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Studies in Cyclocopolymerization. XIV. Cyclocopolymerization Study of Certain Maleoylamino Acids with Divinyl Ether

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Studies in Cyclocopolymerization. XIV. Cyclocopolymerization Study of Certain Maleoylamino Acids with Divinyl Ether

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ABSTRACT

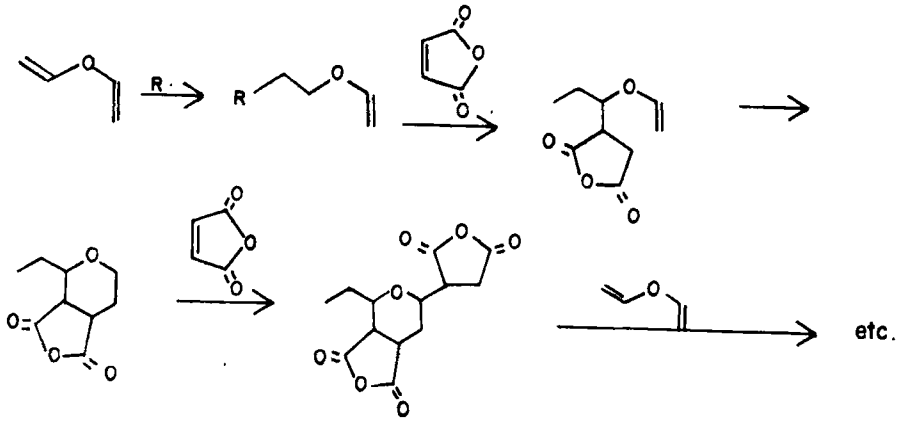
N-Carbethoxymaleimide (I), a convenient intermediate to maleoylamino acids in aqueous solution, also undergoes facile reaction with amines in ether. Thermolyses of the resulting imide-amides at 170-180°C gave N-substituted maleimides. N,N-Dimethyl-1,3-propanediamine reacted with I to give XI directly. Copolymers prepared and studied were those of divinyl ether with N-carbethoxymaleimide, maleoylglycine, maleoyl-DL-alanine, maleoyl-DL-phenylalanine, maleoyl-L-phenylalanine, maleoyl-DL-methionine and maleoyl-DL-leucine. These copolymers were characterized by elemental analyses to establish the comonomer ratios, by viscosity measurements, and in certain instances by determination of molecular weight by membrane osmometry after esterification with diazomethane.

INTRODUCTION

The study of biomaterials and their potential use in medicine is rapidly expanding. Within recent years a number of synthetic polyanions have been found to have antineoplastic activity. Perhaps the most promising polycarboxylate anion is the 1:2 divinyl ether–maleic anhydride copolymer originally prepared by Butler [1]. This copolymer, in addition to its potent antitumor activity, has been found to have a broad spectrum of biological activity and has been the subject of several recent reviews [2-4].

Antitumor activity of varying degree appears to be the general property of copolymers related in structure to the divinyl ether–maleic anhydride copolymer. The corresponding 1:2 divinyl ether–citraconic anhydride copolymer [5] (NSC 133788), the 1:1 furan–maleic anhydride copolymer [6] (NSC 119166) and its half-amide half-ammonium salt [7] (NSC 119167); the 1:1 furan–itaconic anhydride copolymer (NSC 119165) and its half-amide half-ammonium salt [7] (NSC 119168) are all active against lymphoid leukemia cells.

Further efforts to modify the structure of the divinyl ether-maleic anhydride copolymer by the post reaction of the preformed copolymer with several primary amines have been made [8]. Alternately, the copolymerization of divinyl ether with maleimide derivatives, where the imide nitrogen is bonded to an amino acid moiety, was of particular interest since polyelectrolytes could be obtained having potential biological activity.



(1)

It has been well established that certain 1,4-dienes undergo a bimolecular, alternating inter-intramolecular copolymerization with certain alkenes, as illustrated in Eq. (1). It was the object of this work to extend the cyclocopolymerization studies to the copolymerization of maleoylamino acids with divinyl ether, to determine the physical properties of the copolymers, and to have the copolymers evaluated for their antitumor activity.

