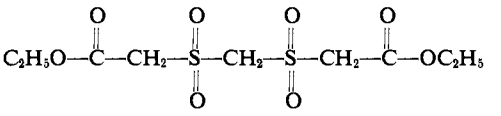
85% purity) of *m*-chloroperbenzoic acid in 250 ml of ether. The reaction mixture was stirred overnight at room temperature, washed with saturated sodium bicarbonate solution (three 100-ml portions), dried, and evaporated to yield a semisolid. This material was chromatographed on silica gel (elution with ether–petroleum ether) to give initially 0.80 g (6.1%) of **31a**, followed by 5.0 g (40%) of **35**: mp 73–74°; $\nu_{max}^{CHCl_3}$ 1735, 1342, and 1120 cm^{-1}; $\delta_{TMS}^{CDCl_3}$ 5.25 (s, 2), 4.38 (s, 4), 4.28 (q, J = 7.0 Hz, 4), and 1.32 (t, J = 7.0 Hz, 3).

Anal. Calcd for $C_9H_{16}O_8S_2$: C, 34.17; H, 5.10; S, 20.27. Found: C, 34.35; H, 5.20; S, 20.19.



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Acknowledgment. We are indebted to the National Science Foundation for their partial support of this research through Grant No. GP-2939.

Aminoacylhydroxamates. A Case of Slow Proton Transfer between Electronegative Atoms in Solution

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Abstract: Aminoacylhydroxamic acids can exist in two forms that migrate with characteristic mobility on the same chromatogram under standard chromatographic conditions in acid aqueous butanol; they are designated fast and slow hydroxamates. Chromatographic mobility is shown to depend on pretreatment of the compound in aqueous solution and is not a chromatographic artifact. The fast and slow forms of hydroxyprolylhydroxamate have been analyzed by various means including ir and nmr spectroscopy. The only chemical difference that can be demonstrated is that in the slow form the basic amino nitrogen is protonated while in the fast form it is not. It is suggested that the nonprotonated amine in acid solution may be stabilized through internal hydrogen bonding; the same may be true of the protonated amine in basic solution.

It is considered almost axiomatic that "proton transfer between electronegative atoms, such as nitrogen, oxygen, sulfur and the halogens, occur so rapidly in solution that they cannot be kinetically examined."[1] The purpose of this paper is to describe a case of slow proton transfer between acidulated water and the amino nitrogen of aminoacylhydroxamates.

The work to be described grew out of an observation of aberrant chromatographic behavior in prolylhydroxamate which was subsequently confirmed for other aminoacylhydroxamic acids.[2] Many of these compounds, it was found, can exist in two forms designated as fast and slow because each form migrates with characteristic mobility under standard chromatographic conditions (15% formic acid in aqueous butanol)[3] (Figure 1). Typical mobilities are given in Table I. Since routine chromatographic analysis on paper is by an "overflow" method, mobilities are reported as R_{PH}, *i.e.*, relative to the mobility of fast prolylhydroxamate (PH).[4] Preparation of aminoacylhydroxamic acid from the corresponding methyl ester under standard conditions (*i.e.*, in slightly basic medium)[5] usually gives only the fast product. After pretreatment with aqueous HCl at pH 2–3, the fast form is converted into the slow form. In the case of PH and HPH treated with mineral acid, this conversion appears quantitative as judged by appearance of the chromatograms. In other cases, for example isoleucyl- and tryptophanylhydroxamic acid, most of the fast hydroxamate is converted to the slow form by mild mineral acid treatment, but some of it still migrates in the fast form. In such cases an equilibrium may be established between forms since prolonged acid treatment does not appear to affect the distribution further and trailing is commonly seen. Lysylhydroxamic acid gives only one (relatively very slow) spot during chromatography after preparation in the usual way; its migratory rate is not affected by mild mineral acid pretreatment. Histidylhydroxamic

Table I. Chromatographic Mobility of Hydroxamic Acids[a]

Hydroxamic acid	Mobility (R_{PH})[b] Fast	Slow
N-Acetylprolyl-	1.57	1.57
Benzoyl-	2.2	2.2
Glycyl-	0.52	0.29
Histidyl-	0.35	0.17
Hydroxyprolyl-	0.70	0.39
Isoleucyl-	1.48	1.00
Leucyl-	1.60	1.30
Lysyl-		0.14
Methionyl-	0.37	0.14
Prolyl-	1.00	0.64
Propionyl-	0.13	0.14
Tryptophanyl-	0.96	0.60
Tyrosyl-	0.88	0.54

