Mechanistic Aspects of Oxazolone Reactions with α Nucleophiles

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The amino acid derived oxazolone, 2-phenyl-L-4-benzyloxazolone, was used in the continuation of our studies on the reactions of α nucleophiles (species containing two adjacent nucleophilic centers). Our experiments indicate that those α nucleophiles which show an enhanced nucleophilicity in relationship to their basicity are capable of a biphilic (electrophilic-nucleophilic) interaction with the carbonyl group of an oxazolone. Racemization may compete with ring opening for substituted hydrazines where biphilic pathways may be involved. Where this bifunctional attack is impossible, as, for example, in 1,1-dimethylhydrazine, no enhancement was found and a totally racemized product was isolated. We found that temperature, solvent, and concentration of reactants are important factors in ring opening *vs.* racemization reactions. For hydroxylamine-derived α nucleophiles, such as N-hydroxypiperidine and N-hydroxysuccinimide, which form active esters, racemization does not compete favorably with ring opening and products with high optical purity are obtained. These reactions can be understood as involving biphilic pathways. The N-hydroxypiperidine reaction may proceed through a zwitterionic attack, while the N-hydroxysuccinimide can be viewed as proceeding by a concerted pathway involving the carbonyl group of the N-hydroxy compound.

Goodman and McGahren[2a] reported that hydrazine hydrate reacts without racemization with the peptide oxazolone, 2-(1′-benzyloxycarbonylamino-1′-methyl)-ethyl-L-4-benzyloxazolone to give the ring-opened product, benzyloxycarbonylaminoisobutyryl-L-phenylalanine hydrazide. This is in contrast to the result found with amino acid esters and other nucleophiles in reaction with oxazolones,[2] where substantial racemization is generally found. Hydrazine belongs to a special class of compounds known as α nucleophiles, *i.e.*, compounds which possess two adjacent nucleophilic centers. Its extremely high nucleophilicity to basicity ratio prompted us to explore the nature of reactions of other α nucleophiles with the oxazolone moiety. We believe that insight in this area would lead to new and improved methods for the synthesis of peptides without racemization.

Synthesis of Various Hydrazides. A. From 2-Phenyl-L-4-benzyloxazolone.—The reaction of various hydrazine derivatives with 2-phenyl-L-4-benzyloxazolone was studied in a variety of solvents and at several temperatures. The effect of a large excess of nucleophilic reagent was also examined. The crude isolated material was compared by thin layer chromatography, infrared spectroscopy, and, in some cases, nuclear magnetic resonance spectroscopy to the product prepared *via* a nonracemizing route. The specific rotation of the crude material prepared from the oxazolone was measured and the value was compared with the specific rotation of the same compound synthesized *via* the nonracemizing route to determine the extent of racemization in each case.

In order to avoid dihydrazide formation,[2a] we added a large excess of hydrazine in anhydrous methanol in one portion to the oxazolone solution in anhydrous methanol (or tetrahydrofuran). An extremely rapid reaction was observed, even at 0°. A tlc taken 1 min after the combination of reactants showed the absence of any oxazolone.

t-Butyloxycarbonyl hydrazide (*t*-butyl carbazate) was allowed to react with the oxazolone in ether at 25°. Tlc indicated a slow reaction. To determine the extent of racemization we converted the isolated carbazate derivative into benzoyl phenylalanine hydrazide, a compound which was prepared in optically pure form by acid hydrolysis of the *t*-butyloxycarbonyl group.

Phenylhydrazines, *o*-methoxyphenylhydrazine, N,N-dimethylhydrazine, and *p*-nitrophenylhydrazine react cleanly with oxazolones to give the expected hydrazide derivatives, although, for the latter two compounds, reaction is slow and the yield of product isolated was low.

B. Synthesis of Optically Pure Hydrazides.—We obtained benzoyl-L-phenylalanine hydrazide as a chromatographically pure material by treatment of the corresponding methyl ester with hydrazine. By comparison with this material, we determined the extent of racemization in the reaction of 2-phenyl-L-4-benzyloxazolone with hydrazine, hydrazine acetate, and *t*-butyl carbazate (indirectly). In addition, this hydrazide was employed in the azide synthesis of the phenylhydrazide, N,N-dimethylhydrazide, and *o*-methoxyphenylhydrazide derivatives.

For *p*-nitrophenylhydrazine, the azide reaction failed to give the desired material in either the sodium nitrite or the modified butyl nitrite approach. The desired compound, benzoyl-L-phenylalanine *p*-nitrophenylhydrazide, was made by an indirect procedure. Benzyloxycarbonyl-L-phenylalanine was coupled with *p*-nitrophenylhydrazine using dicyclohexylcarbodiimide. Hydrogen bromide in glacial acetic acid removed the benzyloxycarbonyl group and the resulting hydrobromide salt was benzoylated in pyridine–dimethylformamide with benzoyl chloride.

The indirect procedure was also used to confirm the azide synthesis for the *o*-methoxyphenylhydrazine and N,N-dimethylhydrazine reactions.

Synthesis of Hydroxylamine Derivatives. A. From 2-Phenyl-L-4-benzyloxazolone.—The parent compound, hydroxylamine, reacts with oxazolone under our reaction conditions to give two initial products. On continued reaction with an excess of hydroxylamine, one material disappears and the second increases in intensity. Apparently both O and N acylation occur. O-acyl derivatives are active esters and on further reaction with hydroxylamine are converted into the stable hydroxamic acids.[3] Spontaneous rearrangement of O-acyl to N-acyl derivatives has also been reported.[4]

(1) Submitted in partial fulfillment of the requirements for the Ph.D. Degree in Chemistry at the Polytechnic Institute of Brooklyn.

(2) (a) M. Goodman and W. J. McGahren, *Tetrahedron*, **23**, 2031 (1967); (b) M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, **86**, 2918 (1964).

(3) W. P. Jencks, *ibid.*, **80**, 4581, 4585 (1958).

(4) S. Bittner, Y. Knobler, and M. Frankel, *Tetrahedron Lett.*, 95 (1965).

N,N-Diethylhydroxylamine, N-hydroxypiperidine, and N-hydroxysuccinimide react rapidly to give product, while N,O-dimethylhydroxylamine reacts considerably more slowly under similar reaction conditions.

B. Attempted Synthesis of Optically Pure Hydroxylamine Derivatives.—The azide reaction with hydroxylamine gave a low yield of the stable hydroxamic acid. N,N-Diethylhydroxylamine failed to give the desired product by this method. For this reason, and also because little is known about oxygen attack on the carbonyl function of the azide, this route was not pursued for N-hydroxypiperidine or N-hydroxysuccinimide. The indirect procedure was also unsuccessful for the preparation of the optically pure N,N-diethylhydroxylamine ester of benzoyl-L-phenylalanine. This method involves benzoylation of a free amino acid activated ester in the final step. This species can form oligomers, small cyclic peptides, or the diketopiperazine. In addition, the active ester might racemize under these conditions, which would defeat the purpose of preparing the optically pure derivative. These considerations also apply to the active esters of N-hydroxypiperidine and N-hydroxysuccinimide. Therefore, we employed another approach to determine the extent of racemization for these active esters. They were converted with hydrazine (under conditions which do not involve racemization) into benzoylphenylalanine hydrazide, which was examined to ascertain its optical purity.

Finally, we employed the indirect procedure for the preparation of the N,O-dimethylhydroxylamine derivative.

Results and Discussion

It has been possible to correlate reactivities of various nucleophiles by suitable examination of such parameters as polarizability and basicity.[5-8] One class of compounds does not appear to follow these correlations in its reaction with certain electrophilic centers, in particular with activated carbonyl compounds. These nucleophiles are more reactive than would be predicted on the basis of polarizability and basic strength.[9-11] Their common structural feature is the presence of an unshared pair of electrons on the atom adjacent to the nucleophilic atom. Edwards and Pearson[12] noted that these nucleophiles exhibit an enhanced reactivity, which they termed the α effect.

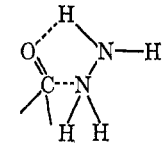
Hydrazine represents an example of this special group of vicinally bifunctional nucleophiles. As mentioned previously, Goodman and McGahren demonstrated that an excess of hydrazine hydrate reacts with the peptide oxazolone from benzyloxycarbonylaminoisobutyryl-L-phenylalanine, yielding optically pure hydrazide.[2a] Siemion and Morawiec[13] reported similar results with the oxazolone from acetyl-L-leucine. In contrast, Siemion and Dzugaj[14] reported that the ammonolysis of the oxazolone from acetyl-L-leucine gives completely racemic product.

