65.3 g (0.4 mole) of N-acetyl-L-cysteine (NAC). After stirring the solution for 2 hr and diluting with 500 ml of H_2O, the product was isolated by lyophilization. A 91% yield (102 g) of white solid was obtained; mp *ca.* 162–168° dec, $[\alpha]^{25}D$ +1.13° (*c* 3, H_2O). *Anal.* ($C_5H_9NO_3S \cdot C_3H_7NO_2S$) C, H, N; SH: calcd, 23.2; found, 22.7.

It is noted that the melting point of **2** is intermediate between those of the two reactants: NAC, 109–110°, and L-cysteine, 221.5–223° dec. In contrast, a mechanical mixture of equimolar quantities of the two reactants showed a depressed melting point, *ca.* 101–150° dec, and an ir spectrum significantly different from that of **2**. The components of this salt are, however, loosely bound to one another. L-Cysteine was precipitated in 97% recovery from a 50% aqueous solution of **2** by diluting with EtOH. A 50% recovery of recrystallized NAC was obtained from the filtrate.

I. **Calcium N-Acetyl-L-cysteinate Acetate (7).** (**a**) To a stirred mixture of 163.2 g (1 mole) of NAC, 60 g (1 mole) of HOAc, and 600 ml of H_2O, was added, in portions, 110 g (1.1 moles) of $CaCO_3$. A rapid evolution of CO_2 followed each addition. After stirring overnight, the reaction mixture was freed of dissolved CO_2, at reduced pressure (aspirator), and filtered (Celite bed). The filter bed was washed with 1 l. of H_2O. Evaporation of the filtrate by lyophilization gave 243 g (93%) of product. Further drying in a vacuum oven at 90–95° lowered the weight to 234 g (89.5%), $[\alpha]^{25}D$ +12.4° (*c* 5, H_2O). *Anal.* $[C_5H_8NO_3(C_2H_3O_2)Ca]$ C, H, N, SH.

(**b**) To a stirred solution of 16.3 g (0.1 mole) of NAC in 105 ml of 85% aqueous MeOH was added 17.6 g (0.11 mole) of $Ca(OAc)_2 \cdot H_2O$. The suspension was stirred at room temperature for 3 hr and at 40° for 2 hr. After standing overnight, the suspension was diluted with 15 ml of H_2O and warmed to 40°. The resulting solution was cooled and filtered. Evaporating the filtrate under reduced pressure (*ca.* 2–3 mm) gave 25.5 g (97%) of a white spongy solid. Purification was achieved by reprecipitating the product from a solution in 50% MeOH (1 g/ml) by the addition of a double volume each of acetone and 2-PrOH. The resulting sticky mass was converted to a finely divided, white suspension by slight warming. The pure product was collected on a filter, washed with warm 2-PrOH, and dried in a vacuum oven at 70°; yield, 16 g (61%) of white powder of mp *ca.* 244° dec. The nmr spectrum was consistent with the structure. *Anal.* N, SH, Ca.

The Anticancer and Antimalarial Properties and Preparation of Arylazopyrimidines[1a]

ROBERT E. HARMON,[1b] FREDERIC E. DUTTON,[1c] AND H. DALE WARREN

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001

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Since Lythgoe and coworkers[2] reported that azo coupling occurs in the 5 position of the pyrimidine ring, various substituted 5-arylazopyrimidines have been synthesized and their mode of action in various biological systems has been studied. However, very few data were available on the activity of these compounds against tumors in animals or man.

Modest and coworkers[3] found that at least one amino group adjacent to the arylazo link is necessary for optimum activity. They concluded that the aryl group should be unsubstituted or contain electron-releasing substituents for maximum microbiological activity. The work of Tanaka and co-workers,[4] and of Roy-Burman and Sen[5] substantiates these findings. Tanaka and co-workers tested a number of 5-arylazo-2,4,6-triaminopyrimidines containing electron-withdrawing substituents such as sulfonic acid, sulfonamide, carboxylic acid, phosphonic acid, or arsonic acid on the aryl group and they found these compounds to be less active in microbiological systems. However, compounds containing electron-withdrawing substituents on the phenylazo group were effective against Yoshida ascites sarcoma and Ehrlich ascites carcinoma. They concluded that these compounds interfere with nucleic acid synthesis. The inhibitory actions by two of the most active compounds, 5-phenylazo-2,4,6-triaminopyrimidine, and 5-phenylazo-2,4-diamino-6-hydroxypyrimidine were not reversed by most bases and nucleotides involved in nucleic acid synthesis. Roy-Burman and Sen[5] found that the inhibitory effects of arylazopyrimidines in the *Streptococcus faecalis* (ATCC 8043) system could be more efficiently reversed by 5-formyltetrahydrofolic acid than by folic acid. These findings would seem to indicate that arylazopyrimidines may act as antagonists of folic acid and that they may interfere with the enzymatic conversion of folic acid to 5-formyltetrahydrofolic acid. Recent studies by Hampshire and coworkers[6] on the inhibitory effects of 5-arylazo-2,4,6-triaminopyrimidines on folic acid reductase from rat liver indicate that these compounds have a wide range of activities depending on the substituent present on the aryl group. The low activity of 2,4,5,6-tetraaminopyrimidine indicates that reductive cleavage of the 5-azo linkage does not occur in this system. There is no direct correlation between enzyme-inhibitory activity and toxicity or antitumor activity of the 5-arylazopyrimidines studied.

