Gels, such as 12 % polyacrylamide, swell with this method so that they are not readily removed from their positions, but others are very simply removed by inserting a fine probe beneath the bottom glass rod and lifting. The gels come out easily, without tear or deformation. Even the finest bands are distinct, sharp and flat.

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Use of "lipophilic" Sephadex in peptide synthesis

In the synthesis of peptides the elimination of reaction by-products from the desired peptide derivative is often a cumbersome task. It occurred to us that in cases where the by-products are soluble in organic solvents filtration through methylated Sephadex[1] might be a useful procedure.

As an example we have chosen the widely used "*p*-nitrophenyl ester method" of peptide synthesis[2,3]. One of the advantages of this method is the ease with which it may be ascertained whether or not the main by-product, the yellow *p*-nitrophenol, has been removed. In the early steps of the elongation of a peptide chain, however, the removal of the nitrophenol may be quite time-consuming. It is usually accomplished by repeated extraction of the solution of the reaction mixture in ethyl acetate or ether with aqueous bicarbonate or ammonia. Often some half a dozen extractions are necessary, during which troublesome emulsions may form. Even then traces of nitrophenol are left and for the removal of these filtration through aluminium oxide has been recommended[4].

We have synthesized two peptides, L-leucyl-L-leucine and L-leucyl-L-valine, by the *p*-nitrophenyl ester method, with filtration of the reaction mixtures containing *p*-nitrophenol and benzyloxycarbonyl-L-leucyl-L-leucine methyl ester or benzyloxycarbonyl-L-leucyl-L-valine methyl ester, respectively, through a column of methylated Sephadex. In each case complete separation of the *p*-nitrophenol from the protected dipeptide was achieved. Fig. 1 shows this for the derivative of L-leucyl-L-leucine. The picture was practically identical for benzyloxycarbonyl-L-leucyl-L-valine methyl ester. The latter was isolated in crystalline form. As far as we know it has only been described as a syrup earlier[5].

It is obvious that filtration through lipophilic Sephadex may also find application in analytical work on peptides when derivatives soluble in organic solvents are formed.

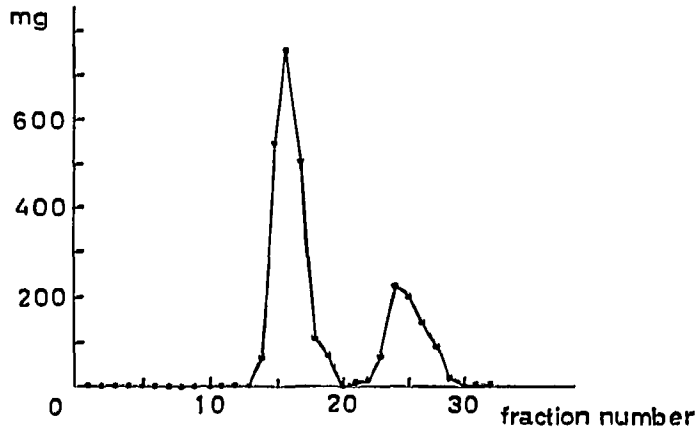

Fig. 1. Filtration of reaction mixture containing *p*-nitrophenol and benzyloxycarbonyl-L-leucyl-

= solvent-free weight of fraction. Fractions 22–28 yellow, the others colourless. For details see text.

Experimental

L-Leucine methyl ester hydrochloride was prepared according to HILL *et al.*[6]. L-Valine methyl ester hydrochloride was obtained from Fluka A.G., Buchs, Switzerland. Paper chromatography in the system of WALEY AND WATSON[7] showed that both materials were contaminated with the corresponding free amino acids. For purification the esters were set free from the hydrochlorides by treatment with ice-cold 50 % w/v aqueous K_2CO_3 and extracted into ether[8,9]. The ether solutions were dried over anhydrous $MgSO_4$ and filtered. Dry HCl was passed through the solutions whereupon the ester hydrochlorides precipitated in a state of high purity. They were reprecipitated with ether from methanol and dried *in vacuo*.

Benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester was obtained from Fluka A.G. It was recrystallized once from ethanol[3].

The synthesis of benzyloxycarbonyl-L-leucyl-L-leucine methyl ester closely followed the description of the synthesis of benzyloxycarbonyl-L-leucyl-glycine ethyl ester given by BODANSZKY AND DU VIGNEAUD[3]: 6 mmoles of L-leucine methyl ester hydrochloride and 5 mmoles of benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester were dissolved in 5 ml of chloroform and 0.5 mmoles of AcOH[4] and 6.25 mmoles of triethylamine were added to the solution. A precipitate formed but dissolved again. After 24 h at room temperature, 50 ml of ether containing 0.1 ml of concentrated hydrochloric acid were added to the clear, yellow solution. A heavy precipitate formed. After a few hours at +4° this was filtered off and discarded. The ether and chloroform were removed *in vacuo* and the yellow residue taken up in a small volume (*ca.* 5 ml) of MeOH–$CHCl_3$ (1:4). The solution was allowed to sink into a column (4.2 × 43 cm) of methylated Sephadex G-25, fine (*ca.* 36 % —OMe), which had been equilibrated with the methanol–chloroform (1:4) solvent. The column was developed with the same solvent. Fractions of 25 ml each were collected at a flow rate of 5 min per fraction. An 0.5 ml aliquot of each fraction was pipetted into a weighed tube and the solvent evaporated in a current of air at 80°. The tubes were reweighed, the difference in weight permitting the calculation of the weight of the solvent-free material in each fraction. The results are evident from Fig. 1. Fractions 22–28 were yellow,

the others colourless. Fractions 14–19 were combined and taken to dryness *in vacuo*. The residue solidified. It had a melting point of about 80°. Crystallization from ether–petroleum ether (b.p. 30–60°) failed to elevate the m.p. significantly. Recrystallization from methanol–water[5], however, gave a material melting sharply at 99.5°. NYMAN AND HERBST[10] found 97–98°, SMITH *et al.*[5] 97.5–98.5°. Yield: 3.2 mmoles.

The synthesis of benzyloxycarbonyl-L-leucyl-L-valine methyl ester followed the same pattern. The residue from evaporation of the solvent from the methylated Sephadex column did, however, not solidify but was a colourless syrup. It was taken up in ethyl acetate and washed consecutively with 0.2 *M* $NaHCO_3$, 0.2 *M* HCl and water. After drying over $MgSO_4$ the ethyl acetate was removed *in vacuo*. The residue, which was still a syrup, was taken up in ether. On the addition of petroleum ether an oily precipitate formed, which crystallized when kept in the cold with vigorous scratching. The m.p. was 70–71° and this did not change on recrystallization of the material from methanol–water. Analysis of the recrystallized material, yield 2.7 mmoles (Firma Analytika, Sollentuna, Sweden) showed: C 63.3%, N 7.3 %, calculated for $C_{20}H_{30}O_5N_2$, mol.wt. 378.46: C 63.4 % N 7.4 %. The isolation of L-leucyl-L-leucine and L-leucine-L-valine from the respective protected peptides followed the directions of SMITH *et al.*[5] and was uneventful. The m.p. was 103.5° for benzyloxycarbonyl-L-leucyl-L-leucine (reported 98–101°[5]) and 113° for benzyloxycarbonyl-L-leucyl-L-valine (reported 108–109°[5]).

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