Participation of the Anilino Group in Peptide Bond Cleavage. The Use of t-Butyl 3,5-Dinitro-2-fluorocarbanilate as a Peptide Reagent

KENNETH L. KIRK AND LOUIS A. COHEN

Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

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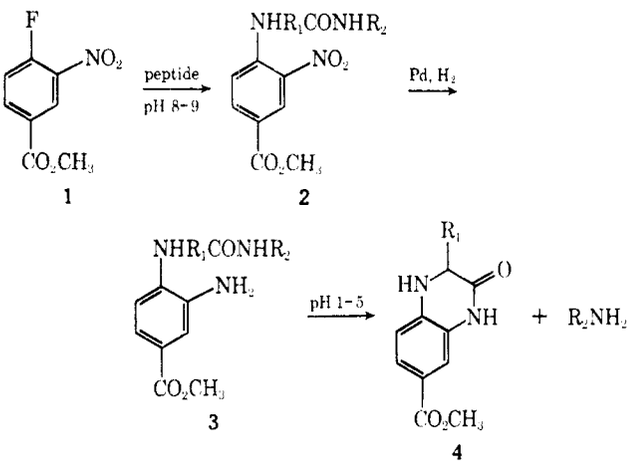
Picramyl fluoride (3,5-dinitro-2-fluoroaniline) has been prepared by the stannous chloride reduction of picryl fluoride and by the Curtius rearrangement of 3,5-dinitro-2-fluorobenzoyl azide. Reaction of picramyl fluoride with peptides (at pH 8) results in replacement of the fluorine atom by the peptide nitrogen. Coupling is followed by rapid intramolecular attack of the anilino group on the neighboring peptide bond (even at pH 8), resulting in cleavage of that bond and the formation of a dihydroquinoxalone derivative of the N-terminal amino acid. By use of the *t*-BOC derivative of picramyl fluoride (*t*-butyl 3,5-dinitro-2-fluorocarbanilate), the coupling and cleavage steps can be separated. Removal of the blocking group by trifluoroacetic acid is followed by rapid cyclization, both reactions proceeding quantitatively. Sequencing of a polypeptide (*e.g.*, oxidized insulin A chain) provides steadily decreasing yields of N-terminal derivatives, owing to benzimidazolone formation during the coupling step. By kinetic analysis, it is shown that the benzimidazolone (**17**) arises from attack of the 2,4-dinitroaniline anion (**16**) on the adjacent *t*-butyl carbanilate group.

The cleavage of amide bonds can be achieved by intramolecular participation of a variety of both nucleophilic and electrophilic species.[1] The protonated forms of pyridine[2] and of benzimidazole[1] nitrogen have been found to be particularly effective, but earlier work of Holley and Holley[3] and of others[4] has suggested than anilinium ions might be even more potent.

Holley and Holley coupled a variety of peptides with 4-carbomethoxy-2-nitrofluorobenzene (**1**) and formed the aniline derivatives (**3**) by catalytic hydrogenation of the nitro group in **2** (Scheme I). A

SCHEME I

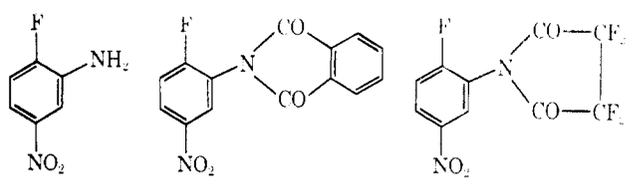


1 2 3 4

facile and rapid conversion of **3** into the dihydroquinoxalone **4**, and a shorter peptide, could then be effected under mildly acidic conditions. In subsequent extensions of the principle, the coupling reagent was modified by variation of the 4 substituent.[4] By use of sulfide ion, the readily accessible 2,4-dinitrophenyl peptide could be reduced selectively at the 2-nitro group.[4a] Despite the ease and efficiency of the cyclization procedure, the method has failed to achieve wide acceptance in the practice of sequential degradation of polypeptides. Principally, the obstacle has been the requirement of a reduction step, which could provide complications, particularly in the case of sulfur-containing peptides or proteins.

