oxalic acid dihydrate in 1 ml of ethanol. The mixture was centrifuged, and the precipitate washed with ethanol (two 1-ml portions) and crystallized from methanol (7 ml) to give 72 mg of II as white needles identical with the crystalline dihydrogen oxalate, prepared as above from natural[10] O-methylpsychotrine: mp 161–163° (effervescence),[19] no mixture melting point depression; tlc (system A), single spot at R_f 0.88 for both synthetic and natural II; optical rotation for synthetic II: $[\alpha]^{20}D$ +39.8°, $[\alpha]^{20}_{578}$ +42.1°, $[\alpha]^{20}_{546}$ +50.0°, $[\alpha]^{20}_{436}$ +133.0°, $[\alpha]^{20}_{365}$ +133.0° (*c* 2, H_2O); optical rotation for natural II:[19] $[\alpha]^{20}D$ +37.3°, $[\alpha]^{20}_{578}$ +39.7, $[\alpha]^{20}_{546}$ +47.6°, $[\alpha]^{20}_{436}$ +130.1°, $[\alpha]^{20}_{365}$ +130.1° (*c* 2, H_2O).

Hydrolysis of O-Methylpsychotrine According to Brindley and Pyman.[5] To 239 mg (0.5 mmole) of natural[10] O-methylpsychotrine in 0.0275 ml (0.5 mmole) of concentrated sulfuric acid and 0.063 ml of water[20] was added 0.049 ml (0.0585 mmole) of concentrated hydrochloric acid, and the mixture was heated in a sealed tube at 170° for 6 hr. The yellow solution was diluted with water (2 ml), concentrated ammonium hydroxide (2 ml) was added, and the precipitate was filtered to give 107 mg of crude as a yellow amorphous solid. Tlc (system A) showed a complex mixture containing nine distinct spots; three of these were individually removed from the plate and separately rechromatographed on plates with pure synthetic samples and thus identified as O-methylpsychotrine (II), psychotrine (I), and 6′-O-methyl-7′-desmethylpsychotrine (XIII), respectively. To a 40-mg aliquot of the above crude isolate dissolved in ethanol (3.5 ml) was added excess oxalic acid dihydrate. The precipitate was removed by filtration and crystallized from methanol to give 28 mg, mp 128–138°, with the same complex mixture on tlc as exhibited by the initial crude isolate.

(19) Reference 9: mp 161–163°, $[\alpha]^{23}D$ +41° (*c* 2.0, H_2O), for dihydrogen oxalate of natural O-methylpsychotrine.

(20) Brindley and Pyman[5] used O-methylpsychotrine sulfate heptahydrate for their hydrolysis experiment.

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Accelerated Polymerization of N-Carboxyamino Acid Anhydrides in Frozen Dioxane[1]

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Abstract: Polymerization of the N-carboxyamino acid anhydrides (NCA's) of phenylglycine, phenylsarcosine, glycine, and several alicyclic amino acids was studied in liquid and frozen dioxane in the presence and absence of added initiator. Rates of NCA reaction were at least 10 times as high in frozen solutions between +5 and −26° as in liquid systems. Solubility, infrared, and end-group analyses confirmed the formation of polyamino acids. The NCA solubilities, the inverse relationship between concentration and the fraction of NCA molecules reacting, and the apparent absence of adventitious catalysts suggest the possible importance of juxtaposition and alignment in the frozen matrix.

Polymerization of N-carboxyamino acid anhydrides (4-substituted 2,5-oxazolidinediones, NCA's) has added much to our understanding of protein configuration.[2] The reactions are carried out in bulk at elevated temperatures or, more frequently, in inert solvents in the presence of initiators. The possibility of a new approach to the synthesis of polyamino acids from NCA's was suggested by the rate increases observed on carrying out a number of bimolecular reactions in frozen solutions[3–5] and by the synthesis in ice of poly-6-aminopenicillanic acid.[6] This paper reports a study of the polymerization of several NCA's in frozen dioxane solutions. These represent NCA's which polymerize readily in the presence of an initiator and a less thoroughly studied group whose members show unusual stability.

