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Base-catalyzed Formation of Cyclic Compounds from Poly-β-benzyl-L-aspartate and Poly-γ-benzyl-L-glutamate[1,2]

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Poly-succinimide is formed from high molecular weight poly-β-benzyl-L-aspartate in dimethyl sulfoxide and dimethylformamide when the polypeptide reacts with catalytic quantities (less than 0.01 molar equivalents) of anhydrous $NaOCH_3$. The poly-imide displays an apparent negative Cotton effect with its inflection point at 242 mμ, the center of an imide absorption band. In the same solvents poly-γ-benzyl-L-glutamate fails to react with catalytic amounts of $NaOCH_3$, but with equimolar quantities yields the sodium salt of D,L-2-pyrrolidone-5-carboxylic acid. In chloroform solution both polymers are debenzylated by equimolar $NaOCH_3$, without formation of cyclic compounds. There is no correlation between the reaction products and the conformations of the polypeptides in various solvents. The results are discussed in terms of their possible relations to the active site of some hydrolytic enzymes.

Introduction

In recent years many studies have been made of the conformational properties of high molecular weight synthetic polypeptides as models for the structure of proteins. However, relatively little attention has been directed toward the analogy between the chemical reactions of these polypeptides and the catalytic functions of enzymes.[4] In several hydrolytic enzymes the reactive serine at the active site lies adjacent to an aspartyl (for example, in chymotrypsin) or a glutamyl (as in liver aliesterase) residue.[5] Because these dibasic acid residues are so often found next to the active serine, it is thought that they, as well as the serine, may have a function in enzymatic hydrolysis. This hypothesis provided motivation for the present investigation of de-esterification reactions of benzyl esters of poly-L-aspartic and poly-L-glutamic acids. We were particularly interested in reactions promoted by catalytic amounts of base, because such reactions suggest a coöperative effect among the dibasic acid residues. A similar effect could conceivably be the manner in which such residues help hydrolyze a neighboring O-serine ester in an acyl-enzyme intermediate.

Morawetz and co-workers[6] have described the unusually rapid hydrolysis of ester bonds when available for intramolecular catalysis by an ionized carboxyl. In the present research under certain conditions formation of cyclic compounds was found to accompany the de-esterification of poly-β-benzyl-L-aspartate and poly-γ-benzyl-L-glutamate, due to condensation of the aspartate or glutamate residues with the neighboring peptide bonds. Therefore, mention of previous work[7-11] on the formation of imide and acylpyrrolidone rings from peptides containing aspartate or glutamate is relevant here. All previous studies employed equimolar, or nearly equimolar, not catalytic, quantities of base or of condensing reagents. Bernhard, *et al.*,[7] measured the rates of appearance of succinimide derivatives formed as intermediates in the base-catalyzed hydrolysis of benzyl esters of α-aspartate peptides. Sondheimer and Holley[8] recorded that esters of both asparagine and glutamine form cyclic imides upon treatment with alkali. Glutamyl residues are theoretically capable of producing either 6-membered glutarimide rings or 5-membered acylpyrrolidone rings by condensation with peptide linkages, and both types of rings were found[9] after treatment of appropriate peptides with thionyl chloride. Battersby and coworkers found that esterified glutamyl as well as aspartyl peptides cyclized to imides in NaOH or $NaOC_2H_5$,[10] but that $SOCl_2$ could cause either type of ring to form from the glutamyl compounds, depending upon the nature of substituent groups.[11]

Poly-imides have been prepared by similar dehydration or de-esterification of polypeptides,[12,13] but the conditions employed were quite severe, and could not be considered catalytic. Furthermore, the resulting polymers were of fairly low molecular weight. Poly-succinimide was produced from poly-aspartic acid by heating at 200° *in vacuo* or at 100° in acetic anhydride,[12] and from poly-β-benzyl-aspartate by treatment with HBr and glacial acetic acid.[13] Poly-glutarimide was prepared from poly-glutamic acid and acetic anhydride at 165°.[13] There has been no report of the formation of poly-2-pyrrolidone-5-carboxylic acid from a polymer.

The present paper is concerned with the reactions through which benzylated polymers of aspartic and glutamic acids are de-esterified by $NaOCH_3$ in several anhydrous solvents. The reaction products vary with the solvent, but do not depend upon the conformation of the polypeptides. Under some conditions catalytic quantities of base are sufficient to cause de-esterification.

Experimental

A. Materials.—The preparation of the polymer samples, poly-β-benzyl-L-aspartate (L-PBA)[14] and poly-γ-benzyl-L-glutamate (L-PBG)[15] has been described. From the reduced specific viscosities in dichloroacetic acid the weight average molecular weights of the samples were estimated[16] as: L-PBA, sample no. P-A6, mol. $wt._w$ 150,000; L-PBG, sample no. ES-508, mol.$wt._w$ 64,000; L-PBG, sample no. GF-D-28-5 (used only for some viscosity studies), mol. $wt._w$ 363,000. Polymer solutions were filtered for measurements of viscosity and optical rotation.

(1) This is Polypeptides XL. For the preceding paper in this series see *J. Am. Chem. Soc.*, **84**, 3971 (1962).

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(4) E. Katchalski, G. D. Fasman, E. Simons, E. R. Blout, F. R. N. Gurd and W. L. Koltun, *Arch. Biochem. Biophys.*, **88**, 361 (1960).

(5) These sequences are summarized in C. Milstein and F. Sanger, *Biochem. J.*, **79**, 456 (1961).

(6) P. E. Zimmering, E. W. Westhead, Jr., and H. Morawetz, *Biochim. et Biophys. Acta*, **25**, 376 (1957).

(7) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).

(8) E. Sondheimer and R. W. Holley, *ibid.*, **76**, 2467 (1954); **79**, 3767 (1957).

(9) D. W. Clayton, G. W. Kenner and R. C. Sheppard, *J. Chem. Soc.*, 371 (1956).

(10) A. R. Battersby and J. C. Robinson, *ibid.*, 259 (1955).

(11) (a) A. R. Battersby and J. C. Robinson, *ibid.*, 2076 (1956). (b) A. R. Battersby and J. J. Reynolds, *ibid.*, 524 (1961).

(12) J. Kovacs, H. Nagy Kovacs, I. Könyves, J. Császár, T. Vajda and H. Mix, *J. Org. Chem.*, **26**, 1084 (1961).

(13) J. Noguchi, *et al.*, *Nippon Kagaku Zasshi*, **81**, 620, 624 (1960).

(14) (a) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **80**, 1259 (1958). (b) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *ibid.*, **82**, 2268 (1960).

(15) E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

(16) P. Doty, J. H. Bradbury and A. M. Holtzer, *ibid.*, **78**, 947 (1956).

All chemicals were of reagent grade purity. N-*n*-Amyl-succinimide was obtained from Eastman Kodak Co., L-2-pyrrolidone-5-carboxylic acid from General Mills. A solution of 0.38 *N* sodium methoxide in methanol–benzene, employed as a basic catalyst, was prepared as previously described[15] and was added to solutions by means of micrometer burets.

