was stirred for 40 min at room temp. The soln was then poured slowly into 500 ml of ice-cold 2 *N* HCl (pH 1.0). The mixture was extracted with EtOAc and the acids were extracted from the EtOAc with a soln of $NaHCO_3$ to pH 7.8. The alkaline soln was reacidified with 2 *N* HCl to pH 2.0 and reextracted with EtOAc; this extract was washed with H_2O and dried ($MgSO_4$). The solvent was evapd and the residue was triturated with petr ether to give a powdered product which crystallized from 50% EtOH. The physical data and yields are summarized in Tables I and II. Ir spectra were consistent with the required structures.

3-Acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic Acid 1-Oxides (VI).—3-Acyloxymethyl-7-[2-(thienyl)-acetamido]-2-cephem-4-carboxylic acid (1 mmole) was dissolved in a minimal amount of $CHCl_3$ at room temp and 0.95 mmole of 85% *m*-chloroperbenzoic acid was added. The mixture was warmed slightly and pptn commenced. After being stirred for 15 min at room temp, the solvent was concd to a small vol; the pptd crystals were filtered and recrystd from EtOH. The physical constants, yields, and other pertinent data are given in Tables I and II.

3-Acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic Acids (VII).—The corresponding sulfoxide VI (1 mmole) and $SnCl_2 \cdot 2H_2O$ (2.5 mmoles) were dissolved in 18 ml of DMF. This solution was cooled in an ice bath, and 2.5 ml of AcCl was added. The mixture was stirred at 18° for 15 min, then poured into 40 ml of cold H_2O, and extracted with EtOAc. The extract was washed with H_2O and then dried ($MgSO_4$). The solvent was evapd, and the residue displayed one spot in tlc. Compound VII could be recrystd from EtOH–H_2O. Isolated compounds VII displayed a single biologically active spot on bioautograph. The physical data and yields are listed in Tables I and II. Biological data are shown in Table III.

Acknowledgment.—The author is grateful to G. Maciak and his staff for microanalyses; to D. O. Woolf, L. A. Spangle, and their associates for spectral data; to J. L. Ott, J. Westhead, and W. E. Wick for biological data; to C. T. Pugh for bioautography; and to A. I. Ellis for technical assistance.

Carcinostatic Activity of Thiosemicarbazones of Formyl Heteroaromatic Compounds. VI. 1-Formylisoquinoline Derivatives Bearing Additional Ring Substituents, with Notes on Mechanism of Action[1]

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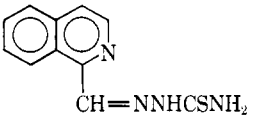
Received March 20, 1970

Twenty-two thiosemicarbazones of 1-formylisoquinolines bearing additional substituents in the pyrido or benzo ring were synthesized and tested against a number of mouse tumor systems *in vivo* in an attempt to improve on the parent compound. The major test tumors were L-1210 leukemia, sarcoma 180 (ascites), L-5178Y lymphoma, C-1498 myelogenous leukemia, and the Lewis lung carcinoma. Occasional screening was performed using B-16 melanoma, Ehrlich ascites carcinoma, and sarcoma 180 (solid). The additional substituents studied were: 2-oxide, 3-methyl, 4-acetoxy, 4-hydroxy, 5-cyano, 5-sulfonic acid, 5-sulfonic acid ammonium salt, 5-chloro, 5-fluoro, 5-nitro, 5-acetoxy, 5-hydroxy, 5-trifluoromethyl, 5-*n*-perfluoropropyl, 5-carboxy, 6-methoxy, 7-acetoxy, 7-fluoro, 7-chloro, 7-hydroxy, 7-methoxy, and 8-fluoro. No simple parametric rationale could be found for the effect of additional substituents on activity against a given tumor system. The order of substituent effects changed markedly from one tumor system to another. This negates the validity of any generalized parametric statements. A complicating factor was that 17 of these 22 compounds were more or less poorly absorbed. A number of active compounds in the isoquinoline series and in the corresponding pyridine series have been studied in other laboratories as inhibitors of DNA synthesis in cell-free systems, in cellular systems, and *in vivo*. The powerful inhibition of DNA synthesis ($\sim 10^{-7}$ *M* for 50% inhibition) in all cases studied involves a blockade of mammalian tumor derived ribonucleoside diphosphate reductase (RDR). These compounds are tridentate ligands for transition metals including iron. As a consequence, a detailed model is proposed for the functioning of RDR and for inhibition of this activity by these ligands.

Since the discovery that 1-formylisoquinoline thiosemicarbazone (**1**) is very active against a variety of mouse tumors,[2] there has been considerable interest in this laboratory and in others to find more active derivatives.[3-6]



1

In this study 22 derivatives substituted in either the pyrido or benzo ring have been synthesized and tested on a variety of mouse tumor systems.

Chemistry.—Some of the 5-substituted 1-formylisoquinoline thiosemicarbazones included in the present study (Table IV) have been described previously.[3] The unreported 5- and 8-substituted 1-methylisoquinolines (Table I) were prepared from 1-methyl-5-nitroisoquinoline (I, Scheme I).

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(1) (a) This investigation was supported by Grant CA-03287 from the National Cancer Institute; (b) presented in part at the 169th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract MEDI-76.

(2) F. A. French and E. J. Blanz, Jr., *Cancer Res.*, **25**, 1454 (1965).

(3) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, **11**, 700 (1968).

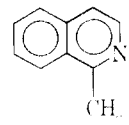
(4) K. C. Agrawal and A. C. Sartorelli, *ibid.*, **12**, 171 (1969).

(5) K. C. Agrawal and A. C. Sartorelli, *J. Pharm. Sci.*, **57**, 1948 (1968).

(6) K. C. Agrawal and A. C. Sartorelli, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, MEDI-39.