EXPERIMENTAL

Melting points and softening points were taken on a Fisher-John or Thomas Hoover melting point apparatus and are uncorrected. Boiling points are uncorrected. Intrinsic viscosities were measured by using an Ubbelohde type viscometer. Molecular weights were obtained on a Mechrolab Model 502 high-speed membrane osmometer. The proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian A-60-A high-resolution instrument with tetramethylsilane as internal standard. Infrared spectra were recorded on a Beckmann IR8 or IR10 spectrophotometer. Polarimetric measurements were done on a Rudolph polarimeter. Elemental analyses were carried out by Galbraith Laboratories, Inc. (Knoxville, Tenn.) or by Heterocyclic Chemical Corp. (Harrisonville, Mo.).

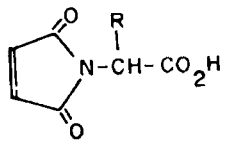
N-Carbethoxymaleimide (I)

I was prepared in 96% yield by reacting maleimide with ethyl chloroformate in the presence of triethylamine in ethyl ether [9]. Purification was carried out by distillation (bp 116°C/0.3 Torr); mp 59-61°C (lit. [9] mp 58-59°C).

Maleoylamino Acids (II-VI)

Monomers II-VI (Table 1) were prepared by using a modification of the method reported by Keller and Rudinger [9] and is illustrated by the following example. To a stirred mixture of 5.30 g (0.05 mole) Na_2CO_3 (in the case of IV and VI, a combination of 0.05 mole NaOH and 0.05 mole $NaHCO_3$ was used) and 4.45 g (0.05 g mole) DL-alanine in 50 ml H_2O was added 8.46 g (0.05 mole) of I while the reaction was cooled by an ice-water bath. After dissolution (10-15 min), the water was removed on the rotary evaporator (ca. 50°C) and the residue dried in a vacuum oven at 50°C. The resulting solid was triturated

TABLE 1. Amino Acid Derivatives of Maleimide



No.	R	Isomer	Mp (°C) and solvent[a]	Lit. mp (°C) and reference	Yield (%)	$[\alpha]_D^{25}$
II	H	–	114-116 EtOAc/PE	113-113.5 [10][b]	60	
IIIa	CH_3	DL	114-116 EtOAc/PE; C_6H_6	–	61	
IIIb	CH_3	L	c	97-98 [10][b]	33	-21° (c = 3.16, MeOH)
IVa	CH_2-C_6H_5	DL	146-148.5 C_6H_6	–	44	
IVb	CH_2-C_6H_5	L	165-166.5 $CHCl_3/C_6H_{12}$	168-169 [9]	49	-127° (c = 8.68, MeOH)
Va	$CH_2CH_2SCH_3$	DL	96-97 C_6H_6/PE	83-85 [10][b]	39	
Vb	$CH_2CH_2SCH_3$	L	c	–	36	-31° (c = 11.63, MeOH)

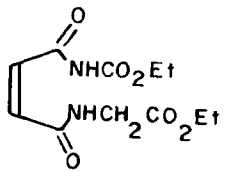
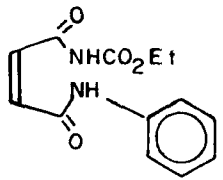
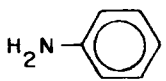
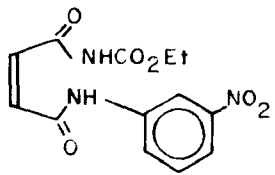
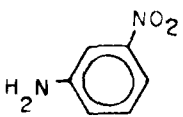
VI	$CH_2CH(CH_3)_2$	DL	104-106 CCl_4	–	28

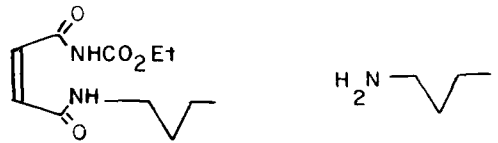
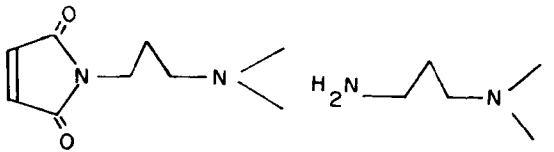
[a]Recrystallization solvents.

[b]While this manuscript was in preparation, an alternate synthesis of maleoylamino acids was reported [10].

[c]Oil having IR and ^1H NMR spectra identical to those of the DL isomer.