A logical alternative would be the use of a reagent with a preexisting anilino or potential anilino group. Such a goal is immediately thwarted by the electron-releasing and fluorine-deactivating properties of the anilino nitrogen. Thus, 2-fluoro-5-nitroaniline (**5**) is inert to peptide nucleophiles under any reasonable reaction conditions, nor does acylation of the nitrogen atom negate the deactivating effect.[5] In cyclic diacylimide derivatives, such as **6** and **7**, the imide carbonyl is more susceptible to nucleophilic attack than the fluorine-bearing carbon atom.[6]

Since it has been shown[7] that 2,4-dinitrofluorobenzene is considerably more reactive toward nucleophiles than 2- or 4-nitrofluorobenzene, we considered the possibility that an additional nitro group in **5** might counteract the negative effect of the anilino function. Earlier work also suggested that an *o*-nitro substituent may have a significant effect in promoting a favorable orientation of the amide bond for hydrolysis.[1,2] A



5 6 7

(1) For an extensive bibliography, see K. L. Kirk and L. A. Cohen, *J. Org. Chem.*, **34**, 390 (1969).

(2) A. Signor and E. Bordignon, *ibid.*, **30**, 3447 (1965); A. Signor, E. Bordignon, and G. Vidali, *ibid.*, **32**, 1135 (1967).

(3) R. W. Holley and A. D. Holley, *J. Amer. Chem. Soc.*, **74**, 5445 (1952).

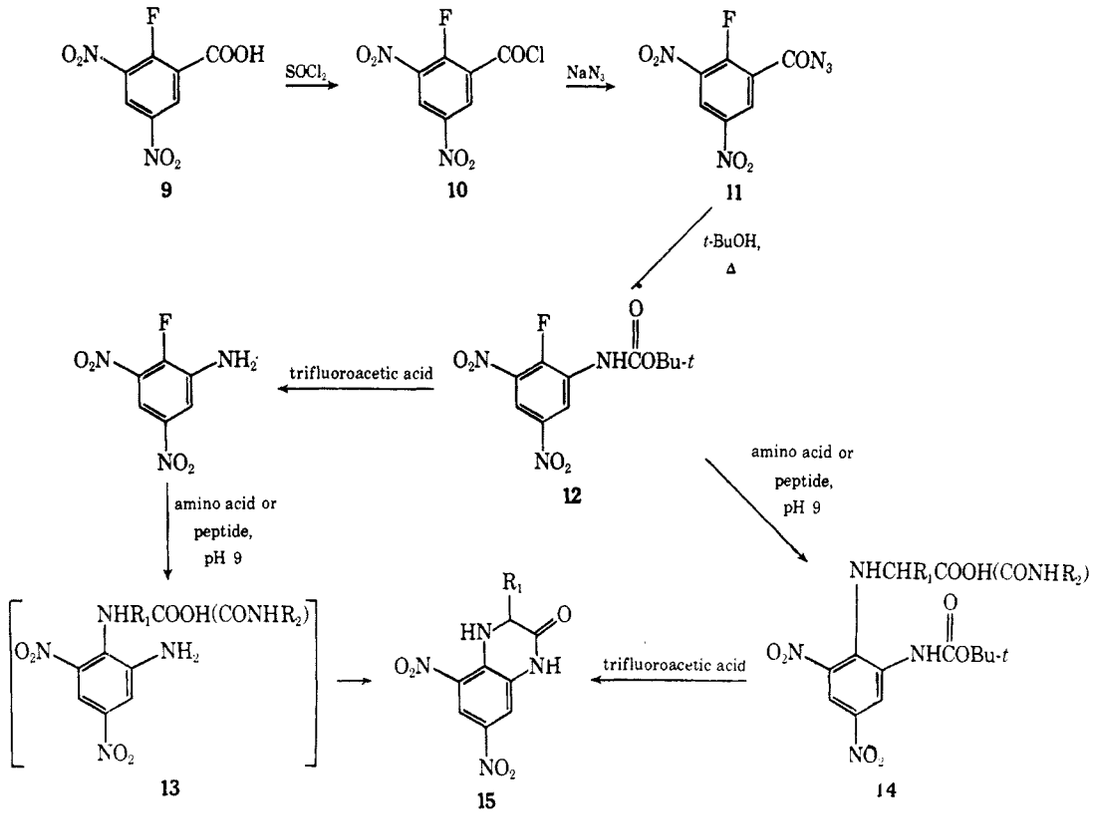
(4) (a) E. Scoffone, E. Vianello, and A. Lorenzini, *Gazz. Chim. Ital.*, **87**, 354 (1957); (b) L. Scarso, E. Scoffone, and D. Chillemi, *ibid.*, **87**, 1348 (1957); (c) P. de la Llosa, M. Jutisz, and E. Scoffone, *Bull. Soc. Chim. Fr.*, 1621 (1960).

