield shift of 0.09 ppm occurs upon O-deprotonation of $Me_2N^+CH_2COOH$ to form $Me_3N^+CH_2COO^-$. The methyl groups of $Me_2N^+HCH_2COOH$ have a chemical shift of 2.84 ppm. Based on the models, complete N-deprotonation should cause a 0.61 ppm upfield shift to 2.23 ppm while complete O-deprotonation should cause only a 0.09 ppm shift to 2.75 ppm. The chemical shift for neutral dimethylglycine in Me_2SO was 2.59 ppm, indicating predominant N-deprotonation to form the zwitterion. The fraction of zwitterion is calculated as (2.59–2.23)/(2.75 – 2.23) = 0.69.

The NMR experiment indicates that the assumption of $pK_E = pK_{NH^+}$ is not valid in Me_2SO solution, for the zwitterion content calculated from eq 3 was 20% (K_Z = 0.25) compared to the actual value of 69% (K_Z = 2.2). Using the actual K_Z of 2.2 and the pK_{app} of 6.3, the pK_a of the N^+H of $Me_2N^+HCH_2COOH$ is calculated to be 6.8, about 0.4 unit higher than the 6.38 value measured for N^+H acidity of the ester. A summary of pK_a values is given in eq 4–7. Since $K_{app} = K_{COOH} + K_{NH^+}$ and pK_{app} = 6.3 and pK_{NH^+} = 6.8, the pK_{COOH} of dimethylglycine is calculated to be 6.45 (eq 6).

$$Me_2N^+HCH_2COOEt \rightleftharpoons Me_2NCH_2COOEt + H^+ \qquad pK_E = 6.38 \quad (4)$$

$$Me_2N^+HCH_2COOH \rightleftharpoons Me_2NCH_2COOH + H^+ \qquad pK_{NH^+} = 6.8 \quad (5)$$

$$Me_2N^+HCH_2COOH \rightleftharpoons Me_2N^+HCH_2COO^- + H^+ \qquad pK_{COOH} = 6.45 \quad (6)$$

$$Me_3N^+CH_2COOH \rightleftharpoons Me_3N^+CH_2COO^- + H^+ \qquad pK_{COOH} = 6.40 \quad (7)$$

The 0.4 unit higher pK_a for the N^+H acidity of $Me_2N^+HCH_2COOH$ compared to $Me_2N^+HCH_2COOEt$ indicates that H-bonding such as that shown in **3** cannot be important in Me_2SO solution, since this stabilization would cause the amino acid to be more acidic than the ester. In

(8) (a) Sutherland, G. B. B. M. *Adv. Protein Chem.* **1952**, *7*, 291–318. (b) Koegel, R. J.; Greenstein, J. P.; Winitz, M.; Birnbaum, S. M.; McCallum, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 5708–5720.

(9) (a) Marsh, R. E.; Donohue, *J. Adv. Protein Chem.* **1967**, *22*, 235–256. (b) Koetzle, T. F.; Lehmann, M. S. *Hydrogen Bond*; Shuster, P., Zundel, G.; Sandorfy, C., Eds.; North Holland: Amsterdam, 1976; Vol. 2, p 457.

(10) Tse, Y.-C.; Newton, M. D.; Vishveshwara, S.; Pople, J. A. *J. Am. Chem. Soc.* **1978**, *100*, 4329–4331.

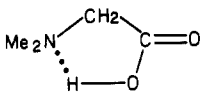
(11) Oegerle, W. R.; Sabin, J. R. *J. Mol. Struct.* **1973**, *15*, 131.

(12) Brown, R. D.; Godfrey, P. D.; Storey, J. W. V.; Bassez, M.-P. *J. Chem. Soc., Chem. Commun.* **1978**, 547–548.

(13) (a) Locke, M. J.; Hunter, R. L.; McIver, R. T., Jr. *J. Am. Chem. Soc.* **1979**, *101*, 272–273. (b) Locke, M. J.; McIver, R. T., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 4226–4232.

(14) Tam, J. W.-O; Nash, C. P. *J. Phy. Chem.* **1972**, *76*, 4033–4037.

(15) Bryson, A.; Davies, N. R.; Serjeant, E. P. *J. Am. Chem. Soc.* **1963**, *85*, 1933–1938.



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the gas phase a reversal occurs, for $N^+H_3CH_2COOH$ is a stronger acid than $N^+H_3CH_2COOEt$ by 3.8 kcal/mol.[13] H-bond stabilization such as that depicted in 3 would account for this reversal compared to aqueous and Me_2SO solution.

Effect of Solvation on Zwitterion/Uncharged Form Equilibrium of Amino Acids. From the data in Table I and eq 3 and 8, K_Z values were determined for the amino acids in Me_2SO solution and are given in the final column of Table I. These data show that amino acids in Me_2SO

$$pK_E = pK_{NH^+} - 0.4 \quad (8)$$

solution exist in 90–97% zwitterionic form, except for *N,N*-dimethylglycine as discussed above. In this respect, the behavior of amino acids in Me_2SO ($K_Z = 10^1$–10^2) is more similar to water ($K_Z = 10^4$–10^5) than to the gas phase ($K_Z \sim 100^{-100}$). The reversal upon transferring the amino acid from the gas phase (uncharged form) to Me_2SO solution (zwitterionic form) is due to solvation of the NH_3^+ and COO^- groups in Me_2SO solution. Solvation of NH_3^+ reduces the acidity of the NH_3^+ group, while solvation of COO^- increases the acidity of the COOH group, as shown in eq 9 and 10. In the gas phase, lack of solvation of $MeNH_3^+$ makes this compound a strong acid, while lack of solvation of $PhCOO^-$ makes PhCOOH a weak acid.

