Potential Antitumor Agents. I. A Series of 5-Substituted 1-Formylisoquinoline Thiosemicarbazones[1]

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A series of 5-substituted derivatives of 1-formylisoquinoline thiosemicarbazone (XI), a potent antineoplastic agent, has been synthesized to determine the effect of various substituents on tumor-inhibitory potency and host toxicity. 1-Methylisoquinoline was substituted on the 5 position with either NO_2, NH_2, $NHCOCH_3$, OH, $OCOCH_3$, or SO_3H; the methyl group was subsequently oxidized to the corresponding carboxaldehyde with selenium dioxide and these carboxaldehydes were treated with thiosemicarbazide, except in the case of the sulfonic acid derivative. 1-Formylisoquinoline-5-sulfonic acid thiosemicarbazone was obtained by direct sulfonation of XI. The antineoplastic activity and the host toxicity of these compounds were assessed in mice bearing Sarcoma 180; the results indicated that substitution of an additional group at the 5 position reduced carcinostatic activity in the case of the NO_2, $NHCOCH_3$, and SO_3H derivatives, whereas the insertion of an NH_2, OH, or $OCOCH_3$ group resulted in derivatives possessing tumor-inhibitory activity comparable to the parent compound. Furthermore, the substitution in the isoquinoline ring system of OH and $OCOCH_3$ yielded compounds that were considerably less toxic than XI to the host.

Several heterocyclic aldehyde thiosemicarbazones have been shown to be potent inhibitors of the growth of transplanted rodent neoplasms. 2-Formylpyridine thiosemicarbazone was first reported by Brockman, *et al.*,[2] to possess antileukemic activity in mice. More recently, French and Blanz[3] extended these observations by testing a series of formyl heteroaromatic thiosemicarbazones; several of these derivatives, especially 1-formylisoquinoline thiosemicarbazone[4] (XI) and 2-formyl-3-hydroxypyridine thiosemicarbazone,[5] showed significant antineoplastic activity when tested against a wide spectrum of transplanted tumors. A conjugate N*-N*-S* tridentate ligand system was found to be a common feature of compounds with carcinostatic potency. Employing structure–activity relationships it was postulated that (a) the π-electron density at the point of attachment of the aldehyde moiety should be low and (b) the ring nitrogen should be a reasonably good donor to the transition metals for formation of octahedral coordination compounds (chelates). It was also deemed necessary that the carbonyl attachment be in a position α to the heteroaromatic nitrogen atom.[3]

The biochemical basis for the growth-inhibitory activity of XI in neoplastic cells has been studied;[6] this agent caused marked inhibition of the synthesis of DNA. Blockade of the formation of RNA and protein was also produced by XI; however, these processes were considerably less sensitive to drug-induced inhibition. Since these heterocyclic aldehyde thiosemicarbazones constitute in essence a new class of compounds with potent antineoplastic properties, it was of interest to evaluate the effects of various structural modifications on biological activity, particularly seeking those changes that result in an increase in the water solubility of these extremely insoluble compounds.

The present investigation reports (a) the synthesis of a series of 5-substituted 1-formylisoquinoline thiosemicarbazones and (b) the antineoplastic potency and host toxicity of these compounds in mice bearing Sarcoma 180 ascites cells.

Chemistry.—1-Methylisoquinoline (I) was nitrated (Scheme I) to the corresponding 5-nitro derivative. Elderfield, *et al.*,[7] have shown that nitration of 3-methylisoquinoline occurs predominantly at the 5 position; smaller amounts of the 8-substituted derivative, however, are also formed. Nitration of 1-methylisoquinoline resulted in only one isomer (II), the structure of which was shown by oxidation with SeO_2 to 5-nitroisoquinoline-1-carboxaldehyde (III), which was further oxidized by sodium dichromate to 5-nitroisoquinoline-1-carboxylic acid; heating this acid resulted in decarboxylation to a known compound, 5-nitroisoquinoline (IV).

The conversion of 1-methyl-5-nitroisoquinoline to the desired amino compound (VI) was followed by acetylation to 5-acetamido-1-methylisoquinoline (VII). Oxidation of VII with SeO_2 furnished the corresponding carboxaldehyde (VIII). The preferential reduction of the nitroaldehyde (III) to the corresponding aminoaldehyde was attempted employing a number of relatively mild reducing agents, but these procedures uniformly resulted in poor yields. 5-Aminoisoquinoline-1-carboxaldehyde (XVIII), however, was readily obtained by acid hydrolysis of VIII.

