Registry No.—2a, 22483-35-8; 2b, 22483-36-9; 2c, 22483-37-0; 3 (X = 4-CN), 22483-38-1; 5 (X = picrate), 22483-39-2; 6, 22483-42-7; 7, 22483-40-5; 8, 22483-41-6; N-[4-(3-cyanophenoxy)phenyl]pyridinium picrate, 22483-43-8; 2-(4-nitrophenoxy)ethyl tosylate, 22483-44-9.

Stable Carbonium Ions. LXXXII.[1] Protonation and Cleavage of N-Alkoxycarbonyl-Substituted Amino Acids in Strong Acid Solution

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Protonation and cleavage of N-alkoxycarbonyl-substituted amino acids have been studied in SbF_5–FSO_3H–SO_2 solution. Complete alkyl–oxygen cleavage at −76° was observed for all the benzyloxycarbonyl and *t*-butyloxycarbonyl derivatives studied, to give dications having both protonated carboxylic and protonated carbamic functions. In the latter case, the trimethylcarbonium ion was observed. N-*n*-Butyloxycarbonyl DL-alanine did not cleave at −20°; N-*sec*-butyloxycarbonyl cleaved slowly at −50°; and N-vinyloxycarbonyl glycine and N-allyloxycarbonyl DL-alanine underwent complex change in the strong acid solution. The spectrum of di-O-protonated N-formylglycine was observed.

The benzyloxycarbonyl group is an important protecting group for the amino function in amino acids and peptides owing to its stability under amino acid coupling conditions, as well as the many methods available for its removal.[3] On removal, the free amino group is obtained, carbon dioxide is liberated, and a benzyl compound is formed. The *t*-butyloxycarbonyl group is

$$PhCH_2OCONHCHRCO_2H + 2HX \longrightarrow PhCH_2X + CO_2 + HXNH_2CHRCO_2H$$

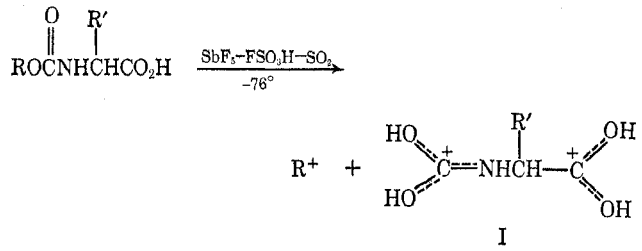
also widely used in peptide synthesis as an amino function protecting group. It is similarly removed under nonhydrolytic mild acid conditions to yield the free amino group, although, as is not the case for the benzyloxycarbonyl group, it is resistant to catalytic hydrogenation and treatment with sodium in liquid ammonia.

The following is the order of ease of removal of some amino-protecting groups for amino acids and peptides under nonhydrolytic acid conditions with evolution of carbon dioxide: *t*-butyloxycarbonyl > benzyloxycarbonyl > allyloxycarbonyl > *sec*-butyloxycarbonyl.[4,5]

Protonated carboxylic acids[6] and protonated alkyl carbamates and carbamic acids[7] have been investigated in our previous studies by nmr spectroscopy in the strong acid system SbF_5–FSO_3H–SO_2. It was of interest to extend these studies to the behavior of N-alkoxycarbonyl-substituted amino acids in SbF_5–FSO_3H–SO_2 solution and to investigate, over a range of temperature, their cleavage reactions.

Results and Discussion

N-Benzyloxycarbonyl and N-*t*-Butyloxycarbonyl Amino Acids.—When solutions of N-benzyloxycarbonyl and N-*t*-butyloxycarbonyl amino acids were prepared in SbF_5–FSO_3H–SO_2 solution at −70°, only at the products of alkyl–oxygen cleavage were observed. In the



I

R = *t*-butyl or benzyl (R′ was not observed for R = benzyl)

case of the N-*t*-butyloxycarbonyl amino acids, the cleaved trimethylcarbonium ion was observed as a singlet absorption at δ 4. In the case of the N-benzyloxycarbonyl amino acids, very broad, weak absorptions were observed at δ 7–10, probably corresponding to polymeric products of the cleaved benzyl cation.

