High-Pressure Studies. XX. Deacylation of Acyl-α-chymotrypsins[1,2]

Robert C. Neuman, Jr.,* Dennis Owen, and George D. Lockyer, Jr.[3]

Contribution from the Department of Chemistry, University of California, Riverside, California 92502. *Received September 2, 1975*

Abstract: Effects of pressure on the deacylation rate constants of dimethyl- and trimethylacetyl-α-chymotrypsin in 0.05 M Tris buffer and of indoleacryloyl-α-chymotrypsin in 0.1 M phosphate buffer have been determined. The ΔV^*_{deacy} values of −5 and −3 cm³/mol for $Me_2CHC(O)$-α-CHT and $Me_3CC(O)$-α-CHT, respectively, support previous conclusions derived from steady-state kinetic studies of hydrolysis of a series of *p*-nitrophenyl esters by α-CHT [*J. Am. Chem. Soc.*, **96**, 7303 (1974)]. The data obtained for indoleacryloyl-α-CHT in phosphate buffer show that inherent effects of pressure on these deacylation rates can be separated from effects on rate due to pressure-induced changes in the pH of the medium. They also suggest that the use of Tris buffer does not lead to complications in these pressure experiments.

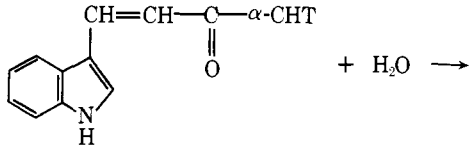
Pressure accelerates the rate of enzymatic hydrolysis of simple ester substrates by α-chymotrypsin.[4] The rate accelerations are small, but an apparent difference in the pressure effect was found as the substrate was varied. The activation volumes for α-CHT hydrolysis of the *p*-nitrophenyl esters of acetic, dimethylacetic, and trimethylacetic acid are −5, −3, and −2 cm³/mol (0.05 M Tris, pH 7.8, 20 or 25 °C).

The rate-determining step for α-CHT hydrolysis of these esters at atmospheric pressure is the deacylation reaction (eq 1b).[5]

$$E + RC(O)OAr \rightleftarrows E{\cdot}S \xrightarrow{k_2} EC(O)R + ArOH \quad (1a)$$

$$EC(O)R + H_2O \xrightarrow{k_3} E + RCO_2H \quad (1b)$$

On this basis we proposed that the values of ΔV^*_{cat} given above reflect the volume change for this step (i.e., $\Delta V^*_{cat} \equiv \Delta V^*_3$).[4] Looking for support of this proposal we directly determined the pressure dependence of the rate of deacylation of an unrelated acyl-α-chymotrypsin [indoleacryloyl-α-chymotrypsin (**1**)] and found that the activation volume for this process was −7 cm³/mol (0.05 M Tris, pH 7.3, 20 °C).[4] We took the similarity in sign and magnitude of ΔV^* for deacylation of **1** to those of ΔV^*_{cat} as supportive evidence of our proposal.



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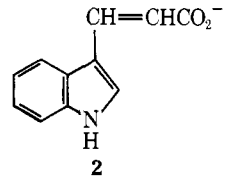

+ α-CHT (2)

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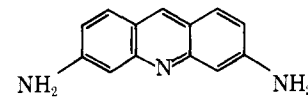
The acyl-enzyme **1** was chosen for study because it and **2** have substantially different uv absorption spectra (**1**, λ_{max} 365 nm; **2**, λ_{max} 313 nm),[6] and the deacylation reaction could easily be followed using a high-pressure optical cell. No such spectral change accompanies the deacylation reactions of methyl-, dimethyl-, and trimethyl-α-chymotrypsin (eq 1b; R = Me, Me_2CH, and Me_3C). However, procedures have been developed for following such reactions by spectroscopic techniques using chromophoric organic molecules which bind to the free enzyme and have different spectra in their free and bound states. Such a molecule which is suitable for α-CHT is proflavin **3**. It does not react with acylated α-chymotrypsin, but it does bind to the active site of α-CHT when it becomes vacant, and the stoichiometry of the binding reaction is 1:1.[7]



