hexane. Evaporation of the extract followed by chromatography on alumina gave several fractions. The hexane eluate was evaporated *in vacuo* under nitrogen to give a yellow oil. The oil was sublimed at 120° (0.5 mm.) giving a yellow oil (*ca.* 0.1 g.), which was identified as anhydrovitamin A by ultraviolet absorption maxima (351, 368, and 389 mμ) and by its infrared spectrum.

Anal. Calcd. for $C_{20}H_{28}$: C, 89.49; H, 10.51. Found: C, 89.08; H, 10.67.

The sublimation residue was a yellow solid which gave yellow crystals, m.p. 120–125°. This compound showed an ultraviolet maximum at 323 mμ and showed the presence of an acetoxy group by the infrared spectrum.

Anal. Found: C, 74.25; H, 9.35; mol. wt. in CH_2Br_2, 713.

Reaction of Vitamin A Acetate with Chromium Hexacarbonyl.—Vitamin A acetate (1.0 g., 3.01 mmoles) was allowed to react with chromium hexacarbonyl (1.0 g., 4.55 mmoles) in 7 ml. of *n*-butyl ether and 3 ml. of hexane. The addition of hexane to the reaction mixture was to prevent sublimation of chromium hexacarbonyl during the reaction. The reaction was continued for 18 hr. under nitrogen at gentle reflux. During the reaction 250 ml. of a gas was evolved. Greenish brown precipitates were formed and the solution was yellow. The solution was decanted and the solvent was removed *in vacuo*. The greenish yellow oily residue was separated by chromatography on deactivated alumina. Elution with hexane gave a yellow semisolid which on sublimation at 110° (0.5 mm.) gave *ca.* 0.2 g. of a yellow liquid. This was found to have a composition near $C_{20}H_{30}$ as indicated by elemental analysis.

Anal. Calcd. for $C_{20}H_{30}$: C, 88.81; H, 11.19. Found: C, 88.09; H, 11.13.

The infrared spectrum showed neither terminal vinyl nor vinylidene groups. The ultraviolet spectrum exhibited a maximum at about 324 mμ, showing that 5 conjugated double bonds are present as in vitamin A acetate (absorption maximum at 327 mμ). The greenish brown precipitate from the reaction mixture turned to a deep brown color on exposure to air. This precipitate was washed with acetone and the color changed again to yield a mixed green and red precipitate. The mixed precipitate was found to be soluble in water resulting in the formation of a blue-green aqueous solution. On the addition of aqueous alkali, the solution gave a blue precipitate which was probably chromic hydroxide. Therefore the original greenish brown precipitate from the reaction mixture may contain chromous acetate.

The benzene eluate of the chromatography yielded a yellow semisolid on evaporation. In the ultraviolet region it had maxima at 313 and 300 mμ and in the infrared it showed absorption due to an acetoxy group. The infrared spectrum indicated similarity of its structure to that of vitamin A acetate, but the ultraviolet maxima are different from those of vitamin A acetate.

Nuclear Magnetic Resonance Results.—Table I shows the peak position, splitting, relative ratio of the areas, coupling constants, and assignment of protons to vitamin A acetate and its iron tricarbonyl π-complex (I).

TABLE I

Peak position (τ)	Splitting	Relative ratio of area	Coupling const., *J* (c.p.s.)	Assignment
		Vitamin A Acetate		
3.68	d	1	13	=CH—
3.95	s	2		=CH—
4.50	d	1	13	=CH—
5.35	d	2	14	$—CH_2—O—$
8.02	s	3		$CH_3—CO—$
8.10	s	6		$CH_3—C$
8.14	s	3		$CH_3—C$
8.30	s	4		$—CH_2—$
8.5	multi.	2		$—CH_2—$
9.00	s	6		$(CH_3)_2C$
		Vitamin A Acetate Iron Tricarbonyl		
4.0	multi.	2	?	=CH—
5.75	multi.	4	?	—CH—
7.8	s	3		$CH_3—C=$
8.0	s	6		$CH_3—CO$ and $CH_3—C$
8.2	s	3		$CH_3—C$
8.32	multi.	4		$—CH_2—$
8.42	multi.	2		
9.0	s	6		$(CH_3)_2C$

Acknowledgment.—The authors wish to express their appreciation for financial support from the National Institutes of Health, United States Public Health Service, Grant RG-8921, and to Hofmann-LaRoche and the U. S. Vitamin Co. for samples of crystalline vitamin A acetate.

Potential Anticancer Agents. I. Schiff Bases and Hydrazone Derivatives of Pyrimidine-4-carboxaldehydes[1]

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A series of hydrazone derivatives and Schiff bases have been prepared by the interaction of the appropriate amine, hydrazine, or hydrazide with substituted and unsubstituted 6-hydroxy-2-thiopyrimidine-4-carboxaldehydes. Several 5- and N-1-substituted thiopyrimidine-4-carboxaldehydes have also been prepared, and in addition the ultraviolet spectra of the N-methylpyrimidine acetals have been determined. These compounds were tested *vs.* the Ehrlich ascites carcinoma in mice.

The interesting biological properties of pyrimidine derivatives, notably as antimetabolites and as potential inhibitors of cancerous growth, have resulted in the synthesis of a large number of related compounds.[2]

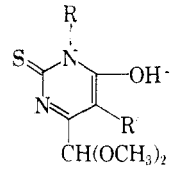
The present investigation was prompted by the possibility that examination of a wider spectrum of pyrimidine-4-carboxaldehyde derivatives might lead to more potent inhibitors of nucleic acid metabolism of the cells. Consequently, a series of Schiff bases and hy-

(1) This investigation was supported by Public Health Service Research Grants C-6364, CA-06364-02, and CA-02756(C7) from the National Cancer Institute, and in a small part from the American Cancer Society Institutional Grant to the University of North Carolina.