Experimental Section

Most of the experiments were carried out with no addition of an initiator. It was therefore important for quantitative reproducibility to avoid the adventitious introduction of initiators with the anhydride or solvent. The anhydrides were synthesized, recrystallized, and analyzed as described previously.[7,8] The *p*-dioxane used was Matheson Coleman and Bell Spectroquality with an analysis of 99+% and an infrared spectrum showing no bands near 3300 cm^{-1}. A new bottle, generally from the same lot, was opened for each experiment; our determination showed a sharp freezing point at 11.8°, the same value as that reported by Grubb and Osthoff[9] and by Teague and Felsing.[10]

In one of the runs described below, initial freezing was carried out in a Dry Ice–acetone bath, and the tubes were then transferred to various constant-temperature chambers. In all other runs at temperatures below the freezing point, the solutions were initially frozen rapidly in an ice–salt mixture at −11°. (With the NCA of 1-aminocyclobutanecarboxylic acid, rate constants at −11°

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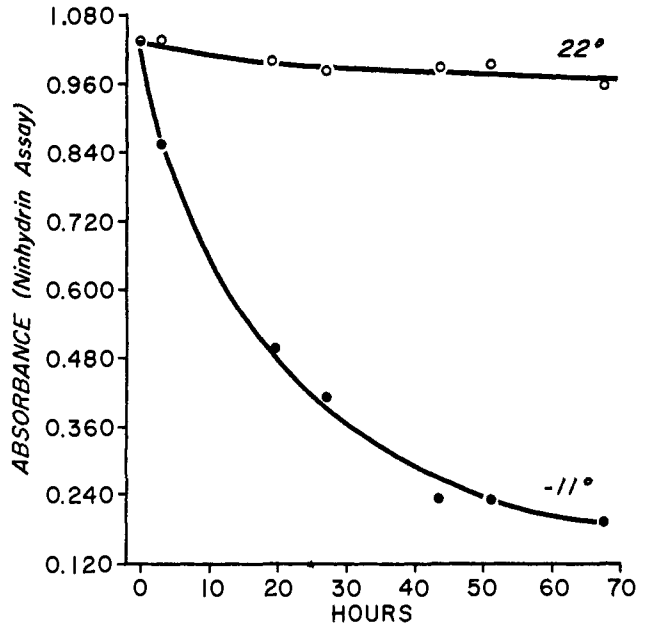

Figure 1. Polymerization of D-phenylglycine NCA; initial concentration, 0.056 *M*; assay for NCA by ninhydrin method.

agreed within 3% for systems frozen in Dry Ice–acetone and systems placed unfrozen in the −11° room.) The reaction volume was always 1 ml, permitting temperature equilibrium to be attained rapidly. Samples were thawed in a room temperature bath, usually in the presence of hydroxylamine, and mixed on a Vortex mixer.

Unreacted NCA's were routinely assayed by formation and determination of the hydroxamic acid. For the NCA's of glycine, D-phenylglycine, and DL-phenylsarcosine, this procedure is carried out at pH 5.0 as follows. To 1 ml of sample there is added 2 ml of a solution containing 0.25 *M* $NH_2OH \cdot HCl$, 0.16% NaOH, and 0.125% potassium acetate; after at least 15 min there is added 2 ml of a solution containing 15% ferric ammonium sulfate in 1.0 *N* H_2SO_4, the system is centrifuged for 5 min, and the color is read at 540 mμ. For the NCA's of the 1-aminocycloalkylcarboxylic acids the procedure of Niedermayer, *et al.*,[11] employing hydroxylaminolysis at neutrality, was followed. In one experiment, unreacted NCA was assayed by a ninhydrin method, consisting of acidification with 1 volume of 0.5 *N* HCl, dilution, and color formation, and determination by the method of Rosen.[12]

Infrared spectra were obtained from the solids using the Perkin-Elmer Model 21 spectrophotometer and KBr disks. Titrations of acid and basic groups were carried out in dimethylformamide by the methods of Sela and Berger.[13]

Results

N-Carboxy Anhydrides of Phenylglycine, Phenylsarcosine, and Glycine. Figure 1 shows the striking facilitation by freezing of the polymerization of D-phenylglycine NCA. This anhydride is highly susceptible to polymerization in the presence of low concentrations of an initiator,[8,14] and failure to react in 70 hr at room temperature indicates the absence of traces of initiators.

The data plotted in Figure 1 represent changes in the anhydride concentration as determined by conversion to the free amino acid followed by quantitative ninhydrin assay. At −11°, the reaction, assayed in this way, was first order up to the disappearance of 78% of the NCA, and the rate constant was 5.81×10^{-4} min^{-1}. Similar experiments, using a higher concentration of D-phenylglycine NCA (0.1 *M*) and assayed by hydroxamic acid formation, also showed no reaction occurring at 22° but significant rates in frozen solutions; first-order constants [$10^4 k_{obsd}$ (min^{-1})] were: 3.71 at 5°, 4.39 at 1°, 2.65 at −11°, and 1.87 at −18°.

