polymer produced is isotactic. Presumably with both cadmium D-tartrate and cadmium *dl*-tartrate there are two equally reactive types of site, one specific to the polymerization of the *R* monomer, the other to the polymerization of the *S* monomer. The polymer should therefore be racemic, *i.e.*, resolvable. With zinc D-tartrate, on the other hand, there is some degree of stereoelection, as also with $ZnEt_2$/(−)-DMBD. The isotactic dyad content of the polymers, however, is well below 100% and will be determined by a number of factors: (i) the number of types of catalyst site; (ii) the degree of stereoelection at each type of site; and (iii) the yield. More data are required before a correlation of optical activity and nmr data can be attempted. Knowing the specific rotation of the pure isomers (32.6°)[5,6] we may estimate that, in the experiment using $ZnEt_2$/(−)DMBD as catalyst, the average rate of polymerization of the *R* monomer is about three times that of the *S* monomer during the first 30% polymerization.

The polymer obtained using a mixture of aluminium triethyl and water as catalyst differed from the other polymers in that it contained a small amount of low molecular weight material which formed as small crystalline particles within the amorphous polymer. It was possible to separate these particles by hand with the aid of a needle. Molecular weight determinations indicated an approximately hexameric species,[11] giving sharp lines in the nmr spectrum. The spectrum of the main polymer showed slight contamination with this material but the isotactic dyad content could be seen to be approximately 50%.

Acknowledgment. We thank the Ministry of Education, Northern Ireland, for the award of a studentship (E. D. L.).

(11) P. Dumas, Thèse 3e Cycle, Paris, 1970.

The Effect of Isomer Ratio on the Properties of Bis(4-aminocyclohexyl)methane Polyamides

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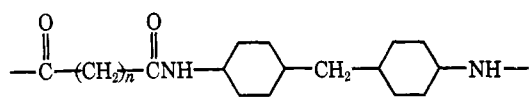
ABSTRACT: Differential thermal analysis data for bis(4-aminocyclohexyl)methane polyamides showed that glass transition temperatures and melting points increased with increasing trans,trans content of the diamine. These values all decreased with increasing chain length of the diacid used. There was also a polymerization rate dependence on the trans,trans content of the diamine. A conformational analysis based upon accessibility of the amino groups and relative stability of the various conformers was used to explain this rate phenomenon. X-Ray powder diagrams showed a change in interplanar spacings for the polyadipamides when the trans,trans diamine content reached 70%.

Bis(4-aminocyclohexyl)methane (PACM) can exist in three different geometric configurations, trans,trans (tt), cis,trans (ct), and cis,cis (cc). Each isomer would adopt a different configuration when incorporated in the backbone of a polyamide chain. This difference should be reflected in the polyamide properties, and the properties should vary with isomer content. There are several publications and patents on the polyamides of PACM,[1-10] but few discuss the effect of isomer ratio on polymer properties. In one paper it was shown that the softening temperatures of poly(PACM-adipamides) increased with increasing tt diamine content.[4] However, this was the only property discussed and none of the PACM samples contained more than 26 mol % of the tt diamine. The tt diamine has the highest degree of symmetry of the three isomers, and one would expect it to have the greatest effect upon the melting point, crystallinity, and glass transition temperature (T_c) of PACM-polyamides. It would be interesting to demonstrate this effect and also that of the chain length of the diacid on the properties of PACM-polyamides.

Discussion

We prepared three sets of polyamides from PACM samples of different isomer ratios and adipic, sebacic, and dodecanoic acids (Scheme I). The designations and isomer contents of

SCHEME I



I

[a] $n = 4, 8, 10$.

the three PACM samples are as follows: PACM[1] 51% tt, 40% ct, 9% cc, ±2%; PACM[2] 70% tt, 25%, ct, 5% cc, ±2%; PACM[3] 98% tt, 2%, ct, 0% cc, ±2%. PACM[1] is the normal hydrogenation product of 4,4′-diaminodiphenylmethane,[11] although other isomer mixtures could be obtained by changing the hydrogenation conditions.[12] PACM[2] was obtained by fractional crystallization of PACM[1] from *n*-