Hydroxylamine and its derivatives are also α nucleophiles and have been found to have enhanced nucleophilicity.[9-11] Diethylhydroxylamine,[4] N-hydroxypiperidine,[15-17] N-hydroxyphthalimide,[18,19] N-hydroxysuccinimide,[20-23] and benzohydroxamic acid[24] have all been used in recent years as racemization-resistant activating agents in peptide coupling reactions.

Bruice and coworkers[11] outlined various proposed explanations for the α effect. They can be summarized briefly as follows: (A) stabilization of the transition state owing to overlap of the orbitals of the lone-pair electrons in the α position; (B) diminished solvation, *e.g.*, of HOO^- compared with OH^-; (C) ground-state destabilization resulting from nonbonding electron-pair repulsions; (D) intramolecular general base catalysis; (E) simultaneous push–pull mechanisms resulting from the "biphilic" nature of the reagent.

Most of the available evidence supports biphilic pathways for the α effect: (A) α nucleophiles which cannot participate in push–pull transition states are found to have normal reactivity;[9-11] (B) the α effect is inoperative in amine general base catalyzed ionization of nitroethane;[25] (C) the α effect is inoperative for displacements on sp^3 carbon (CH_3I);[26] (D) phenylhydroxylamine has a higher rate, lower E_a, and a high negative $\Delta S^{\pm}$ in relation to other nucleophiles in its attack on acetyl peroxide.[27] The high negative $\Delta S^{\pm}$ is indicative of a cyclic transition state.

This bifunctional pathway can be illustrated for the reaction of hydrazine with an activated carbonyl compound (ester, acid halide, acylisourea, anhydride, etc.) as follows.



In the transition state, hydrazine acts in a dual capacity by both supplying electron density for nucleophilic attack at the carbonyl carbon and withdrawing electron density from the electrophilic site by hydrogen bonding to the carbonyl oxygen.

Our studies on 2-phenyl-L-4-benzyloxazolone support these conclusions (Tables I and II). Oxazolones can be viewed in their reactions as members of the general

(5) J. O. Edwards, *J. Amer. Chem. Soc.*, **78**, 1819 (1956).
(6) R. G. Pearson, *Chem. Brit.*, **3** (3), 103 (1967).
(7) R. F. Hudson, *Chimica*, **16**, 173 (1962).
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(11) T. C. Bruice, A. Donzel, R. W. Hoffman, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967).
(12) J. O. Edwards and R. G. Pearson, *ibid.*, **84**, 16 (1962).
(13) I. Z. Siemion and J. Morawiec, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, **12**, 295 (1964).
(14) I. Z. Siemion and A. Dzugaj, *Rocz. Chem.*, **40**, 1699 (1966).
(15) S. M. Beaumont, B. O. Handford, and G. T. Young, *Proc. 7th Eur. Peptide Symp.* (Budapest), 37 (1964).
(16) F. Weygand and W. König, *Z. Naturforsch.*, **20b**, 710 (1965).
(17) J. H. Jones, B. Liberek, and G. T. Young, *Proc. 8th Eur. Peptide Symp.* (Noordwijk), 15 (1966).
(18) G. H. L. Nefkens and G. I. Tesser, *J. Amer. Chem. Soc.*, **83**, 1263 (1961).
(19) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. Trav. Chim. Pays-Bas*, **81**, 683 (1962).
(20) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **85**, 3039 (1963); **86**, 1839 (1964).
(21) E. Wünsch and F. Drees, *Chem. Ber.*, **99**, 110 (1966).
(22) F. Weygand, D. Hoffman, and E. Wünsch, *Z. Naturforsch.*, **21b**, 426 (1966).
(23) J. E. Zimmerman and G. W. Anderson, *J. Amer. Chem. Soc.*, **89**, 7151 (1967).
(24) E. Taschner, B. Rzeszotarska, and L. Lubiewska, *Chem. Ind.* (London), 402 (1967).
(25) M. J. Gregory and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 2327 (1967).
(26) M. J. Gregory and T. C. Bruice, *ibid.*, **89**, 4400 (1967).
(27) K. M. Ibne-Rasa and J. O. Edwards, *ibid.*, **84**, 763 (1962).

TABLE I

REACTIONS OF 2-PHENYL-L-4-BENZYLOXAZOLONE WITH HYDRAZINE AND ITS DERIVATIVES

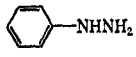
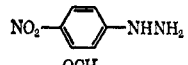
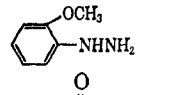
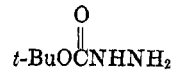
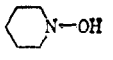
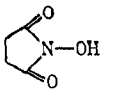
Nucleophile	Solvent	Temp, °C	Racemization, %	Nucleophile/ oxazolone
NH_2NH_2	THF–MeOH (1:1)	0	0	Large excess
	THF–MeOH (1:1)	25	14	Large excess
	THF–MeOH (1:1)	44	32	Large excess
$NH_2NH_2 \cdot HOAc$	THF	0	33	2.6:1
	THF	25	35	2.6:1
	Et_2O	0	35	1.1:1
	Et_2O	0	75	5:1
	Et_2O	25	70	1.1:1
	THF	0	100	5:1
	$CHCl_3$	0	33	1.1:1
	$CHCl_3$	25	59	1.1:1
	THF	25	100	1.1:1
	Et_2O	25	40	1.1:1
	Et_2O	25	75	1.2:1
$(CH_3)_2NNH_2$	Et_2O	25	100	1.1:1
	$CHCl_3$	0	100	1.1:1
	$CHCl_3$	25	100	1.1:1
	$CHCl_3$	25	100	8:1

TABLE II

REACTIONS OF 2-PHENYL-L-4-BENZYLOXAZOLONE WITH HYDROXYLAMINE AND ITS DERIVATIVES

Nucleophile	Solvent	Temp, °C	Racemization, %	Nucleophile/ oxazolone
NH_2OH	MeOH	25	0	2.5:1
	Et_2O	0	<5	1.1:1
	Et_2O	25	<10	1.1:1
	THF	25	<10	1.1:1
	THF	0	0	1.1:1
	THF	25	0	1.1:1
	THF	0	0	4:1
CH_3NHOCH_3	Et_2O	25	55	1.1:1
$(CH_3CH_2)_2NOH$	Et_2O	0	42	1.1:1
	Et_2O	25	56	1.1:1
	THF	0	42	1.1:1

class of activated carbonyl compounds. Those α nucleophiles which can participate in biphilic attack were found to react more rapidly and to give products with a considerably higher degree of optical purity than α nucleophiles which cannot participate in biphilic attack.

A large excess of hydrazine reacts instantaneously with oxazolone, even at 0°, and at this temperature no racemization is found. As the temperature is raised, racemization begins to compete more favorably. This may be a reflection of the highly ordered cyclic transition state necessary for bifunctional attack leading to ring-opened product.

In the series hydrazine, phenylhydrazine, and *p*-nitrophenylhydrazine, the increase in electron-withdrawing effect on the substituted nitrogen atom leads to a decrease in the ring-opening rate and more racemization. Apparently, the weakened nucleophilicity of the primary nitrogen atom is more important than the increased ability for electrophilic attack by the hydrogen atom on the substituted nitrogen. This also serves to explain the high degree of racemization found with *t*-butyl carbazate. A separate experiment was carried out with this nucleophile in which a first crop of product was isolated after 2 hr and a second crop at the completion of reaction. The first crop exhibited a considerably higher optical purity. This is consistent with two separate mechanisms for racemization and ring opening. The reagent, in this case *t*-butyl carbazate, may react with the oxazolone to form the ring-opened hydrazide. It may also act as a base, racemizing the oxazolone by removing the acidic proton from the asymmetric carbon atom. This racemized material may subsequently be ring opened to form product. For this reason, the product formed early in the reaction will have the highest specific rotation.

For phenylhydrazine, we can see that in ether at 0° a fivefold excess of the nucleophile causes substantially more racemization than a 10% excess causes. This is expected because of the increased polarity of the solution.

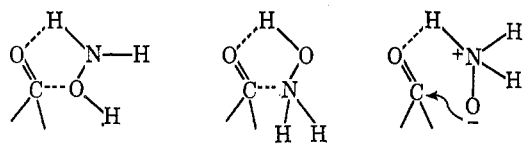
N,N-Dimethylhydrazine is a slightly weaker base than hydrazine in aqueous media.[28] Nevertheless, on reaction with oxazolone under a variety of conditions, complete racemization is found in a relatively slow reaction. No biphilic mechanism leading to product can be viewed for this nucleophile. Attack by the primary amine function does not allow for hydrogen bonding. Hydrogen bonding is possible for attack by the dimethyl nitrogen atom. This cannot lead to product, however, because the dimethyl nitrogen atom has no proton to expel and cannot eliminate the positive charge acquired during nucleophilic attack. Steric hindrance may also play a role in this reaction.