Several solvents required additional purification: Crown-Zellerbach dimethyl sulfoxide (DMSO) was heated with KOH, filtered, and distilled under vacuum through a column packed with glass helices. The hygroscopic DMSO contained 0.03% water by weight (Karl Fischer determination), and was stored in a Drierite-protected siphon bottle. Sulfolane was vacuum distilled. N,N-Dimethylformamide (DMF) was treated with $NaHCO_3$ and distilled from phthalic acid at reduced pressure.

B. **Isolation of Products. Poly-L-succinimide from L-PBA.**—L-PBA (300 mg.) was dissolved in 30 ml. of DMF. To this was added a catalytic amount, 0.005 mole equivalent, of anhydrous $NaOCH_3$ (0.02 ml., 0.381 *N*). After 30 min. an infrared absorption spectrum showed the reaction to be completed, and the mixture was poured into 300 ml. of anhydrous ether. The fluffy precipitate was dissolved in 2 ml. of DMF and was reprecipitated with 10 ml. of ether. The residue was washed with ether and then dried 16 hr. at 100° *in vacuo* over P_2O_5; yield 45 mg. (30%), $[\alpha]^{23}D$ $-29 \pm 2°$ (*c* 1% in DMSO).

Anal. Calcd. for $(C_4H_3O_2N)_n$: C, 49.4; H, 3.1; N, 14.4. Found[17]: C, 48.6; H, 3.9; N, 14.3.

Poly-L-succinimide was soluble in DMSO, DMF and dilute NaOH. It was insoluble in water, lower alcohols, ether, dioxane, chloroform and HCl.

D,L-2-Pyrrolidone-5-carboxylic Acid from L-PBG.—To 600 mg. of L-PBG in 60 ml. of DMSO was added 1.1 equivalents of $NaOCH_3$ (7.86 ml., 0.381 *N*). After 15 min. (reaction completed) the solution was poured into 300 ml. of anhydrous ether. The precipitate was filtered, washed with ether and dissolved in 1.6 equivalents of HCl (1.1 ml., 4 *N*). This solution was evaporated to dryness *in vacuo* over NaOH and P_2O_5; 20 ml. of acetone was added to the brown oily residue, and the mixture was boiled with charcoal. The filtered acetone solution was evaporated to 8 ml. and addition of 5 ml. of ether caused the formation of a crystalline precipitate which was collected and recrystallized slowly from acetone–ether. The product was dried *in vacuo* 20 hr. at 70°; yield 70 mg. (20%), m.p. 179–181°, $[\alpha]^{23}D$ $-0.2 \pm 0.2°$ (*c* 1% in water).

Anal. Calcd. for $C_5H_7O_3N$: C, 46.5; H, 5.5; N, 10.9. Found[17]: C, 47.0; H, 5.7; N, 11.2.

C. **Physical Methods. Spectral Measurements.**—Infrared spectra were obtained using a Perkin-Elmer double-beam instrument (model 21). Stoppered NaCl cells with 0.2-mm. path length were used for solution spectra; sample concentrations were 1%. Alternatively, some samples were cast onto AgCl disks and the films were dried with a hair drier. Visible and ultraviolet absorption spectra were measured, usually in 1-cm. or 1-mm. fused quartz cells, in a Cary model 11 recording spectrophotometer. Several films of poly-succinimide and of sodium-polyaspartate were employed for both ultraviolet absorption and rotatory dispersion. These films, of fairly constant thickness, were prepared as follows: Samples of poly-succinimide (0.3 to 2 mg.) were placed on fused quartz disks, dissolved in DMSO or in aqueous NaOH, and agitated during drying. All spectral and rotational measurements were made at 24 ± 1°.

Optical Rotatory Dispersion. Optical rotations were measured with a Rudolph photoelectric polarimeter (model 80Q3/200AS/650) equipped with a Beckman DU monochromator. The light source was a Hanovia (Newark, N. J.) 225-watt xenon-mercury arc. The symmetrical angle used was 3.5° or 5°, cell path lengths ranged from 1 mm. to 4 dm., and the longest wave length used, for all sets of rotatory dispersion data, was 589 mμ. The shortest wave lengths employed were: 260 mμ for 1% L-PBA in DMSO, 355 mμ for 0.5% L-PBG in DMSO, 313 mμ for 1% L-PBA in DMF, 365 mμ for 0.2% L-PBA in pyridine, 218 mμ for the thinner poly-imide film, 230 mμ for the thicker poly-imide film, and 236 mμ for the sodium poly-aspartate film. The estimated uncertainty in each reading of angle of rotation was about ±0.002°, corresponding to relative errors of ±0.1% to ±4% in each angle measured in solution.

The rotatory dispersion data for polypeptides in solution were analyzed in terms of the Moffitt equation; b_0 was used as a measure of α-helix content. This method has been outlined elsewhere[14]; λ_0 was taken as 212 mμ, the refractive index of the solvent as n_D. Non-helical conformations were always confirmed by the linearity of the plot of $[\alpha]\lambda\lambda^2$ *vs.* $[\alpha]\lambda$.[18] The effective residue rotation, $[R']\lambda$, was calculated for samples of poly-succinimide, to serve as a measure of rotatory power per chromophoric imide residue. In the case of polyimide films an approximate value for $[R']\lambda$ was obtained from

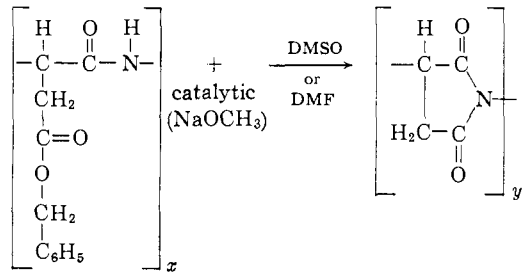
$$[R'] = [\alpha]\lambda \times \frac{\text{residue wt.}}{100} \times \frac{3}{n^2+2} = \alpha\lambda \text{ obsd.} \times \frac{100\,\epsilon_{242}}{\text{O.D.}_{242}} \times \frac{3}{n^2+2},$$

where O.D._{242} is the optical density of the film at the 242 mμ imide absorption peak. The extinction coefficient, ϵ_{242} (113 l. mole^{-1} cm.$^{-1}$), was measured for N-*n*-amyl-succinimide in methanol; 1.5 was assumed as a value for *n*.

Viscometry and Chromatography.—Viscosities of 0.2% polymer solutions were measured in Ostwald viscometers at 25 ± 0.05°. Flow times were greater than 2 minutes. The solvent used for the paper chromatograms was 4:1:5 1-butanol–acetic acid–water. Since most of the compounds of interest were ninhydrin-negative, duplicate chromatograms were developed with ninhydrin and with chlorine and tolidine.[19]

Formation of Poly-L-Succinimide from Poly-β-Benzyl-L-Aspartate by Use of Catalytic Quantities of Base

In certain anhydrous solvents a small amount of $NaOCH_3$ (0.001 to 0.05 mole equivalent) catalyzed the debenzylation of poly-β-benzyl-L-aspartate (L-PBA) with formation of the cyclic compound, poly-succinimide



This reaction was found to proceed, upon the addition of base, in N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), the closely related sulfolane (thiophan sulfone) and possibly N-methylacetamide. In several other solvents (chloroform, pyridine, hydrazine, acetonitrile, diethyl phosphite and some esters and alcohols) there was no reaction of L-PBA with catalytic $NaOCH_3$ concentrations, and no ring formation.