(2) (a) A. Giner-Sorolla, I. Zimmerman, and A. Bendich, *J. Am. Chem. Soc.*, **81**, 2515 (1959); (b) H. C. Koppel, R. H. Springer, R. K. Robins, F. H. Schneider, and C. C. Cheng, *J. Org. Chem.*, **27**, 2173 (1962); (c) L. O. Ross, E. M. Action, W. A. Skinner, L. Goodman, and B. R. Baker, *ibid.*, **26**, 3395 (1961); (d) R. H. Wiley, A. B. Canon, and K. F. Hussung, *J. Med. Chem.*, **6**, 333 (1963).

TABLE I

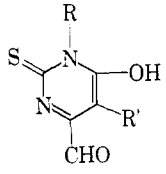
4-(DIMETHOXY)METHYL-6-HYDROXY-6-THIOPYRIMIDINES (III)



No.	R	R'	M.p., °C.	Yield, %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1	H	H	180–181	85	$C_7H_{10}N_2O_3S$	41.57	41.26	4.98	4.77	13.85	13.81	15.85	15.79
2	H	CH_3	155–156	80	$C_8H_{12}N_2O_3S$	44.43	44.31	5.59	5.48	12.95	12.90	14.83	14.81
3	CH_3	H	148–149	65	$C_8H_{12}N_2O_3S$	44.43	44.36	5.59	5.17	12.95	12.98	14.83	14.78
4	H	C_2H_5	135–136	82	$C_9H_{14}N_2O_3S$	46.94	47.00	6.13	6.09	12.16	12.14	13.92	13.90
5	C_2H_5	H	103–104	24	$C_9H_{14}N_2O_3S$	46.94	46.19	6.13	6.86	12.17	12.22	12.92	12.00
6	CH_3	CH_3	93–94	48	$C_9H_{14}N_2O_3S$	46.94	46.37	6.13	6.40	12.17	12.35	13.92	14.29
7	C_3H_7	H	106–107	66	$C_{10}H_{16}N_2O_3S$	49.16	49.01	6.60	6.06	11.47	11.48	13.12	13.25
8	C_2H_5	CH_3	98–100	39	$C_{10}H_{16}N_2O_3S$	49.16	48.88	6.60	6.57	11.47	11.50	13.12	13.02
9	C_3H_7	CH_3	88–90	63	$C_{11}H_{18}N_2O_3S$	51.14	51.34	7.02	7.28	10.84	10.84	12.41	12.55
10	C_6H_5	H	161–162	16	$C_{13}H_{14}N_2O_3S$	56.10	57.43	5.07	4.49	10.07	10.06	11.52	11.38
11	C_6H_5	CH_3	156–157	22	$C_{14}H_{16}N_2O_3S$	57.52	57.77	5.52	5.38	9.58	9.53	10.97	11.00

drazone derivatives of substituted and unsubstituted 6-hydroxy-2-thiopyrimidine-4-carboxaldehyde (A) were prepared in an effort to obtain compounds with better therapeutic indices and to establish more fully their carcinostatic potentiality. The suggested essential role of the azomethine linkage in certain biochemical reactions[3] and the fact that the hydrazino group frequently confers activity upon a given structure,[4] makes these compounds of considerable interest, and should serve as a fruitful field for further biological investigations.

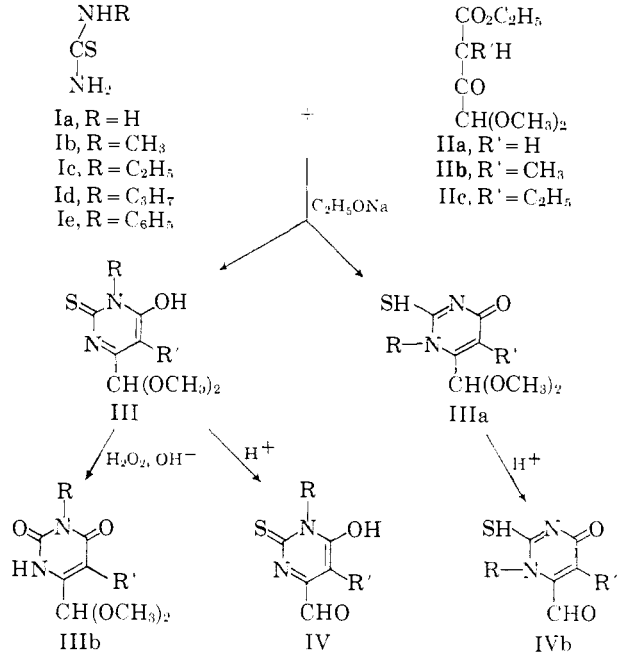
There is a paucity of information concerning derivatives of A, in spite of the fact that the parent aldehyde has been previously reported.[5] Since A bears a structural relationship to orotic acid, which is a precursor of nucleic acid pyrimidines, these compounds might interfere with the normal utilization of uracil-carboxylic acid and consequently result in biological interference with *in vivo* DNA synthesis, or with some other vital metabolic pathway.



A

The pyrimidine-4-carboxaldehyde (A) was prepared by a modification of the procedure described by Johnson and Cretcher[5] (Chart I). The 5- and N-1-substituted thiopyrimidine aldehydes have not been described previously. It was found that 6-hydroxy-2-thiopyrimidine-4-carboxaldehyde could be prepared most conveniently by the reaction of ethyl γ,γ-dimethoxyacetoacetate (IIa) with the appropriate substituted thiourea (I). Subsequent cleavage of the actal linkage (III) afforded the corresponding pyrimidine-4-carboxaldehyde (IV). The 5-substituted derivatives of Table I were prepared from ethyl α-methyl-γ,γ-dimethoxyacetoacetate (IIb) or ethyl α-ethyl-γ,γ-dimethoxyacetoacetate (IIc) and (Ia).