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The pressure effects for deacylation of dimethyl- and trimethyl-α-chymotrypsin, determined using the proflavin technique (0.05 M Tris, pH 7.7–7.8, 20–25 °C), support the proposal that the activation volumes determined for the α-CHT catalyzed hydrolyses of the simple esters above do correspond to the deacylation step, and the results are reported here. Additionally, we have included comparative data for the pressure effects on deacylation of indoleacryloyl-α-chymotrypsin (1) in both Tris and phosphate buffers. It has been found that Tris influences the activation parameters for α-chymotrypsin reactions.[8] Our results indicate that, at least in the case of IA-α-CHT (**1**), any participation by Tris in the deacylation step does not alter the observed activation volume for deacylation.

Results and Discussion

Deacylation of Dimethylacetyl- and Trimethylacetyl-α-chymotrypsin. Bernhard showed that the λ_{max} of proflavin changes from 444 to 458 nm when it binds to α-CHT.[7] The maximum difference in optical density occurs at 465 nm where $\Delta\epsilon$ is greater than 15 000. However, we followed the spectral change at 475 nm ($\Delta\epsilon$ 7500) because the long pathlength of our cell and the requisite high proflavin concentration gave a background absorbance which was too great at 465 nm.

While proflavin rapidly binds to free α-CHT, the binding is reversible and the equilibrium between enzyme (E), proflavin (D), and enzyme–proflavin complex (ED) must be taken into consideration in the kinetic equations used to obtain k_3 for deacylation of acyl-enzyme (EA). Proflavin also dimerizes in solution, but this is not important under our conditions.[9] From the mechanism shown in eq 3–5

$$EA \xrightarrow{k_3} E + A \quad (3)$$

$$E + D \overset{\text{fast}}{\rightleftarrows} ED \quad (4)$$

$$K_D = [E][D]/[ED] \quad (5)$$

it can be shown that

$$[EA] = (1 + K_D/[D_\infty])[ED_\infty] - (1 + K_D/[D])[ED] \quad (6)$$

Table I. Rate Constants for Deacylation of Acyl-α-chymotrypsins (RC(O)-α-CHT) and Comparative Values of k_{cat} for α-CHT

Hydrolysis of RC(O)—O—⟨⟩—NO_2

R	P, atm	Temp, °C	pH[a]	k_{deacy}, 10^4 s^{-1}	k_{cat},[b] 10^4 s^{-1}
Me_3C	1	25	7.70	1.78 ± 0.02	1.79
	340			2.01 ± 0.06	
	680			2.12 ± 0.05	1.88
	1021			2.13	
	1361			2.18 ± 0.03	1.92
	1701			2.27 ± 0.02	2.00
	2041			2.29 ± 0.01	2.22
Me_2CH	1	20	7.84	20.6 ± 0.2	18.8
	680			23.6 ± 0.9	20.8
	1021			24.8 ± 2.0	
	1361			27.2	22.6
	1701			29.7 ± 1.0	
	2041			32.2	24.5

[a] 0.05 M Tris buffer. [b] Reference 4.

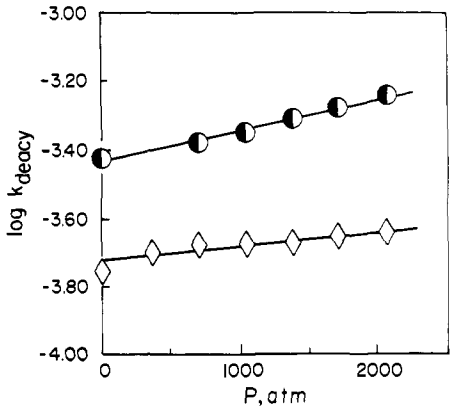

Figure 1. Pressure dependence of log k_{deacy} for deacylation of dimethylacetyl-α-chymotrypsin at 20 °C, 0.05 M Tris, pH 7.84 (◐) and trimethylacetyl-α-chymotrypsin at 25 °C, 0.05 M Tris buffer, pH 7.70 (◇). The curve for dimethylacetyl-α-chymotrypsin has been displaced downward by 0.75 log units.