Sulfonation of 1-methylisoquinoline, which occurred at the 5 position, was proved in the following manner. The alkali fusion of 1-methylisoquinoline-5-sulfonic acid (XIII) yielded the corresponding hydroxy compound (XIV). This was converted to VI by a Bucherer reaction. The product of this reaction was identical with a sample of VI synthesized by the reduction of II as described earlier. Acetylation of XIV gave 5-acetoxy-1-methylisoquinoline (XV) which was oxidized with SeO_2 to the carboxaldehyde XVI. Compound XVI on acid hydrolysis gave 5-hydroxyisoquinoline-1-carboxaldehyde (XX). This was also obtained in poor yield by direct oxidation of XIV with SeO_2. Oxidation of the methyl group of XIII to the corre-

(1) This work was supported by Grant T-23J from the American Cancer Society and Grant CA-02817 from the National Cancer Institute, U. S. Public Health Service.

(2) R. W. Brockman, J. R. Thomson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, **16**, 167 (1956).

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

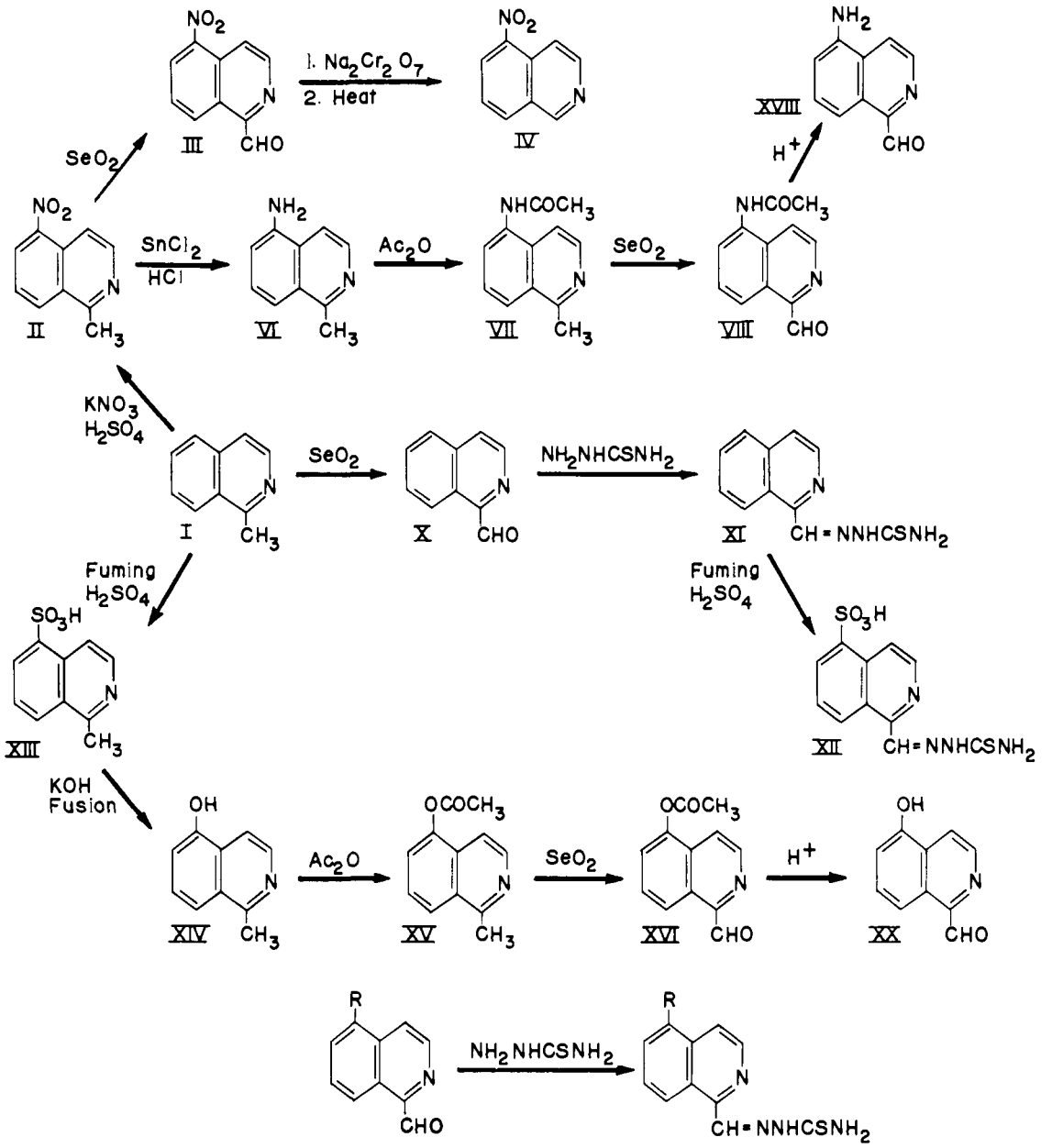
(4) F. A. French and E. J. Blanz, Jr., *Cancer Res.*, **25**, 1454 (1965).