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(3) D. C. Pease and C. T. Handy, U. S. Patent 2,585,163 (1952).
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TABLE I
Dta DATA FOR PACM-POLYAMIDES

Diacid	PACM[1] Time,[a] min	$\eta_{red.}$	$\eta_{red./time} \times 10^2$	T_m, °C	T_g, °C	PACM[2] Time, min	$\eta_{red.}$	$\eta_{red./time} \times 10^2$	T_m, °C	T_g, °C	PACM[3] Time, min	$\eta_{red.}$[b]	$\eta_{red./time} \times 10^2$	T_m, °C	T_g, °C
C_6	40	0.41	1.02	355	165	20	0.32	1.6	365	170	12	0.49	4.08	385	185
C_{10}	12[c]	0.34	0.05	290	130	4[c]	0.56	0.23	300	144	15	0.35	2.33	310	144
C_{12}	12[c]	0.40	0.06	264	125	4[c]	0.34	0.14	290	135	18	0.41	2.27	300	135

[a] Time required for polyamide to crystallize from polymer melt at 250°. [b] Determined in trifluoroacetic acid. Polymers insoluble in 90% formic acid, trifluoroethanol, DMF, etc. [c] Hours.

hexane, and PACM[3] was obtained in a similar manner from PACM[2]. The more crystalline, insoluble tt diamine crystallizes more readily from solution to give tt-enriched PACM samples. PACM samples rich in tt diamines could also be obtained by isomerization of the ct and cc diamines.[13]

We observed a polymerization rate dependence on the chain length of the diacid and the isomer ratio of the diamine. The polymerization rate increased with decreasing chain length of the diacid and increasing trans,trans content of the diamine. For example, the reduced viscosity which is a function of molecular weight is much lower for the PACM[1]-sebacamide than for the PACM[2]-sebacamide even though it was allowed to polymerize three times as long (Table I). Also the C_{10} and C_{12} diacids condensed with PACM[3] to give high molecular weight polyamides in less than 20 min, whereas a similar change required 12 and 4 hr with PACM[1] and PACM[2], respectively.

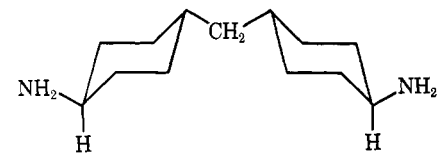
One of the measures for rates of polymerization is the change in viscosity as a function of time. These data for the PACM-polyamides are shown in Table I. In every case the ratio of reduced viscosity to polymerization time is larger for the polyamide (of a given diacid series) with the highest tt diamine content.

As the tt diamine content increased the polyamide solidified from the polymerization melt at 250° several times faster even though the melting points of the polymers are very close. For example, PACM[2]-sebacamide has a melting point of 300° and required 4 hr to crystallize from the polymerization melt, whereas the PACM[3]-sebacamide with a melting point only 10° higher crystallized from the melt in just 15 min. This could stem from a polymerization rate difference. Using the ratio of reduced viscosity to polymerization time as a measure of polymerization rate, the approximate order of diamine polymerization with sebacic acid is (PACM[3]):(PACM[2]):(PACM[1]) 50:5:1. A comparison of the polymerization times for the PACM[1]-polyamides shows that as an approximation it required 18 times as long for dodecanoic acid to polymerize to the same viscosity as adipic acid. This demonstrates the increase in polymerization rate with decreasing chain length of the diacid.