Hydroxylamine and its derivatives are considerably less basic than the corresponding hydrazine compounds;

(28) R. L. Hinman, *J. Org. Chem.*, **23**, 1587 (1958).

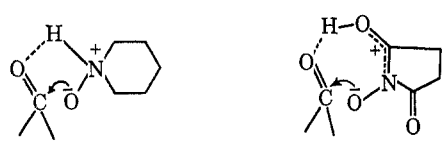
nevertheless, reactions of bifunctional reagents hydroxylamine, N-hydroxypiperidine, N-hydroxysuccinimide, and N,N-diethylhydroxylamine with oxazolone are very fast and the optical purity of the compounds obtained in the first three cases is high (90–100%).

Reaction of the parent compound, hydroxylamine, involves competition between oxygen and nitrogen attack on the carbonyl function. Either reaction may involve bifunctional attack by the neutral hydroxylamine molecule (transition state **1a,b**). In addition, oxygen anion attack by the zwitterionic form of the molecule may also be involved in a push–pull mechanism (transition state **1c**). This latter possibility can account for the initial formation of considerable O-acyl derivative.



1a **1b** **1c**

In the reaction of N-hydroxypiperidine, no biphilic mechanism is possible for the neutral species. However, in the zwitterionic form, reaction may proceed *via* attack by the oxygen anion and simultaneous hydrogen bonding (transition state **2a**). The greater degree of racemization found for N,N-diethylhydroxylamine (42–56%) compared with N-hydroxypiperidine (5–10%) can be explained by steric considerations which would retard the ring-opening reaction. Racemization by proton abstraction should be much less sterically dependent. The alkyl substituents on the N atom of N-hydroxypiperidine are restrained by the ring. These restrictions on rotation do not apply to N,N-diethylhydroxylamine. Consequently, the approach of N,N-diethylhydroxylamine to the oxazolone system should be more sterically hindered and causes more racemization. For N-hydroxysuccinimide, a simple zwitterion (analogous to **2a**) is not likely because of the electronegative effect of the two carbonyl functions adjacent to the nitrogen atom. However, in the delocalized zwitterionic structure illustrated (transition state **2b**), the dispersal of positive charge through three atoms makes possible an enhanced rate of reaction *via* a nucleophilic–electrophilic mechanism. This zwitterionic structure need only be present in extremely low concentration in equilibrium with the more stable unchanged form. It should be noted that hydroxamic acids normally react in aqueous solution as anions. Perhaps the anionic form is the reacting species of N-hydroxysuccinimide in organic media. N,O-Dimethylhydroxylamine can neither participate in a biphilic attack in the transition state nor form a zwitterionic intermediate. Consequently, it ring opens oxazolone considerably more slowly and the product derived is substantially racemized.



2a **2b**

The results of Siemion[29] are consistent with our biphilic interpretation of α-nucleophilic effects involving hydrogen bonding to the carbonyl oxygen of the oxazolone ring. However, he proposed an alternate biphilic mechanism based on the influence of the weakly basic nitrogen atom in the oxazolone ring. Siemion suggests that as the oxazolone ring opens, the basicity of the ring nitrogen is strongly enhanced, leading to racemization by abstraction of hydrogen from the adjacent carbon atom. According to this explanation, attack by hydrazine involves hydrogen bonding to the nitrogen atom of the ring and accounts for the lack of racemization.

We believe that the mechanism proposed by Siemion is incorrect because oxazolone racemization is extremely facile during peptide coupling. The strong bases present abstract the proton from the asymmetric carbon. The weakly basic nitrogen of the oxazolone ring cannot compete. Ring opening and racemization can thus be seen as two distinct and coexisting processes.

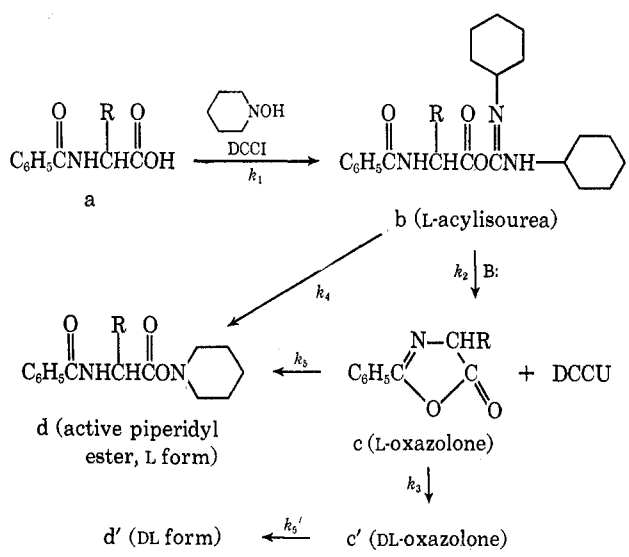
(A) The wide variance in k_{ro}/k_{rac} found for different amino acid esters in reaction with the peptide oxazolone 2-(1′-benzyloxycarbonylamino-1′-methyl)-ethyl-L-4-benzyloxazolone[2a] implies two separate processes for ring opening and racemization. It follows from Siemion's proposal that each of these reactions should have similar k_{ro}/k_{rac} ratios.

(B) For highly hindered nucleophiles such as methyl aminoisobutyrate, $k_{rac} \gg k_{ro}$, and in general $k_{rac} \geq k_{ro}$.[2a] Siemion's proposal leads to a prediction that $k_{rac} \leq k_{ro}$ in all cases.

(C) Tertiary amines,[2a] and even the much less basic dicyclohexylcarbodiimide, lead to rapid racemization of the oxazolone, even though ring opening is impossible for these compounds.

(D) The experiment on partial isolation of product with *t*-butyl carbazate, described in the previous section, is consistent only with separate processes for racemization and ring opening.

It appears that biphilic attack involving hydroxylamine-derived activating groups makes possible the preparation of the active ester in a higher degree of optical purity than would otherwise be found. For example, we can illustrate the preparation of an N-



(29) I. Z. Siemion, *Rocz. Chem.*, **42**, 237 (1968).

hydroxypiperidine active ester from a benzoyl amino acid using dicyclohexylcarbodiimide. (An analogous argument could be used employing the mixed anhydride route.)

Analysis of the reaction after formation of the initial species (b) leads us to the following predictions.

(A) The k_4/k_2 value will be substantially higher for N-hydroxypiperidine, where a biphilic mechanism is operative, than for *p*-nitrophenol, where it is not. Little oxazolone (c) formation is expected in the first case, whereas substantial oxazolone may be formed in the second.

(B) Even if L-oxazolone (c) were to form extensively, biphilic attack of N-hydroxypiperidine would lead to optically active d. Thus k_5/k_3 will be much higher than for *p*-nitrophenol, where no biphilic route is possible.

It cannot be stated at this time which of these factors is most important, but the net effect is clear. Intermediates which are obtained *via* biphilic reactions will have a high degree of optical purity.

As part of a comprehensive study on the chemistry of carbodiimides,[30-32] DeTar and his associates studied the reactions of peptide acids with carbodiimides.[30] They found that the rate of reaction between benzoylphenylalanine and dicyclohexylcarbodiimide is the same with or without *p*-nitrophenol present. Oxazolone is the first identifiable intermediate, confirming earlier results.[33] Under the reaction conditions, the rate of reaction to form oxazolone from benzoylphenylalanine is 1000 times faster than the reaction of oxazolone with *p*-nitrophenol. As we noted earlier, Goodman and Levine[2b] found that the reaction of isolated oxazolone from benzoyl-L-phenylalanine and *p*-nitrophenol is reversible, and racemization proceeds much more rapidly than ring opening for attack by *p*-nitrophenylate anion. There have been several reports of racemization during the preparation of *p*-nitrophenyl esters of acylated derivatives from both the dicyclohexylcarbodiimide[2,30,34,35] and tris-*p*-nitrophenyl phosphite methods.[36]

Conclusions

Our research has centered on the chemistry of oxazolones, particularly in their relationship to racemization and peptide coupling reactions. Stable, optically active oxazolones offer the chemist a unique system for studying the nucleophilicity and basicity of various compounds in organic solvents. Examining this system with α nucleophiles, we explored the special nature of these reagents, which allows for a stronger nucleophilic effect than would otherwise be expected. Participation in a concerted mechanism involving nucleophilic–electrophilic attack can account for the enhanced rate of ring opening. The implication for the formation of peptide-active esters derived from α nucleophiles was discussed.