(5) F. A. French and E. J. Blanz, Jr., *ibid.*, **26**, 1638 (1966).

(6) (a) A. C. Sartorelli, *Biochem. Biophys. Res. Commun.*, **27**, 26 (1967); (b) A. C. Sartorelli, *Pharmacologist*, **9**, 192 (1967).

(7) R. C. Elderfield, J. M. Lagowski, O. L. McCurdy, and S. L. Wytle, *J. Org. Chem.*, **23**, 435 (1958).

SCHEME I



sponding carboxaldehyde could not be effected directly; however, 1-formylisoquinoline-5-sulfonic acid thiosemicarbazone (XII) was conveniently obtained by direct sulfonation of XI.

Biological Results and Discussion

The effects of 1-formylisoquinoline thiosemicarbazone (XI) and its 5-substituted derivatives on the survival time of Sarcoma 180 tumor-bearing mice are shown in Table I. Compound XI caused a pronounced lengthening of the life span of tumor-bearing mice over a relatively wide range of doses (5–60 mg/kg/day); at maximum effective levels (30–60 mg/kg/day), 30–44% of treated animals survived at least 50 days. Substitution of an additional group at the 5 position of the parent molecule (XI) markedly altered the tumor-inhibitory potency and host toxicity of the compound. Thus, substitution of the electron-withdrawing nitro group not only resulted in reduced antineoplastic activity, but also appeared to increase the toxicity of the compound to the host. The latter parameter was reflected by a lowering of the survival time of tumor-bearing mice receiving daily dose levels of V greater than 10 mg/kg. Similarly, substitution of a sulfonic acid group (XII), which resulted in a water-soluble compound, decreased both antineoplastic potency and host toxicity. The findings, which indicate that the substitution of these electron-withdrawing groups at the 5 position results in a decrease in tumor-inhibitory potency, do not appear to be consistent with the postulation of French and Blanz[3] that a low π-electron density at the point of attachment of the aldehyde moiety is required for carcinostatic activity, since the substitution of electron-withdrawing groups would be expected to decrease the π-electron density at the 1 position.

TABLE I

EFFECT OF 5-SUBSTITUTED 1-FORMYLISOQUINOLINE THIOSEMICARBAZONES ON THE SURVIVAL TIME OF MICE BEARING SARCOMA 180 ASCITES CELLS

