TABLE I
ANTIMALARIAL SCREENING DATA[a] FOR COPOLYMERS IIa–c

System	Dose level, mg/kg	Monomer act. (I)[b]	Polymer act. (II)[b]
Ia, IIa	80	Inactive	Inactive
	160	Inactive	Inactive
	320	Active	Inactive
	640	Active	Curative
	1280	Active	Curative
Ib, IIb	40	Curative	Inactive
	160	Curative	Toxic
	640	Curative	Toxic
Ic, IIc	40	Inactive	Curative
	160	Curative	Curative
	640	Curative	Curative

Antimalarial testing was done by Dr. Leo Rane at the University of Miami Medical School. Tests were carried out employing *Plasmodium berghei* in young ICR/Ha Swiss mice. [b] Active = mice in a treated group survive at least 14 days; curative = mice in a treated group survive to 30 days; toxic = deaths occurring through day 5 after infection are attributed to drug action; Control animals do not die before day 6.

Sulfabenzamide–Formaldehyde Copolymer (IIb).—A mixture of 1.37 g (0.005 mole) of sulfabenzamide (Ib), 50 ml of water, 1.1 ml of 37% aqueous HCl, and 1 ml of 37% aqueous formaldehyde solution was refluxed for 5.5 hr. A pale yellow resin was precipitated which adhered to the container walls. The reaction mixture was cooled and the liquid was decanted off. The resin was dissolved in the minimum amount of hot dimethylformamide (DMF) and the product precipitated from the DMF solution by addition of water. The product was filtered, reprecipitated again from DMF with water, filtered, and air dried. The yield of pale yellow solid softening at 193–197° was 0.95 g (66%). The intrinsic viscosity of the product in DMSO at 25° was 0.24; infrared data (cm^{-1}): 3600 w, 3550 m, 3375 s, 3220 m, 3050 w, 2875 w, 2315 w, 1775 s, 1590 s, 1500 m, 1460 s, 1425 s, 1380 w, 1340 s, 1250 m, 1175 s, 1160 s, 1085 s, 1065 m, 1030 w, 1000 w, 950 w, 885 m, 830 m.

Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 58.31; H, 4.79; N, 9.72. Found: C, 58.39; H, 5.11; N, 9.02.

Sulfacetamide–Formaldehyde Copolymer (IIc).—The procedure for the preparation of IIb was repeated employing 1.99 g (0.01 mole) of sulfacetamide (Ic) in place of Ib and 1 ml of 3.7% aqueous HCl instead of 1.1 ml of 37% aqueous HCl. The yield of product softening at 230–235° was 0.3 g (14.2%). The intrinsic viscosity of the copolymer in DMSO at 25° was 0.10; infrared data (cm^{-1}): 3585 m, 3535 m, 3350 s, 3230 s, 3050 m, 2860 w, 2610 w, 2320 w, 1700 w, 1695 m, 1595 s, 1500 m, 1445 m, 1375 w, 1325 s, 1240 w, 1220 w, 1165 s, 1155 s, 1095 m, 1000 w, 950 w, 900 w, 835 w.

Anal. Calcd for $C_9H_{10}N_2O_3S$: C, 47.77; H, 4.45; N, 12.39; S, 14.15. Found: C, 46.45; H, 4.26; N, 13.01; S, 14.95.

Hydrolysis of IIa.—Hydrolysis of IIa was carried out as reported previously for a sulfapyridine–formaldehyde copolymer.[1b] When the reaction mixture was made basic, the odor of ammonia was apparent. The solution was evaporated to dryness after reacidification. The residue was thoroughly washed with water to remove the salt present. The brown solid left was identical with the sulfonic acid hydrolysis product derived from hydrolysis of the sulfapyridine–formaldehyde copolymer.[1a]

Hydrolysis of IIb.—IIb (6.5 g) was dissolved in 50 ml of 10% aqueous NaOH and refluxed for 8 hr. The solution was cooled and the pH was adjusted to 8 with dilute aqueous HCl. The gummy precipitate (3.5 g) was washed with HCl after filtration and dried. The infrared spectrum of this material was comparable to that of IIa, and acid hydrolysis of this product[1a] yielded III. The filtrate at pH 8 was made strongly acidic with HCl, and 2.2 g (96%) of benzoic acid (identified by infrared comparison) was precipitated.

Hydrolysis of IIc.—IIc (2.0 g) was dissolved in 12 ml of 10% aqueous NaOH and refluxed 24 hr. The solution was cooled and carefully neutralized to pH 8. A precipitate appeared which was collected by filtration, washed with water, and dried to yield 1.45 g of material having an infrared spectrum comparable to IIa, and acid hydrolysis of this product yielded III. The filtrate at pH 8 was made strongly acid with aqueous HCl. The odor of acetic acid was apparent, but no attempt was made to achieve quantitative isolation.

