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Bifunctional Synthetic Enzyme Candidates via Alternating Copolymerization. II. Copolymers Containing Alternating Imidazole and Phenolic Hydroxyl Functions

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Bifunctional Synthetic Enzyme Candidates via Alternating Copolymerization. II. Copolymers Containing Alternating Imidazole and Phenolic Hydroxyl Functions

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ABSTRACT

Alternating copolymerization offers an ideal method for introducing certain pairs of functional groups into copolymer chains in a controlled manner. In a previous paper from this laboratory, a novel copolymer containing alternating imidazole and hydroxamic acid functional groups was reported, and its evaluation as a synthetic enzyme was described. In an extension of this work, the present paper reports the synthesis, characterization, and evaluation of those novel copolymers containing alternating imidazole and phenolic hydroxyl functional groups. The results of this study indicate that "cooperativity" between this pair of functional groups is somewhat restricted, probably for reasons related to the conformation of the copolymer chains or the proximity of the functional groups to the copolymer backbone.

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*Dedicated to Professor Georg Manecke on the occasion of his 70th birthday, June 20, 1986.

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INTRODUCTION

A previous paper [1] from this laboratory included a brief summary of the experimental and theoretical aspects of a variety of synthetic enzyme studies [2-8] on which basis the reported investigation was conceived and justified. The concept involved utilization of the known tendency of certain pairs of vinyl monomers to undergo alternating copolymerization to introduce pairs of functional groups which have been shown in earlier studies to exhibit synthetic enzyme activity. This enzyme-like capability has been attributed to a certain degree of "cooperativity" between the pair of functional groups and the substrate. A copolymer containing the pair in an alternating manner was predicted to enhance the probability of this "cooperativity" to contribute to the overall process. A novel copolymer containing alternating imidazole and hydroxamic acid functions was reported along with its characterization and evaluation. This paper reports on the application of this concept to another pair of functional groups which has been shown to exhibit the above referred to "cooperativity." The synthesis of three novel copolymers containing the imidazole and phenolic hydroxyl functions in alternating sequences and their evaluation are reported here.

The carboxylic acid group has also been incorporated into synthetic enzymes [5, 8] and plays a key role in the "charge-relay system" of the natural enzyme chymotrypsin [9]. The synthesis of a copolymer containing carboxylic acid and imidazole functions and its evaluation as a bifunctional synthetic enzyme are also reported.

EXPERIMENTAL

The conditions for selection and purification of starting materials, reagents, and solvents for this work, as well as instrumentation and techniques used for monomer and polymer synthesis and characterization, are identical to those reported in the previous paper.

Monomers

N-(β-Vinyloxyethyl)imidazole/1-[2(Ethenyloxy)ethyl]-1H-imidazole (1)

The general procedure for N-alkylation of imidazole described by Fournari et al. [10] was used to synthesize 1. The spectral data and characterization of 1 are reported in the previous paper.

Histamine/1H-Imidazole-4-ethanamine [51-45-6] (2)

The free base (2) was prepared from histamine dihydrochloride by procedures reported in the previous paper.

2-Propenylphenol/(E) and (Z)-2-(1-Propenyl)-phenol [6380-21-8] (3)

Compound 3 was synthesized via the isomerization of 2-allylphenol as reported in the literature [11]. The product consisted of both E and Z isomers (87%), bp 115-123°C (17 torr), [literature bp 110-115°C (15-16 tor)] [11]. ^{1}H-NMR, ^{13}C-NMR, and IR spectral data confirmed the structure.

Isoeugenol/2-Methoxy-4-(1-Propenyl)-phenol [97-54-1] (4)

Compound 4 was obtained from Aldrich Chemical Co. and was distilled before use, bp 138.5-140.5°C (9 torr), [literature bp 140°C (12 torr)] [12]. The structure was confirmed by ^{1}H-NMR, ^{13}C-NMR, and IR.

trans-Anethole/(E)-1-Methoxy-4-(1-propenyl)-benzene [4180-23-8] (5)

Compound 5 was purchased from Aldrich Chemical Co. and was used without further purification. ^{1}H-NMR, ^{13}C-NMR, and IR spectral data confirmed the structure.

N-Ethylmaleimide/1-Ethyl-1H-pyrrole-2,5-dione [128-53-0] (6)

Compound 6 was purchased from the Aldrich Chemical Co. (Gold Label) and was used without further purification.

Diethylfumarate/(E)-2-Butenedioic Acid, Diethyl Ester [623-91-6] (7)

Compound 7 was obtained from Borden Chemical Co. and was distilled from CaH_2 under reduced pressure, bp 83.5-84°C (4.3 torr) [literature bp 75°C (5 torr)] [13]. ^{1}H-NMR spectral data confirmed its structure.

Fumaronitrile/(E)-2-Butenedinitrile [764-42-1] (8)

Compound 8 was purchased from Aldrich Chemical Co. Recrystallization from benzene gave white needles, mp 94-96.5°C (literature mp 95-97°C [14]. The structure was confirmed by ^{1}H-NMR spectral data.

N-Vinylimidazole/1-Ethenyl-1H-imidazole [1072-63-5] (9)

Compound 9 was purchased from Polysciences, Inc. and distilled from CaH_2 before use, bp 89-90°C (17 torr). ^{1}H- and ^{13}C-NMR spectral data confirmed its structure.

Maleic Anhydride/2,5-Furandione [108-31-6] (10)

Maleic anhydride was obtained from Fisher Scientific Co. and was sublimed at atmospheric pressure (80°C) prior to use, mp 50.5-53°C (literature mp 52.8°C) [12].

N-[2-(4-Imidazolyl)ethyl]succinamic Acid/4-([2-(1H-Imidazol-4-yl)ethyl]amino)-4-oxo-2-butanoic Acid (11)

To an Erlenmeyer flask containing a solution of 0.248 g (2.48 mmol) of succinic anhydride in 5 mL of acetone was added dropwise a solution of 0.275 g (2.47 mmol) of 2 in 3.5 mL of water, and the solution was stirred overnight. Absolute ethanol and acetone were added, and the precipitate was collected and dried in vacuo, giving 0.239 g (45.8%) of white solid, mp 159-159.5°C.

