produced a linear plot, did it emerge that a structure other than **3** would be required. Stereochemical considerations[9] then led to the postulation of **5a** and **5b** and to the test of these against the proton inventory as shown in Figure 1. Postulations of catalytic interactions stereochemically equivalent to that in **3** have not been uncommon although transition state structures have not been established for any such case, simplicity apparently having been generally the guiding principle. It is not yet clear whether the nonlinear catalytic bridges which would be required in such structures are truly "forbidden" or are sufficiently accessible in energy that other factors could compensate for their inclusion. The proton-inventory technique may on occasion serve to discriminate among these possibilities. It should be noted that both pathways (*via* **5a** and **5b**) are probably catalytic routes, **5b** having a solvation bridge[5] and **5a** enjoying intramolecular electrostatic stabilization.

Experimental Section

Materials. Sodium acetate and acetic acid were purified[12] and stored in a desiccator until needed. Potassium chloride, Analytical Grade, was dried in an oven and stored in a desiccator until needed. Acetyl-3,5-dinitrosalicylic acid was prepared according to the previous procedure.[4] Purification was accomplished by low-temperature recrystallization from ether and gave material with mp 92.5–94.0° (lit.[4] 93–94°).

Anal. Calcd for $C_9H_6N_2O$: C, 40.00; H, 2.22; N, 10.38. Found: C, 39.73; H, 2.21; N, 10.27.

Buffer Solutions. Solvents were prepared from distilled water which had been passed through a mixed-bed ion-exchange column and 99.8% deuterium oxide (Diaprep Corp.). Mixed isotopic solvents were prepared gravimetrically. Addition of buffer components, potassium chloride, and acetyl-3,5-dinitrosalicylic acid had a negligible effect (<0.2%) on the mole fraction of deuterium in the mixed solvents. The atom fraction of deuterium in the "pure" deuterium oxide was determined by an nmr technique in which the integral of the water protium signal was compared to the integral of a protium signal from dioxane in known concentration. The solutions were prepared volumetrically and all components of every buffer solution determined gravimetrically. The pH(D) was measured with a pH meter as a check.

Kinetics. The release of 3,5-dinitrosalicylate ion at 337 nm was followed with a Cary 16 ultraviolet–visible spectrophotometer equipped with a constant temperature apparatus. The reaction was initiated by injecting 100 μl of a stock solution of dinitroaspirin in the appropriate solvent mixture into 3.00 ml of the thermally equilibrated buffer solution in a 3-ml quartz cuvette. The cuvettes were kept in the thermostated bath and briefly (<50 sec) removed for null determination of the absorbance in the thermostated cell compartment. At least 20 points were determined for each run and the rate for each buffer concentration was measured in duplicate. The reaction was followed for at least three half-lives and the first-order rate constants were calculated by a nonlinear least-squares computer program from given time and absorbance values. Internal standard deviations of the rate constants (within one run) were consistently less than ±0.8%.

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Rigid Active Esters in Peptide Synthesis[1]

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Abstract: The rates of aminolysis of *o*-nitrophenyl esters of protected amino acids are higher, less solvent dependent, and less sensitive to steric hindrance than the aminolysis rates of the corresponding *p*-nitrophenyl esters. The higher reactivity of the ortho isomers can be readily explained by the electron-withdrawing effect of the nitro group, which is more efficient in the ortho than in the para position. The differences observed in the influence of solvents and of steric hindrance were traced to an intramolecular interaction between the nitro group and the amide group in *o*-nitrophenyl esters. The resulting rigid, cyclic conformation is revealed in the high optical activity and a pronounced effect of temperature on the rotation of a series of *o*-nitrophenyl esters.

At the time of the introduction of nitrophenyl esters[3] as reactive intermediates in peptide synthesis, the esters of *o*-, *m*-, and *p*-nitrophenol and also of 2,4-dinitrophenol were examined. The choice of *p*-nitrophenyl esters as practical reagents was dictated by their relative readiness to crystallize. The more reactive 2,4-dinitrophenyl esters were not favored because of their sensitivity to hydrolysis. Many years later, when the application of active esters in solid-phase peptide synthesis was studied,[4,5] *p*-nitrophenyl esters were found, contrary to earlier, preliminary observations of Merrifield,[6] useful in the preparation of peptides on a solid support. Nevertheless, only the *p*-nitrophenyl esters of (protected) asparagine and glutamine were generally accepted for this purpose, because by acylation with the purified active esters dehydration of the side-chain carboxamides[7] can be avoided. The reluctance toward adopting active esters of other amino acids is probably due to the moderate reaction rates obtainable with *p*-nitrophenyl esters: rates that lag considerably behind those of coupling with dicyclohexylcarbodiimide.[8] A seemingly easy solution to this difficulty would be the use of more reactive esters such as penta-

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chlorophenyl esters.[9] Yet, their high reactivity determined in solution was not evident when pentachlorophenyl esters were applied for the acylation of a polymer-bound nucleophile.[10] Obviously, the higher reactivity of pentachlorophenyl esters is more than counterbalanced by the bulkiness of the electron-withdrawing group, the pentasubstituted nucleus. In the course of investigations aimed at finding active esters more suitable for solid-phase peptide synthesis, the unexpected observation was made that *o*-nitrophenyl esters behave differently from their widely used para isomers. With *o*-nitrophenyl esters, not only higher rates of aminolysis were noted[10] but also a lesser dependence of these rates on the nature of the solvent.[11] Finally, *o*-nitrophenyl esters retain more of their reactivity under hindered conditions.[10-12] An attempt is being made to exploit these advantages and to employ *o*-nitrophenyl esters for the acylation of resin-bound peptides[12] and of peptides in solution, including an expedient new approach, the *in situ* technique.[13,14] The practical application of *o*-nitrophenyl esters left the questions about their character, so different from that of the *p*-nitrophenyl esters, unanswered. We are reporting here the results of a series of experiments that were carried out to explore the reasons for these differences.

