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Hydrazinolysis of Peptidyl Resins of Phenacyl Ester-type Supports for Solid-phase Peptide Synthesis

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The polymer support having a phenacylalkyl side chain for the solid-phase peptide synthesis was found to give a better hydrazinolysis yield than the ordinary polymer support.

Mizoguchi, *et al.*[2] have made an excellent polymer support for the solid-phase peptide synthesis,[3] which has the phenacyl group instead of the benzyl group on the Merrifield type support for anchoring. This Mizoguchi-type peptidyl support is almost unchanged in acidic media but is easily cleaved in basic media. We have examined various hydrazinolysis conditions for this phenacyl ester-type peptidyl support, the hydrazinolysis products of which are useful intermediates for the fragment condensation of peptide synthesis.[4]

The yield from hydrazinolysis was determined mainly from the difference of the amino titration values on a polymer before and after hydrazinolysis by Dorman's method[5] and partly by the ninhydrin colorimetry of the cleaved amino acid hydrazide. As shown by Group I in Table I, the best solvent for hydrazinolysis is dimethylformamide (DMF).[6] The cleavage of Boc-alanyl resin I of the Merrifield type with hydrazine hydrate in DMF showed a relatively low yield (Group I in Table I) and the yield became moderate only when anhydrous hydrazine was used. On the other hand, the cleavage of Boc-alanyl resin II of the Mizoguchi type gave an excellent yield with hydrazine hydrate in DMF. Nevertheless, when sterically hindered peptidyl resins II of the Mizoguchi type were examined the yield dropped remarkably[7] (Group II in Table I). It may be concluded from these results that steric effect of the side chain of amino acid directly attached to a polymer plays an important role in the cleavage reaction. Comparison of Groups II and IV in Table I indicates that 8% cross-linked polymer II gave a better result in cleavage than 2% cross-linked polymer II. It may be assumed that 8% cross-linked polymer prevents the formation of a peptidyl ester or the introduction of a phenacyl group inside the polymer. Only the surface of the polymer can form peptidyl ester.

An attempt was made to avoid these interferences by modification of the Mizoguchi-type polymer. For this purpose, the effect of chain length between phenacyl group and polymer support was examined. Syntheses of the modified polymers were as follows. Polymers IV and V were prepared by the Friedel-Crafts acylation with divinylbenzene–styrene copolymer III and phenylacetyl chloride or ε-phenylcaproyl chloride followed by another Friedel-Crafts

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[6] The ammonolysis of peptidyl resins in DMF was studied by W. Parr, C. Yang, and G. Holzer [*Tetrahedron Letters*, **1972**, 101].

[7] The same kind of steric effect was found by E. Bayer, E. Breitmaier, G. Jung, and W. Parr [*Hoppe-Seyler's Z. Physiol. Chem.*, **352**, 759 (1971)].

acylation with bromoacetyl bromide, respectively. Polymer VI was prepared by the Friedel-Crafts alkylation of III with benzyl chloride followed by the Friedel-Crafts acylation with bromoacetyl bromide. As shown by Group III in Table I, the amino-acylated polymer IV showed a moderate cleavage yield and the amino-acylated polymers V and VI showed a high hydrazinolysis yield. The amino-acylated phenacyl group on the end of the side chain of V. which has a longer side chain than IV, might come out of the polymer matrix more than that of IV. Also, VI might have a relatively longer -($C_6H_4CH_2$)- chain by multiple alkylation and might give the same effect on the cleavage reaction as V. This kind of a sidechain effect appears in the case of affinity chromatography.[8]

TABLE I. Hydrazinolysis Yield of Peptidyl Resins (with 80% $N_2H_4 \cdot H_2O$ or anhyd. N_2H_4)

Group	Amino acid (mmole/g)	Polymer type (% cross-linkage)	Solvent	Mol. eq. of hydrazine	Reaction time (hr)	Yield (%)
I	Ala (0.77)	I (2)	DMF	50	2	48
	Ala (0.77)	I (2)	DMF	50 (anhyd.)	2	70
	Ala (0.64)	II (2)	DMF	50	2	100
	Ala (0.64)	II (2)	DMF	10	1	83
	Ala (0.64)	II (2)	EtOH	50	2	94
	Ala (0.64)	II (2)	Diox.	50	2	100
	Ala (0.64)	II (2)	Diox.	10	1	58
II	His (0.28)	II (2)	DMF	50	2	52
	Ile (0.16)	II (2)	DMF	50	2	52
	Ile-Gly (0.22)	II (2)	DMF	50	2	95
	Ile-Ile (0.08)	II (2)	DMF	100	2	25
III	Ala (0.95)	V (2)	DMF	10	1	90
	Ala (0.88)	IV (2)	DMF	10	1	85
	Ala (0.89)	VI (2)	DMF	10	1	100
	Ile (1.01)	V (2)	DMF	50	2	92
	Ile (1.01)	VI (2)	DMF	50	2	94
IV	Ala (1.00)	V (8)	DMF	10	1	86
	Ala (0.64)	IV (8)	DMF	10	1	72
	Ala (0.80)	II (8)	DMF	10	1	83
	Ile (0.60)	II (8)	DMF	50	2	72

DMF=dimethylformamide; Diox.=dioxane

Experimental

The titration of amino group on a polymer support was carried out by Dorman's procedure.[5] Polymers I and II were synthesized by Merrifield's[3] and Mizoguchi's methods,[2] respectively. The amino-acylation of brominated polymers and the peptide elongation were carried out also by the methods of Merrifield[3] and of Mizoguchi.[2]

Preparation of Modified Polymers——One gram of polymer III (2% or 8% cross-linked) was suspended in 20 ml of the nitrobenzene solution which contained BzCl or the corresponding acid chloride (BzCl, 2.5 g; $C_6H_5CH_2COCl$, 0.77 g; $C_6H_5(CH_2)_5COCl$,[9] 1.1 g). To the chilled mixture, 0.8 g of powdered anhyd. $AlCl_3$ was added in small portions with stirring. Then the mixture was stirred for 1 hr under ice-cooling and overnight at room temperature. After treatment with water, the resulting resin was washed with DMF, MeOH and $CHCl_3$, and dried *in vacuo*. The yield did not depend on the ratio of cross-linkage or reagents and showed almost a similar value of 1.5 g. Polymers IV and V showed the carbonyl absorption at 1675 cm^{-1} in their infrared spectra.

To the suspended solution of 2 g of the above resin and 2.7 g of $BrCH_2COBr$ in 20 ml of nitrobenzene, 1.6 g of powdered anhyd. $AlCl_3$ was added in small portions with stirring under ice-cooling. Further stirring

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[8] P. Cuatrecasas, *J. Biol. Chem.*, **245**, 3059 (1970).
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was continued for 30 min under cooling and overnight at room temperature. After washing successively with water, DMF, MeOH, and $CHCl_3$, the resin obtained was dried *in vacuo*. The yield was 2.5 g.

Analysis for Br:

2% cross-linked resin		8% cross-linked resin	
IV	17.1%	IV	16.6%
V	10.8%	V	18.0%
VI	22.1%	VI	20.5%
		II	7.2%

Hydrazinolysis of Amino-acyl and Peptidyl Resins——Terminal N-Boc-peptidyl (or amino-acyl) resin (150 mg) was suspended in the solution of a suitable amount of hydrazine (see Table I) in 4 ml of DMF. The mixture was shaken for the period given in Table I at room temperature. After filtration, the residual resin was washed with DMF and EtOH, and treated with 1 N HCl–AcOH for removing Boc group. The resin was neutralized with the solution of Et_3N in CH_2Cl_2 and used for titration.