benzene. Dry nitrogen was forced up through the disk so that the reaction was blanketed at all times by an inert atmosphere. Methanesulfenyl bromide (0.01 mol) was added to the silver salt solution from a dropping funnel. When reaction was complete, as indicated by the disappearance of the red color of the sulfenyl bromide, the direction of the nitrogen stream was reversed, and a partial vacuum was placed on the bottom flask. In this manner the solution of sulfenium ion was filtered free of silver bromide under nitrogen. The solution was then concentrated *in vacuo* in the same flask to a volume of about 10 ml. Samples were withdrawn under nitrogen with a syringe and injected into nitrogen-flushed nmr tubes through a small stopple. Spectra were recorded on a Varian Associates A-60 nmr spectrometer at 60 MHz. After the spectrum was recorded, 10 μl of pyridine or acetonitrile was introduced and the spectrum was recorded again.

Generation of Methanesulfenium Ion in the Presence of Dimethyl Sulfide. On the porous disk of Figure 1 was placed a solution of 2.0 g (0.031 mol) of dimethyl sulfide and 2.1 g (0.004 mol) of silver 2,4,6-trinitrobenzenesulfonate–acetonitrile complex in 10 ml of nitromethane. From the dropping funnel was added a solution of methanesulfenyl bromide (0.004 mol, prepared *in situ* from 0.002 mol of dimethyl disulfide and 0.002 mol of bromine) in 50 ml of dichloromethane. When the reaction was complete, the solution was filtered free from silver bromide as previously described. Precipitation of the product with anhydrous ether yielded 0.8 g (0.002 mol, 50%) of dimethyl(methylthio)sulfonium 2,4,6-trinitrobenzenesulfonate, mp 195–196°. The melting point, mixture melting point, and nmr and infrared spectra of this compound were identical with those of the compound prepared by the alkylation of dimethyl disulfide with trimethyloxonium 2,4,6-trinitrobenzenesulfonate.[3,4]

The Reaction of Hydroxamic Acids with Water-Soluble Carbodiimides. A Lossen Rearrangement

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Abstract: A method for the conversion of hydroxamic acids to the amines under mild conditions has been developed. Treatment of the hydroxamic acid with a water-soluble carbodiimide at pH 5 and room temperature was found to give a quantitative conversion to the amine by means of a Lossen rearrangement. The mechanism of the reaction was established by kinetic data and product identifications. Since carboxylic acids can be readily converted to hydroxamic acids, the ready conversion of carboxylic acid groups to amino groups is possible by this method.

The conversion of carboxylic groups to amines can be effected in a number of ways.[2–5] Most of these methods, however, involve drastic conditions, such as strong alkali or highly reactive reagents. Since this reaction could be of great potential importance in biological investigations if it could be performed under mild conditions, a study of the conditions for such an interconversion was initiated. Hydroxamic acids exist in nature[6] and can be formed fairly readily from carboxylic acids.[7,8] Therefore, the first stage of our studies was to develop a method for the rearrangement of the hydroxamic acid at moderate pH's in aqueous solution at room temperature. The method which was found to be successful involves the treatment of the hydroxamic acid with a water-soluble carbodiimide to achieve a Lossen rearrangement (eq 1).

$$\mathrm{RC(=O)NHOH} \xrightarrow{k_1} \mathrm{CO_2} + \mathrm{RNH_2} \qquad (1)$$

In order to establish the mechanism of the reaction, the conditions to obtain quantitative yields, and the possibilities of side reactions, various kinetic measurements were made. These data together with a discussion of the advantages and limitations of the procedure are presented below.

Experimental Section

The authors are indebted to Dr. J. Kirsch for a sample of acetohydroxamic acid. The water-soluble reagent, 1-benzyl-3-dimethylaminopropylcarbodiimide (BDC), was prepared as its *p*-toluenesulfonate as described previously.[9] Glycylhydroxamic acid was made by the method of Cunningham,[10] mp 135° dec (lit.[10] value 137° dec).

All the reactions requiring titration or pH control were conducted in a Radiometer TTTlc pH-Stat with the ABUlb autoburet. A water-jacketed vessel was used, maintaining the temperature to better than ±0.1°.