$MeNH_3^+$ pK_a:

203 kcal/mol (gas)[13b] → 15 kcal/mol (Me_2SO)[16]

$$\Delta pK_a \text{ (gas} \rightarrow Me_2SO) = 188 \text{ kcal/mol} \quad (9)$$

PhCOOH pK_a:

333 kcal/mol (gas)[13b] → 15 kcal/mol (Me_2SO)[28]

$$\Delta pK_a \text{ (gas} \rightarrow Me_2SO) = 318 \text{ kcal/mol} \quad (10)$$

The reduced level of zwitterion in Me_2SO ($K_Z = 10^1$–10^2) as compared to water ($K_Z = 10^4$–10^5) is due to the lack of hydrogen-bond donation in Me_2SO. This is exemplified in comparing the transfers of $MeNH_3^+$ and PhCOOH from water to Me_2SO solution (eq 11 and 12).

$MeNH_3^+$ pK_a:

10.65 (water)[29] → 11.0 (Me_2SO)[16]

$$\Delta pK_a = 0.35 \quad (11)$$

PhCOOH pK_a:

4.2 (water)[29] → 11.0 (Me_2SO)[28]

$$\Delta pK_a = 6.8 \quad (12)$$

The pK_a's of ammonium ions are similar in Me_2SO and water (eq 11), indicating solvation of the cation by polar and hydrogen-bonding effects are similar in the two solvents. However, carboxylic acids are about 7 units less acidic in Me_2SO than in water due to the strong hydrogen-bond donating ability in water that is absent in Me_2SO. The result is that, in Me_2SO, deprotonation of COOH to form the zwitterion (eq 1a) is only slightly favored over deprotonation of NH_3^+ to afford the uncharged form (eq 1b).

Reasoning further, the zwitterionic content in amino acids is a question of how much an α-NH_3^+ group increases the acidity of the COOH group of acetic acid vs. how much an α-COOH group increases the acidity of the NH_3^+ group of methylammonium ion.

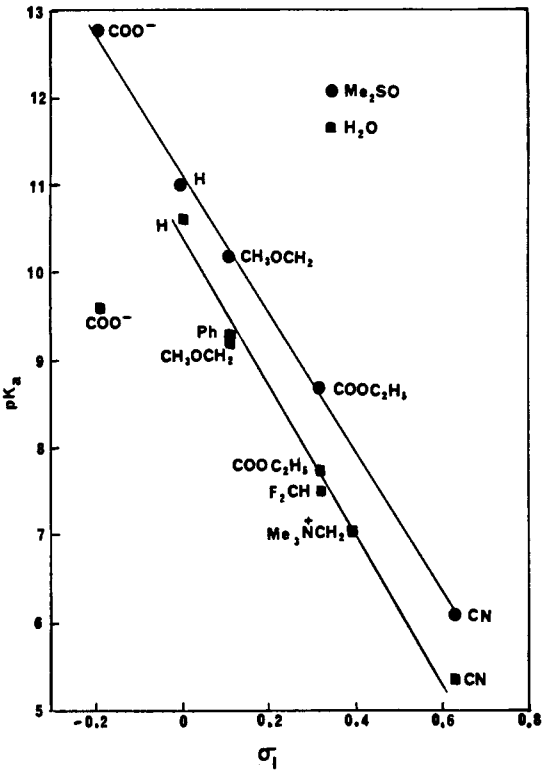


Figure 1. Plot of pK_a (water) and pK_a (Me_2SO) vs. σ_I for aliphatic amines ($GCH_2N^+H_3$).

H—CH_2COOH pK_a (COOH) in Me_2SO[31] = 12.6

H_3N^+—CH_2COOH pK_a (COOH) in Me_2SO = 7.6

$$\Delta pK_a = 5.0 \quad (13)$$

H—$CH_2NH_3^+$ pK_a (NH_3^+) in Me_2SO[16] = 11.0

HOOC—$CH_2NH_3^+$ pK_a (NH_3^+) in Me_2SO = 9.1

$$\Delta pK_a = 1.9 \quad (14)$$

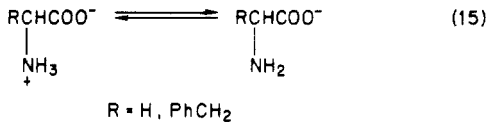
Equations 13 and 14 indicate that the acidifying effect of the α-NH_3^+ group is 3.1 pK_a units greater than the α-COOH group. Thus, the greater inductive effect of $-NH_3^+$ vs. COOH accounts for the increased acidity of $-COOH$ vs. NH_3^+ in amino acids in Me_2SO solution.