It is recognized that our studies represent the most unfavorable situation possible in peptide coupling, *i.e.*, where all the acyl peptide is present as oxazolone. For this reason, the very low degree of racemization found for hydroxylamine compounds in cases where biphilic attack is possible is quite significant.

Experimental Section

All melting points are uncorrected. They were obtained on a Kofler hot stage melting point apparatus. Analyses were carried out by Alfred Bernhardt Mikroanalytisches Laboratorium, Max Planck Institut, Mülheim, West Germany. Solvents used in reactions involving oxazolone were anhydrous. Gelman type SG silica chromatograms were used for tlc with iodine as the developing agent. Chloroform and chloroform–hexane mixtures were used for chromatographic separation.

N-Benzoyl-L-phenylalanine.—This compound was prepared in 59% yield by the procedure of Greenstein and Winitz,[37] mp 142–143°, $[\alpha]^{25}D$ +38.50° (*c* 1.5, dioxane) [lit.[2b] mp 142–143°, $[\alpha]^{25}D$ +38.74° (*c* 1.6, dioxane)].

2-Phenyl-L-4-benzyloxazolone. A. Using Dicyclohexylcarbodiimide.—A solution of dicyclohexylcarbodiimide (1.65 g, 8.0 mmole) in 5 ml of anhydrous ether was added to benzoyl-L-phenylalanine (2.15 g, 8.0 mmol) in 30 ml of anhydrous ether at 0.5°. Precipitation of dicyclohexylurea began almost immediately. After 15 min the solution was filtered and the ether was removed at room temperature under reduced pressure to give a solid material. Fresh ether (20 ml) was added, the solution was filtered again, and the solvent was removed. The solid product was recrystallized from *ca.* 1:1 ether–petroleum ether (bp 30–60°). *Ca.* 30 ml of total volume was sufficient. No cloud point was noticed on addition of petroleum ether, but 1.50 g (75%) of crystalline material formed on cooling for 1–2 hr in the refrigerator, mp 86.0–87.5°, $[\alpha]^{25}D$ −67.0° (*c* 2, dioxane).

A second recrystallization was sufficient to raise the rotation to −71.0° and the melting point to 88–89°, but the overall yield was reduced to 1 g (50%). After drying over P_2O_5 under reduced pressure, this compound was stored in the refrigerator. No decomposition or racemization was found under these conditions after several months [lit.[2b] mp 86.6–87.2°, $[\alpha]^{25}D$ −71.20° (*c* 0.5, dioxane)]. This is the preferred method of preparation.

B. Using Acetic Anhydride.—A solution of 1.0 g of benzoyl-L-phenylalanine in 5.5 ml of dioxane and 5.5 ml of acetic anhydride was prepared, and the change in rotation was followed in the polarimeter (Table III).

TABLE III

Time, min	Rotation, deg	Time, min	Rotation, deg
2.33	+1.534	57.83	−4.510
3.87	+1.249	60.00	−4.570
5.43	+0.967	64.83	−4.680
8.00	+0.500	66.50	−4.730
10.93	+0.041	68.00	−4.744
15.00	−0.600	70.00	−4.760
22.93	−1.659	75.08	−4.790
31.00	−2.534	77.50	−4.785
36.00	−3.018	84.50	−4.717
41.17	−3.467	94.00	−4.620
43.25	−3.607	99.50	−4.513
53.75	−4.317	107.33	−4.393

After 70 min, the solvent was removed under reduced pressure at room temperature. Dry toluene (15 ml) was added twice to the remaining crude material and the solution was evaporated to dryness each time. Recrystallization was effected from ether–hexane to give a yield of 40%, mp 86–87°, $[\alpha]^{25}D$ −68.0° (*c* 2, dioxane).

Benzoyl-L-phenylalanine Methyl Ester.—Diazomethane prepared from 10.0 g of 80% N-nitroso-N-methylurea (20% acetic acid) was added to benzoyl-L-phenylalanine (7.50 g, 26.5 mmol) in 35 ml of methylene chloride at 0°. The yellow ether–methylene

(30) D. F. DeTar, R. Silverstein, and F. F. Rogers, Jr., *J. Amer. Chem. Soc.*, **88**, 1024 (1966).
(31) D. F. DeTar and R. Silverstein, *ibid.*, **88**, 1013 (1966).
(32) D. F. DeTar and R. Silverstein, *ibid.*, **88**, 1020 (1966).
(33) M. M. Botvinik, S. N. Kara-Murza, S. M. Avaeva, and V. Ya. Nikitin, *Dokl. Akad. Nauk. SSSR*, 88 (1964).
(34) J. Kovacs, unpublished results.
(35) W. D. Cash, *J. Org. Chem.*, **27**, 3329 (1962).
(36) M. Goodman and K. C. Stueben, *ibid.*, **27**, 3409 (1962).
(37) J. P. Greenstein and M. W. Winitz, "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York, N. Y., 1961, p 1267.

chloride solution was evaporated to dryness after 2 hr in a stream of nitrogen. Fresh ether (50 ml) was added and the process was repeated. The white solid was taken up in ether, extracted with 10% K_2CO_3, saturated KCl, 5% HCl, and saturated KCl again, and then dried. After filtration, evaporation of solvent, and trituration with hexane, 6.3 g of crude material was obtained which gave a first crop of 5.0 g of pure product on recrystallization from ether–hexane, mp 82–83°, $[\alpha]^{25}D$ +24.0° (*c* 1, dioxane) [lit.[2] mp 83.6–84.6°, $[\alpha]^{25}D$ +24.2° (*c* 1, dioxane)]. A second crop of 550 mg of pure material was also recovered, total yield 67%.

Benzoyl-L-phenylalanine Hydrazide. A. From Benzoyl-L-phenylalanine Methyl Ester.—To benzoyl-L-phenylalanine methyl ester (5.00 g, 17.6 mmol) in 20 ml of methanol at the boiling point, 6 ml of anhydrous hydrazine (97%, large excess) was added and the solution was allowed to stand for 24 hr, being cooled in the ice box before filtration. Crystallization was observed on cooling to room temperature; 4.40 g of crude product was obtained. Recrystallization from methanol yielded 3.55 g (71%) of material, mp 193–198°, $[\alpha]^{25}D$ −45.8° (*c* 1.5, dimethylformamide).

Anal. Calcd for $C_{16}H_{17}O_2N_3$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.85; H, 6.04; N, 15.11.

B. From 2-Phenyl-L-4-benzyloxazolone. Procedure 1.—Oxazolone (320 mg, 1.27 mmol) in 10 ml of anhydrous methanol was added to 1 ml of anhydrous hydrazine (97%) in 10 ml of anhydrous methanol at 0°. A crystalline precipitate was noted instantaneously. After 15 min the solvent was removed at room temperature over sulfuric acid under reduced pressure to remove traces of hydrazine. The solid was washed with ether and filtered to give 300 mg (84%) of crude product, mp 190–197°, $[\alpha]^{25}D$ −45.3° (*c* 1, dimethylformamide). Recrystallization from methanol gave chromatographically pure product, mp 192–196°, $[\alpha]^{25}D$ −45.6° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{16}H_{17}O_2N_3$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.86; H, 5.99; N, 14.95.

This experiment was repeated using tetrahydrofuran as a solvent for the oxazolone and with an inverse order of addition to the above. The results obtained were identical with those reported. In addition, hydrazine hydrate (85%) was used in both methanol and the mixed methanol–tetrahydrofuran solvent system. In all cases, at 0°, no racemization was found.

Procedure 2.—Hydrazine hydrate (3 ml, large excess) in 5 ml of methanol at 25° was added to oxazolone (251 mg, 1 mmol) in 5 ml of tetrahydrofuran. Work-up as described above gave product in 90% yield, $[\alpha]^{25}D$ −39.2° (*c* 1, dimethylformamide).

A similar run with anhydrous hydrazine (97%) in fivefold excess afforded a product, $[\alpha]^{25}D$ −39.5° (*c* 1, dimethylformamide).

Procedure 3.—Anhydrous hydrazine (250 mg, 7.8 mmol) in 5 ml of anhydrous methanol was added to 2-phenyl-L-4-benzyloxazolone (251 mg, 1 mmol) in 5 ml of tetrahydrofuran at 44°. Work-up gave product in 87% yield, $[\alpha]^{25}D$ −30.7° (*c* 1, dimethylformamide).