CHART I



Interaction between Ib and IIa could afford, in principle, two cyclization products, III and IIIa. Support for structure III as opposed to IIIa is given by comparison of the ultraviolet spectrum of III (Fig. 1) and its oxidized product IIIb (Fig. 2) to the spectra reported for related pyrimidines such as 3-ethyl-2-thiouracil[6a] and 3-methyluracil,[6b] which contain the same chromophore as III and IIIb. The spectral properties of III (Fig. 1) resembled the observed spectrum of 3-ethyl-2-thiouracil.[7] Comparison of III with the observed spectrum of 1-ethyl-2-thiouracil[6a] showed a distinct difference. Compound III was then oxidized carefully with alkaline hydrogen peroxide to IIIb and its ultraviolet spectrum was found to be similar to that of 3-

(3) (a) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954); (b) B. Witkop and T. W. Beiler, *ibid.*, **76**, 5589 (1954).

(4) E. Jucker, *Angew. Chem.*, **71**, 321 (1959).

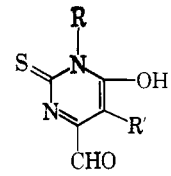
(5) (a) T. B. Johnson and L. H. Cretcher, *J. Am. Chem. Soc.*, **37**, 2144 (1915); (b) T. B. Johnson and E. F. Schroeder, *ibid.*, **53**, 1989 (1931).

(6) (a) D. Shugar and J. J. Fox, *Bull. Soc. Chim. Belges*, **61**, 293 (1952); (b) *Biochim. Biophys. Acta*, **9**, 199 (1952).

(7) Based on the numbering system used, the alkylated nitrogen in this structure corresponds to the N-1 of the pyrimidine-4-carboxaldehyde (IV).

TABLE II

6-HYDROXY-2-THIOPYRIMIDINE-4-CARBOXALDEHYDES (IV)[a]



No.	R	R'	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1[b]	H	H	251 dec.	$C_5H_4N_2O_2S$	38.46	38.41	2.58	2.54	17.94	17.89	20.53	20.44
2[c]	H	CH_3	233–234	$C_6H_6N_2O_2S$	42.35	42.15	3.56	3.50	16.47	16.32	18.81	18.79
3	CH_3	H	215–216	$C_6H_6N_2O_2S$	42.35	42.20	3.56	3.87	16.47	16.48	18.81	18.84
4	H	C_2H_5	148–150	$C_7H_8N_2O_2S$	45.64	45.61	4.38	4.36	15.21	15.19	17.41	17.40
5	C_2H_5	H	155–157	$C_7H_8N_2O_2S$	45.64	46.25	4.38	4.75	15.21	15.20	17.41	17.90
6	CH_3	CH_3	213–215 dec.	$C_7H_8N_2O_2S$	45.64	45.21	4.38	4.38	15.21	15.28	17.41	17.45
7	C_3H_7	H	134–135	$C_8H_{10}N_2O_2S$	48.47	48.11	5.08	5.48	14.13	14.11	16.17	16.31
8	C_2H_5	CH_3	180–182	$C_8H_{10}N_2O_2S$	48.47	47.66	5.08	5.08	14.13	13.98	16.17	16.03
9	C_3H_7	CH_3	152–153	$C_9H_{12}N_2O_2S$	50.92	51.61	5.70	5.63	13.20	13.31	15.11	15.00
10	C_6H_5	H	259–260 dec.	$C_{11}H_8N_2O_2S$	56.88	57.32	3.47	3.70	12.06	12.06	13.81	13.67
11	C_6H_5	CH_3	225–227	$C_{12}H_{10}N_2O_2S$	58.52	58.65	4.09	4.17	11.38	11.42	13.02	12.92

[a] Hydrolysis of the acetal (III) was carried out by refluxing for 10 min. in 10% aqueous sulfuric acid. The yields in all cases were 100%. Compounds 1 to 11 were recrystallized from water. [b] See ref. 5b. [c] S. Borodkin, Thesis, University of North Carolina, 1961, p. 132, m.p. 232–233°.

TABLE III

SCHIFF BASE DERIVATIVES OF SUBSTITUTED AND UNSUBSTITUTED 6-HYDROXY-2-THIOPYRIMIDINE-4-CARBOXALDEHYDES (IV)

No.	Amine used	R	R'	M.p., °C.	Yield, %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1	Ethanolamine	H	H	150–151	66	$C_7H_9N_3OS$	42.21	42.14	4.52	4.44	21.10	21.00	16.08	16.11
2	Allylamine	H	H	171–172	71	$C_8H_9N_3OS$	49.23	49.01	4.62	4.58	21.54	21.41	16.41	16.33
3	Isopropylamine	H	H	141–142	63	$C_8H_{11}N_3OS$	48.73	48.94	5.58	5.41	21.32	21.24	16.24	16.00
4	Propylamine	H	H	147–148	71	$C_8H_{11}N_3OS$	48.73	48.70	5.58	5.49	21.32	21.30	16.24	16.15
5	Isobutylamine	H	H	149–150	76	$C_9H_{13}N_3OS$	51.18	51.11	6.16	6.09	19.91	19.98	15.16	15.00
6	3-Methoxypropylamine	H	H	115–116	70	$C_9H_{13}N_3O_2S$	47.57	47.55	5.72	5.84	18.50	18.46	14.09	14.01
7	Aminoacetaldehyde dimethylacetal	H	H	118–119	65	$C_9H_{13}N_3O_2S$	44.26	44.07	5.73	5.70	17.21	17.09	13.11	13.00
8	3-Dimethylamino-propylamine	H	H	110–111	62	$C_{10}H_{16}N_4OS$	49.79	49.65	7.05	7.00	23.24	23.16	13.28	13.16
9	Cyclopropylamine	H	C_2H_5	211–212	60	$C_{10}H_{13}N_3OS$	53.79	53.71	5.87	5.81	18.82	18.79	14.36	14.24
10	Cyclohexylamine	H	H	169–170	66	$C_{11}H_{15}N_3OS$	55.69	55.63	6.33	6.24	17.72	17.68	13.50	13.47
11	Benzylamine	H	H	132–133	68	$C_{12}H_{11}N_3OS$	58.77	58.69	4.48	4.39	17.14	17.05	13.06	3.00
12	Cyclopentylamine	H	C_2H_5	172–173	62	$C_{12}H_{17}N_3OS$	57.34	57.31	6.81	6.80	16.72	16.64	12.76	12.69
13	*p*-Aminobenzoic acid	H	CH_3	318–319	69	$C_{13}H_{11}N_3O_3S$	53.97	53.86	3.83	3.79	14.53	14.46	11.08	11.01
14	*p*-Anisidine	H	CH_3	295 dec.	71	$C_{13}N_{13}N_3O_2S$	56.50	56.43	5.10	5.00	15.21	14.98	11.60	11.49
15	*p*-Anisidine	H	C_2H_5	279–280	73	$C_{14}H_{15}N_3O_2S$	57.91	57.86	5.55	5.41	14.47	14.31	11.04	10.96
16	Cycloheptylamine	H	C_2H_5	134–135	76	$C_{14}H_{21}N_3OS$	60.18	60.09	7.57	7.49	15.04	15.00	11.48	11.33
17	Cyclooctylamine	H	C_2H_5	157–158	68	$C_{15}H_{25}N_3OS$	60.98	60.90	8.53	8.50	14.32	14.20	10.93	10.76
18	Cyclopentylamine	C_6H_5	CH_3	186–187	52	$C_{17}H_{19}N_3OS$	65.15	65.01	6.11	5.96	13.40	13.24	10.23	10.09
19	Oleylamine	H	C_2H_5	100–101	65	$C_{24}H_{41}N_3OS$	68.41	68.66	9.78	9.63	10.02	10.00	7.65	7.58
20	Octyldecylamine	H	C_2H_5	97–98	72	$C_{25}H_{46}N_3OS$	68.97	68.83	10.33	10.21	9.65	9.48	7.36	7.21