TABLE I

SUBSTITUTED 1-METHYLISOQUINOLINES



Compd	Mp, °C	Yield, %	Crystn solvent[a]	Formula	Analyses
5-Cl	93–94	61	A	$C_{10}H_8ClN$	C, H, Cl, N
5-CN	145–146	36	A	$C_{11}H_8N_2$	C, H, N
5-COOH	297–300 dec	86		$C_{11}H_9NO_2 \cdot HCl$	C, H, Cl, N
5-F	54.5–55.5	54	A	$C_{10}H_8FN$	C, H, F, N
5-I	109.5–110.5	34	A	$C_{10}H_8IN$	C, H, I, N
5-CF_3	84–85	44	A	$C_{11}H_8F_3N$	C, H, F, N
5-*n*-C_3F_7	63–64	34	A	$C_{13}H_8F_7N$	C, H, F, N
7-Cl	65.5–66	36	A	$C_{10}H_8ClN$	C, H, Cl, N
7-AcO	53–54	71	A	$C_{12}H_{11}NO$	H, N; C[b]
7-OH	240.5–241	96	B	$C_{10}H_9NO$	C, H, N
7-NH_2	200–201	95	C	$C_{10}H_{10}N_2$	C, H, N
7-F	36.5–38	78	A	$C_{10}H_8FN$	C, H, F, N
8-NH_2	141–142	81	C	$C_{10}H_{10}N_2$	C, H, N
8-F	43–45	75	A	$C_{10}H_8FN$	C, H, F, N
5-Cl, 8-NO_2	173–175.5	70	C	$C_{10}H_7ClN_2O_2$	C, H, Cl, N

[a] Crystallization solvents: A = petroleum ether; B = EtOH; C = PhH. [b] C: calcd, 71.62; found, 72.23.

Misani and Bogart[7] reported the hydrogenation of 5-nitroisoquinoline to 5-aminoisoquinoline using Pd–C catalyst. 5-Amino-1-methylisoquinoline (II) was prepared by the same method instead of by the $SnCl_2$ reduction used by Agrawal.[5] 5-Amino-1-methylisoquinoline (II) was converted into III, VII, VIII, and XI by suitable diazotization reactions described in the literature for 5-aminoisoquinoline.[8–10] 1-Methyl-5-perfluoro-*n*-propylisoquinoline (XII) was prepared by treating 5-iodo-1-methylisoquinoline (XI) with activated Cu bronze[11] and perfluoro-*n*-propyl iodide in DMSO at 120°.[12]

Osborn *et al.*[13] converted 5-chloro- into 8-aminoisoquinoline by nitration to 5-chloro-8-nitroisoquinoline and subsequent reduction of NO_2 and removal of Cl by H_2 with 6% Pd–$CaCO_3$ as a catalyst. Similarly, 5-chloro-1-methylisoquinoline (III) was converted into 8-amino-1-methylisoquinoline (V). The resulting amine V was diazotized and converted into 8-fluoro-1-methylisoquinoline (VI) by a Schiemann reaction.

5-Cyano-1-methylisoquinoline (VIII) was transformed in 2 steps to 1-methylisoquinoline-5-carboxylic acid (IX). The cyano derivative could not be hydrolyzed directly to the acid derivative with NaOH. The hydrolysis stopped at the amide stage. The amide was then hydrolyzed in concd HCl at refluxing temperature to IX. The acid IX was allowed to react with SF_4 and HF at 150° to yield 1-methyl-5-trifluoromethylisoquinoline (X).

The 6- and 7-substituted 1-methylisoquinolines were prepared from suitable *N*-acetyl-β-phenethylamines by the Bischler–Napieralski synthesis (Scheme II). The preparations of 3,4-dihydro-6-methoxy-1-methylisoquinoline (XIV) and the 7-methoxy derivative (XVII) are described in the literature.[14] The dehydrogenation of XIV and XVII was accomplished with Ph_2S_2 by slowly distilling the benzenethiol from the reaction mixture.[15]

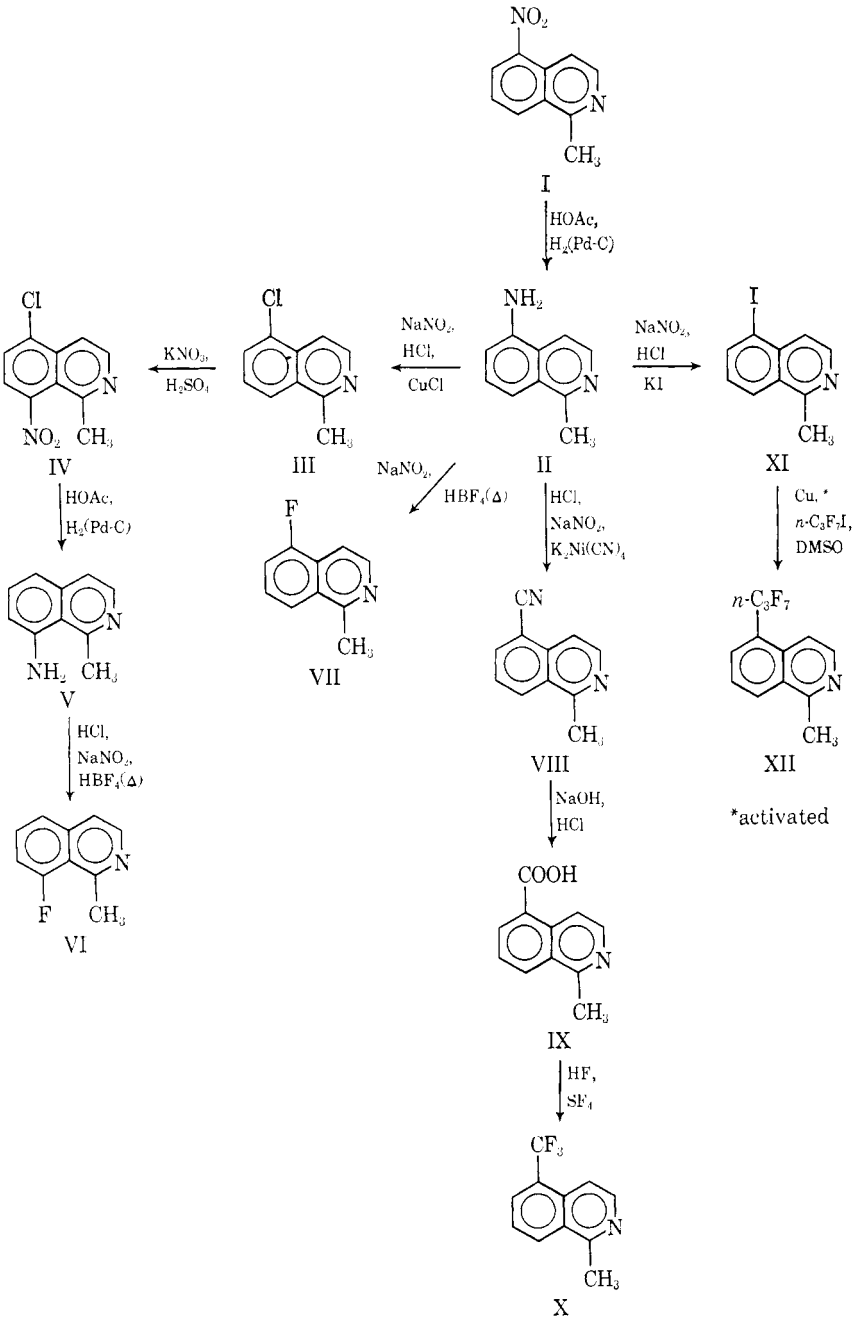
7-Methoxy-1-methylisoquinoline (XVIII) was converted into 7-hydroxy-1-methylisoquinoline (XIX) by refluxing in 50% HBr followed by acetylation to 7-acetoxy-1-methylisoquinoline (XX). 7-Amino-1-methylisoquinoline (XXI), synthesized from XIX by typical Bucherer reaction conditions, was used to prepare 7-chloro-1-methylisoquinoline (XXII)[16] and 7-fluoro-1-methylisoquinoline (XXIII) by the same method used for the 5-halogenated analogs.