Compd	Daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival, days ± SE
Control	None	+17.3	12.5 ± 0.3
1-Formylisoquinoline thiosemicarbazone (XI)	5	+5.9	23.4 ± 3.3 (10)
	10	+1.4	25.9 ± 1.9
	20	−2.3	32.5 ± 3.0 (10)
	30	−3.5	39.9 ± 2.7 (30)
	40	−10.8	39.7 ± 3.3 (44)
	60	−12.4	32.9 ± 2.5 (13)
	80	−15.8	27.4 ± 4.9
	100	−19.9	19.4 ± 5.5
1-Formyl-5-nitroisoquinoline thiosemicarbazone (V)	5	+19.3	11.4 ± 0.4
	10	+8.2	21.7 ± 3.6 (10)
	20	−5.4	16.0 ± 1.9
	40	+5.5	15.7 ± 3.0
	80	−8.0	3.2 ± 0.2
1-Formylisoquinoline-5-sulfonic acid thiosemicarbazone (XII)	10	+16.4	15.4 ± 0.7
	20	+19.2	13.9 ± 0.6
	40	+17.1	15.9 ± 0.9
	60	+19.2	16.6 ± 0.7
	80	+12.7	17.0 ± 0.8
	120	−4.4	11.6 ± 1.4
	160	−14.4	11.8 ± 0.7
1-Formyl-5-hydroxyisoquinoline thiosemicarbazone (XXI)	10	+6.8	18.2 ± 1.2
	20	+6.1	27.2 ± 2.5
	40	+1.6	22.6 ± 1.4
	80	+5.8	30.1 ± 2.8 (10)
	120	+10.4	35.4 ± 3.1 (20)
	160	+12.1	31.6 ± 3.2 (10)
	200	+11.5	33.0 ± 3.2
1-Formyl-5-aminoisoquinoline thiosemicarbazone (XIX)	10	−5.4	27.2 ± 5.7 (20)
	20	−5.5	36.0 ± 3.5 (30)
	40	−8.5	37.3 ± 3.2 (30)
	60	−22.0	18.4 ± 7.5
	80	−21.7	27.0 ± 5.7
	120	−20.9	4.2 ± 0.2
1-Formyl-5-acetoxyisoquinoline thiosemicarbazone (XVII)	10	+11.5	20.5 ± 3.4 (10)
	20	+0.6	23.8 ± 3.7 (10)
	40	+1.2	34.1 ± 4.7 (40)
	80	−4.2	35.9 ± 3.6 (30)
	120	−14.5	30.4 ± 3.6 (10)
	160	−19.5	23.8 ± 8.3 (20)

[a] Administered once daily for 6 consecutive days, beginning 24 hr after tumor implantation; each value represents the results obtained with 5–15 animals. [b] Average weight change from onset to termination of drug treatment. [c] The number in parentheses indicates the per cent of the tumor-bearing animals that survived at least 50 days; these mice were calculated as 50-day survivors in determination of the average survival time.

Furthermore, the insertion in position 5 of XI of an electron-donating group such as NH_2 or OH resulted in derivatives capable of prolonging the life span of tumor-bearing animals to the same extent as that produced by the parent compound. A similar result was obtained with the 5-acetoxy derivative (XVII), which also possessed antitumor activity comparable to the parent compound. Substitution of an acetamido moiety (IX) resulted in a compound completely devoid of carcinostatic activity.

The toxicity of these compounds was estimated in tumor-bearing animals by measuring the drug-induced loss in body weight. A comparison of the toxicity of three of the most active compounds, XI, XVII, and XXI, indicated that the latter two 5-substituted derivatives are on a molar basis much less toxic than the parent compound. A daily dose of 100 mg/kg of XI caused about 20% loss in the body weight of treated animals. To accomplish a similar degree of toxicity (*e.g.*, a 20% loss in body weight) required a dose of 160 mg/kg/day of XVII. In contrast to these two agents, animals receiving injections of compound XXI at dosage levels up to 200 mg/kg/day did not experience any drug toxicity as expressed by weight loss. Compound XIX, an extremely effective antineoplastic agent, appeared to be more toxic than XI, a 22% loss in body weight occurring with a daily dose of 60 mg/kg.

Experimental Section

Melting points were determined using a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental analyses[8] were performed by the Schwarzkopf Microanalytical Laboratory, New York.

Biological Methods.—Transplantation of Sarcoma 180 ascites cells was carried out by withdrawing peritoneal fluid from a donor CD-1 mouse bearing a 7-day growth. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, a 15-fold dilution with isotonic saline was made, and 0.1 ml (approximately 4×10^6 cells) of the resulting cell suspension was injected intraperitoneally into each animal.

Drugs were administered by intraperitoneal injection, beginning 24 hr after tumor implantation, once daily for 6 consecutive days. Thiosemicarbazones were injected as fine suspensions following homogenization in absolute EtOH (adjusted so that the final concentration of the drug solution was 5% with respect to EtOH) and 2–3 drops of 20% aqueous Tween 80 and then made up to volume with isotonic saline, except for XII, which was solubilized in isotonic saline with NaOH and adjusted to pH 8 with HCl. All drugs were administered in volumes of 0.25–0.5 ml. For any one experiment, animals were distributed into groups of five to ten mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water *ad libitum*. Experiments with more than five mice were performed at least twice. Controls given injections of comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiment, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

Determination of the sensitivity of ascitic neoplasms to these agents was based both on the prolongation of survival time afforded by drug treatments and on the number of these animals surviving 50 days.