(5) Similarly, acylation fails to negate the *ortho–para*-directing influence of aniline in electrophilic substitution [H. C. Brown and Y. Okamoto, *J. Amer. Chem. Soc.*, **80**, 4979 (1958)].

(6) L. A. Cohen and W. M. Jones, unpublished observations.

(7) C. W. L. Bevan and G. C. Bye, *J. Chem. Soc.*, 3091 (1954).

SCHEME II



first approach to the synthesis of 3,5-dinitro-2-fluoroaniline (picramyl fluoride) (**8**) was based on the stannous chloride reduction of picryl fluoride. Although a small yield of **8** was obtained, following chromatography of the complex reaction mixture, the method was unsatisfactory for preparative purposes.

In an alternative procedure, 2-fluorobenzoic acid was nitrated to form 3,5-dinitro-2-fluorobenzoic acid (**9**),[8] which, in turn, was converted into the acid chloride (**10**) and the azide (**11**) (Scheme II). Despite the reactivity of the activated fluorine atom in **10**, the acyl azide was the principal product formed in the presence of equimolar amounts of azide ion. Curtius rearrangement of the azide, by heating its solution in *t*-butyl alcohol, provided the protected aniline derivative, *t*-butyl 3,5-dinitro-2-fluorocarbanilate (**12**). The blocking group was readily removed by brief exposure of **12** to trifluoroacetic acid at room temperature. Following this sequence, picramyl fluoride (**8**) was obtained in an over-all yield of 50%; the preparation of **8** *via* **12** was preferred over the direct Curtius rearrangement of **11** in aqueous media. Nmr spectra supported the structures assigned to both **8** and **12** (see Experimental Section).

Although somewhat less reactive than 2,4-dinitrofluorobenzene, picramyl fluoride (**8**) coupled readily with peptide nucleophiles, as we had hoped. When alanylglycylglycine was treated with excess reagent for 3 hr at 35° and pH 8, the dihydroquinoxalones (**14**) corresponding to alanine and glycine were isolated, in 67 and 44% yield, respectively, *prior* to acidification of the reaction mixture. In earlier studies with 2-amino-4-methanesulfonylphenyl derivatives of peptides, it was found that the rate of cyclization decreased sharply above pH 6.[4b,c] In the present series, participation and cyclization proceeded readily, even at pH 8. In more alkaline media, the reaction was complex and led to intractable materials. When **8** was coupled with simple amino acids, such as alanine, the amino acid derivative, **13**, could not be obtained, cyclization to the dihydroquinoxalone occurring even at the pH of the coupling reaction.

To effect a separation of coupling from cyclization, the N-protected derivative, **12**, was utilized. In the reaction with alanylglycine at pH 8 (25°), **12** was found slightly more reactive than 2,4-dinitrofluorobenzene, the second-order rate constants being 7.1×10^{-3} and 6.5×10^{-3} M^{-1} sec^{-1}, respectively. Subsequent to coupling and removal of excess reagent, the anilino group was liberated by exposure of **15** to trifluoroacetic acid for 15 min at room temperature. Under these conditions, cyclization proceeded rapidly and quantitatively.

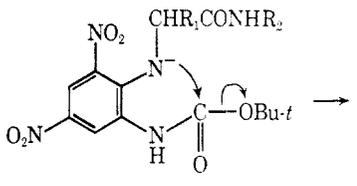
In contrast to the ease of oxidation of other dihydroquinoxalones,[9] those of the dinitro series were stable during isolation and thin layer chromatography (tlc); the unalkylated **14**, derived from glycine, showed a slight tendency to darken on thin layer plates. On the basis of their infrared (ir) spectra (5.9 μ, KBr), the dihydroquinoxalones are properly formulated as lactams, rather than the enolic lactims.[10]

(8) H. Goldstein and A. Giddey, *Helv. Chim. Acta*, **37**, 1121 (1954).

(9) J. C. E. Simpson, "Condensed Pyridazine and Pyrazine Rings," Interscience Publishers, New York, N. Y., 1953, Chapter 37.

(10) D. G. O'Sullivan and P. W. Sadler, *J. Chem. Soc.*, 2916 (1957).

Application of the protected reagent, **12**, to the degradation of several peptides, including oxidized insulin A chain, led to dihydroquinoxalone recoveries which were significantly less than quantitative. In the latter case, the first three degradation cycles gave recoveries of the glycine, isoleucine, and valine derivatives in yields of 72, 55, and 40%, respectively. Since removal of the blocking group and the cyclization reaction had been shown to proceed to completion in trifluoroacetic acid, the efficiency of the coupling reaction was reexamined. When the coupled derivative, **15**-glycylalanine, was exposed to media more alkaline than pH 8, the peak at 370 mμ disappeared and the yellow color of the solution faded. The product of the reaction was found to be the benzimidazolone, **17**.