Almost all the substituted 1-formylisoquinolines were prepared by the oxidation of the corresponding 1-methylisoquinoline with SeO_2, followed by conversion into the thiosemicarbazone derivatives (Tables II and III). The two exceptions were 1-formylisoquinoline-5-sulfonic acid thiosemicarbazone and 1-formylisoquinoline-5-carboxylic acid thiosemicarbazone which were prepared by the Kröhnke method (Scheme III). Agrawal, *et al.*,[3] synthesized 1-formylisoquinoline-5-sulfonic acid thiosemicarbazone by direct sulfonation of 1-formylisoquinoline thiosemicarbazone.

Structure–Activity Correlation.—Typical screening data are presented in Table IV. These data, in the case of positive compounds, are not chosen as the best values but are representative of a large amount of data. Using L-1210 as the test system it was found that the decreasing order of substituent effects in active compounds was: 5-fluoro (**10**) = none (**1**) > 5-trifluoromethyl (**14**) = 7-fluoro (**19**) = 8-fluoro (**23**) > 5-acetoxy (**12**) = 7-methoxy (**22**). With L-5178Y lymphoma the order of substituent effects was: none (**1**) > 5-nitro (**11**) = 7-fluoro (**19**) > 5-fluoro (**10**) > 5-carboxy (**16**)

(7) F. Misani and M. T. Bogart, *J. Org. Chem.*, **10**, 347 (1945).
(8) R. H. F. Manske and M. Kulka, *Can. J. Res.*, **27B**, 161 (1949).
(9) A. Edinger, *J. Prakt. Chem.*, **161**, 375 (1896).
(10) A. Roe and C. E. Teague, *J. Amer. Chem. Soc.*, **73**, 687 (1951).
(11) A. I. Vogel, "Practical Organic Chemistry," Longmans, Green and Co., Ltd., London, 1951, p 128.
(12) Method taken from V. C. R. McLaughlin, J. Thrower, M. A. H. Hewin, J. S. Pippett, and M. A. White, Royal Aircraft Establishment Technical Report No. 66341, Oct 1966.
(13) A. R. Osborn, K. Schofield, and L. N. Short, *J. Chem. Soc.*, 4191 (1956).
(14) M. B. Moore, H. B. Wright, M. Vernstein, M. Freifelder, and R. K. Richards, *J. Amer. Chem. Soc.*, **76**, 3656 (1954).
(15) F. W. Starks, private communication, 1966.
(16) R. A. Robinson, *J. Amer. Chem. Soc.*, **69**, 1939 (1947).

Scheme I



= 7-methoxy (**22**) = 8-fluoro (**23**) = 5-hydroxy (**13**) = 5-acetoxy (**12**) = 5-chloro (**9**). In C-1498 only the parent compound **1** showed significant activity. In the Lewis lung carcinoma only the parent compound **1** and the 5-fluoro derivative **10** showed activity. With the B-16 melanoma only the 7-fluoro derivative **19** was active. No compounds were active on sarcoma 180 (solid). In marked contrast, most of the thiosemicarbazones were active on sarcoma 180 (ascites) and most of the active compounds yielded a significant cure rate. The parent compound was the most active. Hence S-180 (ascites) may be regarded as a sensitive tool for demarking the borderlines of activity but it is a poor tool for meaningful comparisons within that domain.

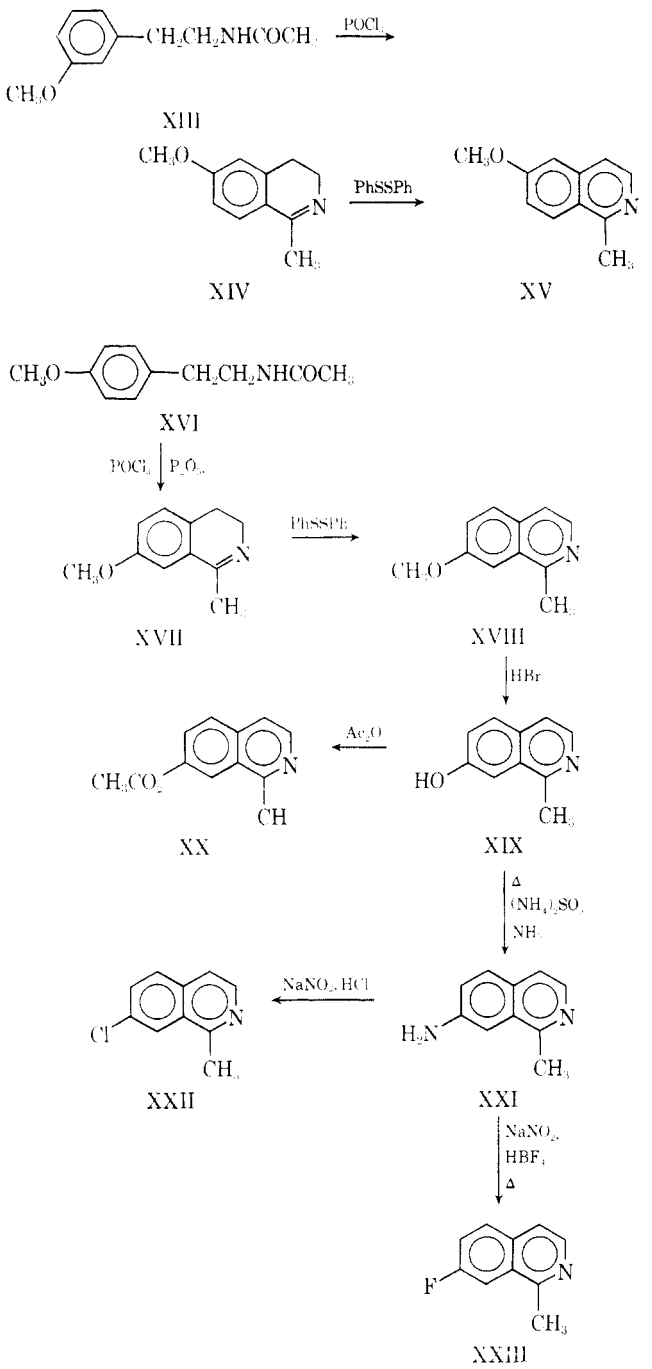
The order of toxicity is, roughly: 5-sulfonic acid NH_4 salt (**8**) = 7-hydroxy (**21**) = 7-chloro (**20**) < 6-methoxy (**17**) = 5-chloro (**9**) = 5-hydroxy (**13**) = 7-fluoro (**19**) < 5-sulfonic acid (**7**) < 4-acetoxy (**4**) = 4-hydroxy (**5**) = 5-cyano (**6**) < 7-methoxy (**22**) < 3-methyl (**3**) < *N*-oxide (**2**) = none (**1**) = 5-acetoxy (**12**) = 5-perfluoropropyl (**15**) = 8-fluoro (**23**) = 5-fluoro (**10**) = 7-acetoxy (**18**) < 5-carboxy (**16**) = 5-trifluoromethyl (**14**) = 5-nitro (**11**).