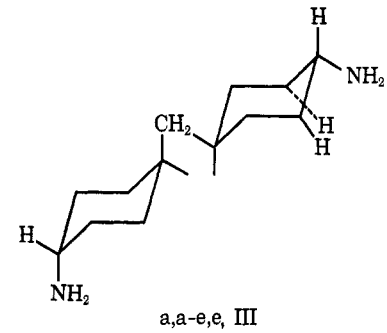
Conformational Analysis

Individual polymerization rates of the isomers of PACM will be dependent upon the conformational stability of the isomer and the accessibility of the amino groups. If we assume a chair conformation, then a trans-disubstituted cyclohexane ring must have both of its substituents in either the axial (a,a) or equatorial (e,e) position. A cis-disubstituted cyclohexane ring would have one of its substituents in the axial position and the other in the equatorial position. This particular conformer will be designated a,e. Since there are two disubstituted cyclohexane rings in each diamine molecule, there are four positions to be designated by a or e. There are three possible conformations for the tt diamine molecule—e,e-e,e (II), a,a-e,e (III), and a,a-a,a (IV); two for the ct diamine—a,e-e,e (V), and a,e-a,a (VI); and three for the cc diamine—a,e-a,e (VII), a,e-e,a (VIII), and e,a-a,e (IX) (Charts I, II). Conformers with substituents in the equational position are more stable than those with substituents in the axial position because of 1,3 diaxial shielding of the latter. For the same reason, an amino group in the equatorial position is more accessible than in the axial position. On the basis of 1,3 diaxial shielding the order of conformational stability and reactiveness is as follows: tt diamine e,e-e,e > a,a-e,e > a,a-a,a; ct diamine a,e-e,e > a,e-a,a; cc diamine e,a-a,e > a,e-a,e > a,e-e,a. Also on this basis the tt diamine is the most stable conformer and the order of

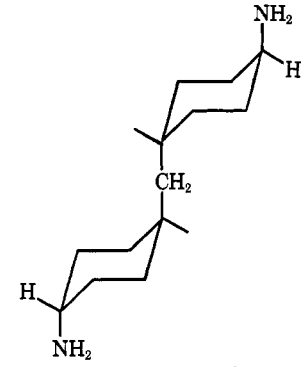
CHART I



e,e-e,e, II



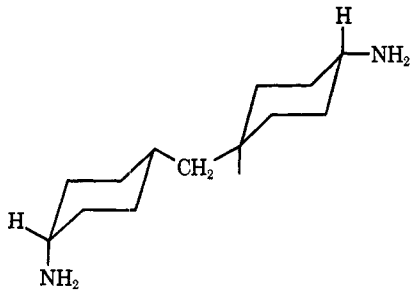
a,a-e,e, III



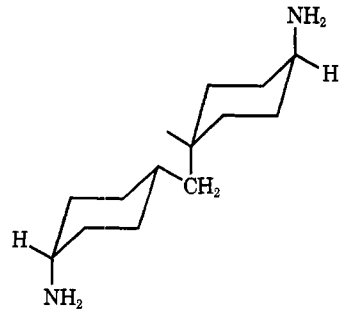
a,a-a,a, IV

(13) E. I. du Pont de Nemours and Co., Netherlands Patent 6,409,630 (1966).

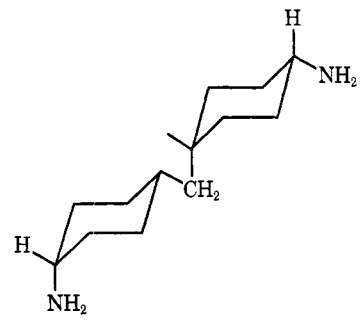
CHART II



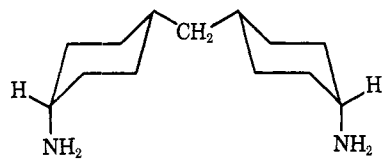
a,e-e,e,V



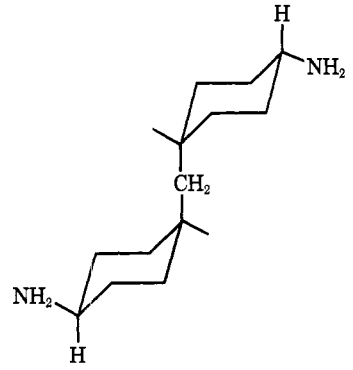
a,e-a,a,VI



a,e-a,e,VII



a,e-e,a,VIII



e,a-a,e,IX

diamine stability is tt > ct > cc. For the same reasons the order of decreasing accessibility of amino groups is tt > ct > cc. This also appears to be the order of individual isomer polymerization rate.