^{1}H-NMR (DMSO-d_6, 2.49): 2.37 (m, 4H), 2.63 (m, 2H), 3.26 (m, 2H), 6.84 (s, 1H), 7.68 (s, 1H), 7.98 (t, 1H).

^{13}C-NMR (DMSO-d_6, 39.5): 26.68, 29.75, 30.44, 38.87, 117.19, 134.00, 134.73, 171.34, 174.41.

IR (KBr): 3240 (w), 3155 (m), 3100 (m), 3000 (m), 2940 (m), 2855 (m), 1635 (vs), 1610 (s), 1570 (s), 1450 (w), 1420 (s), 1352 (s), 1285 (w), 1205 (s), 1140 (m), 1110 (m), 1065 (w), 1035 (w), 975 (w), 940 (w), 905 (w), 865 (m), 820 (m, br), 770 (m), 720 (m), 640 (m).

N-[2-(4-Imidazolyl)ethyl]succinimide/1-[2-(1H-Imidazol-4-yl)ethyl]-2,5-pyrrolidinedione (12)

A 25-mL three-necked round-bottomed flask fitted with a stir bar, condenser, and gas inlet tube was assembled hot and was cooled by flushing the apparatus with argon. To the cool flask were introduced 0.734 g (7.34 mmol) of succinic anhydride and 2 mL of distilled DMF. To the stirred solution was added by syringe a solution of 0.820 g (7.38 mmol) of 2 in 3 mL of DMF. A white solid formed which dissolved when the mixture was heated. The solution was refluxed for 2.5 h and allowed to cool. DMF was removed in vacuo, leaving a brown solid which was recrystallized from $CHCl_3$ mp 164-165°C.

^{1}H-NMR (DMSO-d_6, 2.49): 2.58 (s, 4H), 2.67 (m, 2H), 3.56 (m, 2H, 6.83 (s, 1H), 7.57 (s, 1H), 8.87 (br, 1H).

^{13}C-NMR (DMSO-d_6, 39.5: 24.82, 28.00, 38.04, 116.36, 133.86, 134.88, 177.57.

IR (KBr): 3440 (w), 3120 (w), 3080 (w), 3035 (w), 2990 (w), 2940 (2), 2830 (m), 2750 (w), 2640 (m), 1765 (m), 1690 (vs), 1575 (m), 1485 (m), 1450 (m), 1438 (m), 1433 (m), 1405 (s), 1330 (s), 1318 (m), 1289 (m), 1260 (w), 1248 (s), 1230 (m), 1150 (s), 1090 (m), 1055 (m), 1030 (w), 1000 (sh, m), 990 (m), 950 (m), 910 (br, m), 840 (m), 825 (m), 798 (m), 772 (m), 660 (m), 630 (m), 608 (w).

Copolymers

Isoeugenol–Maleic Anhydride Copolymer (13)

To a dry heavy-walled polymerization tube were added 2.758 g (0.0168 mol) of 4 and a solution of 1.652 g (0.0168 mol) of 10 and 0.0555 g (0.338 mmol) of AIBN in 10 mL of distilled acetone. The

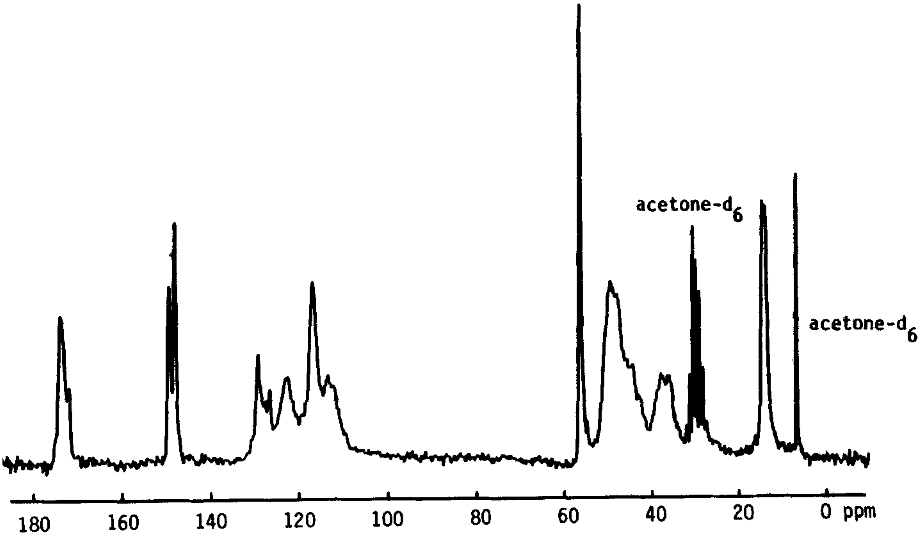


FIG. 1. ^1H decoupled ^{13}C-NMR spectrum of isoeugenol–maleic anhydride copolymer (13) in acetone-d_6 at 45°C.

solution immediately turned yellow in color. The tube contents were degassed on a high-vacuum line and the tube sealed at ~10^{-5} torr. Polymerization was carried out at 60°C for 72 h. The viscous acetone solution was added dropwise to a beaker of rigorously stirred CH_2Cl_2, the precipitate filtered and dried in vacuo, affording 2.725 g (62% conversion) of white solid (13). The ^{13}C-NMR spectrum is shown in Fig. 1.

IR (KBr): 3580-3300 (m), 2965 (w), 2940 (w), 1855 (m), 1775 (vs), 1605 (m), 1515 (s), 1460 (m), 1430 (m), 1370 (m), 1275 (s), 1235 (s), 1215 (sh, s), 1155 (m), 1130 (m), 1080 (m), 1030 (m), 920 (s), 825 (m), 785 (w), 645 (w).

Elemental analysis: Calculated for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38%. Found: C, 63.47; H, 5.61%.

VPO (acetone): $\overline{M}_n$ = 6950 g/mol. Intrinsic viscosity (acetone, 30.0°C): $[\eta]$ = 0.183 dL/g.