No simple correlation based on electronic, steric or hydrophilic parameters is apparent. This was also noted in the pyridine series.[17] We feel the available evidence points to the necessity of knowing more about the detailed structure of mammalian tumor-derived ribonucleoside diphosphate reductase necessary for DNA synthesis.

The foregoing commentary and the data in Table IV apply only to the single daily dose mode of administra-

(17) E. J. Blanz, Jr., F. A. French, J. R. DoAmaral, and D. A. French *J. Med. Chem.*, **13**, 1124 (1970).

Scheme II



Scheme III

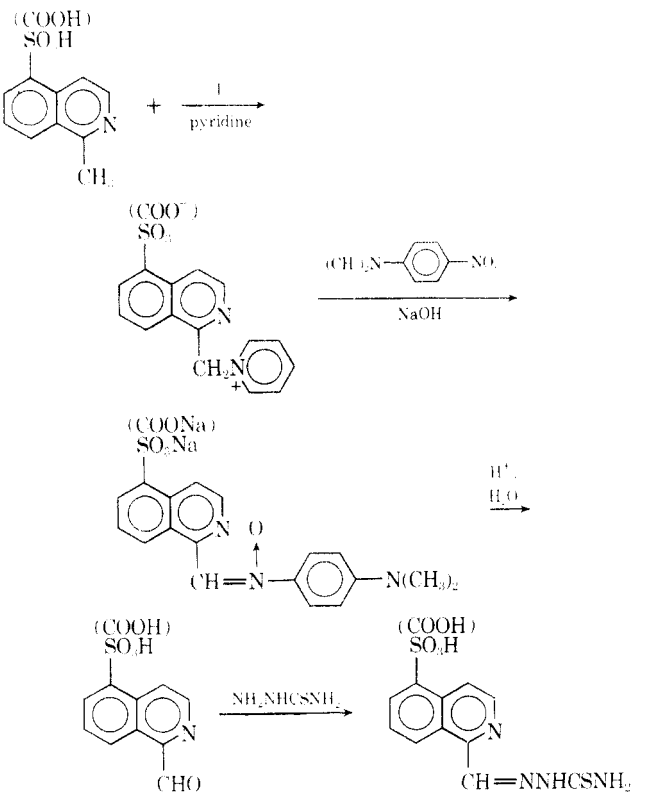
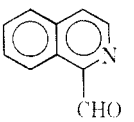


Table II

Substituted 1-Formylisoquinolines



Compd[a]	Mp, °C	Yield, %	Formula	Analyses
5-Cl	134–135	47	$C_{10}H_6ClNO$	C, H, Cl, N
5-CN	185–187	51	$C_{11}H_6N_2O$	C, H, N
5-F	117–118	55	$C_{10}H_6FNO$	C, H, F, N
5-CF_3	117–118	51	$C_{11}H_6F_3NO$	C, H, F, N
5-*n*-C_3F_7	74–75	68	$C_{13}H_6F_7NO$	C, H, F, N
6-OCH_3	74–76	13	$C_{11}H_9NO_2$	C, H, N
7-AcO	89.5–91	51	$C_{12}H_9NO_3$	C, N; H[b]
7-Cl	128.5–129.5	49	$C_{10}H_6ClNO$	C, H, Cl, N
7-F	119–120	50	$C_{10}H_6FNO$	C, H, F, N
7-OCH_3	100–101	43	$C_{11}H_9NO_2$	C, H, N
8-F	89.5–91	32	$C_{10}H_6FNO$	C, H, F, N

[a] All compounds were crystallized from petroleum ether (60–110°). [b] H: calcd, 4.22; found, 3.67.

tion. With the hydroxylated derivatives, both in the pyridine and isoquinoline series, it has been observed in this laboratory,[17,18] at CCNSC,[19] and by Agrawal and Sartorelli[5] that very different and improved results are sometimes obtained using multiple daily dosage. In the study by Agrawal and Sartorelli[5] it is especially noteworthy that with the highly insoluble 5-hydroxy-1-formylisoquinoline thiosemicarbazone (**13**) conversion into the monosodium salt yields greatly improved results on L-1210 leukemia. With the more soluble thiosemicarbazones bearing phenolic OH groups the differences in the results with the phenolic *vs.* the salt forms are much less significant and sometimes the phenolic form appears to be slightly more active. Nevertheless, the Na salts would appear to be, in general, the preferred dosage form.

A complicating factor in the interpretation of the data in Table IV is the fact that many of these compounds were so insoluble *in vivo* that they left small to large drug deposits. This was the case, in varying degrees, with **2–6**, **9–11**, **13**, **15**, and **17–23**.

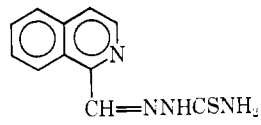
Also, it is important to note that while the L-1210 strain used in this laboratory is the same as that used at CCNSC it is apparently significantly different from the Yale strain. This adds to the complications of interlaboratory comparison of results.

Mechanism of Action.—Since we are proposing not only a provisional structured model of the mecha-

(18) This laboratory, unpublished data.
(19) Dr. Harry B. Wood, Jr., private communication, 1969.