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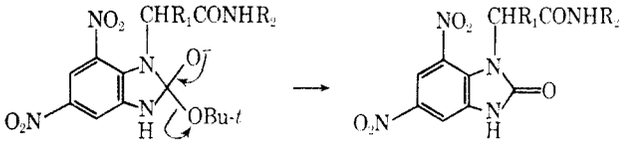

17

The reaction may proceed by nucleophilic attack of the nitrogen anion of **16** on the urethan carbonyl, with displacement of *t*-butoxide ion. An alternative pathway to **17**, involving collapse of the *t*-butyl carbanilate to an isocyanate *via* β elimination, followed by addition of the neighboring anilino nitrogen, was partially excluded by the demonstration that both **12** and **15**-proline were stable under the same alkaline conditions. However, the possibility remains that isocyanate formation is initiated by intramolecular proton transfer from the carbanilate nitrogen to the nitrogen anion of **16**.

Based on the two relationships which follow, eq 1

$$\mathrm{ArNHR} \overset{K_a}{\rightleftharpoons} \mathrm{ArN^-R} + \mathrm{H^+} \text{ and } \mathrm{ArN^-R} \xrightarrow{k_{rate}} \text{products}$$

$$1/k_{obsd} = 1/k_{rate} + [\mathrm{H^+}]/K_a k_{rate} \qquad (1)$$

may be formulated as shown below. The linearity of a plot of $1/k_{obsd}$ *vs.* H^+ (Figure 1) confirmed the assumption that the cyclization reaction depends on a rate limiting concentration of **16**. Once benzimidazolone formation has occurred, participation and cleavage of the peptide bond are no longer possible.

Although further modification of the reagent will be necessary to eliminate such side reactions in coupling, the facility and completeness of the cyclization step, as well as the ease of indentifying and assaying the resulting amino acid derivative, are features which indicate that further effort is warranted.

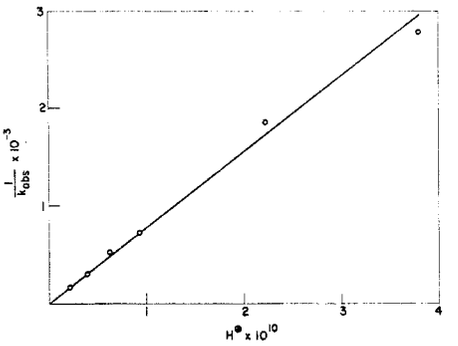

Figure 1.—Rate of benzimidazolone formation as a function of pH.

Of additional interest is the facility with which lactam formation occurs between the 3,5-dinitroanilino and carboxyl groups and the facility of peptide bond cleavage, *even* in mildly alkaline media. Assuming, as previous work has suggested,[4b,c] that the anilinium ion is the reactive species, and considering the low basicity of the nitrogen atom in **15** (pK_a = 1–2), the specific rate constants for such reactions should be impressive; appropriate kinetic studies are in progress.

Experiment Section[11]

3,5-Dinitro-2-fluoroaniline (**8**).—Anhydrous stannous chloride (9.5 g)[12] was dissolved in 80 ml of glacial acetic acid by saturation with hydrogen chloride at 25° and with exclusion of moisture. Dilution to 100 ml with acetic acid provided a reducing reagent which was 0.5 *M* in stannous chloride.

To a stirred solution of 462 mg (2.0 mmol) of picryl fluoride[13] in 5 ml glacial acetic acid was added 13 ml (6.5 mmol) of stannous chloride reagent dropwise over 5 min at room temperature. The original yellow color of the solution was transformed to green and finally to red. After 4 hr, the solvent was removed *in vacuo* and the residual material taken up in water. Colored material was extracted with several portions of chloroform; the combined extracts were dried and evaporated giving 483 mg of red oil. The material was purified by chromatography on 50 g of silica gel and elution with benzene. The desired compound was located in the yellow fractions by exposure of thin layer samples to ammonia vapor; the rapid transformation of a spot from yellow to red revealed the presence of **8**. Appropriate fractions were combined to give 105 mg (26%) of yellow crystals which were recrystallized from benzene–cyclohexane: mp 105–106°, λ_{max}^{EtOH} 375 mμ (ε 2150).

