Does water suppress the racemization and decomposition of amino acids?

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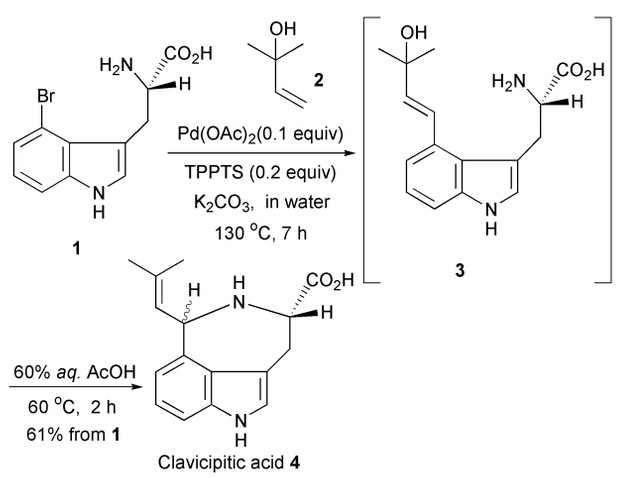
Received (in Cambridge, UK) 9th March 2001, Accepted 1st May 2001
First published as an Advance Article on the web 30th May 2001

Several neutral free amino acids, phenylglycine, leucine, phenylalanine and tryptophan, are heated at 100–130 °C under alkaline or acidic conditions in water or various polar organic solvents. Although serious racemization and decomposition occur in polar organic solvents such as DMF, DMSO, or ethylene glycol under alkaline conditions (K_2CO_3, KOH, Et_3N), these phenomena do not occur, or are largely decreased, in water or water-containing organic solvents under the same alkaline conditions. Serious racemization and decomposition of free phenylglycine are also observed in AcOH, while water or aqueous AcOH (<90%) largely suppress the racemization and decomposition. We discuss the possible reasons for this suppression effect of water in terms of differential solvation of bases and states of dissociation of amino acids in aqueous and organic solvents.

Introduction

Amino acids are indispensable to certain synthetic reactions, as inexpensive members of chiral pools, and numerous chemical transformations of amino acids have been reported.[1] However, most such transformations have been performed using protected amino acids, in order to increase solubility in organic solvents and avoid side reactions, and there have been only a few reports of synthetic reactions using unprotected (free) amino acids.[2–4] We feel that development of reactions involving free amino acids might be useful because of the possibility of shortening the time required for synthesis by omitting protection and deprotection steps. In the course of our efforts to develop such reactions,[5] one of which was one-pot transformation of optically active 4-bromotryptophan **1** to clavicipitic acid **4**, we found that racemization did not occur during the Heck reaction of **1** with 1,1-dimethylallyl alcohol **2** in spite of the strong alkaline conditions used (3 eq. K_2CO_3, 130 °C, 7 h) (Scheme 1). We were interested in this result, because amino



Scheme 1

acids are easily racemized under basic conditions in an organic solvent.

Although there have been many reports[6] of racemization of free amino acids in aqueous solution, there have been few reports that water suppressed the racemization of amino acids in comparison to that observed in polar organic solvents. It has been reported that racemization does not occur in coupling reactions of α-amino acid *N*-carboxyanhydrides (NCA) in aqueous media,[7] and that hydrolysis of pseudoephedrine amides of amino acids under alkaline conditions give[8] corresponding amino acids without racemization. Since these are specific examples of racemization of amino acids, we systematically investigated the racemization of some neutral free amino acids under acidic and basic conditions in water and organic solvents.

Results

Various amino acids were heated in the presence of 3 eq. of K_2CO_3, in DMF and water, and the resulting racemization was evaluated. Table 1 shows the results of these experiments.

