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Transesterification of the Benzyl Ester Protecting Group During Purification of a Protected Pentapeptide

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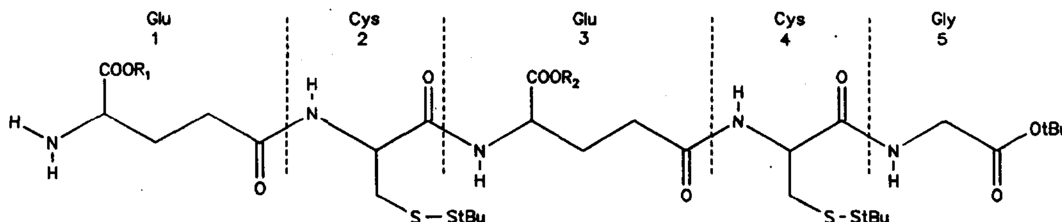
Abstract: Partial transesterification of the benzyl ester protecting group of the *N*-terminal glutamic acid residue of a protected pentapeptide during gel chromatographic purification using methanol as eluting solvent is described.

One of the main advantages of the classical solution synthesis of peptides in comparison with the solid phase method is that in the former procedure protected fragments can be purified before assembling them to a final product. Purification procedures should not, however, damage the side-chain or the *C*- and *N*-terminal protecting groups.

In the solution synthesis of phytochelatins, a class of cysteine-rich peptides with the general structure H-[-γ-Glu-Cys]$_n$-Gly-OH, we employed the Fmoc-strategy and benzyl protection for the side-chain carboxyl groups of glutamic acid residues.

Since its introduction in peptide synthesis in 1933[1], benzyl ester has been one of the most popular protecting groups for the side chains of aspartic acid and glutamic acid, both in solution synthesis and in solid phase method[2,3]. Its deprotection is affected by hydrogenolysis or acidolysis using strong acids[2,3]. Like other primary alkyl esters, benzyl esters are also subject to transesterification by bimolecular alcoholysis under acidic or basic conditions[4], or under neutral conditions in the presence of catalysts such as phosphorus ylides[5] or over alumina at higher temperatures[6]. Uncatalyzed bimolecular alcoholysis of esters can also proceed, however, it is very slow. In peptide synthesis, there has been warning about possible transesterification during hydrogenation using primary alcohols as solvents[3], but there has been no concrete report about this side-reaction.



Peptide I: $R_1 = R_2 = CH_2C_6H_5$
Peptide II: $R_1 = CH_3$, $R_2 = CH_2C_6H_5$
Peptide III: $R_1 = R_2 = CH_3$

Pentapeptide I was synthesized according to the classical procedures then it was attempted to purify it by chromatography on a column of Sephadex LH-20 and eluting with methanol. TLC on silica gel and HPLC (Nucleosil 120 C18 5μm, 2.5 mm x 36 cm, A: 0.1% TFA in water, B: 0.1%TFA in acetonitrile, 60% - 85% of B in 20 min, Fig. 1) analyses of the material obtained from the column showed the presence of a new product. Ion-spray MS showed that this extra compound has a molecular mass of 876 Da, corresponding to peptide II which must have been produced by transesterification of the benzyl ester of Glu 1 of peptide I during methanol elution, although no acid or base has been added. After its separation by preparative HPLC, MS-MS, ^{1}H- and ^{13}C-NMR analyses confirmed the correctness of the structure of peptide II. This

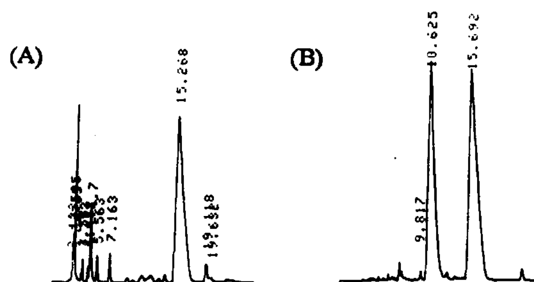
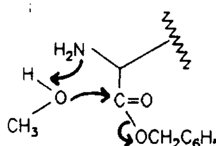


Fig. 1 HPLC Chromatogram of products before (A) and after (B) running through the LH-20 column using methanol as elution solvent.

partial transesterification also took place by dissolving peptide I in methanol and allowing it to stand for 24 h, indicating that Sephadex LH-20 apparently did not influence this reaction. By allowing the same solution to stand at room temperature for 5 days, peptide I was completely converted to compound II, and a very small amount of peptide III was also produced.

The fact that transesterification of the benzyl ester of Glu 1 took place, and was exceedingly faster than that of Glu 3, is presumably due to the presence of the neighboring amino group of Glu 1. Thus, the neighboring amino group has apparently assisted the attack of methanol on the carbonyl group according to the mechanism proposed below.



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