methyluracil[7] and distinctly different from that of 1-methyluracil.[6b] Thus, the ultraviolet absorption spectra contributed confirmatory evidence for the structure assigned (IV).

The corresponding Schiff bases and hydrazones of IV were prepared in the usual manner, utilizing absolute ethanol or glacial acetic acid as the solvent (Tables III and IV).

Screening Results.—The various pyrimidine derivatives were tested *vs.* the Ehrlich ascites carcinoma in Swiss–Webster white mice by procecures described previously.[8] The results are recorded in Table V in which compounds are designated by the table number (II–IV) and compound number (Arabic numerals). The rapid increase in body weight of control mice is a measure of the accumulation of tumor cells and ascitic fluid (column 4). However, the total packed-cell volume of tumor cells (TPCV) (columns 5 and 6) determined on the 6th day after intraperitoneal transplantation of the tumor is the most reliable index of the multiplication of the tumor cells. The dosages recorded in column 2 were divided into two intraperitoneal injections per day commencing 24 hr. after transplantation of the tumor and continuing for 4.5 days.

Judged on the basis of significantly lower TPCV in the treated mice, with relatively low toxicity, several of the compounds listed in Table V showed sufficient activity to warrant further study. The majority of the Schiff bases of 2-mercapto-6-oxopyrimidine-4-carboxaldehyde showed similar or less activity against the tumor than did the parent aldehyde. A number of the hydrazones exhibited greater activity than did the parent aldehyde. However, the majority of the hydrazones had low solubilities in water, and this factor probably tended to limit their effectiveness. Of the Schiff bases, III-1, III-4, III-6, III-10, III-17, and III-19 had the greatest activity. The oleylamine

(8) (a) J. E. Wilson, J. L. Irvin, J. E. Suggs, and K. Liu, *Cancer Res.*, **19**, 272 (1959); (b) J. E. Wilson, J. E. Suggs, and J. L. Irvin, *Cancer Res. Supplement*, **21**, 692 (1961).

TABLE IV

HYDRAZONE DERIVATIVES OF SUBSTITUTED AND UNSUBSTITUTED 6-HYDROXY-2-THIOPYRIMIDINE-4-CARBOXALDEHYDES (IV)