Although decomposition occurred in both DMF and the aqueous solution, it occurred to a greater extent in DMF. For example, phenylglycine was significantly decomposed during reaction in DMF solvent when using the bases K_2CO_3, KOH and Et_3N, and (its protected ester) was recovered in 5, 22 and 1% respectively (runs 1, 3 and 5). In contrast, the ester was recovered to the extent of 74–78% from reactions in aqueous solutions (runs 2, 4 and 6). The other amino acids, phenylalanine, tryptophan and leucine, showed similar tendencies (Table 1, runs 7–13), while the protected amino acid, Boc-Trp-OMe, was stable under basic conditions, even in DMF solvent (run 14). Addition of water remarkably improved the recovery of amino acids (run 11).

There were large differences in optical purity of recovered amino acids between reactions in DMF and in water. In DMF, enantiomeric excess of recovered phenylglycine, phenylalanine and tryptophan was less than 85% ee (runs 1, 7, and 9). In aqueous solution, enantiomeric excess was higher than 94% ee, even for phenylglycine [the amino acid which underwent the greatest degree of racemization in DMF (run 2)]. Racemization of the other amino acids, leucine, tryptophan and phenylalanine, was almost negligible (runs 8, 10 and 13). Racemization was also nearly completely suppressed in aqueous DMF (water–DMF = 1 : 1) (run 11). The protected amino acid Boc-Trp-OMe was completely racemized in DMF, as predicted (run

DOI: 10.1039/b102254g

Table 1 Racemization of various amino acids under basic conditions in DMF and water

Run	Amino acid	Solvent	Base (mole eq.)	Conditions [a, b]	Protected ester product [c] Yield (%)	ee (%)
1	L-Phenylglycine	DMF	K_2CO_3 (1.2)	100 °C, 2 h	5	56
2		H_2O			78	94
3		DMF	KOH (2.4)		22	33
4		H_2O			74	90
5		DMF	$^iPr_2NEt$ (2.4)		1	7
6		H_2O			75	98
7	L-Phenylalanine	DMF	K_2CO_3 (1.2)	130 °C, 1.5 h	42	76
8		H_2O			71	98
9	L-Tryptophan	DMF	K_2CO_3 (1.2)	130 °C, 2 h	18	85
10		H_2O			84	99
11		DMF–H_2O (1 : 1)			61	98
12	L-Leucine [d]	DMF	K_2CO_3 (1.2)	100 °C, 2 h	14	94
13		H_2O			41	>99
14	Boc-L-Trp-OMe	DMF	K_2CO_3 (1.2)	130 °C, 2 h	92	1

[a] Amino acid (65 mg) was heated in the presence of base in 1 cm^3 of solvent. [b] Indicated temperature was inner temperature measured in sealed tube. [c] Yield was calculated by isolation of the recovered amino acid, after converting it to corresponding *tert*-butoxycarbonyl (Boc)-protected amino acid methyl ester, and its enantiomeric excess (ee) was determined by HPLC. [d] Yield and optical purity were determined after conversion to Z-Leu-OMe.

Table 2 Effect of solvents on racemization of L-phenylglycine with heating at 100 °C

Run	Amino acid [a]	Solvents	Base	Protected ester product Yield (%)	ee (%)
1	L-Phenylglycine [b]	H_2O	K_2CO_3	78	94
2		DMF	K_2CO_3	5	56
3		DMSO	K_2CO_3	1	66
4		DMI [d]	K_2CO_3	7	87
5		Ethylene glycol	K_2CO_3	23	2
6		Ethylene glycol–water (1 : 1)	K_2CO_3	49	96
7	L-Tryptophan [c]	AcOH	None	81	48
8		AcOH–water (1 : 1)	None	90	99

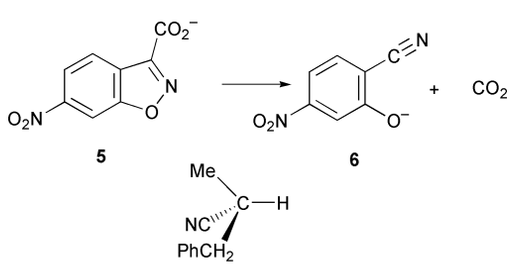
[a] Reaction time 2 h. [b] Concentration 6.5%. [c] Concentration 0.3%. [d] 1,3-Dimethylimidazolidin-2-one.