Reactivity in Aminolysis. The fundamental studies of Pless and Boissonnas[15] on the aminolysis rates of different substituted aryl esters pointed to a direct correlation between their reactivity and the acidity of their phenol components. Exceptions were found when bulky substituents, ortho to the phenolic hydroxyl, were present, especially when both the 2 and 6 positions were substituted; in these cases, steric hindrance of the reaction became evident. Since the dissociation constants of *o*-nitrophenol and *p*-nitrophenol are practically identical (pK in H_2O, 7.17 and 7.15, respectively[16]), equal electron-withdrawing effects and hence about equal enhancement of the electrophilic character of the carbonyl carbon should be expected in *o*- and *p*-nitrophenyl esters. Yet, not only different aminolysis rates were determined,[10] but the stronger negative inductive effect of the ester component was evident in the ir and nmr spectra of the members of the ortho series. The carbonyl stretching frequencies in *p*-nitrophenyl esters range from 1772 to 1782 cm^{-1}, and from 1780 to 1788 cm^{-1} in *o*-nitrophenyl esters (Table I). In the nmr spectra the electron-withdrawing effect of the nitrophenyl group causes deshielding of protons on the α-, β-, and even γ-carbon atoms of active esters of protected amino acids. This deshielding is stronger in the ortho than in the para series and consequently the signals of these protons of *o*-nitrophenyl esters appear further downfield than corresponding signals in the para-substituted series (Table II).

Table I. Stretching Frequencies in the Ir Spectra of Nitrophenyl Esters[a]

Active ester of	CO active —NH— ONO	—NH— ONP	—ester— ONO	—ester— ONP	—NO_2— ONO	—NO_2— ONP
Boc-Gly	3454	3458	1788	1782	1349	1350
Boc-Ala	3450	3452	1786	1778	1352	1350
Z-Ala	3450	3450	1786	1780	1353	1350
Boc-Val	3455	3451	1782	1772	1353	1350
Z-Val	3452	3449	1780	1773	1353	1350
Boc-Leu	3452	3452	1784	1776	1353	1351
Z-Met	3441	3442	1783	1773	1350	1349
Boc-Phe	3450	3448	1782	1774	1352	1350
Boc-Tyr (Bzl)	3452	3450	1782	1772	1351	1349

[a] In cm^{-1}.

Table II. Chemical Shifts (in ppm from TMS) in the Nmr Spectra of Active Esters

Active ester of	N-H ONO	N-H ONP	α-CH ONO	α-CH ONP
Boc-Gly	5.20	5.28	4.27	4.20
Z-Gly	5.48	5.52	4.32	4.23
Boc-Ala	5.11	5.15	4.57	4.52
Z-Ala	5.43	5.45	4.68	4.62
Z-Val	5.62	5.35	4.58	4.51
Boc-Leu	4.99	5.02	4.59	4.55
Z-Leu	5.16	5.22	4.65	4.60
Z-Ile	5.59	5.30	4.61	4.57
Boc-Phe	4.92	5.08	4.80	4.78
Z-Met	5.60	5.62	4.85	4.78

The obvious explanation for these differences is the known fact (*cf.*, *e.g.*, ref 17) that substituents at the ortho position exert a larger electronic effect on the phenolic hydroxyl than para substituents. Accordingly, a more pronounced enhancement of the electrophilic character of the carbonyl carbon should be found in *o*- than in *p*-nitrophenyl esters. That indeed this is the case is shown by the markedly higher aminolysis rates in the ortho series. The cause of the discrepancy with expectations based on the almost equal acidity of the two phenols lies in the intramolecular hydrogen bond in *o*-nitrophenol. This bond, by reducing the availability of the phenolic proton, renders the phenol less acidic and by coincidence the decrease in acidity compensates the enhancement of the negative effect due to the ortho position. In the esters, however, no such compensation is present and the *o*-nitrophenyl group is a more efficient activator. Simply, the acidity constants of the phenols cannot be applied for the estimation of activation when esters of *o*- and *p*-nitrophenol are considered.

Since the ir and nmr spectra provide evidence for higher activation in *o*-nitrophenyl esters than in *p*-nitrophenyl esters in the absence of nucleophiles, it seems to be unwarranted to invoke alternative explanations, such as anchimeric acceleration,[18] for the difference in the aminolysis rates observed in the two series.

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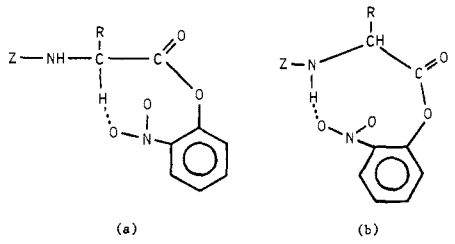

Figure 1. Possible hydrogen bonded cyclic structures of *o*-nitrophenyl ester of protected amino acids.