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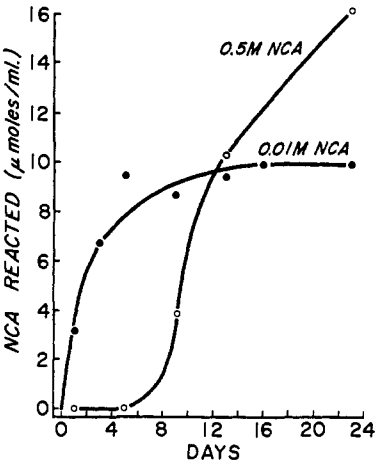

Figure 2. Polymerization of 1-aminocyclopentanecarboxylic acid NCA at −11°.

The NCA's of DL-phenylsarcosine and glycine also reacted much faster in frozen than in liquid dioxane. Portions of a 0.01 *M* DL-phenylsarcosine NCA solution were stored at −11° (after freezing in a Dry Ice–acetone bath) and at 22°. Both systems gave clean first-order kinetics throughout the 3-hr run, during which 66% of the NCA reacted at −11°. The rate constants were $k_{22} = 3.46 \times 10^{-4}$ min^{-1} and $k_{-11} = 59.4 \times 10^{-4}$ min^{-1}, with $k_{-11}/k_{22} = 17$.

A glycine NCA solution (0.003 *M*) showed the following levels of disappearance after 5 hr: 2% at 22°, 22% at 5°, 97% at −11°, 90% at −18°, 66% at −26°, and 13% at −76°. Kinetic analyses showed half-lives of 4.4 hr at −26°, 2.9 hr at −18°, 2.1 hr at 1°, and approximately 40 hr at +22°.

N-Carboxy Anhydrides of 1-Aminocyclopentanecarboxylic Acid, Cycloleucine, and Other Alicyclic Amino Acids. In 48 hr, none of the cycloleucine NCA (0.01 *M*) reacted at 22°, but during the same period considerable amounts reacted in frozen solutions: 35% at 5°, 41% at −11°, and 27% at −18°.

A marked discrepancy also appeared in the fraction of cycloleucine NCA at different initial concentrations which reacted in frozen dioxane. Figure 2 shows a typical example in which reaction began immediately at low levels but only after a lag at higher levels. In 5 days, at −11°, the following amounts of various concentrations of NCA reacted: 0.5 *M*, 0%; 0.25 *M*, 0%; 0.1 *M*, 4%; 0.05 *M*, 3%; 0.01 *M*, 95%. The situation in liquid dioxane was entirely different. With molarities of 2.0, 1.4, 1.0, 0.5, 0.1, 0.05, or 0.01, no reaction at all occurred within 4 days at 22°. Moreover, both 0.25 and 0.005 *M* NCA failed to show any polymerization within 5 days at 50°.

Exactly the same relationship between concentration and fraction of NCA undergoing reaction appeared with frozen solutions of 1-aminocyclobutanecarboxylic acid NCA: at −24°, 99% of the NCA in a 0.005 *M* solution reacted in 5 days, while only 3% of the NCA in a 0.125 *M* solution reacted in 9 days. In a liquid solu-

tion heated at 50° for 5 days, a 0.005 *M* solution of this NCA reacted to the extent of only 13%.

In another experiment over a period of 5 days, the NCA's of 1-aminocyclobutanecarboxylic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclohexanecarboxylic acid, and 1-aminocycloheptanecarboxylic acid (initial concentration, 0.005 *M*) all remained intact at 22°. However, polymerization occurred at −12.5°, following excellent first-order kinetics throughout the experimental period (complete reaction in the case of the cyclobutane compound). The rate constants [$10^5 k_{obsd}$ (min^{-1})] for the NCA's containing various size rings were: C_4, 20.8; C_5, 2.50; C_6, 2.69; and C_7, 3.26. Thus, the smallest alicyclic NCA reacted fastest, but the compounds with five-, six-, and seven-membered rings reacted at about the same rate.

The Addition of Initiators. Marked acceleration occurred in frozen dioxane with, as well as without, added initiator. Isobutylamine and triethylamine gave similar results when added to 0.01 and 0.2 *M* cycloleucine NCA at an NCA:amine ratio of 20. Figure 3 shows the results with isobutylamine. In frozen solutions (−11°), a much higher fraction of NCA reacted in a given time at the lower initial concentration; this contrasted with the situation in the liquid solution (22°) where the fraction reacting in a given time at the higher concentration exceeded that at the lower concentration.