In Me_2SO solution tertiary ammonium ions are 2–3 pK_a units more acidic than primary ammonium ions (Me_3NH^+ = 8.4[16] vs. $MeNH_3^+$ = 11.0,[16] and $Me_2NHCH_2COOEt^+$ = 6.4 vs. $NH_3CH_2COOEt^+$ = 8.7). The result of this is that the pK_a of the ammonium group in dimethylglycine is closer to the pK_a of the carboxyl group, so K_Z is only 2.2 for dimethylglycine compared to 10–50 for the other amino acids.

C. pK (2) Values for Amino Acids in Me_2SO. In 17% H_2O/Me_2SO (mol/mol) Edward and co-workers[17] have shown that pK_a (2) value for amino acids (12.5 for glycine) are higher than the pK_a's for aliphatic amines (11.0 for $MeNH_3^+$),[16] in contrast to the behavior in water (pK_a (2) for glycine is 9.6, pK_a for $MeNH_3^+$ is 10.65). The pK_a (2) values of phenylalanine and glycine (eq 15) measured in Me_2SO solution are 12.2 and 12.8, respectively, similar

(16) Mucci, A.; Domain, R.; Benoit, R. L. *Can. J. Chem.* **1980**, *58*, 953–958.
(17) Edward, J. T.; Farrell, P. G.; Halle, J.-C.; Kirchnerova, J.; Schaal, R.; Terrier, F. *J. Org. Chem.* **1979**, *44*, 615–619.

to the pK_a values in 17% H_2O/Me_2SO. Dissociation



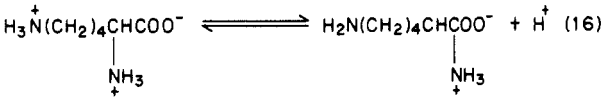
(15)

R = H, $PhCH_2$

constants for $GCH_2NH_3^+$ in Me_2SO and water are given in Table III. A plot of pK_a vs. δ_I for $GCH_2NH_3^+$ in Me_2SO and water is shown in Figure 1 and indicates that the COO^- group behaves normally in Me_2SO solution. The ρ_I in Me_2SO is 8.0. In water the COO^- group falls about 3 pK_a units below the line; otherwise the plot is similar to the one in Me_2SO with a ρ_I of 8.5.

The similarity in pK_a's and ρ_I values in Me_2SO and water for ammonium ions is unusual. For phenols, aliphatic and aromatic carboxylic acids, benzenethiols, and phenylnitromethanes, ρ or ρ_I values are 2–3 times larger in Me_2SO than in water. The reason for the larger ρ in Me_2SO for these cases is that anion stabilization by water H-bond donation reduces the sensitivity of the system to substituent effects in water. For ammonium ions, however, it is primarily solvation of the cation that determines pK_a. Me_2SO is a good H-bond accepting solvent (β = 0.76 on Kamlet–Taft scale) and is apparently able to solvate the ammonium ion to a similar extent as water.

The pK_a (2) of lysine is 10.8, which corresponds to the ionization shown in eq 16 and is nearly equivalent to that of an aliphatic amine in Me_2SO (n-$BuNH_3^+$ = 11.0).[16] Because of the acid-weakening inductive effect of the COO^- group in Me_2SO solution, in contrast to water, the ϵ-NH_3^+ group is more acidic than the α-NH_3^+ group.



(16)

II. Ambident Reactivity of Amino Acids. Although amino acids exist in water as zwitterions ($K_Z \sim 10^5$), O-alkylatiyon of unprotected amino acids is rare in aqueous solution and appears to be limited to a single instance in which phenylalanine was O-alkylated with 2-chloroethyl methyl sulfide and $PhCH_2Cl$.[18] Significant amounts of N-alkylation also occurred, indicating that the free amino group, although present in small quantities, was so much more reactive than the carboxylate anion in water that N-alkylation was still competitive. Hence a general procedure for direct O-alkylation of amino acids has never been accomplished. Instead the three-step procedure involving N-protection, O-alkylation, and deprotection must be used to obtain high yields of the desired esters.[27]

In the 1960s Parker showed that S_N2 reactions of anions are several magnitude faster in dipolar aprotic solvents than in protic solvents.[19] For example, acetate is 10^7 more reactive in DMF than in MeOH. In accord with these findings, Saari and co-workers[20] had some success in O-alkylating amino acids in dipolar aprotic solvents without N-protection. Thus, alkylation of α-methyldopa with $PhCH_2Cl$ in Me_2SO gave a crude 61% yield of O-benzyl ester, while reaction with 1-(chloroethyl)succinimide, (chloromethyl)succinimide, and chloromethyl pivalate gave 35–40% O-alkylated products.[20] Budavari has described

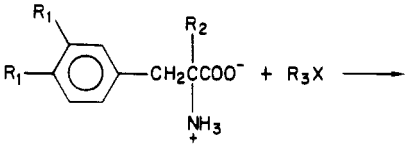
Table III. Dissociation Constants of $GCH_2NH_3^+$ in Me_2SO

G	pK_a (water)[a]	pK_a (Me_2SO)
COO^-	9.6	12.8
H	10.65	11.0[b]
Ph	9.37	
CH_3OCH_2	9.20	10.2[c]
$COOCH_2CH_3$	7.83	8.7
F_2CH	7.52	
$Me_3N^+CH_2$	7.0	
CN	5.3	6.1