N-(β-Vinyloxyethyl)imidazole–Fumaronitrile Copolymer (14)

To a heavy-walled glass polymerization tube were added 1.878 g (0.0136 mol) of 1, 1.116 g (0.0143 mol) of 8, 0.0466 g (0.284 mmol) of AIBN, and 25 mL of distilled CH_2Cl_2. The tube was transferred to a high-vacuum line, degassed via several freeze-pump-thaw cycles, and sealed at ~10^{-5} torr. The polymerization was carried out at 60°C for 44 h, resulting in a red solution containing an oily dark precipitate. The tube contents were poured into ether. The oily precipitate was taken up in acetone-methanol and precipitated into chloroform. The solid was repricipated from acetone into carbon tetrachloride and dried in vacuo (room temperature) overnight, affording 0.367 g of mustard-brown powder. The mother liquors (from precipitations) were combined and reduced in volume on a rotary evaporator. Precipitation into chloroform gave an additional 0.525 g of dark-brown powder.

IR (KBr): 3150 (m), 2970 (m), 2940 (m), 2250 (m), 2200 (s), 2140 (m), 1620 (s), 1545 (m), 1440 (m), 1420 (m), 1355 (w), 1330 (m), 1290 (m), 1170 (m), 1080 (m), 1035 (w), 830 (m), 750 (m), 665 (m), 625 (m).

Elemental analysis: Calculated for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91%. Found: C, 60.53; H, 4.38; N, 28.62%.

N-(β-Vinyloxyethyl)imidazole–Diethylfumarate Copolymer (15)

To a polymerization tube were added 1.202 g (8.70 mmol) of 1, 1.199 g (6.96 mmol) of 7, 0.0114 g (0.0694 mmol) of AIBN, and 25 mL of distilled acetone. The tube contents were degassed (four freeze-pump-thaw cycles) and the tube sealed at ~10^{-5} torr. Polymerization was carried out at 60°C for 90 h.

2-Propenylphenol–Maleic Anhydride Copolymer (16)

To a dry heavy-walled polymerization tube were added 2.331 g (0.0174 mol) of 3 and a solution of 1.703 g (0.0174 mol) of 10 and 0.0542 g (0.330 mmol) of AIBN in 10 mL of distilled acetone. The solution became yellow, and the color persisted throughout polymerization. The tube contents were degassed and the tube sealed at ~10^{-5} torr. Polymerization was carried out at 60°C for 44 h. The viscous acetone solution was added dropwise to a beaker of vigorously stirred CH_2Cl_2. The precipitate was filtered and dried in vacuo, affording 3.510 g (87% conversion) of white solid (16).

IR (KBr): 3660-2500 (m, br), 1855 (m), 1770 (s, br), 1610 (m), 1585 (m), 1485 (m), 1365 (m, br), 1225 (s), 1150 (s, br), 920 (m, br), 755 (s).

Elemental analysis: Calculated for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21%. Found: C, 63.95; H, 5.69%.

VPO (acetone): $\overline{M}_n$ = 21,200 g/mol. Intrinsic viscosity (acetone, 30.0°C): $[\eta]$ = 0.231 dL/g. The ^{13}C-NMR spectrum was also in agreement with the proposed structure.

Isoeugenol–N-[2-(4-Imidazolyl)ethyl] maleimide Copolymer (17)

A 25-mL three-necked round-bottomed flask fitted with a stir bar, condenser, and gas inlet tube was assembled hot and cooled by flushing with Ar. To the flask were introduced 0.3463 g (1.32 mmol of repeating units) of 13 and ~2 mL of distilled DMF. To this solution was added 0.1707 g (1.54 mmol) of 2 in 0.5 mL of DMF. A white precipitate was observed. The mixture was refluxed for 6 h and allowed to cool. The viscous solution was added dropwise to a beaker of rapidly

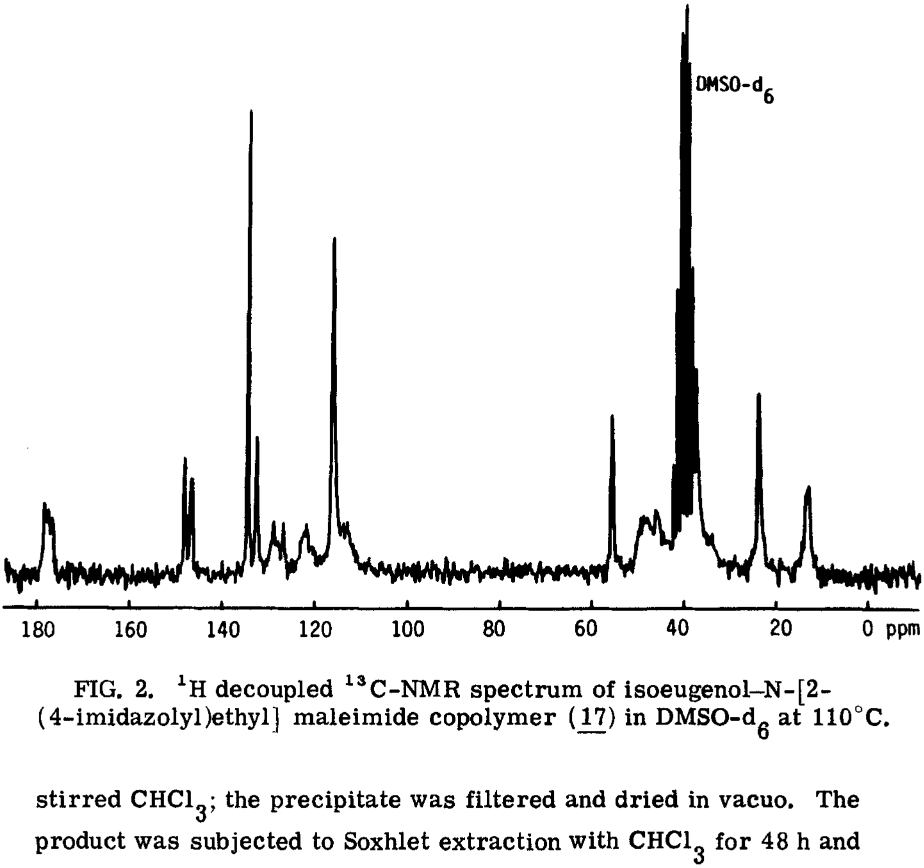


FIG. 2. ^{1}H decoupled ^{13}C-NMR spectrum of isoeugenol–N-[2-(4-imidazolyl)ethyl] maleimide copolymer (17) in DMSO-d_6 at 110°C.

stirred $CHCl_3$; the precipitate was filtered and dried in vacuo. The product was subjected to Soxhlet extraction with $CHCl_3$ for 48 h and dried in vacuo, affording 0.473 g of off-white solid (17). The ^{13}C-NMR spectrum is shown in Fig. 2.

