Tetrahedron Letters No.23, pp. 1819-1820, 1969. Pergamon Press. Printed in Great Britain.

ESTERIFICATION OF PEPTIDES IN AQUEOUS SOLUTION[1]

Osamu Yonemitsu, Tatsuo Hamada and Yuichi Kanaoka

Faculty of Pharmaceutical Sciences, Hokkaido University

Sapporo, Japan

(Received in Japan 24 March 1969; received in UK for publication 8 April 1969)

In our recent report it was demonstrated that sodium borohydride in aqueous solution is a good reagent for the selective reduction of the ester group of some peptides.[2] It is therefore desirable that esters of peptide and protein are prepared under mild conditions in aqueous solution. Although the treatment with hydrogen chloride in absolute methanol or ethanol provides a generally applicable esterification procedure of amino acids and peptides,[3] and diazo compounds are also useful for the esterification of carboxyl groups in some enzymes under specified conditions,[4] some limitations in each case are unavoidable.[5]

In the course of chemical studies on triethyloxonium fluoroborate, Meerwein reagent (MR), Meerwein et al. reported that a very concentrated aqueous solution of sodium benzoate was treated with MR to give ethyl benzoate,[6] whereas MR is rather unstable in water.[7]

When acyl amino acids and peptides in aqueous sodium bicarbonate were treated with excess of MR at room temperature, corresponding esters were easily obtained in good yield. As indicated in preliminary experiments with Bz.DL-Ala-Gly and Bz.Gly-L-Val, fifteen to twenty fold excess of MR was enough to give satisfactory results. The pH dependence of this reaction was also examined; esterification of Bz.Gly with twenty fold excess of MR at pH 2.0, 3.0, 4.0, 5.5, 6.0 and 7.0 gave Bz.GlyOEt in yield of 18, 48, 73, 82 and 82%, respectively.

Following is a typical procedure; 1 mmole of acyl amino acid or peptide in 10 ml of water, 21 mmole of sodium bicarbonate and 20 mmole of MR in solid or in a solution of acetonitrile are alternately added portionwise with stirring over a period of 10 min. After 20 min the mixture is extracted with ethyl acetate which is evaporated to leave corre-

sponding ester in almost pure state. Some results are summerized in yields of esters; Bz.-Gly (82-92%), Bz.L-Glu (90), Bz.L-Asp (83), Bz.L-Thr (93), Bz.L-Lys (82), Bz.L-Arg (90), Bz.-L-Tyr (86), Bz.DL-Met (98),[a] Bz.L-His (58),[b] Cbz.DL-Ser (90), Cbz.DL-Thr (89), Cbz.L-Arg (96), Bz.DL-Ala-Gly (89), Bz.Gly-L-Val (91), Cbz.DL-Ala-Gly (90), Cbz.Gly-Gly (87), Cbz.Gly-L-Ala (88), Cbz.L-Leu-Gly (92), Phth.Gly-Gly (92). a) sulfonium salt. b) quaternary ammonium salt.

Functional groups of alcoholic OH, phenolic OH, NH_2 and NH-C(NH_2)=NH of side chains of amino acids were completly nonreactive in the above conditions, though Met and His were esterified followed by alkylation on S of Met and N in imidazole ring of His to yield sulfonium salt and quaternary ammonium salt, respectively. In spite of this accompanying reaction, the esterfication with MR may provide a potential new method in the area of peptide chemistry, particularly in chemical modification of proteins, because it is carried out in aqueous solution by the simple and convenient procedure. The authors are indebted to Dr. T. Oishi for helpful discussions.

REFERENCES

1. Presented at the Annual Meeting of the Pharmaceutical Society of Japan, Apr. 1968, Tokyo.
2. O.Yonemitsu, T.Hamada and Y.Kanaoka, Tetrahedron Letters, 3575 (1968).
3. J.P.Greenstein and M.Winitz,"Chem. of Amino Acids", John Wiley & Sons, pp. 925 (1961).
4. A.C.Chibnall, J.L.Nangan and M.W.Rees, Biochem. J., 68, 114 (1958); M.S.Duscher, P.E.Wilcox, J. Biol. Chem., 236, 1328 (1961); J.P.Riehm and H.A.Scheraga, Biochemistry, 4, 772 (1965); V.M.Stepanov and T.I.Vaganova, Biochem. Biophys. Res. Commun., 31, 825 (1968).
5. P.E.Wilcox,"Methods in Enzymology", Academic Press, Vol 11, pp. 605 (1967).
6. H.Meerwein, G.Hinz, P.Hofmann, E.Kroning and E.Pfeil, J. Prak. Chem., 147, 257 (1937).
7. Recently, Kemp et al. briefly reported that "Cbz.Gly-L-Phe-Gly was dissolved in aqueous base and converted with triethyloxonium ion to its ethyl ester in 60% yield."; D.S.Kemp and S.W.Chien, J. Am. Chem. Soc., 89, 2743 (1967).