L-alanine copolymers^{26,27} have indicated that the mesopolymer is not predominantly helical. Nevertheless, the results with poly-L-alanine described here make it difficult to imagine that no helical structure would be present. Moreover, Wada²⁸ has recently shown that as a consequence of the greater affinity of a growing D,Lpolymer chain for a monomer unit of the same configuration as the terminal group, poly- γ -benzyl-D,L-gluta-mate is substantially helical, being composed of many short regions of right- and left-handed helices. It is consistent with the observations made here that a similar situation holds for poly-D,L-alanine. The failure of the molecule to take up a completely helical conformation could be due to the fact that the closest methyl–methyl distance is such a helix, 3.3 Å., requires too much interpenetration of van der Waals radii, resulting thereby in steric hindrance rather than an attractive interaction.

The tentative conclusion that poly-D,L-alanine has perhaps one-third of its residues in the form of short helices carries with it the corollary that the helical form is indeed very stable. This follows from the fact that for a given polypeptide-solvent system, helix

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(27) A. Elliott, ref. 2, p. 119.

(28) A. Wada, J. Mol. Biol., 3, 507 (1961).

stability decreases with decreasing molecular weight.^{22,23,29} Thus for quite short regions of the chain to form helices requires that longer chains of a pure enantiomorph will form very stable helices indeed.

The conclusions concerning the very considerable stability of the α -helical conformation in the two polymers of alanine have a direct bearing on the problem of the basis for the stability of this conformation in proteins. As previously remarked, the enthalpy of formation for the helix must be much greater than around 1–2 kcal./mol in order to account for the existence of short helical regions in proteins.²⁹ The present work suggests that the source of this greater stability resides in the polypeptide backbone.

If further experiments support the view developed here, the helical conformation will have to be considered as quite stable and the role of side chains as being primarily sources of weakness or instability.

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The Elucidation of the Reaction of Benzohydroxamic Acid with Benzenesulfonyl Chloride and with Diisopropyl Phosphofluoridate Using Oxygen-18 as Tracer

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The reaction of benzohydroxamic acid with benzenesulfonyl chloride and with diisopropyl phosphofluoridate has been investigated using O¹⁸-enriched water as solvent. The absence of oxygen-18 in any of the products confirms the mechanism in which the hydroxamic acid is first sulfonated or phosphorylated to form an unstable intermediate which breaks down by a Lossen type rearrangement to give the products.

A number of compounds have recently been studied in an attempt to counter the toxicity of certain organophosphorus compounds.¹ Among the most effective of these are the anions of hydroxamic acids first suggested by Wagner-Juaregg and co-workers.^{2a,b}

It was found² that various substituted hydroxamic acids cause a rapid increase in the rate of breakdown of benzenesulfonyl chloride and of diisopropyl phosphofluoridate in slightly basic solutions. The increase in rate at room temperature and pH 7.6 was found^{2b} to be many hundredfold. The products of the reaction were benzenesulfonic acid (or diisopropyl hydrogen phosphate) and the "dihydroxamic acid," namely, phenylcarbamoyl benzohydroxamate. On the basis of the earlier work of Hurd and Bauer on the Lossen rearrangement of hydroxamic acids³ the following mechanism for this reaction in aqueous alkaline solution was suggested.²

(A) $C_6H_6CONHOH + ArSO_2CI \xrightarrow{-HCI}$ $C_6H_5CONHOSO_2Ar \xrightarrow{-ArSO_3H} C_6H_5NCO \xrightarrow{PhCONHOH}$ I $C_6H_5CONHOCONHPh$

(B)
$$C_{6}H_{5}CONHOH + (i-PrO)_{2}P(O)F \xrightarrow{-HF}$$

 $C_{6}H_{5}CONHOP(O)(i-PrO)_{2} \xrightarrow{-(i-PrO)_{2}PO_{2}H}$
II
 $C_{6}H_{5}NCO \xrightarrow{PhCONHOH} C_{6}H_{5}CONHOCONHPh$

The unstable condensation intermediates (I or II) were not isolated, since they immediately undergo the reaction found by Hurd and Bauer³ giving the free oxy-acid and an aryl isocyanate which condenses with another molecule of hydroxamic acid.

