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.

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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,

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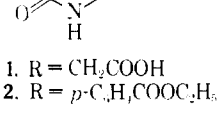
interest has been focused on the possibility of inhibiting this enzyme with analogs of N^5,N^{10}-methylenetetrahydrofolic acid (CH_2-THFA).[3] This stems from studies on the enzyme that demonstrate a sequential order in binding; the initial complex formed between the enzyme and the cofactor CH_2-THFA is followed by formation of a ternary complex with dUMP. Stepwise dissociation leads to the products TMP and 7,8-dihydrofolic acid (DHFA).[4] The source of the methyl group is firmly established as coming from formaldehyde *via* the cofactor. Reduction of the carbon transferred to dUMP is accomplished by oxidation of tetrahydrofolic acid to 7,8-dihydrofolic acid. Although there is a question of the position of label (tritium at C_6 or C_7) on the isotopic reduction of DHFA to THFA by enzymatic and chemical methods, it has been established that the hydrogen (tritium) introduced in reduction of DHFA is transferred to the methyl group of TMP.

Several folate analogs have been studied as thymidylate synthetase inhibitors and found to be effective agents. Kisliuk examined the relative inhibitory effect of reduced aminopterins on both dihydrofolate reductase and thymidylate synthetase.[3d] Reduced homofolate derivatives also have been shown to inhibit thymidylate synthetase.[3f]

The nature of the intermediate in the transfer of the carbon unit from the enzymatically bound cofactor to the bound substrate necessitates the proper spatial positioning of these units on the enzyme. Although an intermediate has been proposed for the methyl transfer,[4d] several questions have arisen regarding the linkage from N_5 of the cofactor to the substrate and the position of loss of the hydrogen from the cofactor (C_6 or C_7) to the product.[5]

In an effort to examine the possibility of combining the binding sites for both cofactor and substrate in one inhibitor a series of analogs of the intermediate were synthesized and tested *in vitro*.[3i] Although these compounds were more effective inhibitors of thymidylate synthetase than dihydrofolate reductase, the anticipated "bridging of the binding sites" was not realized. Two additional compounds in this series were synthesized and tested: N-thymidylglycine (**1**) and ethyl *p*-(N-thymidyl)aminobenzoate (**2**).



1. R = CH_2COOH
2. R = *p*-$C_6H_4COOC_2H_5$

Cline and co-workers[6] reported the detection of a product giving a positive ninhydrin test by treating 5-hydroxymethyluracil with glycine. Examples of uracil undergoing the Mannich reaction at C-5 have been reported.[7] Under these conditions the condensation of 5-hydroxymethyluracil with glycine gave **1** and with ethyl *p*-aminobenzoate gave **2**.

The *in vitro* testing was carried out according to reported procedures on thymidylate synthetase and dihydrofolate reductase.[3i] Compounds **1** and **2** failed to inhibit the former enzyme in a concentration ratio of (inhibitor) : (deoxyuridine 5′-monophosphate) of 30.

Against dihydrofolate reductase in an (inhibitor) : (dihydrofolic acid) ratio of 10 compound **1** showed 20% inhibition and **2** was less than 10% inhibitory.

Experimental Section

Melting points were obtained on a calibrated Thomas–Hoover Unimelt and are corrected. Ultraviolet spectra were recorded on a Cary 14 spectrophotometer. Microanalyses were carried by Drs. G. Weiler and F. B. Strauss, Oxford, England, and using an F and M Model 185 CHN chromatograph.

N-Thymidylglycine (**1**).—5-Hydroxymethyluracil[6] (2.3 g, 0.015 mole) and 3.1 g of glycine hydrochloride (0.03 mole) in 25 ml of water were heated at 90° for 24 hr. After cooling, a precipitate was collected which was purified by repeated precipitation from base by titration to pH 6.8–7.1 and recrystallized from water to give 0.2 g (7%) of product that did not melt below 340°, $\lambda_{max}^{0.1\ M\ HCl}$ 262 mμ (ε 12,000), $\lambda_{max}^{0.1\ M\ NaOH}$ 286 mμ (ε 9200).

Anal. Calcd for $C_7H_9N_3O_4$: C, 42.21; H, 4.55; N, 21.10. Found: C, 42.48; H, 4.39; N, 21.19.

Ethyl *p*-(N-thymidyl)aminobenzoate (**2**) was prepared by the procedure used for **1** from 2.8 g (0.02 mole) of 5-hydroxymethyluracil and 4.0 g (0.2 mole) of ethyl *p*-aminobenzoate in a solution of 1% HCl in 85 ml of 30% aqueous ethanol. After refluxing for 19 hr the hot suspension was filtered and the solid was recrystallized several times from ethanol to give 2.1 g (36%) of product melting at 261–262°; $\lambda_{max}^{0.1\ M\ HCl}$ 267 mμ (ε 10,000), 303 mμ (ε 7200); $\lambda_{max}^{0.1\ M\ NaOH}$ 287 mμ (ε 20,000).

Anal. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.12; H, 5.23; N, 14.53. Found: C, 57.88; H, 5.14; N, 14.20.

The authors are indebted to Mrs. William Riggs for the enzyme inhibition studies.

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Chemistry of Cephalosporin Antibiotics. X.[1] Synthesis of Methyl 3-Formyl-7-(thiophene-2-acetamido)-3-cephem-4-carboxylate, a New Cephalosporin Derivative

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The 3-acetoxymethyl side chain of the cephalosporin nucleus has been subjected to modification in the search

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