Anal. Calcd for $C_6H_4FN_3O_4$: C, 35.83; H, 2.00; F, 9.45; N, 20.89. Found: C, 35.88; H, 1.93; N, 20.82; F, 9.74.

(11) Melting points are uncorrected. The uv spectra were measured on a Cary recording spectrophotometer, Model 14, nmr spectra with a Varian A-60 spectrometer, and ir spectra with a Perkin-Elmer spectrophotometer, Model 421. Mass spectra were measured on an Hitachi double-focusing spectrometer, Model RMU-6E. Microanalyses were performed by Dr. W. C. Alford and his associates of this institute.

(12) H. Stephen, *J. Chem. Soc.*, 2786 (1930).

(13) G. Olah, A. Pavlath, S. Kuhn, and G. Varsanyi, *Acta Chim. Acad. Hung.*, **7**, 431 (1955); S. J. Kuhn and G. A. Olah, *J. Amer. Chem. Soc.*, **83**, 4564 (1961). For other methods of preparation, see G. C. Shaw and D. L. Seaton, *J. Org. Chem.*, **26**, 5227 (1961); R. E. Parker and T. O. Read, *J. Chem. Soc.*, 9 (1962); N. N. Vorozhtsov, Jr., and G. G. Yakobson, *Zh. Obshch. Khim.*, **31**, 3705 (1961); J. Murto, *Acta Chem. Scand.*, **20**, 303 (1966); H. L. Sharma, V. N. Sharma, and R. L. Mital, *Can. J. Chem.*, **44**, 1327 (1966).

TABLE I

PROPERTIES OF DIHYDROQUINOXALONES[a]

Amino acid	Yield, %	Mp, °C	m/e^b	λ_{max}^{EtOH}, mμ (ε)	R_f[c]
Glycine	98	225–250 dec	238	374 (10,800), 266 (14,100)	0.37
Alanine	95	206–235 dec	252	374 (9,800), 264 (13,500)	0.56
Isoleucine	92	184–187	294	374 (10,000), 265 (13,300)	0.78
Valine	87	175–180	280	374 (10,200), 268 (13,800)	0.70
Proline	98	205–209 dec	278	392 (9,500), 276 (10,900)	0.65

[a] All of the dihydroquinoxalones show an ir band (KBr) at 5.9 μ (carbonyl). [b] Composition was confirmed by m/e of parent peak in mass spectrum. [c] On silica gel GF, with ether as solvent.

The nmr spectrum ($CDCl_3$) showed two quartets (area 1:1) centered at 8.11 and 7.71 ppm (J_{HH} = 3 Hz and J_{HF} = 5 Hz).

3,5-Dinitro-2-fluorobenzoyl Azide (11).—The nitration of 2-fluorobenzoic acid and conversion of the dinitro derivative (**9**) into the acid chloride (**10**) were performed according to published procedures.[8] Comparable yields (60–70%) were obtained on a fivefold scale. The acid chloride was converted into the acid azide by exchange with azide ion. A sample of crude acid chloride (2.75 g, 11 mmol) was dissolved in 10 ml of glacial acetic acid and to the stirred solution was added 0.73 g (11 mmol) of powdered sodium azide over 15 min.[14] The mixture was kept an additional 45 min at room temperature and poured onto ice. Crystallization was induced by scratching. The pale yellow solid was filtered, washed with water, and dried, to give 2.08 g (75%) of **11**, which was used directly for Curtius rearrangement. Attempts to purify the material were unsuccessful. The compound began to melt at 55° with gas evolution.

The ir spectrum ($CHCl_3$) showed azide bands at 4.50 (w) and 4.65 (s), carbonyl absorption at 5.85, and nitro bands at 6.48 and at 7.43 μ.

***t*-Butyl 3,5-Dinitro-2-fluorocarbanilate (12).**—A solution of 2.00 g (7.8 mmol) of crude azide in 20 ml of anhydrous *t*-butyl alcohol was heated slowly and maintained at reflux for 1 hr. Following removal of solvent *in vacuo*, the partially crystalline material was purified by chromatography on 70 g of silica gel and elution with benzene, to give 1.72 g (73%) of pale yellow crystals. The product was recrystallized from benzene–cyclohexane: mp 149–151°; λ_{max}^{EtOH} 330 mμ (ε 2240).

Anal. Calcd for $C_{11}H_{12}FN_3O_6$: C, 43.86; H, 4.02; F, 6.31; N, 13.95. Found: C, 44.05; H, 3.82; N, 13.75; F, 6.80.