Table III

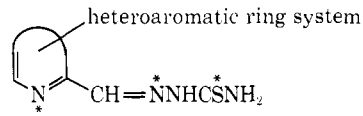
Substituted 1-Formylisoquinoline Thiosemicarbazones



Compd	Mp, °C dec	Formula	Analyses
N-Oxide[a]	234 235	$C_{11}H_{10}N_4OS$	C, H, N, S
5-COOH[b]	245–246	$C_{12}H_{10}N_4O_2S \cdot 0.25C_2H_5OH$	C, H, N, S
5-Cl	247–248	$C_{11}H_9ClN_4S$	C, H, Cl, N, S
5-CN	247–248	$C_{12}H_8N_5S$	C, H, N, S
5-F	231–232	$C_{11}H_9FN_4S$	C, H, N
5-CF_3	212–213	$C_{12}H_9F_3N_4S$	C, N; H[c]
5-*n*-C_3F_7	224–225	$C_{12}H_8F_7N_4S$	C, H, N
6-OCH_3	238–239	$C_{12}H_{12}N_4OS$	C, H, N, S
7-Cl	245–246	$C_{11}H_9ClN_4S$	C, H, Cl, N, S
7-F	239.5–240.5	$C_{11}H_9FN_4S$	C, H, N
7-OCH_3	228–229	$C_{12}H_{12}N_4OS$	C, H, N, S
7-AcO	225–226	$C_{13}H_{12}N_4O_2S$	C, H, N, S
7-OH[d]	250–251	$C_{11}H_{10}N_4OS$	C, H, N, S
8-F	232–232.5	$C_{11}H_9FN_4S$	C, H, N

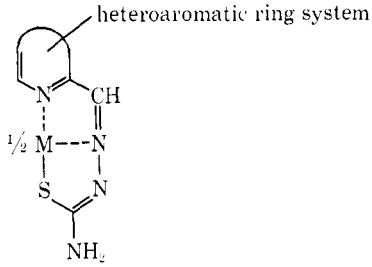
[a] Prepared from crude 1-formylisoquinoline *N*-oxide. [b] Prepared by hydrolysis of nitrone. [c] H: calcd, 3.04; found, 3.61. [d] Compound was obtained by directly treating the acid hydrolyzed solution of 7-acetoxy-1-formylisoquinoline with a solution of thiosemicarbazide followed by neutralization (NaOAc).

nism of action of this generic class of compounds but also a model of the mode of action of the target enzyme, a brief review of the experimental observations and speculations leading to these models is in order. The essential structural features of the molecule are shown in A. It was postulated by French and Freedlander[20]



A

that compounds of this generic class could act as tridentate ligands for suitable metal ions of the first transition series (B). This postulation has been verified by



B

the precise X-ray crystal structure determination of bis(isoquinoline-1-carboxaldehyde thiosemicarbazanato)nickel (II) monohydrate by Mathew and Palenik.[21] At the *in vivo* level the first evidence was the fact that 2-formylpyrazine thiosemicarbazone not only possessed antitumor activity but caused urinary and fecal excretion of Fe in mice and rats as the green ferrous complex.[22]

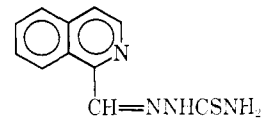
(20) F. A. French and B. L. Freedlander, *Cancer Res.*, **18**, 1290 (1958).

(21) M. Mathew and G. J. Palenik, *J. Amer. Chem. Soc.*, **91**, 6310 (1969).

(22) F. A. French, A. E. Lewis, E. J. Blanz, Jr., and A. H. Sheena, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **24**, 402 (1965).

Table IV

Antitumor Activity of Substituted 1-Formylisoquinoline Thiosemicarbazones[a]



		L-1210		L-5178Y		S-180 ascites	
No.	Substituent	Dose, mg/kg	% *T/C*	Dose, mg/kg	% *T/C*	Dose, mg/kg	% *T/C*
1	None	67	163	67	165	35	326 (50)[b]
2	*N*-Oxide	150	116	75	105	100	169
3	3-CH_3[c]	100	107	71	105	100	174
4	4-AcO[d]	400	99			200	238 (10)
5	4-OH[d]	400	115			200	182 (20)
6	5-CN	400	111	400	104	200	109 (10)
7	5-SO_3H[e,f]	400	107			400	140
8	5-SO_3NH_4	400	107			400	131 (10)
9	5-Cl	100	104	75	125	75	246 (10)
10	5-F	50	164	40	133	40	227 (20)
11	5-NO_2[f]	60	118	40	140	40	289 (30)
12	5-AcO[f]	75	136	50	125	80	254 (10)
13	5-OH[f]	200	124	200	125	200	249 (30)
14	5-CF_3	30	140	25	122	25	160
15	5-*n*-C_3F_7	30	103	30	112	20	95 (10)
16	5-COOH	60	118	30	129	50	106
17	6-OCH_3	200	111			150	100 (20)
18	7-AcO	100	110	50	119	50	198 (10)
19	7-F	200	139	150	139	100	285 (10)
20	7-Cl	400	103			200	128
21	7-OH	400	104			200	111
22	7-OCH_3	141	134	71	127	50	267 (20)
23	8-F	100	140	150	126	100	238 (10)

[a] Six to ten mice were used in each experiment. See Experimental Section for antitumor test procedures. Detailed data too numerous to be reported here will be published in Cancer Chemotherapy Reports. [b] No. in parentheses are per cent 60-day cures. [c] K. C. Agrawal and A. C. Sartorelli, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1969, MEDI-16. [d] See ref 6. [e] This compound was neutralized with $NaHCO_3$ before administration. [f] See ref 3.

Moore and Reichard[23] noted the stimulatory effect of Fe^{3+} on crude cytidine diphosphate reductase isolated from the Novikoff hepatoma. Sartorelli[24] made the important observation that **1** is a very potent inhibitor of the synthesis of DNA by sarcoma 180 ascites cells *in vivo* and has relatively little effect on RNA and protein synthesis. He also found that Fe is involved in this interaction. It has since been established in part by Moore[25] and by Sartorelli and colleagues[26–29] that ribonucleoside diphosphate reductase derived from the mammalian tumors studied has an obligatory requirement for Fe^{2+}. These observations have been independently verified at Southern Research Institute using a partially purified ribonucleoside diphosphate reductase (RDR) derived from H. Ep.-2 cells (human epidermoid carcinoma cells).[30,31] It cannot be asserted with

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(24) A. C. Sartorelli, *Biochem. Biophys. Res. Commun.*, **27**, 26 (1967).

(25) P. Reichard, "The Biosynthesis of Deoxyribose," Wiley, New York, N. Y., p 46, ref 46; E. C. Moore, private communication, 1968.

