# α-(N)-Formylheteroaromatic Thiosemicarbazones. Inhibition of Tumor-Derived Ribonucleoside Diphosphate Reductase and Correlation with *in Vivo* Antitumor Activity†

Frederic A. French,* Erwin J. Blanz, Jr.,

*Chemotherapy Research Laboratory, Mount Zion Hospital and Medical Center, San Francisco, California 94115*

Sue C. Shaddix, and R. Wallace Brockman

*Kettering-Meyer Laboratories, Southern Research Institute, Birmingham, Alabama 35205.* *Received August 8, 1973*

An internally consistent set of evaluations was made of inhibition constants of a large number of α-(*N*)-formylheteroaromatic thiosemicarbazones against the enzyme ribonucleoside diphosphate reductase (RDR) of human tumor origin (H.Ep.-2 cells) *in vitro* and as inhibitors of the growth of three mouse neoplasms, L1210 leukemia, sarcoma 180 ascites, and the Lewis lung carcinoma *in vivo*. The *in vivo* doses tabulated are those which gave maximum antitumor effect without toxic signs. Of 27 compounds which were highly active inhibitors of RDR ($-\log C_{50} \geq 6.0$) 25 were active against one or more mouse tumors *in vivo*. Of 51 compounds of intermediate activity as inhibitors of RDR [$6.0 > (-\log C) > 4.0$] 33 were active *in vivo* against one or more experimental neoplasms. Of compounds of low inhibitory activity against RDR ($-\log C < 4.0$) only 2 out of 19 showed *in vivo* activity. These data affirm the general utility of enzyme studies as one of the guides in designing these drugs. *In vitro* activities of thiosemicarbazones and their relation to *in vivo* antitumor results and to toxicity data are discussed. Physicochemical parameters, structure–activity relationships, and enzyme interaction models are explored. The outlook is optimistic for the development of compounds more useful *in vivo* and as potential candidates as anticancer agents.

This line of antitumor drug investigation was launched by the discovery by Brockman, *et al.*,[1] that 2-formylpyridine thiosemicarbazone (Table I, 1), but not its 3 and 4 isomers, was active on L1210, L82T, and L4946 leukemias in mice. The L1210 results were verified by French and Freedlander,[2] who postulated that compounds which combined the N*–N*–S* pattern of 1 could act as tridentate chelators of divalent metals of the first transition series. Qualitative observations by French, *et al.*,[3-5] showed that the most likely physiological metal involved was Fe(II) and that octahedral coordination of ferrous by two ligands was a function of pH and structure. Much later Mathew and Palenik[6] showed, by precision X-ray diffraction studies, the octahedral formulation to be correct for bis(isoquinoline-1-carboxaldehyde thiosemicarbazanoto)nickel(II) monohydrate. They found the two ligands tridentately bound in two orthogonal planes. Ferrous was not used because corresponding chelates of this class of ligands yield compounds vulnerable to oxidation and decomposition under unprotected aerobic conditions.

We launched a concerted attack in 1963, involving broad synthetic and *in vivo* testing studies, which immediately resulted in more active, less toxic compounds with broader antitumor spectra.[7-13] Also, a very interesting series of papers was generated by the Yale group.[14-22]

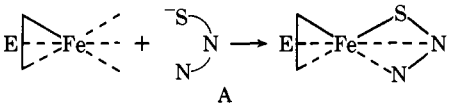
It was apparent, once significantly improved antitumor activities were found, that the early structural postulations were validated and that a very large number of potentially active derivatives could be synthesized. Insight into mechanism(s) of action was not only highly desirable *per se* but could have utility in drug design. In a series of investigations Sartorelli[23-25] showed that DNA synthesis was strongly inhibited in L1210 cells and especially S180A cells in mice while RNA and protein synthesis were affected much less by 1-formylisoquinoline thiosemicarbazone ("IQ-1," **62**). Since Moore and Reichard[26] noted the stimulatory effect of Fe(III) on ribonucleoside diphosphate reductase (RDR) from Novikoff hepatoma, this tended to focus attention on interference with this enzyme. Moore developed methods of isolation and partial purification of mammalian RDR.[27] The RDR system is responsible for the conversion (CDP, UDP, ADP, GDP) → (dCDP, dUDP, dADP, dGDP), respectively, and hence ultimately to DNA.

The basic work on RDR, especially the highly purified system from *Escherichia coli* B which is Fe dependent, is described by Reichard.[28] This pure enzyme consists of two subunits, $B_1$ and $B_2$. These work in conjunction with the small protein thioredoxin $(SH)_2$ (mol wt 12,000) to convert the 2′-CHOH group in the ribosyl portion of the substrates stereospecifically to 2′-$CH_2$ in the deoxyribosyl moiety of the products. Thioredoxin (SS) is formed; the amino acids at the active thioredoxin site are


in the oxidized form. The evidence, then[28] and later,[29] indicates two irons in the $B_2$ protein and that these are required for enzyme activity. The spectral peaks at 410 and 360 mm disappear on Fe removal and reappear on Fe reactivation. Herman and Moore[30] purified rat Novikoff heptoma thioredoxin and found similarities to the *E. coli* form. Hopper[31] found with 200-fold purified rabbit bone marrow RDR that two subunits are present, paralleling the behavior noted in the *E. coli* enzyme. The complex allosteric nucleotide regulatory effects are similar for H.Ep.-2 cells used in this study, in the Novikoff rat tumor,[32] and in *E. coli*.[28] There is, however, a marked difference in inhibitor response. The bacterial enzyme is unresponsive to the bulky tridentate ligands under discussion[33] and it is much more difficult to remove Fe from the bacterial enzyme, which is inhibited by the small bidentate ligand hydroxyurea.[28,34]

Before considering the bulk of the *in vitro* data in Tables I–III it is necessary to discuss detailed studies of a few of these inhibitors, some of the fundamental differences between the *in vitro* and *in vivo* test systems, the nature of studies of these ligands in simple chemical systems, and some fundamentals of ligand chemistry. French, *et al.*,[11] first proposed an addendal ligand blocking model shown in structure A. This was based on adaptation of a model proposed in 1952 by Martell and Calvin[35] and amplified in the reviews of Ulmer and Vallee[36]

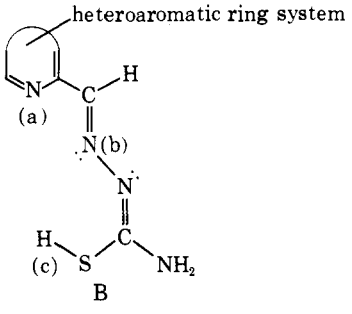
†The investigation at Mount Zion Hospital was supported by Grants CA03287 and RR05472 from the National Institutes of Health and the work at Southern Research Institute by Contract PH43-66-29, Division of Cancer Treatment, NCI, NIH. This material was presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, Abstract MEDI-19.



A

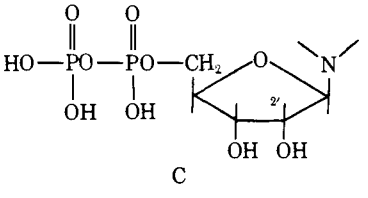
and especially Vallee's studies on the effects of addendal ligands on the spectral degeneracy of pure metallo enzymes.[37] Moore, *et al.*,[38] also offer the suggestion that the Fe chelates could be the inhibitor. We feel that only sophisticated studies with pure enzymes can absolutely settle these points. Using octahedral scale models of Fe chelates of drugs in this series, it is difficult to reconcile the octahedral complex hypothesis with the observed activity differences from one compound to another. In a series of papers Sartorelli and colleagues[39-41] present much detailed evidence on the *in vitro* interaction of 1, 16, 27, 61, and some other compounds with components of the enzyme reaction with RDR and with other enzymes. These studies show that there are important and subtle differences between members of this series. Perhaps most interesting is the finding by Sartorelli and Booth[42] that S180A resistant to IQ-1 (**61**) is cross resistant to 5-hydroxy-2-formylpyridine thiosemicarbazone ("5-HP," **27**) but is susceptible to 3-hydroxy-2-formylpyridine thiosemicarbazone ("3-HP," **16**).