TABLE 2. Reaction of N-Carbethoxymaleimide with Primary Amines

No.	Product[a]	Amine[b]	Mp (°C)	Solvent	Time (hr)	Yield (%)	Analysis calcd and found[c] C (%)	H (%)	N (%)
VII		$H_2NCH_2CO_2Et$	133-134.5	$CHCl_3$/ether	2	84	(48.53) 48.55	(5.92) 5.85	(10.29) 10.29
VIII			151.5-154	$CHCl_3$	4	70	(59.54) 59.55	(5.38) 5.29	(10.68) 10.69
IX			159-160.5	THF/ether	72	50	(50.82) 51.44	(4.26) 4.56	(13.67) 13.41

X		113-115	$CHCl_3$/ pentane	2	79	(54.53) 54.56	(7.49) 7.51	(11.56) 11.62
XI				3	18			

[a]The methyl esters of L-leucine, L-phenylalanine and the dimethyl ester of L-glutamic acid gave products which failed to crystallize; however, their NMR spectra were consistent with those of other maleic acid derivatives. These products were used in subsequent reaction without purification.

[b]o-Nitroaniline failed to react at 15 days at 25°C.

[c]Calculated values in parentheses.

[d]Bp 82-83°C/0.55 Torr (lit. [11] bp 67°C/0.5 mm).

with boiling ether (three times, 50 ml) to remove the urethane and acidified with 50 ml 3 N HCl. The mixture was concentrated on the rotary evaporator (below 50° C/1.5 Torr) and dried under vacuum at room temperature. In the case of IV and V, the crude product was isolated by filtration and decantation, respectively. The residue was treated with hot EtOAc (two times, 50 ml), the organic extract concentrated to ca. 30 ml, and the product precipitated with petroleum ether (PE). Recrystallization from benzene gave 5.2 g (61%) of IIIa.

Reaction of N-Carbethoxymaleimide I with Primary Amines

Products VII-X (Table 2) were prepared as illustrated by the following example. To a stirred solution of 5.3 g (31 mmole) of I in 150 ml of ether at 0-3° C was added a solution of 3.2 g (31 mmole) of ethyl glycinate in 5 ml ether. After stirring for 2 hr at 0-3° C, the product was collected by filtration, thoroughly washed with ether, and vacuum-dried to yield 7.2 g (84%) of N-(2-carbethoxy-1-methyl)-N'-carbethoxymaleamide [VII]; mp 133-134.5° C. Recrystallization from $CHCl_3$/ether did not raise the melting point. The NMR spectrum ($CDCl_3$) showed δ 1.32 (2 overlapping t, 6, $2CH_3$), 4.17 (s, 2, CH_2), 4.04-4.46 (2 overlapping q, 4, $2CH_2$), 6.30 and 6.54 (2d, 2, HC=CH, J = 17 Hz).

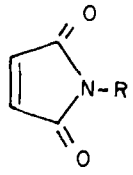
A solution of 0.5 g (1.8 mmole) of VII in 5 ml dimethylformamide (DMF) was heated at 110° C for 30 min; the reaction mixture was poured into 50 ml H_2O and the resulting white platelets collected by filtration and vacuum-dried to yield 0.25 g (50%) of N-(2-carbethoxy-1-methyl)-N'-carbethoxyfumaramide, mp 191° C (dec.). Recrystallization from ethanol did not alter the melting point. NMR (DMSO-d_6) showed δ 1.21 (t, 3, CH_3), 1.25 (t, 3, CH_3), 3.97 (d, 2, $NHCH_2$, J = 6 Hz), 4.12 (q, 2, CH_2), 4.16 (q, 2, CH_2), 6.97 and 7.25 (2d, 2, HC=CH, J = 15 Hz), 8.88 (t, 1, $NHCH_2$, J = 6 Hz), and 10.89 (s, 1, $NHCO_2Et$).

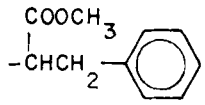
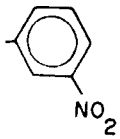
Analysis. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53%; H, 5.92%; N, 10.29%. Found: C, 48.98%; H, 6.24%; N, 10.59%.