(26) A. C. Sartorelli, M. S. Zedeck, K. C. Agrawal, and E. C. Moore, *Fed. Proc., Fed Amer. Exp. Biol.*, **27**, 650 (1968).

(27) R. L. Michaud and A. C. Sartorelli, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, N-54.

(28) A. C. Sartorelli, B. A. Booth, and E. C. Moore, *Proc. Amer. Ass. Cancer Res.*, **10**, 76 (1969).

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(30) R. W. Brockman, personal communications, 1969 and 1970.

(31) R. W. Brockman, R. W. Sidwell, G. Arnett, and S. Shaddix, *Proc. Soc. Exp. Biol. Med.*, **133**, 609 (1970).

absolute certainty that inhibition of RDR is the only mechanism of antitumor activity of these compounds. Since there is a considerable amount and variety of functional and of storage iron *in vivo*, toxicity effects involving iron not in RDR can easily be envisioned. However, the available data published and to be published builds a strong case for the inhibition of RDR as the mechanism of antitumor activity. Since it has already been established that RDR's of different origins are not necessarily identical in the structure of regions adjacent to the active site, and because there are so many differences between the cell-free *in vitro* and *in vivo* systems it is not at all surprising that the structure–activity correlations are different in the *in vitro* and various *in vivo* situations.

The reductases involved in these studies are in relatively crude form. Nonheme Fe is required and is at the active site, apparently in ferrous form. The valence state of the bound Fe cannot be asserted absolutely because the nature of the binding to the enzyme protein is not known. While the potential for the normal aquated ferrous–ferric couple is -0.771 V, this potential for bound iron ions can easily vary from 0.0 to -1.0 V depending upon the nature of the attached ligands. As a consequence it is, at present, hazardous to assert that this reductase is acting as a ferrous–ferric reductant. Additionally, the reduction of a ribonucleoside to a deoxyribonucleoside involves a 2-electron transformation while the ferrous–ferric shift is a 1-electron process. To obviate the complications posed by this formulation we are proposing an alternative template model illustrated in eq 1, 3, 4, and especially eq 5. Suitable SH compounds are required and all of the riboside diphosphates leading to DNA can serve as substrates. The total system interactions involved are exceedingly complex and will not be dealt with here. We shall consider only those aspects pertinent to the formulation of a reasonable model. In the *in vitro* assay system, in addition to the crude enzyme and sundry materials, Fe^{2+} is present in a concentration of 6×10^{-5} *M* and dithioerythritol is present at 6.2×10^{-3} *M*.

Salient to the argument is the fact that the better inhibitors corresponding to A are active (50% inhibition) in the range 10^{-6} to 10^{-7} *M*.[31] Compounds not bearing the N*–N*–S* tridentate ligand such as the thiosemicarbazones of 3-formylpyridine, 4-formylpyridine, or isatin are either inactive or require concentrations on the order of 10^{-3} *M* or greater and they are not active on these tumor systems *in vivo*.

The overall reaction the reductase participates in may be written as eq 1:

$$>\text{CHOH} + 2\text{RSH} \xrightarrow{\text{enzyme } Fe^{2+} \text{ system}} >\text{CH}_2 + \text{RSSR} + \text{H}_2\text{O} \quad (1)$$

Reviewing available electrode potential data, ΔF may be estimated at approximately -12.7 kcal. Pauling bond energies give $\Delta H = -13.9$ kcal and hence $\Delta S = 4.0$. Even allowing for the uncertainties in these values and considering the nature of the net reaction, ΔS must be small. The agreement is good, the driving force is adequate, and the reaction does happen. Any compound which can efficiently block access to the active site by the ribotide, the SH compound, or both, may be an effective inhibitor.

Let us consider the dissociative mechanism shown in eq 2:

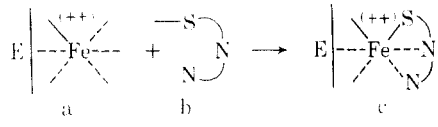
$$E{:}Fe^{2+} + 2L^- \not\longrightarrow E + FeL \quad (2)$$

In the case of **1**, which is 50% inhibitory at 1.7×10^{-7} *M*, and recalling that the free Fe^{2+} concentration is 6×10^{-5} *M*, the amount of ligand present is insufficient to complex the available free Fe^{2+} by a factor of 7×10^2. Consequently, the dissociative mechanism can be ruled out. This leaves, as the only reasonable alternative, an associative blockade of the active site as indicated in eq 3:

$$E{:}Fe^{2+} + L^- \longrightarrow E{:}Fe^{2+}{:}L^- \quad (3)$$

Since all indications are that the ligand L is acting in a tridentate manner and the Fe is hexacoordinate, only three coordination sites remain for binding the Fe to the enzyme. Some of these must be strong, sterically protected loci, or dissociation would result. If the ligand were too good a coordinating agent for free ferrous and could not adequately match the geometry of the enzymes active site and immediate adjacent regions, much higher concentrations would be required. The importance of the regions adjacent to the active site is not inconsistent with the peculiar ordering of substituent effects shown in Table IV, previous data on various ring systems,[32] and similar effects noted in the pyridine system.[7] Also, the changing order of substituent effects from one tumor system to another is suggestive of differences in regions adjacent to the active site in different isozymes.

One may write, schematically, eq 4 as an inhibition model:



(4)

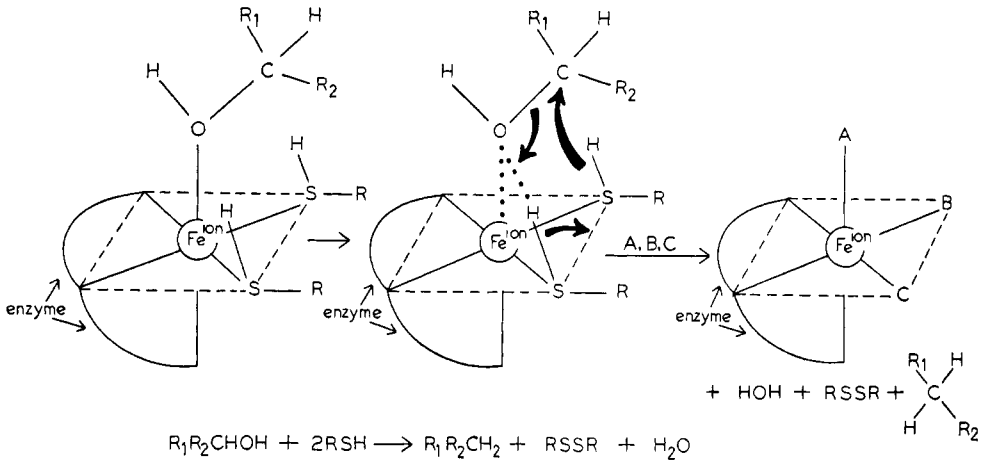
In the free enzyme **4a** there are undoubtedly ligands, even if some of these are H_2O or loose associations with the enzyme protein, occupying all octahedral positions. The inhibitor **4b**, taken from the class represented by A, would interact in the SH form and hence affect some charge neutralization in the complex **4c**. In the blockaded enzyme complex **4c** the tridentate inhibitor would occupy either an octahedral edge or face with more or less distortion. Some ligand rearrangement in the protein–Fe bonds may be required in the process of reaction with the inhibitor.