Hydroxamic acids were assayed by a method based on the procedure of Goldenberg and Spoerri.[8] Aliquots of 0.5 ml at pH <4 were added to 2.5 ml of 0.10 *M* ferric chloride in 0.08 *M* HCl, and the absorbance (280 mμ, 1 cm) was measured; calibration indicated absorbance (280 mμ, 1 cm) = 0.321/μmol of acetohydroxamic acid. Control experiments indicated that O-acetylhydroxylamine yielded no hydroxamic acid under the assay conditions, even when the concentration of hydroxylamine hydrochloride in the assay solution was as high as 0.16 *M*. Diacetylhydroxylamine yielded 1 mol of hydroxamic acid per mol of diacetylhydroxylamine (*cf.* below).

Formaldehyde was estimated by a chromotropic acid procedure similar to that of Tompsett and Smith.[11] The sample (in 3 ml) was heated to 100° for 4 hr with 5.0 ml of chromotropic acid reagent (0.2 g of chromotropic acid in 2 ml of water plus 48 ml of 13 *M* H_2SO_4), cooled, and diluted with 2.0 ml of 9 *M* H_2SO_4. The formaldehyde was estimated from the absorbance (565 mμ, 1 cm). Calibration with hexamethylenetetramine gave absorbance (565 mμ, 1 cm) = 1.65/μmol of formaldehyde.

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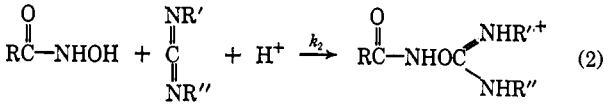
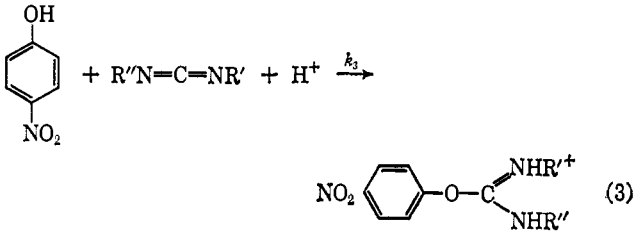
Results

The expected initial reaction between a carbodiimide and a hydroxylic compound according to Khorana[12] is addition to one of the C=N double bonds. In the case of a hydroxamic acid the course of the reaction would be as shown in eq 2.



(2)

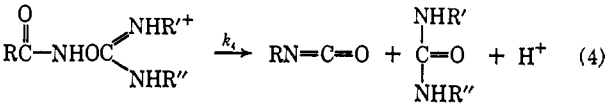
Direct evidence for this initial step is difficult to obtain because of the subsequent rapid reactions which are described below. However, support may be adduced from previous studies[13] with nitrophenol, where addition is not followed by other rapid reactions. In that case, it was shown that the phenol forms the adduct shown in eq 3 (an O-arylisourea), with concomitant uptake of 1 mol of hydrogen ion.[13]



(3)

When acetylhydroxyamic acid is treated with BDC in excess, the rate of uptake of acid is shown in Figure 1. The uptake of acid is clearly biphasic in this case beginning with an accelerating phase for about 8 min, after which the rate of uptake of acid diminishes as the titer approaches a limiting value of 1 equiv of acid per mol of hydroxamic acid. The reaction occurring is clearly not the simple first-order reaction of the phenol,[13] but an explanation of the complex behavior can be obtained if one condsiers the possibility of Lossen rearrangement.

The product of the reaction shown in eq 2 has the general structure R–CO–NHOX, and compounds of this type are known to undergo a Lossen rearrangement if OX^- is a good leaving group. For example, rearrangement has been observed under very mild conditions when OX^- is benzoate or substituted benzoate,[14] *p*-toluenesulfonate,[15] or dialkylphosphate.[16] The reaction scheme for a Lossen rearrangement with subsequent hydrolysis of the isocyanate is shown in eq 4 and 5. From the velocities of reactions when *p*-toluene-



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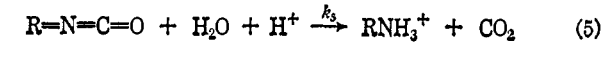

(5)

sulfonate or dialkylphosphate are leaving groups, it seems probable that step 2 (eq 4) will be relatively