C. From Indirect Route Using *t*-Butyloxycarbonyl Hydrazide. Procedure 1. Benzoylphenylalanine *t*-Butyloxycarbonyl Hydrazide.—*t*-Butyloxycarbonyl hydrazide (380 mg, 2.9 mmol, prepared by the method of Carpino[38]) in 5 ml of ether was added to 2-phenyl-L-4-benzyloxazolone (600 mg, 2.4 mmol) in 10 ml of anhydrous ether at room temperature. Thin layer chromatography indicated a slow reaction. After 5 hr, substantial oxazolone remained. Precipitation of product began after 1 hr.

In the first run, 260 mg of material was filtered off after 2 hr, and the material gave one spot with tlc after washing with cold ether. A second crop of 500 mg with an identical thin layer chromatogram and infrared spectrum was obtained on completion of reaction: fraction 1, 260 mg, mp 165–167°, $[\alpha]^{25}D$ −63.7° (*c* 2, dimethylformamide); fraction 2, 500 mg, mp 100–105°, $[\alpha]^{25}D$ −22.9° (*c* 2, dimethylformamide). Obviously, as time passes, the material formed is more highly racemized.

In the second run, the reaction was allowed to run overnight to completion at room temperature. Crude product (800 mg, 87.3%) was obtained on filtration and washing with cold ether. No additional product could be seen in the tlc of the solution. The product gave one spot with tlc, mp 148–150°, $[\alpha]^{25}D$ −31.8° (*c* 2, dimethylformamide). This material was used in the hydrolysis step.

(38) (a) L. A. Carpino, *J. Org. Chem.*, **28**, 1909 (1963); (b) *J. Amer. Chem. Soc.*, **82**, 2725 (1960).

Procedure 2. Benzoylphenylalanine Hydrazide.—Benzoyl phenylalanine *t*-butyloxycarbonyl hydrazide (500 mg, 1.30 mmol) was added to 7 ml of a dry, saturated solution of hydrochloric acid in tetrahydofuran at 0°. Evolution of carbon dioxide was noticed. The solution was allowed to warm to room temperature, and after 2 hr the cloudy mixture was precipitated by adding it to 200 ml of ether. After cooling in the refrigerator for 1 hr, 270 mg of crude product (65%) was obtained after filtration and washing with ether. Neutralization was accomplished by adding a concentrated solution of $NaHCO_3$ to this material. After 1 hr, the product was removed by filtration, washed with water, and dried under reduced pressure over P_2O_5. The hydrazide was obtained, mp 180–188°, $[\alpha]^{25}D$ −12.7° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{16}H_{17}O_2N_3$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.78; H, 5.99; N, 15.00.

D. From 2-Phenyl-L-4-benzyloxazolone and Hydrazine Acetate.—A solution of acetic acid (660 mg, 1.0 mmol) in 10 ml of tetrahydrofuran was added to anhydrous hydrazine (320 mg, 1.0 mmol) in 5 ml of tetrahydrofuran. The immediate formation of an insoluble material was noted. This was filtered off, washed with tetrahydrofuran, dried *in vacuo* over P_2O_5, and used without further characterization.

The hydrazine acetate salt (240 mg, 2.6 mmol), suspended in 10 ml of tetrahydrofuran, was added to L-oxazolone (251 mg, 1.0 mmol) in 5 ml of tetrahydrofuran at room temperature and stirring was continued for 4 hr. The solvent was evaporated at room temperature with a stream of nitrogen, water was added, and the product was filtered and dried *in vacuo* over P_2O_5 and concentrated H_2SO_4. Product (210 mg, 74%) was obtained, identifiable from its thin layer chromatogram and infrared spectrum, $[\alpha]^{25}D$ −39.5° (*c* 1.5, dimethylformamide).

The same reaction was repeated at 0° for 6 hr. Identical work-up afforded 215 mg (76%) of product, $[\alpha]^{25}D$ −29.6° (*c* 1.5, dimethylformamide).

In both cases, thin layer chromatography showed only one material, while the infrared spectra indicated minor impurities.

Benzoylphenylalanine Phenylhydrazide. A. Azide Route.—To benzoyl-L-phenylalanine hydrazide (566 mg, 2 mmol) in 0.1 *N* hydrochloric acid (40 ml, 4 mmol), 10 ml of glacial acetic acid was added to bring about solution. The solution was cooled to −5°, and a solution of $NaNO_2$ (280 mg, 4 mmol) in 15 ml of H_2O at 0° was added dropwise over 3 min. The azide formed immediately as an insoluble, white solid. After 10 min, the azide was extracted into 40 ml of a 1:1 mixture of methylene chloride–ether, and this solution was extracted with ice-cold solutions of saturated KCl, 5% $NaHCO_3$ (until basic), and once again with KCl and dried ($MgSO_4$) at −15°. The dry organic solution was filtered and added in one portion to phenylhydrazine (0.3 ml, 0.329 g, 3 mmol) in 10 ml of ether at 0°. The solution was stirred overnight and allowed to warm to room temperature after *ca.* 8 hr. After 50 ml of petroleum ether had been added, 400 mg (55.7%) of crude product was isolated on cooling. Recrystallization from methanol gave 287 mg (40%) of product, mp 197–208°, $[\alpha]^{25}D$ −69.9° (*c* 1.5, dimethylformamide).

Anal. Calcd for $C_{22}H_{21}O_3N_3$: C, 73.52; H, 5.89; N, 11.69. Found: C, 73.30; H, 5.94; N, 11.99.

B. From Oxazolone.—Experiment B was run as follows. Phenylhydrazine (120 mg, 1.1 mmol) in 5 ml of ether at room temperature was added to 2-phenyl-L-4-benzyloxazolone (251 mg, 1.0 mmol) in 10 ml of ether. Precipitation of product began within 5 min. After 2.5 hr at room temperature, the solvent was removed and petroleum ether was added. Product (350 mg, 92.0%) was obtained on filtration and washing with cold ether–petroleum ether, $[\alpha]^{25}D$ −21.1° (*c* 1, dimethylformamide). One spot was obtained with tlc, which corresponded to that from the azide reaction. Recrystallization from ethanol afforded an analytical sample, mp 199–207°.

Anal. Calcd for $C_{22}H_{21}O_3N_3$: C, 73.35; H, 5.88; N, 11.79. Found: C, 73.52; H, 5.89; N, 11.69.

This reaction was repeated under several conditions (Table IV).

C. From Succinimide Active Ester.—Benzoyl-L-phenylalanine succinimide ester (365 mg, 1 mmol) in 15 ml of tetrahydrofuran was allowed to react with phenylhydrazine (260 mg, 2.5 mmol) in 5 ml of tetrahydrofuran for 4 hr at room temperature. The solvent was removed several times with a stream of nitrogen. Water was added to remove N-hydroxysuccinimide, and the product was filtered and dried over P_2O_5 and H_2SO_4 in the vacuum desiccator overnight. Crude product (330 mg, 92%) was obtained. The infrared spectrum and thin layer chromatogram

TABLE IV

Solvent	Temp, °C	Racemization,[a] %	Nucleophile/ oxazolone
Et_2O	0	35	1.1:1
Et_2O	0	75	5:1
THF	0	100	5:1
$CHCl_3$	0	33	1.1:1
$CHCl_3$	25	59	1.1:1

[a] Based on azide run.

showed it to be the desired material, $[\alpha]^{25}D$ −66.6° (*c* 1.5, dimethylformamide).

The succinimide active ester used was 3–4% racemic, $[\alpha]^{25}D$ −51.5° (*c* 1.5, THF). Therefore, the active ester reacted to give phenylhydrazide product with no racemization (compared with the azide method).

Benzoylphenylalaine Dimethylhydrazide. A. From Azide.—The general method used for the preparation of benzoylphenylalanine phenylhydrazide gave 33% crude product on addition of petroleum ether and cooling. Recrystallization from ethyl acetate gave a first crop of 125 mg (21%) of product, mp 202–203°, one spot on tlc, $[\alpha]^{25}D$ −16.9° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{18}H_{21}O_2N_3$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.37; H, 6.73; N, 13.70

B. From Oxazolone.—To 2-phenyl-L-4-benzyloxazolone (1.04 g, 4 mmol) in 15 ml of anhydrous ether was added N,N-dimethylhydrazine (264 mg, 4.4 mmol) (freshly distilled under nitrogen) in 10 ml of ether. After 40 min crystals formed in the flask. After 6 hr (last hour in the refrigerator), 930 mg (75%) of product was filtered off, $[\alpha]^{25}D$ 0° (*c* 1, dimethylformamide). The filtrate had no optical activity. The thin layer chromatogram had one spot which corresponded to the pure product from the azide reaction, and the infrared spectra of the two samples were the same. One recrystallization from ethyl acetate and two from chloroform gave the analytical sample, mp 170–171°.