[a] Hydroxamic acids applied to Whatman no. 1 paper from neutral solution (fast) or solution made to pH 2–3 with HCl (slow). Descending chromatograms developed in 2-butanol–formic acid–water (75:15:10, v/v) for 18–20 hr and air-dried; spots visualized with $FeCl_3$ spray. [b] Relative to fast prolylhydroxamate.

(1) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 723; *cf.* G. O. Dudek and E. P. Dudek, *J. Amer. Chem. Soc.*, **86**, 4283 (1964); **88**, 2407 (1966).

(2) M. L. Bade and B. S. Gould, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 2, 173 (1969).

(3) Th. Wieland and H. Fritz, *Chem. Ber.*, **86**, 1186 (1953).

(4) Abbreviations used are: PH, prolylhydroxamate; HPH, hydroxyprolylhydroxamate; R_{PH}, mobility of chromatographed spot relative to fast PH; R_f, mobility of chromatographed spot relative to solvent front.

(5) K. G. Cunningham, *et al.*, *J. Chem. Soc.*, 2091 (1949).

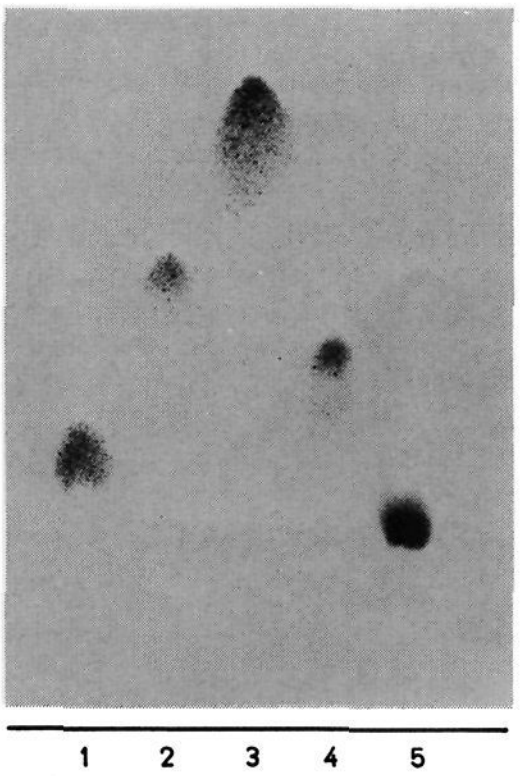


Figure 1. Photograph of actual chromatogram of aminoacyl hydroxamates. Descending 18-hr chromatography on Whatman no. 1 paper; solvent, 2-butanol–formic acid–water, 75:15:10 (v/v); spots visualized with Fe^{3+}: 1, slow hydroxyprolylhydroxamate; 2, fast hydroxyprolylhydroxamate; 3, fast prolylhydroxamate (reference compound); 4, fast glycylhydroxamate; 5, slow glycylhydroxamate.

acid gives both a fast and a slow spot from slightly basic solution; all of it migrates as the slow compound after mild mineral acid treatment.

The conversion of fast to slow aminoacylhydroxamic acid, and the reverse, appears to depend on the precise conditions employed. Slow PH migrates at R_{PH} = 0.64 after 35 min at pH 6.9; it moves as fast PH after pretreatment above pH 9 for the same length of time. Fast PH is stable for at least 1 year at pH 7.4 in the frozen state. Slow crystalline HPH dissolved in 1 *N* NH_4OH moves as the slow compound even if the solution is briefly heated to 60°. A solution of slow crystalline HPH neutralized with 1 *N* NaOH moves as the fast compound. Fast crystalline HPH dissolved in 0.1 *N* HCl, if spotted immediately, migrates as fast HPH (R_{PH} = 0.70). If the solution is allowed to stand a few seconds before spotting, however, the slow form has been regenerated. The R_{PH} of PH is the same (0.64) whether H_2SO_4 or HCl is used in the prechromatographic acid treatment. If the solution of iminoacylhydroxamate is brought to pH 2–3 with perchloric acid, on the other hand, both fast and slow forms appear on the chromatogram; it is known from experiments with ^{14}C-labeled PH and HPH that this simultaneous appearance of both forms is accompanied by little if any trailing on the chromatogram.[2]