The poly-imide-forming condensation is unusual because of its catalytic nature; smaller aspartate peptides require equimolar amounts of base for similar reactions.[7,8,10]

A. **Characterization of the Product.**—The following evidence led to the identification of the reaction product of L-PBA with catalytic $NaOCH_3$ in DMSO and in DMF as poly-succinimide: (i) The proper elemental analysis was obtained (see Experimental). (ii) The infrared absorption spectrum of the product (see Table I) was very similar to that of the model imide, N-*n*-amyl-succinimide, and to spectra reported for Nujol mulls of poly-succinimide.[13,20] (iii) The ultraviolet spectra of films of the isolated product consisted of a weak band at 242 mμ and intense absorption farther in the ultraviolet (maximum below 204 mμ). Complete debenzylation was indicated by the lack of absorption at 250–270 mμ. The ultraviolet spectrum of the product was identical to that of N-*n*-amylsuccinimide in methanol. (iv) The chemical reactions of the product were those of an imide (see below).

The extent of depolymerization during imide formation, as estimated from viscometry and paper chromatography, was not large. The isolated poly-succinimide did not move during chromatography. Measurement of the decrease in viscosity of L-PBA during re-

(17) The analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(18) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

(19) C. G. Greig and D. H. Leaback, *Nature*, **188**, 310 (1960).

(20) A. Vegotsky, K. Harada and S. W. Fox, *J. Am. Chem. Soc.*, **80**, 3361 (1958).

TABLE I

INFRARED ABSORPTION BANDS

No.	Compound	State of sample	Carbonyl band frequencies[a] (cm.$^{-1}$) and assignments
		Reaction products	
1	L-PBA + 0.01 equiv. $NaOCH_3$ in DMSO or DMF	DMSO reacn. soln., or films of isolated products	1782(w) imide, 1715(s) imide
2	Prod. from (1) + 1.5 equiv. aq. NaOH	Film	1650(m, sh) amide I, 1595(s) COO^-, 1535(m, sh) amide II, 1400(s) COO^-
3	Prod. from (2) + aq. or anhyd. HCl	Film	1723(s) COOH, 1660(s) amide I, 1532(m) amide II
4	L-PBA + 1.1 equiv. $NaOCH_3$ in DMSO or DMF	Reacn. soln., or film	1587(s) COO^-, 1550(w, sh)?, 1400(s) COO^-
5	Prod. from (4) + HCl	Film	1720(s) COOH, 1535(w)?
6	L-PBG + 1.1 equiv. $NaOCH_3$ in DMSO or DMF	DMSO reacn. soln., or films of isolated products	1685(s) pyrrolidone, 1600(s) COO^-, 1405(s) COO^-
7	Prod. from (6) + HCl	Film	1725(s) COOH, 1670(s) pyrrolidone
8	L-PBA + 1.1 equiv. $NaOCH_3$ in $CHCl_3$	Reacn. soln.	1660(m) amide I, 1600(s) COO^-, 1565(m, sh) amide II
9	L-PBG + 1.1 equiv. $NaOCH_3$ in $CHCl_3$	Film	1650(m, sh) amide I, 1610(m) COO^-, 1550 (m) amide II, 1395(s) COO^-
10	Prod. from (9) + HCl	Film	1725(s) COOH, 1650(s) amide I, 1545(m, s) amide II
		Reference compounds	
11	L-PBA	DMSO soln.	1738(s) ester, 1668(m, s) amide I, 1530(m) amide II
12	L-PBG	DMSO soln.	1727(s) ester, 1645(m, s) amide I, 1548(m, s) amide II
13	N-*n*-Amylsuccinimide	DMSO or CH_3OH soln.	1775(w) imide, 1700(s) imide
14	2-Pyrrolidone-5-carboxylic acid	DMSO soln., or film	1722(s) COOH, 1668(s) pyrrolidone
15	Sodium pyrrolidonecarboxylate	DMSO soln., or film	1680(s) pyrrolidone, 1598(s) COO^-, 1410(s) COO^-

[a] Abbreviations used: (s) strong band, (m) medium, (w) weak, (sh) shoulder.

action with various amounts of $NaOCH_3$ in DMSO and in DMF showed that in both solvents the extent of chain cleavage increased with the fraction of imide formed (as estimated by infrared). At the completion of the reaction the observed viscosities equalled those calculated[21] on the assumption that each molecule of $NaOCH_3$ eventually caused one chain scission. That is, the final number-average degree of polymerization of poly-succinimide was about 100 when 0.01 mole equivalent of $NaOCH_3$ was used as the catalyst. Thus, the poly-imide product was still a high polymer.

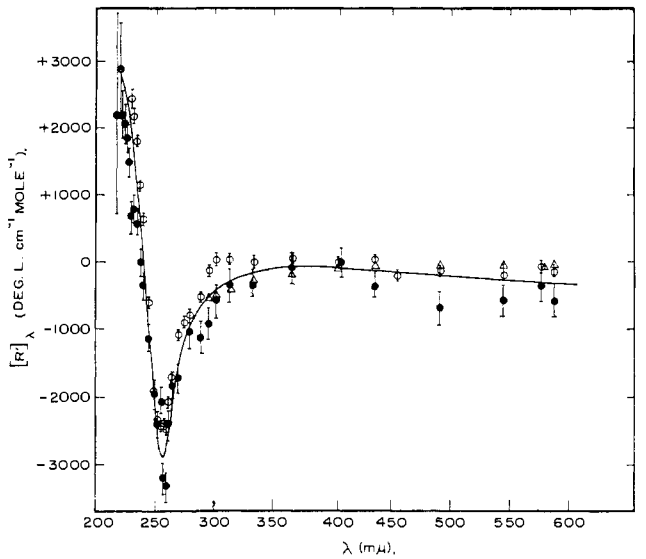
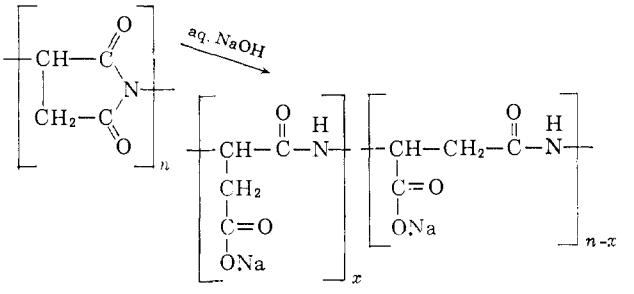


Fig. 1.—Optical rotatory dispersion of poly-succinimide: ⊙, film from 1 mg. of isolated poly-imide; ●, film from 0.3 mg. of isolated poly-imide; ▲, L-PBA (*c* 1%) + 0.01 equiv. $NaOCH_3$ in DMSO solution. The uncertainties in $[R']_\lambda$ were calculated from the reading errors in α_λ obsd.