Solvent Effects. The marked influence of solvent on the rates of aminolysis of active esters is well known (*cf.*, *e.g*, ref 15). Still, it was intriguing to note that while the solvent effects are qualitatively similar in *o*- and in *p*-nitrophenyl esters, they are significantly less pronounced in the ortho isomers.[11] The assumption that the nitro group interacts with the solvents in *p*-nitrophenyl esters, but because of some intramolecular involvement is less able to do so in the ortho series, prompted an investigation into the nature of this involvement. An inspection of molecular models offers the obvious explanation that the close proximity of the nitro group to the active ester grouping prevents, at least on one side of the nitro group, close contact with solvent molecules. An alternative possibility is intramolecular hydrogen bonding in which the *o*-nitro group can participate while the *p*-nitro group is unable to do so. In the two cyclic structures shown in Figure 1, one (a) involves the hydrogen on the α carbon, the second (b) the hydrogen atom of the amide group. The α carbon could play the role of atoms with greater electron affinity, such as O, N, or F, because it is under the influence of considerable electron-attracting forces. Therefore it is not impossible to assign to it the function of donor in a hydrogen bond. In fact, a hydrogen-bonded cyclic structure similar to the one shown in Figure 1a was postulated for guaiacyl esters by Bankowski and Drabarek[19] and was supported by racemization studies. A brief study of the racemization of *o*-nitrophenyl esters revealed no analogy with guaiacyl esters in this respect. Addition of *o*-nitrophenol did not reduce racemization in the coupling of acetyl-L-isoleucine[20] to glycine ethyl ester with dicyclohexylcarbodiimide as condensing agent. Also, the *o*-nitrophenyl ester of *N*-*tert*-butyloxycarbonyl-*S*-benzyl-L-cysteine rapidly lost optical activity when exposed to the effect of triethylamine.[21] Furthermore, an inspection of space filling (Pauling–Corey–Koltun) models revealed that the eight-membered ring shown in Figure 1a, while possible, is unlikely. The model can be constructed only with some difficulty and the angle of the bonds connecting the bonding hydrogen atom with the bridgehead atoms, C, and O, differs considerably from the optimal 180°. It is easier to build a model of the molecule depicted in Figure 1b; the hydrogen bond in the nine-membered ring is closer to linear. In order to examine further the likelihood of structures such as shown in Figure 1, the influence of solvents on the aminolysis rates of active esters of N-protected proline was determined. Proline is an imino acid which does not have an amide hydrogen in its N-protected form. Thus, a hydrogen bond stabilized ring that would involve the nitro group of its *o*-nitrophenyl ester could correspond only to Figure 1a. The reaction of benzyloxycarbonyl-L-proline *o*-nitrophenyl ester with benzylamine was found to be faster than that of the corresponding *p*-nitrophenyl ester (*cf.* Experimental Section). However, both rates diminished to about the same extent when the solvent, ethyl acetate, was replaced by dichloromethane. The same change of solvents resulted in a drastic loss of reactivity in the *p*-nitrophenyl ester of benzyloxycarbonyl-L-leucine and in a more moderate decrease in its *o*-nitrophenyl ester. Thus an intramolecular involvement of the nitro group in the *o*-nitrophenyl ester of protected leucine is indeed indicated in the influence of solvents on the reaction rates, but this is less obvious in the corresponding derivative of proline. Therefore, the hydrogen-bonded structure that involves the amide nitrogen of acylamino acids (Figure 1b) could be a possible explanation for the solvent effects. However, the experiment with the proline derivatives is inconclusive, because the *individuality* of the amino acids leads to serious inconsistencies: *e.g.*, the *p*-nitrophenyl esters of the two protected amino acids do not react at the same rate, neither do the two *o*-nitrophenyl esters and, as a consequence, the solvent effect on the reaction rate of benzyloxycarbonyl-L-leucine *o*-nitophenyl ester is greater than the influence of solvents on the aminolysis rate of benzyloxycarbonyl-L-proline *p*-nitrophenyl ester. Therefore, while a comparison of solvent effects on two different esters of the *same amino acid* is justified, a similar comparison between derivatives of *different amino acids* may not be warranted. Because of these difficulties, the study of solvent effects on reaction rates was discontinued and more attention was given to the physical properties of the active esters in question.[22]

Infrared Spectra. The ir spectra of a series of *o*- and *p*-nitrophenyl esters were recorded (Table I) in solution (CCl_4) with the help of a high-resolution instrument. With respect to N–H stretching frequencies, no significant differences were found between the ortho- and para-substituted esters of the same protected amino acid; neither was a broadening of the N–H band observed in *o*-nitrophenyl esters.[23] Similarly, the spectra of such pairs revealed no change in the frequencies of the various modes of the NO_2 group. Furthermore, in the high-resolution spectra of pairs of active esters, the C–H stretching bands were superimposable. Hence, the assumption of hydrogen band stabilized ring structures shown in Figure 1 is not tenable.

Optical Activity. The values of the specific rotation of *o*-nitrophenyl esters of protected amino acids are significantly higher than the values determined for corresponding *p*-nitrophenyl esters (Table III). The derivatives of valine, isoleucine, and proline are notable

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(21) M. Bodanszky and C. A. Birkhimer, *Chimia*, **14**, 368 (1960).

(22) The weaker interaction with solvents of *o*-nitrophenyl esters (as compared with *p*-nitrophenyl esters) is indicated also in their solubilities. The *o*-nitrophenyl ester of *tert*-butyloxycarbonylglycine is six times less soluble in tetrachloromethane than the *p*-nitrophenyl ester of the same amino acid. In the corresponding derivatives of alanine, the solubilities are different by a factor of 3, and in the esters of phenylalanine by a factor of 2.

(23) The stretching frequencies of the NH bands in the ir spectra of the nitrophenyl esters of benzyloxycarbonyl-L-methionine and the slight broadening of these bands suggest hydrogen bonding to the sulfur atom.