so that

$$-d[EA]/dt = k_3[EA] = k_3(1 + K_D/[D_\infty])[ED_\infty] - k_3(1 + K_D/[D])[ED] \quad (7)$$

If $(1 + K_D/[D])$ is approximately constant during the reaction, eq 7 can be simplified to that shown as eq 8

$$d[ED]/dt = k_3([ED_\infty] - [ED]) \quad (8)$$

Since the relationship between [ED] and the absorbance (A) at 475 nm is given by eq 9, the simple expression shown in

$$[ED] = (A - \epsilon_D[D_0])/(\epsilon_{ED} - \epsilon_D) \quad (9)$$

eq 10 arises and can be used to obtain k_3 from the measured

$$\log (A_\infty - A_0) - \log (A_\infty - A) = k_3 t \quad (10)$$

change in A at 475 nm.

Under conditions similar to those used in our study, Bernhard determined that $K_D = 3.7 \times 10^{-5}$ M (Tris, pH 8.0, 25 °C).[7] The initial concentrations of acyl-enzyme and proflavin that we used were about 2.5×10^{-5} and 8.4×10^{-5} M, respectively. Thus, in our experiments, $1 + K_D/[D]$ varied from about 1.44 to 1.53 (an increase of about 6%).[9d] While higher concentrations of proflavin or lower acyl-enzyme concentrations would have aided in making $(1 + K_D/[D])$ more constant, neither was experimentally possible with our optical cell.

The rate data (Table I, Figure 1) obtained using eq 10, based on the approximation that $1 + K_D/[D]$ is constant, suggest that this necessary assumption did not lead to major errors. The atmospheric pressure value of k_{deacy} (k_3) for R = Me_3C is essentially the same as the value of k_{cat} determined from the earlier turnover experiments under comparable conditions but without proflavin. The high pressure values of k_{deacy} are also quite close to those for k_{cat} for the trimethylacetyl system.

The values of k_{deacy} for dimethylacetyl-α-chymotrypsin are lower than the comparable values of k_{cat} by amounts ranging from about 9 to 24%. It was difficult to follow the deacylation reaction for this system because the rate was so rapid. The half-life for deacylation of this acyl-enzyme was on the order of 5–6 min, and it took at least this long to load the high-pressure cell and place it in the Cary. After allowing for a bare minimum time for temperature equilibration, as much as 75% of the reaction had gone to completion before the collection of kinetic data was begun. Thus, our confidence in the rate data for deacylation of dimethylacetyl-α-chymotrypsin is less than in those for trimethylacetyl-α-chymotrypsin.

The resultant activation volumes for deacylation of $Me_3CC(O)$-α-CHT and $Me_2CHC(O)$-α-CHT are −3 and −5 cm^3/mol, respectively. The comparable values of ΔV^*_{cat} for α-CHT hydrolyses of *p*-nitrophenyl trimethylacetate and *p*-nitrophenyl dimethylacetate were determined to be −2 and −3 cm^3/mol, respectively, and that for *p*-nitrophenyl acetate was −5 cm^3/mol.[4] It was not possible to determine ΔV^*_{deacy} for acetyl-α-chymotrypsin because its rate was simply too fast to study by our procedure. Considering the experimental uncertainty of these data, we believe that the similarity in the respective values of ΔV^*_{deacy} and ΔV^*_{cat} clearly supports our earlier assumption that the values of ΔV^*_{cat} are those for the deacylation reaction (i.e., ΔV^*_3, eq 1b).[4] Additionally, the difference between the values of ΔV^*_{cat} for the various substrates appears to be further confirmed by these results for deacylation. In both studies the absolute value of ΔV^* is smaller for R = Me_3C than for R = Me_2CH.

We are in the process of attempting to further clarify the origin of the differences in ΔV^* as a function of substrate variation. Since our first report we have learned that there is evidence which suggests that Tris buffer participates in the catalysis reactions of α-CHT and that the extent of this participation varies as the substrate is varied.[8] The results in the next section are pertinent to this question.