For the general species I, the protonated carboxylic function was observed as a singlet at *ca.* δ 13.1. The carboxylic protons are deshielded by *ca.* δ 0.9 compared with the carboxylic protons of protonated aliphatic carboxylic acids[6] owing to the protonated carbamic group on the α-carbon atom. Compared with the carboxylic protons of the simple diprotonated α-amino acids $RCH(NH_3^+)C^+(OH)_2$,[8] they are shielded by δ 0.9.

Only carbonyl oxygen protonation was observed for the carbamic group of I. The proton on nitrogen was observed as a broad absorption at *ca.* δ 7.7. Two resonances were seen for the two protons on the carbamic oxygen atoms. This is most likely a result of hindered rotation about the C_α–N bond and relatively free rotation about the C_α–O bonds. Both resonances appear in the region δ 10.5–11.0, and there is allylic coupling between the lower field resonance and the hydrogen atom on nitrogen. For the proton on the hydroxyl group *trans* to the proton on nitrogen with respect to the C–N bond, there is the possibility of a favorable planar W coupling path with the protons on nitrogen.[9] Consequently, the coupled lower field resonance is assigned to

(1) Part LXXXI: G. A. Olah and M. B. Comisarow, *J. Amer. Chem. Soc.*, **91**, 2955 (1969).

(2) National Institutes of Health Postdoctoral Research Investigator, 1967–1968.

(3) See, *e.g.*, M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience Publishers, Inc., John Wiley & Sons, Inc., New York, N. Y., p 25.

(4) R. A. Boissonnas and G. Preitner, *Helv. Chim. Acta*, **36**, 875 (1953).

(5) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York, N. Y., 1961, pp 887–901 and 1187–1257.

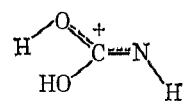
(6) G. A. Olah and A. M. White, *J. Amer. Chem. Soc.*, **89**, 3591, 4752, 7072 (1967).

(7) G. A. Olah and M. Calin, *ibid.*, **90**, 401 (1968).

(8) G. A. Olah, D. L. Brydon, and R. D. Porter, *J. Org. Chem.*, **35**, 317 (1970)

(9) S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964).

the *trans* carbamyl oxygen proton. The following N-alkoxycarbonyl derivatives were studied in SbF_5–



FSO_3H–SO_2 solution: N-benzyloxycarbonyl and N-*t*-butyloxycarbonyl glycine, L-alanine, L-valine, L-leucine, and L-proline; N-benzyloxycarbonyl L-aspartic acid; N,N′-dibenzyloxycarbonyl L-lysine; and ϵ-N-benzyloxycarbonyl L-lysine. The nmr parameters at −60° of the protonated carbamic–carboxylic species I, obtained by alkyl–oxygen cleavage, are summarized in Table I.

After the temperature was raised to −20° and then relowered to −60°, the original spectra of I could be observed unchanged. When the temperature was raised from −60° it was observed that the carboxylic protons exchanged more rapidly than the protons on the carbamic oxygen atoms, which were unaffected by exchange processes at −20°.