Table III. Specific Rotations[a] of Nitrophenyl Esters of Protected Amino Acids

Active ester of	[α]^{25}D ONO	[α]^{25}D ONP	Active ester of	[α]^{25}D ONO	[α]^{25}D ONP
Boc-L-Ala	−79	−52.5	Z-L-Gln	−39	−24
Boc-L-Ala	−36.5[b]	−21[b]	Z-L-Ile	−16	−15.5
Boc-L-Asn	−55	−44	Z-L-Leu	−47	−33.5
Boc-L-Asp (Bzl)	−42	−36	Z-L-Phe	−63	−25
Boc-L-Cys (Bzl)	−75	−38	Z-L-Phe	−49[d]	−19[d]
Boc-L-Gln	−55	−35	Z-L-Phe	−53[e]	−19[e]
Boc-L-Leu	−68	−48	Z-L-Phe	−28[f]	−8[f]
Boc-L-Met	−73	−48	Z-L-Phe	−41[g]	−17[g]
Boc-L-Phe	−65	−21	Z-L-Pro	−71	−68
Boc-L-Phe	−30[c]	−13[b]	Z-L-Ser (Bzl)	−18	−12
Boc-L-Tyr (Bzl)	−51	−2	Z-L-Trp	−65	−4.5
Z-L-Asp (Bzl)	−32	−16.5	Z-L-Tyr (Bzl)	−55	−9
Z-L-Asn	−42	−31.5	Z-L-Val	−25	−25
Z-L-Cys (Bzl)	−105	−43			

[a] *c* 2, DMF containing 1% AcOH. [b] *c* 2, CCl_4. [c] *c* 0.6, CCl_4. [d] *c* 0.4, CH_3OH. [e] *c* 2, AcOH. [f] *c* 2, $CHCl_3$. [g] *c* 2, TFA.

exceptions. According to Kauzmann and Eyring,[24] "those influences which tend to restrict freedom of rotation and of orientation about bonds will tend to increase the order of magnitude of the optical activity." They also call attention to increased optical activity, when an asymmetric center is one of the atoms of a ring. The conspicuously high specific rotation of proline and proline-containing peptides demonstrates the validity of their statement for amino acids and their derivatives. Nevertheless, the higher specific rotation of *o*-nitrophenyl esters could be a consequence of a larger contribution to optical activity by the *o*-nitrophenyl than by the *p*-nitrophenyl group. Therefore, the assumption that the differences observed in the specific rotations are indeed due to some kind of conformational restriction present only in the ortho series was tested by determining the values of specific rotation at different temperatures (Figure 2). The high values measured at or below room temperature in *o*-nitrophenyl esters of protected alanine, leucine, and phenylalanine decrease considerably when the temperature is raised. In the para isomers, the change of temperature causes less change in rotation.

To allow more meaningful comparisons of temperature effects on optical activity in addition to the conventionally used temperature coefficient (tc), a new term, the relative temperature coefficient (rtc), was introduced. This term also corresponds to the change in rotation due to the change in temperature, but the change is expressed as per cent of the rotation determined at 0°. The expression rtc has a positive

$$\text{rtc} = \frac{(\alpha^{t_1} - \alpha^{t_2}) \cdot 100}{\alpha^{t_0}(t_1 - t_2)}$$

value when raising the temperature increases the absolute value of rotation, while a negative coefficient indicates decreased optical activity at higher temperatures. An advantage of the relative term is its independence from precision in determining the concentration of the solutions and therefore from the optical purity of the compounds examined.

The rtc values determined for the *o*- and *p*-nitrophenyl esters of benzyloxycarbonyl-L-leucine, or *tert*-butyloxycarbonyl-L-alanine (Table IV), suggest a more rigid conformation in the ortho than in the para isomers. At higher temperatures, some conformational restriction that results in high optical activity is relaxed; there is more rotation around bonds and therefore a closer to statistical distribution of rotamers.

The rigidity in the molecules of these *o*-nitrophenyl esters is caused by the electronic structure of the nitro group, and not by its bulk. This is clearly demonstrated by the nearly identical rtc values of the *o*- and *p*-cresyl esters and also of the phenyl ester of *tert*-butyloxycarbonyl-L-alanine. Thus a methyl substituent in the aryl ester group does not interfere with the conformational freedom of the molecule, not even when it is ortho to the ester grouping. On the other hand, the *o*-nitrophenyl ester of the same protected amino acid has an rtc value several times higher than its

(24) W. Kauzmann and H. Eyring, *J. Chem. Phys.*, 9, 41 (1941).

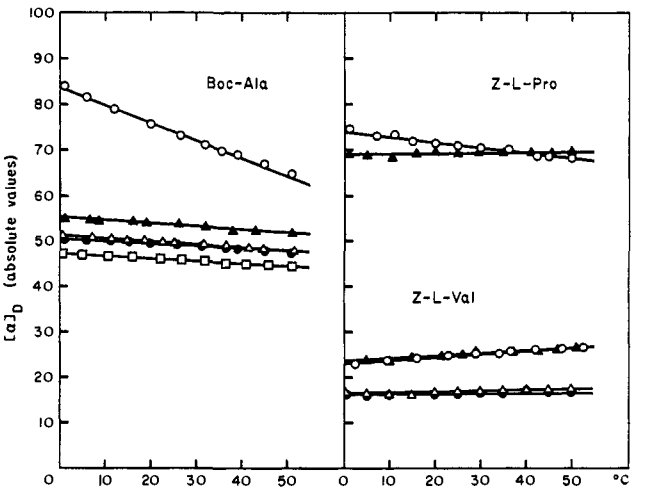

Figure 2. Optical rotations were determined in dimethylformamide solutions containing 1% acetic acid at 2–4% concentration of the aryl esters. The same values were obtained when the temperature, after having reached *ca.* 50°, was lowered to room temperature: (○) *o*-nitrophenyl esters; (▲) *p*-nitrophenyl esters; (●) *o*-cresyl esters; (△) *p*-cresyl esters; (□) phenyl ester.