No.	Reagent used	R	R′	M.p., °C.	Yield, %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1[a]	Hydroxylamine HCl	H	H	250 dec.	92	$C_5H_5N_3O_2S$	35.07	35.01	2.94	2.73	24.50	24.31	18.79	18.67
2[b]	Hydroxylamine HCl	H	CH_3	245 dec.	80	$C_6H_7N_3O_2S$	38.91	38.77	3.80	3.79	22.69	22.53	17.31	17.21
3[c]	Thiosemicarbazide	H	H	260 dec.	90	$C_6H_7N_5OS_2$	31.43	31.32	3.07	2.97	30.55	30.23	27.97	27.69
4	Semicarbazide HCl	H	H	278–279	88	$C_6H_7N_5O_2S$	33.80	33.64	3.30	3.24	32.85	32.64	15.03	14.96
5[d,e]	Nitroaminoguanidine[f]	H	H	295 dec.	77	$C_6H_8N_7O_3S$	27.91	27.88	3.12	3.00	37.96	37.91	12.42	12.40
6[g]	Thiosemicarbazide	H	CH_3	300 dec.	89	$C_7H_9N_5OS_2$	34.52	34.39	3.73	3.91	28.79	28.66	26.36	26.30
7	Semicarbazide HCl	H	CH_3	290–291	81	$C_7H_9N_5O_2S$	36.99	36.93	3.99	3.94	30.82	30.81	14.11	14.09
8	Semicarbazide HCl	CH_3	H	292–293	82	$C_7H_9N_5O_2S$	36.99	36.83	3.99	4.02	30.82	30.69	14.10	14.05
9	Thiosemicarbazide	H	C_2H_5	280–281	85	$C_8H_{11}N_5OS_2$	37.34	37.21	4.31	4.09	27.22	27.05	24.92	24.81
10	Semicarbazide HCl	CH_3	CH_3	277–278	87	$C_8H_{11}N_5O_2S$	39.66	39.59	4.99	5.01	28.91	28.89	13.23	13.10
11[d]	Nitroaminoguanidine	H	C_2H_5	279–280	88	$C_5H_{12}N_8O_3S$	35.25	35.21	4.14	4.31	30.87	30.74	11.78	11.64
12	Thiosemicarbazide	C_3H_7	H	252–253	77	$C_9H_{13}N_5OS_2$	39.68	39.63	5.18	5.09	25.72	25.69	23.55	23.41
13	2,4-Dinitrophenylhydrazine	H	H	300 dec.	79	$C_{11}H_9N_9O_8S$	30.05	30.00	2.98	3.00	24.84	24.76	9.48	9.59
14[h]	Isonicotinic acid hydrazide	H	H	300 dec.	75	$C_{10}H_9N_5O_2S$	48.00	47.89	3.29	3.19	25.45	25.32	11.62	11.59
15[i]	*p*-Hydrazinobenzoic acid	H	H	349–350	71	$C_{12}H_{12}N_4O_3S$	49.31	49.28	4.03	4.01	19.17	19.11	10.97	10.83
16[h]	Isonicotinic acid hydrazide	CH_3	H	293–294	90	$C_{12}H_{10}N_6O_2S$	49.82	49.81	3.83	3.76	24.21	24.09	11.08	11.00
17[h]	Isonicotinic acid hydrazide	H	CH_3	325–326	89	$C_{12}H_{11}N_5O_2S$	49.82	49.77	3.83	3.81	24.21	24.13	11.08	10.97
18	Semicarbazide HCl	C_6H_5	H	279–280	79	$C_{12}H_{12}N_5O_2S$	49.64	49.59	4.16	4.09	24.13	24.01	11.01	11.00
19	Thiosemicarbazide	C_6H_5	H	263–264	78	$C_{12}H_{12}N_5OS$	47.04	47.00	3.95	4.00	22.68	22.74	20.93	21.00
20[d,j]	Benzenesulfonylhydrazide	CH_3	H	192–193	88	$C_{12}H_{12}N_4O_3S_2$	44.43	44.39	3.73	3.68	17.27	17.09	19.77	19.70
21[i]	*p*-Hydrazinobenzoic acid	CH_3	H	323–324	88	$C_{13}H_{12}N_4O_3S$	51.31	51.20	3.97	3.99	18.41	18.39	10.54	10.45
22[i]	*p*-Hydrazinobenzoic acid	H	CH_3	345 dec.	90	$C_{13}H_{12}N_4O_3S$	51.31	51.29	3.97	4.01	18.41	18.36	10.54	10.50
23	4-Fluorophenylhydrazine HCl	H	C_2H_5	264–265	93	$C_{13}H_{13}N_4OFS$	53.41	53.32	4.48	4.39	19.17	19.11	10.96	10.75
24	*p*-Nitrophenylhydrazine	H	C_2H_5	300 dec.	79	$C_{13}H_{14}N_5O_3S$	48.75	48.69	4.41	4.29	21.87	21.74	10.01	10.00
25	Thiosemicarbazide	C_6H_5	CH_3	260–261	84	$C_{13}H_{14}N_5OS_2$	46.41	46.33	4.19	4.00	20.82	21.00	19.06	19.09
26[b]	Isonicotinic acid hydrazide	H	C_2H_5	304–305	83	$C_{13}H_{13}N_6O_2S$	51.48	51.50	4.31	4.26	23.09	22.96	10.55	10.62
27[d,j]	Toluenesulfonylhydrazine	H	CH_3	178–179	61	$C_{13}H_{14}N_4O_3S_2$	46.14	46.09	4.17	4.05	16.56	16.31	18.95	19.01
28[d,j]	Toluenesulfonylhydrazine	CH_3	H	194–195	70	$C_{13}H_{14}N_4O_3S_2$	46.14	46.12	4.17	4.11	16.56	16.42	18.95	18.91
29[d,j]	Benzenesulfonylhydrazide	H	C_2H_5	165–166	78	$C_{13}H_{14}N_4O_3S_2$	46.14	46.11	4.17	4.12	16.56	16.41	18.95	18.88
30	4-Chlorophenylhydrazine	H	C_2H_5	260–261	74	$C_{13}H_{13}N_4OClS$	50.56	50.50	4.24	4.19	18.15	18.06	10.38	10.22
31[i]	3-Nitrophenylhydrazine	H	C_2H_5	274–275	83	$C_{13}H_{13}N_5O_3S$	48.89	49.01	4.10	4.02	21.93	22.00	10.03	9.95
32	2,4-Dinitrophenylsemicarbazide	H	C_2H_5	254–255	73	$C_{11}H_{13}N_7O_5S$	41.28	41.08	3.22	3.10	21.06	21.01	7.87	7.73
33	4-Phenylsemicarbazide	H	C_2H_5	279–280	81	$C_{11}H_{13}N_5O_2S$	52.98	52.76	4.76	4.59	22.07	22.00	10.19	9.95

[a] Recrystallized from 50% ethanol. [b] Recrystallized from 80% ethanol. [c] Recrystallized from water. [d] Recrystallized from ethanol. [e] Reaction solvent was glacial acetic acid. [f] Prepared by the method described by R. Philips and J. F. Williams, *J. Am. Chem. Soc.*, **50**, 2465 (1928). [g] Recrystallized from dimethylformamide–water; all other compounds listed were recrystallized from DMF–H_2O unless otherwise noted. [h] Not recrystallized, obtained analytically pure. [i] Reaction solvent was an equal mixture of ethanol and glacial acetic acid. [j] Recrystallized from acetic acid.