Let us consider briefly a slightly more detailed model of RDR as a metallo- or metal-dependent enzyme and some basic coordination chemistry before discussing the tabular data in detail. Ferrous ion in aqueous solution is coordinated to six $H_2O$ molecules with an energy of about 60 kcal per coordinate bond. From Palenik's data[6] and current stereo nomenclature rules,[43] the most probable combining form of the ligand is essentially inner conjugated and *E* form relative to the aldimine bond (structure B) and the *Z* form about the outer double bond. Two such




B

ligands combine with Fe(II) to produce an octahedral complex with the two tridentate ligands surrounding the metal to produce a neutral complex.[5] Then a and a′, c and c′ are cis and b and b′ are trans for the two coordinating molecules. There is a tendency for solvent molecules to occupy the space between the heteroaromatic rings. In the enzyme the Fe is probably at or very near a surface which is in a complexly contoured region to meet the very specific structural requirements for its enzymatic function. All substrates have in common the configuration shown in structure C. For the enzyme to function the



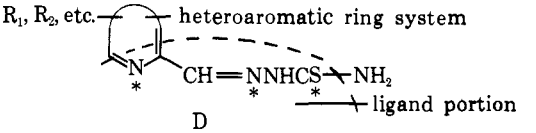
C

phosphates must be directly or indirectly (*via* $Mg^{2+}$, for example) bound to its surface so that the 2′-OH, only, is positioned to interact with the template Fe. In the action sequence, a suitable disulfhydryl donor must come in close proximity to the Fe, donating an H in place of the leaving OH of the ribosyl group. The Fe, to stay put reasonably well, must be attached firmly to three ligating atoms from protein residues (such as imidazole, glutamate, etc.). At such a protein "surface" the Fe is in a completely different environment from that in free solution or singly ligated to a single candidate (tridentate) drug molecule. The other covalent positions may be, *a priori*, aquated or weakly ligated to other protein side chains or suitable components in the environment.

At pH 7 1 reacts with free $Fe(II)\cdot(H_2O)_6$ to form a quite stable complex (2:1). Compound **15** forms a 2:1 complex with difficulty and this dissociates rapidly on modest dilution. Yet **15** is active both *in vitro* and *in vivo*. Compounds **79**, **81**, and especially **80** form, in increasing order, extremely stable Fe(II) complexes in neutral $H_2O$. Models indicate strong stabilizing H-bonding forces. The *in vitro* test medium contains $6 \times 10^{-5}$ *M* Fe(II). For a ligand with a high $K_1K_2$ for bisoctahedral complex formation relative to $K_3$ for interaction with the enzyme, this concentration of Fe(II) could tie up as much as a concentration of $1.2 \times 10^{-4}$ *M* of the drug, leaving at most a very low concentration of free drug. On the basis of the blocking addendal ligand hypothesis the observed ($-\log C$) values are substantiative. *In vivo*, the concentration of free ferrous is generally orders of magnitude below that used in the *in vitro* test system. Compounds **79**–**81** are active *in vivo* and this constitutes further support and tends to rule out the hypothesis that it is the Fe(II) (2:1) complex of the test drug that is active.

*In Vitro* **Data and** *in Vivo* **Comparisons.** The *in vitro* 50% inhibition levels in Tables I–III were determined from a number of points, as described in the Experimental Section, for partially purified RDR derived from H.Ep.-2 cells (human epidermoid carcinoma). The results are expressed as ($-\log C_{50}$), the conventional thermodynamic form used in correlative work.[44-46] At a glance, using ($-\log C$) and $\Delta(-\log C)$ (R–H), one can compare intrinsic maximal potencies of these compounds by this mechanism of action and note the magnitude of simple and complex substituent effects. Since in all reported studies only partially purified mammalian tumor enzymes were used, the absolute magnitudes of ($-\log C_{50}$) are open to question. However, due to the care with which these values were obtained, they have a high degree of internal consistency as a set.

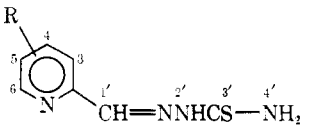
It can be seen from structure D that the direct liganding structure is only a portion of these molecules, especially those bearing large or complex substituents. Hence ($-\log C$) is a summation of this interaction and interactions with portions of the enzyme less proximal to the active Fe site.




D

First we can look for correspondence and deviations from additivity using the values for 1 and the alkyl group Δ's (11–15) to estimate ($-\log C$) for the benzopyridines. The comparisons (found − calculated) are quinoline-2 (**88**), −0.01; isoquinoline-1 (**62**), −0.14; isoquinoline-3 (**63**), −0.24, showing a close approximation to additivity on a space-filling basis. Analogously, for ring systems involving additional heteroatoms, one computes Δ = −0.53 for replacement of CH=CH in quinoline-2 (**88**) by S giving benzothiazole-2 (**96**). Comparing the cinnoline-3 derivative **89** with isoquinoline-3 (**63**), Δ = −1.12 for introduction of the second ring (1,2-diazine) N. Comparing quinoxaline-2 (**90**) and quinoline-2 (**88**), the second (1,4-di-

**Table I.** *In Vitro* and *in Vivo* Activity of 2-Formylpyridine Thiosemicarbazone Derivatives and Related Compounds



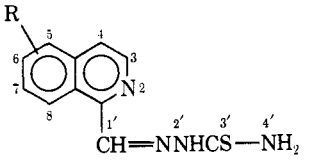
2-Formylpyridine TSC, $C_{50} = 2.8 \times 10^{-7}$