IR (KBr): 3550-2500 (by, m), 3140 (m), 1765 (m), 1690 (s), 1615 (m), 1595 (m), 1510 (m), 1445 (m), 1400 (m), 1360 (m), 1270 (m), 1225 (br, m), 1160 (m), 1130 (m), 1080 (w), 1025 (m), 900 (br, w), 820 (m), 785 (m), 650 (w), 620 (m).

Elemental analysis: Calculated for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82%. Found: C, 58.75; H, 5.66; N, 10.68%.

2-Propenylophenol–N-[2-(4-Imidazolyl)ethyl] maleimide Copolymer (18)

Copolymer 18 was prepared from 16 by the same method as copolymer 15. Thus, 0.383 g (1.65 mmol of repeat units) of 16 was combined with 0.198 g (1.78 mmol) of 2 in refluxing DMF to give 0.470 g of off-white product (18).

IR (KBr): 3650-2500 (br, m), 2960 (m), 1765 (m), 1690 (s), 1590 (m), 1483 (m), 1450 (m), 1400 (m), 1360 (m), 1255 (m), 1220 (m), 1160 (m), 1100 (m), 980 (w), 935 (w), 830 (w), 755 (m), 660 (w), 615 (w).

Elemental analysis: Calculated for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91%. Found: C, 62.00; H, 5.56; N, 10.55%.

trans-Anethole–Maleic Anhydride Copolymer (19)

To a polymerization tube were added 1.630 g (0.0166 mol) of 10, 0.0534 g (0.325 mmol) of AIBN, 2.464 g (0.0166 mol) of 5, and 10 mL of distilled acetone. The tube contents were degassed in the usual manner and sealed at ~10^{-5} torr. Polymerization was carried out for 20 h at 60°C. The gelatinous mass was dissolved in DMF and precipitated into ether. The precipitate was filtered and dried in a vacuum oven (90°C, 1 torr) for 48 h, affording 2.065 g (50% conversion) of white solid (19).

IR (KBr): 2960 (m), 2940 (m), 2840 (w), 1860 (m), 1780 (vs), 1610 (m), 1580 (w), 1510 (s), 1465 (m), 1440 (m), 1390 (w), 1335 (m), 1305 (m), 1255 (s), 1180 (s), 1080 (m), 1030 (m), 920 (s), 830 (m), 815 (sh, m), 738 (m).

Elemental analysis: Calculated for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73%. Found: C, 68.11, H, 5.77%.

The ^{13}C-NMR spectral data also supported the proposed structure.

trans-Anethole–N-[2-(4-Imidazolyl)ethyl]maleimide Copolymer (20)

Copolymer 20 was prepared from 19 by the same method as copolymers 17 and 18. Thus, 0.422 g (1.71 mmol of repeat units) of 19 was combined with 0.200 g (1.80 mmol) of 2 in refluxing DMF to give 0.402 g of off-white product (20).

IR (KBr): 3440-2800 (br, m), 2940 (m), 2840 (m), 1770 (m), 1695 (s), 1610 (m), 1580 (w), 1510 (s), 1460 (sh, m), 1440 (m), 1400 (m) 1355 (m), 1300 (w), 1250 (m), 1180 (m), 1160 (m), 1105 (w), 1085 (w), 1030 (m), 975 (w), 930 (w), 830 (m), 730 (w), 660 (w), 615 (m).

Isoeugenol–N-Ethylmaleimide Copolymer (21)

To a polymerization tube were added 1.5315 g (0.0122 mol) of 6, 0.0397 g (0.242 mmol) of AIBN, 2.010 g (0.0122 mol) of 4, and 10 mL of distilled acetone. The tube contents were degassed and the tube sealed at ~10^{-5} torr. Polymerization was carried out at 60°C for 38 h. The acetone solution was added dropwise to a vigorously stirred beaker of ether. The precipitate was filtered and dried in vacuo, affording 3.117 g (88% conversion) of white solid (21).

The ^{13}C-NMR spectrum is shown in Fig. 3.

IR (KBr): 3680-3100 (by, m), 2970 (m), 2940 (m), 2880 (w), 2840 (w), 1770 (m), 1690 (vs), 1600 (m), 1510 (s), 1450 (m), 1405 (s), 1375 (m), 1350 (m), 1270 (m), 1225 (s), 1130 (m), 1030 (m), 940 (w), 895 (w), 860 (w), 810 (m), 790 (m), 770 (w), 730 (w), 650 (m).

Elemental analysis: Calculated for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84%. Found: C, 65.66; H, 6.64; N, 5.03%.