Racemization of the α-carbon atom during imide formation was not extensive, if it occurred at all, as was shown by the optical rotatory dispersion of the poly-imide and of the poly-sodium aspartate formed from it. Thus, the imide can be referred to as poly-L-succinimide.

B. Properties of Poly-L-succinimide. Chemical Reactions.—The poly-imide dissolved in 1.5 equivalents of cold aqueous NaOH (0.1 or 1 *N*) and was rapidly hydrolyzed to form sodium polyaspartate in a reaction typical of cyclic imides.[22] The imide ring did not reform under acid conditions even after 24 hours.[23] As indicated above, a mixture of α- and β-aspartyl residues appears likely[12] but was not investigated. (Because of the possibility that this product is a mixture, and because of the partial degradation discussed above, the formation of poly-succinimide from L-PBA followed by hydrolysis of the imide ring does not constitute a good method for the preparation of highly polymeric poly-α,L-aspartic acid.)



(21) Other assumptions made for this calculation: (i) the cleavage is random; (ii) the calibration curve given in ref. 16 for mol. wt.$_w$ *vs.* η of randomly-coiled L-PBG is approximately valid for polysuccinimide.

(22) S. S. G. Sircar, *J. Chem. Soc.*, 600, 1252 (1927).

(23) This finding can be compared to the statement in ref. 10 that acid does not cause the cyclization of peptides to imides.

Cotton Effect.—The optical rotatory dispersion of films and solutions of poly-L-succinimide is shown in Fig. 1. A negative Cotton effect is apparent, having a trough at 258 ± 2 mμ, coinciding with the 242 mμ cyclic-imide absorption band. That the Cotton effect was not an artifact caused, perhaps, by scattering of light by the films was shown as follows: A similar polyimide film was cast and was treated with aqueous NaOH. This sodium polyaspartate film displayed continuously decreasing negative rotation from 400 to 240 mμ with no Cotton effect.

The Cotton effect of poly-L-succinimide signifies that the electrons of the chromophoric imide group probably interact with the asymmetric α-carbon atom. However, an asymmetric macromolecular conformation of the poly-imide cannot be ruled out as a possible contributary cause of the Cotton effect; the imide residues may be oriented in a rigid and asymmetric manner with respect to one another. However, the rotatory dispersion data for the poly-imide in DMSO (which agree with the film data) cannot be fitted well by a Moffitt plot, thus indicating that the poly-imide is not involved in an α-helix. The origin of this Cotton effect might be further examined by comparing the rotatory dispersion of poly-L-succinimide with that of a monomeric asymmetric N-substituted imide. The possibility should be noted that the increase in rotation of the poly-imide at $\lambda < 260$ mμ may be the result of a large positive Cotton effect at short wave length; this uncertainty could be resolved if improvements in instrumentation permitted measuring meaningful rotations of such films at $\lambda < 220$ mμ.

C. Characteristics of the Imide-forming Reactions. Kinetics.—Some crude kinetic data were obtained by means of an infrared absorption method. Various amounts of $NaOCH_3$ (0.001 to 0.03 mole equivalent) were added to 1% L-PBA in DMSO, and the rate of formation of poly-L-succinimide in each case was followed by running infrared spectra at intervals ranging from 3 minutes to 2 hours. From each spectrum the fraction of imide formed was calculated from the intensity of the imide bands at 1780 and 1717 cm.$^{-1}$, and the fraction of L-PBA remaining from the ester and amide bands at 1734, 1673 and 1527 cm.$^{-1}$; these five results were averaged. The data could be fitted to first-order rate curves but with considerable scatter. For each $NaOCH_3$ concentration a first-order rate constant, $k_{(1)} = d[\text{imide}]/[\text{L-PBA}]dt$, was obtained. These constants are shown in Fig. 2. The estimated error in each value of extent of reaction, [imide]/([L-PBA] + [imide)], is 0.1–0.15; the errors in $k_{(1)}$ are indicated in Fig. 2.

The data are sufficient to reveal that $k_{(1)}$ is directly proportional to $[NaOCH_3]$. Thus, OCH_3^- is involved before or during the rate-determining step of the cyclization. The $NaOCH_3$ appears to act as a true catalyst; it or some species, such as $C_6H_5CH_2O^\ominus$, capable of performing the same function kinetically, is continually regenerated. (If catalyst were used up appreciably during the reaction, the observed first-order rate constants would have decreased with the progress of the reaction.) The failure of the line in Fig. 2 to go through the origin may be caused by the reaction of some of the catalyst with a trace impurity in the DMSO, present as 1 mole impurity per 3×10^7 moles of solvent.

A "second-order" rate constant, defined as $k_{(2)} = k_{(1)}/[NaOCH_3]$, was calculated from the slope of Fig. 2 to be $k_{(2)} = 70 \pm 25$ min.$^{-1}$ mole^{-1} l. for poly-imide formation from L-PBA in DMSO. This value is about 2 orders of magnitude smaller than the second-order constant found by Bernhard, *et al.*,[7] for the formation of imide from NaOH and β-benzyl-N-carbobenzoxy-L-aspartyl-methylamide in dioxane–water. This comparison is probably not very significant because of the catalytic nature of the polymer reaction and the difference in solvents.

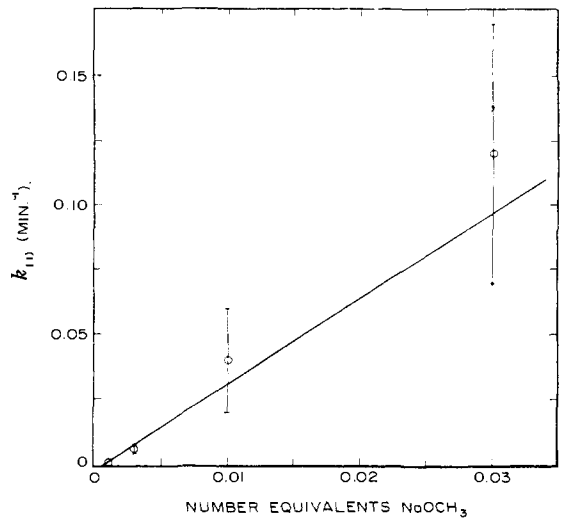


Fig. 2.—Effect of catalyst concentration upon first-order rate constant for poly-succinimide formation from L-PBA in DMSO.

Mechanism.—Sondheimer and Holley[8] and Bernhard, *et al.*,[7] have proposed a mechanism for the reaction through which small peptides containing esterified aspartyl residues react with base to form imides. The first step is the abstraction of the amide proton by a base; the second step is the attack of the amidate ion upon the aspartyl carboxyl, with subsequent formation of a succinimide ring. The same type of mechanism may well apply to poly-succinimide formation from L-PBA. Although methoxide ion would be depleted in the first step, regeneration of the alkoxide-type catalyst in the polymer reaction could be effected if $C_6H_5CH_2O^\ominus$ were formed as a product of the reaction.