Table IV. Temperature Coefficients of the Optical Activity of Active Esters[a]

	tc	rtc
Boc-L-Ala phenyl ester	−0.05	−0.11
Boc-L-Ala *o*-cresyl ester	−0.05	−0.10
Boc-L-Ala *p*-cresyl ester	−0.07	−0.14
Boc-L-Ala *o*-nitrophenyl ester	−0.39	−0.47
Boc-L-Ala *p*-nitrophenyl ester	−0.07	−0.13
Z-L-Leu *o*-nitrophenyl ester	−0.14	−0.28
Z-L-Leu *p*-nitrophenyl ester	−0.02	−0.05
Z-L-Pro *o*-nitrophenyl ester	−0.13	−0.17
Z-L-Pro *p*-nitrophenyl ester	+0.02	+0.03
Z-L-Phe *o*-cresyl ester	−0.14	−0.32
Z-L-Phe *p*-cresyl ester	−0.09	−0.34
Boc-L-Phe *o*-nitrophenyl ester	−0.51	−0.65
Boc-L-Phe *p*-nitrophenyl ester	−0.05	−0.20
Z-N-methyl-L-Phe *o*-nitrophenyl ester	−0.30	−0.38
Z-N-methyl-L-Phe *p*-nitrophenyl ester	−0.41	−0.41
Z-L-Val *o*-cresyl ester	+0.02	+0.11
Z-L-Val *p*-cresyl ester	+0.02	+0.11
Z-L-Val *o*-nitrophenyl ester	+0.07	+0.32
Z-L-Val *p*-nitrophenyl ester	+0.06	+0.26
Boc-L-Ile *o*-nitrophenyl ester	+0.08	+0.49[b]
Boc-L-Ile *p*-nitrophenyl ester	+0.07	+0.80[b]

[a] tc, temperature coefficient; rtc, relative temperature coefficient (*cf.* text). [b] When specific rotations are small, the rtc values are high and exaggerate minor differences in the tc values.

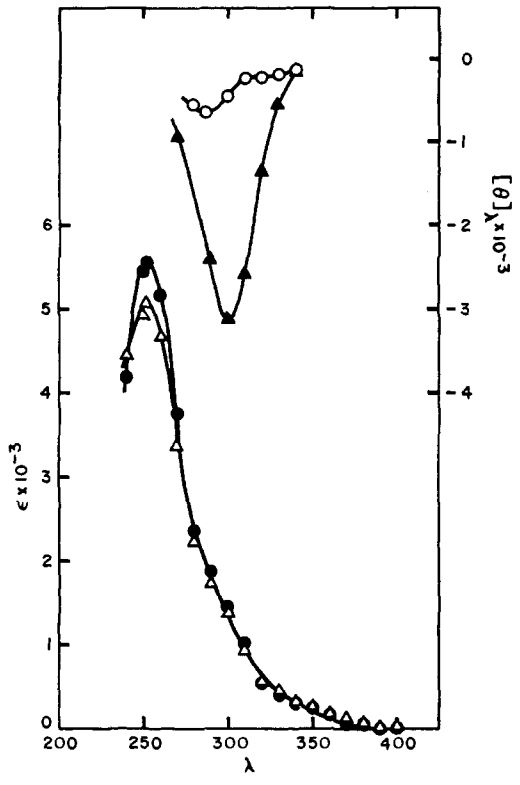

Figure 3. Ultraviolet absorption spectra of Boc-Val-ONO (△) and Boc-Ala-ONO (●) in 95% EtOH; CD spectra of Boc-Val-ONO (○) and Boc-Ala-ONO (▲) in 95% EtOH.

p-nitrophenyl ester. These comparisons do not clarify the nature of the intramolecular interaction. They leave no doubt about the existence of an attractive force between the nitro group and a second position of the molecule, a force that can stabilize a rigid, and probably cyclic conformation.

While the rtc values of *o*- and *p*-nitrophenyl esters of protected alanine and leucine permit a simple interpretation, the esters of phenylalanine seem to belong to a different class. Here the steric effect of the aryl ester group seems to be superimposed on the preferred conformation stabilized by the known[25-29] interaction between the amide group and the aromatic ring in the side chain of the amino acid. Such a combination of two effects, both limiting the conformational freedom of the molecule, might explain the conspicuously large differences between the specific rotations of *o*- and *p*-nitrophenyl esters of protected phenylalanine, tyrosine, and tryptophan (Table III). The optical activity of the ortho isomers was found to be two to several times higher than that of the para-substituted derivatives, and this relationship persisted when the rotations were determined in different solvents. The rtc values of *o*- and *p*-cresyl esters of benzyloxycarbonyl-L-phenylalanine are quite similar[30] (Table IV), and this again points to the role of the polarity of the nitro group in the determination of the molecular architecture, since the rtc values of the *o*- and *p*-nitrophenyl esters are very different. The similar rtc values of benzyloxycarbonyl-*N*-methyl-L-phenylalanine *o*- and *p*-nitrophenyl esters point to the amide group as the second participant in the intramolecular interaction in question. The methyl group on the amide nitrogen prevents this interaction and thereby also the unique distortion of the molecule recognized in *o*-nitrophenyl esters with an unhindered amide.

The active esters of valine and isoleucine belong to yet a third cass, in which the branching of the amino acid side chain at the β-carbon atom determines the geometry of the molecule to such an extent that no further restriction can be introduced by a nitro substituent in the ortho position of the aryl ester group. This is already indicated by the values of specific rotations which in this group are practically the same in the *o*- and *p*-nitrophenyl esters[31] (Table III). More significantly, the rtc values are the same in the ortho- and para-substituted derivatives (Table IV). The positive sign of the coefficient suggests a frozen conformation, that on relaxation at elevated temperatures can produce higher optical activity.

Not unexpectedly, the esters of proline are a class in themselves. The rotation of the *p*-nitrophenyl ester of benzyloxycarbonyl-L-proline is almost independent of temperature in the range examined, while the *o*-nitrophenyl ester has a small but definite negative temperature coefficient (Figure 2). The values of specific rotations are quite similar in the two esters (Table III).