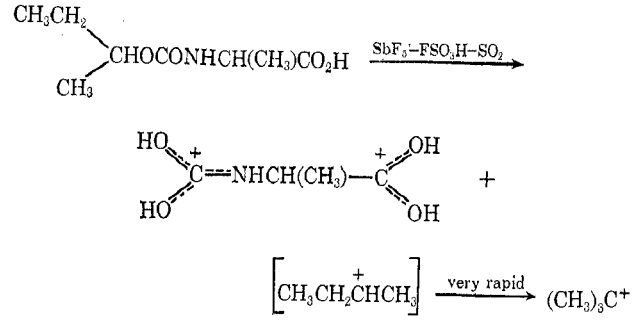
The diprotonated N-carboxy amino acids II–V derived from the N-benzyloxycarbonyl and N-*t*-butyloxycarbonyl derivatives of glycine, L-alanine, L-valine, and L-leucine, can be exemplified by that obtained from L-alanine. The carboxylic protons (2 H) appear as a singlet at δ 13.69 while the two carbamic OH groups appear at δ 10.70 (1 H) and 11.10 (1 H, J_{OH-NH} = 3 Hz). The NH hydrogen (1 H) gives rise to a broad doublet at δ 7.77. This chemical shift reflects the partial positive charge carried by the nitrogen atom. The methyl resonance (3 H) is a doublet at δ 2.23 (J_{CH_3-CH} = 7 Hz), while the methine proton, being vicinal to both a nitrogen and a carbon atom carrying some positive charge, appears as a multiplet at δ 5.66.

The triprotonated species VI resulting from the cleavage of N-benzyloxycarbonyl aspartic acid has to be cooled to −80° to lower the exchange rates before the resonances of both protonated carboxylic groups at δ 13.93 (2 H) and 14.57 (2 H) can be observed. The lower field resonance is assigned to the protons on the carboxylic group on the tertiary carbon atom. Both the CH (1 H) and NH (1 H) protons, at δ 6.27 and 8.00, respectively, are shifted downfield compared with their counterparts in the species derived from L-alanine. Also the carbamic OH resonances are at lower field [δ 11.13 (1 H) and 11.72 (1 H)] with the latter resonance broadened but not quite resolved as a doublet.

The nitrogen atom in the diprotonated carbamic derivatives VII of proline carried no hydrogen atoms. The carbamic OH resonances appear as two singlets, with a lower separation and a higher field than usual, at δ 10.43 (1 H) and 10.57 (1 H).

The triprotonated species IX, possessing two O-protonated carbamic functions, produced from N,N′-dibenzyloxycarbonyl lysine, demonstrates the fact that the alkoxycarbonyl group need not necessarily be linked to an amino group on the α atom of a carboxylic acid for alkyl–oxygen cleavage, with the production of a protonated carbamic group, to occur. The OH protons of the protonated carbamic group adjacent to the protonated carboxylic group are assigned to the two resonances at δ 10.77 (1 H) and 11.17 (1 H), while the NH proton (1 H) of this group appears at δ 7.7. For the other carbamic function, the OH protons appear at δ 9.63 (1 H) and 9.87 (1 H), with the NH proton (1 H) at δ 7.17. These assignments are based on comparison with the nmr parameters of the triprotonated species VIII obtained from the cleavage of ϵ-N-benzyloxycarbonyl lysine. In this case, the carbamic OH protons are seen at δ 9.70 (1 H) and 9.93 (1 H), while the NH proton and NH_3^+ protons appear as a broad peak at δ 7.17 (4 H). No cleavage reaction was observed even when a solution of N-*n*-butyloxycarbonyl DL-alanine in SbF_5–FSO_3H–SO_2 was maintained at −20° for 1 hr. The di-O-protonated N-*n*-butyloxycarbonyl DL-alanine (X) was observed with the carboxylic protons (2 H) appearing as two resonances, δ 13.47 and 13.52, at −60°. The proton on the carbonyl oxygen appeared as a singlet at δ 10.60 and as a doublet at δ 10.66 (J_{OH-NH} = 3 Hz). As before, there is assumed to be a greater energy barrier to rotation about the C–N bond than about the C–O bond. The singlet corresponds to the isomer in which the OH group is *cis* to the proton on nitrogen with respect to the C–N bond, and the doublet to the *trans* isomer.[9]

It was observed that N-*sec*-butyloxycarbonyl DL-alanine had undergone slight cleavage (as evidenced by the trimethyl carbonium ion at δ 4) after the solution in SbF_5–FSO_3H–SO_2 had been prepared at −78°. When the temperature is raised to between −60 and −35°, alkyl–oxygen cleavage takes place in the following manner.