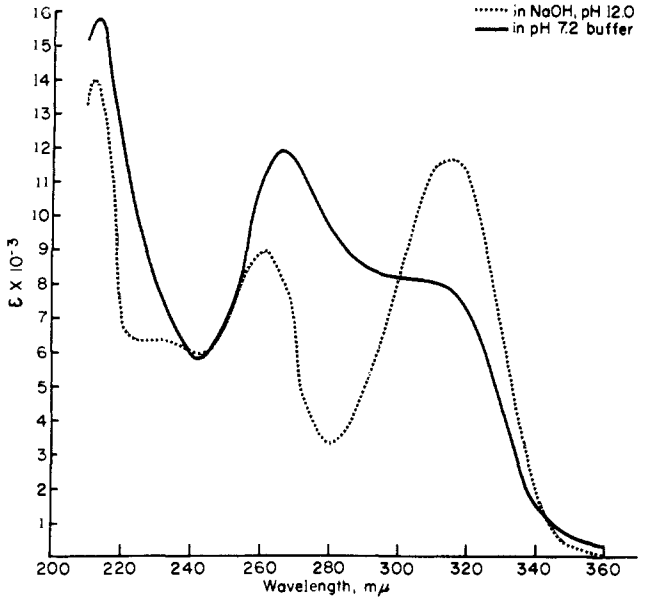


Fig. 1.—4-(Dimethoxy)methyl-1-methyl-6-oxo-2-thiopyrimidine in aqueous solution at pH values indicated.

Schiff base, III-19, was the most active of that series. In the hydrazone series, IV-3, IV-4, IV-15, IV-18, IV-20, IV-25, IV-27, and IV-28 were most active. The benzenesulfonylhydrazone, IV-20, and the toluenesulfonylhydrazone, IV-28, showed the greatest activity of both series of compounds. In general, substitution of the pyrimidines in the 5-position with methyl or ethyl groups tended to decrease activity slightly in comparison with corresponding pyrimidines which were unsubstituted in that position, but there were some exceptions to this generality. Further studies of these and related pyrimidine derivatives will be undertaken.

Experimental[9]

Alkylthioureas.—This methyl compound was obtained by a method previously described in the literature,[10] m.p. 120–121° (lit.[10] m.p. 120.5–121°). The ethylthiourea, m.p. 104–106° (lit.[10] m.p. 103–106°) and propylthiourea, m.p. 109–110° (lit.[11] m.p. 110°) were prepared in an analogous manner. Phenylthiourea, m.p. 153–154°, was obtained commercially.

Ethyl γ,γ-Dimethoxyacetoacetate.—This compound was prepared by a modification of the procedure reported in the literature[12] for ethyl γ,γ-diethoxyacetoacetate. To a refluxing mixture of 140 g. (0.91 mole) of methyl dimethoxyacetate[13] and 138 g. (1.57 mole) of ethyl acetate, 24 g. (1.04 g.-atom) of metallic sodium was added slowly. An additional 139 g. of ethyl acetate and 24 g. of metallic sodium were introduced into the reaction

(9) Elementary microanalyses by Weiler and Strauss, Oxford, England, and Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points are uncorrected but were determined using a "Mel-Temp" apparatus with a thermometer calibrated for exposed stem. This work was completed before the requirement for corrected melting points was introduced by American Chemical Society Journals. The ultraviolet absorption spectra were determined with a Beckman DK-1 spectrophotometer. Sorensen phosphate buffers were employed to obtain pH 7.2 and 0.01 *N* NaOH was taken as essentially equal to pH 12.0. The pH values were checked with a calibrated glass electrode.

(10) M. L. Moore and F. S. Crossley, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, New York, N. Y., 1955, p. 617.

(11) O. Hecht, *Ber.*, **23**, 281 (1890).

(12) T. B. Johnson and L. A. Mikeska, *J. Am. Chem. Soc.*, **41**, 810 (1919).

(13) We wish to thank Max G. Gergel, Columbia Organic Chemical Co., Inc., Columbia, S. C., for making this compound available to us.

TABLE V

RESULTS OF SCREENING TESTS *vs.* THE EHRLICH ASCITES CARCINOMA[a]