Thermolysis of the Imide-amide Derivatives of Maleic Acid

Compounds XII-XVI (Table 3) were obtained as illustrated by the following example. A 25-ml round-bottomed flask equipped with a magnetic stirrer and an air cooled condenser was charged with 3.00 g (11 mmole) of VII. The flask was submerged into a preheated oil bath and kept at 180° C for 30 min. After cooling the product mixture was

TABLE 3. Thermal Products from the Imide-amide Derivatives of Maleic Acid



No.	R	Bp (°C/Torr)	Mp (°C)	Yield (%)
XII	$-CH_2CO_2Et$	75-78/0.05		36
XIII		123/0.05		9[a]
XIV	$-CH_2CH_2CH_2CH_3$	76-79/3.0[b]		24
XV			87.5-89[c]	50
XVI			129-130[d]	51

[a]Satisfactory combustion and spectral analyses were obtained for this compound.

[b]Lit. [12] bp, 104-105°C/12 Torr.

[c]Lit. [13] mp, 89-89.8°C.

[d]Lit. [14] mp, 134°C.

treated with 25 ml ether, filtered, the filtrate washed with 10 ml of H_2O and dried ($MgSO_4$). Removal of ether on the rotary evaporator and short path distillation gave 0.73 g (36%) of XII as a clear liquid.

Copolymerization of Maleoylamino Acids with Divinyl Ether

Copolymers XVII-XXIII (Table 4) were obtained by employing the technique represented by the following example. To a 30 ml Pyrex

TABLE 4. Divinyl Ether-Maleimide Copolymers

No.	Divinyl ether copolymer	Polymerization solvent	Precipitation solvent	Conversion (%)[a]	$[\eta_{in}]$ (dl/g)[b]	Mp (°C)[c]	Analysis, calcd and found[d] C (%)	H (%)	N (%)
XVII	Maleoylglycine	DMF	Ether	93	0.33	235	(50.53)	(4.24)	(7.37)
							46.39	4.67	7.28
XVIII	Maleoyl-DL-alanine	DMF	Ether	97	0.27	244	(52.94)	(4.94)	(6.86)
							52.49	5.02	6.60
XIX	Maleoyl-DL-phenylalanine	DMF	Ether	80	–	215	(64.28)	(5.03)	(5.00)
							63.49	4.97	4.93
XX[e]	Maleoyl-L-phenylalanine	DMF	Ether	88	0.06	218	(64.28)	(5.03)	(5.00)
							60.52	5.27	4.72
XXI	Maleoyl-DL-methionine	DMF	Ether	90	0.14	177	(49.99)	(5.34)	(5.30)
							48.70	5.53	5.30
XXII	Maleoyl-DL-leucine	DMF	2:1 Ether-pentane	75	–	230	(58.53)	(6.55)	(5.69)
							57.08	6.76	5.61
XXIII[f]	Maleoyl N-carbethoxy	C_6H_6	Benzene	80	0.19[g]	191	(52.94)	(4.94)	(6.86)
							52.62	5.25	6.94

[a] After 24 hr at 50 C; AIBN was used as initiator at 10^{-3} mole/liter concentration.

[b] Sodium salt in aqueous NaCl (1.03 M) at 21.7° C.

[c] Temperature at which the polymer began to soften.

[d] Calculated from the assumption that two monomers enter the polymer chain for each diene. Calculated values in parentheses.

[e] $[\alpha]_D^{25} = 155$ (c = 8.35, MeOH).

[f] $\overline{M}_n = 1.7 \times 10^5$ determined by membrane osmometry in 1,2-dichloroethane solution.

[g] In acetone at 26.7° C.

polymerization tube was added 1.55 g (10 mmole) of II, 16 mg of 2,2'-azobisisobutyronitrile (AIBN), and 15 ml of DMF. To the solution 0.92 ml (10 mmole) of freshly distilled divinyl ether (DVE) was added; the tube was degassed three times by the freeze-thaw technique and sealed. The polymerization tube was placed in a constant temperature bath and the reaction allowed to proceed for 24 hr at 50° C. The contents were then poured with vigorous stirring into 400 ml ether. The white powder was collected by filtration, dried, and reprecipitated by pouring an acetone solution into ether. The complete removal of DMF was achieved by heating the copolymer under vacuum (110° C/0.01 Torr) to yield 1.78 g (93%) of XVII.

Esterification of the Maleoylamino Acid-Divinyl Ether Copolymers with Diazomethane

The esterified copolymers XVIIa-XXIIIa (Table 5) were obtained as represented by the following general procedure. To 0.5 g of the copolymer dissolved in 25 ml methanol was added, in parts via a pipet an etheral solution of diazomethane [15]. The addition of the diazomethane solution was continued until the characteristic yellow color persisted. Upon loss of color, the insoluble methyl esters were collected by filtration, reprecipitated from ether, and dried (110° C/0.01 Torr). All methyl esters except XVIIa and XXIa were soluble in acetone. These insoluble copolymers, however, were soluble in DMF.