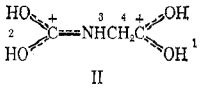
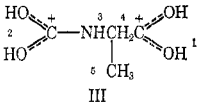
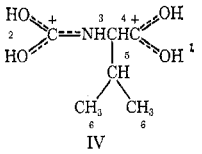
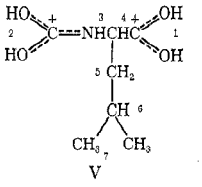
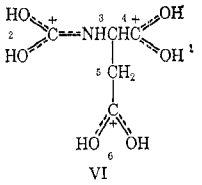
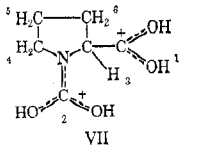
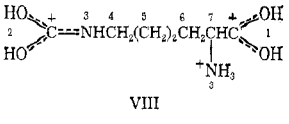
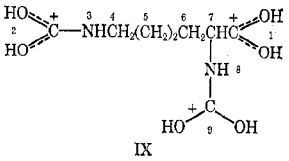
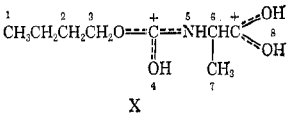
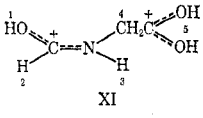
However, when the temperature is raised to −20°, secondary reactions occur; and, after the solution had been kept at −20° for 1 hr, the trimethyl carbonium ion absorption disappears.

The stability of the three isomeric N-butyloxycarbonyl alanines in the superacid system is inversely related to stabilities of the respective butyl cations initially formed by alkyl–oxygen cleavage. The *n*-butyl isomer is not cleaving at −20°, while the *t*-butyl isomer cleaves rapidly and completely at −78°. The *sec*-butyl isomer behaves in an intermediate fashion, cleaving in the range −60 to −35°.

N-Allyloxycarbonyl DL-alanine, when dissolved in SbF_5–FSO_3H–SO_2 at −76°, underwent a reaction, as evidenced by the two high-field doublets at δ 1.83 and 2.20. The nmr spectrum at −60° and below is consistent with an intramolecular cyclization having taken place between the carbonyl oxygen and the secondary carbon atom of the allyl group. When the temperature is raised from −60°, the spectrum becomes more complex, but at no time, even after the solution was left at room temperature for 2 hr, was the diprotonated N-carboxy alanine (III) expected from alkyl–oxygen

TABLE I[a]

NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS (HERTZ)[b] OF PROTONATED CARBAMIC–CARBOXYLIC ACIDS (PRODUCED BY CLEAVAGE OF N-ALKOXYCARBONYL PRECURSORS) AND PROTONATED *n*-BUTYLOXYCARBONYL DL-ALANINE AND N-FORMYLGLYCINE AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