Compound	Dosage, mg./kg./day	Mortality treated group	Average wt. change, T/C (g.)	—Average TPCV— T/C (ml.)	% of Controls
II-1	122	0/8	3.4/8.6	1.8/2.9	63
	201	3/8	−0.8/4.4	0.3/1.6	19
III-1	65	3/10	6.5/6.8	1.7/2.5	68
	120	2/8	4.1/5.9	0.8/2.4	33
III-2	57	2/10	2.0/6.8	2.5/2.5	100
III-3	66	6/10	5.4/6.2	1.0/1.5	67
III-4	113	1/16	2.5/2.4	0.8/1.4	57
	237	0/8	1.7/3.3	0.7/1.4	50
	282	0/8	2.8/7.7	0.6/2.4	25
III-5	66	3/10	3.9/6.2	1.3/1.5	87
III-6	183	2/8	7.0/7.7	1.7/2.3	74
III-7	57	2/10	4.2/6.2	1.1/1.5	73
III-8	66	4/10	4.3/6.8	2.1/2.5	84
III-9	125	2/8	4.0/1.9	1.8/1.6	100
	135	3/8	2.3/3.9	1.7/0.8	100
III-10	126	0/8	2.4/1.4	1.4/2.5	56
	258	0/8	2.1/3.3	0.7/1.4	50
III-11	186	3/8	2.1/3.3	1.8/1.4	100
	190	1/8	2.3/1.4	2.5/2.5	100
III-12	68	3/8	2.5/4.4	1.8/2.2	82
III-13	132	2/8	1.7/3.3	1.8/1.4	100
III-14	155	0/8	3.1/2.4	2.3/2.5	92
III-15	150	2/8	1.2/2.4	1.6/2.5	64
III-16	75	1/8	3.5/4.4	1.6/2.2	73
III-17	80	1/8	2.5/4.4	1.5/2.2	68
III-18	73	4/10	7.2/6.8	2.0/2.5	80
III-19	69	1/10	0.9/6.8	1.3/2.5	52
	120	1/10	1.1/5.4	0.5/2.3	22
III-20	119	0/8	7.6/1.9	2.5/1.6	100
	144	2/8	1.8/3.9	1.9/0.8	100
IV-1	71	3/10	3.7/6.8	2.1/2.5	84
IV-2	58	6/10	3.9/6.8	2.1/2.5	84
IV-3	43	2/8	−0.4/5.7	1.5/2.3	65
	79	1/8	−1.1/2.4	1.6/2.5	64
	153	2/8	−2.7/8.0	1.3/2.5	52
IV-4	34	0/8	−3.5/5.7	1.5/2.3	65
	145	1/8	−4.3/8.0	1.0/2.5	40
	153	5/8	−4.2/8.8	0.6/2.0	30
IV-5	57	3/10	2.1/6.8	2.5/2.5	100
IV-6	61	0/10	0.4/6.8	2.2/2.5	88
IV-7	60	2/10	0.3/6.2	1.3/1.5	86
IV-8	67	4/10	1.9/6.8	2.1/2.5	84
IV-9	76	0/8	−1.3/2.4	2.1/2.5	84
	153	3/8	−1.5/8.8	1.5/2.0	75
IV-10	64	4/10	4.0/6.8	2.4/2.5	96
IV-11	63	0/10	−0.6/6.8	1.9/2.5	76
IV-12	62	0/10	2.6/6.2	1.8/1.5	100
IV-13	60	3/10	6.7/6.2	2.4/1.5	100
IV-14	62	3/10	4.8/6.8	3.5/2.5	100
IV-15	147	0/8	−4.6/5.0	1.0/2.2	45
IV-16	60	1/10	−3.7/6.8	2.2/2.5	88
IV-17	63	2/10	2.0/6.2	1.8/1.5	100
IV-18	75	3/8	−2.5/2.4	1.0/2.5	40
IV-19	74	1/8	−1.3/2.4	2.8/2.5	100
IV-20	59	2/10	1.4/6.8	0.6/2.5	24
	120	2/8	1.0/5.9	0.3/2.6	12
IV-21	66	3/10	4.9/6.8	4.3/2.5	100
IV-22	66	4/10	−4.7/6.2	1.4/1.5	92
IV-23	124	1/8	5.3/1.9	2.9/1.6	100
	132	5/8	4.7/1.9	2.6/1.6	100
IV-24	65	0/10	4.7/6.8	2.9/2.5	100
IV-25	66	3/10	−2.3/6.8	1.6/2.5	64
IV-26	65	2/8	2.4/1.9	1.5/1.5	100
	147	3/8	−5.6/3.3	1.0/1.4	72
IV-27	65	1/10	6.9/6.8	1.8/2.5	71
	130	2/10	5.1/6.5	1.2/2.3	53
IV-28	68	1/10	4.4/6.8	1.0/2.5	40
IV-29	67	3/10	7.6/6.8	1.5/2.5	60
IV-30	133	2/8	2.1/3.3	1.8/1.4	100
IV-31	135	2/8	2.8/3.3	2.2/1.4	100
IV-32	125	2/8	3.1/6.1	2.0/2.3	87
	132	0/8	3.6/1.9	1.6/1.6	100
IV-33	127	1/8	2.5/6.1	2.0/2.3	87

[a] T = treated group; C = control group; TPCV = total packed-cell volume of tumor cells; average mortality of control group to day of assay = 40%.

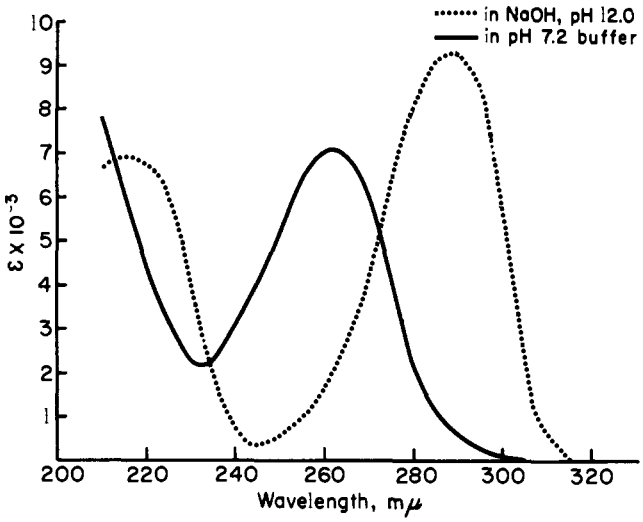


Fig. 2.—4-(Dimethoxy)methyl-2,6-dioxo-1-methylpyrimidine in aqueous solution at pH values indicated.

mixture, and stirring and heating were continued until all the sodium dissolved. The solution was allowed to remain overnight at room temperature, poured over crushed ice, and acidified with concentrated hydrochloric acid. The oil was removed and the aqueous layer extracted 3 times with 300-ml. portions of ether. These washings were added to the organic phase and the ethereal solution washed with 2% sodium carbonate, dried over sodium sulfate, and the ether removed *in vacuo*. Subsequent fractionation gave 80% of a nearly colorless liquid which distilled at 98–100° (5 mm.).

Anal. Calcd. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.34; H, 7.30.

The following were prepared in an analogous manner:

(a) **Ethyl α-methyl-γ,γ-dimethoxyacetoacetate** (70% yield), b.p. 115–117° (8 mm.).

Anal. Calcd. for $C_9H_{16}O_5$: C, 52.93; H, 7.89. Found: C, 52.88; H, 7.76.

(b) **Ethyl α-ethyl-γ,γ-dimethoxyacetoacetate** (70% yield), b.p. 110–112° (5 mm.).

Anal. Calcd. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.97; H, 8.26.

4-(Dimethoxy)methyl-6-hydroxy-1-methyl-2-thiopyrimidine (III).—To 11 g. (0.47 g. atom) of sodium in 250 ml. of absolute ethanol, 45 g. (0.5 mole) of dry methylthiourea and 95 g. (0.5 mole) of ethyl γ,γ-dimethoxyacetate were added, and the solution was refluxed for 2 hr. After about 1.5 hr. a solid had formed. The mixture was cooled to room temperature, the solid removed by filtration, washed with two 30-ml. portions of ethanol, and dissolved in 200 ml. of water. The solution was filtered, and upon acidifying with 10% hydrochloric acid, a pure white crystalline solid separated, which was collected by filtration, washed with 100 ml. of water, dried, and recrystallized from ethanol, yielding 70 g. (65%) of product, m.p. 148–149°. Table I lists the compounds prepared by a similar procedure. Table

II lists the compounds prepared by hydrolyzing the pyrimidine acetal.