While the rtc values reveal a conformational restriction in the *o*-nitrophenyl esters of several protected amino acids and demonstrate an intramolecular interaction between the nitro group (or nitroaryl group) and the amide grouping in these compounds, the anomalies detected in the derivatives of valine and isoleucine serve as warning against disregarding the individuality of the amino acids. Moreover, the nature of the interaction itself remains problematic. Since cyclic conformations stabilized by hydrogen bonds (Figure 1) were already excluded, other possibilities such as dipole–dipole interaction, dipole–induced dipole interaction, or the formation of charge-transfer complexes had to be considered.

The ultraviolet absorption spectra of *o*-nitrophenyl esters were examined to discern evidence for possible intramolecular charge-transfer complexes. A comparison of the uv spectrum of *tert*-butyloxycarbonyl-L-alanine *o*-nitrophenyl ester, in which an intramolecular attraction was described earlier in this paper, with the spectrum of the analogous derivative of L-valine, in which no such interaction was found, revealed no significant differences.[32] Therefore a weak Cotton effect in the *optical rotatory dispersion spectrum* of the alanine derivative and the corresponding negative band in its *circular dichroism spectrum* (Figure 3) had to be

(25) G. Gawne, G. W. Kenner, N. J. Rogers, R. C. Sheppard, and K. Titlestad, *Peptides, Proc. Eur. Symp., 9th*, 28 (1968).
(26) K. D. Kopple and D. H. Marr, *J. Amer. Chem. Soc.*, **89**, 6193 (1967).
(27) K. D. Kopple and M. Onishi, *J. Amer. Chem. Soc.*, **91**, 962 (1969).
(28) C. M. Deber and H. Joshua, *Bipolymers*, **11**, 2493 (1972).
(29) C. F. Lin and L. E. Webb, *J. Amer. Chem. Soc.*, **95**, 6803 (1973).
(30) However, the specific rotations of *o*- and *p*-cresyl esters of this protected amino acid are fairly different.
(31) The practically identical specific rotations of benzyloxycarbonyl-L-valine *o*- and *p*-nitrophenyl esters underline our contention that the differences found between *o*- and *p*-nitrophenyl esters of other protected amino acids are indeed not due to different contributions by the *o*-nitrophenyl and *p*-nitrophenyl groups to optical rotation. Also, closely similar rotations were determined for the *o*- and *p*-cresyl esters and for the *o*- and *p*-bromophenyl esters of benzyloxycarbonyl-L-valine (*cf.* Experimental Section).
(32) The slight difference in the absorption at the maxima (254 nm) is probably due to nonidentical angles between the nitro group and the plane of the aromatic ring in the two compounds.

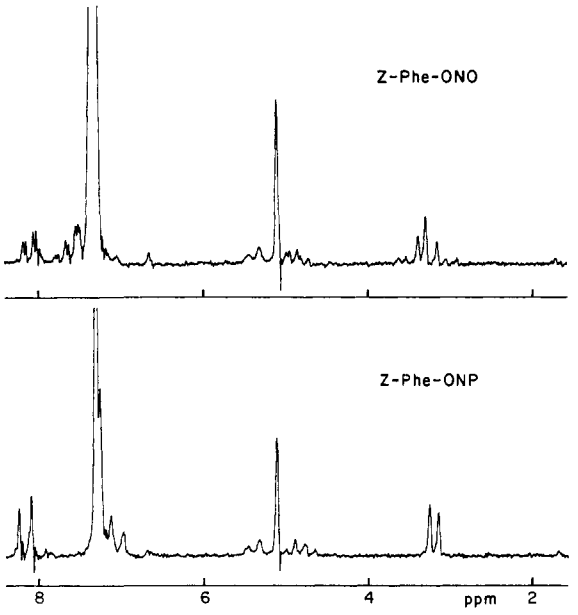

Figure 4. Nmr spectra of the *o*- and *p*-nitrophenyl esters of benzyloxycarbonyl-L-phenylalanine.

regarded merely as indications for a chromophore in a nonsymmetrical environment and not as evidence for a charge-transfer complex.[33] The absence of similar effects in the ORD and CD spectra of *tert*-butyloxycarbonyl-L-valine suggests a less distorted molecule.

The nmr spectra provided some limited insight into the conformation of active esters. The electron-withdrawing–deshielding effect of the *o*-nitrophenyl group, that resulted in α-CH and even β- and γ-CH resonances at lower field in *o*-nitrophenyl esters than in the para derivatives (Table II), did not influence the amide protons in the same way: in most *o*-nitrophenyl esters the NH protons appear 0.02–0.08 ppm higher field than in the corresponding *p*-nitrophenyl esters. Thus, shielding by a group that is not directly bonded to the nitrogen can be assumed. It is very suggestive that in the *o*-nitrophenyl esters of benzyloxycarbonyl-L-valine and benzyloxycarbonyl-L-isoleucine, the signals of the NH protons appear at unusually low field, about 5.6 ppm. In these compounds, an absence of intramolecular interaction between the nitro group and the amide was already indicated by their optical properties. Therefore it is not unreasonable to attribute the upfield shift of NH resonances in *o*-nitrophenyl esters to a through-space shielding influence of the nitroaryl group. A brief study of the temperature dependence of the chemical shifts of *o*- and *p*-nitrophenyl esters of *tert*-butyloxycarbonyl-L-alanine again indicated the absence of hydrogen bonding. The NH resonances shifted downfield when the temperature was lowered to −40°, but no difference was found in this respect between the ortho and para derivatives.