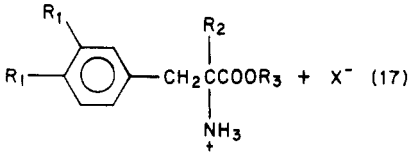
[a] Reference 29. [b] Reference 16. [c] Ritchie, C. D. *J. Am. Chem.* **1983**, *105*, 3573–3578.

the reaction of α-methyldopa with 1-chloromethyl pivalate in tetramethylurea in yields ranging from 38% to 75%.[21]

We have expanded on these preliminary entries into the field of unprotected amino acid alkylations, primarily with α-methyldopa (α-MD) as the nucleophile. Yields for O-alkylation of α-methyldopa (**4a**) and phenylalanine (**4b**) with MeX, $PhCH_2X$, and 1-chloroethyl pivalate (eq 17) in several solvents are given in Table IV. The yields are



4a, R_1 = OH, R_2 = CH_3
4b, R_1 = R_2 = H



(17)

based on HPLC assays by using a purified standard of product as reference. For alkylation of **4a** we established that alkylation was occurring at the carboxyl group and not at the phenolic hydroxyls on the following basis: (1) the product made by direct alkylation with 1-chloroethyl pivalate matched that produced by Saari[20] where the hydroxyl groups were protected during alkylation, and (2) the methyl ester was prepared from HCl/MeOH and matched that produced by direct alkylation with MeX.

Inspection of Table IV shows that ester yields are moderate (50–70%); N-alkylation of the ester is the major byproduct. The following are noteworthy points: (1) Hard–soft acid–base interactions affect N- vs. O-alkylation. Since O^- is a harder base than N, increasing the hardness of the leaving group increases the proportion of O-alkylation: Cl = I = 58%; OPO_3Me_2 = 67%; OTs = 76%; OSO_3Me = 85%. This is in line with the results of Sonstad and Engemyr, who found that the rate ratio K_{MeOTs}/K_{MeI} increased as the nucleophile became progressively harder.[22] (2) A decrease in temperature slightly favors O-alkylation. (3) N-Methylpyrrolidinone and tetramethylurea are the best solvents for O-alkylation. Reactions in hydroxylic solvents are sluggish and give little of the desired O-alkylated product. Ueda[23] reports that amino acids can be O-alkylated by using EtOTs in EtOH by an S_N2 mechanism. However, using MeOTs and Me_2SO_4 in EtOH, we find the major product to be the ethyl ester. Apparently, solvolysis to form small quantities of p-TsOH and H_2SO_4 occurs, thus catalyzing the esterification with EtOH.

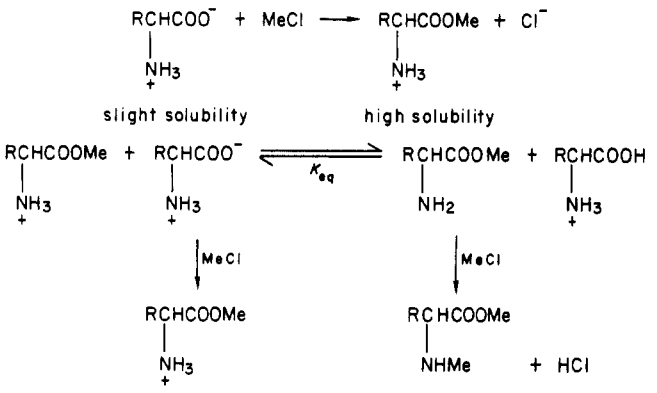
(18) Rydon, H. N.; Willett, J. E. *Peptides, Proceedings of the European Symposium, 5th*; Young, G. T., Macmillan: New York, 1963; pp 23–26.
(19) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1–32.
(20) Saari, W. S.; Freedman, M. B.; Hartmann, R. D.; King, S. W.; Raab, A. W.; Randall, W. C.; Engelhardt, E. L.; Hirschmann, R.; Rosegay, A.; Ludden, C. T.; Scriabine, A. *J. Med. Chem.* **1978**, *21*, 746–753.
(21) Budavari, J. U.S. Patent 4 440 942, April 13, 1984.
(22) Engemyr, L. B.; Sonstad, J. *Acta Chem. Scand.* **1972**, *26*, 4179–4180.
(23) Ueda, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1879–1880.

Table IV. Product Distribution in Alkylation Reactions of Unprotected Amino Acids in the Absence of Salts