Anal. Calcd for $C_{18}H_{21}O_2N_3$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.33; H, 6.80; N, 13.42.

Three similar runs were made with freshly distilled $CHCl_3$ (from $CaCl_2$): a 10% excess of dimethylhydrazine was used at 25°; a 10% excess of dimethylhydrazine was used at 0°; and an 8:1 ratio of dimethylhydrazine to oxazolone was used at 25°. All three runs gave the same clearly identifiable product, but in each case with complete racemization.

Benzoylphenylalanine *p*-Nitrophenylhydrazide. A. Attempted Preparation from Azide.—The major product isolated was a yellow, crystalline material, mp 210–212°. The melting point, thin layer chromatogram, infrared spectrum, and nuclear magnetic resonance spectrum did not correspond to material obtained from the oxazolone, mixed anhydride, or indirect approaches. Furthermore, the analysis obtained did not correspond to the desired product, $[\alpha]^{25}D$ −24.3° (*c* 1.5, dimethylformamide).

Anal. Calcd for $C_{22}H_{20}O_4N_4$: C, 65.32; H, 4.99; N, 13.85. Found: C, 63.03; H, 5.19; N, 16.55.

One possibility is the rearrangement of azide to isocyanate, followed by reaction with *p*-nitrophenylhydrazine.

Anal. Calcd for $C_{22}H_{21}N_5O_4$: C, 62.99; H, 5.05; N, 16.69.

B. From Oxazolone.—A solution of *p*-nitrophenylhydrazine (336 mg, 2.2 mmol) in 8 ml of dry tetrahydrofuran was added to 2-phenyl-L-4-benzyloxazolone (502 mg, 2.0 mmol) in 2 ml of tetrahydrofuran at 25°. After 6 hr in the dark, precipitation of product was noted. After 48 hr, the solution was cooled and filtered, giving 435 mg (54%) of a crude yellow solid, $[\alpha]^{25}D$ 0° (*c* 1.5, dimethylformamide). The mother liquor had no optical activity. Two recrystallizations from ethanol gave an analytical sample, mp 226–235°.

Anal. Calcd for $C_{22}H_{20}O_4N_4$: C, 65.32; H, 4.99; N, 13.85. Found: C, 65.43; H, 5.06; N, 13.90.

C. Indirect Route. Procedure 1. Benzyloxycarbonyl-L-phenylalanine *p*-Nitrophenylhydrazide.—A crude product in 71% yield was obtained *via* the dicyclohexylcarbodiimide method. Recrystallization from ethanol gave a first crop of chromatographically pure material in 53% yield, mp 194–197°, $[\alpha]^{25}D$ −8.3° (*c* 2, dimethylformamide).

Procedure 2. L-Phenylalanine *p*-Nitrophenylhydrazide Hydrobromide.—To benzyloxycarbonyl-L-phenylalanine *p*-nitrophenylhydrazide (2.3 g, 5.6 mmol) was added 15 ml of a dry, saturated solution of hydrogen bromide in glacial acetic acid. The mixture was stirred for 3 hr and then added slowly with stirring to 300 ml of cold, anhydrous ether. Crude product (1.75 mg, 85%) was filtered off. Recrystallization from methanol–ether gave a first crop of 1.30 g (63%), $[\alpha]^{25}D$ +48.5° (*c* 1, dimethylformamide).

This material was used directly in the next step without further characterization.

Procedure 3. Benzoylation of L-Phenylalanine *p*-Nitrophenylhydrazide.—To L-phenylalanine *p*-nitrophenylhydrazide hydrobromide (860 mg, 2.35 mmol) in 15 ml of pyridine and 8 ml of dimethylformamide at 0°, benzoyl chloride (316 mg, 2.47 mmol) in 5 ml of ether was added dropwise over 15 min. After 1 hr at 0° and 2 hr at room temperature, the solution was added to 450 ml of 1 *N* hydrochloric acid in the cold. Crude product (560 mg, 63%) was obtained after filtration and washing with water and ether. Recrystallization from ethanol gave a first crop of 300 mg (34%), mp 234–238°, $[\alpha]^{25}D$ −56.8° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{22}H_{20}O_4N_4$: C, 65.32; H, 4.99; N, 13.85. Found: C, 65.16; H, 4.93; N, 13.97.

Benzoyl-L-phenylalanine *o*-Methoxyphenylhydrazide. A. From *o*-Methoxyphenylhydrazine.—*o*-Methoxyphenylhydrazine hydrochloride was added to a saturated sodium bicarbonate solution and the solution was extracted many times with ether. The ether solution was dried over $MgSO_4$ and filtered and the solvent was removed. The hydrazine was distilled at 110° (0.5 mm).

The product solidified rapidly at room temperature and could be handled as a solid, mp 43–44°. This material was used in subsequent reactions.

B. From Oxazolone.—*o*-Methoxyphenylhydrazine (304 mg, 2.2 mmol) in 10 ml of ether was added to 2-phenyl-L-4-benzyloxazolone (502 mg, 2.0 mmol) in 10 ml of ether at 25°. Precipitation of product began within 5 min. After 4 hr, 710 mg (91%) of material was filtered off. No additional material was obtained on evaporation. Thin layer chromatography indicated a single product, $[\alpha]^{25}D$ −43.8° (*c* 1, tetrahydrofuran). Recrystallization from ethyl acetate–hexane gave an analytical sample, mp 186–189°.

Anal. Calcd for $C_{23}H_{23}O_3N_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.85; H, 5.91; N, 10.78.

C. From Azide.—The general procedure described in part A for benzoyl phenylalanine phenylhydrazide was employed. Crude product in 36% yield was obtained by filtration of the reaction mixture after stirring overnight at room temperature. Recrystallization from ethyl acetate–hexane gave pure material in 26% yield, mp 187–188.5°, $[\alpha]^{25}D$ −73.9° (*c* 1, tetrahydrofuran).

Anal. Calcd for $C_{23}H_{23}O_3N_3$: C, 70.85; H, 5.91; N, 10.78. Found: C, 70.97; H, 5.90; N, 10.89.

D. Indirect Method. Procedure 1. Benzyloxycarbonyl-L-phenylalanine *o*-Methoxyphenylhydrazide.—Crude material was obtained in 68% yield by the dicyclohexylcarbodiimide method. Recrystallization from ethyl acetate–hexane gave a pure product, mp 162–165°, $[\alpha]^{25}D$ −22.1° (*c* 2, dimethylformamide). This material was used directly in the next reaction.

Procedure 2. L-Phenylalanine *o*-Methoxyphenylhydrazide Hydrobromide.—The crude material was obtained in 91% yield on treatment of the benzyloxycarbonyl compound with hydrogen bromide in acetic acid. Recrystallization from ethanol–ether gave a first crop in 52% yield, $[\alpha]^{25}D$ −64.7° (*c* 1, dimethylformamide). No attempt was made to recover a second crop. This material was used directly in the next step.

Procedure 3. Benzoylation of L-Phenylalanine *o*-Methoxyphenylhydrazide.—Treatment with benzoyl chloride in a pyridine dimethylformamide mixture gave product in 22% yield after recrystallization from ethanol, mp 185–187°, $[\alpha]^{25}D$ −72.3° (*c* 1, tetrahydrofuran.)

Anal. Calcd for $C_{23}H_{23}O_3N_3$: C, 70.85; H, 5.91; N, 10.78. Found: C, 71.00; H, 5.97; N, 10.84.

Benzoylphenylalanine Hydroxamic Acid.—The procedure of Hurd[39] was used for the preparation of free hydroxylamine. The product was stored under butanol–ether in the refrigerator until use. The material was rapidly filtered, weighed, and dissolved in anhydrous methanol. Aliquot portions were taken from the methanolic solution for each reaction.

A. From Oxazolone.—A solution of hydroxylamine (330 mg, 10.0 mmol) in 10 ml of anhydrous methanol was added to 2-

(39) C. D. Hurd, "Inorganic Syntheses," Vol. 1, McGraw-Hill Book Co., Inc., New York, N. Y., 1939, p 87.

phenyl-L-4-benzyloxazolone (1.0 g, 4.0 mmol) in 20 ml of anhydrous methanol at 0°. After stirring for 12 hr, the solvent was removed in a cold nitrogen stream. A solid product weighing 1.05 g (93%) showing one spot in the thin layer chromatogram was obtained.