To a 1.5 g. sample of III, dissolved in 20% sodium hydroxide, 50 ml. of 3% hydrogen peroxide was added and the mixture heated for 5 min. After cooling to 0°, it was acidified with hydrochloric acid and the resulting solids were collected by filtration and washed with cold water. Crystallization from ethanol–ether produced 90% of 4-(dimethoxy)methyl-2,6-dioxo-1-methylpyrimidine (IIIb), a white crystalline solid, m.p. 137° (cor.). The ultraviolet spectrum was consistent with the proposed structure.[14]

Anal. Calcd. for $C_8H_{12}N_2O_4$: C, 47.99; H, 6.04; N, 13.99. Found: C, 47.90; H, 5.98; N, 14.00.

Schiff Bases from Pyrimidine-4-carboxaldehydes.—These compounds were prepared by the interaction of the substituted and unsubstituted pyrimidine-4-carboxaldehyde with the appropriate amine. A solution of 0.01 mole of the aldehyde in a minimum amount of hot absolute ethanol was prepared. To this was added 0.01 mole of the amine and the mixture refluxed for 30 min. on a steam bath. The reaction mixture was placed in a refrigerator overnight, the product collected on a filter, and recrystallized from ethanol. The Schiff bases are shown in Table III.

Hydrazones from Pyrimidine-4-carboxaldehydes.—These derivatives were prepared by the interaction of the pyrimidine aldehydes with the appropriate hydrazine or hydrazide. To a solution of 0.01 mole of the aldehyde in a minimum amount of hot absolute ethanol or glacial acetic acid was added 0.01 mole of the hydrazine or hydrazide reagent dissolved in a minimum amount of the same solvent. The mixture was refluxed for 10 min. and then placed in a refrigerator overnight. The product was collected by filtration and recrystallized from ethanol or acetic acid. The hydrazones are shown in Table IV.

(14) According to D. Shugar and J. J. Fox (see ref. 6b) 3-methyluracil has λ_{max} 258.5 mμ (ε 7300) occurring between pH 3.0 and 7.2 and λ_{max} 218 mμ (ε 7060) at pH 12.0. The 1-methyluracil has λ_{max} 267.5 mμ (ε 9750) at pH 7.2 and λ_{max} 265 mμ (ε 7020) occurring between pH 12.0 and 14.0.

Acknowledgment.—We wish to thank Mr. Robert Morris for help in preparing some of the intermediate compounds required during the course of this work and to Mr. Carl W. Anderson for assistance in the screening tests. We are grateful to Dr. J. J. Fox and Dr. A. F. Hirsch, of the Sloan-Kettering Institute for Cancer Research, for their interest and suggestions on the ultraviolet studies.

The Synthesis and Antiinflammatory Activity of Some Derivatives of 1,3-Diphenylbarbituric Acid

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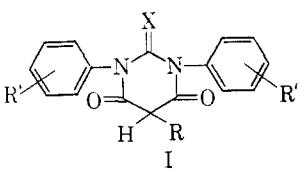
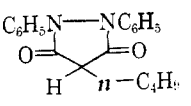
A series of 1,3-diphenylbarbituric acid derivatives has been prepared and evaluated for antiinflammatory activity. The compounds were found to be less toxic and less active than phenylbutazone by the testing procedures used. The most potent member of the series was 1,3-diphenyl-5-(3-methyl-2-butenyl)barbituric acid. Its activity compared quite favorably with that of phenylbutazone on parenteral administration but, when given orally, it proved active only at high dosages.

The recent communication by Scarborough and McKinney[1] describing the preparation and uricosuric activity of some substituted 1,3-diphenylbarbituric acids prompts us to report the results of a related investigation.

As part of a research program devoted to the synthesis and evaluation of organic compounds as potential antiinflammatory agents, it was of interest to us to examine a series of 1,3-diaryl-5-substituted barbituric and 2-thiobarbituric acids (I), whose relationship to phenylbutazone (II) is evident.



I



II

X = O or S
R = alkyl, cycloalkyl, aryl, alkenyl, aralkyl
R′ = H, CH_3, OCH_3

A search of the literature disclosed that, while there had been sporadic reports[2] on the preparation of N,N′-diarylbarbituric and 2-thiobarbituric acids, little interest had been shown in the 5-substituted analogs of these compounds. Whiteley[2a,b] reported the preparation of several compounds of structure I by means of the zinc–acetic acid reduction of the corresponding 5-alkylidene derivatives. Although we were able to obtain one compound (**19**, Table I) in good yield by this method, a more general and reliable procedure, and one which we used almost exclusively for the preparation of the compounds listed in Table I even though yields were low, proved to be the condensation of carbanilide or thiocarbanilide with substituted malonic acids in the presence of acetyl chloride.

It has been reported[2c] that sodiomalonic ester and carbanilide or thiocarbanilide in absolute alcohol or benzene fail to form N,N′-disubstituted barbituric acid derivatives. We found that the desired reaction will occur under conditions of elevated temperature and succeeded in preparing 1,3-diphenyl-5-amylbarbituric acid (I, R = *n*-C_5H_{11}, R_1 = H, X = O) from the appropriate reactants in xylene at 140–145°. However, purification of the product proved troublesome, eventually requiring vacuum distillation to give a low yield of pure compound, and this procedure therefore received limited attention as a potential alternate route to I.

Pharmacology.—All of the compounds synthesized were evaluated for antinflammatory activity by means

(1) H. C. Scarborough and G. R. McKinney, *J. Med. Pharm. Chem.*, **5**, 175 (1962).

(2) (a) M. A. Whiteley, *J. Chem. Soc.*, **91**, 1330 (1907); (b) M. A. Whiteley and H. Mountain, *Proc. Chem. Soc.*, **25**, 121 (1909); (c) N. V. Koshkin, *Zh. Obshch. Khim.*, **5**, 1460 (1935); (d) I. N. D. Dass and S. Dutt, *Proc. Indian Acad. Sci.*, **8A**, 145 (1938).