| | | *In vitro* activity[a] | | *In vivo* activity[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | L1210 | | S180 ascites | | Lewis lung Ca | |
| No. | R | −Log *C* | Δ(−log *C*) (R–H) | Dose, mg/kg | % T/C[c] | Dose, mg/kg | % T/C[d] | Dose, mg/kg | % T/C |
| 1* | None (pyridine-2)[z] | 6.55 | | 8 | 139* | 6 | 97 | 10[f] | 47 |
| 2 | Pyridine-3[e,g] | 2.70 | −3.85 | 106 | 104 | 75 | 131 (10) | 75 | 98 |
| 3 | Pyridine-4[e,g] | 3.30 | −3.25 | 200 | 99 | 200 | 85 | 200 | 104 |
| 4 | 1′-$CH_3$[e,h] | 5.82 | −0.73 | 5 | 110 | 5 | 101 | 5 | 79 |
| 5 | 2′-$CH_3$ | 2.28 | −4.27 | 150 | 100 | 100 | 105 | 150 | 82 |
| 6 | 4′-$CH_3$[e] | 5.52 | −1.03 | 150 | 111 | 50 | 139 | 100 | 106 |
| 7 | 4′-*n*-$C_4H_9$ | 5.66 | −0.89 | 50 | 99 | 10 | 136 (10) | 50 | 59 |
| 8 | 4′-$C_6H_5$[e] | 4.96 | −1.59 | 100 | 104 | 20 | 108 | 75 | 94 |
| 9 | 4′-(2-Pyridyl) | 5.34 | −1.21 | 100 | 107 | 40 | 109 | 50 | 74 |
| 10* | 4′,4′-$(CH_3)_2$ | 5.74 | −0.81 | 10 | 131* | 10 | 133 | 10 | 14* |
| 11* | 3-$CH_3$[i] | 6.59 | +0.04 | 12 | 125* | 8 | 119 | 8 | 58 |
| 12* | 4-$CH_3$[i] | 6.57 | +0.02 | 20 | 125* | 20 | 100 | 15 | 63 |
| 13* | 5-$CH_3$[i] | 6.51 | −0.04 | 50 | 146* | 25 | 128 | 35 | 64 |
| 14* | 5-$C_2H_5$[i] | 6.66 | +0.11 | 20 | 160* | 5 | 134 | 10 | 20* |
| 15* | 6-$CH_3$[e] | 5.11 | −1.44 | 150 | 125* | 150 | 141 | 150 | 30* |
| 16* | 3-OH[e] | 5.19 | −1.36 | 100 | 184* | 71 | 278 (30)* | 71 | 10* |
| 17* | 3-$OCH_3$ | 5.89 | −0.66 | 15 | 135* | 5 | 147 (10) | 10 | 60 |
| 18* | 3-$OC_2H_5$[e,j] | 6.04 estd | −0.51 estd | 100 | 141* | 75 | 174* | 75 | 42 |
| 19* | 3-$OOCCH_3$[e,k] | 5.44 estd | −1.11 estd | 50 | 164* | 50 | 266 (30)* | 50 | 16* |
| 20* | 3-F[i] | 5.42 | −1.13 | 25 | 140* | 10 | 147 | 25 | 13* |
| 21 | 3-COOH[i] | | | 200 | 104 | 100 | 93 | 100 | 63 |
| 22* | 5-F[i] | 5.92 | −0.63 | 10 | 139* | 10 | 170 (10)* | 10 | 17* |
| 23* | 5-Cl[i] | 6.25 | −0.30 | 10 | 139* | 5 | 122 | 15 | 7* |
| 24* | 5-Br[i] | 6.30 | −0.25 | 25 | 122 | 20 | 191* | 20 | 52 |
| 25 | 5-I[i] | 6.39 | −0.16 | 60 | 114 | 30 | 113 (10) | 30 | 83 |
| 26* | 5-$CF_3$[i] | 5.62 | −0.93 | 30 | 158* | 10 | 149 | 25 | 9* |
| 27* | 5-OH[i] | 5.17 | −1.38 | 141 | 268* | 100 | 309 (50)* | 141 | 19* |
| 28* | 5-$OCH_3$ | 5.92 | −0.63 | 250 | 213* | 100 | 269 (10) | 200 | 43 |
| 29 | 5-$OCF_3$[i] | 5.60 | −0.95 | 20 | 124 | 10 | 142 | 15 | 71 |
| 30* | 5-$OC_2H_5$[l] | 6.07 estd | −0.48 estd | 100 | 149* | 50 | 144 | 100 | 44 |
| 31 | 5-$CH_3SO_2$[i] | | | 141 | 104 | 71 | 134 | 71 | 97 |
| 32* | 5-$OC_2H_4N(CH_3)_2$ | 4.62 | −1.93 | 50 | 128* | 50 | 112 | 50 | 62 |
| 33* | 5-$O(C_2H_4O)_2C_2H_5$ | 5.69 estd | −0.86 estd | 100 | 145* | 25 | 154* | 100 | 42 |
| 34* | 5-$OOCCH_3$[i] | 5.44 | −1.11 | 238 | 152* | 50 | 209* | 150 | 4* |
| 35* | 5-$OOCC_2H_5$ | 5.28 | −1.27 | 150 | 151* | 50 | 217* | 100 | 19* |
| 36* | 5-*n*-$OOCC_3H_7$ | 5.17 | −1.38 | 150 | 167* | 50 | 158* | 100 | 18* |
| 37* | 5-*n*-$OOCC_{15}H_{31}$ | 3.96 | −2.59 | 200 | 127* | 175 | 196* | 100 | 114 |
| 38* | 5-$OOCC(CH_3)_3$ | | | 100 | 178* | 100 | 320 (20)* | 150 | 17* |
| 39* | 5-$OOCCH_2OCH_3$ | 5.30 | −1.25 | 150 | 153* | 50 | 197* | 150 | 13* |
| 40* | 5-$OOCCH_2OC_2H_5$ | 5.25 | −1.30 | 200 | 158* | 50 | 238 (10)* | 200 | 10* |
| 41* | 5-$OOCCH_2N(CH_3)_2$ | 5.24 | −1.31 | 50 | 168* | 50 | 199 (40)* | 100 | 25* |
| 42* | 5-$OOCCH_2OC_6H_5$ | 4.89 | −1.66 | 100 | 213* | 25 | 300 (10)* | 150 | 27* |
| 43* | 5-$OOCC_6H_5$ | | | 200 | 150* | 100 | 239 (20)* | 150 | 83 |
| 44* | 5-$OOCCH_2OC_6H_4$-*o*-Cl | | | 100 | 173* | 100 | 267 (10)* | 150 | 49 |
| 45* | 5-$OOCCH_2OC_6H_4$-*p*-Cl | | | 150 | 201* | 50 | 283 (10)* | 150 | 35 |
| 46* | 5-$OOCCH_2OC_6H_3Cl_2$ (2″,4″) | | | 150 | 152* | 150 | 328* | 200 | 25* |
| 47* | 5-$OOCCH_2OC_6H_2Cl_3$ (2″,4″,5″) | | | 150 | 153* | 25 | 232 (20)* | 200 | 28* |
| 48 | 5-[(2′,3′,4′,6′-Tetraacetyl)-1-β-D-glucopyranosyl] | | | 300 | 109 | 200 | 103 (10) | 200 | 58 |
| 49* | 5-$NHCOCH_3$ | 5.92 | −0.63 | 75 | 143* | 50 | 191* | 100 | 39 |
| 50* | 5-$N(CH_3)_2$[i] | 6.40 | −0.15 | 50 | 179* | 30 | 247* | 35 | 23* |
| 51 | 3-OH, 4-$CH_3$ | <4.00 | >−2.55 | 400 | 122 | 150 | 108 | | |
| 52 | 3-OH, 6-$CH_3$ | 3.73 estd | −2.82 estd | 400 | 122 | 200 | 98 | 400 | 74 |
| 53* | 3-Cl, 5-OH | 5.20 | −1.35 | 200 | 144* | 150 | 260 (30)* | 100 | 106 |
| 54 | 3-OH, 3′-$CH_3$ | 3.72 | −2.83 | 200 | 123 | | | 200 | 41 |
| 55 | 3-OH, 3′,4′-$(CH_3)_2$ | 4.10 | −2.45 | 150 | 107 | 150 | 93 | 150 | 90 |
| 56* | 5-OH, 4′-*n*-$C_4H_9$ | 4.18 | −2.37 | 200 | 104 | 50 | 174* | 100 | 102 |
| 57* | 5-OH, 4′-$C_6H_5$ | 4.24 | −2.31 | 400 | 135* | 100 | 160* | 200 | 45 |
| 58* | 5-OH, 4′-(2-pyridyl) | 4.23 | −2.32 | 50 | 101 | 50 | 173* | 50 | 71 |
| 59 | 3-OH, $N^1$-methylpyridinium iodide[i] | | | 200 | 101 | | | 100 | 87 |
| 60* | 5-$OCH_2C_6H_5$, 4-OH | | | 400 | 100 | 400 | 163 (10)* | 400 | 83 |
| 61 | 3,5-$(OC_2H_5)_2$ | 5.68 | −0.87 | 400 | 108 | 75 | 104 | 400 | 69 |

*Footnotes to Table I*

[a]Compound concentrations yielding 50% inhibition of RDR from H.Ep.-2 cells, expressed in thermodynamic form. A compound is designated "inactive" against RDR if the value of $-\log C$ is 4 or below (molar concentration = $1 \times 10^{-4}$). [b]The drugs were given ip daily, at approximately maximum tolerated doses, starting 24 hr after tumor inoculation; six to ten mice were used in each experiment. [c]% T/C = treated/control × 100. Criteria for activity: L1210, % T/C ≧ 125, S180 ascites ≧ 150, LLCa ≦ 30. As asterisk indicates significant activity. [d]Numbers in parentheses are % 60-day survivors. These mice are not included in the calculation of mean survival time. [e]See ref 9. Pyridine-3, etc., designates the 3-formylpyridine derivative. [f]Drug given every other day. [g]These isomers are included for comparative purposes. [h]More toxic than parent compound. [i]See ref 16. [j]Included to compare with **17**. [k]This compound hydrolyzes rapidly to **16** in $H_2O$. [l]Included to compare with **28**.