Presence of the α-amino group in protonable form appears necessary for the effect. *N*-Acetylprolylhydroxamate, where the ring nitrogen is in amide linkage, migrates as one spot the R_{PH} of which is not affected by mild mineral acid pretreatment. The same is true of benzoyl- and propionylhydroxamates in which the α-amino group is lacking. Commercially obtained glutamoylmonohydroxamate also gave only one $FeCl_3$-positive spot; by its reaction with ninhydrin,[3] this compound was shown to be the γ-hydroxamate. In PH and HPH on the other hand, where the effect is very pronounced, the imino nitrogen is sufficiently basic to permit separation of these derivatives from aminoacylhydroxamates on the weakly acidic ion exchange resin Biorex 70.[6]

A number of chromatographic artifacts have been elucidated in recent years and it had to be considered whether any of them could explain the observations cited above. During paper chromatography with neutral solvent, organic acids and bases often form long trails. This so-called comet formation, attributed to gradual ionization of molecules in the traveling spot, is customarily suppressed by including acid in the solvent mixture.[7] The solvent mixture here used does contain an appreciable amount of acid; comet formation therefore did not seem to explain the experimental observations. Another artifact encountered in paper chromatography, the formation of two or more spots in the course of the run, is attributed to nonequilibrium between various complex and ionized forms[7] or to deposition of molecules of different degrees of hydration.[8] The mobility of the single spot obtained with iminohydroxamates, however, clearly depends on pretreatment of the compound in solution and not on its reaction subsequent to encountering solvent and paper. Thus, none of the possible explanations enumerated appeared to be obviously applicable in this case.

In ionizable molecules, the R_f of the un-ionized form generally is higher than that of the ionized form. However, it did not seem reasonable to attribute the mobility of the fast compound to persistence, through an 18-hr run in acid medium, of wholly unprotonated amine groups. Nevertheless, this is the conclusion drawn from the further experimental work now to be detailed.

In order to discover the chemical basis for the phenomenon, both the fast and the slow form of HPH were crystallized, the latter from a water–ethanol mixture made acid with HCl. Both forms gave a wine-red color with Fe^{3+}. Both, when dissolved in water, gave the appropriate R_{PH}, *i.e.*, slow HPH traveled with an R_{PH} of 0.39 and fast HPH with R_{PH} 0.70. The conversion by added mineral acid of fast HPH in solution into slow HPH, and the reverse reaction, were confirmed for the crystalline material. Hydrolysis (15 min) of fast HPH in 1 *N* HCl at 100° gave 100.2% of the theoretical amount of hydroxyproline. Acid hydrolysis of slow HPH under identical conditions led to only partial recovery of free hydroxyproline (10–25%).[9] Only slow HPH gave a precipitate when dissolved in aqueous $AgNO_3$. Elementary analysis was consistent with the empirical formula $C_5H_{10}N_2O_3 \cdot H_2O$ for the fast form[10] and $C_5H_{10}N_2O_3$–$HCl \cdot H_2O$ for the slow form.[11] An attempt to remove the water of crystallization by exhaustive drying in an Abderhalden gun failed; apparently the molecule breaks down as soon as the water is driven off.

On the basis of the elementary analysis, it was concluded that the only difference between fast and slow

(6) W. H. Elliott and G. Coleman, *Biochim. Biophys. Acta*, 57, 236 (1962); *cf.* ref 2.

(7) E. Lederer and M. Lederer, "Chromatography," Van Nostrand, Princeton, N. J., 1957, pp 121, 122.

(8) Sam Aronoff, "Techniques of Radiochemistry," Hafner Publishing Co., New York, N. Y., 1967 , p 172.

(9) A similar observation had been made earlier with ^{14}C-labeled PH.[2]

(10) *Anal.* Calcd: C, 36.60; H, 7.31; N, 17.08. Found: C, 36.53; H, 7.32; N, 17.00.