amino acid	alkylating agent	solvent	T, °C	product distribution, %		
				starting material	O-alkyl ester	N,O-dialkyl product
α-MD(4a)	MeI	NMP[a]	23	28	56	16
α-MD	MeI	NMP	50	16	58	26
α-MD	MeI	NMP[b]	50	18	58	24
α-MD	MeCl	NMP	50	25	57	16
α-MD	MeCl	NMP	75	14	55	24
α-MD	Me_3PO_4	NMP	63	8	67	22
α-MD	Me_3PO_4	NMP	100	16	60	20
α-MD	MeOTs	NMP	23	13	76	10
α-MD	Me_2SO_4	NMP	23	8	85	6
α-MD	Me_2SO_4	NMP	50	13	76	10
α-MD	Me_2SO_4	TMU[c]	23	5	87	4
α-MD	MeI	CH_3CN	75	97	3	0
α-MD	MeI	H_2O	130	90	10	0
α-MD	MeI	*i*-PrOH	100	88	12	0
α-MD	MeOTs	EtOH	100	42	25[d]	0
α-MD	Me_2SO_4	EtOH	90	27	22[d]	0
α-MD	1-CEP[e]	NMP	75	25	60	15
α-MD	1-CEP	TMU	90	28	60	13
α-MD	$PhCH_2Cl$	NMP	75	20	66	14
α-MD	$PhCH_2Cl$	Me_2SO	60	12	62	10
α-MD	$PhCH_2Br$	NMP	65	18	67	13
Phe[f]	$PhCH_2Cl$	NMP	100	40	44	
Phe	$PhCH_2Br$	NMP	65	40	40	

[a] NMP = *N*-methylpyrrolidinone. [b] 1 equiv of 18-crown-6 added. [c] TMU = tetramethylurea. [d] Ethyl ester is major product. [e] 1-CEP = 1-chloroethyl pivalate. [f] Phe = phenylalanine.

Scheme I



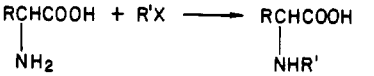
Therefore, the reported esterification using EtOTs in EtOH occurs primarily via *p*-TsOH-catalyzed EtOH esterification, not by the S_N2 alkylation of EtOTs.

The reasons for the low O-alkylation yields shown in Table IV are the following: (1) the N,O-dialkylated product is formed in 10–25% yield, and (2) 10–25% of the amino acid remains unreacted. Addition of base to force the reaction to completion increased the production of N,O-dialkylated product. Rationalization of these findings is shown in Scheme I, using MeCl as a typical alkylating agent.

The amino acid is only slightly soluble in dipolar aprotic solvents, while the ester and its hydrochloride are highly soluble (solubility of α-MD is 2 mg/mL at 70 °C in TMU and 15 mg/mL at 25 °C in NMP). Our pK_a data from the first section indicated that the esters are about one pK_a unit less acidic than the amino acid. However, the low concentration of the amino acid in solution combined with the high concentration of ester means that much of the amino aicd is protonated (unreactive) even though K_{eq} is 0.1. Assuming that the ester hydrochloride concentration is 10-fold greater than the amino acid zwitterion and that K_{eq} = 0.1, calculations indicate that roughly equal amounts of the ester free base and the amino acid zwitterion will be present in solution, both competing for MeCl. Consequently, once an appreciable quantitity of ester is formed, then the rates of formation of the ester and dialkylated product will be comparable, assuming that the N- and O-alkylation rate constants are comparable. A rough comparison of rates of reaction of α-MD with 1-chloroethyl pivalate (O-alkylation) and of the free base of the pivaloylethyl ester of α-MD with 1-chloroethyl pivalate (N-alkylation) showed that the N- and O-alkylation rate constants were similar under the conditions used synthetically.[30]

N-alkylation also generates an equivalent of HCl, which further protonates the amino acid to prevent completion of the reaction.

Only a small amount (2–3%) of the N-alkylated product (eq 18) is formed in the reactions, as judged by HPLC of a reaction sample of phenylalanine and $PhCH_2Cl$ compared to an authentic sample of $PhCH_2CH(COOH)$-$NHCH_2Ph$.[24] This means that O-alkylation is occurring



(18)

as desired; however, poor solubility of the amino acid and the closeness in acidity of the ester and amino acid causes dialkylation and incomplete reaction to occur, as discussed above.

Effect of Salts on Ambident Reactivity. To increase the solubility of the amino acid and to enhance the zwitterion/uncharged form present in solution, salts were added to the alkylation reactions described in the previous section. A wide variety of salts were tried, with the following results: (1) $NaBF_4$, $LiClO_4$, NH_4NO_3, $NaNO_3$, $MgCl_2$, and NH_4I caused a reduction in yield of the ester; (2) NaI, NaBr, KBr, LiCl, and $PhCH_2NMe_3Cl$ gave 5–10% improvement of ester yield; (3) *n*-Bu_4NI, Et_4NI, LiI, KI, and LiBr gave a 20–30% yield boost. With 2 equiv of LiBr per 1 equiv of α-MD in NMP solution at 25 °C, yields for the reaction with 1-chloroethyl pivalate were 87–90%, with only 5–7% unreacted α-MD and 5% N,O-dialkylated product. Table V summarizes yields of O-alkylated

(24) Prepared by T. Lamanec by reduction of the *N*-benzoyl derivative.