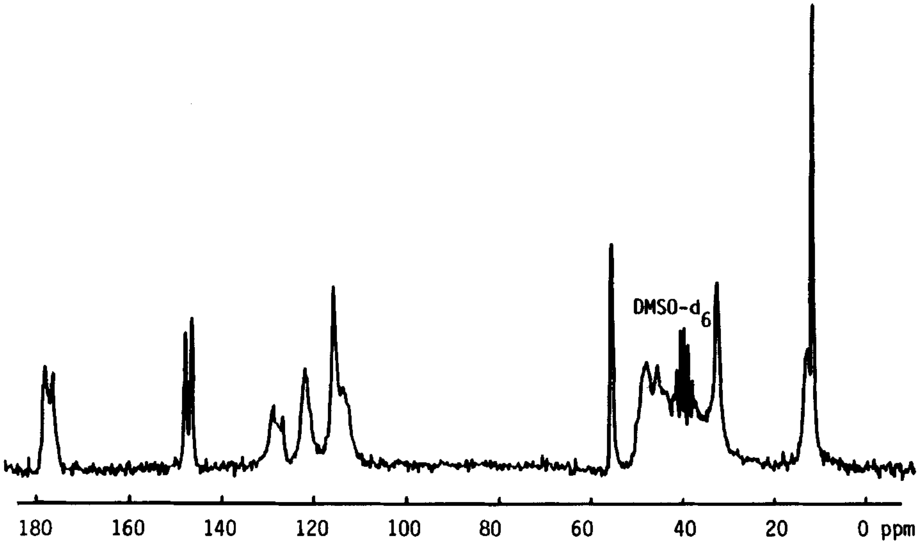


FIG. 3. ^1H decoupled ^{13}C-NMR spectrum of isoeugenol–N-ethylmaleimide copolymer (21) in DMSO-d_6 at 110°C.

VPO (acetone): $\overline{M}_n$ = 25,200. Intrinsic viscosity (acetone, 30.0°C): $[\eta]$ = 0.276 dL/g.

2-Propenylphenol–N-Ethylmaleimide Copolymer (22)

To a polymerization tube were added 1.8594 g (0.01486 mol) of 6, 0.0485 g (0.295 mmol) of AIBN, 1.994 g (0.01486 mol) of 3, and 10 mL of distilled acetone. The tube contents were degassed in the usual manner and the tube sealed ($\sim 10^{-5}$ torr). Polymerization was carried out at 60°C for 38 h. The acetone solution was added dropwise to ethyl ether, the precipitate filtered and dried in vacuo, affording 1.286 g (33% conversion) of white solid (22).

The NMR and IR spectral data were in agreement with the proposed structure.

Elemental analysis: Calculated for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40%. Found: C, 67.01; H, 6.51; N, 6.25%.

Intrinsic viscosity (acetone, 30.0°C): $[\eta]$ = 0.083 dL/g.

trans-Anethole–N-[2-(4-Imidazolyl)ethyl]maleimic Acid Copolymer (23)

Copolymer 23 was prepared from 19 by the same method as was used to prepare 11 from succinic anhydride and 2. The ^{13}C- and ^1H-NMR and IR spectral data confirmed its structure.

Kinetic Measurements

Pseudo first-order kinetics were measured on Cary 17-D or Perkin-Elmer 330 spectrophotometers. Temperature control was provided by a Lauda K-2/R (40.0 ± 0.2°C) or a Haake A80 (25.0 ± 0.2°C) constant-temperature apparatus. A Corning-125 pH meter fitted with a Ag/AgCl pH electrode was used to measure the pH of solutions before and after the reaction with substrate. p-Nitrophenyl acetate (PNPA) was obtained from the Aldrich Chemical Co. and was recrystallized from cyclohexane before use, mp 77-78°C (literature mp 81-82°C) [15]. 2,4-Dinitrophenyl benzoate (DNPB) was kindly supplied by Ann Mobley [16]. Deionized water was distilled in glass before use. DMSO and THF were purified as previously described. Tris(hydroxymethyl)-aminomethane (Tris) was obtained from Fisher Chemical Co. and was used without further purification.

Two 80% DMSO:H_2O (v/v) stock solutions were prepared, both 0.02 M in Tris or Tris·HCl, $\mu = 0.02$ (KCl). The first solution was prepared by adding 1.2114 g (0.010 mol) of Tris, 0.7455 g (0.010 mol) of KCl, and 100 mL of distilled water to a 500-mL volumetric flask and diluting to the mark with distilled DMSO. The second solution was prepared in the same manner, substituting 100 mL of 0.1 N HCl (aq) for 100 mL of distilled water. Again, the solutions were combined to give buffer solution of the desired pH. Thus, a 4:1 volume ratio of Tris · HCl: Tris gave a pH of 8.9. Stock solutions of PNPA (1.60×10^{-3} M) in DMSO and DNPB (1.60×10^{-3} M) in THF were employed.

Catalyst solutions were prepared according to the concentration of functional groups. Due to difficulty in determining the exact composition of copolymers, it was assumed that the copolymers studied were strictly 1:1 alternating copolymers. The contribution to the molecular weight by endgroups was also neglected in all polymer catalysts. Thus, stock solutions of all copolymer catalysts studied were $\sim 5.33 \times 10^{-3}$ N in repeat units dissolved in 0.02 M Tris buffer of the desired pH.

The kinetic method using the Cary 17-D spectrophotometer was the same as was used with the Perkin-Elmer 330 spectrophotometer, except that each sample cell required a corresponding reference cell. To each of six 1-cm path length quartz cells was added 3.0 mL of buffer solution by pipet. To four of the cuvettes was added 150 μL of catalyst solution by micropipet; to the other two cuvettes was added 150 μL of buffer solution. To one of the latter cuvettes was added 50 μL of substrate solvent (CH_3CN, DMSO, or THF), and it was placed in the reference beam of the spectrophotometer. The remaining five cuvettes were placed in the sample compartment to equilibrate thermally. The sample cuvettes were then each charged with 50 μL of substrate solution, agitated by inverting the sample holder, and replaced in the sample compartment. The release of 2,4-dinitrophenolate ion (370 nm) was observed at constant wavelength at constant time intervals.

The reaction was followed for at least 10 half-lives as judged by the constancy of the absorbance readings (A_∞). A plot of ln (A_∞ - A_t) vs time (t) was constructed, and the negative slope of the best straight line as determined by the least squares program of a Texas Instruments TI-55-II calculator gave the desired rate constants (k_{meas}), the sum of the catalyzed (k_{obs}) and uncatalyzed (k_{blank}) rate constants. Furthermore, the second-order rate constant (k_{cat}) was calculated from the relation $k_{cat} = k_{obs}/[\text{catalyst}]$ [17]. In the case of slow reactions, where A_∞ was not obtained in a reasonable time, k_{meas} was determined by the method described in the literature [18].