(11) *Anal.* Calcd: C, 30.00; H, 6.50; N, 14.00. Found: C, 30.42; H, 6.69; N, 13.84.

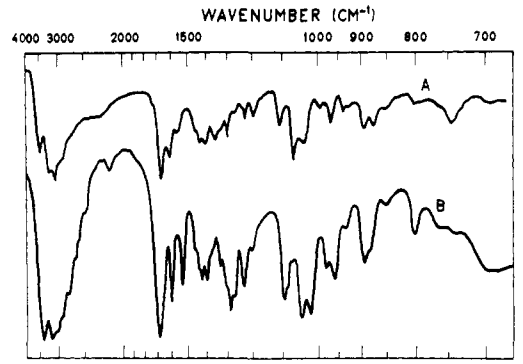


Figure 2. Infrared spectra (in KBr) of fast and slow hydroxyprolylhydroxamic acids: A = fast HPH; B = slow HPH.

HPH was that the latter was the salt of the former. This tentative conclusion has been confirmed by ir and nmr spectroscopy.[12]

The ir spectra for fast and slow HPH are shown in Figure 2. The two spectra are clearly similar though not identical, especially in the fingerprint region. Strong absorption bands at 1530 and 1600 cm^{-1} in the slow HPH spectrum are characteristic for an amine salt.[13] The peak at 1530 cm^{-1} is also consistent with a secondary amide such as C(=O)NHOH but its absence from the spectrum of fast HPH argues against that assignment. The spectrum of slow HPH also shows the complex series of absorptions between 2000 and 2800 cm^{-1} which Bellamy cites as characteristic for secondary amine hydrochlorides.[14] At wave numbers below 2000 cm^{-1}, the strongest absorption in both spectra is at approximately 1670 cm^{-1}; the band appears to shift from about 1665 cm^{-1} in fast HPH to 1685 cm^{-1} in the salt. This is somewhat higher than might be expected for the amide I band in the solid state[15] but in a hydroxamic acid the additional hydroxyl group on the nitrogen might well operate to bring about a shift to higher wave numbers. On the other hand, C=N stretching would also be expected in this region.[13,16]

Figure 3 shows the 100-MHz nmr spectra for fast and slow HPH; the numerical values are summarized in Table II. Assignment of protons in fast HPH to their respective carbons is based on the following considerations: (1) high-field protons must be C_3 since all others have N or O α to them; (2) the proton at τ 6.36 shows a single coupling of 8 Hz and must therefore be on C_2 rather than C_4 since the latter proton is also coupled to C_5; (3) this leaves by elimination the AB pattern as the C_5 protons, both coupled to C_4 (J = 2, 4.5 Hz). The assignments are confirmed by comparison to the pattern exhibited by slow HPH. If N_1 is protonated in slow HPH, C_2 and C_5 protons should be more strongly affected than those on C_3 and C_4. In fact C_2 and C_5 proton signals are shifted downfield more (0.4–0.6 ppm) than those of C_3 and C_4 (0.2–0.3 ppm).

(12) I thank Professor A. T. Blomquist, Department of Chemistry, Cornell University, Ithaca, N. Y., for infrared spectroscopy and Dr. Bryan Jennings, Department of Chemistry, University of Texas, for nmr spectra.

(13) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall, Englewood Cliffs, N. J., 1969, p 37.

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen, London, 1958, p 260.

(15) See ref 14, p 203.

(16) See ref 14, p 268.