The NH signals appear at about 5.6 ppm in both the *o*- and the *p*-nitrophenyl ester of benzyloxycarbonyl-L-methionine. An intramolecular hydrogen bond[34] between the amide hydrogen and the sulfur atom was

(33) A possible intramolecular charge-transfer complex is suggested by the orange color of the crystals of benzyloxycarbonyl-L-tryptophan *o*-nitrophenyl ester.

(34) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, London, 1969, p 103.

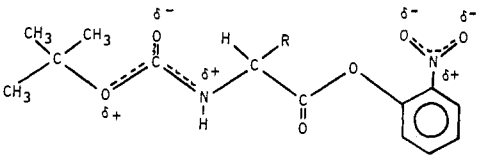

Figure 5. Polar groups in *o*-nitrophenyl esters of protected amino acids.

already indicated in the optical properties of these esters. A definitive explanation of these observations, however, will require separate studies. Similarly, detailed investigations are needed for information on the conformational aspects of active esters of amino acids with functional side chains.

The nmr spectra of the active esters of benzyloxycarbonyl-L-phenylalanine and also of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine reveal nonequivalent β hydrogens in the *o*-nitrophenyl derivatives, but equivalent β protons in the *p*-nitrophenyl esters (Figure 4). This was interpreted as a consequence of the conformation-limiting effect of the aromatic side chain. The introduction of the *o*-nitrophenyl group into a molecule of a protected amino acid that exists in a preferred conformation should produce more anisotropy than the addition of the symmetrical *p*-nitrophenyl group. The observation that the *o*- and *p*-cresyl esters of benzyloxycarbonyl-L-phenylalanine have different rotations (the ortho derivative has the higher absolute value) is in line with this rationale. The closely similar rtc values of these cresyl esters demonstrate the absence of attracting forces.

Conclusions

The electron-withdrawing effect of the nitro group that is stronger in the ortho than in the para position is a plausible explanation for the higher reactivity of *o*-nitrophenyl esters of protected amino acids. A comparison of the ir and nmr spectra of pairs of active esters of the same protected amino acid provided convincing evidence for higher activation in the ortho-substituted series. The lower sensitivity of *o*-nitrophenyl esters to the influence of solvents should be due in part to the proximity of the nitro and the ester groups (solvent molecules cannot enter the space between them), but probably also to an intramolecular involvement of the nitro group when in ortho position. A study of the optical activity of *o*- and *p*-nitrophenyl esters demonstrated generally higher specific rotations in the ortho series. This observation, together with the differences in the relative temperature coefficients of the optical activity, pointed to a cyclic conformation in the *o*-nitrophenyl esters of most common amino acids. The nature of the intramolecular interaction could not be established. No indication for intramolecular hydrogen bonding was found in the ir spectra of *o*-nitrophenyl esters, nor for charge-transfer complexes in their uv spectra. A possible explanation for the involvement of the nitro group is an intramolecular dipole–dipole (or dipole–induced dipole) interaction with the amide groupings of the protected amino acids (Figure 5). The polar character of the arylnitro group and that of the urethane amide[35] are complementary to each other

(35) *Cf.* M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Wiley, New York, N. Y., 1966, p 141.

and it is conceivable that one, two, or even three pairs of charged atoms are in sufficient proximity to provide attractive forces that stabilize a cyclic geometry.

The individuality of the amino acids precludes statements that would be valid across the board; *e.g.*, conformational restrictions caused by bulky side chains with branching at the β-carbon atom prevent ring formation in derivatives of valine and isoleucine. In amino acids with aromatic side chains, an attractive interaction between the side chain aryl group and the amino group lessens their conformational freedom, and interferes with the interpretation of the optical activity and the effect of temperature on this activity. Nevertheless, in the aromatic amino acid derivatives, the tendency for the development of a cyclic geometry still can be recognized.

Notwithstanding such differences between the analogous derivatives of the individual amino acids, *o*-nitrophenyl esters were found to have rigid preferred conformations. Only one side of their active ester carbonyl can be exposed to nucleophilic attack by the amino components in the peptide bond forming reaction; yet, because of the rigidity of the molecule, this side should be available all the time during acylation. Moreover, the rigid, compact molecules of *o*-nitrophenyl esters can probably better penetrate crowded environments, such as the matrix of polymeric supports, than acylating agents that possess more conformational freedom.

These observations made on *o*- and *p*-nitrophenyl esters of different protected amino acids point to the need for further extensive studies, which might lead to the development of optimal active esters. It is our view that for different amino acids, different activated derivatives will turn out to be most suitable.

Experimental Section

Infrared spectra were recorded on a Beckman IR9 instrument in KBr cells of 1-mm path length; 2% solutions in CCl_4 were used. Ultraviolet spectra were taken on a Cary 14 spectrophotometer; ORD–CD spectra on a Cary 60 spectropolarimeter.

Specific rotations were determined with the help of a Perkin-Elmer 141 automatic polarimeter in 1-dm jacketed cells. The nmr spectra were recorded on a Varian A-60 instrument, in $CDCl_3$ as solvent. For the definitive assignment of the signals of the NH protons, the active esters were dissolved in CD_3COOD, evaporated, and redissolved in the same solvent. The NH protons were exchanged in this process and were absent from the spectrum.