14). In aqueous solutions, use of a stronger base, KOH, resulted in nearly the same ee for phenylglycine as observed when K_2CO_3 was used (runs 2 and 4). Interestingly, use of a weaker base, $^iPr_2NEt$, resulted in complete racemization in DMF but almost negligible racemization in water (runs 5 and 6).

Table 2 shows the effect of solvents on racemization of L-phenylglycine. Serious racemization and decomposition occurred in polar aprotic organic solvents such as DMSO and 1,3-dimethylimidazolidin-2-one (DMI) (runs 3 and 4), results similar to those observed in DMF. The result of racemization in ethylene glycol was interesting, because nearly complete racemization occurred (run 5) in spite of its similar polarity and structure to water. Serious racemization occurred in another protic solvent, AcOH (without base) (run 7). As with DMF (Table 1, run 11), addition of water completely suppressed racemization in both ethylene glycol and AcOH (Table 2, runs 6 and 8).

Discussion

The results of the present investigation clearly indicate that neutral amino acids are more stable and less easily racemized in water or aqueous solutions than in polar organic solvents such as DMF, ethylene glycol and AcOH. Although decomposition of amino acids with reactive side chains (*e.g.*, tryptophan, † tyrosine, serine and cysteine) is known [10] to occur during hydrolysis of peptides under aqueous alkaline or acidic conditions, there has as yet been no report of the chemistry of decomposition of other amino acids, especially in polar organic solvents. Because the decomposed products could not be isolated in the present study, we were unable to determine with certainty the reason for their instability in organic solvents. However, decarboxylation is considered one possible pathway in the degradation of amino acids. ‡ Kemp and Paul reported [9] that the rate of decarboxylation of 6-nitrobenzoisoxazole-3-carboxylate **5** in the presence of tetramethylguanidine (as base) (Scheme 2) was slowest in water, and that it was enhanced in polar aprotic solvents such as DMSO and HMPT by a factor of up to 10^7. The explanation given for these results was that hydrogen bonding between water and the carboxylate group had suppressed decarboxyl-



Scheme 2

† It is known that tryptophan is markedly decomposed during acidic hydrolysis of peptides but is relatively stable under alkaline hydrolysis. See ref. 10.

‡ Amino acids are known to be decarboxylated under mild conditions: for example, the presence of a mildly oxidizing reagent or heating with carbonyl compounds such as pyridoxal or ninhydrin. See ref. 2.

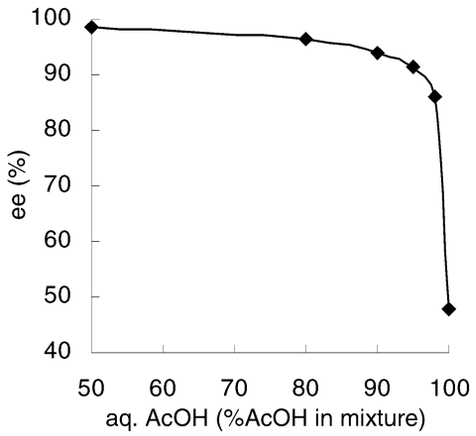


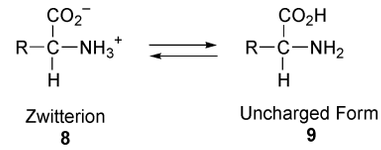
Fig. 1 Racemization of L-tryptophan in aq. AcOH. L-Tryptophan was heated at 100 °C for 2 h.

ation. In the future, we will attempt to characterize the structures of the decomposed products, in order to clarify the reasons for the instability of amino acids in DMF.

