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CLEAVAGE OF ESTERS USING CARBONATES AND BICARBONATES OF ALKALI METALS: SYNTHESIS OF THYMOPENTIN

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Summary: A novel method for hydrolysis of primary esters using aqueous alkali carbonates or bicarbonates and an alcohol as cosolvent is described. Several peptide esters, including a Cbz-Arg-Lys(Cbz)-Asp(OBzl)-Val-Tyr-OBzl (sequence corresponding to the active site of thymic immunoregulatory hormone, thymopoietin), were hydrolyzed to demonstrate the utility of this method.

Hydrolysis of esters with retention of chiral integrity using common reagents under ambient conditions is highly desirable in the synthesis of peptides and other biological molecules.[1] Treatment with an alkali or acid is a commonly used procedure for ester hydrolysis. Use of other reagents, such as $AlCl_3$-alkyl sulfides,[2] sodium benzeneselenoate,[3] nitrosonium tetrafluoroborate,[4] lithium methylmercaptide,[5] potassium tert-butoxide,[6] and iodotrimethylsilane[7] in peptide synthesis is restricted due to side reactions, loss of chirality, and difficulty in handling. Mild reagents such as alkali metal carbonates have been used to cleave carboxamidomethyl,[8] N-benzhydryl-glycolamide,[9] and aryl[10] esters. In spite of these developments, methyl, ethyl, benzyl, and t-butyl esters continue to be widely used for carboxyl group protection. While the methyl and ethyl esters are usually cleaved by treatment with alkali, the benzyl esters are readily deprotected by catalytic hydrogenation or catalytic transfer hydrogenation.[11] The potential of alkali carbonates and bicarbonates for the cleavage of methyl, ethyl or benzyl esters has not been evaluated, and is the subject of this report.

The cleavage of methyl, ethyl, and benzyl esters by aqueous alkali metal carbonates or bicarbonates was studied using several model esters. Analysis of reaction aliquots at regular time intervals indicated that the alkali carbonates hydrolyzed esters faster than the corresponding bicarbonates.[12] An organic cosolvent, such as methanol or ethanol, was required to keep the esters in solution. The hydrolysis rate was independent of the nature of the alkali cation except for lithium, possibly due to the low solubility of lithium carbonate in the solvent medium. Considerable transesterification was noted when the reactions were performed in anhydrous alcohols, particularly in methanol. The rate of hydrolysis and the level of racemization depended upon the water:alcohol ratio used in the reaction. Efficient hydrolysis with negligible

racemization was observed with > 30% water in the reaction medium.

The cleavage of Ac-Phe-OMe by M_2CO_3(M = Li, Na, K, Rb, or Cs) in methanol-water was examined.[13] Reversed-phase high performance liquid chromatography (RP-HPLC) analysis[14] of reaction aliquots at regular intervals indicated that the cleavage reaction was uniform with all carbonates tested except with Li_2CO_3. After 4 h, 37% Ac-Phe-OH was formed in the presence of Li_2CO_3 in contrast to 88% for all other carbonates (Na, K, Rb or Cs). The efficiency of hydrolysis and the level of racemization were evaluated using Boc-L-Leu-L-Phe-OBzl.[15] Ester hydrolysis was uniform for all carbonates (except Li_2CO_3) as mentioned above with a product distribution of 91% Boc-L-Leu-L-Phe-OH, 2% Boc-L-Leu-D-Phe-OH, 1% Boc-L-Leu-L-Phe-OMe, and 6% Boc-L-Leu-L-Phe-OBzl. With Li_2CO_3 the product distribution was 41% Boc-L-Leu-L-Phe-OH, 0.5% Boc-L-Leu-D-Phe-OH, 52% Boc-L-Leu-L-Phe-OMe, and 6% Boc-L-Leu-L-Phe-OBzl. Substitution of alkali carbonates with the corresponding bicarbonates[16] gave a product distribution of 45% Boc-L-Leu-L-Phe-OH, 0.5% Boc-L-Leu-D-Phe-OH, 49% Boc-L-Leu-L-Phe-OMe, and 5.5% Boc-L-Leu-L-Phe-OBzl. However, the product distribution in the case of cesium bicarbonate was found to be comparable to that observed for the M_2CO_3 (M = Na, K, Rb or Cs). Studies using more hydrophilic esters, for example Boc-L-Ala-L-Ala-OMe,[17] revealed that a higher water:alcohol ratio yielded products virtually free of racemization.