RESULTS AND DISCUSSION

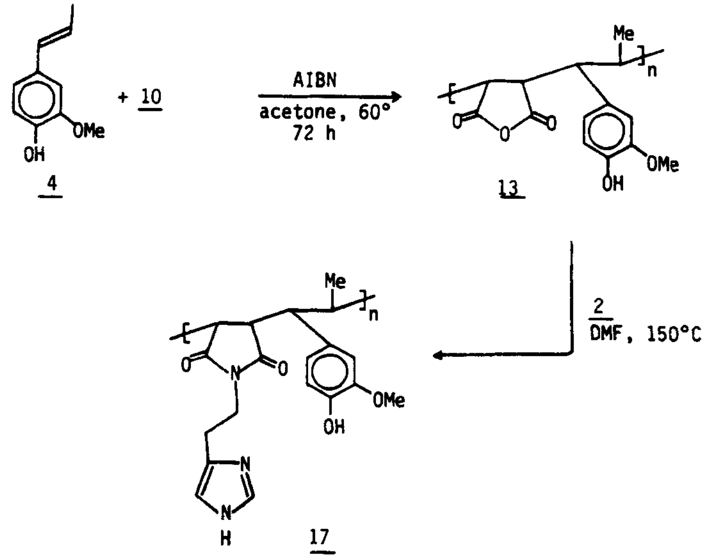
Copolymer Synthesis

Considerable difficulty was encountered in copolymerization of 1 with maleimide derivatives, as reported in the previous paper. Since both fumaronitrile (8) and diethylfumarate (7) are electron-deficient monomers (8 (e = +2.73) [19] and 7 (e = +2.26) [19]), copolymerization with vinyl ether (1) would be predicted to produce copolymers. It was envisioned that hydroxamic acid groups could be introduced following copolymerization via transformation of the nitrile and ester groups.

Copolymerizations of 1 with 7 and 8 were carried out in solution at 60°C using AIBN as initiator to produce low yields of low molecular weight copolymers 15 and 16, respectively. The ^{1}H-NMR spectrum of copolymer 15 indicated that it was rich in diethyl fumarate (~2.3:1) as determined by comparing the integration of methyl protons to imidazole protons. These data are supported by elemental analysis for nitrogen, the value obtained being ~2.8% lower than expected for a 1:1 copolymer. Copolymer 14 was suspected of being rich in fumaronitrile on the basis of nitrogen analysis, found to be ~2.7% higher than expected for a 1:1 alternating copolymer. Since alternating copolymers were not obtained in either case, no attempt was made to transform either the nitrile or ester group to the hydroxamic acid group.

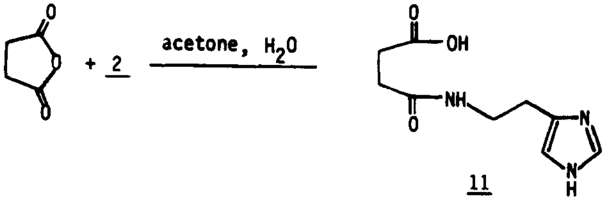
Cooperative interactions between imidazole and phenol hydroxyl groups have been demonstrated previously. Thus, it was decided to synthesize and evaluate alternating copolymers containing these functional groups. Since considerable difficulty had been encountered with the direct polymerization of imidazole-containing monomers, introduction of the imidazole group into preexisting alternating copolymers appeared to be a promising approach to the desired polymer.

A 1:1 alternating copolymer resulting from copolymerization of isoeugenol (4) and maleic anhydride (10) had been reported. This reaction was repeated to give copolymer 13 in 62%conversion. Treatment of 13 with histamine (2) in refluxing DMF gave a quantitative yield of copolymer 17 containing both phenol and imidazole functionalities (Eq. 1).

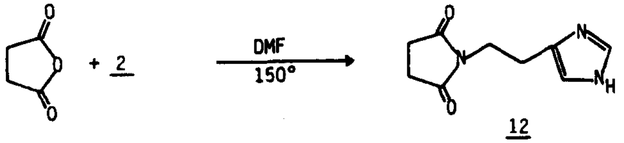


(1)

In order to know that 17 contained succinimide and not succinamic acid units, model compounds 11 and 12 were synthesized (Eqs. 2 and 3).



(2)



(3)

Imide (26) displayed carbonyl stretching frequencies at 1765 and 1690 cm^{-1} which were in agreement with those of copolymer 17. Compound 11 displayed IR absorptions at 1 635 and 1 610 cm^{-1}.

Copolymers 16 and 19 were synthesized in a similar manner by copolymerization of 10 and 3 to yield 16 and 10 and 5 to yield copolymer 19. Copolymers 18 and 20 were synthesized from 16 and 19, respectively, in a similar manner to that used to convert copolymer 13 to 17 (Eq. 1). It was predicted that either 17 or 18 would exhibit cooperativity in the hydrolysis of an ester substrate, while 20 would exhibit catalysis by the imidazole group only. Copolymer 21 was synthesized by copolymerization of 6 and 4, and copolymer 22 was synthesized by copolymerization of 6 and 3. These copolymers, being alternating copolymers of N-ethylmeleimide and a phenol, were synthesized as model catalysts which would demonstrate catalysis by the phenol group only.

Observations support an alternating structure for these copolymers. Mixing 10 or 6 with 3, 4, and 5 produces a yellow color, indicative of a charge-transfer complex [21]. It has been shown [20] that 4 and 10 form a 1:1 copolymer regardless of monomer feed ratios. Elemental analysis of copolymer 19 gives excellent agreement for a 1:1 copolymer, although the others do not. Finally, neither propenylphenols nor 10 homopolymerize under the copolymerization conditions employed.

Some properties of copolymers 13 and 16 through 22 are presented in Table 1.

Copolymer 23 was synthesized from copolymer 19 to represent a catalyst possessing imidazole and carboxylic acid functions, only, and for comparison with copolymer 20 to determine whether ring closure during its synthesis was incomplete or ring opening had occurred by hydrolysis during its evaluation as a catalyst.