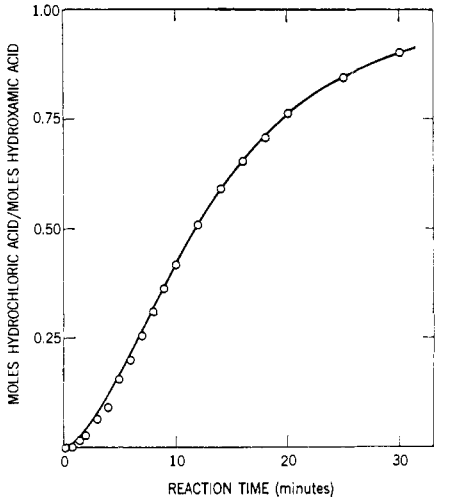

Figure 1. The uptake of acid during reaction of acetohydroxamic acid with BDC. Conditions: 0.08 *M* BDC, 2.7×10^{-3} *M* acetohydroxamic acid, pH maintained at 4.75 by titration with 0.05 *M* HCl, 28°. The solid line represents a theoretical curve calculated assuming a two-step process: A → B → C in which the respective rate constants are 4.1×10^{-3} sec^{-1} and 1.54×10^{-3} sec^{-1}. These constants can be identified with eq 2 and 5 as discussed in the text.

rapid compared to steps 1 (eq 2) and 3 (eq 5). In that case the reaction scheme would reduce to two kinetically observable steps as far as measurement by means of proton uptake is concerned. If the first step is slow followed by a rapid rearrangement, the sum of these first two steps involves zero net uptake of protons. This would, therefore, account for the small initial rate of uptake of acid in Figure 1. The third step involving the hydrolysis of the isocyanate involves the uptake of 1 equiv of acid and accounts for the latter part of the rate curve. If this interpretation is correct, the two rate constants obtained from Figure 1, *i.e.*, 4.1×10^{-3} sec^{-1} and 1.54×10^{-3} sec^{-1}, can be identified with k_2[BDC] and k_5, respectively.

The magnitude of k_2[BDC] can be measured independently by the rate of consumption of free acetohydroxamic acid during the course of the reaction. The results of such an experiment are shown in Figure 2. Using the ferric chloride colorimetric test for hydroxamic acids,[8] the rate of loss of acetohydroxamic acid is seen to follow a first-order reaction with a rate constant of 4.1×10^{-3} $sec^{-1} \pm 0.5 \times 10^{-3}$ sec^{-1}. This is in excellent agreement with the rate constant of the first step of the reaction shown in Figure 1 which was also identified with the constant k_2[BDC].

The second constant obtained in Figure 1 is identified with the hydrolysis of methyl isocyanate, and this also can be checked independently. The values for the hydrolysis of methyl isocyanate under the same conditions are summarized in Table I and are in

Table I. The Rate of Hydrolysis of Methyl Isocyanate[a]

(CH_3NCO), *M*	% acetonitrile	*k*, sec^{-1}	Mol of H^+/mol of isocyanate
2.5×10^{-3}	1.0	1.51×10^{-3}	1.02
5.0×10^{-3}	7.0	1.57×10^{-3}	1.03

[a] Conditions: 0.08 *M* NaCl, pH 4.75, maintained by automatic titration with 0.05 *M* HCl, 28°.

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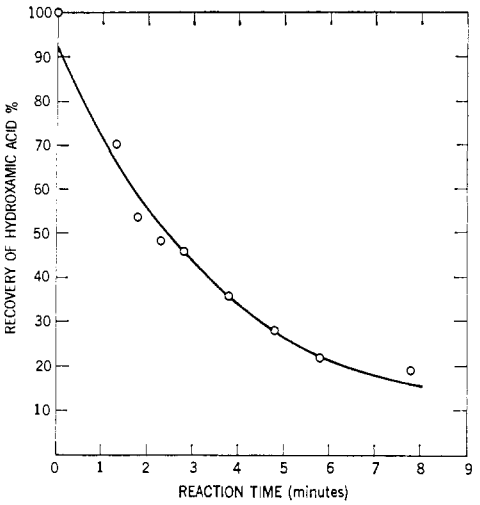

Figure 2. The disappearance of acetohydroxamic acid during reaction with BDC. Conditions: 0.10 M BDC, 2.7×10^{-3} M acetohydroxamic acid, pH maintained at 4.75 with 0.5 M HCl, 25°. Disappearance of hydroxamate is determined by ferric chloride color assay. The solid line represents a theoretical curve with a constant of 4.1×10^{-3} sec^{-1}. Standard error of $\pm 0.5 \times 10^{-3}$ sec^{-1} can be calculated.