Since in mildly basic solution this reaction competes with the hydrolysis of the acyl halides, it appears that hydroxamic acids can, in effect, catalyze the decomposition of arylsulfonates and of diisopropyl phosphofluoridate in aqueous solution. The kinetics of the reaction of analogous phosphofluoridates with benzohydroxamic acid were determined by Swidler and Steinberg,⁴ who found that the stoichiometry and the rate of formation of fluoride and of acid were consistent with the scheme shown above. A more extensive kinetic investigation of the reaction related⁵ the dissociation constant of various nucleophilic reagents including hydroxamic acids with their activity in the hydrolysis of toxic organophosphorus compounds.

(4) R. Swidler and G. M. Steinberg, ibid., 78, 3594 (1956).

⁽¹⁾ R. D. O'Brian, "Toxic Phosphorus Esters," Academic Press, Inc., New York, N. Y., 1960.

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⁽³⁾ C. D. Hurd and L. Bauer, ibid., 76, 2791 (1954).

⁽⁵⁾ A. L. Green, G. L. Sainsbury, B. Saville and M. Stansfield, J. Chem. Soc., 1583 (1958).

TABLE I Atom % Excess Oxygen-18

Reactant	Solvent, water	Benzenecarbamoyl benzohydroxamate	Benzenesulfonic acid	Diisopropyl phosphate
Benzenesulfonyl chloride	5.27 ± 0.03	0.00 ± 0.01	0.00 ± 0.01	• • •
Benzenesulfonyl chloride (control)	3.34 ± 0.03		$3.21 \pm 0.01^{\circ}$	
Diisopropyl phosphofluoridate (DFP)	5.27 ± 0.03	0.00 ± 0.01		0.00 ± 0.01

^a Calculated for one oxygen atom.

This reaction has now been investigated using oxygen-18 labeled water as solvent. In straightforward hydrolysis the product acid should contain one labeled oxygen atom per molecule. A control hydrolysis experiment was run without the addition of benzohydroxamic acid and the results are given in Table I. However, in the presence of a hydroxamic acid, if the mechanism proposed is correct, the oxygen atom replacing the halogen is that originally in the hydroxyl group of hydroxamic acid. The oxygen atoms in the carbamoyl hydroxamate are those originally in the hydroxyl and carbonyl groups of hydroxamic acid, since the former is formed by condensation of the isocyanate formed by Lossen rearrangement with unreacted hydroxamic acid. Under the conditions of the reaction there is found to be no isotopic exchange of oxygen between the reactants or the products and water. The cyanate ion is known not to undergo isotopic exchange with water and it is reasonable to assume that the transiently formed aryl isocyanate does not readily undergo exchange either.

The reaction between benzenesulfonyl chloride and benzohydroxamic acid was run in water containing ca. 5% O¹⁸ keeping the *p*H constant at 7.6. The products, benzenesulfonic acid and O-phenylcarbamoyl benzohydroxamate, were isolated and analyzed for their isotopic oxygen content. Since sulfonic acids often present problems in isotopic analysis, this compound was condensed⁶ with dicyclohexylcarbodiimide in dry dioxane, to form N,N'-dicyclohexylurea. Both disubstituted urea and carbamoyl hydroxamate were analyzed by decomposition on heating with copper bronze, and the carbon dioxide formed was analyzed mass-spectrometrically As is seen from the table neither product contains any excess oxygen-18. It thus appears that the mechanism involving a Lossen rearrangement is correct.

An analogous reaction was run between diisopropyl phosphofluoridate and benzohydroxamic acid in O18enriched water at pH 7.6. The phenylcarbamoyl benzohydroxamate was filtered off and the diisopropyl phosphate isolated by lyophilization and condensed with dicyclohexylcarbodijmide in dry dioxane. Both products were again analyzed for their isotopic oxygen content and found to be unenriched. Thus the breakdown of diisopropyl phosphofluoridate in aqueous solution in the presence of hydroxamic acids follows an analogous mechanism. The results given in Table I show that the reaction with hydroxamic acid in both cases is much faster than the competing hydrolysis, since virtually no oxygen-18 is found in the products. The results also confirm the assumption that no isotopic exchange of oxygen took place between water and any of the reagents.