**Table II.** *In Vitro* and *in Vivo* Activity of 1-Formylisoquinoline Thiosemicarbazone Derivatives and Related Compounds




1-Formylisoquinoline TSC; $C_{50} = 1.7 \times 10^{-7}$

| | | *In vitro* activity[a] | | *In vivo* activity[b] L1210 | | S180 ascites | | Lewis lung Ca | |
|---|---|---|---|---|---|---|---|---|---|
| No. | R | $-\log C$ | $\Delta(-\log C)$ (R–H) | Dose, mg/kg | % T/C[c] | Dose, mg/kg | % T/C[d] | Dose, mg/kg | % T/C |
| **62*** | None[e] | 6.77 | | 67 | 163* | 35 | 326 (50)* | 67 | 20* |
| **63*** | Isoquinoline-3[f,g] | 6.59 | −0.18 | 100 | 108 | 75 | 248 (20)* | 100 | 72 |
| **64*** | 4-OH (Na salt)[h] | 4.70 | −2.07 | 44[i] | 140* | 200[i] | 182 (20)* | 200[i] | 78 |
| **65*** | 5-OH[k] | 6.64 | −0.13 | 200 | 124 | 100 | 360 (20)* | 100 | 88 |
| **66*** | 5-F[l] | 6.46 | −0.31 | 50 | 164* | 40 | 227 (20)* | 40 | 22* |
| **67*** | 5-Cl[l] | 5.77 | −1.00 | 100 | 104 | 75 | 246 (10)* | 75 | 35 |
| **68*** | 5-$CF_3$[l] | 5.40 | −1.37 | 30 | 140* | 25 | 160* | 35 | 40 |
| **69*** | 5-$NO_2$[k] | 5.75 | −1.02 | 60 | 126* | 40 | 289 (30)* | 60 | 74 |
| **70** | 5-CN[l] | 5.66 | −1.11 | 400 | 111 | 200 | 109 (10) | 200 | 81 |
| **71** | 5-COOH[l] | 5.00 | −1.77 | 60 | 118 | 50 | 106 | 40 | 121 |
| **72** | 5-$SO_3H$[k] | <5.00 | >−1.77[m] | 400 | 107 | 400 | 140 | 400 | 134 |
| **73** | 6-$OCH_3$[l] | 6.68 | −0.09 | 200 | 111 | 150 | 100 (20) | 100 | 65 |
| **74*** | 7-OH[l] | 6.72 | −0.05 | 400 | 104 | 50 | 233* | 100 | 66 |
| **75*** | 7-$OCH_3$[l] | 6.02 | −0.75 | 141 | 134* | 50 | 267 (20)* | 60 | 61 |
| **76*** | 7-F[l] | 6.75 | 0.02 | 200 | 139* | 100 | 285 (10)* | 150 | 31 |
| **77*** | 8-F[l] | 6.16 | −0.61 | 100 | 140* | 100 | 238 (10)* | 150 | 63 |
| **78*** | 2-Oxide[l] | 5.80 | −0.97 | 150 | 116 | 100 | 169* | 100 | 95 |

[a]See footnote *a*, Table I. [b]See footnote *b*, Table I. [c]See footnote *c*, Table I. [d]See footnote *d*, Table I. [e]See ref 7. [f]See ref 9. [g]Isomer for comparison with **62**. [h]See ref 16. [i]Dose given twice daily. [j]Free base. [k]See ref 14. [l]See ref 11. [m]No activity at $C = 1.0 \times 10^{-4}$.

**Table III.** *In Vitro* and *in Vivo* Activity of Formylthiosemicarbazones of Other Mono- and Bicyclic Ring Systems

| | | *In vitro* activity[a] | | *In vivo* activity[b] L1210 | | S180 ascites | | Lewis lung Ca | |
|---|---|---|---|---|---|---|---|---|---|
| No. | R | $-\log C$ | $\Delta(-\log C)$ (R–H) | Dose, mg/kg | % T/C[c] | Dose, mg/kg | % T/C[d] | Dose, mg/kg | % T/C |
| **79*** | Pyrimidine-4 | 5.00 | | 50 | 121 | 35 | 166* | 50 | 81 |
| **80*** | Pyrazine-2[e] | <4.00[f] | | 75 | 151* | 75 | 127 | 75 | 29* |
| **81*** | Pyridazine-3[g] | 4.30 | | 75 | 139* | 40 | 150* | 75 | 6* |
| **82*** | 6-$CH_3$-pyridazine-3 | 5.36 | +1.06 | 50 | 141* | 25 | 123 | 50 | 39 |
| **83** | *v*-Triazole-4[e] | <4.00[f] | | 200 | 99 | | | 200 | 67 |
| **84** | Isothiazole-5[h] | <4.00[f] | | 150 | 99 | 50 | 107 | 75 | 93 |
| **85** | *p*-Anisaldehyde[i] | <4.00[f] | | 100 | 102 | 100 | 87 | 100 | 100 |
| **86** | Salicylaldehyde[i] | <4.00[f] | | 800 | 107 | 800 | 78 | 400 | 104 |
| **87** | 1-Methylisatin-3[j] | <5.00[f] | | 75 | 92 | 50 | 79 | 100 | 77 |
| **88** | Quinoline-2[e] | 5.36 | | 100 | 91 | 25 | 108 | 150 | 53 |
| **89** | Cinnoline-3[e] | 5.47 | | 100 | 101 | | | 100 | 87 |
| **90** | Quinoxaline-2[e] | 4.48 | | 100 | 101 | 100 | 92 | 100 | 79 |
| **91** | 3-OH-quinoxaline-2[e] | 5.40 | +0.92 | 200 | 95 | 100 | 121 | 100 | 114 |
| **92** | 3-$OCH_3$-quinoxaline-2[e] | 4.80 | +0.32 | 250 | 109 | | | 100 | 90 |
| **93*** | Quinazoline-4[e] | 6.50 | | 40 | 162* | 20 | 89 (20) | 21 | 9* |
| **94** | 2-OH-quinazoline-4[e] | 5.70 | −0.80 | 200 | 100 | 150 | 86 | 150 | 69 |
| **95** | 4-OH-quinazoline-2[e] | <3.30 | | 200 | 100 | 200 | 89 | 200 | 73 |
| **96** | Benzothiazole-2[e] | 4.85 | | 25 | 103 | 25 | 97 (10) | 25 | 70 |
| **97*** | Purine-6[e] | 6.50 | | 35 | 135* | | | 30 | 55 |

[a]See footnote *a*, Table I. [b]See footnote *b*, Table I. [c]See footnote *c*, Table I. [d]See footnote *d*, Table I. [e]See ref 9. [f]No activity detected at lowest concentration studied. [g]M. Kumagai, *Nippon Kagaku Zasshi*, **81**, 492 (1960). [h]M. P. L. Caton, D. H. Jones, R. Slack, and K. R. H. Woolridge, *J. Chem. Soc.*, 446 (1964). P. Grammaticakis, *Bull. Soc. Chim. Fr.*, 504 (1950). [i]A. Hantsch, *Chem. Ber.*, **54**, 1244 (1944).

azine) N gives $\Delta = -0.88$. However, converting isoquinoline-1 (**62**) to quinazoline-4, the second N decrement (1,3-diazine) is only −0.27. Reversing the calculation back to monocyclics one obtains $\Delta = -0.03$ for the 6-methylpyridazine-3 derivative **82**. Models indicate the 6-methyl group in this case tends to block the hydration effects which give rise to *in vitro* anomalies. This is borne out by the anomalously low (found − calculated) Δ's for pyridazine-3 (**81**), −1.13; pyrimidine-4 (**79**), −1.28; and especially pyrazine-2 (**80**) where (−log *C*) calcd = 5.77 and Δ is >−1.77. The $\Delta = -1.44$ for the 6-methylpyridine-2 derivative **15** is thought to be a steric effect due to interference with the ring N chelation and correlates well with the value for the similarly hindered quinoline-2 derivative **88**. Another instance of apparent additivity is noted for the 3,5-diethoxypyridine-2 derivative **61** where Δ (found − calculated) = +0.12, computed from the monomethyl ethers (**17** and **28**) and the methylene increments (**14**–**13**).