D. Reaction of Poly-L-succinimide with an Equimolar Amount of Anhydrous Base.—When anhydrous $NaOCH_3$, in excess of the amount required to form the poly-imide, was added to PBLA in DMSO or in DMF, a stoichiometric quantity of an uncharacterized, low molecular-weight compound was formed. Addition of more than one residue-equivalent of base did not affect the nature of this reaction. Its infrared spectrum (Table I) showed that the product contained a COO^- group (which formed COOH upon acidification) but no amide linkage; a negative ninhydrin reaction revealed the absence of amine groups. The compound was dark red in basic solution, and had absorption bands (480, 383, 300 mμ) identical to those for the product of N-*n*-amylsuccinimide plus $NaOCH_3$ in DMSO. Viscometry and chromatography showed the compound, which may be an oxidation product, to be largely degraded.

Formation of Sodium D,L-2-Pyrrolidone-5-Carboxylate From Poly-γ-Benzyl-L-Glutamate by Use of Equimolar Amounts of Base

In some hydrolytic enzymes a glutamyl residue instead of an aspartyl is found adjacent to the active serine. For this reason we were interested in determining whether the debenzylation of L-PBG could be a catalytic reaction like that of L-PBA, and whether cyclic compounds were formed during the reaction in the same solvents.

No solvent was found in which L-PBG could be debenzylated with only catalytic quantities of base. When larger amounts of $NaOCH_3$ were added to L-

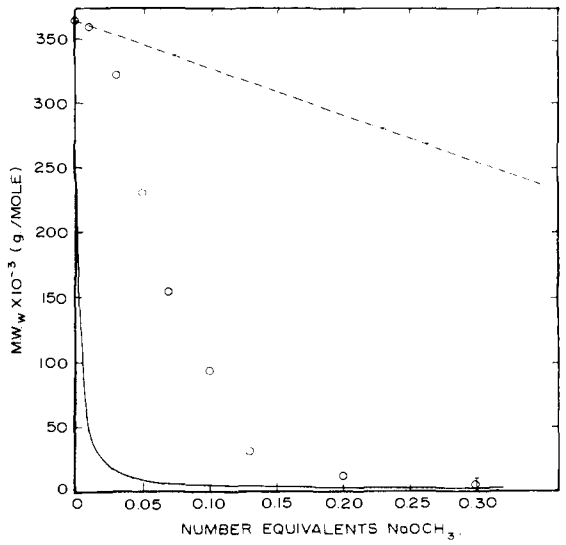
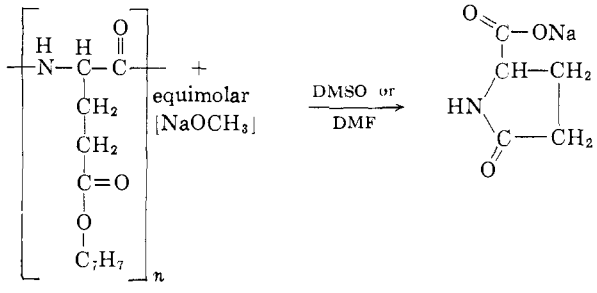


Fig. 3.—Depolymerization of L-PBG during pyrrolidonecarboxylate formation in DMSO; effect of base concentration: ———, calculated mol. wt.$_w$, assuming random cleavage; - - - -, calculated mol. wt.$_w$, assuming that only terminal residues are split off; ⊙, experimental points. The number of equivalents of base equals the extent of reaction.

PBG in DMSO or in DMF, debenzylation proceeded with rapid (less than 5 minutes) formation of the ring compound sodium D,L-2-pyrrolidone-5-carboxylate in stoichiometric quantities, until the addition of a full molar equivalent of base resulted in a completed reaction



This ring formation was not a general reaction in all solvents; in chloroform L-PBG, like L-PBA, was simply debenzylated by equimolar $NaOCH_3$.

Monomeric pyrrolidone-carboxylate was not an anticipated product. The expected product from ring formation was one of the two possible polymers, either poly-glutarimide or poly-acylpyrrolidone; the pyrrolidone ring could be predicted to be the more stable.[24] However, Battersby and co-workers[11] have shown that an acyl-pyrrolidone, once formed, could then be split by base at either the cyclic or the non-cyclic amide bond, depending upon the polarity of substituents on the acyl–carbon atom. The latter type of split could produce pyrrolidone-carboxylic acid.

A. Evidence for the Product.—When a slight molar excess of $NaOCH_3$ was added to L-PBG in DMSO or in DMF the resultant reaction mixture had an infrared spectrum identical to that of an authentic sample of the sodium salt of L-2-pyrrolidone-5-carboxylic acid (see Table I). The spectrum was not altered by isolation of the product; thus, only one compound was formed in the reaction. The spectrum was quite different from those reported[13] for either of the two polymeric ring compounds which could be derived from L-PBG. Elemental analysis of the isolated product, its ready solubility in water, its negative color test with ninhydrin and positive test with tolidine, and its reactions with HCl and with NaOH to form –COOH and $-COO^{\ominus}$, respectively (see Table I), were all to be expected of pyrrolidonecarboxylic acid. Its R_f value on paper chromatography, 0.73, was identical to that of a sample of the L-acid. The melting point and optical rotation of the acid form of the reaction product showed that it was D,L-2-pyrrolidone-5-carboxylic acid (m.p. 179°, $[\alpha]_D$ 0),[25] and not the L-isomer (m.p. 162°, $[\alpha]_D$ −11.5°, c 1% in water).[25]

B. Discussion of the Reaction. Racemization and Chain Scission.—A sample of L-pyrrolidonecarboxylic acid (with $[\alpha]^{24}D$ −11.2°) was carried through the same procedure used for the preparation and isolation of the reaction product (see Experimental). The acid thus treated had $[\alpha]^{24}D$ −10.9°, which showed that only negligible racemization took place during the isolation and purification procedure. Therefore, the racemization of the α-carbon atom of L-PBG must actually occur during the cyclization reaction with $NaOCH_3$; D,L-pyrrolidonecarboxylic acid is the initial product of the reaction, and is not formed from subsequent racemization of the L-acid.

The experiments to be described now were designed to study the depolymerization of L-PBG during its reaction with $NaOCH_3$ to form pyrrolidonecarboxylate. We wished to see whether chain cleavage proceeded in a random manner, or whether the terminal residues of L-PBG were unusually susceptible to attack by base. The method consisted of measuring the viscosity of 0.2% solutions of L-PBG (sample GF-D-28-5) in DMSO after adding small amounts of $NaOCH_3$ (0.01 to 0.3 mole equivalent) to cause partial reaction. Infrared spectra showed that pyrrolidonecarboxylate was formed in stoichiometric quantities, and control experiments proved that the small volumes of benzene-methanol added with the base as solvent had, by themselves, no effect upon the viscosity of the polymer.