The ir spectrum ($CHCl_3$) was devoid of azide bands and showed carbonyl absorption at 5.78 μ ($CHCl_3$). The nmr spectrum ($CDCl_3$) showed a singlet at 1.57 (*t*-butyl) and two quartets centered at 8.47 and 9.30 ppm (J_{HH} = 3 Hz and J_{HF} = 6 Hz).

3,5-Dinitro-2-fluoroaniline (8).—To a solution of 200 mg of the carbanilate (**12**) in 5 ml of benzene was added 2 ml of trifluoroacetic acid. The mixture was kept at room temperature for 3 hr and the solvent was removed *in vacuo* to give 137 mg (97%) of crystalline material which was recrystallized from benzene–cyclohexane: mp 105–106°. On the basis of chromatographic behavior, nmr spectrum and mixture melting point, the product was identical with that prepared by partial reduction of picryl fluoride.

Preparation of 3-Substituted 3,4-Dihydro-5,7-dinitro-2(1H)-quinoxalones (15). A.—The following general procedure was used to couple the N-protected reagent (**12**) with amino acids and to generate the dihydroquinoxalones therefrom. A mixture of 10 mg of **12** and 15 mg of amino acid in 0.4 ml of dioxane–0.2 *M* sodium carbonate buffer (pH 9.2) (1:1) was kept at 45° for 1 hr. At this point, tlc indicated the total consumption of reagent. The solution was cooled, diluted with 0.5 ml of water, and acidified to pH 3 with 1 *N* hydrochloric acid. The mixture was extracted with ethyl acetate and the extract was dried and evaporated. The residual material, **14**, was shown to be homogeneous by tlc but was not characterized further. The product was dissolved in 5 ml of trifluoroacetic acid, the solution was kept at room temperature for 15 min, and the solvent was removed, providing **15** as a yellow crystalline solid. Properties of dihydroquinoxalones, prepared in this manner, are recorded in Table I.

B.—To a solution of 10 mg of alanine in 0.2 *M* sodium bicarbonate was added 5 mg of 3,5-dinitro-2-fluoroaniline (**8**). The mixture was kept at 60° for 30 min; tlc indicated the formation of the dihydroquinoxalone derivative of alanine. Upon cooling of the solution, yellow needles separated. The ir spectrum of the material (KBr) was identical with that of **15**-alanine prepared above.

Reaction of 12 with Glycylalanine.—A mixture of 50 mg of glycylalanine and 25 mg of **12** in 2 ml of dioxane–0.2 *M* phosphate buffer (pH 8.0) (1:1) was kept at 45° for 1 hr. The solution was cooled, diluted with 5 ml of water, and acidified to pH 3 with 1 *N* hydrochloric acid. The mixture was extracted with several portions of ethyl acetate until the organic layer remained colorless. The combined ethyl acetate extracts were dried and evaporated to give 35 mg (96%) of a yellow solid: mp 102–123° dec; λ_{max}^{EtOH} 354 mμ (ε 9800). The product, **14**-glycylalanine, was shown to be homogeneous by tlc. For analysis, the compound was dried at 50° *in vacuo*.

Anal. Calcd for $C_{16}H_{21}N_5O_9$: C, 44.96; H, 4.95; N, 16.39. Found: C, 45.21; H, 4.95; N, 16.49.

Reaction of 3,5-Dinitro-2-fluoraniline (8) with Alanylglycylglycine.—To 10 mg of reagent (**8**) in 0.2 ml of dioxane was added 2.5 mg of alanylglycylglycine in 0.2 ml of 0.2 *M* phosphate buffer (pH 8) and the mixture was maintained at 35° for 3 hr. The reaction mixture was cooled, diluted with 1 ml of water, and extracted with portions of ethyl acetate until the extract remained colorless. The combined extracts were dried and evaporated to yield a solid yellow residue. In addition to excess reagent, tlc showed two yellow spots, corresponding to **15**-alanine and **15**-glycine. Following purification of the dihydroquinoxalones by preparative tlc, the yields of the alanine and glycine derivatives, based on optical density at 374 mμ, were 67 and 44%, respectively. Work-up of the aqueous phase, by evaporation and exposure to trifluoroacetic acid, provided less than 5% additional material.

Reaction of 12 with Alanylglycylglycine.—To a solution of 2.4 mg of alanylglycylglycine and 18 mg of **12** in 0.5 ml of dioxane–water (1 : 1) was added 0.5 ml of 2,6-lutidine and the mixture was maintained at 40° for 2 hr. The solvent was evaporated and the residue was dissolved in 5 ml of trifluoroacetic acid. After 15 min at room temperature, the solvent was removed and the residue was extracted with ethyl acetate. Tlc showed the ethyl acetate extract to contain **15**-alanine and picramyl fluoride (**8**). The yield of **15**-alanine, following preparative thin layer purification, was 63%.