Table V. Effect of LiBr on O-Alkylation of Amino Acids in *N*-Methylpyrrolidinone Solution

amino acid	alkylating agent	LiBr, equiv	T, °C	HPLC yield, %	isolated yield, %
α-MD[a]	1-CEP[b]	0	75	56	
α-MD	1-CEP	2	25	89	74
α-MD	1-CEP	2	50	85	
α-MD	1-CEP	2	80	81	
α-MD	1-CEP	2	120	53	
α-MD	$PhCH_2Br$	0	65	67	
α-MD	$PhCH_2Br$	2	25	85	
α-MD	MeI	0	23	56	
α-MD	MeI	2	50	75	
phenylalanine	$PhCH_2Br$	0	65	40	
phenylalanine	$PhCH_2Br$	2	25	86	74
serine	$PhCH_2Br$	2	50	86	47
alanine	$PhCH_2Br$	2	55	89	40

[a] α-MD = α-methyldopa. [b] 1-CEP = 1-chloroethyl pivalate.

product for amino acids reacting with alkylating agents, with and without 2 equiv LiBr in NMP solution. The yields were determined by HPLC assay of a sample solution compared to a pure standard.

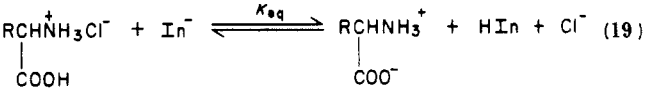
Table V indicates that the yield of O-alkylated product is substantially increased by addition of LiBr. Part of this increased yield is due to a "salting in" of the zwitterionic form of the amino acid, as the solubility of α-MD in NMP is increased about 10-fold in the presence of LiBr. Lack of solubility was pinpointed in the last section as a major cause of poor ester yields. Increased amino acid solubility also speeds the reaction so that the alkylations may be run at a lower temperature, another factor which favors O-alkylation.

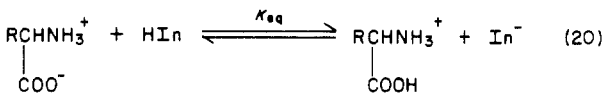
Isolation of the ester products proved difficult, with oils often being obtained. From the reaction of α-MD and 1-chloroethyl pivalate, the ester was crystallized as the phosphate ethanolate in 75% overall yield with 98% purity.[25] The phosphate salt of the ester from phenylalanine and $PhCH_2Br$ was obtained in a 74% recrystallized yield. However, benzyl esters of serine and alanine were obtained in only 47% and 40%, respectively, recrystallized yields. Further workup studies would undoubtedly improve these isolated yields.

Experimental Section

NMR Spectra were taken on a Varian XL-300 spectrometer at 20 °C.

Acidity Measurements. Most of the pK_a's of the amino acids and their esters were measured spectrophotometrically by equilibrating the acid hydrochloride with an indicator anion of known pK_a (eq 19), according to the method of Matthews, Bordwell, et al.,[4] except that tetramethylguanidine was used to generate In^- instead of the conjugate base of Me_2SO. In a few





cases the pK_a's were measured by equilibrating the amino acid with the neutral indicator (eq 20). A description of the latter procedure follows: (1) A Beer's Law titration of In^- was made at a fixed wavelength by adding 25-μL aliquots of HIn solution to a solution of tetramethylguanidine in Me_2SO in a cuvette and measuring the absorbance of In^- after each addition. From this ϵ for In^- was determined. (2) After the cuvette was emptied 2 mL of a 1–2 mM solution of the amino acid of unknown pK_a was added. Several aliquots of the indicator solution were added to establish the equilibrium in eq 20, the absorbance being measured after each addition. In^- concentration (which is equal to the concentration of $RCH(COOH)NH_3^+$ was determined from the absorbance and previously measured ϵ and the concentrations of HIn and $RCH(COO^-)NH_3^+$ were then determined from mass balance consideration.

Activity coefficients of the neutral species were assumed to be 1, while the activity coefficients for the ions were calculated with the Debye–Hückel nonlimiting law with a = 4 Å.[26]

Good agreement was obtained between the two methods. When Me_2NCH_2COOEt·p-TsOH was measured by using the first method, a pK_a of 6.43 was obtained with 2-chloro-4-nitrophenol as indicator and 6.34 with 2,5-dinitrophenol as indicator. When Me_2NCH_2COOEt was measured by using the second method, a pK_a of 6.35 was obtained with pentachlorophenol as indicator.

The following indicators were used: 2-nitrophenol, 10.8; 4-methyl-2-nitrophenol, 11.55; 2-chloro-4-nitrophenol, 8.0; 2,5-dinitrophenol, 7.55; pentachlorophenol, 6.85. In addition a pK_a of 6.1 was measured for aminoacetonitrile hydrochloride.

Alkylation Reactions were run with the following mole ratios: 50 mmol of amino acid, 55 mmol of alkylating agent, 100 mmol of LiBr, and 50 mL of solvent. With 1-chloroethyl pivalate, molecular sieves[21] (powdered 4 Å) were also added to keep the reaction mixture dry, since water decomposed the alkylating agent and ester product. The following is a typical procedure. α-Methyldopa (**4a**) (10.5 g, 50 mmol), powdered 4 Å molecular sieves (5 g), anhydrous LiBr (8.7 g, 100 mmol), 1-chloroethyl pivalate (9.0 g, 55 mmol), and 50 mL of anhydrous *N*-methyl-2-pyrrolidinone were stirred for 8 h at 50 C. The cooled reaction mixture was diluted with 100 mL of EtOAc and washed with 3 × 100 mL saturated $NaHCO_3$.[21] The combined bicarbonate washes were extracted with 2 × 100 mL of EtOAc, and the combined EtOAc, and the combined EtOAc extracts were concentrated under vacuum to 150 mL. HPLC assay at this point showed 14.1 g of product (88%). Phosphoric acid (5.2 g, 85%, 52 mmol) in 30 mL of EtOH was added over a 2-h period at ambient temperature, and the mixture stored 2 h at 5 °C. Isolated was 16.1 g (74%) of the phosphate salt containing 0.25 mol of EtOH and 0.75 mol of water.[25]