A large number of 5-O-substituted pyridines, notably esters, were synthesized because this is the only stable aromatic position, especially for acyloxy substituents, in this class of compounds where no steric proximity problems are found. These are frequent in the corresponding 3-derivatives. Looking at O attachments in the 3 and 5 positions on pyridine, one notes a decrement of about −0.65 for the methyl ethers and this increases to −1.11 for 5-acetoxy (**34**), probably due to dipolar and π resonance effects in the esters. An added methylene decrement is −0.16 (**35**), −0.11 (**36**), and then averages −0.10 per added $CH_2$ group clear to palmitoxy (**37**). Introduction of phenoxy (**42**) yields an additional $\Delta = -0.55$ relative to **34**. In the acetyl esters, however, introduction of $-OCH_3$ (**39**), $-OC_2H_5$ (**40**), or $-N(CH_3)_2$ (**50**) yields an average decrement of only −0.28. In the ethers, the polyether side chain (**33**) has only a small effect but a dimethylamino group (**22**) yields −1.45 relative to **30**. Halogen substituents yield about the effect one would expect except for the large difference (−0.50) between the 3- and 5-fluoro derivatives **20** and **22**.

The only two N-linked derivatives presented, the 5-acetylamino (**49**) and especially dimethylamino (**50**), give quite small decrements indicating the desirability of ring N linkage for more complex drugs. The *in vitro* and *in vivo* activity of the 5-acetylamino derivative **49** is in sharp contrast to the finding of Agrawal, *et al.*,[14] who stated (for the isoquinoline series), "Substitution of a 5-acetamido moiety resulted in a compound completely devoid of carcinostatic activity."

The hydroxy derivatives require special consideration. *In vivo* in mice, they have high therapeutic indices and are highly active on a variety of tumors. *In vitro*, the (−log *C*) values of the 3- and 5-hydroxy compounds **16** and **27** are unexpectedly low, $\Delta = -1.36$ and −1.38, respectively. However, their solubility–pH characteristics are very favorable. Spectroscopic–pH variation studies of 3-hydroxypyridine show that 50% is in zwitterion form.[47-50] Also, Metzler and Snell[51] found that pyridoxal is 92% zwitterion at physiological pH, leaving only 8% in the phenolic form. One would expect similar coupling of OH and aldimine groups in **16** and **25**, probably through π-electron effects. This, along with the generally adverse effect of ring O substitution, easily accounts qualitatively for the observed values. The anomalously high value for **52** (found − calculated = +0.31) can be explained by the effect of halogenation increasing enolization in hydroxy heterocycles.[52-54] The very low value for **51** (found − calculated = −1.21) is easily explained using precision models. The gross cascading interaction of 4-methyl → 3-hydroxy → 2-aldimine yields configurations unfavorable to metal coordination.

In the isoquinoline series (Table II) the 5- and 7-hydroxy derivatives **65** and **74** are virtually purely phenolic and this is in accord with the favorable (−log *C*) values. In the case of the 4-hydroxy derivative **64** the zwitterion form is even more strongly favored than in the case of the two hydroxypyridines.[48,50] This correlates with the low (−log *C*) value. By inspection it can be noted that the effect of substituents in the benzo ring is quite position dependent and very different from what is observed in the pyridine ring. The two reversals, **66** *vs.* **67** and **73** *vs.* **74**, are especially noteworthy. For other cyclic systems (Table III) the tabular data are clear but the data are sparse for detailed interpretation.

In the pyridine series the simple side-chain studies *per se* are not encouraging in the RDR mechanistic context. There are, however, three potentially important lessons here. The mono-4′-substituted derivatives **6**–**9** show an average decrement of −1.18 and are inactive *in vivo*. However, when a 5-hydroxy is added to **7**–**9** generating **56**–**58** with an even greater average decrement of −2.33 (relative to 1), modest but significant *in vivo* activity appears. In **5** the 2′-methyl substituent abolishes *in vitro* and *in vivo* activity of any significance. A 2′ substituent renders impossible the overall conjugation effect, illustrated in structure B and indicated in the X-ray diffraction work.[6] To amplify this fact is the increase in *in vitro* activity and the appearance of *in vivo* activity in the 4,4′-dimethyl derivative **10**. This compound can only fully act as a tridentate ligand as indicated by structure B.

Sartorelli and colleagues have published $ID_{50}$ values for some 30+ compounds using RDR partially purified from the Novikoff rat tumor. Their values for compounds 1, 11–13, **15**, **62**, **63**, and **88**,[41] **16** and **27**,[40] and **65**[18] may be compared with those in Tables I–III. With the exception of **62**, the average deviation in (−log $C_{50}$) of Sartorelli's work is +0.14 greater than our findings. This is fortuitously good agreement. Using Δ(−log $C_{50}$) (R–H), and comparing the two sets of data, certain different trends appear which may be real, consistent, and indicative of a valid isozyme difference. Thus our Δ values for **12**, **13**, and **15** are +0.02, −0.04, and −1.44 while theirs are +0.41, +0.41, and −0.97, respectively. This is consistent with their value of (−log *C*) = 5.60 *vs.* the value 5.36 in Table III for **88** (quinoline-2). More striking is their larger value for **62** (isoquinoline-1), 7.40 *vs.* our value of 6.77, but this is in accord with the same trend noted above.

*In Vivo* **Activity.** The *in vivo* data given in Tables I–III are representative maximum antineoplastic effects at doses of drug that do not result in toxicity to the host. The drug dose is based on evaluation of any toxicity in both tumor-bearing and nontumorous mice.

In the pyridine series 42 out of 61 compounds are active *in vivo* on at least one tumor. Of the acyloxy derivatives **19** and **34**–**47**, 11 compounds are significantly active on all three tumors and 4 give a significant cure rate on S180 ascites. Aside from the acyloxy derivatives only four compounds (**16**, **22**, **27**, and **50**) are active on all three tumors. Of the nonacyloxy derivatives 24 compounds are active on L1210 and only 10 of these are active on S180A; 6 compounds that are not active on S180A are active on the Lewis lung carcinoma (LLCa). Similarly, four compounds (**24**, **56**, **58**, and **60**) active on S180A are inactive on L1210.

The relative antitumor spectra are quite different for the isoquinolines. Out of 17 compunds 13 are active on S180A and of these 8 produced a quite significant cure rate. Also 10 of the compounds active on S180A are active on L1210 but only 2 are active on LLCa. No compounds not active on S180A were active on the other tumors.

Concerning the compounds in Table III, the monocyclic diazines may be a promising point of departure. The in-

trinsic activities of **93** and **97** are good and they are active *in vivo*. The quinazolines pose formidable design and synthetic problems. In the purines the nephrotoxicity of **97** is a problem.

There have been a few publications concerning compounds with complex substituents on pyridine or isoquinoline that indicate both potentialities and problems. Sartorelli, *et al.*,[19] found that of six 5- or 3-*m*-(hydroxy, acetylamino, and amino)benzyloxy-2-formylpyridine thiosemicarbazones only one, the 3-*m*-(aminobenzyloxy) derivative was highly active on S180A *in vivo* (twice daily). Some remarkable chemical problems were encountered in this study. In another study,[18] largely involving 5-NHCO, $NHSO_2$, $OCO_2$, and OSO linked $C_6H_4SO_2F$ groups on the 5 position of 1-formylisoquinoline thiosemicarbazone, all had ($-\log C_{50}$) values in the range 6–8 but were inactive. However, the $OCOOC_2H_5$ and $OCOOC_6H_5$ derivatives were quite active *in vitro* and *in vivo*. In a third study[55] it was found that 4- and 5-(*m*-aminophenyl)-2-formylpyridine thiosemicarbazone, but not the other isomers, are significantly active *in vitro* and *in vivo* (S180A). In the same study, in concordance with our pyridine data, the 5-amino- and 5-methylaminoisoquinoline-1 derivatives were found to be highly active *in vitro* and *in vivo*. One of their findings, which is most significant from a design point of view,[22] is that replacement of the 4′-amino group by morpholine (4′-diethyleneoxy) in the side chain yields a reduction in anti-RDR activity with a large increase in inhibition of RNA synthesis. Two of these compounds, the 4-hydroxyisoquinoline-1 and the 5-hydroxypyridine-2 derivatives, were active *in vivo* (S180A). This represents essentially a change in mechanism of action.