Partial Sequential Degradation of Oxidized Insulin A Chain.—Conditions for coupling with **12** and cleavage were the same as those used for alanylglycylglycine. Prior to treatment with trifluoroacetic acid, the residue was washed with ethyl acetate to remove excess reagent. The yield of **15**-glycine, following thin layer purification, was 72%. Repetition of the cycle gave **15**-isoleucine in 55% yield, and a second repetition gave **15**-valine in 40% yield. In each cycle, the dihydroquinoxalone derivative was devoid of contamination by derivatives of other amino acids.

Formation of Benzimidazolone (17) from 14-Glycylalanine.—A solution of 30 mg of **14**-glycylalanine in 5 ml of 0.2 *M* sodium carbonate buffer (pH 10.2) was stored at room temperature for 20 min, during which time the yellow color of the solution faded. Tlc ($CHCl_3$–*t*-amyl alcohol–acetic acid, 70:30:5) on silica gel GF revealed the total disappearance of starting material and the formation of a new, colorless product. The reaction mixture was acidified with 1 *N* hydrochloric acid, precipitating a pale yellow solid, which was collected and dried: 17 mg (95%); mp 243–245°; $\lambda_{max}^{pH\ 10.07}$ 380 mμ (sh) (ε 5000) and 310 (10,000), λ_{max}^{EtOH} 350 mμ (ε 6900) and 275 (8800).

Anal. Calcd for $C_{12}H_{11}N_5O_8$: C, 40.79; H, 3.14; N, 19.83. Found: C, 41.07; H, 3.32; N, 19.62.

The ir spectrum (KBr) showed carbonyl bands at 5.85 and 5.95 μ. The nmr spectrum showed a loss of the *t*-butyl peak at 1.5 ppm.

Rate of Reaction of 12 with Alanylglycine.—A 500 μl aliquot of a 6.7×10^{-4} *M* solution of **12** in ethanol was diluted to 4.50 ml with 0.2 *M* phosphate buffer (pH 7.95). To this solution was added 500 μl of 1.07×10^{-2} *M* alanylglycine in the same phosphate buffer. The rate of reaction, at 25°, was followed by the increase in optical density at 370 mμ. The observed second-

(14) The use of excess sodium azide led to partial replacement of the fluorine atom as well.

order rate constant was found to be 7.09×10^{-3} l. mol^{-1} sec^{-1}; under the same conditions, the second-order rate constant for the reaction of alanylglycine with 2,4-dinitrofluorobenzene was 6.52×10^{-3} l. mole^{-1} sec^{-1}.

Kinetics of Benzimidazolone (17) Formation.—Aliquots (500 μl) of a solution of 2.091 mg of 14-glycylalanine in 10 ml of ethanol were diluted to 5.00 ml with 0.2 *M* carbonate buffer at various pH values. Benzimidazolone formation was followed at 25°, by the decrease in optical density at 370 mμ. Observed first-order rate constants for benzimidazolone formation follow (pH, $k \times 10^3$ in sec^{-1}): 9.42, 0.36; 9.65, 0.54; 10.04, 1.41; 10.20, 1.96; 10.41, 3.52; 10.68, 5.70.

Registry No.—**8,** 18646-02-1; **11,** 18646-13-4; **12,** 18646-03-2; **14**-glycylalanine, 18646-04-3; **15**-glycine, 18646-05-4; **15**-alanine, 18646-06-5; **15**-isoleucine, 18646-07-6; **15**-valine, 18646-08-7; **15**-proline, 18646-09-8; **17,** 18646-10-1.

