anol mixture gave minute white needles (4 mg) with m.p. 218–219°C (uncorr.). Mixed m.p. with authentic (–)-16α-hydroxykaurane (I) 217–218°, $[\alpha]_D^{25} = -46°$ (*c* 0.40, $CHCl_3$), I: $[\alpha]_D^{25} = -45°$ (*c* 1.0, $CHCl_3$). The molecular weight found (mass spectrometry) was 290 ($C_{20}H_{34}O$: m.w. 290.5). The mass spectrum differed from that of I (*cf.* Ref. 4) only in the relative intensities of some peaks. Infrared spectra (KBr phase) of the two samples were indistinguishable.

NMR spectra were recorded on a Varian A60 instrument equipped with a C 1024 time averaging computer. Signals from the four methyl groups occurred at the following δ-values (TMS as internal reference, solvent $CDCl_3$).

| | | | | |
|---|---|---|---|---|
| I | 0.80 | 0.84 | 1.02 | 1.35 |
| Moss substance | 0.80 | 0.83 | 1.04 | 1.35 |

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# Studies on the Coupling Step in Solid Phase Peptide Synthesis. Some Preliminary Results from Competition Experiments

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This preliminary study deals with the reactivity of Boc *-protected amino acids under the standard coupling conditions used in solid phase peptide synthesis as described by Merrifield.[1] The aim has been to estimate roughly the relative coupling rates of properly protected derivatives in order to have a rational basis for considerations by which differences in reactivity can be compensated for in some way or another. Such differences in reactivity may be assumed to exist for sterical and other reasons but have not received due attention so far, though there are some cases already known when certain amino acids have caused special difficulties in the coupling step.[2,3]

We recently started an investigation, the first results of which are reported below. Thus polymer-bound amino acid ** (1 equiv.) was reacted with altogether 4 equiv. of 4 generally *different* Boc-amino acids and the same quantity of dicyclohexylcarbodiimide for 2 h at room temperature. Boc-glycine was present in all experiments to make a rough comparison possible. From a practical point of view, the general procedure was essentially that of Merrifield. The Boc-group was removed from the dipeptide resin with 50 % (v/v) trifluoroacetic acid in methylene chloride before the dipeptide mixture was split off from the resin with liquid hydrogen fluoride,[4] and a peptide aliquot after acid hydrolysis analyzed for free amino acids.[5] One series of experiments was performed with polymer-bound alanine (Table 1), another with polymer-bound valine (Table

* Boc stands for α-*t*-butyloxycarbonyl.

** Prepared from Bio-Beads S·X-1, 200–400 mesh, chloromethylated, capacity 0.75 mequiv./g, obtained from Bio-Rad Laboratories, Richmond, Calif., USA, following the standard procedure.

*Table 1.* Competitive coupling experiments with alanine-resin.[a]

| Experiment No. | Boc-amino acids used (incorporation, %) | | | | Total incorporation (%) |
|---|---|---|---|---|---|
| 1 | Gly | Phe | Leu | Val | |
| | 39.7 | 29.6 | 24.8 | 4.8 | 98.6 |
| 2 | Gly | Tyr[b] | Ser[b] | Ile | |
| | 45.7 | 30.3 | 26.5 | 4.2 | 106.7 |
| 3 | Gly | Asp[b] | Cys[b] | Glu[b,c] | |
| | 36.7 | 29.2 | 25.6 | 12.9 | 104.4 |
| 4 | Gly | Met | Pro | Thr[b] | |
| | 42.0 | 26.0 | 17.8 | 9.4 | 95.2 |
| 5A | Gly | Ile (3 equiv.) | | | |
| | 66.1 | 33.0 | | | 99.1 |
| 5B[d] | Gly | Ile (3 equiv.) | | | |
| | 63.8 | 32.1 | | | 95.9 |
| 5C[e] | Gly | Ile (3 equiv.) | | | |
| | 61.7 | 37.8 | | | 99.5 |
| 6[f] | Gly | Phe | Asn | Gln | |
| | 41.0 | 21.2 | 16.0 | 13.7 | 91.9 |

[a] Results were normalized with the originally resin-bound amino acid = 100. [b] The side-chain functional group was protected by benzyl. [c] The value for this amino acid was not reproducible and is therefore tentative. [d] In this experiment, the peptide mixture was hydrolyzed for 48 h in constant boiling hydrochloric acid before analysis. In all other experiments of this table except 5C, a hydrolysis time of 24 h was used. [e] Concerning special conditions used for hydrolysis, see the text. [f] Boc-amino acid *p*-nitrophenyl esters were used in this experiment.