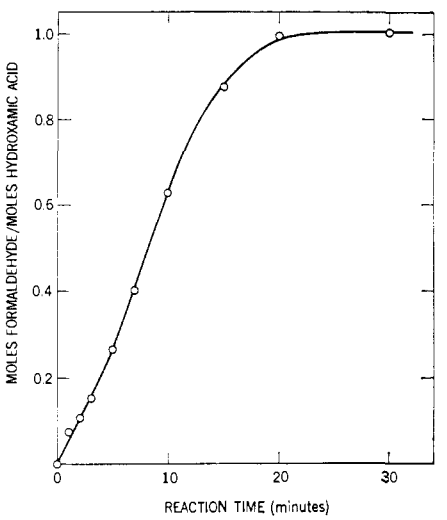

Figure 3. The yield of formaldehyde obtained from the reaction of glycylhydroxamic acid with BDC. Conditions: 0.08 M BDC, 2.9×10^{-3} M glycylhydroxamic acid, pH maintained at 4.75 with 0.5 M HCl, 25°. Aliquots were quenched with four volumes of 1.0 M formic acid before assay. Formaldehyde determined by the chromotropic assay (see Experimental Section).

close agreement with the 1.54×10^{-3} sec^{-1} value used in the theoretical curve of Figure 1.

An independent check on the proposed mechanism of reaction can be obtained by allowing BDC to react with glycylhydroxamic acid instead of acetohydroxamic acid. A Lossen rearrangement of glycylhydroxamic acid should produce formaldehyde under the acid conditions used here. The results of this experiment, summarized in Figure 3, confirm these conclusions. A quantitative yield of formaldehyde is obtained, and the rate curve for the production of formaldehyde is similar to that of Figure 1. A slight difference occurs in the rate of titration of acid. There appears to be an initial uptake of acid, then a liberation of acid, and a final kinetically first-order uptake of acid. This would be consistent with the three-step mechanism outlined above, if, in the case of the glycylhydroxamic acid, the respective rate constants k_2[BDC], k_4, and k_5 were all approximately equal.

These results show that treatment of a simple hydroxamic acid with a carbodiimide leads to a quantitative conversion to the amine. In a polyfunctional molecule, however, competitive intramolecular reactions can occur, and therefore it is of interest to determine the rate of reaction of the intermediate isocyanate with potential reactants such as those found in the side chains of proteins.

Table II shows the results of some exploratory experiments in which methyl isocyanate was allowed to react with solutions containing methylamine and ethanol. The proportion of the isocyanate reacting with these compounds was calculated from the total number of equivalents of acid taken up per mole of isocyanate using eq 5, 6, and 7.

$$\text{R-N=C=O} + \text{R'OH} \longrightarrow \text{R-NH-CO-OR'} \quad (6)$$

$$\text{R-N=C=O} + \text{R'NH}_3^+ \longrightarrow \text{R-NHCONHR'} + \text{H}^+ \quad (7)$$

The results indicate that ethanol is not particularly reactive being approximately of equal reactivity to water. Reaction with serine and threonine hydroxyl groups would, therefore, not be expected unless an unusually reactive serine were present such as the serine at the active site of chymotrypsin.

The reaction with methylammonium ion is fairly small at low concentrations of ammonium ion and therefore intramolecular reactions of the intermediate isocyanate with lysine residues in other protein molecules would be unlikely. However, if the concentration of the methylammonium ion is increased, significant concentrations of dimethylurea are obtained. Thus, a lysine group held in a favorable position might indeed form a cross-link with the isocyanate intermediate. From the rate constants, only a very favorable orientation would lead to reaction, and therefore this side reaction (a) should not occur frequently, and (b) should be interesting in regard to the structure of the protein if it does occur.