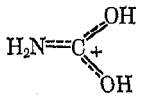
Species	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
 II	13.66	10.66 11.06 d ($J_{2,3}$ = 3)	7.7 br	5.23 d ($J_{3,4}$ = 6)					
 III	13.69	10.70	7.7 br	5.66 m	2.23 d ($J_{4,5}$ = 7)				
 IV	13.8	10.47 11.06 d ($J_{2,3}$ = 3)	7.8 br	5.4 br	2.90 br	1.43 br			
	13.66	10.43 11.00 d ($J_{2,3}$ = 3)	7.8 br	5.37 d ($J_{3,4}$ = 5) ($J_{4,5}$ = 8)	2.90 m	1.52 d ($J_{5,6}$ = 6.5) 1.43 d ($J_{5,6}$ = 6.5)			
 V	13.66	10.70 11.10 ($J_{2,3}$ = 3)	7.63 br	5.47 m	2.10 m	2.10 m	1.16 m		
 VI	14.57[c]	11.13 11.72	8.0 br	6.27 br	4.47 br	13.93			
 VII	13.7	10.43 10.57	5.60 dd	4.23 t	2.8 m	2.8 m			
 VIII	14.53[c]	9.70 9.93	7.17 br	3.87 br	2.07 br	2.66 br	5.23 br		
 IX	13.80	9.63 9.87	7.17 br	3.73 br	1.97 br	2.47 br	5.47 br	7.7 br	10.77 11.17
 X	1.07[d] m	1.7[d] m	4.9[d] m	10.60[d] 10.66 d ($J_{4,5}$ = 3)	7.43[d] m	5.50[d] m	2.10[d] d ($J_{6,7}$ = 8) 2.17 d ($J_{6,7}$ = 8)	13.47 13.52	
 XI	11.95[d] dd ($J_{1,2}$ = 5) ($J_{1,3}$ = 2.25)	9.23[d] ($J_{1,2}$ = 5) ($J_{2,3}$ = 5)	9.53[d] br	5.68[d] d ($J_{3,4}$ = 6)	13.93				

[a] Spectra of compounds in this table may be obtained by ordering document no. 00602 from ASIS National Auxiliary Publications Service, % CCM Information Services, Inc., 22 W. 34 Street, New York, N. Y. 10001, remitting $1.00 for microfiche or $3.00 for photocopies. [b] Coupling constants are in parentheses. Multiplicity is indicated as follows: d, doublet; t, triplet; m, multiplet; br, broad. [c] At −80°. [d] At −20°.

cleavage observed. Complex changes were observed when N-vinyloxycarbonyl glycine was dissolved in the strong acid solution at −76° and the temperature was raised. However, after the solution had been maintained at 55° for 1 hr, a small amount of diprotonated N-carboxy glycine (II) was observed in the nmr spectrum when the solution was cooled back to −40°.

The behavior of vinyl carbamate in SbF_5–FSO_3H–SO_2 was also investigated. The nmr spectrum of the solution prepared at −76° was complex, but resonances

corresponding to the alkyl–oxygen cleavage product, protonated carbamic acid[7]



were observed. The OH protons (2 H) were observed at δ 10 and the NH protons (2 H) at δ 7.47 as broad singlets. After the solution was heated to 60°, complex spectral changes took place, but, when the solution was cooled back to below −60°, the resonances attributed to protonated carbamic acid were observable.

Diprotonated N-formylglycine (XI) is an aldehydic analog of the diprotonated N-carboxy amino acids of type I and is therefore an interesting model compound. N-Formylglycine was di-O-protonated in SbF_5–FSO_3H–SO_2 solution, and no decomposition was observed to occur at −20°. At −60° the carboxylic protons (2 H) appear at δ 13.93 as a singlet, and at −20° they exchange too rapidly to be seen. The methylene protons (2 H) are seen as a doublet (J_{CH_2-NH} = 6 Hz) at δ 5.68 and the proton on nitrogen (1 H) appears as a broad peak at δ 9.53, which is comparable with the chemical shift of the proton on nitrogen in protonated imines.[10] The proton on the aldehydic oxygen (1 H) appears at δ 11.95 and is the same δ 3 upfield compared with the proton on oxygen in aliphatic aldehydes,[11] while the proton on the aldehydic carbon atoms appears at δ 9.23. These spectral observations might suggest that the positive charge attributable to aldehydic O-protonation is delocalized over the oxygen, carbon, and adjacent nitrogen atoms. Also, as in protonated, carbamic acids,[7] there is hindered rotation about the C–N bond and relatively free rotation about the C–O bond. Only one isomer is observed and that observed CH–NH coupling is 5 Hz, suggesting that the CH and NH protons bear a cisoid relationship, as depicted in Table I. The CH resonance (1 H) is observed as a triplet (J_{CH-NH} = Hz, J_{CH-OH} = 5 Hz), and the proton on the aldehydic oxygen atom is seen as a doublet of doublets due to a 5-Hz coupling with the proton on the vicinal carbon and an allylic-type coupling (J_{OH-NH} = 2.25 Hz) with the proton on nitrogen. Analogously with the protonated carbamic acid cases, the coupled NH and OH protons can assume a planar W coupling path and the hydroxyl group and the NH proton bear a *trans* relationship with respect to the C–N bond. However, in the case of protonated formylglycine, the long-range coupling is 0.55–0.75 Hz less than in the case of the protonated carbamic groups.

