The mechanism of the reaction of hydroxylamine with activated acyl groups

The low yield of hydroxamic acid obtained in the reaction of dilute solutions of hydroxylamine with enzymically and chemically activated acyl groups[1] stimulated an investigation into the mechanism of hydroxylamine acylation.

The reaction of *p*-nitrophenyl acetate with 0.1*M* hydroxylamine at neutral pH was found to result in the immediate liberation of *p*-nitrophenol followed by a slow formation of hydroxamic acid (Fig. 1). This suggested that hydroxamic acid formation proceeds in part through an intermediate compound. Similar results were obtained with *p*-nitrophenyl benzoate, acetyl imidazole, diacetohydroxamic acid, acetic anhydride, and 2,4-dinitrophenyl benzoate, but not with benzoyl chloride. The acetyl intermediate could not be isolated because of its lability, but after reaction with benzoyl chloride in dilute solution, O-acetyl N-benzoyl hydroxylamine, m.p. and mixed m.p. 128–129° was isolated. The more stable benzoyl intermediate was prepared from *p*-nitrophenyl benzoate and hydroxylamine in alcohol and crystallized from ether–petroleum ether at —78° as large plates, m.p. 8°. Anal.: C 61.0%, H 5.3%, N 10.2%; calc. for $C_7H_7O_2N$: C 61.3%, H 5.2%, N 10.2%; carbonyl infrared band in $CHCl_3$ at 5.78 μ (*cf.* perbenzoic acid 5.77 μ[2]).

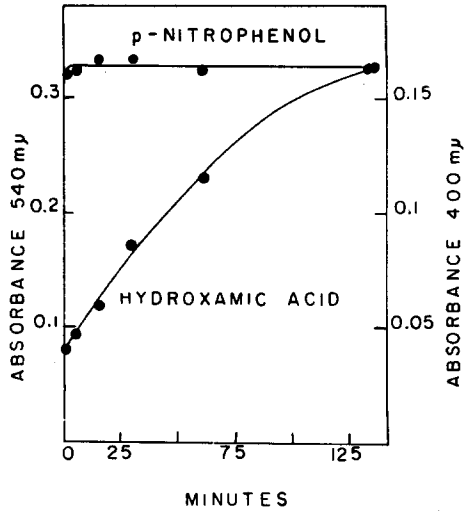


Fig. 1. The time course of *p*-nitrophenol liberation and hydroxamic acid formation from the reaction of $10^{-3}M$ *p*-nitrophenyl acetate and 0.1*M* NH_2OH, pH 6.9, 25°. Hydroxamic acid determined as the ferric complex at 540 mμ[11]; *p*-nitrophenolate measured directly at 400 mμ.

Reaction with acetic anhydride gave O-benzoyl N-acetyl hydroxylamine, m.p. and mixed m.p. 98°–99°, and reaction with *p*-nitrobenzaldehyde in acetic acid gave *p*-nitrobenzaldoxime benzoate, m.p. and mixed m.p. 192°–192.5°. These data establish that the intermediate compound is O-acyl hydroxylamine (acyl oxyamine).

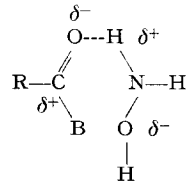
O-benzoyl hydroxylamine reacts with 1.6*M* hydroxylamine to form hydroxamic acid and is rapidly decomposed in the presence of 0.01*M* NaOH or traces of heavy metals at room temperature. The pure substance at room temperature is converted to benzohydroxamic acid and dibenzohydroxamic acid in a few hours, but is stable in dilute solution or at —78° for days. The acetyl compound undergoes similar reactions, but is considerably more reactive. Neither compound gives a color with $FeCl_3$.

O acylation of hydroxylamine has been described previously only in the reaction of isatoic anhydride with hydroxylamine to give O anthranoyl hydroxylamine[3]. It is of interest, however, that LOSSEN in 1872 pointed out that both N and O monoacylation of hydroxylamine should be possible[4], and the reactions of hydroxylamine with chlorosulfonic acid[5] and, on the basis of indirect evidence, with certain phosphate anhydrides[6], give O substituted products.

Formation and decomposition of labile O acyl hydroxylamine provides an explanation for the low yields of hydroxamic acid observed in the acylation of hydroxylamine at neutral pH[1] and formation of the analogous carbamyl ester, $H_2NOCONH_2$, from potassium cyanate and hydroxylamine would account for the alkali-labile, low melting isomer of hydroxyurea observed in this reaction[7].

The rapid rate of O acylation of hydroxylamine is unexpected in view of the generally very low nucleophilic reactivity of the hydroxyl group; *p*-nitrophenyl acetate, for instance, takes days

to decompose in aqueous or alcoholic solution. It appears that the nucleophilic attack by the hydroxylamine oxygen atom, which may be expected to carry a partial negative charge because of the high electronegativity of oxygen, is aided by an increase in the polarization of the carbonyl group induced by simultaneous electrophilic attack by the hydroxylamine amino group (*cf.* [8]).



Alternatively, the reactive species of hydroxylamine in solution may be the zwitterion $H_3\overset{+}{N}-O^-$, although spectroscopic evidence has been interpreted to indicate that this form is not present in the hydroxylamine crystal[9].

The occurrence of this reaction provides experimental support for the concept that the hydroxyl group of serine may react to form O-acyl serine as an intermediate in the hydrolysis and transfer of activated acyl groups by chymotrypsin and similar enzymes[10], provided that the serine reactivity is enhanced by neighboring electrophilic groups on the enzyme.

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Converse Memorial Laboratory, Harvard University, Cambridge, Mass. (U.S.A.) WILLIAM P. JENCKS*

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* U.S. Public Health Service Fellow. Present address: Graduate Department of Biochemistry, Brandeis University, Waltham 58, Mass. (U.S.A.).

The amino acid composition of the collagen fractions of rabbit skin

Three collagen fractions are now recognized: neutral salt-soluble collagen[1,2], citrate-soluble collagen[3] and insoluble collagen which constitutes the bulk of the collagen in connective tissue and can be rendered soluble by transformation into gelatin.

A number of complete amino acid analyses of mammalian insoluble collagen and of gelatin have been made[4,5], and BOWES *et al.*[4] have analysed citrate-soluble collagen from calf-skin. However no analysis is available for neutral salt-soluble collagen nor have analyses been made of all three types of collagen from the same source. This paper reports a method for the isolation and purification of neutral salt-soluble collagen and the amino acid analysis of all three types of collagen isolated from the skins of young growing rabbits.

All extractions were carried out at 2° in the presence of *sec.*-octanol as preservative. Neutral salt-soluble collagen as prepared by HIGHBERGER *et al.*[7] is soluble only with considerable difficulty after purification and may become completely insoluble[6]. It was therefore prepared by the following method, some of the physical properties of this fraction having already been described[1].

The skins were extracted several times with 0.2*M* NaCl, pH 7.4, for 24 h and the extracts pooled and filtered after centrifuging for 30 min at 12,000 *g*. NaCl to a final concentration of 20% (w/v) was added and the precipitate removed by centrifuging at 12,000 *g* for 30 min. The precipitate was redissolved in 0.2 *M* NaCl, pH 7.4, and the insoluble residue removed by centrifuging at 12,000 *g*. An equal volume of 5 *M* NaCl was added and the precipitate removed by centrifugation, redissolved in 0.2 *M* NaCl and this step repeated. The final solution in 0.2 *M* NaCl was faintly opalescent, and was clarified by centrifuging at 100,000 *g* for 1 h. 5 *M* NaCl was added