Chemical Methods.—The compounds which were synthesized by utilizing standard procedures are listed in Table II.

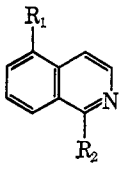
General Procedure for Oxidation Reactions.—To a solution of 0.01 mole of the corresponding methyl derivative in 100 ml of dioxane, a suspension of freshly sublimed SeO_2 (0.01 mole) in 25 ml of dioxane was added slowly and refluxed for 2 hr. The precipitate of Se was removed by filtration and the filtrate was evaporated under vacuum. The residue was extracted with dilute HCl and filtered, and the filtrate was made alkaline with $NaHCO_3$ to precipitate the carboxaldehyde derivative.

1-Methylisoquinoline-5-sulfonic Acid (XIII).—1-Methylisoquinoline (2.86 g, 0.02 mole) was added dropwise to 25 g of fuming (30%) H_2SO_4 cooled to 0°. The solution was stirred for 2 hr at 45–50° and then was allowed to attain room temperature. The reaction mixture was maintained at room temperature for 18 hr with occasional stirring and then decomposed in 100 g of chopped ice; the solution was adjusted to pH 4 with 20% NaOH and cooled. The resulting precipitate of the sulfonic acid derivative was collected by filtration, washed (H_2O, EtOH), and dried to give 3.1 g (70%). *Anal.* ($C_{10}H_9NO_3S$) C, H, N, S.

1-Methyl-5-hydroxyisoquinoline (XIV).—Compound XIII (4.46 g, 0.02 mole), KOH (1.5 g), and 6 ml of H_2O were mixed; 16.5 g of KOH was added and the mixture was heated at 280–290° for 15 min. The mixture was stirred vigorously; during the fusion the color of the mixture became dark brown and frothing occurred. The reaction mixture was then cooled, dissolved in 100

(8) Where analyses are indicated only by symbols of the elements or functions, the analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

TABLE II



Compd	R_1	R_2	Recrystn solvent	Yield, %	Mp, °C	Formula	Analyses
II[a]	NO_2	CH_3	EtOH	70	150–151	$C_{10}H_8N_2O_2$	N
III[b]	NO_2	CHO	50% EtOH	50	175–176	$C_{10}H_6N_2O_3$	
VI[a]	NH_2	CH_3	C_6H_6	92	213–214	$C_{10}H_{10}N_2$	N
VII[a]	$NHCOCH_3$	CH_3	Dioxane	90	226–227	$C_{12}H_{12}N_2O$	C, H, N
VIII[b]	$NHCOCH_3$	CHO	C_6H_6	75	206–207	$C_{12}H_{10}N_2O_2$	C, H, N
XV[a]	$OCOCH_3$	CH_3	Hexane	94	99–100	$C_{12}H_{11}NO_2$	C, H, N
XVI[b]	$OCOCH_3$	CHO	Hexane	70	100–101	$C_{12}H_9NO_3$	C, H, N

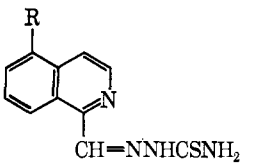
[a] Compounds were synthesized utilizing standard procedures and reactants as described in the Chemistry section. [b] The general procedure used for the oxidation reactions is given in the Experimental Section.

ml of H_2O, and filtered, and the filtrate was made acidic with 10% HCl. The undissolved material was removed by filtration using Celite and the filtrate was made alkaline with $NaHCO_3$. The precipitate of XIV was filtered, washed (H_2O), and dried to give a light brown product; yield 2.4 g (75%). Crystallization from EtOH after treatment with Norit A gave white crystals, which collapsed at 268–270° and finally melted at 295–300° dec. *Anal.* ($C_{10}H_9NO$) N.