This rearrangement of the hydroxamic acid under the influence of the carbodiimide is of great significance in regard to attempts to synthesize hydroxamic acids by the direct reaction of carboxylic acids with carbodiimides and hydroxylamine. Figure 4 shows the yields obtained during the direct reaction of acetic acid, BDC,

Table II. The Reactions of Methyl Isocyanate with Aqueous Solutions of Methylamine and Ethanol[a]

Expt	$CH_3N-H_3^+Cl^-$, M	C_2H_5OH, mol fraction	Yield of product,[b] %	H^+ uptake[c] 10^3k, sec^{-1}
1	0.2	...	5.3	1.42
2	0.6	...	20.5	1.54
3	1.0	...	33	1.75
4	...	0.017	~0	1.42
5	...	0.25	32	1.28
6	...	...	...	1.65

[a] Conditions: 0.02 M CH_3NCO, 2% acetonitrile, pH 4.75, maintained with 0.4 M HCl, 25°. [b] $CH_3NHCONHCH_3$ in the case of $CH_3NH_3^+Cl^-$ and $CH_3NHCOOC_2H_5$ in the case of C_2H_5OH. [c] From HCl titration.

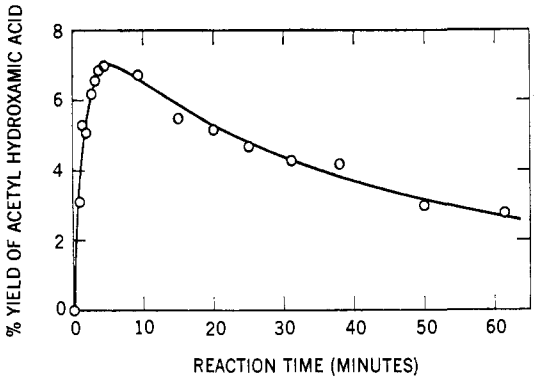

Figure 4. The apparent yield of hydroxamic acid from the reaction of acetate with BDC and hydroxylamine in excess. Conditions: 0.02 *M* acetate, 0.10 *M* hydroxylamine, 0.10 *M* BDC *p*-toluenesulfonate, pH maintained at 4.75 with 2.0 *M* NaOH, 25°. Hydroxamic acid determined by the ferric chloride assay.

and hydroxylamine. The coupling of a nucleophile to a carbodiimide-activated carboxyl (*i.e.*, an O-acylisourea) has been shown to occur,[12,13] and the analogous reaction of hydroxylamine would be that of eq 8. It

$$CH_3COOH + \underset{\underset{NR'}{\|}}{\overset{NR}{\|}}{C} + NH_2OH \longrightarrow CH_3CONHOH + \underset{NHR'}{\overset{NHR}{|}}{CO} \quad (8)$$

is seen that the yield of hydroxamic acid as determined by the ferric chloride color test rises to a maximum and then decreases. The yield at the maximum is extremely small, approximately 7% under these conditions. This is reasonable in view of the studies reported above since the acetohydroxamic acid formed initially would react with BDC and undergo the Lossen rearrangement. In fact at first sight it seems surprising that Franzblau, *et al.*,[17] were able to obtain even a small yield of hydroxamic acid from the reaction of acetic acid with hydroxylamine and cyclohexyl-2-morpholinoethylcarbodiimide in excess.

The explanation can probably be found in the formation of O-acetylhydroxylamine and possibly diacetylhydroxylamine as products in addition to acetohydroxamic acid. Jencks[18] has found that the reaction of hydroxylamine with acetylating agents can yield O-acetylhydroxylamine and that this intermediate can be converted to acetohydroxamic acid by the action of hydroxylamine. The presence of products of this type could therefore be demonstrated by adding hydroxylamine to the product of the reaction shown in Figure 4. Thus, after 90 min under the conditions shown in Figure 4 there was an apparent yield of approximately 2% acetohydroxamic acid as determined by the ferric chloride colorimetric procedure. On incubation of this mixture with hydroxylamine buffer at pH 6 a 31% yield was obtained. The added yield was undoubtedly caused by the conversion of some O-acetylhydroxylamine (which does not give the ferric chloride color) to acetohydroxamic acid (which does).

This can be shown more clearly in the reaction of BDC with an excess of acetate and hydroxylamine, in which case secondary reactions between BDC and the

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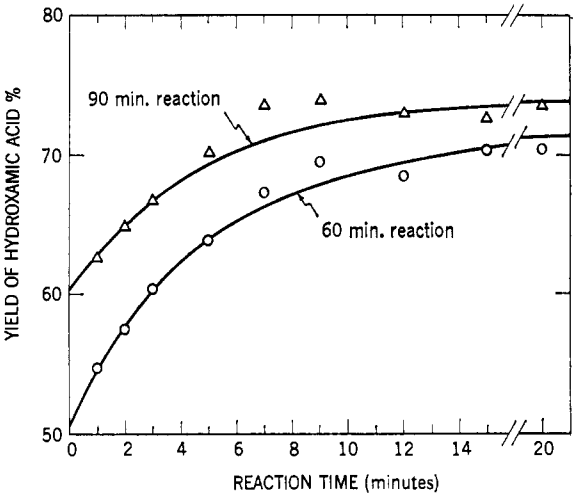