HPLC Assays were performed isocratically by using an Altex ultrasphere 5-μm ODS ion-pairing column, 25 cm × 4.6 mm. The eluent consisted of mixtures of MeOH and water that contained 0.01 M sodium octanesulfonate and 0.2% phosphoric acid. For the methylation of α-methyldopa, the eluent was 20% MeOH/80% aqueous, for the benzylation of phenylalanine, 60% MeOH/40% aqueous, and for the reaction of α-methyldopa with 1-chloroethyl pivalate, 50% MeOH/aqueous.

Registry No. α-MD, 555-30-6; α-MD methyl ester, 18181-08-3; α-MD *N,O*-dimethyl deriv., 102108-08-7; α-MD pivaloylethyl ester, 81660-38-0; α-MD *N,O*-bis(pivaloylethyl) deriv., 102108-09-8; α-MD benzyl ester, 58780-60-2; α-MD *N,O*-dibenzyl deriv., 102108-10-1; α-MD ethyl ester, 6014-30-8; Phe, 63-91-2; Phe-OCH_2Ph, 962-39-0; Ser-OCH_2Ph, 1738-72-3; Ala-OCH_2Ph, 17831-01-5; 1-CEP, 40258-80-8; $H_3N^+CH_2COOH$, 20813-04-1; $MeNH_2^+CH_2COOH$, 102108-04-3; $Me_2NH^+CH_2COOH$, 102108-05-4; $Me_3N^+CH_2COOH$, 6915-17-9; $HOCH_2CH(H_3N^+)COOH$,

(25) Budavari, et al. (Budavari, J.; Czaja, R.; Grabowski, E.; Shukis, W.; Zambito, A. *U.S. Patent* pending) have described the isolation of α-MD pivaloylethyl ester salts.

(26) Gordon, J. E. *The Organic Chemistry of Electrolyte Solutions*; Wiley: New York, 1975; pp 37–42.

(27) (a) Kotodziejczyk, A. M.; Slebioda, M.; *Synthesis* **1984**, 865–866. (b) Maclaren, J. A. *Aust. J. Chem.* **1978**, *31*, 1865–1868. (c) Bocchi, V.; Casnati, G.; Dossena, A.; Marchelli, R. *Synthesis* **1979**, 957–960.

(28) Ritchie, C. D.; Uschold, R. E. *J. Am. Chem. Soc.* **1968**, *90*, 2821.

(29) Jencks, W. P.; Regenstein, J. *Handbook of Biochemistry and Molecular Biology*, Sober, H. A., Ed.; Chemical Rubber Co.: Cleveland, p J150.

(30) Rates were measured under the conditions used synthetically; no attempt was made to obtain precise kinetic measurements at high dilution and with controlled salt effects since these results would not pertain to the conditions used synthetically.

(31) Kolthoff, I. M.; Chantooni, M. K.; Bhowmik, S. *J. Am. Chem. Soc.* **1968**, *90*, 23–28.

17829-41-3; $PhCH_2CH(H_3N^+)COOH$, 19665-03-3; 3,4-$(HO)_2PhCHC(CH_3)(H_3N^+)COOH$, 102108-06-5; $H_3N^+(CH_2)_4CH(H_3N^+)COOH$, 17829-44-6; $HOOCCH_2CH_2CH(H_3N^+)COOH$, 17806-34-7; $H_3N^+CH_2CH_2CH_2COOH$, 21029-90-3; $Me_2NH^+CH_2COOEt$ p-TsO^-, 102108-07-6; Me_2NCH_2COOEt, 33229-89-9; $Me_3N^+CH_2COOH$ Cl^-, 590-46-5; $Me_3N^+CH_2COO^-$, 107-43-7; $Me_2NH^+CH_2COOH$ Cl^-, 2491-06-7; $Me_2NH^+CH_2COO^-$, 1118-68-9; $H_3N^+CH_2COO^-$, 56-40-6; $H_3N^+CH_2COOEt$, 33888-04-9; $H_3N^+CH_2CN$, 73900-04-6; MeI, 74-88-4; MeCl, 74-87-3; Me_3PO_4, 512-56-1; MeOTs, 80-48-8; Me_2SO_4, 77-78-1; $PhCH_2Cl$, 100-44-7; $PhCH_2Br$, 100-39-0; LiBr, 7550-35-8; serine, 56-45-1; alanine, 56-41-7.