Effect of Buffer on Deacylation of Indoleacryloyl-α-chymotrypsin. Tris buffer was originally chosen for these enzymatic pressure studies because, unlike acetate or phosphate buffer systems, it maintains an almost pressure invariant hydrogen ion concentration.[10] The ΔV^*_a for the Tris acid-base equilibrium (eq 11)

$$RNH_3^+ \rightleftarrows RNH_2 + H^+ \quad (11)$$

is about +1 cm^3/mol, while that for the appropriate acid-base reaction in phosphate buffer (pH 6–8) (eq 12) is about −24 cm^3/mol. This dramatic change in K_a for $H_2PO_4^-$ with

$$H_2PO_4^- \rightleftarrows HPO_4^= + H^+ \quad (12)$$

pressure leads to a *decrease* in pH of more than 0.7 over a pressure range of 2000 atm.

In principle we felt that it should be possible to separate the inherent effects of pressure on enzymatic reaction rates from those associated with a pH change induced by pressure. To test this we have determined the rates of deacylation of indoleacryloyl-α-chymotrypsin as a function of pressure in phosphate buffer, calculated the apparent activation volume ($\Delta V^*_{phosphate}$), calculated an effective activation volume for the rate change associated with the pressure-induced pH change (ΔV^*_{pH}), and used these data to obtain the inherent

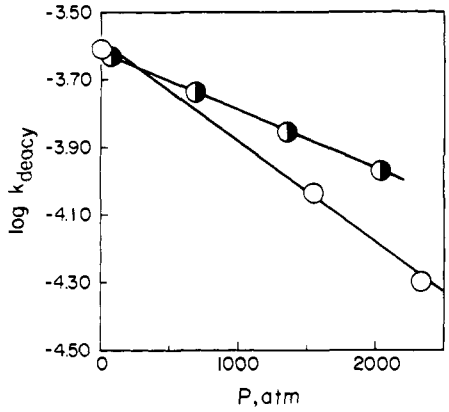

Figure 2. Pressure dependence of log k_{deacy} for deacylation of indoleacryloyl-α-chymotrypsin at 20 °C in 0.1 M phosphate buffer, pH 7.23 (◐). pH dependence (converted to "effective" pressure dependence, see text) of log k_{deacy} for IA-α-CHT at 20 °C in 0.1 M phosphate buffer at atmospheric pressure (○).

Table II. Rate Constants for Deacylation of Indoleacryloyl-α-chymotrypsin in Phosphate Buffer[a,b]

P, atm	pH[c]	k_{deacy}, 10^4 s^{-1}
51	7.23	2.32
681		1.83
1361		1.39
2042		1.07
1 (1)[d]	7.23	2.44
1 (1558)[d]	6.67	0.918
1 (2328)[d]	6.43	0.500

[a] 0.1 M phosphate buffer, 20 °C. [b] Errors ca. ±5%. [c] pH at atmospheric pressure. [d] Runs done at atmospheric pressure; values in parentheses are the "effective" pressures at which a pH 7.23 buffer would have the pH value shown in the next column.

activation volume for the deacylation reaction (ΔV^*_{deacy}) presumably reflecting a pressure effect uncomplicated by pH variation (eq 13). The value of ΔV^*_{pH} was obtained by determining rates of deacylation of IA-α-CHT in phosphate

$$\Delta V^*_{phosphate} - \Delta V^*_{pH} = \Delta V^*_{deacy} \quad (13)$$

buffer at various pH values *at atmospheric pressure*, converting these pH values into effective pressures using the known pressure dependence of the pH of phosphate buffer, and then calculating ΔV^*_{pH} from a plot of log k vs. effective pressure.

The rate data used for this test are given in Table II and Figure 2. They were determined by monitoring the spectral change at 335 nm associated with the loss of IA-α-CHT (see eq 2). In phosphate buffer the rate of deacylation of IA-α-CHT *decreases* with pressure, giving a value for $\Delta V^*_{phosphate}$ equal to +10 cm^3/mol. In contrast, we previously found that in Tris buffer, the deacylation rate *increased* corresponding to a ΔV^* of −7 cm^3/mol.[4] Bernhard has shown that deacylation of IA-α-CHT is retarded by decreasing pH,[6] and since the pH in phosphate buffer decreases with pressure, the comparative gross results in phosphate and Tris buffers are qualitatively consistent.