Chadwick and coworkers[7] used spectrophotometric methods with a rat liver homogenate fortified with a NADPH-generating system to determine the ease of reduction of the azo linkage in azopyrimidines and azobenzenes. They found that the azobenzenes were rapidly reduced under these conditions while the arylazopyrimidines were recovered unchanged. They postulated that 5-arylazopyrimidines are inhibitors rather than substrates of liver azo reductase.

Hibino[8] reported that the remission rates in cases of acute leukemia initially treated with a combination of three of the four agents, 6-purinethiol, steroid hormones, mitomycin C, and 4-(2,4,6-triamino-5-pyrimidinylazo)benzenesulfonic acid, were as high as 85.7%. The remission rates achieved by using steroidal hormones, mitomycin C, and 6-purinethiol either alone or in combination were much lower. This indicated that 4-(2,4,-6-triamino-5-pyrimidinylazo)benzenesulfonic acid has a high synergistic effect when used in combination therapy.

(1) (a) This work was supported in part by a grant from the Michigan Cancer Foundation and Grant CA-06140-06 from the National Cancer Institute. (b) To whom inquiries should be addressed. (c) Abstracted from the thesis of Frederick E. Dutton, submitted as partial fulfillment of Master of Arts Degree.

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In this investigation, diazotized sulfonamide drugs and arsanilic acid were coupled with various trisubstituted pyrimidines. The coupling reactions were carried out at 0° and at low pH. Under these conditions a pyrimidine bearing a 4-methyl substituent in place of an amino or hydroxy group failed to couple. These compounds were evaluated for their anticancer activity (see Table I) against tumors in animals by the Cancer Chemotherapy National Service Center.[9]

TABLE I
ANTICANCER RESULTS

No.	Test[a] system	Dose, mg/kg	No. of survivors	Animal wt dif T − C	Tumor wt (g) or survival days T/C	Stage index or % T/C
IX	SA	500	5/6	−4.7	587/1695	34
	LL	400	6/6	−4.2	352/1214	28
	LE	400	6/6	−4.2	8.8/8.9	98

[a] SA = Sarcoma 180, LL = Lewis lung carcinoma, LE = L1210 lymphoid leukemia.

Compounds I–III and V showed little or no activity against L1210 lymphoid leukemia, P1798 lymphosarcoma, and Walker carcinosarcoma 256 (intramuscular). Compounds IV and VI were inactive against L1210 lymphoid leukemia and P1798 lymphosarcoma, and VII was inactive in the L1210 lymphoid leukemia. It showed little or no activity against Sarcoma 180, Lewis lung carcinoma, and L1210 lymphoid leukemia. Compound II was also inactive against HS1 human sarcoma (rat, egg) and VI was inactive against Dunning leukemia (ascites).

The compounds were also evaluated for their antimalarial activity (see Table II) by the Walter Reed Army Medical Center.[10] Compounds I, V, and VI showed no antimalarial activity.

Furst[11] has reviewed the activity of many biological agents which may be due to their ability to complex certain metal ions vital to normal biological processes. It has been found in this investigation that the phenylazopyrimidines which were biologically active complexed with the following ions. Fe^{2+}, Fe^{3+}, Co^{2+}, but did not complex with Mg^{2+}, Ca^{2+}, Mn^{2+}, or Zn^{2+} except in certain cases (see Table III). A more extensive investigation on metal complexation by arylazopyrimidines may allow us to correlate more rigorously metal complexing ability of a given arylazopyrimidine with its biological activity.