Differential Thermal Analysis

Dta data in Table I show that the melting point and T_g increased with increasing trans,trans content of the diamine.

TABLE II
PER CENT CRYSTALLINITY OF PACM-ADIPAMIDES

	Diamine		
	PACM[1]	PACM[2]	PACM[3]
Per cent crystallinity	39	38	32

However, there appears to be a maximum in T_g and T_m for the C_{10} and C_{12} polyamides at 70% tt diamine content. The T_g values for these polyamides remained the same and the melting points changed only slightly in going from 70% (PACM[2]) to 98% tt diamine (PACM[3]) in the polyamide chain. The Dta curves also showed evidence of multiple transitions. This could stem from the existence of polymer chains with different isomer compositions or could possibly occur as a result of conformational flipping of the cyclohexane rings in the main chain.

X-Ray Diffraction

We examined the X-ray powder diagrams of the PACM-polyadipamides to determine the effect of isomer ratio on crystallinity and unit cell dimensions. We found that the lattice spacings of PACM[1]- and PACM[2]-adipamides were identical which indicates that the unit cell dimensions and the crystalline lattices are the same. However, these patterns differed markedly from that of the PACM[3]-adipamide, and two different crystalline structures actually exist. In a recent paper[14] we have shown that the nylon-6 lattice can only accommodate less than 30 mol % 4-aminomethylcyclohexanecarboxylic acid before a new structure develops, and it appears that a similar change in lattice structure occurred with PACM-adipamides.

In our PACM samples the tt diamine is always present in 50 or more mol % and so we shall consider it to be the host unit and the ct and cc diamines as comonomers. One could assume that the 98% tt diamine–polyadipamide (PACM[3]-adipamide) has the same crystalline lattice as the 100% tt diamine–polyadipamide would. An explanation for the X-ray data would then be as follows. Up to about 25 mol %, the ct and cc diamine units dissolve in the crystal lattice of the trans,trans unit in a manner reminiscent of cocrystallization phenomena. At 30 or more mol % the presence of the ct and cc comonomers creates such a large lattice distortion that new crystal structure develops.

The per cents crystallinity (Table II) of PACM[1]- and PACM[2]-adipamides were both slightly higher than that of PACM[3] even though it has the highest content of the more symmetrical tt diamine. However, PACM[3]-polyadipamide has a T_g of 185°, and these samples were only annealed at 190°. The orderly packing of the PACM[3]-adipamide chains into crystallites was probably not complete at this temperature.

We shall continue our investigation of the effect of isomer ratio on the crystalline structure of PACM-polyamides through the use of monofilaments of these polymers.

Experimental Section[15]

Fractional Crystallization of PACM. PACM[1] (Aldrich Chemical Co., 60 g), a waxy solid, was dissolved in *n*-hexane (120 ml) with

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(15) Reduced viscosities were determined in 90% formic acid except the PACM[3] polyamides which were done in trifluoroacetic acid. Dta data were obtained on the Du Pont 900 differential thermal analyzer. X-Ray diffraction powder patterns were obtained on a Norelco X-Ray diffractometer using Cu Kα radiation. The per cent crystallinity was determined from these patterns by dividing the area of the crystalline regions by the crystalline plus amorphous area. The samples were annealed at 190° for 2 hr.

slight warming and the solution allowed to stand overnight in the cold. This gave semicrystalline PACM[2] (33%, mp 45–55°) which was filtered in a nitrogen atmosphere and dried. PACM[2] (25 g) was heated with hexane (200 ml) and the mixture cooled to give white crystals (64%, mp 65–8) of PACM[3].

The diamines were made volatile for gas chromatographic analysis by conversion to their *N,N'*-trifluoroacetyl derivatives. The samples were treated with trifluoroacetic anhydride overnight, and the reagent was removed under vacuum to give a residue which was analyzed without further purification.