Figure 5. The rate of generation of hydroxamic acid on addition of hydroxylamine buffer to the product of a reaction of BDC with acetate and hydroxylamine. Initial reaction 2.0×10^{-2} *M* BDC, 0.10 *M* acetate, 0.10 *M* NH_2OH, pH 4.75, 25°. Time of reaction: 60 or 90 min. The second phase of the reaction was obtained by taking the reaction mixture from the initial phase and adding to nine volumes of a mixture of 0.556 *M* $NH_2OH \cdot HCl$ and 0.278 *M* NaOH. The pH was 6.0 and temperature 25°. Yield of acetohydroxamic acid determined by the ferric chloride color assay. The solid curves are calculated from a first-order rate constant of 3.4×10^{-3} sec^{-1}.

initially formed acetohydroxamic acid are minimized. Figure 5 shows the rate of generation of acetohydroxamic acid when hydroxylamine at pH 6 was added to reactions of this type after 60 min and 90 min of reaction, respectively. In these cases at time zero, the yields of acetohydroxamic acid were 50 and 60%, respectively, and this amount increased as additional acetohydroxamic acid was produced in a first-order process with a rate constant of 3.4×10^{-3} sec^{-1}.

The rate of reaction of O-acetylhydroxylamine and diacetylhydroxylamine with hydroxylamine are quite consistent with this hypothesis. Tables III–V show

Table III. Effect of NH_2OH Concentration on Reaction with Acetic Anhydride[a]

NH_2OH, *M*	Initial yield of $CH_3CONHOH$, %	10^3k for isomerization of CH_3COONH_2, sec^{-1}
0.02	51.7	...
0.10	49.4	6.15
0.50	59.2	1.26
1.00	63.9	2.75
1.80	76.9	4.40

[a] Conditions: 0.0125 *M* acetic anhydride, 10% acetonitrile, pH 4.75, maintained with NaOH, 24°.

Table IV. Effect of pH on Reaction of Acetic Anhydride with Hydoxylamine [a]

pH	Initial yield of $CH_3CONHOH$, %	10^3k for isomerization of CH_3COONH_2, sec^{-1}
4.75[b]	59.2[b]	1.26[b]
5.44	70.5	1.79
5.97	73.4	3.05
6.50	76.1	2.69

[a] Conditions: 0.01 *M* acetic anhydride, 0.5 *M* $NH_2OH \cdot HCl$, 10% acetonitrile, buffers of $NH_2OH \cdot HCl$ + NaOH for pH maintenance, 25°. [b] Acetic anhydride = 0.125 *M* in this experiment and pH maintained with NaOH.

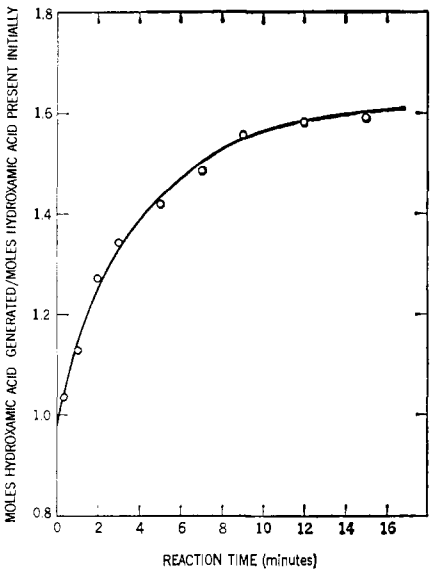

Figure 6. The generation of acetohydroxamic acid from the reaction of diacetylhydroxylamine with hydroxylamine. Initial reaction conditions: 7.7×10^{-3} *M* acetic anhydride, 3.1×10^{-3} *M* acetohydroxamic acid, pH 4.75 maintained with NaOH, 25°. After 40 min (greater than ten half-lives for acetic anhydride hydrolysis) an equal volume of 1 *M* $NH_2OH \cdot HCl$ in 0.5 *M* NaOH was added (pH 6.0) and the yield of hydroxamic acid determined by the ferric chloride color assay. The solid line is calculated for a first-order reaction with a rate constant of 4.04×10^{-3} sec^{-1}.