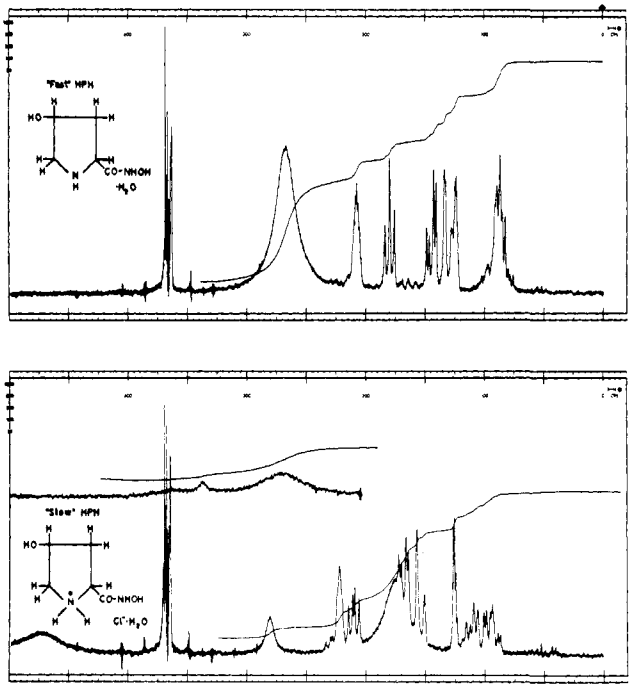


Figure 3. Nuclear magnetic resonance spectra of fast and slow hydroxyprolylhydroxamic acids: solvent, DMSO-d_6 (DMSO-d_5 signal set at τ 7.5); benzene lock at approximately τ 2.6. The upper curve is slow HPH offset 400 Hz.

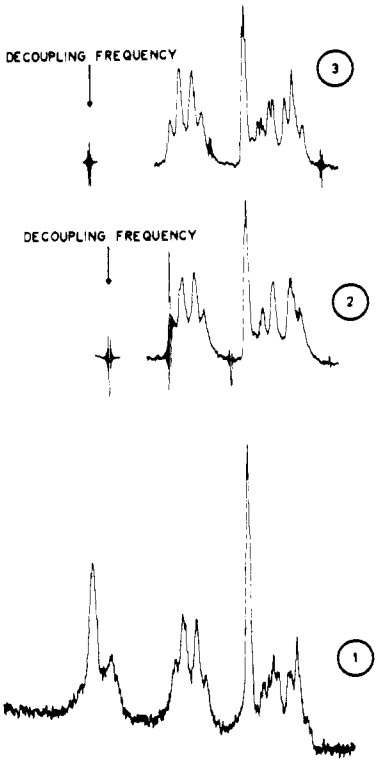


Figure 4. Effect of decoupling on nuclear magnetic resonance spectrum of slow HPH; conditions as in Figure 3; curve 1, slow HPH at 100 MHz (reference spectrum); curve 2, slow HPH after decoupling at τ 5.76; curve 3, slow HPH after decoupling at τ 5.55.

Proton assignments were also confirmed by decoupling experiments (Figure 4). For example, components of the two-proton multiplet assigned to C_3 were sharpened by irradiation both at τ 5.55 and 5.76; this confirms the assignment since C_3 is adjacent to both

Table II. Characterization of Nuclear Magnetic Resonance Spectrum of Fast and Slow Hydroxyprolylhydroxamate in DMSO-d_6[a]

Protons associated with carbon	Fast HPH				Slow HPH			
	Signal	No. of protons	Position, τ	Coupling, Hz	Signal	No. of protons	Position, τ	Coupling, Hz
4	Multiplet	1	5.8	Unresolved	Multiplet	1	5.55	Unresolved
2	Triplet	1	6.36	8	Quartet	1	5.76	7, 10
5	AB	2		11.5	AB	2		12
	A (split)		7.06	4.5	A (split)		6.65	4
	B (split)		7.38	2	B		6.93	0
3	Quartet	2	8.25	4.5, 8	AB multiplet	2		12
					A (split once)		7.78	7
					B (split twice)		8.12	4, 10–12
Other	Broad peak containing four protons at τ 4.65				Five protons distributed in four broad peaks at τ −0.75, +0.5 (two protons), 4.40 and 6.50			

[a] DMSO-d_5 signal set at τ 7.5.

C_2 and C_4 protons. The fine structure of the two-proton AB system assigned to C_5 collapsed on decoupling at τ 5.55 leaving a clean AB quartet. It was unchanged on decoupling at τ 5.76. Thus the AB protons at C_5 must be adjacent to the proton at τ 5.55 which therefore must be on C_4. After decoupling at τ 5.55 the C_3 multiplet signal could be identified as an AB quartet split by an adjacent proton; the latter must therefore be the C_2 proton at τ 5.76.