Following the reaction with thin layer chromatography indicated that two products had formed initially in a rapid reaction. The material with the greater R_f value in the elution medium (*i.e.*, chloroform) disappeared with time, $[\alpha]^{25}D$ −39.8° (*c* 1, dimethylformamide).

The material was recrystallized from ethyl acetate to give an analytical sample, mp 146–154°.

Anal. Calcd for $C_{16}H_{16}O_3N_2$: C, 67.59; H, 5.67; N, 9.86. Found: C, 67.28; H, 5.47; N, 9.50.

B. From Azide.—The azide reaction was carried out on benzoyl-L-phenylalanine hydrazide (1.1 g, 4 mmol) as previously described in part A for benzoylphenylalanine phenylhydrazide. This time, however, the solid azide product was filtered rapidly, washed with cold saturated potassium chloride solution, and quickly dissolved in 20 ml of cold methylene chloride. After drying over $MgSO_4$, the cold solution was filtered quickly and added to a solution of hydroxylamine (330 mg, 10 mmol) in 20 ml of methanol at 0°. Work-up as previously described gave a crude mixture of several components. The product was obtained in 10% yield on fractionation from chloroform–hexane and ether–petroleum ether. Thin layer chromatography and the infrared spectrum corresponded to material prepared from oxazolone, mp 147–154°, $[\alpha]^{25}D$ −39.7° (*c* 0.5, dimethylformamide).

C. Attempted Preparation from Benzoyl-L-phenylalanine Methyl Ester.—Benzoyl-L-phenylalanine methyl ester (566 mg, 2 mmol) was dissolved in a solution of hydroxylamine (330 mg, 10 mmol) in 20 ml of methanol at 0°. The solution was allowed to stand for 24 hr at 0° and 24 hr at room temperature. Evaporation of the product gave a white solid, which was readily identified as unreacted ester.

Benzoylphenylalanine N-Hydroxypiperidine Ester. A. From Oxazolone.—N-Hydroxypiperidine (202 mg, 2.2 mmol) in 5 ml of anhydrous ether was added to 2-phenyl-L-4-benzyloxazolone (502 mg, 2 mmol) in 10 ml of dry ether at 0°. Precipitation of product began in 3 min. After 2.5 hr at 0°, 620 mg (88.5%) of product was filtered off: $[\alpha]^{25}D$ −25.7° (*c* 2, dimethylformamide). Tlc of the mother liquor did not show additional product. Recrystallization from ether–hexane gave an analytical sample, mp 115–125°.

Anal. Calcd for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.56; H, 6.97; N, 7.88.

B. Determination of Racemization of the Piperidine Active Ester by Conversion into Hydrazide.—To 500 mg of benzoyl phenylalanine N-hydroxypiperidine ester dissolved in 10 ml of boiling methanol, 1 ml of anhydrous hydrazine (large excess) was added. The solution was allowed to cool and stand for 24 hr. The solvent was removed in a stream of nitrogen, and the process was repeated with methanol and then with chloroform. Finally, ether was added and 380 mg of crude product was filtered off (94.7%). Infrared analysis and thin layer chromatography indicated the presence of pure hydrazide. The material was thoroughly dried in a vacuum desiccator over P_2O_5 and H_2SO_4 to remove any traces of hydrazine, $[\alpha]D$ −43.5° (*c* 1, dimethylformamide).

Subsequently, the hydrazine treatment was repeated by dissolving the piperidyl ester in tetrahydrofuran at 0° and adding the hydrazine at 0° in methanol. The product obtained on work-up had the same optical activity as above.

The reaction was repeated twice with a 10% excess of N-hydroxypiperidine in ether at 25° and in tetrahydrofuran at 0°. Similar work-up and conversion of the active ester into benzoyl phenylalanine hydrazide at 0° gave a product, $[\alpha]^{25}D$ −42.0° (*c* 1, dimethylformamide) for the ether reaction and $[\alpha]^{25}D$ −41.6° (*c* 1, dimethylformamide) for the tetrahydrofuran reaction.

O-(Benzoylphenylalanyl)-N,N-diethylhydroxylamine.—Freshly distilled N,N-diethylhydroxylamine was used in these experiments, bp 40–41° (10 mm).

To 2-phenyl-L-4-benzyloxazolone (502 mg, 2 mmol) in 15 ml of anhydrous ether at 0° was added N,N-diethylhydroxylamine (196 mg, 2.2 mmol) in 5 ml of ether. After 2 hr the solvent was removed under reduced pressure, washed with petroleum ether several times, filtered, washed with water, and dried over P_2O_5 *in vacuo* to give 625 mg (92%) of chromatographically homogeneous material, mp 109–113.5°, $[\alpha]^{25}D$ −21.8° (*c* 1.5, tetrahydrofuran). Recrystallization from ethyl acetate–hexane gave the analytical sample.

Anal. Calcd for $C_{20}H_{24}O_3N_2$: C, 70.56; H, 7.11; N, 8.23. Found: C, 70.23; H, 6.74; N, 8.03.

To determine the racemization in the ring-opening reaction, the crude active ester (before recrystallization) was converted into the hydrazide as follows.

To active ester (235 mg, 0.95 mmol) in 5 ml of tetrahydrofuran at 0°, 1 ml of anhydrous hydrazine (large excess) in 5 ml of methanol at 0° was added. Standard work-up afforded 225 mg (79%) of material showing one spot in a thin layer chromatogram. The infrared spectrum confirmed the presence of benzoylphenylalanine hydrazide, $[\alpha]^{25}D$ −26.4° (*c* 1.5, dimethylformamide).

The above reaction was repeated at 25° for 3 hr. Work-up gave 85% active ester, which on reaction with hydrazine gave benzoylphenylalanine hydrazide in 93% yield (based on active ester used), $[\alpha]^{25}D$ −20.1° (*c* 1.5, dimethylformamide).

Repetition of the reaction at 0° for 2 hr and at 25° for 1 hr more gave an 88% yield of active ester, which on reaction with hydrazine gave benzoyl phenylalanine hydrazide in 90% yield (based on active ester used), $[\alpha]^{25}D$ −26.7° (*c* 1.5, dimethylformamide).

Racemization of N,N-Diethylhydroxylamine. Active Ester. A.—A solution of 0.0745 *M* in the active ester of benzoyl-L-phenylalanine and 0.0475 *M* in triethylamine racemized to the extent of 13.5% after 40 hr in tetrahydrofuran at 25°.

B.—A solution 0.0685 *M* in the active ester of benzyloxycarbonyl-L-phenylalanine and 0.0475 *M* in triethylamine under the same conditions as above racemized to the extent of 12.4%.

The racemization in both cases is probably due to direct proton abstraction rather than oxazolone formation. If the latter were the case, the benzoyl derivative should racemize much more readily.

N-Methyl-N-(benzoylphenylalanyl)-O-methylhydroxylamine.—N,O-Dimethylhydroxylamine was prepared by the method of Bissot[40] from the hydrochloride salt.

A. From Oxazolone.—A solution of 2-phenyl-L-benzyloxazolone (502 mg, 2.0 mmol) and N,O-dimethylhydroxylamine (134 mg, 2.2 mmol) in 15 ml of ether at room temperature was allowed to react for 4 hr. Oxazolone could still be noted in the thin layer chromatogram. Cooling of the solution to 0° and filtration gave 520 mg (83.8%) of product, mp 113–114.5°, $[\alpha]^{25}D$ −2.41° (*c* 2.5, tetrahydrofuran). Recrystallization from ether gave an analytical sample, mp 114–115°.

Anal. Calcd for $C_{18}H_{20}O_3N_2$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.64; H, 6.22; N, 9.24.

B. Indirect Procedure.—The product was prepared as described above (part C for benzoylphenylalanine *p*-nitrophenylhydrazide) in 24% overall yield, mp 108.5–111.5°, $[\alpha]^{25}D$ −5.38° (*c* 1.0, tetrahydrofuran).

Anal. Calcd for $C_{18}H_{20}O_3N_2$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.96; H, 6.30; N, 8.47.

Benzoylphenylalanine N-Hydroxysuccinimide Ester. A. From 2-Phenyl-L-4-benzyloxazolone. Procedure 1.—To a solution of 2-phenyl-L-4-benzyloxazolone (502 mg, 2 mmol) in 4 ml of tetrahydrofuran, N-hydroxysuccinimide (253 mg, 2.2 mmol) in 4 ml of tetrahydrofuran was added in one portion and the reaction was allowed to stir for 3 hr at room temperature. The solvent was evaporated at room temperature and 50 ml of water was added to dissolve unreacted N-hydroxysuccinimide. Crude product (530 mg, 73%), showing one spot in thin layer chromatography, was obtained after filtration and washing with cold ether (to remove oxazolone), $[\alpha]^{25}D$ −52.2° (*c* 1.5, tetrahydrofuran).

