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N-ALKYLATION OF AMINO ACIDS DURING HYDROGENOLYTIC DEPROTECTION

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Summary: *Partial N-alkylation of amino acids was observed during hydrogenolytic deprotection.*

Partial N-methylation of threonine during catalytic hydrogenation of methanol-acetic acid solutions of Fmoc-(Gal α or β)Thr-Lys(Z)-Pro-Arg (NO_2)-OBzl has been demonstrated by proton n.m.r. and FAB mass spectroscopy (1). N-Alkylation of α-amino acids by reaction with aldehydes followed by catalytic hydrogenation (2) and conversion of primary and secondary alcohols to aldehydes or ketones in the presence of palladium catalyst (3) were previously described. Hydrogenolytic deprotection in methanol, or in methanol-containing solutions, is widely used in peptide synthesis. It is thus surprising that, to our knowledge, this side reaction has not been described to date.

To evaluate the effect of different parameters on this side reaction simple model compounds were hydrogenated, in various conditions, in methanol-acetic acid (1:1 v/v). Proton n.m.r. spectra at 200 MHz of the hydrogenation products were recorded, at definite times, at 298° K on a Bruker WP 200 SY spectrometer. Concentration of the samples was in the range 1.3-2% in D_2O (99.7%), chemical shifts (δ) are expressed relative to HOD set at 4.8 ppm in D_2O. Proton assignments were determined by selective homo-spin decoupling. The amount of N-alkylation was calculated either from the ratio of the signals of N-CH_3 and γ-CH_3 or the ratio of the α-protons of threonine and its N-methylated derivative. Though the reproducibility of the experiments proved low, the results obtained showed that neither the type of catalyst (Pd black or Pd/C), nor the presence of oxygen significantly influence the N-methylation extent which depends on the hydrogenation time and is reduced, or even eliminated, by addition of water. Catalytic hydrogenation of Fmoc-Thr-OH, or Z-Thr-OH, (48 h hydrogenation time, 10% Pd/C 160 mg, substrate concentration 20

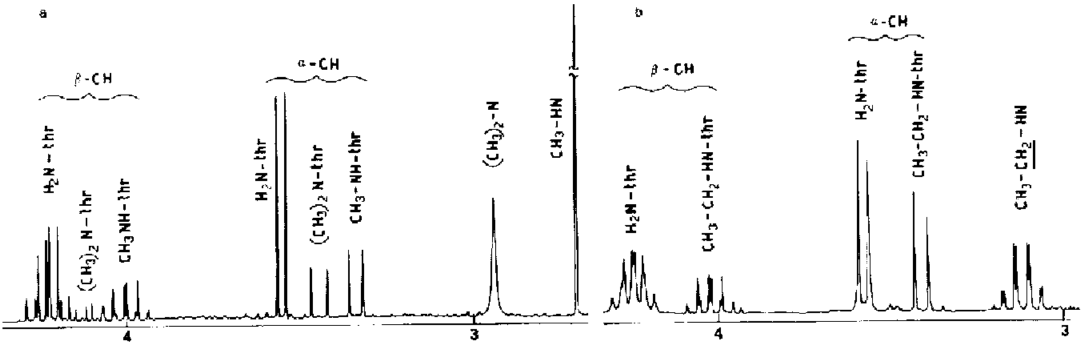

Fig.1.-^{1}H n.m.r.spectra of hydrogenated Fmoc-Thr-OH in: a) methanol-acetic acid, and b) ethanol-acetic acid.

mmol) yielded 20-40% of N-methylated threonine. A consistent amount of N,N-dimethyl-threonine was also formed (Fig. 1a). When methanol was replaced with ethanol N-ethylation occurred to a similar extent (Fig. 1b) but diethylation was never observed.

Hydrogenation of H-Thr-OH gave less than 10% of the N-methyl derivative. Addition of 2 mmol 9-methyl-fluorene, toluene, or even fluorene, to the threonine solution before catalytic hydrogenation, rose the methylation extent to 20-25% and N,N-dimethyl-threonine was also formed. Comparable results were obtained by catalytic hydrogenation of H-Ala-OH. A partial N$^{\varepsilon}$-alkylation also occurred during catalytic hydrogenation of Boc-Lys-OH and Ac-Lys-OH. The fact that N-methylation during hydrogenolytic deprotection steps in peptide synthesis has not been previously described, could be due to the fast removal of the N-protecting group(s) normally occurring in a time shorter than that needed for a significant occurrence of the side reaction. Moreover the solvents used often contain some water and ^{1}H n.m.r. spectroscopy is not routinely utilized in the characterization of synthetic peptides.

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