(10) G. A. Olah and P. Kreinbuhl, *J. Amer. Chem. Soc.*, **89**, 4756 (1967).
(11) G. A. Olah, D. H. O'Brien, and M. Calin, *ibid.*, **89**, 3582 (1967).

Experimental Section

Materials.—N-Benzyloxycarbonyl and N-*t*-butyloxycarbonyl α-amino acids were commercially available and were obtained as pure compounds from Schwarz Bioresearch, Inc., and Nutritional Biochemicals Corp.

N-Formylglycine was prepared by slowly adding acetic anhydride to a cooled solution of glycine in formic acid (90%). The product was recrystallized from water,[12] mp 148–150° (lit. mp 153–154°).

N-*n*-butyloxycarbonyl and N-allyloxycarbonyl DL-alanine were prepared from DL-alanine and the appropriate chloroformates according to the general procedure of Stevens and Matanabe.[13] Both compounds were recrystallized from benzene–pentane, and the latter compound had a melting point of 61.9–62.3° (lit. mp 60–61°). N-*n*-butyloxycarbonyl DL-alanine was obtained in 53% yield, mp 67.7–68°.

Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.91; N, 7.40. Found: C, 50.89; H, 7.72; N, 7.38.

N-*sec*-Butyloxycarbonyl DL-alanine, mp 55° (lit. mp 59°), was prepared from DL-alanine and *sec*-butyl chloroformate,[4] after the chloroformate was first prepared from phosgene and *sec*-butyl alcohol.[13]

Nmr Spectra.—A Varian Associates Model A-56/60A nmr spectrometer equipped with a variable-temperature probe was used for all spectra. Coupling constants are believed accurate to ±0.1 Hz. The reference standard used was capillary TMS.

Preparation of Superacid Solution.—Solutions were usually in the following molar proportions: amino acid derivative (1); sulfur dioxide (15); 1:1 *M* antimony pentafluoride–fluorosulfonic acid (6).

The amino acid derivative was dissolved in sulfur dioxide and added slowly at −76° with vigorous agitation to the 1:1 SbF_5–FSO_3H solution diluted with sulfur dioxide. The few amino acid derivatives which did not dissolve in sulfur dioxide were added slowly to the SbF_5–FSO_3H–SO_2 solution with vigorous agitation.

There was usually a large peak at *ca.* δ 11 owing to excess acid. All the solutions were either colorless or faintly colored except for the benzyloxycarbonyl solutions, which were yellow at −76° and darkened rapidly with rise in temperature.

Registry No.—II, 22483-22-3; III, 22486-00-6; IV, 22486-01-7; V, 22486-02-8; VI, 22486-03-9; VII, 22486-04-0; VIII, 22493-24-9; IX, 22486-05-1; X, 22486-06-2; XI, 22483-23-4.

Acknowledgment.—Professor M. Bodanszky is thanked for stimulating discussions and the arousal of our interest in this study. The research was made possible through a grant of the National Institutes of Health. Professor R. A. Olofson is thanked for samples of N-vinyloxycarbonyl glycine and vinyl carbamate.

(12) Reference 5, p 921.
(13) C. M. Stevens and R. Matanabe, *J. Amer. Chem. Soc.*, **72**, 725 (1950)