Procedure 2.—The above reaction was repeated at 0°. After identical work-up, 600 mg (82%) of product was obtained, $[\alpha]^{25}D$ −53.1° (*c* 1.5, tetrahydrofuran).

Procedure 3.—2-Phenyl-L-4-benzyloxazolone (1.0 g, 4 mmol) and N-hydroxysuccinimide (900 mg, 7.8 mmol) were stirred in 10 ml of tetrahydrofuran at 0° for 2 hr and at room temperature for an additional 2 hr. This work-up yielded 1.25 g (86%) of crude material, $[\alpha]^{25}D$ −53.4° (*c* 2, tetrahydrofuran). Recrystallization twice from chloroform gave an analytical sample, mp 164–165°, $[\alpha]^{25}D$ −54.3° (*c* 2, tetrahydrofuran).

Anal. Calcd for $C_{20}H_{18}O_5N_2$: C, 65.52; H, 4.92; N, 7.65. Found: C, 65.41; H, 5.11; N, 7.64.

B. Determination of Optical Purity of Succinimide Active

(40) T. C. Bissot, R. W. Parry, and D. H. Campbell, *J. Amer. Chem. Soc.*, **79**, 796 (1957).

Ester.—To a solution of benzoylphenylalanine N-hydroxysuccinimide ester (240 mg, 0.66 mmol) in 20 ml of tetrahydrofuran at 0°, hydrazine hydrate (150 mg, 3 mmol) in 5 ml of methanol was added at 0°. Precipitation of product was noted immediately. After 2 hr, the solvent was removed under a stream of nitrogen at room temperature. After addition of water to remove N-hydroxysuccinimide, filtration, and drying over P_2O_5, 160 mg (85%) of chromatographically pure paterial was obtained, $[\alpha]^{25}D$ −45.7° (*c* 1, dimethylformamide).

C. Racemization by Methanol.—Benzoyl-L-phenylalanine succinimide ester (250 mg) was boiled in 20 ml of methanol for 20 min. Evaporation of solvent gave completely racemized starting material as determined by infrared spectroscopy thin layer chromatography, and polarimetry. No methanolysis took place under these conditions.

Benzyloxycarbonyl-L-phenylalanine Succinimide Ester.—The crude product was obtained in 68% yield by the dicyclohexylcarbodiimide method. Recrystallization from ethyl acetate–hexane gave a first crop of 1.8 g (45%) of crystalline material, mp 136–138.5°, $[\alpha]^{25}D$ −21.5° (*c* 1.5, tetrahydrofuran).

Anal. Calcd for $C_{21}H_{20}O_6N_2$: C, 63.63; H, 5.08; N, 7.07. Found: C, 63.53; H, 5.33; N, 6.93.

Racemization by Methanol.—The succinimide active ester (250 mg) was boiled for 20 min in methanol and then the solvent was removed at room temperature. The product was washed with hexane and filtered. Infrared and thin layer analyses showed no methanolysis, $[\alpha]^{25}D$ −19.4° (*c* 1.5, tetrahydrofuran).

Instruments and Apparatus.—All measurements of optical activity were made on a Model 80 Rudolph polarimeter equipped with a Model 200A oscillating polarizer. Monochromatic light was obtained by a prism monochromator equipped with an independent Xenon light source (Hanovia 901B). Center-fill, 2-dm polarimeter tubes with a bore of 3 mm in diameter were used (Polarimeter tube type 14, catalogs of O. C. Rudolph and Sons, Caldwell, N. J.). The temperature of the tube compartment was kept constant at 25 ± 0.2° by a circulating pump connected to a constant-temperature bath. The voltage applied to the photoelectric cell was controlled by a Keithley Voltage Supply Model 240.

High-resolution nuclear magnetic resonance spectral measurements were made with the Cary A-60 megacycle instrument at room temperature and resonances are expressed in units relative to tetramethylsilane as an internal standard.

Infrared spectra were determined with a Perkin-Elmer Model 132, 21, or 521 spectrophotometer from Nujol mulls or potassium bromide pellets.

Registry No.—2-Phenyl-L-4-benzyloxazolone, 5874-61-3; benzoyl-L-phenylalanine methyl ester, 3005-61-6; benzoyl-L-phenylalanine hydrazide, 23912-50-7; benzoylphenylalanine *t*-butyloxycarbonyl hydrazide, 23912-51-8; benzoylphenylalanine phenylhydrazide, 23912-53-0; benzoylphenylalanine dimethylhydrazide, 23912-54-1; benzoylphenylalanine *p*-nitrophenylhydrazide, 23912-55-2; benzoyloxycarbonyl-L-phenylalanine *p*-nitrophenylhydrazide, 23912-56-3; L-phenylalanine *p*-nitrophenylhydrazide hydrobromide, 23912-57-4; benzoyl-L-phenylalanine *o*-methoxyphenylhydrazide, 23912-58-5; benzoyloxycarbonyl-L-phenylalanine *o*-methoxyphenylhydrazide, 23912-59-6; L-phenylalanine *o*-methoxyphenylhydrazide hydrobromide, 23912-60-9; benzoylphenylalanine hydroxamic acid, 23912-61-0; benzoylphenylalanine N-hydroxypiperidine ester, 23967-35-3; *o*-(benzoylphenylalanyl)-N,N-diethylhydroxylamine, 23912-62-1; N-methyl-N-(benzoylphenylalanyl)-O-methylhydroxylamine, 23912-63-2; benzoylphenylalanine N-hydroxysuccinimide ester, 23912-64-3; benzyloxycarbonyl-L-phenylalanine succinimide ester, 3397-32-8.

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Reaction of Aldehydes with N-Hydroxybenzenesulfonamide. Acetal Formation Catalyzed by Nucleophiles

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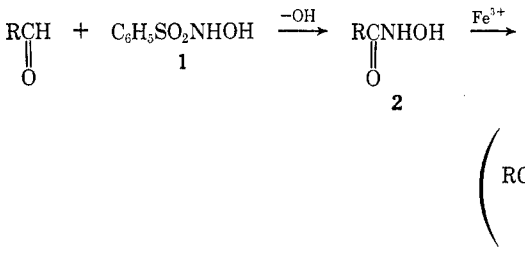
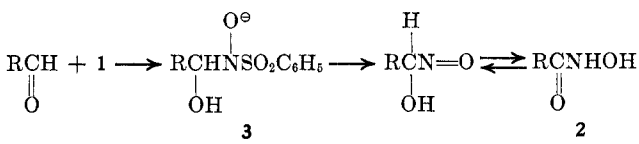
The reaction of N-hydroxybenzenesulfonamide (**1**) with aldehydes was studied. In the presence of strong base, hydroxamic acids are formed. In methanol in the absence of base, rapid acid catalysis by **1** takes place, leading to dimethyl acetals. In this manner acetal formation or hydrolysis can be catalyzed by the mild acids **1** or its O-benzyl ether **6**. Treatment of **1** or **6** with base does not appear to furnish nitrenes, as indicated by lack of reaction with olefins.

The reaction of aldehydes with N-hydroxybenzenesulfonamide (**1**) under basic conditions constitutes the basis for a well-known spot test used in the qualitative identification of aldehydes.[1] This test, known as the Angeli–Rimini test, involves the formation of a hydroxamic acid **2** which forms characteristically colored complexes with ferric ions.[2]



purple color

A proposed mechanism for hydroxamic acid formation involves the following scheme.[3] Alternatively, 1,2 elimination of benzenesulfinic acid from **3** would lead to **2**.



Since α-elimination reactions have been used to generate nitrenes,[4] we considered the possibility that the

(1) F. Feigl, "Spot Tests In Organic Chemistry," 2nd ed, Elsevier Publishing Co., New York, N. Y., 1966, p 196.

(2) A. Angeli, *Gazz. Chim. Ital.*, **26** (II), 17 (1896); E. Rimini, *ibid.*, **31** (II), 84 (1901).

(3) P. A. S. Smith and G. E. Hein, *J. Amer. Chem. Soc.*, **82**, 5732 (1960).

(4) W. Lwowski, *Angew. Chem., Int. Ed. Engl.*, **6**, 897 (1967); D. Carr, T. P. Seden, and R. W. Turner, *Tetrahedron Lett.*, 477 (1969).