Polyamides (I). These were prepared by melt condensation of equimolar mixtures of the PACM sample and diacid in sealed tubes, under vacuum, at 250°. The polymerizations were allowed to proceed until the polymer melt solidified. The solid was ground, washed with water and methanol, and dried overnight at 75° in a vacuum oven. Polymer yields were generally 85% of the theoretical.

Acknowledgment. We wish to acknowledge the contributions of Professor Murray Goodman for helpful discussions on the conformations of PACM, Mr. Ralph Hetterley, who did the gas chromatographic analysis of the diamines, Dr. Robert J. Fredricks and Mr. Donald Richardson for the X-ray powder diagrams, Mrs. Edith Turi and Mr. Will Wenner for obtaining the dta data, and Dr. John Haylock for the fractional crystallization procedure for PACM.

Polyacrylamide Derivatives of Amino Acid Acylase and Trypsin

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ABSTRACT: Water-insoluble acylase and trypsin polyacrylamide derivatives were prepared by coupling the enzymes through an amide bond by reaction with a water-insoluble polyazide derived from a copolymer of acrylamide, *N,N'*-methylenebisacrylamide, and methyl acrylate. Water-insoluble acylase derivatives showed hydrolytic activities per unit weight of bound protein corresponding to 50–100% of that of the original acylase against *N*-acetyl-DL-methionine, *N*-acetyl-DL-alanine, and *N*-acetyl-DL-norleucine. Water-insoluble acylase derivatives were much more stable to heat inactivation at neutral pH than was free acylase. Optically active amino acids were easily isolated in high yields from the reaction mixtures containing *N*-acetyl derivatives of the corresponding DL-amino acids and the acylase derivatives. Water-insoluble trypsin derivatives showed an esteratic activity, per unit weight of bound protein, 40% of that of crystalline trypsin when assayed with *N*-benzoyl-L-arginine ethyl ester as substrate. Activity of the trypsin derivatives was retained after repeated use at 25°. Trypsin heated in a pH 7 phosphate buffer at 100° for 10 min lost 87% of its initial esteratic activity, whereas the trypsin derivative heated in the same way lost only 23% of its initial activity.

Recent reviews[2] show how extensively the conversion of soluble enzymes into water-insoluble forms has been studied. Water-insoluble enzyme derivatives are of interest from a practical point of view in that they may be used repeatedly to induce specific chemical reactions in relatively large amounts of substrates either by batchwise or continuous column processing. Since water-insoluble enzyme derivatives can be readily filtered from reaction mixtures, such processes reduce the amount of enzyme required and keep reaction products free from contamination by enzyme protein.

A number of papers have appeared on enzyme carriers such as diazotized poly(*p*-aminostyrene),[3] diazobenzylcellulose,[4] carboxymethylcellulose azide,[5,6] and nitrated copolymer of methacrylic acid and methacrylic acid *m*-fluoroanilide.[7] However, none of the enzymes coupled to these carriers was treated with large amounts of substrates because of either low activity or poor stability.

Diazotized copolymers of *p*-amino-DL-phenylalanine and L-leucine were also employed for coupling of various enzymes.[8–11] Among these enzyme derivatives, papain,[2a] urease,[10] and polytyrosyl trypsin[12] derivatives were used for the preparation of enzyme columns.

A proteolytic enzyme, trypsin, has been used frequently in such studies.[2a,5,6] The well-defined water-insoluble trypsin derivatives provide good model systems for comparison of the effects of various carriers on coupled trypsin.

Amino acid acylase was used in an attempt to couple it to poly(methacrylic acid).[13] The coupled product was reported unstable on storage. Apart from the method of covalent binding to water-insoluble carriers, insolubilization of acylase by its copolymerization with *N*-carboxy-γ-methyl-L-glutamate anhydride has been reported as another method for the preparation of water-insoluble acylase derivatives.[14]

Acylase was chosen because its derivative might be useful for the resolution of racemic amino acids.[15] To characterize the new water-insoluble acylase derivatives, their properties

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