Kinetic Studies with Copolymer Catalysts 17, 18, 20, 21, and 23

Pseudo first-order kinetics were measured using copolymer catalysts 17, 18, 20, 21 and 22 and model compound 26 as previously described (see Experimental). The water insolubility of these copolymers necessitated the use of an 80% $DMSO:H_2O$ (V/V) medium. Copolymer 22 was insoluble in this solvent system and, therefore, was not studied as a catalyst. PNPA was initially used as substrate, but its hydrolysis proceeded too slowly to obtain rate constants in a reasonable time. The more reactive substrate DNPB was subsequently used. The results obtained with copolymer 21 are presented in Table 2. The results of the measurements on copolymers 17, 18, and 20 are shown in Fig. 4.

Copolymer 21 exhibited little or no catalytic activity toward hydrolysis of DNPB (Table 2), which implies that the phenol group or phenol-

TABLE 1. Properties of Copolymers 13 and 16 through 22

Copolymer	$\overline{M}_n$, g/mol	$[\eta]$, dL/g[a]	Conversion, wt%	IR carbonyl stretch, cm^{-1}
13	6 950	0.183	62	1 855, 1 775
16	21 200	0.231	87	1 855, 1 770
17	-	-	100	1 765, 1 690
18	-	-	88	1 765, 1 690
19	-	-	50	1 860, 1 780
20	-	-	69	1 770, 1 695
21	25 200	0.276	88	1 770, 1 690
22	-	0.083	33	1 770, 1 695

[a] In acetone at 30.0°C.

TABLE 2. Results of Esterolysis[a] of DNPB with Copolymer Catalyst

Catalyst	pH	k_{obs}, min^{-1}	k_{cat}, $L \cdot mol^{-1} min^{-1}$
21	8.4	0	0
Blank	"	1.00×10^{-3}[b]	-
21	8.9	0.06×10^{-3}	0.24
Blank	"	1.61×10^{-3}[b]	-
21	9.5	0.01×10^{-3}	0.04
Blank	"	3.84×10^{-3}[b]	-
21	10.0	0.36×10^{-3}	1.44
Blank	"	5.79×10^{-3}[b]	-

[a] Conditions: [Tris buffer] = 0.02 M; $\mu = 0.1$ (KCl); 0.015:0.197:0.788 = THF:H_2O:DMSO (v/v); initial substrate concentration = 2.5×10^{-5} N; catalyst concentration = 2.5×10^{-4} N (of repeat units); T = 25.0°C.

[b] k_{meas} of uncatalyzed reaction.

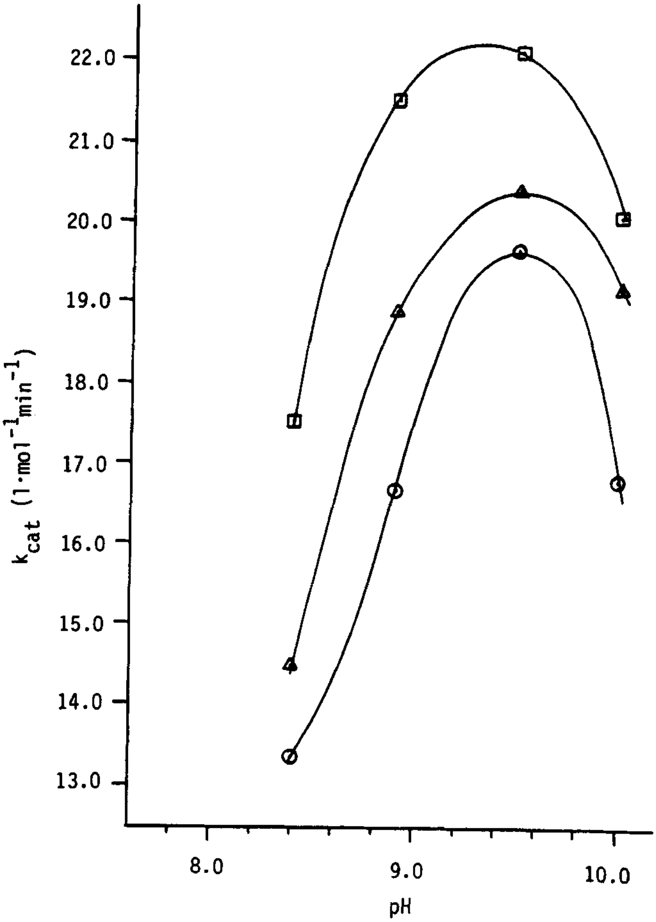


FIG. 4. pH-rate profile for the esterolysis of DNPB using 17 (○), 18 (△), and 20 (□) as catalysts. (Conditions for measurements are described in Footnote a of Table 2).

ate ion is catalytically inactive. All of the imidazole-containing polymers (17, 18, and 20) catalyze the esterolysis of DNPB at about the same rate. No cooperative interactions between imidazole and phenol groups were observed by comparison of the rates for 17 and 18 vs 20. Indeed, copolymer 20 was the most efficient catalyst in this group. The differences in the rate constants for 17, 18, and 20 are most likely the result of unequal concentrations of imidazole groups.

As can be seen in Fig. 4, copolymers 17, 18, and 20 display a bell-shaped pH-rate profile. Such behavior is often interpreted as a result of simultaneous participation by a general acid (BH^+) and a weak base (nucleophilic or general). The concentration of BH^+ would decrease with increasing pH, which would account for the decrease of k_{cat} at pH = 10.0. A reasonable candidate for BH^+ is the imidazolium ion. This argument is unsatisfactory, however, because of the extreme reactivity of DNPB, i.e., no assistance by a general acid should be required to effect hydrolysis. Also, the pK_a of the 4-methylimidazolium ion is 7.61 [22], which would indicate that the concentration of imidazolium ion in the polymer is very low in the pH range studied. Without knowledge of the pK_a of the imidazolium ion in the copolymers studied, this latter argument is speculative.