In eq 5 there is portrayed a model of how the enzyme may perform its normal reductase function. This is not unlike similar reductions occurring at metal surfaces. In this model one may envision a concerted reaction wherein the carbinol moiety and the close-coupled disulfhydryl entity approach three contiguous octahedral facial sites resulting in the hydrogenolysis of the C–O bond, formation of H_2O, and a disulfide. This model obviates any necessity of complex valence considerations. The Fe–enzyme locus may simply be regarded as a template, *i.e.*, an assembly zone.

Experimental Section

Antitumor Tests.—The mice used in these experiments were obtained from Simonsen Laboratories, Gilroy, Calif. In L-1210

(32) F. A. French and E. J. Blanz, Jr., *J. Med. Chem.*, **9**, 585 (1966).



(5)

$R_1R_2CHOH + 2RSH \longrightarrow R_1R_2CH_2 + RSSR + H_2O$

A, B, and C are ligands

$\Delta F = \sim -12.7$ K Cal (Estimated)

Pauling bond energies, $\Delta H = -13.9$ K Cal (ΔS small)

leukemia, L-5178Y lymphoma, and C-1498 myelogenous leukemia BDF_1 mice were inoculated intraperitoneally with 10^6 tumor cells. In sarcoma 180 (ascites) and the Ehrlich ascites carcinoma Swiss mice were inoculated intraperitoneally with 2×10^6 and $10–20 \times 10^6$ tumor cells, respectively. In the above tumors increase in survival time is the index of activity. In L-1210, C-1498, and L-5178Y $\%T/C$ (treated/control) ≥ 125 is considered positive; in S-180 (ascites) and the Ehrlich ascites carcinoma the $\%T/C$ must be ≥ 150. In the Lewis lung carcinoma and the B-16 melanoma BDF_1 mice were given inoculations in the groin of 0.2 ml of a suspension of approximately 40 mg of tumor tissue in normal saline. In sarcoma 180 (solid) the same procedure was followed, however Swiss mice were used. The tumors were excised and weighed (in 11 days with the Lewis lung carcinoma, 13 days with B-16 melanoma, and 7 days with S-180 solid). Inhibition of tumor weight is the index of activity. A $\%T/C \leq 30$ is considered positive. The finely ground drugs were suspended in a solution of sterile, distilled water with a drop of Tween 80. Doses were determined with preliminary toxicity tests. The drugs were given daily, intraperitoneally, at approximately the maximum tolerated doses starring 24 hr after tumor inoculation.

Chemical Procedures.—Melting points are corrected and were measured on a Thomas-Hoover capillary melting point apparatus. Microanalyses were performed by Berkeley Analytical Laboratory, Berkeley, Calif. and by Micro-Analysis, Inc., Wilmington, Del. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within $\pm 0.4\%$ of the theoretical values. Ir spectra were determined on a Perkin-Elmer Model 700 spectrophotometer.

5-Cyano-1-methylisoquinoline (VIII).—5-Amino-1-methylisoquinoline (II, 15.8 g, 0.1 mole) was suspended in 250 ml of H_2O and 20 ml of concd HCl. The stirred suspension was cooled to 0–5° and $NaNO_2$ (7 g, 0.1 mole) dissolved in 25 ml of H_2O was added dropwise to form the diazonium salt solution. This was neutralized with Na_2CO_3. PhMe (250 ml) was added to a solution of $K_2Ni(CN)_4$ (16 g, 0.062 mole) in 200 ml of H_2O. The above neutral diazonium salt was added dropwise to this stirred suspension. N_2 evolved immediately and the total addition time was 0.5 hr. After stirring for 1 hr longer the temp was slowly raised to 50°. The reaction mixture was filtered; the organic layer was sepd from the aq layer and dried ($MgSO_4$). The residue that was obtained from the evaporation of PhMe was sublimed at 150° *in vacuo* and finally crystallized from petroleum ether to give 6.0 g (36%) of product, mp 145–146°; ir (KBr) 2230 cm^{-1} (C≡N).

1-Methyl-5-trifluoromethylisoquinoline (X).[33]—5-Carboxy-1-methylisoquinoline (50 g, 0.27 mole), SF_4 (90 g, 0.83 mole), and HF (54 g, 2.7 moles) were charged into a 1.4-l. Hastelloy autoclave and heated with rocking at 150° for 16 hr. The autoclave was then cooled to ambient temp and vented. The volatile material in the autoclave was then vacuum pumped through a NaF trap to remove HF. The brown residual mixture of solids and liquid was then made basic with 10% aq NaOH and extracted with Et_2O. The Et_2O extract was evap on a warm H_2O bath with the aid of a water aspirator. The resulting brown solid residue, which appeared to be crystalline, weighed 53 g. A portion of this material (14 g) was sublimed and crystallized from petroleum ether (60–110°): yield, 10.6 g; mp 84–85°; ir (Nujol) 1200–1350 cm^{-1} (CF_3).

1-Methylperfluoro-*n*-propylisoquinoline (XII).—Perfluoro-*n*-propyl iodide (18.3 g, 0.062 mole), activated Cu bronze (16 g), and 150 ml of DMSO were heated on an oil bath at 90°. The reaction vessel was equipped with a thermometer, stirrer and a reflux condenser that was cooled to approx −60°. The temp of the oil bath was gradually increased to 120°, at which time an exothermic reaction occurred. After stirring for an additional hour, 16.2 g (0.062 mole) of 5-iodo-1-methylisoquinoline (XI) was added to the reaction mixture and heated and stirred 1 hr more. The reaction mixture was cooled and a yellow solid crystallized, which was filtered and washed with H_2O. The DMSO solution was diluted to 1 l. with H_2O and the ppt was collected and washed thoroughly with H_2O. Both solids were combined and dried over P_2O_5 in a vacuum desiccator. The solid material was sublimed and then crystallized from petroleum ether (60–110°) to yield 7.4 g of product, mp 61–62.5°.