The influence of organic solvents on the rate of hydrolysis of Ac-Phe-OEt by Cs_2CO_3 was studied.[18] The amount of Ac-Phe-OH formed after 24 h was 99% (MeOH), 81% (EtOH), 68% (2-PrOH), and 59% (t-BuOH). In contrast, insignificant hydrolysis occurred when DMF, CH_2Cl_2, CH_3CN, or THF was used as the cosolvent.

The compatibility of the various side-chain protecting groups used in peptide synthesis under the conditions of carbonate-mediated ester hydrolysis was evaluated to establish the preparative potential of the method. Cs_2CO_3 was chosen because of its high solubility in H_2O. Our analysis indicated that S-4-MeBzl (Cys), N^{π}-Bom (His), N^{g}-NO_2 (Arg), N^{g}-Tos (Arg), Boc, Cbz, 2-Chloro-Z, 2,6-Cl_2Bzl (Tyr), and Bzl (Ser, Thr, Tyr) groups were stable, whereas 2-BrZ (Tyr), N^{im}-Tos (His), and N-Fmoc groups were completely cleaved.

The usefulness of this method in the synthesis of biologically active peptides is demonstrated by its application to the synthesis of the immunomodulatory peptide, thymopentin.[19] Thus, Cs_2CO_3-mediated hydrolysis of the benzyl ester groups in Cbz-Arg(HCl)-Lys(Cbz)-Asp(OBzl)-Val-Tyr-OBzl yielded the corresponding acid (92% yield). This compound, upon subsequent catalytic transfer hydrogenation, gave a product which was found to be identical to an authentic sample of thymopentin and possessed full biological activity.[20,21] Further, HPLC analysis confirmed that the final product was free of epimeric contaminants.[22]

In this report, we have demonstrated the usefulness of alkali metal carbonates for ester hydrolysis. The utility of this method for the release of peptides from Merrifield type resins will be described in a separate report.

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12. Typical ester cleavage procedure: An aqueous solution of alkali carbonate or bicarbonate (2 mmol) is added to a solution of the ester (1 mmol) in an alcohol or water (10 mL) and stirred for 4 to 24 h. The reaction was monitored by RP-HPLC or TLC. The organic solvent is removed under reduced pressure, and the product isolated after acidification with dilute HCl either by filtration or extraction in to a suitable organic solvent.
13. Ac-Phe-OMe (1 mmol), methanol:water (10 mL, 1:1, v/v), M_2CO_3 (2 mmol), gradient t_r (Ac-Phe-OH) 6.8 min and t_r (Ac-Phe-OMe) 12.2 min.

14. RP-HPLC conditions: All peaks were assigned by comparison with authentic samples. Vydac 218TP54, flow = 1 mL/min; A = 0.1% TFA/H_2O, B = 0.1% TFA/CH_3CN-H_2O (4:1 v/v); Gradient 1:20% B to 60% B over 20 min. Gradient 2:40% B to 80% B over 20 min followed by an isocratic hold at 80% B for 10 min. Gradient 3:10% B to 30% B over 20 min followed by 30% B to 50% B over 10 min. Gradient 4: 10% B to 30% B over 20 min.
15. Boc-L-Leu-L-Phe-OBzl (1 mmol), methanol (10 mL), M_2CO_3 (2 mmol, M = Li, Na, K, Rb, or Cs), and water (5 mL) for 21 h. Gradient 2^{14}:t_r (Boc-L-Leu-L-Phe-OH) 15.8 min, t_r (Boc-L-Leu-D-Phe-OH) 17.0 min, t_r (Boc-Leu-Phe-OMe) 22.3 min, and t_r (Boc-Leu-Phe-OBzl) 24.5 min.
16. Boc-L-Leu-L-Phe-OBzl (0.5 mmol), methanol (10 mL), $MHCO_3$ (4 mmol, M = Na, K, or Cs), water (5 mL) for 48 h.
17. Gradient 3^{14}:t_r (Boc-L-Ala-L-Ala-OH) 17.9 min; t_r (Boc-L-Ala-D-Ala-OH) 19.1 min, and t_r (Boc-L-Ala-L-Ala-OMe) 23.3 min.
18. Ac-Phe-OEt (1 mmol), Cs_2CO_3 (2 mmol), water (5 mL), and organic solvent (5 mL) for 24 h.
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22. Gradient 4^{14}:t_r (Thymopentin) 13.3 min, t_r (D-Tyr-Thymopentin) 15.1 min.

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