The NCA of 1-aminocyclobutanecarboxylic acid (0.125 *M*) was stored at 22 and −11° with 0.025 and 0.005 *M* isopropylamine. The reactions at 22° were very slow and gave poor kinetics; at the higher amine level 61% of the NCA remained intact after 4 hr, and at the lower amine level 63% of the NCA remained intact after 4 days. However, in the frozen solutions the reactions were much faster and gave good first-order kinetics to 75% completion; the rate constant for 0.025 *M* amine was 19.0×10^{-3} min^{-1}, while that for 0.005 *M* amine was 6.03×10^{-3} min^{-1}.

Characterization of the Products. All of the NCA's in this study led, on freezing, to dioxane-insoluble products. The derivatives of D-phenylglycine, cycloleucine, and 1-aminocyclobutanecarboxylic acid were examined by infrared analysis and by titration of their free carboxyl and amino groups.

Each preparation showed intense absorptions at 3320–3250, 1670–1650, and 1540–1510 cm^{-1}. Each showed weak absorptions at 3100–2920 and 1470–1450 cm^{-1}. Spectra of D-phenylglycine polymers prepared in frozen dioxane and D-phenylglycine polymers prepared in various liquid solvents in the presence of initiators disclosed many similarities: NH stretching bands near 3320 and 3100 cm^{-1}, the amide I band near 1670 cm^{-1}, the amide II band near 1510 cm^{-1}, and a CH deformation band near 1470 cm^{-1}. Resemblances also appeared in the "fingerprint" region: a doublet at 1240–1170 cm^{-1} and other bands near 1035, 735, and 700 cm^{-1}.

None of the dioxane-insoluble products of freezing showed the infrared absorptions characteristic of their precursors: NH_3^+ bands near 2900 and 200 cm^{-1} and COO^- bands near 1610 and 1400 cm^{-1}, present in the spectra of the amino acids; and a CO stretching doublet at 1860–1780 cm^{-1}, present in the spectra of the NCA's.

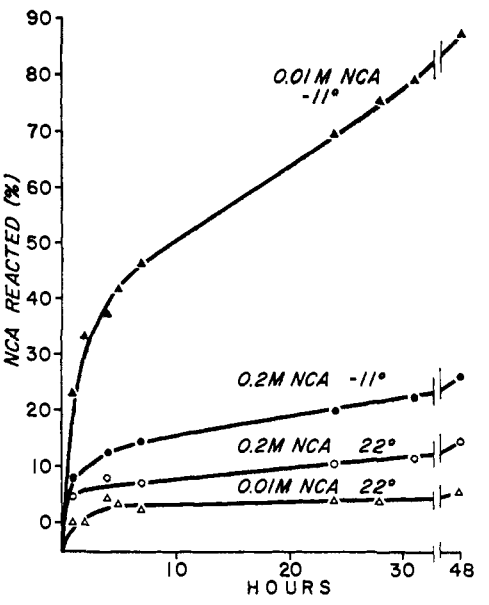

Figure 3. Polymerization of 1-aminocyclopentanecarboxylic acid NCA in the presence of isobutylamine; initiator concentration 1/20 that of the NCA.

Determinations of the degree of polymerization were carried out on the following materials: poly-D-phenylglycine prepared from storage of 0.004 *M* NCA at −23° for 3 days, poly-1-aminocyclobutanecarboxylic acid prepared from storage of 0.004 *M* NCA at −12° for 3 days, and poly-1-aminocyclopentanecarboxylic acid prepared from storage of 0.005 *M* NCA at −12° for 5 days. Thawed systems were found by hydroxamate assay to be free of NCA, and precipitates were collected and washed thoroughly with dioxane.

Table I, summarizing the titrations performed on the dioxane- and dimethylformamide-insoluble products, shows degrees of polymerization of 13 and 23. In none of the cases did the number of carboxyl groups exceed that of amino groups. Sela and Berger observed such an excess with each of their water-initiated and bulk polymers.[13]

Table I. End-Group Determination of Polyamino acids[a]

Poly-	*A*	*B*	*DP*
D-Phenylglycine	0.041	0.115	13
1-Aminocyclobutane-carboxylic acid	0.019	0.068	23
1-Aminocyclopentane-carboxylic acid	0.079	0.078	13

[a] *A* and *B* represent the number of moles of terminal carboxyl and terminal amino groups, respectively, per mole of amino acid residue. *DP*, the number-average degree of polymerization, is calculated from $DP = 2/(A + B)$, as discussed by Sela and Berger.[13]

Discussion

Polymerization of several N-carboxyamino acid anhydrides proceeds at a negligible rate in liquid dioxane but speeds up markedly in frozen dioxane between +5 and −26°. Although the polyamino acids so far examined have low average degrees of polymerization, there may be synthetic utility in carrying out such reactions in solvents at low temperatures and without the addition of initiators.

