Note

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Studies on Peptides. XXXVII.[1] Suppressed Racemization in Peptide Synthesis by the Use of *p*-Chloro or *p*-Nitrobenzenesulfohydroxamic Acid

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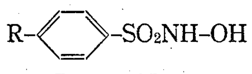
The use of esters of N-hydroxyphthalimide and N-hydroxysuccinimide in peptide synthesis was introduced by Nefkens and Tesser[3] and Anderson, *et al.*,[4] respectively. It was pointed out by Weygand, *et al.*,[5] that peptide synthesis with dicyclohexylcarbodiimide (DCC) in the presence of N-hydroxysuccinimide 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 succinimidoxycarbonyl-β-alanine-N-hydroxysuccinimide ester[6] and in the presence of amino components, succinimidoxycarbonyl-β-alanine amide derivatives[7] 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.



I: R=H
II: R=Cl
III: $R=NO_2$

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.

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Table I. Degree of Racemization

Reagent	Benzenesulfohydroxamic acid	*p*-Chlorobenzenesulfohydroxamic acid	*p*-Nitrobenzenesulfohydroxamic acid	N-Hydroxysuccinimide
Racemization[a] %	22.3	9.0	8.1	14.8

[a] lit. DCC 27.4%[1] 37%[9]

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 acylpeptide 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,[11] 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 H_2O, dried over Na_2SO_4 and then evaporated. The solid residue was recrystallized from ether; yield 6.1 g (61%), mp 128—129°. *Anal.* Calcd. for $C_6H_6O_3NSCl$: 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°. *Anal.* Calcd. for $C_6H_5O_5N_2S$: 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 P_2O_5 *in vacuo*, was hydrolyzed by 6 N HCl and the hydrolysate was submitted for quantitative amino acid analysis. The results were listed in Table I.

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[11] L. Gattermann, "Die Praxis des Organischen Chemikers," Walter De Gruyter & CO., Berlin, 1962, p. 169.