Another factor which might account for a bell-shaped pH-rate profile is the conformation of the copolymers in solution. If ring closure following derivatization of the maleic anhydride copolymers with histamine did not proceed quantitatively, a small number of succinamic acid units would result, giving rise to a polyelectrolyte. Alternatively, ring opening might occur under the conditions of the kinetic experiments. To check this hypothesis, copolymer 20 was recovered after standing in buffer solution at pH = 10.0 for 15 days. The IR spectrum of recovered 20 was identical to the spectrum of untreated copolymer, indicating that no ring opening had occurred in buffer solution.

Copolymer 20 did exhibit polyelectrolyte behavior as determined by an increase in η_{sp}/c on dilution (Fig. 5). The bell-shaped pH-rate profile might then be explained by chain expansion, allowing greater access of DNPB to imidazole residues and chain contraction at higher pH leading to a decrease in observed rate.

To test this hypothesis, compound 12 and copolymer 23 were evaluated as catalysts. The results are shown in Fig. 6. Model compound 12 exhibited a similar bell-shaped pH-rate profile, which gave evidence that this behavior could not be attributed to copolymer conformation.

In view of the small differences in k_{cat} with increasing pH, it became evident that the bell-shaped pH-rate profile might not be meaningful. Indeed, there seems to be only a slight dependence of catalytic activity on pH. Therefore, the bell shape might be the result of error associated with the determination of pH and k_{obs}.

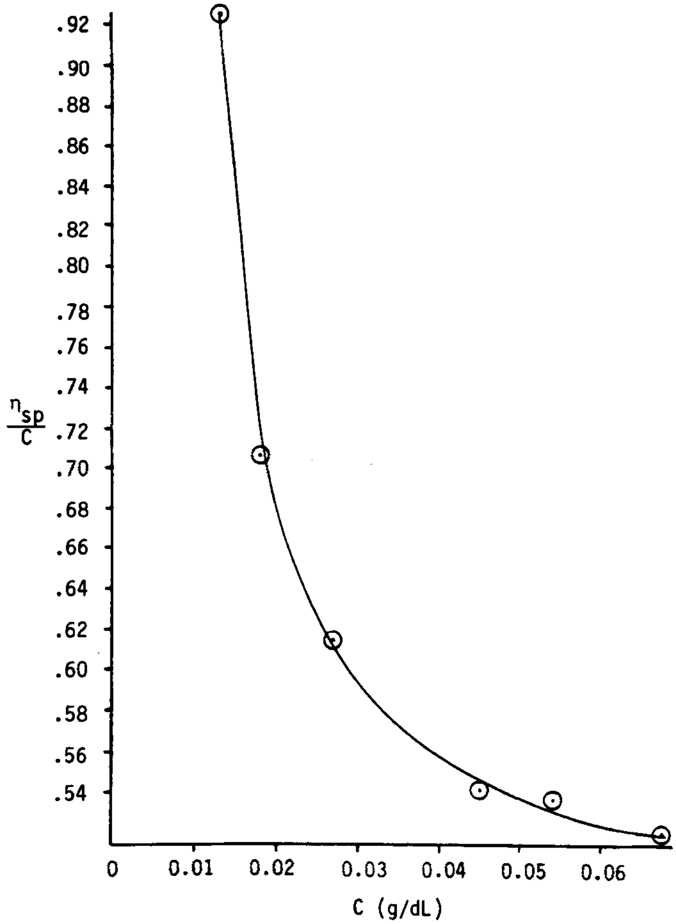


FIG. 5. Plot of η_{sp}/c vs c for copolymer 20, 0.02 M Tris buffer, $\mu = 0.02$ (KCl), pH = 9.5.

CONCLUSION

The inability to observe cooperativity between imidazole-hydroxamic acid and imidazole-phenol groups is surprising in light of results by other workers. However, cooperativity would be expected in alternating copolymers only when a precise stereochemical fit can be achieved between substrate and functional groups. Cooperativity reported by other workers in random bifunctional copolymers could be the result of a

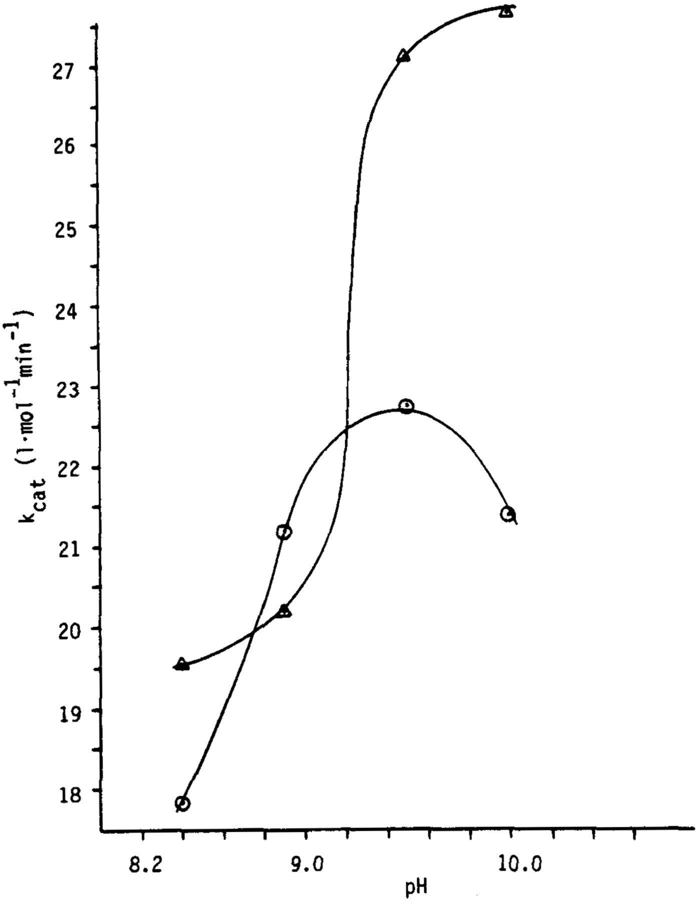


FIG. 6. pH-rate profile for the esterolysis of DNPB using 12 (○) and 23 (△) as catalysts. (Conditions for measurement as described in Footnote a of Table 2.)

serendipitous alignment of functional groups as a result of chain conformation and/or other unknown factors.

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