Several mechanisms have been proposed to account for accelerations in frozen solutions. These are (1) a concentration effect, involving an increased concentration of reactant molecules in liquid microinclusions; (2) a positional effect, involving a constrained juxtaposition and orientation of reactants; (3) catalysis at surfaces of the frozen solvent lattice; and (4) enhanced proton transfer resulting from enhanced proton mobility in the frozen solution.

Pincock and Kiovsky[5] presented kinetic evidence that the reactions of *t*-butyl peroxyformate with 2,6-lutidine in frozen *p*-xylene and that of methyl iodide with triethylamine in frozen benzene occur in concentrated liquid regions. They proposed for frozen-state reactions in general that if the reactants are soluble enough and the reaction has a low activation energy, an acceleration due to the concentration effect will be observed. Of the reactions studied in frozen aqueous systems, some are adequately explained on this basis,[4a] while others appear to be more completely explained by catalyses involving the ice structure.[3c,4b] In the present study with an inert organic solvent, solubility data and the effects of concentration variation may be relevant to the interpretation.

A solute concentration raised to 1.4 *M* could lower the freezing point of dioxane to about +5° (taking K_f as 4.9), but we observed that a concentration of 1.4 *M* cycloleucine NCA fails to react in liquid dioxane at +5 or at +22°. As the temperature for such experiments is lowered, the possibility of reactant precipitation becomes germane. Maintenance of a liquid state at −18° requires a solute concentration of at least 6.3 *M*, but this is more than three times the saturation level (1.9 *M*) for cycloleucine NCA at a *higher* temperature, +5°. Maintenance of a liquid state at −12.5° requires a solute concentration of at least 5.1 *M*, but this concentration exceeds the solubility of the NCA of 1-aminocyclobutanecarboxylic acid at +5° and the solubilities of the NCA's of 1-aminocyclohexanecarboxylic acid and 1-aminocycloheptanecarboxylic acid at room temperature.

If adventitious catalysts were present and if the NCA were soluble enough to account for liquid inclusions at the temperatures studied, then a concentration effect might explain an inverse relation between the initial NCA concentration and the fraction of molecules reacting. However, there is no evidence pointing to the presence of adventitious catalysts and much evidence (such as stability for 5 days at 50°) pointing to their absence. At temperatures above the eutectic, freezing unquestionably concentrates the reactants as relatively pure solvent crystallizes. In liquid inclusions, initially low solute concentrations could be expected to approach, but never exceed, levels which are initially high. The data in Figures 2 and 3, and observations such as almost complete reaction at 0.01 *M* cycloleucine NCA contrasted with no reaction at 0.25 *M* NCA, can be interpreted as suppression of the reaction by high concentrations.

An inverse relation between the fraction of molecules reacting and initial concentration suggests the phenomenon of substrate inhibition occasionally met in enzyme kinetics. Although the mechanism of substrate inhibition is unclear and may vary in different cases, the idea of a catalytic site is implicit; assuming the requirement of a precise arrangement of reacting molecules, a crowding in at the catalytic site is envisaged as hindering the reaction.[15] In the present case, if it is assumed that the initiator is a form of the NCA, such as the enol suggested by Bamford, *et al.*, for spontaneous polymerization at high temperatures,[16] then the concept of specific ordering could apply. Formation of a linear polymer in a truly solid state implies either a linear alignment of properly oriented NCA molecules on freezing or a considerable degree of mobility of the molecules within the frozen matrix. The fact that freezing an initially more dilute solution of NCA results in reaction of a larger fraction of the molecules suggests that alignment may occur in a channel of molecular dimensions during the simultaneous crystallization of solvent and solute. The phenomenon could resemble clathrate formation, with the solvent acting as host and template partially or fully enclosing solute molecules.

Acknowledgment. We are indebted to Dr. W. Dvonch and Mr. H. Fletcher for synthesizing the N-carboxyamino acid anhydrides used in this study.

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