The Behavior of 4-Alkyl-4-bromo-2,5-cyclohexadienones Formed during the Aqueous Bromination of *p*-Alkylphenols

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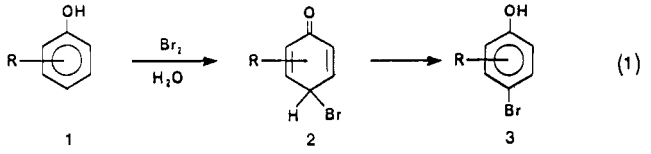
The title compounds ("ipso-dienones") **5** have been observed during the reaction of bromine with six *p*-alkylphenols **4** (R = Me, Et, *n*-Pr, *i*-Pr, *t*-Bu, 3,4-Me_2) in aqueous solutions of pH 0–3. Their formation by ipso bromine attack on **4** accounts for about 10% of the initial consumption of bromine. The decomposition of **5**, which is catalyzed by H^+ and by Br^-, is attributed to debromination. The rates of this reaction and of the attack of bromine on **4** are not very sensitive to the nature of the alkyl substituents. Studies of the behavior of **5** (R = Me) in buffers give curved buffer plots which provide additional support for the debromination mechanism and also demonstrate general acid catalysis. Decomposition of **5** (R = Me) in the presence of a trap for liberated bromine give straight buffer plots from which a Brønsted $\alpha \simeq 0.27$ is deduced. The ipso-dienone **8**, derived from 5-methylsalicyclic acid, shows intramolecular catalysis by the carboxyl group (EM = 58 M) and no catalysis by buffer acids.

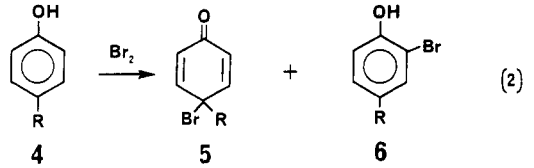
Recently, we showed that transient 4-bromo-2,5-cyclohexadienones **2** (eq 1) can be observed in the aqueous bromination of phenol and phenols bearing methyl groups at positions 2, 3, 5, and/or 6 (**1**).[1,2] This ability allowed



(1)

1 2 3

us to carry out a detailed study of the enolization of such dienones (**2** → **3**).[2] Our initial studies[1] also revealed that some ipso attack occurs in the bromination of *p*-cresol, resulting in the formation of a kinetically unstable 4-alkyl-4-bromo-2,5-cyclohexadienone (**5**, eq 2). The present paper describes more extensive studies on the breakdown of such "ipso-dienones" in dilute aqueous acidic solution.

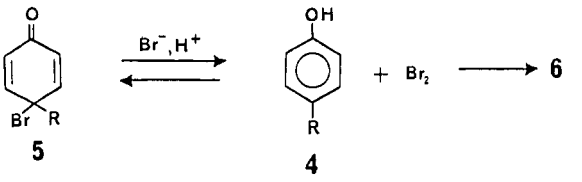


(2)

4 5 6

The occurrence of ipso halogen attack on 2,4,6-trisubstituted phenols has long been known.[3] Moreover, such attack must occur in electrophilic substitution reactions

Scheme I



5 4

in which halogen replaces a substituent other than hydrogen.[6] That it can also be of significance in the halogenation of simple *p*-alkylphenols has recently been demonstrated by Fischer and Henderson.[4,5] They were able to isolate chloro analogues of **5** from various organic media[4] but not the bromo derivatives which proved to be too labile.[5] These studies suggested to us that the dienones **5** are probably formed during the aqueous bromination of *p*-alkylphenols but that they had escaped attention up to now because of their lability. Our findings support this view and provide information about their mode of breakdown.

Results

The transient dienones **2** have absorption maxima at 230–260 nm with extinction coefficients of about 10 000, in accord with the values found for analogous stable structures.[1,2] Therefore, using stopped-flow UV spectrophotometry, we monitored the region around 250 nm during the course of the reaction of bromine with an excess of *p*-cresol (**4**, R = Me) in aqueous solutions of pH 0–3. After the initial fast consumption of bromine ($k_2 = 6.2 \times 10^5$ M^{-1} s^{-1}),[7] there is a slower decrease in absorbance which

(1) Tee, O. S.; Iyengar, N. R.; Paventi, M. *J. Org. Chem.* **1983**, *48*, 759.
(2) Tee, O. S.; Iyengar, N. R. *J. Am. Chem. Soc.* **1985**, *107*, 455.
(3) (a) de la Mare, P. B. D. *Electrophilic Halogenation*; Cambridge University: Cambridge, England, 1976. (b) de la Mare, P. B. D. *Acc. Chem. Res.* **1974**, *7*, 361. (c) Waring, A. J. *Adv. Alicyclic Chem.* **1966**, *1*, 129. (d) Ershov, V. V.; Volod'kin, A. A.; Bogdanov, G. N. *Russ. Chem. Rev. (Engl. Transl.)* **1963**, *32*, 75. (e) Brittain, J. M.; de la Mare, P. B. D. In *The Chemistry of Functional Groups*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1983; Supplement D, Chapter 12. (f) Additional background references are given by Fischer and Henderson.[4,5]
(4) Fischer, A.; Henderson, G. N. *Can. J. Chem.* **1979**, *57*, 552.
(5) Fischer, A.; Henderson, G. N. *Can. J. Chem.* **1983**, *61*, 1045.
(6) Norman, R. O. C.; Taylor, R. *Electrophilic Substitution in Benzenoid Compounds*; Elsevier: London, England, 1965.

0022-3263/86/1951-2585$01.50/0