There has been some discussion³⁻⁵ on the reason for the marked instability of the sulfonated (or phosphorylated) benzohydroxamic acid intermediates which, in the course of all these studies, have never been isolated. It is known^{3,7} that the tendency for substituted hydroxamic acids III to undergo Lossen rearrangement is in-

(7) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 499.

RONHCOC₆H₅ RNHP(O)(OH₂) III IV

creased by electron-withdrawing properties of the group R. Both sulfonyl and phosphoryl groups have marked electron-withdrawing properties due to the polarity of the S=O and P=O bonds (structures I and II). The effect of these groups is reflected^{8a,b} in the sensitivity of N-substituted phosphoramidic acids (IV) to acid catalysis. The hydrolysis of N-benzoylphosphoramidic acid (IV, $R = C_6 H_5 CO$) is acid catalyzed in concentrated acid solution, due to protonation of the amido nitrogen. On the other hand, the rates of hydrolysis of diphenyl-N-dihydroxyphosphinylphosphoramidic acid^{8a} (IV, R = $(C_6H_5O)_2PO)$ and of N-benzene-sulfonylphosphoramidic acid^{8b} (IV, $R = C_6 H_5 SO_2$) are unaffected by quite high acidities (up to 8 N HClO₄ in the latter case). Presumably the strong electron withdrawal by the polar groups makes the nitrogen of the phosphoraniidic acid less susceptible to protonation. In the substituted hydroxamic acids (III, $R = C_6H_5SO_2$ - and $R = (i-PrO)_2PO-$) this electron withdrawal increases the tendency to positive charge on nitrogen, thereby facilitating migration of the phenyl group. This accounts for the lability of the intermediates I and II, which rearrange before they can be isolated.

Experimental

Materials.—O¹⁸-Enriched water was obtained from the Separation Plant of the Weizmann Institute. Benzohydroxamic acid was synthesized by the method of Hauser and Renfrow⁹ (m.p. 129-130°, lit. 125-128°). Diisopropyl phosphofluoridate was prepared as described by Saunders and Stacey,¹⁰ and benzene-sulfonyl chloride was a commercial sample.

Reaction of Benzenesulfonyl Chloride with Benzohydroxamic Acid.—Benzenesulfonyl chloride (0.5 ml.) was added to a solution of benzohydroxamic acid (0.5 g.) in O^{18} -enriched water (50 ml., 5.27% O^{18}) in a beaker with a magnetic stirrer. The *p*H was kept at 7.6 by means of a TTI Radiometer *p*H-meter using a 0.1 N solution of NaOH in O^{18} -enriched water. After a time equal to about five times the half-life,¹ the benzenecarbamoyl benzohydroxamate was filtered off and dried *in vacuo*. The filtrate was treated with Dowex 50, filtered and lyophilized. The solid of dicyclohexylcarbodiimide (0.5 g.) in dry dioxane (10 ml.) was added. After a few minutes a dense white precipitate of the urea was formed which was filtered off, washed with dry dioxane, and dried *in vacuo* (m.p. 220–223°, lit.¹¹ 220–225°). The control was run under identical conditions of reaction and isolation, without hydroxamic acid.

Reaction of Diisopropyl Phosphofluoridate with Benzohydroxamic Acid.—This reaction was performed in analogous manner to that described above using 0.65 g. of diisopropyl phosphofluoridate and 0.5 g. of benzohydroxamic acid in 40 ml. of O^{18} enriched water taking suitable safety precattions. Benzenecarbamoyl benzohydroxamate was isolated and dried. Diisopropyl hydrogen phosphate was isolated from the filtrate by lyophilization and condensed with dicyclohexylcarbodiimide in dry dioxane. The precipitated urea was filtered off, washed with dry dioxane and dried *in vacuo* (m.p. 220–223°). Isotopic Analysis.—All four products were analyzed by heating areal employed for many with o troop of compart bronze in a sealed

Isotopic Analysis.—All four products were analyzed by heating small samples (25 mg.) with a trace of copper bronze in a sealed tube to 325° for 0.5 hr. The results are presented in the above table. The O¹⁸-enriched water was analyzed by the hypobromite method of Anbar.¹²

(8) M. Halmann, A. Lapidot and D. Samuel: (a) J. Chem. Soc., 4672 (1960); (b) ibid., 3158 (1961).

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