*Table 2.* Competitive coupling experiments with valine-resin.[a]

| Experiment No. | Boc-amino acids used (incorporation, %) | | | | Total incorporation (%) |
|---|---|---|---|---|---|
| 7 | Gly | Ala | Phe | Leu | |
| | 32.3 | 28.8 | 24.0 | 20.1 | 105.2 |
| 8A | Gly | Ser[b] | Tyr[b] | Ile | |
| | 43.3 | 27.5 | 25.2 | 2.8 | 98.8 |
| 8B[c] | Gly | Ser[b] | Tyr[b] | Ile | |
| | 42.8 | 27.2 | 25.4 | 4.6 | 100.0 |
| 9 | Gly | Met | Pro | Thr[b] | |
| | 38.5 | 26.6 | 23.0 | 10.0 | 98.2 |
| 10A | Gly | Ile (3 equiv.) | | | |
| | 68.6 | 29.7 | | | 98.6 |
| 10B[c] | Gly | Ile (3 equiv.) | | | |
| | 62.3 | 39.1 | | | 101.4 |

[a,b] See notes to Table 1. [c] After hydrolysis for 72 h in constant boiling hydrochloric acid. All other values in this table were obtained after hydrolysis for 24 h.

2). Data are included for 16 amino acids, the missing ones being lysine, tryptophan, histidine, and arginine, since they require minor modifications mainly in the analytic procedure.

Two experiments, described in Table 1, were performed differently from the rest. Thus in 5C, a dipeptide resin aliquot was subjected to hydrolysis in a boiling 1:1 mixture of dioxane and conc. hydrochloric acid for 24 h, followed, after the evaporation of the liquid, by further hydrolysis in boiling 6 N hydrochloric acid for another 24 h. The total recovery

of the C-terminal amino acid (alanine) in this experiment was 82 % compared to 62–77 % when the hydrogen fluoride procedure described above was used. Experiment 6 was performed with one equivalent each of Boc-glycine, phenylalanine, asparagine and glutamine *p*-nitrophenyl ester in dimethylformamide for 24 h. The low total incorporation value in this case is supported by a determination [6] of the quantity of free amino groups on a resin aliquot. The value thus obtained was 10 %.

Because of the limited number of experiments performed so far we would like to restrict our comments of the results to the following points. Of the Boc-amino acids used in this investigation, isoleucine and valine have a far lower reactivity than the others. This seems to be in agreement with the experiences of Li and Yamashiro [2] and to some extent of Yajima, Kawatani and Watanabe.[3] On the other hand Boc-leucine does not seem to present special difficulties as reported by the last mentioned authors. With the low reactivity of Boc-isoleucine and -valine in mind we performed the experiments with valine-resin of Table 2, *i.e.* used valine as an amino component in the coupling step. Total as well as relative incorporation values seem to be very similar when alanine- and valine-resins are used, though of course we are aware of the fact that in the first context the experimental error is much too large to permit reliable conclusions. Further work following this approach will show if the nature of the amino component has any influence on the coupling step. Since after all it is a well-known fact that isoleucine and valine are sterically hindered,[7] our experiments demonstrate above all how similar the reactivity is for the other amino acids studied, with the exception of *O*-benzyl-threonine which, like isoleucine and valine, has two $\beta$-substituents.

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# Chlorinated Long-chain Fatty Acids

## Their Properties and Reactions. IV.[1] Quantitative Gas Chromatography of Chlorinated Octadecanoic Acids Prepared from Oleic, Linoleic and Linolenic Acids

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Resin constituents, *i.e.* compounds extracted by neutral organic solvents, that remain in pulp are responsible for serious disturbances during the pulping process and they often have a decisive effect on the quality of the pulp and determine whether it is suitable for conversion into paper or for use as a raw material.[2] For this reason, the deresination of pulp is a very important process, although it is often difficult, especially when the pulp is made from hardwoods like birch.

The main constituents of the fatty acid fractions of the resins of birch, pine, and