RESULTS AND DISCUSSION

Monomer Synthesis

Preliminary studies concerning the preparation of the maleoylamino acid monomers II-VI indicated that purification was facilitated by removing the urethane by-product prior to acidification. After acidification and removal of solvent the product could usually be separated from major impurities by dissolution in hot benzene. However, under these conditions no maleoyl derivative of glutamic acid was detected whereas the maleoyl derivatives of valine and serine could not be isolated. In general, the DL-isomers were found more amenable to crystallization than the L-isomers which in most instances were obtained as oils.

Due to the difficulty encountered to purify these maleimide

TABLE 5. Methyl Esters of the Divinyl Ether-Maleoylamino Acid Copolymers

No.	Divinyl ether copolymer	Mp (°C)[a]	$[\eta_{in}]$ (dl/g)[b]	$\overline{M}_n \times 10^{-4}$[c]	Analysis calcd and found[d]		
					C (%)	H (%)	N (%)
XVIIa	Maleoylglycine	175	–	–	(52.94) 51.97	(4.94) 5.30	(6.86) 6.63
XVIIIa	Maleoyl-DL-alanine	171	–	2.6	(55.04) 52.97	(5.54) 5.78	(6.42) 6.04
XIXa	Maleoyl-DL-phenylalanine	152	0.05	2.8	(65.30) 65.50	(5.58) 5.77	(4.76) 4.69
XXa	Maleoyl-L-phenylalanine	156	0.06	–	(65.30) 63.51	(5.58) 5.50	(4.76) 4.82
XXIa	Maleoyl-DL-methionine	126	–	–	(51.79) 51.96	(5.79) 6.10	(5.03) 4.87
XXIIa	Maleoyl-DL-leucine	174	0.12	5.8	(59.99) 59.01	(6.97) 7.12	(5.38) 5.20

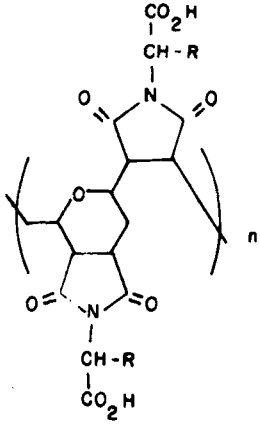
[a]Temperature at which the polymer began to soften.
[b]In acetone at 26.7° C.
[c]Molecular weight determined by membrane osmometry in 1,2-dichloroethane.
[d]Calculated values in parentheses.

(1)

derivatives as well as the limited applicability of the synthetic method it became desirable to investigate alternate synthetic routes [10]. A procedure which showed promise is illustrated by Eq. (1). The imide-amide derivatives of maleic acid (VII-X) were readily obtained in ether at room temperature. The rate of ring opening of I seemed to be in the same order as the basicity of the amine. Formation of XI was surprising, since ring closure of VII was not observed in the presence of triethylamine. In solution at elevated temperature VII was observed to give rise to a number of products with isomerization to the fumaric acid derivative comprising the major process. Maximum isomerization (50%, 110° C) was obtained within 30 min in DMF. Cyclization was finally achieved by heating neat samples of the imide-amide compounds at 170-180° C for 30 min. Table 3 summarizes the results of this study.

Polymer Synthesis

High conversions at a reasonable reaction rate were obtained when the polymerization reactions were carried out in the presence of onefold excess of DVE. The resulting copolymers were soluble in most polar solvents, only XVII being soluble in water. Infrared and nuclear magnetic resonance spectral analyses of the copolymers showed lack of unsaturation or pendant vinyl groups. Solubility of the copolymers demonstrates lack of crosslinked products and, finally, elemental analyses are consistent with a 2:1 repeating unit illustrated by the general formula XXIV. Elemental analyses results



XXIV

and some physical constants of the copolymers are shown in Table 4. In order to further characterize these copolymers they were treated with diazomethane and the molecular weights of some of the methylated copolymers, determined by membrane osmometry, indicate a degree of polymerization of the order of 47-388 for the employed polymerization conditions. Taken together, these facts strongly support the proposed polymeric structure and eliminate other possible structures that could result from the functionality of the monomers.

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