Studies on Peptides. XXXVII.1) Suppressed Racemization in Peptide Synthesis by the Use of \( p \)-Chloro or \( p \)-Nitrobenzenesulfohydroxamic Acid

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The use of esters of N-hydroxyphthalimide and N-hydroxysuccinimide in peptide synthesis was introduced by Nefkens and Tesser\(^9\) and Anderson, *et al.*,\(^9\) respectively. It was pointed out by Weygand, *et al.*,\(^9\) that peptide synthesis with dicyclohexylcarbodiimide (DCC) in the presence of N-hydroxysuccinimide suppresses the rate of racemization during the amide forming step. Despite of this advantageous property, it was found later that the reaction of N-hydroxysuccinimide with DCC gave succinimidocarbonyl-\( \beta \)-alanine-N-hydroxysuccinimide ester\(^9\) and in the presence of amino components, succinimidocarbonyl-\( \beta \)-alanine amide derivatives\(^9\) can be isolated as a side reaction product in some instance. Considering an analogous situation in N-hydroxyphthalimide, a number of other N-hydroxy compounds was investigated.\(^8\)

We have now examined the degree of racemization caused by DCC in the presence of benzenesulfohydroxamic acid (I) and two of its derivatives: \( p \)-chlorobenzenesulfohydroxamic acid (II) and \( p \)-nitrobenzenesulfohydroxamic acid (III). The system of Bodanszky and Conklin\(^9\) was adopted for this purpose. During the coupling reaction of Ac-L-Ile-OH with H-Gly-OEt, racemized Ac-allo-d-Ile-Gly-OEt, can be detected, after acid hydrolysis, by the Spackman-Stein-Moore method\(^10\) of amino acid analysis. The results are listed in Table I.

No remarkable improvement could not be achieved by the addition of benzenesulfohydroxamic acid. However addition of \( p \)-chloro or \( p \)-nitrobenzenesulfohydroxamic acid suppressed the racemization of this coupling reaction in great extent and these values seem comparable to or somewhat better than that of N-hydroxysuccinimide.

2) Location: Sakyo-ku, Kyoto.
### Table I. Degree of Racemization

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Benzenesulfohydroxamic acid</th>
<th>p-Chlorobenzenesulfohydroxamic acid</th>
<th>p-Nitrobenzenesulfohydroxamic acid</th>
<th>N-Hydroxy succinimide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racemization(a)\ %</td>
<td>22.3</td>
<td>9.0</td>
<td>8.1</td>
<td>14.8</td>
</tr>
</tbody>
</table>

\(a\) \text{lit. DCC 27.4\%}^{11} \text{; } 37\%^{11}

In the preceding paper,\(^1\) we described that 5-chloro and 5,7-dichloro-8-hydroxyquinoline are both effective as racemization depressants. Reagents bearing such property possess an ability to suppress the formation of acylurea, the by-product of the DCC condensation reaction of acyl peptide fragment. The use of these adducts in the DCC coupling reaction seems to open a way of peptide synthesis via these types of active esters as intermediates.

**Experimental**

**p-Chlorobenzenesulfohydroxamic Acid**—According to Gattermann,\(^1\) a solution of hydroxylamine (prepared from 10.0 g of the hydrochloride with sodium ethalate) in EtOH (20 ml) was added dropwise to a solution of p-chlorobenzenesulfonyl chloride (10.5 g) in EtOH (20 ml). After stirring for 1 hr, the solution was condensed in vacuo and the residue was dissolved in ether, which was washed with \(\text{H}_2\text{O}\) dried over \(\text{Na}_2\text{SO}_4\) and then evaporated. The solid residue was recrystallized from ether; yield 6.1 g (61\%), mp 128—129\(^\circ\). *Anal.* Calcd. for \(\text{C}_7\text{H}_4\text{O}_2\text{NSCl}\): C, 34.04; H, 2.91; N, 6.75. Found: C, 34.32; H, 2.89; N, 6.63.

**p-Nitrobenzenesulfohydroxamic Acid**—The reaction was performed as described above. Instead of p-chlorobenzenesulfonyl chloride, p-nitrobenzenesulfonyl chloride was employed; yield 65\%, mp 154—155\(^\circ\). *Anal.* Calcd. for \(\text{C}_7\text{H}_4\text{O}_2\text{N}_2\text{S}\): C, 33.04; H, 2.77; N, 12.84. Found: C, 33.27; H, 2.68; N, 12.78.

**Coupling Reaction of Ac-L-Ile-OH with H-Gly-OEt**—Condensation reaction was performed as described previously.\(^1\) The crude product, after drying over \(\text{P}_2\text{O}_5\) in vacuo, was hydrolyzed by 6\(\text{N}\) HCl and the hydrolysate was submitted for quantitative amino acid analysis. The results were listed in Table I.