The effect of pH on the deacylation rate of IA-α-CHT at atmospheric pressure yields a value of ΔV^*_{pH}, calculated as described above, equal to +17 cm^3/mol. Taking these values of $\Delta V^*_{phosphate}$ and ΔV^*_{pH} in conjunction with eq 13 yields a value of −7 cm^3/mol for ΔV^*_{deacy} in phosphate buffer, a result which is in remarkable agreement with that determined in Tris buffer (−7 cm^3/mol).[4]

While generalization can be dangerous based on only a single study, these results certainly suggest that inherent pressure effects on rates and those caused by pressure-induced pH variation may be separable. Additionally, these data indicate that any special involvement of Tris in the catalytic action of α-CHT does not affect the activation volume for the deacylation step of at least the indoleacryloyl derivatized enzyme.

Experimental Section

Dimethyl- and Trimethyl-α-chymotrypsin. The acyl-enzymes were prepared from α-CHT (Worthington Biochemical Co., three times recrystallized and dialyzed; ca. 80% active) and the *p*-nitrophenyl esters of dimethylacetic acid or trimethylacetic acid by the method of Balls.[11] To a 2-ml volumetric flask, 0.08 g (3.2×10^{-6} mol) of α-chymotrypsin was added with about 1 ml of pH 3.7 acetate buffer (0.01 M). To this solution was added 2.5×10^{-6} mol of the appropriate ester substrate in 150 to 200 μl of acetonitrile, and the solution was diluted to the line with acetate buffer. This solution was allowed to incubate for about 30 min at room temperature and then overnight in the refrigerator (5 °C). The resulting solution of acyl-enzyme and *p*-nitrophenol in acetate buffer was suitable for use without further purification in the present studies. It was shown that removal of the *p*-nitrophenol by Sephadex chromatography (G-25, coarse) yielded an acyl-enzyme kinetically identical with that in the solution which had not been chromatographed.

Deacylation kinetics were followed by the proflavin-binding method of Bernhard.[7] To a 5-ml volumetric flask was added 300 μl of proflavin sulfate (ICN Nutritional Biochemical Corp.) stock solution (1.4×10^{-3} M in water), 100 μl of the acyl-enzyme stock solution, enough acetonitrile to bring the final concentration to 2%, and pH 7.8 Tris buffer (0.05 M) to bring the volume to 5 ml, giving a reaction mixture with initial concentrations of proflavin and acyl-enzyme on the order of 8.4×10^{-5} and 2.6×10^{-5} M, respectively. The sample was stirred and loaded into the high-pressure optical cell, and the absorbance increase at 475 nm (due to the binding of free α-chymotrypsin by proflavin) was monitored, after allowing a few minutes for thermal equilibrium. The wavelength of maximal absorbance change due to proflavin binding is 465 nm;[7] however, in this study the binding was studied at 475 nm due to the large background absorbance of proflavin at 465 nm. During a typical run, the reference compartment of the Cary spectrometer contained a screen, filtering out approximately 1.6 absorbance units. All deacylation runs gave apparent first-order rate plots using eq 10.

Indoleacryloyl-α-chymotrypsin. Separate master solutions of α-chymotrypsin in 0.01 M acetate buffer, pH 5.7 (1.6×10^{-3} M), and indoleacryloylimidazole (IAI)[12] (5.0×10^{-3} M) in acetonitrile were prepared. Just prior to the kinetic run, secondary stock solutions were prepared by addition of 250 μl of the substrate (IAI) master solution to 1 ml of the α-chymotrypsin stock solution. This secondary stock solution was stirred and 100 μl was removed and placed in a 5-ml volumetric flask which was then diluted to the line with pH 7.3 Tris buffer (0.05 M). This solution was stirred and placed in the high-pressure optical cell, and the disappearance of the acyl-enzyme absorbance was monitored at 355 nm.[4,6,12] All kinetics were strictly first order, and the secondary stock solutions were destroyed after a single use.

