Butylation of the Tryptophan Indole Ring: a Side Reaction During the Removal of t-Butyloxycarbonyl and t-Butyl Protecting Groups in Peptide Synthesis

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Summary The butylation of tryptophan residues during the trifluoroacetic acid catalysed removal of the t-butyloxycarbonyl and t-butyl protecting groups of peptides is reported.

IN the course of our studies on the mass spectrometric determination of the amino-acid sequence in peptides[1,2] we have found that the mass spectra of synthetic *N*-acyl peptide esters containing a tryptophan residue often have, in addition to the molecular ion, an ion of m/e $(M + 56)$. Moreover, in such spectra, all the fragment ions that contain tryptophan are accompanied by ions 56 a.m.u. to higher mass. Accurate mass measurements of such ions showed that in every case, this mass difference is 56·0626 $\pm$ 0·0030 and corresponds therefore to C_4H_8.

A reinvestigation of the synthetic routes to tryptophan peptides[3] revealed that in all cases when there was an ion at m/e $(M + 56)$ in the mass spectrum of a peptide, one of

the steps in its synthesis had been removal of a t-butyloxycarbonyl protecting group from a tryptophan-containing intermediate. Ions of *m/e* (*M* + 56) were absent from the mass spectra of peptides synthesized without the t-butyloxycarbonyl protecting group and thus it seemed possible that the appearance of ions of *m/e* (*M* + 56) in the mass spectra of tryptophan-containing peptides is caused by the butylation of the tryptophan indole nucleus during the removal of the t-butyloxycarbonyl protecting group.

This hypothesis was proved as follows. The *N*-acetyldipeptide methyl ester, Ac–Trp–Gly–OMe was synthesized in two ways: firstly, by direct condensation of *N*-acetyltryptophan with glycine methyl ester, and secondly, by condensation of *N*-t-butyloxycarbonyltryptophan with glycine methyl ester followed by removal of the *N*-protecting group (CF_3CO_2H, 1 hr., 20°), and acetylation. In the mass spectrum of the first sample there was an intense peak corresponding to the molecular ion (*m/e* 317) and there was no ion at *m/e* 373 (*M* + 56). However, in the mass spectrum of the same compound prepared by the second route, there appeared not only the molecular ion (*m/e* 317, 100%) but also an ion of *m/e* 373 (180%). These data are consistent with butylation of the tryptophan during removal of the t-butyloxycarbonyl group, the ion of *m/e* (*M* + 56) being the molecular ion of the butyltryptophan-containing peptide, *i.e.*, Ac–(Bu)Trp–Gly–OMe in this case. If CF_3CO_2H at 0° for 1 hr. was used to remove the blocking group, the relative abundance of the ion at *m/e* (*M* + 56) falls to 120% and use of 95% CF_3CO_2H gives a product in which this relative abundance is 59% (relative to M^+, 100%)

The degree of butylation is affected by the position of the tryptophan residue in the peptide chain. Thus the abundance of the ion at *m/e* (*M* + 56) in the product formed by removal (CF_3CO_2H, 20°, 1 hr.) of the protecting group from BOC–Gly–Trp–OMe is 43% (relative to M^+, 100%), *cf.* 180% for BOC–Trp–Gly–OMe. We investigated the effectiveness of other methods of removal of the t-butyloxycarbonyl group from BOC–Trp–Gly–OMe. Use of 2N-HCl/EtOAc or 2N-HCl/AcOH led to considerably less butylation of tryptophan, but it was still noticeable, the abundances of the *m/e* (*M* + 56) ions being 10% and 7% relative to M^+ (100%), respectively.

Butylation of the tryptophan indole ring also takes place during acidolysis of *N*-acylpeptide t-butyl esters. Thus the abundance of the ion of *m/e* (*M* + 56) in the mass spectrum of Ac–Trp–Gly–OMe prepared by the treatment of Ac–Trp–Gly–OBu^t with CF_3CO_2H for 1 hr. at 20° and subsequent methylation was 230%, relative to M^+ (100%).

Schwyzer[4] has found that tyrosine residues are also subject to *O*- and *C*-butylation (although to a lesser extent than tryptophan) during removal of the t-butyloxycarbonyl group.

The data reported in this paper show that t-butyloxycarbonyl and t-butyl groups should not be used in the synthesis of tryptophan-containing peptides; a high degree of butylation of tryptophan takes place during the removal of these groups with CF_3CO_2H. This side reaction can lead to a particularly high proportion of impurities in the synthesis of complex natural peptides and their analogues by the solid phase method, where repeated removal of t-butyloxycarbonyl groups will inevitably lead to the accumulation of butyltryptophan-containing peptides.

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[1] M. M. Shemvakin, *Pure Appl. Chem.*, 1968, **17**, 313.
[2] G. W. A. Milne, A. A. Kiryushkin, Yu. B. Alakhov, V. M. Lipkin, and Yu. A. Ovchinnikov, *Tetrahedron*, in the press.
[3] E. I. Vinogradova, V. M. Lipkin, Yu. B. Alakhov, and Yu. B. Shvetsov, *Zhur. obshchei Khim.*, 1968, **38**, 787.
[4] R. Schwyzer, personal communication.