Burchenal and Dowling[56] found that 5-HP (**27**) potentiates asparaginase in several experimental tumor systems *in vivo*. Grindey, *et al.*,[57] noted that IQ-1 (**62**) and 1-β-D-arabinofuranosylcytosine are synergistic against L1210 in DAA/2Ha-DD mice but in irradiated mice this synergism disappears. Laster and Brockman[58] found that deferoxamine mesylate enhances the activity of 5-HP against L1210 *in vivo*, yielding a high percentage of cures. Brockman, *et al.*,[59] noted that several of these compounds, especially 1, **27**, **65**, and **97**, are active against some DNA viruses.

It has only been possible in this study to discuss *in vivo* activities on a standard screening regimen using single daily doses. Skipper, *et al.*,[60] have discussed the results of numerous dose schedules for a number of drugs including the RDR inhibitors 5-HP, hydroxyurea, and guanazole. Some of these regimens give notable cure rates on L1210 leukemia with 5-HP and also with a number of other types of tumor inhibitors.

Etcubanas, *et al.*,[61] studied one member of this series, 5-HP, in man. Unlike the results in rodents, gastrointestinal toxicity severely limited dosage levels. DeConti and colleagues[62] also studied this compound clinically. They found that conjugation (glucuronidation) was rapid and plasma half-life was short. They conclude that studies on other members of this class of drugs are warranted but it would appear that hydroxy derivatives will not be candidates at the human level.

In conclusion, the data most clearly indicate certain design trends to avoid. They also indicate that, aside from attention to the usual considerations such as solubility, polar–nonpolar balance, metabolizability, etc., the outlook for developing more useful drugs is good. There is a large field of addendal complex substituents and multiple substituents that has scarcely been touched. It is clear that highly ionic or readily metabolizable groups, for example phenolic OH, are to be avoided. Suitable complex ring substituents that are C-linked or N-linked and can yield ancillary drug–enzyme interactions appear the most promising. The optimum geometries can only be found experimentally at this time because the exact structure of the enzyme is unknown.

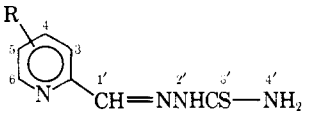
**Chemistry.** All of the thiosemicarbazones and related hydrazones in this study were prepared by standard methods (Tables I–IV). The carbonyl hydrazones in Table IV are not reported in the literature as are many of the heterocyclic aldehydes and all of the thiosemicarbazides. Many of the unreported aldehydes were not reported in Table V because they were used directly to prepare the thiosemicarbazones (Table IV) and were not isolated and purified first.

It has been reported that substituted 2-picoline *N*-oxides rearrange on refluxing with $Ac_2O$ to give substituted 2-acetoxymethylpyridines. Repetition of this reaction with substituted 2-acetoxymethylpyridine *N*-oxides gives substituted picolinaldehyde diacetates in moderate yields.[12,63] We prepared 5-methoxy-2-formylpyridine diacetate and 5-ethoxy-2-formylpyridine diacetate by this method from the corresponding alkoxypicoline *N*-oxides. The diacetates were then hydrolyzed and allowed to react with thiosemicarbazide to form **28** and **30**, respectively. Other 5-alkoxy-2-formylpyridines were prepared by allowing the Na salt of 5-hydroxy-2-formylpyridine to react with the appropriate alkyl halide in DMSO at 70–80°. β-Dimethylaminoethyl chloride, 1-(2-bromoethoxy)-2-ethoxyethane, and α-tetraacetylglucose 1-bromide were allowed to react in this manner to form the corresponding pyridyl ethers. These aldehydes were then treated with thiosemicarbazide to form **32**, **33**, and **48**. 3-Hydroxy-2-formylpyridine diethyl acetal and 3-hydroxy-2-formylpyridine thiosemicarbazone hydrochloride have been prepared by Heinert and Martell.[64] We prepared 3-methoxy-2-formylpyridine by the oxidation of 3-methoxy-2-hydroxymethylpyridine with $Pb(OAc)_4$ in $CHCl_3$.

The syntheses of the 5-acyloxy-2-formylpyridines were accomplished by general procedures. The aldehydes utilized for the synthesis of **35** and **36** were prepared directly by the reaction of boiling propionic anhydride and *n*-butyric anhydride on 5-hydroxy-2-formylpyridine. The aldehydes were used directly in crude form. A better procedure was utilized to prepare the aldehydes for **37–42** and **44–47**. An aqueous solution of the Na salt of 5-hydroxy-2-formylpyridine was treated with $CHCl_3$ solution of the appropriate acid chloride or acid anhydride at 0–5°. To obtain the aldehyde for **43** the Na salt of 5-hydroxy-2-formylpyridine was treated with benzoyl chloride in DMF at room temperature. The aldehydes **33a–47a** (Table V) were isolated in pure form and used to prepare the thiosemicarbazones. The *N,N*-dimethylglycinate ester of 5-hydroxy-2-formylpyridine thiosemicarbazone was prepared from aldehyde **41a** by allowing the 5-chloroacetoxy-2-formylpyridine thiosemicarbazone to react with dimethylamine (see Experimental Section).

The preparation of 5-acetoxy-2-formylpyridine thiosemicarbazone has been reported previously.[12] We were interested in the possibility of finding a general acylation procedure that could be utilized in directly preparing both 3- and 5-acyloxy-2-formylpyridine thiosemicarbazones from 3- and 5-hydroxy-2-formylpyridine thiosemicarbazones. As a model study these hydroxyaldehyde thiosemicarbazones were dissolved in aqueous NaOH at 0–5° and acetic anhydride was added dropwise. 5-Acetoxy-2-formylpyridine thiosemicarbazone and **19** precipitate almost immediately in excellent yield. Compound **19** hydrolyzes very readily and earlier attempts at its preparation failed. Unfortunately, most of the acyloxyaldehyde thiosemicarbazones had already been prepared by the other procedures and this method was not utilized to its maximum.

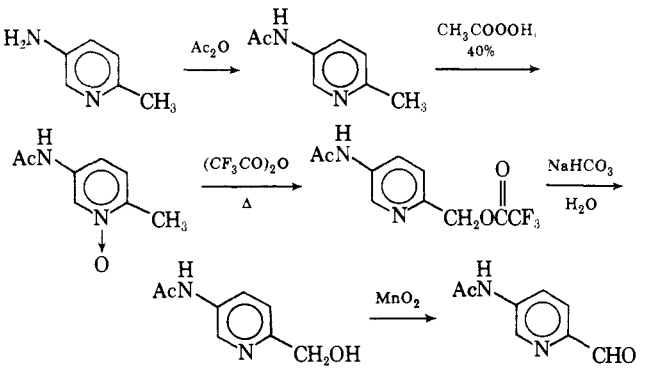
**Table IV.** 2-Formylpyridine Thiosemicarbazone Derivatives and Related Compounds