1-Formylisoquinoline-5-sulfonic Acid Thiosemicarbazone (XII). —Compound XI[4] (1.15 g, 0.005 mole) was mixed slowly in small portions with 20 g of fuming (30%) H_2SO_4 at 0°. Addition of the compound required 15 min to ensure the maintenance of temperature at 0°. The solution was stirred for 2 hr and then kept at room temperature for 18 hr. The mixture was then decomposed in 100 g of ice flakes; the red precipitate was filtered, washed (cold H_2O), and dissolved in a 5% solution of $NaHCO_3$. The resulting yellow solution was filtered to remove insoluble material and the filtrate was made acidic (dilute AcOH). The resulting precipitate was collected by filtration, washed (H_2O, EtOH), and then dried to give 1.2 g (78%). *Anal.* ($C_{11}H_{10}N_4O_3S_2$) C, H, N, S.

Thiosemicarbazones.—The thiosemicarbazones V, IX, XVII, and XXI were prepared by treating alcoholic solutions of the corresponding aldehydes with an aqueous solution of thiosemicarbazide acidified with a few drops of dilute AcOH. Relevant data concerning these compounds are listed in Table III. Compound XIX was best obtained by directly treating the acid-hydrolyzed solution of VIII with a solution of thiosemicarbazide followed by neutralization (NaOAc).

TABLE III



Compd	R	Mp, °C dec	Formula	Analyses
V	NO_2	238–240	$C_{11}H_9N_5O_2S$	C, H, N, S
IX	$NHCOCH_3$	230–232	$C_{13}H_{13}N_5OS \cdot H_2O$	H, N, S; C[a]
XVII	$OCOCH_3$	200–201	$C_{13}H_{12}N_4O_2S$	C, H, N, S
XIX	NH_2	223–225	$C_{11}H_{11}N_5S$	C, H, N, S
XXI	OH	224–226	$C_{11}H_{10}N_4OS$	C, H, N, S

[a] C: calcd, 51.15; found, 51.73.

Studies on Condensed Pyrimidine Systems. XXIII. Synthesis of 2,4-Diaminopyrido[2,3-*d*]pyrimidines from β-Keto Esters[1]

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The condensation of 2,4,6-triaminopyrimidine with β-keto esters gave 5-mono- and 5,6-disubstituted 2,4-diamino-7,8-dihydro-7-oxopyrido[2,3-*d*]pyrimidines (**III**) which were chlorinated by means of thionyl chloride and N,N-dimethylformamide to give 7-chloro-2,4-diaminopyrido[2,3-*d*]pyrimidines (V). Both the 7-oxo and 7-chloro derivatives were thiated to give 2,4-diaminopyrido[2,3-*d*]pyrimidine-7-thiones (IV). Dethiation of the 7-thiones gave 2,4-diaminopyrido[2,3-*d*]pyrimidines having substituents in the 5 or 5,6 positions.

The study[2-6] in this laboratory of pyrimidine and condensed pyrimidine systems as inhibitors of dihydrofolate reductase led to an investigation of 2,4-diaminopyrido[2,3-*d*]pyrimidines. In 1958, Robins and Hitchings[2] reported the synthesis of a number of pyrido[2,3-*d*]pyrimidines. The 2,4-diamino compounds (Ia) have been found to be inhibitors of dihydrofolate reductase. However, only pyrido[2,3-*d*]pyrimidines having alkyl or aryl substituents in the 7 position were prepared. 5,6-Disubstituted derivatives (II) were expected to exhibit greater enzyme binding because they more closely resemble the natural substrate in configuration.

The key intermediates, 5,6-disubstituted 2,4-diamino-7,8-dihydro-7-oxopyrido[2,3-*d*]pyrimidines (III), were prepared by heating a mixture of a β-keto ester and 2,4,6-triaminopyrimidine to a temperature above 200° either alone or in an inert solvent such as diphenyl

(1) The previous paper in this series was by G. B. Elion, *J. Org. Chem.*, **27**, 2478 (1962).

(2) R. K. Robins and G. H. Hitchings, *J. Am. Chem. Soc.*, **80**, 3449 (1958).

(3) R. K. Robins and G. H. Hitchings, *ibid.*, **77**, 2256 (1955).

(4) G. H. Hitchings and K. W. Ledig, U. S. Patent 2,937,284 (1960).

(5) G. H. Hitchings, T. A. Herrmann, B. S. Hurlbert, and S. R. M. Bushby, Proceedings of the IIIrd International Congress of Chemotherapy, Stuttgart, 1963, p 1363.

(6) G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 417 (1965).