the rate constants for the reaction of O-acetylhydroxylamine with hydroxylamine under a range of conditions.[19] Figure 6 shows the rate of reaction of diacetylhydroxylamine with hydroxylamine. Under the conditions that we have used for the reaction of acetate with BDC and hydroxylamine, *i.e.*, 0.1 *M* NH_2OH, pH 4.75, 25°, the half-time for conversion of O-acetylhydroxylamine to acetohydroxamic acid is about 80 min. Thus, a significant fraction of the initial yield of this compound would survive 60 to 90 min and be capable of generating acetohydroxamic acid by subsequent treatment with a hydroxylamine buffer at pH 6 (*cf.* Figure 5). In the more concentrated hydroxylamine buffer, *i.e.*, 0.5 *M* NH_2OH, pH 6, 25°, the rate constant for the conversion of O-acetylhydroxylamine to acetohydroxamic acid is $3.4 \pm 0.2 \times 10^{-3}$ sec^{-1}, which is consistent with the value of $3.4 \pm 0.3 \times 10^{-3}$ sec^{-1} observed in Figure 5. However, the rate constant for the conversion of diacetylhydroxylamine to acetohydroxamic acid under these conditions is $4.04 \pm 0.25 \times 10^{3}$ sec^{-1} so that it is not possible to positively identify the unknown product by rate constants with either O-acetylhydroxylamine or diacetylhydroxylamine or a mixture of the two. In fact a mixture seems most probable since the apparent yield of acetohydroxamic acid shown in Figure 4 could be produced from a yield of diacetylhydroxylamine equivalent to 10–14% of the acetate during acidification and addition of ferric chloride, but not from O-acetylhydroxylamine. However, this yield of diacetylhydroxylamine would by itself be inadequate to account for the 31% yield of acetohydroxamic acid generated by hydroxylamine buffer at pH 6.

Table V. Effect of Acetonitrile Concentration on Reaction of Acetic Anhydride with Hydroxylamine Hydrochloride[a]

% acetonitrile	Initial yield of $CH_3CONHOH$, %	10^3k for isomerization of CH_3COONH_2, sec^{-1}
1	77.0	3.47
10	77.0	3.17
20	77.0	3.61

[a] Conditions: 0.01 *M* acetic anhydride, 0.50 *M* $NH_2OH \cdot HCl$, pH 5.97, 25°.

(19) These data were obtained from the reaction of acetic anhydride with excess hydroxylamine; an initial very rapid reaction produces a mixture of O-acetylhydroxylamine and hydroxamic acid. The O-acetylhydroxylamine is then converted to hydroxamic acid by further reaction with hydroxylamine.

A further complexity of the direct reaction of hydroxylamine with carbodiimide is that the hydroxylamine itself attacks the carbodiimide, apparently with liberation of approximately 0.5 proton per molecule of BDC per hydroxylamine reacting. The rate constant calculated from the rate of titration of alkali for this process is 1.0×10^{-2} M^{-1} sec^{-1} at pH 4.75 and 25°.

Thus, there appears to be no inconsistency between the Lossen rearrangement shown here and the fact that the hydroxamic acids can be obtained in intermediate yields from the reaction of carboxylic acid. Franzblau, *et al.*,[17] used an excess of hydroxylamine over carbodiimide. The present kinetic studies suggest that their reaction fell into three partially overlapping phases: (a) the conversion of a carboxylic acid to hydroxamic acid, O-acetylhydroxylamine, and diacetylhydroxylamine with rapid subsequent reaction removing the hydroxamic acid; (b) the destruction of excess of carbodiimide by reaction with excess hydroxylamine; and (c) the subsequent conversion of the remaining acetylhydroxylamine derivative to hydroxamic acid by reaction with hydroxylamine.