Five samples of L-PBG, ranging in molecular weight from 14,000 to 363,000, were employed for a calibration of mol. wt.$_w$ as a function of viscosity in DMSO. The molecular weights were calculated from viscosity in dichloroacetic acid.[16] A linear graph was obtained for log mol. wt.$_w$ *vs.* log η reduced specific with L-PBG in DMSO. The calibration was nearly identical to that for helical L-PBG in DMSO.

The data collected from the addition of limited amounts of $NaOCH_3$ are displayed in Fig. 3. The calculated values for mol. wt.$_w$ were obtained by utilizing the knowledge[26] that the L-PBG sample used had originally a nearly Poisson mol. wt. distribution. Figure 3 shows that residues are certainly not cleaved only from the ends of the polymer chain during pyrrolidone-ring formation. This conclusion implies that a reacting glutamyl residue can exert no strong cooperative effect upon neighboring residues to help catalyze their debenzylation. However, the location of chain scission (and, hence, of ring formation) does not appear to be purely random; bonds near the ends of the helical L-PBG chain may be somewhat more reactive toward $NaOCH_3$ attack than are internal residues.

Mechanism.—Several compounds will be considered which are *a priori* possible intermediates in the reactions of L-PBG to form pyrrolidonecarboxylate; neither sodium glutamate nor sodium polyglutamate could be

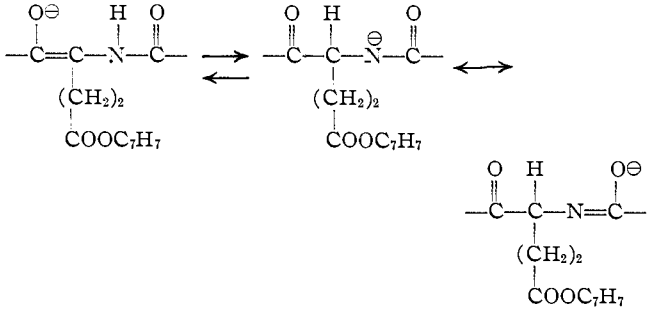
(24) H. C. Brown, J. H. Brewster and H. Schechter, *J. Am. Chem. Soc.*, **76**, 467 (1954).

(25) I. Heilbron and H. M. Bunbury (Ed.), "Dictionary of Organic Compounds," Eyre and Spottiswoode, Ltd., London, 1953, Vol. 11, p. 607.

(26) P. Doty and J. T. Yang, unpublished results.

an intermediate since (i) both compounds were insoluble in a mixture of DMSO and an equivalent of $NaOCH_3$ (and yet no precipitate was noticed in the reaction mixture) and (ii) neither compound reacted with base in DMSO to form a pyrrolidone ring (as shown by infrared). Poly-acylpyrrolidone could well be an intermediate, although there was no direct evidence for it. If formed, this polymer was cleaved to the monomer within 5 minutes, for its infrared spectrum[13] was not observed.

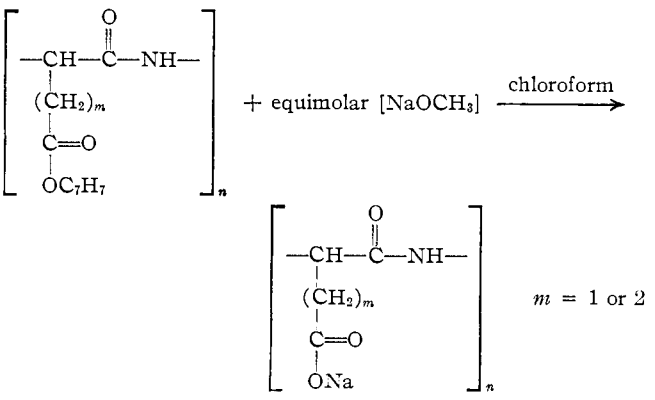
Any mechanism given for this reaction of L-PBG will have to account not only for ring formation and chain cleavage, but also for the racemization of the α-carbon atom during formation of pyrrolidone-carboxylate. For such racemization to occur, the H would have to be removed from the α-carbon atom, perhaps through the formation of a double bond involving this C. One way of attaining this sort of intermediate is through abstraction of a hydrogen ion by $OCH_3^{\ominus}$, and stabilization of the resulting charge by means of



The negative nitrogen could then attack the ester carbonyl –C to form the pyrrolidone ring. Additional mechanisms may be operative in the racemization, as is evidenced by the exceptionally facile racemization of L-glutamic acid itself.[27]

Effect of Various Solvents upon the Course of the Debenzylation Reactions

A. De-esterification of L-PBA and L-PBG in Chloroform.—The base-catalyzed debenzylation of L-PBA and L-PBG in other solvents was not always accompanied by ring formation as in DMSO and in DMF. The reaction of both polymers in chloroform was one of simple de-esterification, yielding sodium polyaspartate and glutamate



The infrared spectral evidence is given in Table I. A full equivalent of $NaOCH_3$ was required for complete debenzylation of both polymers; no reaction occurred in chloroform with catalytic quantities of base, even in the case of L-PBA.

Another way in which debenzylation in chloroform differed from that in DMSO or DMF is that in chloroform there was little, if any, depolymerization. A sample of L-PBG (mol. $wt._w$ 64,000) was de-esterified in chloroform, and the resulting sodium polyaspartate was dissolved (0.2%) in dichloroacetic acid. The mol. $wt._w$ of this acidified reaction product, calculated from the measured viscosity, was about 55,000, provided that the same calibration[16] is valid for polyglutamic acid as for L-PBG. Presumably, chain cleavage was negligible also for L-PBA, since the reactions were so similar in chloroform.

The presence of water in the chloroform reaction mixture (1% water by volume, more than enough to saturate the solvent) was found to cause no change either in the product from chloroform debenzylation of L-PBG or in the essential lack of polymer–chain cleavage. These results show that the presence of a trace of water in a solvent is, by itself, insufficient as a possible explanation for ring formation or chain scission during debenzylation. Therefore, the unusual reactions found in DMSO and in DMF cannot be attributed to water impurities in these hygroscopic solvents; actual properties of the solvents must be responsible.

Many reactions are known which vary in rate upon change of solvent. The base-catalyzed de-esterification of L-PBA and L-PBG is seen to be a case in which the entire course of the reaction is dependent upon the solvent used.

B. Effect of Polymer Conformation upon Reaction Course.—In the next two sections consideration will be given to those differences between solutions of L-PBA and L-PBG in chloroform on the one hand and in DMSO on the other hand which might possibly result in the observed differences in the debenzylation reactions. Polymer conformation is one such solvent-dependent property; a property which will now be shown to lack any correlation with the reaction products in the various solvents.

In Table II are summarized the conformations of the polypeptides in several solvents, along with the products of their reactions with $NaOCH_3$ in the same solvents. All conformations were inferred from optical rotatory dispersion data by means of Moffitt and Drude plots. These methods are discussed in a recent review.[28] The data for all solutions in which the polymers were randomly coiled could be fitted by a single-term Drude equation. The helical conformation of L-PBG in DMSO was confirmed also by viscosity data.