| No. | R | Mp, °C | Crystn solvent | Formula | Analyses |
|---|---|---|---|---|---|
| **5** | 2′-$CH_3$[a] | 174–175 | EtOH | $C_8H_{10}N_4S$ | C, H, N, S |
| **7** | 4′-*n*-$C_4H_9$[b] | 145–146 | MeOH | $C_{11}H_{16}N_4S$ | C, H, N, S |
| **9** | 4′-(2-Pyridyl)[c] | 193–194 | EtOH | $C_{12}H_{11}N_5S$ | C, H, N, S |
| **10** | 4′,4′-$(CH_3)_2$[a] | 121–122 | EtOH | $C_9H_{12}N_4S$ | H, S; C,[d] N[d] |
| **17** | 3-$OCH_3$ | 197.5–198 | | $C_8H_{10}N_4OS$ | C, H, N, S |
| **19** | 3-$OOCCH_3$ | 196–197 | | $C_9H_{10}N_4O_2S$ | C, H, N, S |
| **28** | 5-$OCH_3$ | 234.5–235 dec | | $C_8H_{10}N_4OS$ | C, H, N, S |
| **30** | 5-$OC_2H_5$ | 222–223 dec | | $C_9H_{12}N_4OS$ | C, H, N, S |
| **32** | 5-$OC_2H_4N(CH_3)_2$ | 196–197 | EtOH | $C_{11}H_{17}N_5OS$ | C, H, N, S |
| **33** | 5-$O(C_2H_4O)_2C_2H_5$ | 135–136 | EtOH | $C_{13}H_{20}N_4O_3S$ | C, H, N, S |
| **35** | 5-$OOCC_2H_5$ | 196–197 | EtOH | $C_{10}H_{12}N_4O_2S$ | C, H, N, S |
| **36** | 5-*n*-$OOCC_3H_7$ | 194–195.5 | EtOH | $C_{11}H_{14}N_4O_2S$ | C, H, N, S |
| **37** | 5-*n*-$OOCC_{15}H_{31}$ | 146–148 | EtOH | $C_{23}H_{38}N_4O_2S$ | C, H, N |
| **38** | 5-$OOCC(CH_3)_3$ | 197–198 | EtOH | $C_{12}H_{16}N_4O_2S$ | C, H, N, S |
| **39** | 5-$OOCCH_2OCH_3$ | 170–171 | EtOH | $C_{10}H_{12}N_4O_3S$ | C, H, N, S |
| **40** | 5-$OOCCH_2OC_2H_5$ | 184.5–185.5 | EtOH | $C_{11}H_{14}N_4O_3S$ | C, H, N, S |
| **41** | 5-$OOCCH_2N(CH_3)_2$ | 231.5–232.5 dec | EtOH | $C_{11}H_{15}N_5O_2S \cdot HCl$ | C, H, Cl, N, S |
| **42** | 5-$OOCCH_2OC_6H_5$ | 198.5–199.5 | EtOH | $C_{15}H_{14}N_4O_3S$ | C, H, N, S |
| **43** | 5-$OOCC_6H_5$ | 226–227 | EtOH, $H_2O$ | $C_{14}H_{12}N_4O_2S$ | C, H, N, S |
| **44** | 5-$OOCCH_2OC_6H_4Cl$(o) | 189–190 | EtOH | $C_{15}H_{13}ClN_4O_3S$ | C, H, Cl, N, S |
| **45** | 5-$OOCCH_2OC_6H_4Cl$(p) | 192–193 | EtOH | $C_{15}H_{13}ClN_4O_3S$ | C, H, Cl, N, S |
| **46** | 5-$OOCCH_2OC_6H_3Cl_2$(2″,4″) | 171.5–172 | EtOH | $C_{15}H_{12}Cl_2N_4O_3S$ | C, H, Cl, N, S |
| **47** | 5-$OOCCH_2OC_6H_2Cl_3$(2″,4″,5″) | 200.5–201.5 | EtOH | $C_{15}H_{11}Cl_3N_4O_3S$ | C, H, Cl, N, S |
| **48** | 5-[(2′,3′,4′,6′-tetraacetyl)-1-β-D-glucopyranosyl] | 166–167 | EtOH | $C_{21}H_{26}N_4O_{10}S \cdot 0.5H_2O$ | C, H, N, S |
| **49** | 5-$NHCOCH_3$ | 253–254 dec | EtOH | $C_9H_{11}N_3OS$ | C, H, N, S |
| **51** | 3-OH, 4-$CH_3$ | 223–224 dec | | $C_8H_{10}N_4OS \cdot 0.75H_2O \cdot 0.25C_2H_5OH$ | C, H, N, S |
| **53** | 3-Cl, 5-OH | 239–240 | | $C_7H_7ClN_4OS \cdot 0.5H_2O$ | C, H, Cl, N, S |
| **54** | 3-OH, 3′-$CH_3$[e,f] | 169–170 | EtOH | $C_8H_{10}N_4OS$ | C, H, N, S |
| **55** | 3-OH, 3′4′-$(CH_3)_2$[e,g] | 159.5–160.5 | EtOH | $C_9H_{12}N_4OS$ | C, H, N, S |
| **56** | 5-OH, 4′-*n*-$C_4H_9$[b,h] | 170–171 | MeOH | $C_{11}H_{16}N_4OS$ | C, H, N, S |
| **57** | 5-OH, 4′-$C_6H_5$[h,i] | 188–189 | EtOH, $H_2O$ | $C_{13}H_{12}N_4OS \cdot 0.5H_2O$ | C, H, S; N[i] |
| **58** | 5-OH, 4′-(2-pyridyl)[c,h] | 223–224 | | $C_{12}H_{11}N_5OS$ | C, H, N, S |
| **60** | 5-$OCH_2C_6H_5$, 4-OH | 233–234 dec | | $C_{14}H_{14}N_4O_2S$ | C, H, N, S |
| **61** | 3,5-$(OC_2H_5)_2$ | 207–207.5 | | $C_{11}H_{16}N_4O_2S$ | C, H, N, S |
| **79** | 4-Formylpyrimidine TSC[j,k] | 233–234 dec | EtOH | $C_6H_7N_5S$ | C, S; H,[l] N[l] |
| **82** | 6-$CH_3$-3-formylpyridazine TSC | 241–242 dec | | $C_7H_9N_5S$ | C, N, S; H[m] |

[a] $NH_2N(CH_3)CSNH_2$ and $NH_2NHCS(CH_3)_2$ prepared by the methods of K. A. Jensen, U. Anthoni, B. Kägi, C. Larsen, and T. Pedersen, *Acta Chem. Scand.*, **22**, 1 (1968). [b] $NH_2NHCSNH$-*n*-$C_4H_9$ prepared by the method of E. Lieber, C. N. Pillai, and R. D. Hites, *Can. J. Chem.*, **35**, 832 (1957). [c] $NH_2NHCSNH$-(2-pyridyl) prepared by the method of Farbenfabriken Bayer A.G. (J. Klarer and R. Behenisch), German Patent 832,891 (1952); *Chem. Abstr.*, **47**, 3342 (1953). [d] C: calcd, 51.89; found, 53.31. N: calcd, 26.90; found, 25.74. [e] For aldehyde preparation see ref 65. [f] $NH_2NHC(SCH_3)=NH \cdot HI$ prepared by method of E. Cattelain, *Bull. Soc. Chim. Fr.*, **11**, 249 (1944). [g] $NH_2NHC(SCH_3)=NCH_3 \cdot HI$ prepared by method of A. H. Greer and G. B. L. Smith, *J. Amer. Chem. Soc.*, **76**, 1141 (1954). [h] For aldehyde preparation see ref 12. [i] $NH_2NHCSNHC_6H_5$ prepared by method of M. Tisler, *Experientia*, **12**, 261 (1956). [j] N: calcd, 19.91; found, 18.53. [k] Aldehyde prepared by the method of Y. Ashany, H. Edery, J. Zahavy, W. Künberg, and S. Cohen, *Israel J. Chem.*, **3**, 133 (1965). [l] H: calcd, 3.89; found, 4.35. N: calcd, 38.65; found, 38.14. [m] H: calcd, 4.65; found, 5.2.

Scheme I



5-Acetoamido-2-formylpyridine was prepared as shown in Scheme I. The polyfunctional 2-formylpyridine thiosemicarbazones **51**, **53**, and **61** were prepared as shown in Schemes II–IV. The starting materials, 3-hydroxy-4-picoline,[65] 3,5-diethoxypicolinic acid,[66] and ethyl 2-methyl-5-nitronicotinate[67] were prepared as reported in the literature. In the preparation of ethyl 2-methyl-5-nitronicotinate, Fanta's procedure[68] was modified because the original procedure failed when using commercial ethyl β-aminocrotonate (see Experimental Section).