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Synthetic Biologically Active Polymers. V. 4,4′-Diaminodiphenyl Sulfone–Formaldehyde Copolymer[1]

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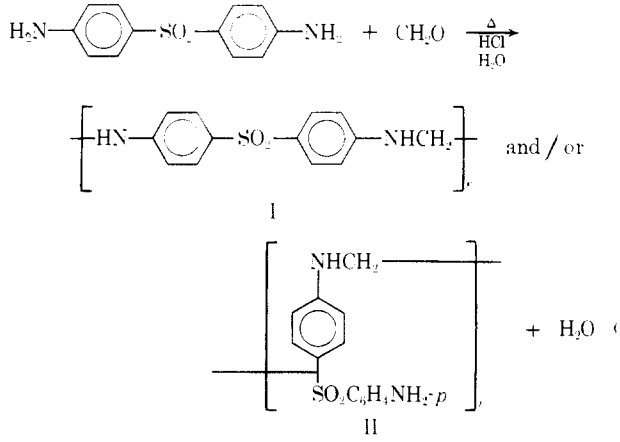
Previous articles in this series[2] have dealt with the preparation, characterization, and properties of new polymeric tropolone derivatives[2a] and a novel sulfapyridine–formaldehyde copolymer.[2b] This report concerns itself with the copolymerization of 4,4′-diaminodiphenyl sulfone (DDS) and formaldehyde. Reaction of DDS and formaldehyde in refluxing aqueous HCl (see eq 1) yielded a solid material which softened at 235° and whose infrared spectrum and analysis indicated that it possessed either structure I or structure II. Structures I and II also would be expected by analogy with similar reactions.[2b] The product was soluble in aqueous HCl, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF).



$$[\eta] = K\bar{M}^{a} \quad (2)$$

$[\eta]$ = intrinsic viscosity
$\bar{M}$ = average molecular weight
K and a = empirical constants

In order to differentiate between the two possibilities, or to recognize the presence of both structures in the product, the purified reaction product was treated with aqueous nitrous acid to diazotize any free amino groups present. Upon warming the solution and col-

(1) This work was taken in part from the thesis to be submitted by Mr. John Razzano in partial fulfillment of the requirements for the Ph.D. degree.

(2) (a) R. J. Cornell and L. G. Donaruma, *J. Med. Chem.*, **8**, 388 (1965); R. J. Cornell and L. G. Donaruma, *J. Polymer Sci.*, **3A**, 827 (1965); (b) L. G. Donaruma and John Razzano, *J. Med. Chem.*, **9**, 258 (1966).

lecting the evolved nitrogen, it was found that the volume of N_2 collected, per unit weight of II used, indicated that only II was present. If the product had been a mixture of I and II, less nitrogen would have evolved, and if I were the structure, no N_2 would have been produced. Thus, it appears that the copolymers possess the structure exemplified by II.

The intrinsic viscosity of II in DMSO at 25° was 0.34 and by light-scattering measurements in DMSO, its average molecular weight was found to be 4700 ± 10%. The polymer system (II) appeared to be monodisperse since fractions of differing inherent viscosities could not be obtained by fractional precipitation from either DMF or DMSO with water or aqueous saline solutions.

Because we wished to establish a viscosity–molecular weight relationship for this DDS–formaldehyde copolymer system, the identical synthetic reaction for the preparation of II was reemployed utilizing a 0.5 *M* and 1.0 *M* excess of formaldehyde, respectively. The reactions went smoothly to yield products similar in all respects to II except that the intrinsic viscosities at 25° in DMSO and the average molecular weights by light-scattering measurements in DMSO proved to be 0.38 and 7600 ± 10% and 0.45 and 10,000 ± 10%, respectively. Having obtained intrinsic viscosity and molecular weight data for three similar samples, we were in a position to obtain the Staudinger constants (see eq 2) which relate viscosity to molecular weight.[3] By plotting the logarithm of $[\eta]$ *vs.* log $\bar{M}$, the slope of the curve obtained and the intercept of the curve were log *a* and log *K*, respectively, and, for this system, $a = 0.37$ and $K = 0.0145$. Thus, we can now determine absolute weight-average molecular weights directly from viscosity measurements, and we are now capable of determining the relationship of molecular weight to biological activity in a much more quantitative fashion than previously.[2b]

Both DDS and II were screened for antimalarial activity under identical conditions as reported previously,[2b] and the results are shown in Table I.

TABLE I
ANTIMALARIAL ACTIVITY[a] OF DDS AND ITS FORMALDEHYDE COPOLYMER (II)

Dose level, mg/kg of body wt	DDS act.[b]	II act.[b]
40	Curative; nontoxic	Inactive; nontoxic
160	Curative; nontoxic	Active; nontoxic
640	Curative; toxic	Curative; nontoxic

[a] Antimalarial testing done by Dr. Leo Rane at the University of Miami Medical School. Tests were carried out employing *Plasmodium berghei* in young ICR/Ha Swiss mice. [b] Active, when mice in a treated group survive at least 14 days; curative, when mice in a treated group survive to 30 days; toxic, deaths occurring through day 5 after infection are attributed to drug action. Control animals do not die before day 6.