The deacylation of indoleacryloyl-α-chymotrypsin was also examined in phosphate buffer. Master solutions of IAI (5×10^{-3} M) in acetonitrile and α-chymotrypsin (5×10^{-4} M) in acetate buffer (pH 5.7) were prepared. Before each run, a secondary stock solution was prepared by combining 200 μl of the IAI stock solution with 1 ml of the α-chymotrypsin stock solution. Deacylation was followed using a solution prepared by addition of 250 μl of this secondary stock solution to a volumetric flask and dilution to 10 ml with the appropriate phosphate buffer. The initial buffer pH for all pressure studies was pH 7.23. For atmospheric pressure studies, the pH ranged from 7.23 to 6.43. Deacylation was again monitored by the disappearance of the indoleacryloyl-α-chymotrypsin absorbance at 355 nm.

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References and Notes

(1) (a) Part XIX: R. C. Neuman, Jr., and G. Lockyer, *Proc. Int. Conf. High Pressure, 4th, 1974*, 635–638, (1975). (b) Part XVIII: R. C. Neuman, Jr., and R. Wolfe, *J. Org. Chem.*, **40**, 3147 (1975). (c) Presented in part at the

4th International Conference on High Pressure, Kyoto, Japan, Nov 25–29, 1974.
(2) Support by the National Science Foundation through Grant MPS 72-05018 is gratefully acknowledged.
(3) Taken from the Ph.D. Dissertation of George D. Lockyer, Jr., University of California, Riverside, June 1975.
(4) G. D. Lockyer, Jr., D. Owen, D. Crew, and R. C. Neuman, Jr., *J. Am. Chem. Soc.*, **96**, 7303 (1974).
(5) M. L. Bender and F. J. Kezdy, *J. Am. Chem. Soc.*, **86**, 3704 (1964).
(6) (a) S. A. Bernhard and Z. H. Tashjian, *J. Am. Chem. Soc.*, **87**, 1806 (1965); (b) G. L. Rossi and S. A. Bernhard, *J. Mol. Biol.*, **55**, 215 (1971).
(7) S. A. Bernhard, B. F. Lee, and Z. H. Tashjian, *J. Mol. Biol.*, **18**, 405 (1966).
(8) See T. H. Marshall and V. Chen, *J. Am. Chem. Soc.*, **95**, 5400 (1973).
(9) (a) The dimerization equilibrium constant for proflavin (25 °C, pH 7.8 phosphate or acetate buffer) is ca. 400 M^{-1} [9b] and the uv λ_{max} of the dimer is at a shorter wavelength than that of the monomer.[9c] However, under our conditions (vide infra), at atmospheric pressure 94% of the proflavin initially present is monomeric, and at the end of the reaction the total percentage of monomeric proflavin (compared with proflavin dimer) is 95%. At 2000 atm, the respective percentages are 91 and 92%.[9d] The high percentage of monomeric proflavin and the very slight change during the deacylation reaction indicate that this equilibrium need not be included in the kinetic analysis. (b) D. H. Turner et al., *Nature (London)*, **239**, 215 (1972); (c) G. Schwarz, S. Klose, and W. Balthasar, *Eur. J. Biochem.*, **12**, 454 (1970). (d) The ΔV for proflavin dimerization is −6.5 cm^3/mol, while that for binding of proflavin to α-CHT is zero; K. A. H. Heremans, J. Snauwaert, H. A. Vandersypen, and Y. V. Nuland, *Proc. Int. Conf. High Pressure 4th, 1974*, 623–626 (1975).
(10) R. C. Neuman, Jr., W. Kauzmann, and A. Zipp, *J. Phys. Chem.*, **77**, 2687 (1973).
(11) C. E. McDonald and A. K. Balls, *J. Biol. Chem.*, **227**, 727 (1957).
(12) S. A. Bernhard, S. J. Lau, and H. Noller, *Biochemistry*, **4**, 1108 (1965).