TABLE II
ANTIMALARIAL ACTIVITY IN *Plasmodium berghei*[a]

No.	Dose, mg/kg	ST[b]	Toxic[c] deaths	MST[d]
II	40	5.6+	0	0
	160	11.7+	2	0
	640		4	0
	40	7.0+	0	0
	160	11.6+	0	0
	640		4	4.5
III	40	1.9+	0	0
	160	3.7+	0	0
	640	10.3+	0	0
	40	2.6+	0	0
	160	4.8	0	0
	640	10.0+	0	0
IV	40	0.5	0	0
	160	4.1+	0	0
	640	7.7+	3	0
	160	5.0+	1	0
	320	7.0+	2	0
	640	9.0+	4	0
VII	40	1.6+	0	0
	160	5.4+	0	0
	640		0	0
	40	2.0+	0	0
	160	5.8	0	0
	640	16.2	0	0
VIII	40	6.9	0	0
	160	12.9	0	0
	640		0	0
	40	4.2+	0	0
	160	12.8	0	0
	640		0	0

[a] See ref 15 for procedures used in evaluating compounds for antimalarial activity. Five mice were used in each test group. [b] The mean survival time of the treated group minus mean survival time of the control group. [c] Deaths occurring on days 2, 3, 4, and 5 after infection are attributed to drug action and counted as toxic deaths. Control animals do not die before day 6. [d] The mean survival time for the mice dying on days 3, 4, and 5 only (toxic deaths).

TABLE III
METAL COMPLEXATION DATA[a]

No.	Mg^{2+}	Ca^{2+}	Mn^{2+}	Co^{2+}	Zn^{2+}	Fe^{2+}	Fe^{3+}
IX	?[b]	?	*[c]	+[d]	+	−[e]	?
VIII	−	−	*	+	+	+	+
II	−	−	+	?	−	+	+
V	−	−	*	?	*	*	−

Reduction

[a] Determinations done on a Beckman Model DB spectrophotometer (see Experimental Section). [b] ?, inconclusive spectral data. [c] *, complex precipitated. [d] +, complex formed as evidenced by a change in the spectra of the ligand solution. [e] −, no spectrophotometric evidence for complex formation.

Experimental Section

Melting points are corrected. Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The uv spectra were determined in 95% ethyl alcohol on a Beckman Model DB spectrophotometer and a Cary Model 14 spectrophotometer (see Table IV). The ir data were obtained from Nujol mulls on a Beckman IR-8 spectrophotometer. They were not found to be very useful in characterizing the azo link (–N=N–) in arylazopyrimidines since this group absorbs at low intensity and is obscured by the strong absorption of the pyrimidine nucleus at 1600 cm^{-1}. The absence of a band at 3700–3500 cm^{-1} (OH) indicates that in the solid phase (Nujol mull) these hydroxypyrimidines probably exist in the tautomeric amide form. This is in agreement with the work of Short and Thompson[12] and Brown and Short.[13] The spectra of these compounds showed medium-to-strong absorption bands characteristic of the pyrimidine and the benzene rings at 1575–1625 cm^{-1}, strong absorption bands at 1150–1320 cm^{-1} (SO_2NH when it was present), medium-to-strong bands at 3100–3300 cm^{-1} (NH_2), and medium bands at 800–860 cm^{-1} (*para*-substituted benzene).

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TABLE IV

TRISUBSTITUTED ARYLAZOPYRIMIDINES

No.	R_1	R_2	R_3	R_4	Mp, °C[a]	Recrystn[b] solvent	Yield,[c] %	Formula[g]
I	NH_2	NH_2	OH	SO_2NH_2	250	A	85	$C_{10}H_{11}N_7O_3S \cdot HCl \cdot 0.5H_2O$[d]
II	NH_2	NH_2	OH	SO_2NH–pyrimidinyl	275	A	83	$C_{14}H_{13}N_9O_3S \cdot HCl \cdot 0.5H_2O$
III	CH_3S	NH_2	OH	SO_2NH_2	215	A	75	$C_{11}H_{12}N_6O_3S_2 \cdot HCl \cdot 0.5H_2O$
IV	NH_2	NH_2	NH_2	SO_2NH–pyrimidinyl	315	A	88	$C_{14}H_{14}N_{10}O_2S \cdot HClO_4 \cdot H_2O$[e]
V	NH_2	NH_2	OH	AsO_3H_2	200	A	80	$C_{10}H_{11}AsN_6O_4 \cdot 0.5H_2O$[e]
VI	CH_3S	NH_2	NH_2	AsO_3H_2	317	A	36	$C_{11}H_{13}AsN_6O_3S$
VII	NH_2	NH_2	OH	—SO_2NH–thiazolyl	240	A	80	$C_{13}H_{11}N_8O_3S_3 \cdot HCl \cdot 0.5H_2O$[f]
VIII	CH_3S	NH_2	OH	—SO_2NH–thiazolyl	230	A	49	$C_{14}H_{13}N_7O_3S_3 \cdot HCl \cdot 0.5H_2O$
IX	CH_3S	NH_2	OH	SO_2NH–pyrimidinyl	280	A	70	$C_{15}H_{14}N_8O_3S_2 \cdot HCl$