Synthesis of Aryl Esters of Protected Amino Acids. The protected amino acids were esterified with the appropriate phenol in pyridine,[12,13] with dicyclohexylcarbodiimide as consensing agent.[36] The crude products were purified by recrystallization from 95% ethanol or, when too soluble in this solvent, by chromatography on silica gel columns with $CHCl_3$ as eluent. The homogeneity of the purified materials was ascertained by thin-layer chromatography. Spots were detected by their uv absorption, and by spraying with *tert*-butyl hypochlorite followed by KI–starch reagent.[37] The protected amino acid aryl esters were characterized by their ir and nmr spectra. Where dimethylformamide was used as solvent in the determination of specific rotation, 1% acetic acid was added to prevent the reaction of active esters with any basic impurities present in or forming from the solvent: *tert*-butyloxycarbonyl-L-alanine phenyl ester, mp 42–45°, $[\alpha]^{25}D$ −46° (*c* 4, DMF); *o*-cresyl ester, mp 56–59°, $[\alpha]^{25}D$ −49° (*c* 4, DMF); *p*-cresyl ester, mp 93–96°, $[\alpha]^{25}D$ −49.5° (*c* 4, DMF); *tert*-benzyloxycarbonylvaline *o*-cresyl ester, mp *ca.* room temperature, $[\alpha]^{25}D$ −7.5° (*c* 4, $CHCl_3$), $[\alpha]^{25}D$ −25.5° (*c* 4, 95% EtOH), $[\alpha]^{25}D$ −16° (*c* 4, DMF), $[\alpha]^{25}D$ −26.5° (*c* 4, AcOH); *p*-cresyl ester, mp *ca.* room temperature, $[\alpha]^{25}D$ −6.5° (*c* 4, $CHCl_3$), $[\alpha]^{25}D$ −27° (*c* 4, 95% EtOH), $[\alpha]^{25}D$ −17° (*c* 4, DMF), $[\alpha]^{25}D$ −28.5° (*c* 4, AcOH); benzyloxycarbonyl-L-valine *o*-nitrophenyl ester, oil at room temperature, crystals at lower temperature, $[\alpha]^{25}D$ −23° (*c* 4, DMF); *p*-nitrophenyl ester, mp 81–83°, $[\alpha]^{25}D$ −22° (*c* 4, DMF); benzyloxycarbonyl-*N*-methyl-L-phenylalanine *o*-nitrophenyl ester, oil, $[\alpha]^{25}D$ −71° (*c* 2, DMF), $[\alpha]^{25}D$ −85° (*c* 1, pyridine); benzyloxycarbonyl-*N*-methyl-L-phenylalanine *p*-nitrophenyl ester, mp 60–61°, $[\alpha]^{25}D$ −90° (*c* 2, DMF), $[\alpha]^{25}D$ −100° (*c* 2, pyridine) (lit.[38] $[\alpha]^{25}D$ −99° (*c* 1.4, pyridine)); benzyloxycarbonyl-L-phenylalanine *o*-cresyl ester, mp 92–94°, $[\alpha]^{25}D$ −40.5° (*c* 4, DMF); *p*-cresyl ester, mp 84–86°, $[\alpha]^{25}D$ −24° (*c* 4, DMF).[39]

Racemization of *N*-*tert*-Butyloxycarbonyl-*S*-benzyl-L-cysteine *o*-Nitrophenyl Ester. Triethylamine (1%) was added to a 2% solution of the active ester in dimethylformamide. The solution was kept at room temperature and the specific rotation was determined from time to time: after 5 min, −71°; 1 hr, −65°; 5 hr, −47°; 24 hr, −20°; 48 hr, −6°. In $CHCl_3$, the initial value, −51°, dropped to −46° in 2 hr, to −20° in 1 day, and to −1.5° in 5 days.

Solvent effects on the rates of aminolysis were studied by mixing 0.004 *M* solutions of the active esters in the appropriate solvent with equal volumes of a 0.008 *M* solution of benzylamine in the same solvent. The reaction mixtures were kept in a constant-temperature bath at 25 ± 0.5°. Samples were taken at preselected intervals and diluted immediately with 95% ethanol containing 1% 1 *N* HCl. The progress of the reaction was determined from the uv spectra[40] of the diluted samples. Half-reaction times in EtOAc and CH_2Cl_2: Z-Leu-ONP, 23 and 720 min; Z-Leu-ONO, 4 and 23 min; Z-Pro-ONP, 11 and 46 min; Z-Pro-ONO, 3.5 and 8 min.

Solubility of Active Ester. Samples (1 ml) were taken from solutions saturated at 24°, the solvent (CCl_4) was removed with the aid of a stream of N_2, and finally *in vacuo*, and the residue was weighed: Boc-Gly-ONO, 18 mg/ml; Boc-Gly-ONP, 114 mg/ml; Boc-L-Ala-ONO, 96 mg/ml; Boc-L-Ala-ONP, 294 mg/ml; Boc-L-Phe-ONO, 8.0 mg/ml; Boc-L-Phe-ONP, 17 mg/ml.

Acknowledgments. The authors express their gratitude to Professor Eugene L. Pace and Mr. Anthony Severdia for their help in the studies involving ir spectra, and to Dr. Henry Lin in those connected with nmr spectra.

(36) D. F. Elliot and D. W. Russell, *Biochem. J.*, **66**, 49P (1957); M. Rothe and F. W. Kunitz, *Justus Liebigs Ann. Chem.*, **609**, 88 (1957).

(37) D. P. Schwartz and M. J. Pallansch, *Anal. Chem.*, 30, 1190 (1958).

(38) M. Goodman and K. C. Steuben, *J. Org. Chem.*, **27**, 3409 (1962).

(39) The difference between the specific rotations of *o*- and *p*-cresyl esters of benzyloxycarbonyl-L-phenylalanine is probably due to the higher anisotropy of the ortho derivative. The rtc values (Table IV) do not indicate a special cohesive force in the *o*-nitrophenyl ester. It is noteworthy in this connection that the *o*-fluorophenyl ester of this protected amino acid has higher specific rotation than its *p*-fluorophenyl ester (ref 15) and an even more pronounced difference exists between the corresponding *o*- and *p*-chlorophenyl esters.

(40) K. Stich and H. G. Leemann, *Helv. Chim. Acta*, **46**, 1887 (1963).