1,4 Additions of Phosphorus Trichloride to Cyclic α,β-Unsaturated Ketones[1a,b]

JAMES A. ROSS[1c] AND MICHAEL D. MARTZ

Department of Chemistry, University of Missouri, Columbia, Missouri

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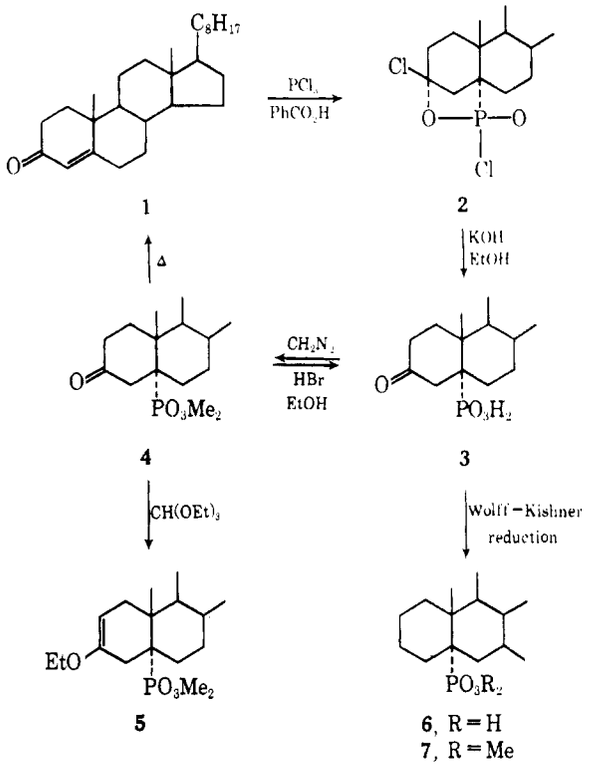
Phosphorus trichloride reacts with 4-cholesten-3-one in the presence of benzoic acid to provide a stable, crystalline intermediate in the normal 1,4-addition reaction of this reagent. Chemical and spectral evidence support the assignment of phostonyl chloride structure **2** to this intermediate with the phosphorus atom in the 5α position. An analogous product (**11**) is obtained from 2-keto-10-methyl-$\Delta^{1,9}$-octalin. A mechanism is proposed involving an initial electrophilic attack of the phosphorus atom on the carbonyl oxygen.

The reactions of phosphorus trichloride with ketones provide methods for forming carbon–phosphorus bonds which we have investigated for use in preparing steroidal phosphonic acids. We have found that the course of the reactions of this reagent with Δ^4-3-keto steroids is dependent upon the reaction conditions, thus the use of acetic acid as the solvent leads to 3-chloro-3,5-dienes, whereas acetic anhydride causes the formation of 3-acetoxy-3,5-dienes.[2] Substituting crystalline phosphorous acid for the acetic acid and using phosphorus trichloride in excess provides 3,5-dien-3-ylphosphonic acids by 1,2 addition of the phosphorus reagent.[3] We now report that the use of benzoic acid in phosphorus trichloride as the solvent allows the isolation of 1,4-addition products of 4-cholesten-3-one and 2-keto-10-methyl-$\Delta^{1,9}$-octalin which are the first fully characterized intermediates obtained from the normal 1,4-addition reaction of this reagent with α,β-unsaturated ketones to give γ-ketophosphonic acids. In addition to providing a route to steroidal C_5-phosphonic acids, therefore, these intermediates have significance in providing evidence about the mechanism of the reaction.

The nonhydrolytic work-up of a solution of 4-cholesten-3-one (**1**), phosphorus trichloride, and benzoic acid allows the isolation in 20–25% yield of a crystalline phosphorus-containing steroid, mp 206–208°, in addition to the major product, 3-chloro-3,5-cholestadiene. The elemental analysis and molecular weight of this new

SCHEME I



compound are consistent with the molecular formula $C_{27}H_{45}Cl_2O_2P$. On the basis of these and the following data, structure **2** is proposed for this compound (Scheme I).[4]

(1) (a) Abstracted from the Ph.D. Dissertation of M. D. Martz, University of Missouri, Jan 1967. Presented in part at the First Midwest Regional Meeting of the American Chemical Society, Kansas City, Mo., Nov 1965. (b) Journal Series Paper No. 5457. Approved by the Director of the Missouri Agriculture Experiment Station. (c) To whom all correspondence should be addressed at the Department of Plant Pathology, University of Missouri, Columbia, Mo. 65201.

(2) J. A. Ross and M. D. Martz, *J. Org. Chem.*, **29**, 2784 (1964).

(3) J. A. Ross and S. S. Wasson, Abstracts of Papers Presented at the First Midwest Regional Meeting of the American Chemical Society, Kansas City, Mo., Nov 4–5, 1965, p 31.

(4) The Chemical Abstracts name for **2** is (3β-chloro-3-hydroxy-5α-cholestan-5-yl)phosphonochloridic acid intramolecular ester. We shall refer to it as a "phostonyl chloride" following Conant's original suggestion: *cf.* A. Eberhard and F. H. Westheimer, *J. Amer. Chem. Soc.*, **87**, 253 (1965).