[a] Melts with decomposition. [b] A = 0.75 N HCl and 95% EtOH (3:1) by volume. [c] Yield was calculated after one recrystallization. [d] Free base is known, see ref 6a. [e] H_2O: calcd, 2.48; found, 2.56. [f] Cl: calcd, 8.10; found, 8.03. [g] All compounds were analyzed for C, H, N. The observed ir, uv, or nmr data were as expected.

These assignments are consistent with those quoted by Bellamy[14] and by Rao.[15]

One of the sulfonamide drugs or arsanilic acid (0.05 mole) was dissolved in 100 ml of 3 N HCl and cooled to −5°; the amine hydrochloride precipitated. It was diazotized by adding 0.05 mole of $NaNO_2$ in 25 ml of H_2O. The temperature was not allowed to rise above 0°. One of the pyrimidines (0.05 mole) was dissolved in 3 N HCl, cooled to −5°, and the solution of the diazonium salt was added. The addition took about 20 min and during this time some coupling occurred. The temperature was not allowed to rise above 10°. After standing 1 hr at 10° the temperature of the reaction mixture was allowed to rise to room temperature and was kept there for 12 hr. A thick slurry containing a bright yellow or orange solid developed. The azo compound was collected and washed (H_2O, 95% EtOH). It was recrystallized from boiling 0.75 N HCl–EtOH (75:25) (see Table IV). The structure of these compounds was elucidated by reductive cleavage of the azo linkage using sodium dithionite in aqueous solution. A sulfonamide drug or arsanilic acid and a tetrasubstituted pyrimidine were obtained from each of the trisubstituted arylazopyrimidines. The identity of the two compounds obtained by the cleavage reaction was established using spectral, melting point, and tlc data.

Metal Complexation Studies.—In order to screen the complexation behavior of the active compounds with selected cations of biological importance, a series of solutions was prepared for each such compound. A ligand (phenylazopyrimidine) concentration was chosen in the range (2.5–5.0) $\times 10^{-5}$ F which gave a maximum absorbance of about 0.7. All solutions for a given ligand were of the same concentration. One contained only the ligand, the others a fourfold formal excess of one of the ions Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, or an equimolar amount of Fe^{2+} or Fe^{3+}, the cations being added as the perchlorates. All solutions were 0.02 F in tris(hydroxymethyl)aminomethane from a stock adjusted to pH 7 with $HClO_4$. The resulting solutions all were at pH 6.86 ± 0.02.

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958.

(15) C. N. R. Rao, "Chemical Applications of Infrared Spectroscopy," Academic Press Inc., New York, N. Y., 1964.

Absorption spectra of all solutions were measured in the range 500–220 mμ with 1-cm silica cells and a H_2O reference. None were observed to absorb above 500 mμ. For comparison, blanks containing the cations and buffer were run. No corrections for the blanks were necessary except for Fe^{2+}, Fe^{3+}, Co^{2+} where corrections were made.

A change of the absorption spectra of the ligand in the presence of a given cation was taken as evidence for complex formation. In a few cases results were inconclusive, where the ligand cation solution exhibited a gradual increase in absorbance, relative to the ligand alone, and this increase was only slightly larger than the cation blank.

A peculiar phenomenon was observed for the solution containing Mn^{2+}. In every case except for II, a brown, flocculent precipitate was observed to form after a few hours or days. The spectrum of the filtrate, filtered after some standing, was very similar to that of the ligand alone, but with diminished absorbance. Inasmuch as the solubility product of $Mn(OH)_2$ was not exceeded in these solutions, the precipitate is thought to be MnO_2, formed by oxidation of the Mn^{2+} either by dissolved O_2, or perhaps by partial reduction of the ligand to a nonabsorbing species.

Compound V showed anomalous results, forming precipitates with both Zn^{2+} and Fe^{2+}. In the former case the ligand was completely precipitated, but in the latter only partial reaction occurred. This behavior is thought to be due to the presence of the arsonic acid group.

It should be noted that in the most favorable case, *i.e.*, the solution of I with Co^{2+}, the absorbance of the Co^{2+}-containing solution differed by no more that 32% from that of the ligand alone. Hence, it would be difficult to study these systems spectrophotometrically, and other methods are under consideration.

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