N-Methylation of Peptides

By G. MARINO and L. VALENTE
(*Laboratorio di Chimica delle Sostanze Naturali, Università di Napoli, Italy*)
R. A. W. JOHNSTONE* and F. MOHAMMEDI-TABRIZI
(*The Robert Robinson Laboratories, Liverpool University* L69 3BX)
and G. C. SODINI
(*SNAM PROGETTI, Laboratori Studi e Recerche di Base, Monterotondo, Rome, Italy*)

Summary A method for the *N*-methylation of peptides (amide bonds) is described and preferred conditions are suggested whereby all amino-acid residues except arginine react without prior modification.

N-METHYLATION of amide bonds in peptides to increase their volatility and to help direct mass spectrometric fragmentation for sequence analysis is well known.[1] Because of undesirable side-chain reactions with the initial methylation method[2,3] (AgI–MeI in $HCONMe_2$), it was superseded by the use of NaH and MeI in $HCONMe_2$,[4] Me_2SO,[5] or $MeCONMe_2$,[6] but the last method required careful control of conditions in the initial preparation of the reagent, and the second method using the dimsyl anion leads to *C*-methylation of some amino-acid residues and low yields. The dimsyl anion is extremely reactive[7] and can give undesirable side products in the *N*-methylation of peptides; *e.g.* a much cleaner product was obtained from the methylation of acetyl dimethylaspartate with NaH–Me_2SO_4–MeCN (see below)[8] than with dimsyl sodium.† Dimsyl sodium is much too powerful a base, and recently it has been shown that the much milder base, Me_4NOH,[9] is effective for methylation of amides with MeI. Dimsyl sodium and a controlled slight excess of MeI may be satisfactory[10,11] for the *N*-methylation of cysteine and methionine residues without sulphonium salt formation, but this procedure requires precise knowledge of the number of methylation sites, which is not readily available on the micro-scale frequently necessary for peptides.

Under the conditions originally described for the NaH–MeI–$HCONMe_2$ reaction, various amino-acid side-chains were adversely affected; *e.g.* sulphonium salt formation with methionine and *C*-methylation of glycine, aspartic acid, and glutamic acid occurred.[12] Using these reagents under the following much milder conditions, we have found no significant amounts of undesirable side-reactions. As an example, the tetrapeptide Ac–Trp–Met–Asp–Phe–OMe (0·06 mmol) was stirred with NaH (3·6 mmol; washed with n-pentane) in $HCONMe_2$ (2 ml) for 15 min at 20°. MeI (3·6 mmol) was added and stirring was continued for a further 20 min at 20°. The mixture was then poured into saturated NaCl solution (5 ml) and the *N*-methylated peptide was extracted with CH_2Cl_2 (5 ml). The CH_2Cl_2 extract was washed with saturated NaCl solution (4 × 4 ml portions) to give the per-*N*-methylated peptide (*M* 737; A-type sequence ions at *m/e* 257, 402, 545, and 706). There was no mass spectrometric evidence for anything less than full methylation (no *M* − 14 ions) nor for over-methylation (no *M* + 14 ions). Methylation of the tetrapeptide, Ac–Trp–Met–Asp–OMe, would normally be considered difficult

† Shown by g.l.c., the product obtained with NaH–Me_2SO_4–MeCN gave only one g.l.c. peak, whereas that with dimsyl sodium gave many peaks due to impurities also.

because of (i) the possibility of sulphonium salt formation, (ii) the possibility of *C*-methylation at the aspartic acid residue and, (iii) the bulky side-chains hindering *N*-methylation.

Under similar conditions, Ac–Met–OMe was fully *N*-methylated with no detectable sulphonium salt formation (g.l.c. evidence with internal standard) and no *C*-methylation of glycine was observed in the peptides Ac–Gly–Gly–Phe–OMe and Ac–Gly–Leu–Gly–Leu–Gly–OMe for example.

MeCN may replace $HCONMe_2$ as solvent, especially where volatile products are formed which could be lost on evaporation of the latter. Initial kinetic experiments on the *N*-methylation of acetylated amino-acid esters have shown that the reaction is slower in MeCN compared with $HCONMe_2$ and is bimolecular in amide anion and MeI. As reaction is slower in MeCN a better leaving group than I^- was needed to effect rapid methylation; Me_2SO_4 was satisfactory. With Me_2SO–NaH in MeCN at 20°, reaction times for *N*-methylation are about the same as with NaH–MeI in $HCONMe_2$; it is essential to use freshly prepared, carefully purified Me_2SO_4 because traces of acid, present in unpurified material, stop methylation on the small scale used here. With NaH–Me_2SO_4–MeCN, the following acetylated amino-acid esters were successfully *N*-methylated in high yield with little or no side-product formation: Gly, Ala, Leu, Val, Asp, Met, Trp. The method was also used with peptides, *e.g.* Ac–Trp–Met–Asp–Phe–OMe, Ac–Phe–Gly–OMe, Ac–Ala–Phe–Leu–OMe, and Z–Val–Ile–Ala–OMe.

Work at the University of Naples was financially supported by SNAM PROGETTI and by the Italian Research Council (C.N.R.). The authors thank EMBO (G.M.) and CENTO (F.M-T) for long-term fellowships.

(*Received*, 28*th January* 1972; *Com.* 132.)

[1] E. Lederer, *Pure Appl. Chem.*, 1968, **17**, 489.
[2] B. C. Das, S. D. Gero, and E. Lederer, *Biochem. Biophys. Res. Comm.*, 1967, **29**, 211.
[3] K. L. Agarwal, R. A. W. Johnstone, G. W. Kenner, D. S. Millington, and R. C. Sheppard, *Nature*, 1968, **219**, 498.
[4] J. R. Coggins and N. L. Benoiton, Abstracts of the 156th Meeting of the American Chemical Society (Biology), 1968, p. 18.
[5] E. Vilkas and E. Lederer, *Tetrahedron Letters*, 3089.
[6] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Amer. Chem. Soc.*, 1969, **91**, 3096.
[7] T. Durst, *Adv. Org. Chem.*, 1969, **6**, 285.
[8] Reported at the "EMBO Meeting on the Mass Spectrometry of Peptides", Paris, Sept. 1971.
[9] C. Cone and R. G. Oldham, Abstracts of the 162nd Meeting of the American Chemical Society (Organic), 1971, p. 94.
[10] M. L. Polan, W. J. McMurray, S. R. Lipsky, and S. Lande, *Biochem. Res. Comm.*, 1970, **38**, 1127.
[11] P. A. Leclerq and D. M. Desiderio, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 308.
[12] D. W. Thomas, *FEBS Letters*, 1969, **5**, 53.