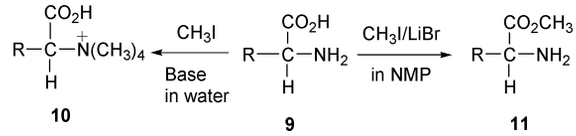
Racemization was also markedly suppressed by water under both basic and acidic conditions. Furthermore, the addition of water to organic solvents markedly suppressed racemization. The mechanism of racemization under basic conditions is thought to involve abstraction of the α-hydrogen by a base.[6] Since basicity is stronger in solutions with organic solvents than it is in aqueous solutions[11] due to poor solvation of OH^-, this could result in a greater degree of racemization. For example, Cram *et al.* reported[12] that the rate of racemization of optically active 2-methyl-3-phenylpropionitrile **7** in the presence of potassium methoxide is higher in DMSO than in MeOH by a factor of 10^9. However, solvation of base cannot be used to explain the results obtained when $^iPr_2NEt$ was used as the base. Marked racemization occurred in the presence of $^iPr_2NEt$ in DMF (Table 1, run 5) despite the fact that this base is weaker than K_2CO_3 or KOH, and racemization did not occur in water solutions of $^iPr_2NEt$ (Table 1, run 6). Racemization in ethylene glycol (Table 2, run 5) was also inconsistent with such an explanation, because it is likely that the ability of ethylene glycol to solvate OH^- is similar to that of water.

The results of racemization in AcOH (Table 2, run 7) were also curious, because free amino acids are known to be racemized more slowly in weakly to moderately acidic solutions (pH ≈ 2) than in alkaline solutions (pH ≈ 13).[6a,13] Matsuo *et al.* observed[14] similar results for AcOH-induced racemization of amino acids; phenylglycine was racemized more rapidly in deuteriated acetic acid (AcOD) than in mineral acids such as 10% aq. D_2SO_4 (which is more strongly acidic than AcOD). They also observed that racemization was suppressed in 50% aq. AcOH. Although Matsuo explained the high rate of racemization in AcOH as the result of abstraction of α-hydrogen by acetate anions,[14] this did not explain why water suppressed racemization. We further investigated the effects of the concentration of water in acetic acid on racemization in order to clarify the role of water. As shown in Fig. 1, racemization suddenly increases when the concentration of water falls below 10%. This result clearly shows that even the presence of a small amount of water inhibits racemization.

All of the above results imply that an explanation other than solvation of OH^- is required. Hughes *et al.* reported[4] that the ratio of zwitterions **8** to uncharged forms **9** of amino acids in aprotic organic solvents such as DMSO ranged from 2 to 40, compared with 10^4–10^5 in water (Scheme 3). They also reported[4] that this tendency affected the selectivity of N- or O-alkylation of free amino acids by alkylating reagents such as methyl iodide. In polar aprotic solvents such as *N*-methylpyrrolidin-2-one (NMP), O-alkylation of amino acids **9** occurred selectively to form esters **11**, while in water, quaternary ammonium salts



Scheme 3



Scheme 4

10 were formed from alkylation of amino acids by CH_3I under basic conditions (Scheme 4). These results are probably relevant to our results, because decomposition and racemization (attack on asymmetric hydrogen by a base) are likely to be affected by the ratio of the ionic and uncharged forms of an amino acid.

It is well known[15] that, while in the gas-phase amino acids exist as neutral forms, zwitterionic ones predominate in aqueous solution. Theoretical calculations revealed[16] that water molecules play an important role in the formation of zwitterionic form in aqueous solution, because water assists proton transfer from COOH to the NH_2 group to form the zwitterion and it also stabilizes the charged form by solvation. Taking this consideration into account, we supposed that charged amino acids in polar aprotic solvents under acidic or basic conditions are more unstable than those in aqueous solvents. Therefore the amino acids were easily decomposed and racemized under acidic and basic conditions compared with results aqueous solution.

Conclusions

Although we tested limited numbers of amino acids for stability and racemization in polar organic solvents and aqueous solvents, the results of the present investigation clearly indicate that racemization and decomposition of free amino acids take place to a far lesser extent in water than in organic solvents, under acidic or basic conditions. Thus, water may play a key role as a solvent in the development of new synthetic reactions involving free amino acids. We are currently working on the development of new reactions involving water-soluble compounds in aqueous media.