Unusual Metalloporphyrin Derivative. Insertion of an Ethoxycarbonylcarbene Fragment into a Ni–N Bond of Nickel(II) *meso*-Tetraphenylporphine. Crystal and Molecular Structure of the Complex

Bernard Chevrier and Raymond Weiss*

Contribution from the Institut de Chimie, Laboratoire de Cristallochimie associé au C.N.R.S., Université Louis Pasteur, BP 296/R8, Strasbourg, France. *Received June 26, 1975*

Abstract: The structure of the title compound has been determined from three-dimensional x-ray counter data. The crystals, grown from a chloroform–methanol solution, have the space group $P\bar{1}$ with a = 13.677 (6) Å, b = 14.212 (8) Å, c = 11.958 (5) Å, and α = 98.85 (8)°, β = 99.21 (8)°, γ = 113.73 (7)°. This triclinic unit cell contains two molecules of the Ni(II) complex and two molecules of chloroform present as uncomplexed solvent of crystallization. Intensity data were collected by θ–2θ scanning with Mo Kα radiation, and 5493 independent data were retained as observed and used for the solution and refinement of structure; the conventional and weighted R values are 0.061 and 0.086, respectively. An ethoxycarbonylcarbene moiety is inserted into a Ni–N bond of the original nickel(II) *meso*-tetraphenylporphine. Thus, the nickel atom is tetracoordinated with nitrogen atoms of only three pyrrole bases and with the extra carbon atom of the carbene fragment. The Ni–C bond length of 1.905 (4) Å is close to that expected for a pure σ bond. The three Ni–N bond lengths are equivalent and equal to 1.916 ± 0.010 Å. The four nitrogen atoms of the pyrrole bases are approximately coplanar. The nickel and carbon atoms are displaced from this (4N) plane by 0.19 and 1.04 Å, respectively. The porphyrin macrocycle is distorted, leading to unusual distances between opposite pyrrole nitrogen atoms (3.81 and 4.49 Å). The pyrrole rings are quite planar but rotated with respect to the (4N) plane. Especially, owing to the insertion of the carbene moiety into the original Ni–N(4) bond, the N(4) pyrrole exhibits a very large angle of 46.4°.

Recent papers have described the reactions of ethyl diazoacetate with diverse metalloporphyrins. It appears from these studies that the nature of the adduct depends on the nature of the coordinating metal and even on the nature of the porphyrin. Thus, with copper(II) octaethylporphine, the reaction gives addition of the carbene fragment to the $\beta\beta$ double bonds to form chlorins together with a little meso substitution.[1] However, with cobalt(II) octaethylporphine reaction yields an unstable 1:1 adduct, a cobalt(III) salt where the carbene is inserted into one of the metal–nitrogen bonds.[2] On the other hand, zinc *meso*-tetraphenylporphine was shown to give mainly addition to the nitrogen atoms.[3,4] Of prime interest, this last compound, if demetalated, reacts with nickel(II) salts to cause spectacular rearrangements,[5,6] finally yielding novel nickel derivatives A and B.

One of them (A) is nickelhomoporphyrin. Its structure recently determined by x-ray analysis[7] shows a severe ruffling of the porphinato core mainly introduced by the carbene insertion between two pyrroles of the macrocyclic ligand. The other derivative (B) was presumed to be analogous to the above-mentioned Co(III) derivative, i.e., the carbene is inserted into a Ni–N bond of nickel(II) *meso*-tetraphenylporphine.[6]

Details of an x-ray examination of such compounds are not yet available.[8] Therefore, it is of interest to determine the structure of the air-stable derivative B and to specify the effects of the carbene moiety on the porphine skeleton and on the coordinated metal.

Experimental Section

The synthesis of this nickel(II) complex B and preliminary results on its structure have already been reported.[6] Suitable crystals were grown by slow evaporation from a cloroform–methanol solution. Preliminary x-ray photographic study established a triclinic unit cell with $P1$ or $P\bar{1}$ as the possible space groups. Lattice constants, a = 13.677 (6), b = 14.212 (8), c = 11.958 (5) Å and α = 98.85 (8), β = 99.21 (8), γ = 113.73 (7)°, came from a least-squares refinement[9] that utilized the setting angles of 12 reflections measured on a Picker four-circle automatic diffractometer with Mo Kα radiation (λ 0.70926 Å). The unit cell volume is 2038 $Å^3$. A unit cell containing just two molecules of complex ($C_{48}H_{34}N_4O_2Ni$, M = 757.5) gives a calculated density of 1.234 g/cm^{-3}. As measured by flotation in aqueous zinc chloride solu-