Discussion

The Quantitative Conversion of Hydroxamic Acids to Amines. The quantitative conversion of a hydroxamic acid to an amine by means of a Lossen rearrangement is, of course, well known. In each case the OH group of the hydroxamic acid is modified to make it a better leaving group and then rearrangement occurs to an isocyanate. The mechanism of the rearrangement has been thoroughly investigated[14–16] and it closely resembles the Hofmann and Curtius rearrangements involving a 1–2 shift of an alkyl group. Apparently a carbodiimide can also be used to bring about rearrangement and the advantage for our purposes is the unusually mild conditions of the reaction. This is particularly valuable for protein reactions but its usefulness may extend to organic chemistry for compounds in which mild conditions in terms of pH, temperature, etc., are desirable. In the case of proteins it is particularly fortunate that the reaction can be carried out at pH ~5 since many of the protein groups which might interfere with the reaction are nucleophilic under alkaline conditions but relatively unreactive at pH <7.

In some cases the conversion of the carboxylic acid to a hydroxamate can be performed readily and this hydroxamate can then be converted to the amine with the carbodiimide. In others a direct reaction of the free carboxyl group, hydroxylamine, and the carbodi-

imide would be preferable since both steps would occur under mild conditions with a selective reagent. Unfortunately, as shown here, this reaction will not produce a quantitative amount of amine. Nevertheless the yields are relatively high and repeated treatment of some proteins has given encouraging results.[20]

From previous studies[13] it is clear that the particular soluble carbodiimide used here is not essential for the reaction, and other compounds, *e.g.*, the commercially available 1-ethyl-3-dimethylaminopropylcarbodiimide, would be equally efficient in these reactions.

Side Reactions. It is clear that the above procedure will produce a quantitative yield of the amine provided side reactions do not occur. These are very easy to avoid in the case of a simple free carboxylic acid in solution but are not so easy to avoid in the case of a complex polyfunctional protein. The possibility of inter- and intramolecular reactions of amino acid residues with reactive intermediates is, of course, not predictable unless the protein structure is known. The three-dimensional shape of the protein may place reactive groups in immediate proximity or it may prevent any such favorable orientation. From the rate constants obtained with methyl isocyanate and ammonium ion or alcohol it is seen that the main intermolecular reactions between two protein molecules would be slow at concentrations $\leq 10^{-3}$ *M* and by further dilution could be eliminated. However, the rate constants are sufficiently great so that the juxtaposition of a lysine side chain or an unusually reactive serine could produce intramolecular cross-linking reactions. Since the data indicate that a favorable conformation would be required for a trapping of the intermediate isocyanate, the finding of such cross-linking reactions would be an interesting clue to the geometry of the molecule. If such intramolecular reactions did occur, chromatographic separation of the cross-linked material from the remaining material might be necessary but the study might be rewarding in regard to its information on the structure of the molecule.

Implications of the Rearrangement. The rearrangement outlined here would allow one to convert the carboxylic acid group in proteins to a group of opposite charge and approximately the same steric size. This should be unusually interesting in studies on the conformational properties of proteins, in problems of the association of protein subunits with each other, in problems involved with the folding of protein, *i.e.*, the determination of three-dimensional structure from primary structure, and in problems related to carboxylic acid groups at active sites, Recently it has been shown that the specificity of a protein can be altered by the modification of protein side chains,[21] in that case a conversion of the hydrophobic methionine side chain to the polar methionine sulfoxide side chain. Perhaps similar results can be obtained by the conversion of the negatively charged carboxyl side chains of glutamic and aspartic acids to the positively charged 2,4-diaminobutyric or 2,3-diaminopropionic acid residues.

The conversion of the C-terminal carboxylic acid groups to an amine would lead to an aldehyde on acid hydrolysis and this might allow the development of a quantitative C-terminal procedure.

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Synthesis and Fluorescent Properties of Some N-Methyl-2-anilino-6-naphthalenesulfonyl Derivatives[1]

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Abstract: N-Methyl-2-anilino-6-naphthalenesulfonyl chloride has been synthesized and the fluorescent properties of its derivatives have been studied. The quantum yield of the sulfonamide is extremely sensitive to the polarity of its environment. A bovine serum albumin derivative has a high quantum yield in the native state which is significantly lowered when the protein is denatured with 5 *M* guanidine hydrochloride. When the protein is denatured with acid, the emission increases. The binding of the chromophore to the protein is covalent.

Weber and Laurence[3] found that the quantum yield of anilinonaphthalenesulfonates was extremely sensitive to the environment. The noncovalent binding of these salts to proteins was accompanied by a large increase in quantum yield.[4-6]

Of the methods of attaching such compounds covalently, that using the sulfonyl chloride suggests itself

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