Experimental

IR spectra were obtained with a JASCO FT/IR-230 spectrometer. ^1H NMR spectra were obtained with a JEOL EX-400 spectrometer for samples in $CDCl_3$. Chemical shifts of protons were reported in δ-values and referenced to tetramethylsilane as internal standard, or the residual chloroform (δ 7.26) was used as the internal reference when measured in $CDCl_3$. Mass spectra were measured using a JEOL JMS-AM II 50. TLC was performed on Merck 25 DC-Platten 20 × 20 cm Kieselgel 60 F_{254} plates (Art 5715).

Procedure for racemization test under basic conditions

A mixture of L-phenylglycine (65 mg, 0.43 mmol) and K_2CO_3 (65 mg, 0.5 mmol) in water (1 cm^3) was put into a sealed tube with a small thermometer (to measure inner temperature) and heated at 100 °C for 2 h. Then, Boc_2O (500 mg, 2.3 mmol), 1,4-dioxane and water (each 1 cm^3) were successively added to the reaction mixture at room temperature, which was stirred for 1 day. After the mixture had been washed with benzene, the aqueous layer was acidified with 10% aq. citric acid and extracted with AcOEt. The organic layer was washed with saturated aq. NaCl and dried over $MgSO_4$. After evaporation

of the mixture, the residual oil was esterified with CH_2N_2 in Et_2O. This solvent was then evaporated off, and the resultant crude mixture was purified by preparative TLC, yielding *N*-Boc-phenylglycine methyl ester as a colorless oil (89.4 mg, 78%). The structure was confirmed by comparison of the product's IR, NMR and mass spectra with those of a corresponding authentic sample. Optical purity was 94% ee, as revealed by high-performance liquid chromatography (HPLC) using a chiral column [CHIRALCELL OD, *n*-hexane–propan-2-ol = 100 : 1, 1.0 cm^3 min^{-1}, t_R 21.0 min (*R*), 23.8 min (*S*)].

Recovered tryptophan, phenylalanine and leucine (Table 1) were converted to Boc-Trp-OMe, Boc-Phe-OMe and Z-Leu-OMe, respectively, utilizing a procedure similar to that described in the preceding paragraph. The structures of these compounds were confirmed by comparing their spectral data with those of corresponding authentic samples. Optical purity was determined by HPLC, using a chiral column according to the following procedure. Boc-Trp-OMe: [CHIRALCELL AS, *n*-hexane–propan-2-ol = 5 : 1, 1.0 cm^3 min^{-1}] t_R 11.6 min (*R*), 14.5 min (*S*); Boc-Phe-OMe: [CHIRALCELL AS, *n*-hexane–propan-2-ol = 30 : 1, 1.0 cm^3 min^{-1}] t_R 9.6 min (*R*), 11.4 min (*S*); Z-Leu-OMe: [CHIRALCELL AS, *n*-hexane–propan-2-ol = 50 : 1, 1.0 cm^3 min^{-1}] t_R 25.1 min (*R*), 32.5 min (*S*).

Procedure for racemization test under acidic conditions (Table 2, run 7)

Tryptophan (23.3 mg, 0.114 mmol) in AcOH (8 cm^3) was heated at 100 °C for 2 h in a sealed tube. After cooling, the solvent was evaporated to dryness, and water (0.4 cm^3), 1,4-dioxane (0.4 cm^3) and K_2CO_3 (75 mg, 0.37 mmol) were added to the residue. Boc_2O (71 mg, 0.33 mmol) was added, and this mixture was stirred vigorously at room temperature overnight before being washed with benzene, and the aqueous layer was acidified with 10% aq. citric acid and extracted with AcOEt. The organic layer was washed with saturated aq. NaCl and dried over $MgSO_4$. After evaporation of the solvent, the residual oil was esterified with CH_2N_2 in Et_2O. This solvent was then evaporated off, and the resultant crude mixture was purified by preparative TLC, yielding Boc-Trp-OMe as a colorless oil (30 mg, 81%). The product's structure was confirmed by comparing its IR, NMR and mass spectra with those of a corresponding authentic sample. The optical purity was 48% ee, as revealed by HPLC using a chiral column [CHIRALCELL OD, *n*-hexane–propan-2-ol = 100 : 1, 1.0 cm^3 min^{-1}, t_R 21.0 min (*R*), 23.8 min (*S*)].

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