The lack of dependence of reaction course upon conformation is apparent. Special attention may be given to the entry in Table II for L-PBA in pyridine; in this solvent the polymer assumed a random coil conformation, as in DMSO and in DMF, and yet did not react with catalytic amounts of base to yield polysuccinimide. L-PBG is seen to be helical in DMSO, DMF and chloroform; however, the course of the debenzylation reaction was very different in the first two solvents from the course in the third.

As has already been noted,[14] L-PBA forms a structurally weaker helix than does L-PBG. This difference is confirmed by the finding that L-PBA has a random conformation in DMSO and in DMF whereas L-PBG is helical. Both of these solvents are capable of forming hydrogen bonds only with the —NH— of the polypeptides, not with the —C=O—. Since intramolecular peptide hydrogen bonds are important in stabilizing the helical conformation of polypeptides, perhaps the disrupting influence of intermolecular

(27) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p. 1950.

(28) E. R. Blout, chapter in "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

TABLE II

EFFECT OF SOLVENT UPON CONFORMATION AND REACTION PATH

Polymer	Solvent	b_0[a]	Conformation	Product formed in reaction with $NaOCH_3$: Catalytic [$NaOCH_3$]	Equimolar [$NaOCH_3$]
L-PBA	DMSO	-110 ± 40	Random coil[b]	Poly-succinimide	(Carboxylate compound)
L-PBA	DMF	-280 ± 15	Random coil[b]	Poly-succinimide	(Carboxylate compound)
L-PBA	Pyridine	0 ± 20	Random coil[b]	No reaction	...
L-PBA	DCA, TFA[c]	-260[d]	Random coil[d]	...	...
L-PBA	Chloroform	$+630$[d]	Helix[d]	No reaction	Sodium polyaspartate
L-PBG	DMSO	-610 ± 20	Helix[b]	No reaction	Sodium pyrrolidonecarboxylate
L-PBG	DMF	-630[e]	Helix[f]	No reaction	Sodium pyrrolidonecarboxylate
L-PBG	Chloroform	-670[e]	Helix[f]	No reaction	Sodium polyglutamate
L-PBG	DCA, TFA[c]	0[e]	Random coil[f]	...	...

[a] Units for b_0: degree cm.^{2} decimole^{-1}; limits of error obtained graphically. [b] Conformations inferred from b_0 and from plot of $[\alpha]\lambda^2$ *vs.* $[\alpha]$. [c] Abbreviations: DCA dichloroacetic acid, TFA trifluoroacetic acid. [d] From ref. 14. [e] From ref. 18. [f] From ref. 16.

solvent —HN— hydrogen bonds is sufficient to destroy the L-PBA helix but not the stronger L-PBG helix.

Hydrazine was considered as another solvent for debenzylation, but L-PBA was found to react with hydrazine, probably to form the hydrazide, even in the absence of $NaOCH_3$.

C. Solvent Properties Affecting the Debenzylation Reactions:—The question may be asked: What factors cause L-PBA and L-PBG to be simply debenzylated by $NaOCH_3$ in chloroform, but to undergo the more unusual reactions (ring formation, chain cleavage and racemization in the case of L-PBG, a catalytic reaction in the case of L-PBA) with the same reagent in DMSO or DMF? Several properties of the solvents can be eliminated from consideration: (i) Solvent-dependent conformation of the polypeptides (see above). (ii) Basicity: DMSO and pyridine are both weakly basic, and yet catalytic imide-formation from L-PBA proceeded in one solvent but not in the other. Furthermore DMF, like chloroform, is neutral but allowed the catalytic reaction. (iii) Dielectric constant: acetonitrile ($\epsilon = 39$) is approximately as polar as DMSO ($\epsilon = 45$), and yet failed to promote any reaction between the polyesters and $NaOCH_3$.

One solvent property which may play a role in the ring-forming reactions is the ability of DMSO and of DMF to hydrogen bond to the NH of peptide groups. DMSO[29,30] and DMF[30] have both been found to be effective in greatly increasing the rates of nucleophilic displacement reactions. Perhaps more relevant to the present investigation is the fact that DMSO enhanced also the rate of a base-catalyzed proton-abstraction reaction.[31] In all of these cases the accelerating ability of the solvents was attributed to their specifically solvating only the cation of the added basic catalyst, leaving the anion (for example $OCH_3^{\ominus}$) free to react. These reports all involve changes in reaction *rates* due to specific solvation effects of DMSO and of DMF. However, the present study is concerned with actual changes in reaction *products*. Whether the same solvation properties are operative here is not clear. Perhaps the ring-forming reactions really occur slowly even in chloroform, and the role of DMSO or DMF may be to accelerate these reactions until they compete successfully with the normal debenzylation.

Fuchs, *et al.*,[29] have given evidence for an additional mechanism involving nucleophilic attack by DMSO on the substrate of the reaction, followed by displacement of the DMSO by an added nucleophilic reagent. Such a mechanism cannot be significant in the reactions of the polypeptides with base: The kinetic data show that $NaOCH_3$ is involved in the rate-determining step of polyimide formation. If a L-PBA sulfonium complex were part of the mechanism, the complex would therefore have to accumulate in DMSO solution before the addition of $NaOCH_3$. But infrared spectra show only unchanged L-PBA even after several hours in DMSO (refer to Table I).

Thus, DMSO operates only *via* some specific solvation effect in the polypeptide reactions under consideration here.

Conclusions

The most striking result of the present investigation is that in certain solvents (DMSO and DMF) L-PBA undergoes debenzylation and cyclizes to form poly-L-succinimide upon the addition of only catalytic amounts of strong base. The catalytic nature of this reaction suggests that a reacting aspartyl residue may be able to assist in breaking the ester bond of the neighboring residue in the polypeptide. This cooperative effect may be related to the role of the dibasic amino acid residue found at the active site of some hydrolytic enzymes.

A somewhat more realistic model for such enzymes is a 1:1 copolymer of O-acetyl-L-serine and β-benzyl-L-aspartate. Preliminary infrared absorption studies have shown that catalytic amounts of $NaOCH_3$ in DMSO cause complete de-esterification of both the aspartyl and seryl groups in this copolymer. The reaction is accompanied by imide formation. This finding makes even more likely the suggestion that aspartyl residues are capable of catalyzing the hydrolysis of adjacent O-serine esters, (as in an acyl-enzyme intermediate). Furthermore, there has been a report that the O-acetylserine ester bond in a copolymer with aspartic acid is hydrolyzed more rapidly than the same bond in a homopolymer.[32]

Since glutamic acid is found instead of aspartic acid at some active sites, one might expect glutamyl polymers to undergo reactions similar to those of aspartyl polymers. However, this research has shown that L-PBG behaves quite differently from L-PBA in its reaction with base in DMSO or in DMF. The glutamate polymer reacts only with stoichiometric amounts of $NaOCH_3$, and yields sodium D,L-2-pyrrolidone-5-carboxylate. This difference in reactions may reflect structural differences between the two polypeptides. Surface-film studies[33] have shown that L-PBG and L-PBA exist in different conformations as monolayers on an aqueous phase. Optical rotatory dispersion has led to the conclusions[14] that the sense of the helix in L-PBA is opposite to that in L-PBG, that the former helix is weaker, and that the ester group of L-PBA may not rotate freely.