1-Pyridiniummethyl-1-isoquinoline-5-sulfonate.—1-Methylisoquinoline-5-sulfonic acid[3] (10 g, 0.045 mole) and I_2 (11.4 g 0.045 mole) was added to 400 ml of pyridine. The dark solution was heated with stirring at reflux temp for 15 min when the product began to crystallize. The resulting mixture was stirred and heated for 45 min longer and then cooled to ambient temp. The solid material was then filtered, washed well with cold H_2O, and dried. The yield of the salt (12.5 g) was 93% mp >300°. *Anal.* ($C_{15}H_{12}N_2O_3S$) N, S; C: calcd, 59.98; found, 59.52; H: calcd, 4.03; found, 4.57.

1-Formylisoquinoline-5-sulfonic Acid Thiosemicarbazone.—1-Pyridiniummethylisoquinoline-5-sulfonate (12 g, 0.04 mole) was added to 400 ml of H_2O containing 3.2 g of NaOH. *N,N*-Dimethyl-4-nitrosoaniline (6.0 g, 0.04 mole) dissolved in 400 ml of pyridine was added to this solution. The dark red-orange solution was allowed to stand overnight at room temp. The solution was evapd *in vacuo* to dryness. The resulting orange nitrone was washed with EtOH and Et_2O and dried. The crude nitrone (10 g) was dissolved in 225 ml of H_2O containing 7.5 ml of concd HCl and 75 ml of EtOH. The deep purple solution was treated with 1 g of Norit and filtered. The purple solution was added to 700 ml of boiling H_2O containing 2.3 g of thiosemicarbazide. After a few sec the solution turned yellow orange and the yellow thiosemicarbazone pptd. The ppt was washed successively with 100 ml of boiling H_2O, 100 ml of boiling EtOH, and 100 ml of Et_2O and dried. The yield of the thiosemicarbazone

(33) This intermediate was purchased from Peninsular Chemresearch, Calgon Corp., Gainesville, Fla. We thank Dr. T. W. Brooks for providing us with the synthetic details for publication.

was 5.8 g (45% based on 1-pyridiniummethylisoquinoline-5-sulfonate), mp 314–316° dec.

General Procedure for Oxidation Reaction.—SeO_2 (1 mole-equiv) was added portionwise to a solution of the Me derivative (1 mole-equiv) in dioxane and heated slowly to reflux temp and maintained at reflux for 2 hr. The Se ppt was removed by filtration and the filtrate evapd under vacuum. The residue was extracted with dilute HCl and filtered and the filtrate made alkaline with $NaHCO_3$ to ppt the carboxaldehyde. The aldehyde was filtered off, washed with H_2O, dried over P_2O_5 in a desiccator, and crystallized from petroleum ether (bp 60–110°).

Thiosemicarbazones.—Except where noted the thiosemicarbazones (Tables III and IV) were prepared by the method of French and Blanz[32] from the corresponding aldehyde and thiosemicarbazide.

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Carcinostatic Activity of Thiosemicarbazones of Formyl Heteroaromatic Compounds. VII. 2-Formylpyridine Derivatives Bearing Additional Ring Substituents[1]

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Sixteen thiosemicarbazones of 2-formylpyridines bearing additional ring substituents were synthesized and tested for antitumor activity against 5 mouse tumor systems *in vivo* and compared with the parent unsubstituted derivative. The major tumors used were L-1210 leukemia, sarcoma 180 (ascites), L-5178Y lymphoma, C-1498 myelogenous leukemia, and the Lewis lung carcinoma. Occasional studies were also performed on sarcoma 180 (solid), B-16 melanoma, and the Ehrlich ascites carcinoma. The substituents studied were: 3-carboxy, 3-fluoro, 3-methyl, 4-methyl, 5-fluoro, 5-chloro, 5-bromo, 5-iodo, 5-methyl, 5-ethyl, 5-trifluoromethoxy, 5-trifluoromethyl, 5-dimethylamino, 5-methylsulfonyl, 5-hydroxy, and 5-acetoxy. The effect of additional substituents on activity against a particular tumor system follows no simple parametric rules. Furthermore, the order of substituent effects changes markedly from one tumor system to another. A number of the compounds studied have been found, in other laboratories, to be extremely potent inhibitors of tumor-derived ribonucleotide diphosphate reductase and hence the synthesis of DNA. The 5-hydroxy derivative is, in general, the most interesting compound studied. On certain dose–time regimens it yields a significant cure rate in L-1210 leukemia.

The original observation of the antitumor activity of 2-formylpyridine thiosemicarbazone[2] stood in isolation for several years. However, preliminary theorizations on possible modes of action were formulated.[3] In 1963 a concerted attack on the overall problem was initiated. This led, in the pyridine series, to the discovery that 3-hydroxy-2-formylpyridine thiosemicarbazone and especially 5-hydroxy-2-formylpyridine thiosemicarbazone displayed markedly superior activity.[4–7] This was not the result of an increase in gravimetric potency *per se* but was essentially due to a large improvement in therapeutic index and hence the practical attainability of much higher and protracted dose levels.

In the companion paper on related isoquinoline derivatives the question of mechanism of action is dealt with in detail.[8] In this paper attention is focused on the pyridine derivatives.

It gradually became apparent, during the course of this investigation, that the pyridine derivatives possessed a strong advantage over the isoquinoline compounds due to the simple fact that, in general, they are more water soluble and readily absorbed *in vivo*. In the pyridine group only 2 out of 17 compounds studied yielded drug deposits in mice. In contrast, 17 out of 23 compounds in the isoquinoline series gave rise to this problem.

Chemistry.—The substituted 2-formylpyridine thiosemicarbazones were prepared from the appropriate substituted 2-picolines (Scheme I,[9] Tables I–VI). Most of the 3- and 5-halogenated 2-picolines (Ia–e) utilized in this study are known compounds and were prepared according to or with slight modification of published procedures.[10,11] 2-Methyl-5-trifluoromethylpyridine (If) was prepared by heating 6-methylnicotinic acid with SF_4 and HF at 120°. 2-Methyl-5-trifluoromethoxypyridine (Ig) was prepared essentially by the method of Sheppard.[12] 3-Hydroxy-6-methylpyridine and COF_2 were allowed to react at 100–150° for 4 hr followed by reaction with SF_4 and anhydrous HF to form the desired product.

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(1) (a) This investigation was supported by Grant CA-03287 from the National Cancer Institute; (b) presented in part at the 169th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract MEDI-76.

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