Experimental Section

Copolymerization of DDS with Formaldehyde.—A mixture of 2.44 g (0.0094 mole) of DDS, 250 ml of water, and 5 ml of 4% aqueous HCl was heated to reflux, and 0.8 ml (0.0098 mole) of 37% aqueous formaldehyde solution was added. The mixture was refluxed for 9 hr, and the precipitated product was removed by filtration, extracted with boiling water to remove unreacted DDS, and dried or, the crude product was dissolved in DMF and reprecipitated by the addition of water, filtered, and air dried. The yield of product softening at 235° was 2.52 g. The intrinsic viscosity of the product in DMSO at 25° was 0.34, and the molecular weight of the product by light-scattering measurements in DMSO was 4700 ± 10%; infrared data (cm^{-1}): 3540 w, 3480 w, 3380 s, 3245 w, 3070 w, 2835 w, 1600 s, 1535 s, 1470 w, 1435 w, 1375 w, 1330 s, 1300 s, 1285 s, 1210 w, 1160 s, 1120 s, 1090 m, 1060 m, 1040 w, 970 w, 845 m, 825 w, 740 m, 710 s.

Anal. Calcd for $C_{13}H_{12}N_2SO_2$: C, 59.99; H, 4.62; N, 10.75; S, 12.30. Found: C, 59.81; H, 4.92; N, 10.20; S, 12.13.

By carrying out the experiment above employing 1.2 ml of 37% aqueous formaldehyde solution (0.5 *M* excess) instead of 0.8 ml, 2.50 g of product softening at 245° was obtained. This material had an intrinsic viscosity of 0.38 in DMSO at 25° and a molecular weight (determined by light scattering measurements in DMSO) of 7600 ± 10%; infrared data (cm^{-1}): 3545 w, 3480 w, 3370 m, 3250 w, 3065 w, 2860 w, 1600 s, 1520 s, 1475 m, 1420 w, 1330 m, 1300 s, 1275 s, 1210 w, 1165 s, 1120 s, 1090 m, 1055 w, 1020 w, 970 w, 845 m, 825 m, 740 m, 710 m.

Anal. Calcd for $C_{13}H_{12}N_2SO_2$: C, 59.99; H, 4.62; N, 10.75; S, 12.30. Found: C, 59.76; H, 4.62; N, 10.53; S, 12.05.

Again, by repeating the experiment for the preparation of II employing 1.6 ml of 37% aqueous CH_2O solution (1.0 *M* excess) in place of 0.8 ml, 2.53 g of product softening at 253° was obtained. This material had an intrinsic viscosity of 0.45 in DMSO at 25° and a molecular weight (determined by light-scattering measurements in DMSO) of 10,000 ± 10%; infrared data (cm^{-1}): 3590 w, 3490 w, 3375 m, 3205 w, 3025 w, 2850 w, 1600 s, 1515 m, 1470 w, 1420 w, 1330 m, 1300 s, 1275 s, 1205 w, 1160 s, 1120 s, 1085 m, 1050 w, 1020 w, 960 w, 840 m, 820 w, 740 w, 710 m.

Anal. Calcd for $C_{13}H_{12}N_2SO_2$: C, 59.99; H, 4.62; N, 10.75; S, 12.30. Found: C, 59.73; H, 4.83; N, 10.56; S, 12.38.

Reaction of II with Nitrous Acid.—A known weight of II was dissolved in enough concentrated HCl to dissolve the copolymer at 25° and enough ice was added to the solution to initiate precipitation. Sufficient concentrated HCl was added to redissolve the precipitate which appeared, and a cold saturated aqueous solution of $NaNO_2$ was added until the copolymer solution gave a positive test for HNO_2 with starch–iodide paper. The solution was carefully warmed, and N_2 was rapidly evolved and collected over N_2-saturated water in a closed system. When N_2 evolution ceased, the reaction flask was cooled to 0°, and the volume of collected N_2 was measured. After corrections for temperature and pressure were made, it was found that 0.95 mole of N_2 was evolved per mole of II employed.

Acknowledgment.—We are indebted to the Public Health Service Service for partial support of this work under Grant no. 5RO1AI06662 and to Drs. Milton Kerker and Josip Kratohvil for the use of their light-scattering equipment.

(3) F. W. Billmeyer, "Textbook of Polymer Chemistry," Interscience Publishers, Inc., New York, N. Y., 1957.

N-Thymidylglycine and Ethyl *p*-N-Thymidylaminobenzoate[1]

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The biological activity and tumor inhibition noted in folate and pyrimidine analogs have been widely explored. All available evidence suggests that the action of the fluoropyrimidines is exerted by inhibition of thymidylate synthetase, the enzyme catalyzing the conversion of deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate (TMP).[2] Recently,

(1) (a) This work was generously supported by Grant CA-7522 of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. (b) National Science Foundation Undergraduate Research Participant.

(2) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965).