Pronounced solvent effects were found which altered

(29) R. Fuchs, G. E. McGrary and J. J. Bloomfield, *J. Am. Chem. Soc.*, **83**, 4281 (1961).

(30) H. E. Zaugg, B. W. Horrom and S. Borgwardt, *ibid.*, **82**, 2895, 2903 (1960).

(31) D. J. Cram, B. Rickborn and G. R. Knox, *ibid.*, **82**, 6412 (1960).

(32) M. Sela and E. Katchalski, *Adv. Protein Chem.*, **14**, 420 (1959).

(33) S. Ikeda and T. Isemura, *Bull. Chem. Soc., Japan*, **34**, 416 (1961).

the course of the debenzylations of L-PBA and of L-PBG. Perhaps such solvent effects have a counterpart in the reactions of enzymes, where amino acid side-chains of residues near the active site may exert an influence upon reactions at the active site by determining the environment at the site.

[CONTRIBUTION FROM THE GORGAS LABORATORY, ROHM & HAAS COMPANY, REDSTONE ARSENAL RESEARCH DIVISION, HUNTSVILLE ALA.]

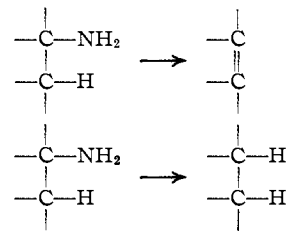
Deamination Reactions of Difluoroamine

BY CARL L. BUMGARDNER, KENNETH J. MARTIN AND JEREMIAH P. FREEMAN

RECEIVED SEPTEMBER 7, 1962

Difluoroamine (HNF_2) converts primary aliphatic amines to alkanes. Nitrogen and alkylammonium fluorides constitute the other major products. The secondary amines, aziridine, azetidine and dibenzylamine react with difluoroamine to yield, respectively, ethylene, cyclopropane and bibenzyl. Butene-1 is obtained from reaction of difluoroamine and cyclopropylcarbinylamine and from treatment of N-cyclopropylmethyl-*p*-toluenesulfonamide with hydroxylamine-O-sulfonic acid in aqueous base. Difluoroamine induces fragmentation of 3,5,5-trimethylpyrazoline into acetonitrile, isobutylene and nitrogen. These transformations are rationalized by postulating the formation of fluorazene (NF), which subsequently leads to intermediates of the type RN=NH and $R_2\overset{+}{N}=\overset{-}{N}$.

Removal of an amino group from an aliphatic carbon atom may be accomplished by applying one of several E_1 or E_2 olefin-forming elimination reactions[1,2], eq. 1, or by a reductive deamination process which results in a saturated hydrocarbon,[3] eq. 2. The only general method for effecting this latter transformation involves



(1)

(2)

conversion of a primary amine to a sulfonamide derivative which is then treated with hydroxylamine-O-sulfonic acid in aqueous base to generate the alkane.[3]

We have observed that difluoroamine[4] functions as an efficient and direct deaminating reagent for aliphatic and aromatic primary amines and certain secondary amines. The over-all reaction with primary amines may be represented by eq. 3.

$$3RNH_2 + HNF_2 \longrightarrow 2R\overset{+}{N}H_3\overset{-}{F} + N_2 + R\text{-}H \quad (3)$$

Representative examples are collected in Table I.

TABLE I

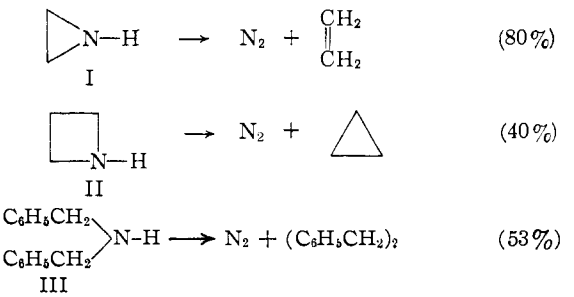
$$RNH_2 \xrightarrow{HNF_2} RH$$

Nature of R	Yield of alkane,[a] %
n-Butyl	61
sec-Butyl	40
t-Butyl	22
Cyclopropyl	77
Cyclopropylmethyl	46[b]
Phenyl	20

[a] Based on HNF_2. Yields are not necessarily the optimum obtainable; see Experimental. [b] Product is butene-1; see Discussion.

The reactions were conducted in a glass vacuum system by condensing difluoroamine into an excess of amine. Volatile products were purified by bulb-to-bulb distillation and identified by infrared and mass spectrometry. Elemental analyses, infrared and F^{19} n.m.r. spectra showed the solid product obtained to be alkylammonium fluoride. Examination of the infrared spectra of the less volatile liquid fractions revealed that small amounts of alkyl azide were usually present. In the case of *t*-butylamine, traces of isobutylene and ammonia were also detected.

When treated with difluoroamine, aziridine (I), azetidine (II) and dibenzylamine (III) released nitrogen and the remaining fragments coupled to form ethylene, cyclopropane and bibenzyl, respectively.



(80%)

(40%)

(53%)

Discussion

The primary amine transformations may be rationalized in terms of intermediate IV, which, in turn, may arise by the sequence

R—N=N—H

IV

Amine-promoted α-elimination of the elements of hydrofluoric acid from difluoroamine can yield fluorazene,[5] NF, reminiscent of the formation of NH by elimination of sulfuric acid from hydroxylamine-O-sulfonic acid[6] and formation of dihalocarbene from haloforms.[7] Attack by the electrophilic azene on nucleophilic amine,[8] followed by a proton shift and loss of the elements of hydrofluoric acid, would produce structure IV. This intermediate, identical to that proposed by Nickon and Sinz[3] to arise from elimination of sulfinic acid from alkyl sulfonhydrazides, would be expected to proceed readily to hydrocarbon and nitrogen. A portion, however, may also act as a trap for fluorazene and, after loss of HF, yield azide.

$$RN_3 \longleftarrow IV \longrightarrow N_2 + R\text{—}H$$

The ammonia and isobutylene observed from reaction of *t*-butylamine and difluoroamine probably arise from direct elimination of ammonia from the aliphatic amine. Ammonia and isobutylene were also obtained when

(1) E. H. White and H. Scherrer, *Tetrahedron Letters*, 758 (1961).
(2) A. C. Cope and E. R. Trumbull, *Org. Reactions*, **11**, 317 (1960).
(3) A. Nickon and A. Sinz, *J. Am. Chem. Soc.*, **82**, 753 (1960).
(4) J. P. Freeman, A. Kennedy and C. B. Colburn, *ibid.*, **82**, 5304 (1960).
(5) For a discussion of nomenclature, see P. A. S. Smith and J. H. Hall, *ibid.*, **84**, 480 (1962).
(6) For examples of this type of reaction, see C. L. Bumgardner and R. L. Lilly, *Chemistry and Industry*, 559 (1962).
(7) J. Hine and R. J. Rosscup, *J. Am. Chem. Soc.*, **82**, 6115 (1960).