Table III compares chemical shifts and coupling constants for hydroxyproline reported in the literature[17] to those found in the present work. Correlation is very good and assignments are again confirmed. Note also that ring nitrogen protonation in slow HPH and absence of protonation in fast HPH are likewise confirmed. Frequency separation between C_2 and C_4 protons is 73 Hz for alkaline hydroxyproline and 56 Hz for fast HPH; by contrast, slow HPH shows a separation of 25 Hz which corresponds to $\Delta\nu$ of 3 and 31 Hz for acid and neutral hydroxyproline, respectively.

Table III. Comparison of Chemical Shifts at 100 MHz and of Coupling Constants

	Hydroxyproline, Hz			HPH, Hz	
	Acid	Neutral	Alkaline	Slow	Fast
Chemical shifts[a,b]					
ν_4	474	465	442	445	420
ν_2	471	434	369	420	364
ν_{5a}	363	348	321	335	294
ν_{5b}	345	334	269	207	262
ν_{3a}	252	240	206	222	175
ν_{3b}	234	212	188	188	175
Constants[c]					
$J_{(2a,3a)}$	7.7	7.1		7	
$J_{(2a,3b)}$	10.4	9.4		10	
$J_{(3a,3b)}$	14.1	13.6		14	
$J_{(3a,4b)}$	1.4	2.4		1	
$J_{(3b,4b)}$	4.3	6.1		4	
$J_{(4b,5a)}$	1.2	3.0		0	
$J_{(4b,5b)}$	4.1	5.3		4	
$J_{(5a,5b)}$	12.7	11.9		12	

[a] Chemical shifts for hydroxyproline at 100 MHz calculated from values given in ref 17; solvent, D_2O. [b] HPH values from present work; solvent, DMSO-d_6. Peak positions obtained from spectra shown in Figure 3; coupling constants from Figure 3 and from 100-MHz spectra obtained during decoupling at τ 5.55 and 5.76 (Figure 4). [c] Assignment to major groups of protons in the spectrum is as discussed in the text; assignment of particular interactions is by similarity with previously established values (*cf.* ref 17).

It is of interest that the protons presumably attached to N and O undergo the most striking change in signals when the patterns of fast and slow HPH are compared. It is also noteworthy that the two protons which are equivalent in fast HPH no longer are so in the slow compound. A possible interpretation of these observations is that in becoming protonated the molecule undergoes a change in shape that involves both the ring and the side chain.

The salient conclusions from these results can be summarized as follows. (1) No gross chemical change and no change in the state of hydration seem to be involved in the conversion; this is implicit in the lack of trailing of chromatographic spots, notably the iminoacylhydroxamates, in the elementary analyses and spectral data, and in the ease with which the reaction may be reversed with alteration in hydrogen ion concentration. (2) The α-amino group as well as the hydroxamate group are necessary for the effect; this is apparent from the data given in Table I. (3) Protons enter into the rearrangement, *i.e.* one of the species is more protonated than the other; this must be so since otherwise addition of a catalyst (H^+) would effect a shift in a chemical equilibrium, *viz.*, that between fast and slow hydroxamate. (4) The barrier to interconversion between fast and slow hydroxamate forms must be rather high; this follows from the relatively appreciable lifetimes of tautomers in solution. (5) The only demonstrable chemical difference between slow and fast hydroxamates is that in the former the amino nitrogen is protonated while in the latter it is not; this is suggested by elementary analyses and confirmed by ir and nmr data for the model compound HPH.

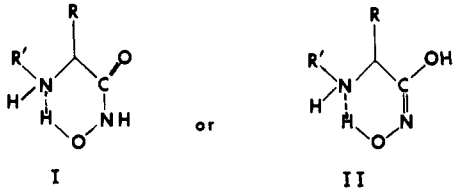
A tentative hypothesis which can account for all these observations is that fast and slow aminoacyl hydroxamic acids are rotational (*i.e.*, geometric) isomers stabilized by internal hydrogen bonds. The hydrogen bonds presumably involve both the α-amino and the hydroxamate group and operate to shield the amino nitrogen from protonation or deprotonation.

Several plausible structures for either fast or slow forms can be suggested on the basis of this hypothesis although structural data at present available do not yet permit a choice as to which is most probably correct. By analogy with amides, hydroxamates may be expected to have predominantly the hydroxylactam structure;[18] nevertheless, the lactim structure has not been ruled out[19] and the existence of an equilibrium between

(17) R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, **5**, 197 (1962).

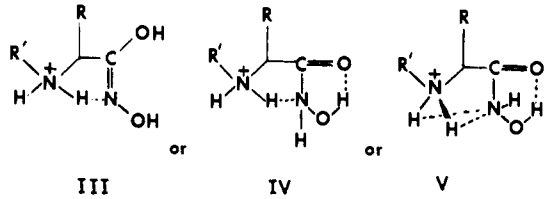
(18) G. W. Wheland, "Advanced Organic Chemistry," 3rd ed, Wiley, New York, N. Y., 1960, pp 706–707.

lactim and lactam is also occasionally assumed.[18] Unfortunately, the ir data do not permit a choice to be made here. Among possible structures for fast hydroxamate are hydroxylactam (I) and -lactim (II).



I or II

A plausible mechanism of acid catalysis for the conversion can be envisaged whichever structure is correct. The ambiguity, however, remains for the slow form which might be written as



III or IV or V

Note that conversion from II to III is also one from syn to anti form. Structure III, which as mentioned corresponds to the form in which hydroxamic acids are commonly written,[18] also seems most plausible as a compound that would tend to migrate slowly in a chromatographic system with water as the stationary phase.

The structures suggested as possibilities are consistent with the experimental evidence here marshalled. Although the experimental facts and the conclusions drawn therefrom would appear to be unequivocal, no more than suggestions appear possible at present for the structural peculiarities that must underlie the facts. It is to be hoped that the rearrangement described will stimulate interest in more definitive work on the structure of hydroxamates.

(19) "Handbook of Chemistry and Physics," 41st ed, Chemical Rubber Publishing Co., Cleveland, Ohio, *e.g.*, p 846, entry no. 1446.

Experimental Section

To prepare the methyl ester of an amino acid, 0.5–1.0 g of the acid was weighed out and suspended in 10 ml of methanol. Dry HCl gas was passed in for 15–30 min with cooling. The liquid was taken off in the flash evaporator without warming and the hydrochloride recrystallized if possible from absolute ethanol in the cold.

To prepare the hydroxamate, 10 mmol of the methyl ester hydrochloride was dissolved in 20 ml of cold methanol and neutralized with the calculated amount of sodium metal dissolved in cold methanol. A stoichiometric amount of NH_2OH–HCl was also dissolved in methanol and neutralized with a slight excess of sodium metal dissolved in methanol. Both solutions were cooled to $-20°$ in a salt–ice bath and the slightly basic hydroxylamine solution was added dropwise with efficient stirring to the methyl ester solution. NaCl usually crystallized out during overnight standing in the refrigerator. It was filtered off and the hydroxamate allowed to crystallize from the methanol solution in the cold. With some compounds, the filtrate was evaporated to dryness in a cold flash evaporator, taken up in the minimum amount of ethanol–water mixture, and placed over CaO to promote crystallization.

Prolylhydroxamate, hydroxyprolylhydroxamate, propionylhydroxamate, *N*-acetylprolylhydroxamate, and lysylhydroxamate were made as indicated. Benzoylhydroxamic acid was purchased from Eastman-Kodak, Rochester, N. Y. A number of aminoacylhydroxamates were purchased from Sigma Chemical Co., St. Louis, Mo. Amino acids were obtained from Calbiochem Co. and were their Grade I.

Hydroxyproline was assayed by method I of Woessner.[20] The elementary analyses were carried out at the Microchemical Laboratory, Massachusetts Institute of Technology.

The chromatographic system used in the separations[3] consisted of *sec*-butyl alcohol, formic acid, and water (75:15:10, v/v). Chromatograms were developed for 18–20 hr along the machine direction on Whatman no. 1 paper. After air drying, spots were visualized by spraying with $FeCl_3$ (1% in 0.1 *N* HCl).

The infrared spectra were obtained with KBr pellets on a Perkin-Elmer infrared spectrophotometer, Model 137. Nmr spectra were recorded at both 60 and 100 MHz frequency on Varian Associates instruments. DMSO-d_6 was used as the solvent and the signal of DMSO-d_6 arbitrarily set at τ 7.5. A little benzene was added to serve as a locking signal.

(20) F. Woessner, *Arch. Biochem. Biophys.*, **93**, 440 (1961).