5-Benzyloxy-2-formyl-4(1*H*)-pyridone was prepared

Scheme II

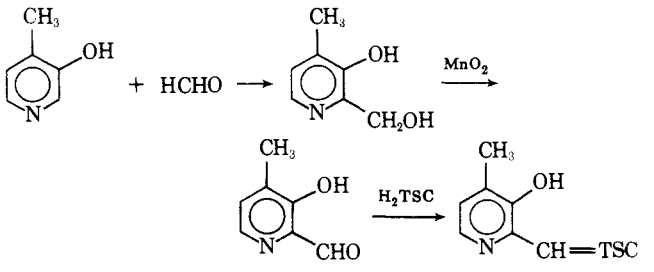
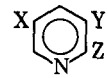


**Table V.** Substituted Pyridines



| No. | X | Y | Z | Mp or bp (mm), °C | Yield, % | Crystn solvent | Formula | Analyses[a] |
|---|---|---|---|---|---|---|---|---|
| **17a** | H | $OCH_3$ | $CH_2OH$ | 73–74 | 18 | Petr ether | $C_7H_9NO_2$ | C, H, N |
| **17b** | H | $OCH_3$ | CHO | 115–118 (1.5) | 68 | | $C_7H_7NO_2$ | C, H, N |
| **30a** | $OC_2H_5$ | H | $CH_3$, *N*-oxide·$H_2O$ | 47–48 | 90 | | $C_8H_{11}NO_2 \cdot H_2O$ | C, H, N |
| **30b** | $OC_2H_5$ | H | $OOCCH_3$ | 100–102 (1) | 68 | | $C_{10}H_{13}NO_3$ | C, H, N |
| **30c** | $OC_2H_5$ | H | $OOCCH_3$, *N*-oxide | 121–123 | 90 | Petr ether | $C_{10}H_{13}NO_4$ | C, H, N |
| **33a** | $O(C_2H_4O)_2C_2H_5$ | H | CHO | 150–155 (1) | 36 | | $C_{12}H_{17}NO_4$ | C, H, N |
| **37a** | *n*-$OOCC_{15}H_{31}$ | H | CHO | 47–48 | 57 | | $C_{22}H_{35}NO_3$ | C, H, N |
| **38a** | $OOC(CH_3)_3$ | H | CHO | 98–101 (1.4) | 34 | | $C_{11}H_{13}NO_3$ | C, H, N |
| **39a** | $OOCCH_2OCH_3$ | H | CHO | 118–120 (1.5) | 32 | | $C_9H_9NO_4$ | C, H, N |
| **40a** | $OOCCH_2OC_2H_5$ | H | CHO | 130–134 (2) | 60 | | $C_{10}H_{11}NO_4$ | |
| **41a** | $OOCCH_2Cl$ | H | CHO | 71.5–73 | 64 | Petr ether | $C_8H_6ClNO_3$ | C, H, Cl, N |
| **42a** | $OOCCH_2OC_6H_5$ | H | CHO | 111.5–112.5 | 44 | $C_6H_6$ | $C_{14}H_{11}NO_4$ | C, H, N |
| **44a** | $OOCCH_2OC_6H_4Cl$(o) | H | CHO | 71.5–72 | 96 | $C_6H_6$–petr ether | $C_{14}H_{10}ClNO_4$ | C, H, Cl, N |
| **46a** | $OOCCH_2OC_6H_3Cl_2$-(2″,4″) | H | CHO | 118–119 | 56 | $C_6H_6$–petr ether | $C_{14}H_9Cl_2NO_4$ | C, H, Cl, N |
| **47a** | $OOCCH_2OC_6H_2Cl_3$-(2″,4″,5″) | H | CHO | 136–137 | 67 | $C_6H_6$ | $C_{14}H_8Cl_3NO_4$ | C, H, Cl, N |
| **51a**[b] | | | | 135–136[c] | | *i*-PrOH | $C_7H_9NO_2 \cdot 0.25H_2O \cdot 0.25C_3H_7OH \cdot HCl$ | C, H, Cl, N |
| **53a** | $NO_2$ | $NH_2$ | $CH_3$ | 191–192.5 | 71 | $C_6H_6$ | $C_6H_7N_3O$ | C, H, N |
| **53b** | $NO_2$ | Cl | $CH_3$ | 35–36 | 88 | Petr ether | $C_6H_5ClN_2O_2$ | C, H, Cl, N |
| **53c** | OH | Cl | $CH_3$ | 170–171 | 50 | $C_2H_5OH$, $H_2O$ | $C_6H_6ClNO$ | C, H, Cl, N |
| **53d** | OH | Cl | $CH_3$, *N*-oxide | 197–198.5 | 89 | $C_2H_5OH$ | $C_6H_6ClNO_2$ | C, H, Cl, N |
| **53e** | $OOCCH_3$ | Cl | $CH_2OOCCH_3$ | 73.5–74.5 | 71 | Petr ether | $C_{10}H_{10}ClNO_4$ | C, H, Cl, N |
| **53f** | OH | Cl | $CH_2OH$ | 184–185.5[c] | 83 | *i*-PrOH | $C_6H_6ClNO \cdot HCl$ | C, H, Cl, N |
| **61a** | $OC_2H_5$ | $OC_2H_5$ | $COOC_2H_5$ | 134–136[c] | 82 | $C_2H_5OH$ | $C_{12}H_{17}NO_4 \cdot HCl$ | C, H, Cl, N |
| **61b** | $OC_2H_5$ | $OC_2H_5$ | $CH_2OH$ | 67.5–68 | 74 | Petr ether | $C_{10}H_{15}NO_3$[d] | C, H, N |
| **61c** | $OC_2H_5$ | $OC_2H_5$ | CHO | 105–105.5 | 87 | Petr ether | $C_{10}H_{13}NO_3$ | C, H, N |

[a] Where no symbols appear, these intermediates were not obtained in a state of analytical purity. [b] 3-Hydroxy-2-hydroxymethyl-4-picoline hydrochloride. [c] Hydrochloride. [d] Analyzed as the picrate, mp 133.5–134.5°.

from the oxidation of the known compound, 5-benzyloxy-2-hydroxymethyl-4(1*H*)-pyridone, with $MnO_2$.[67]

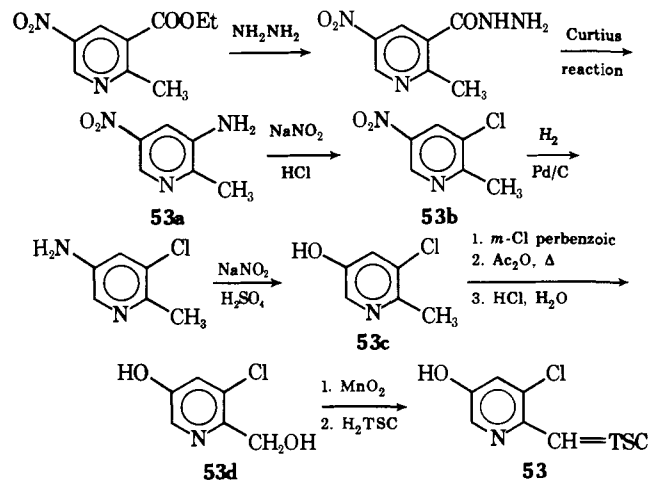
3-Formyl-6-methylpyridazine was prepared from 3,6-dimethylpyridazine *N*-oxide. Heating the *N*-oxide in $Ac_2O$ gave 2-acetoxymethyl-6-methylpyridazine, which was hydrolyzed with aqueous NaOH to 3-hydroxymethyl-6-methylpyridazine and then oxidized with $MnO_2$ to 3-formyl-6-methylpyridazine.

## Experimental Section

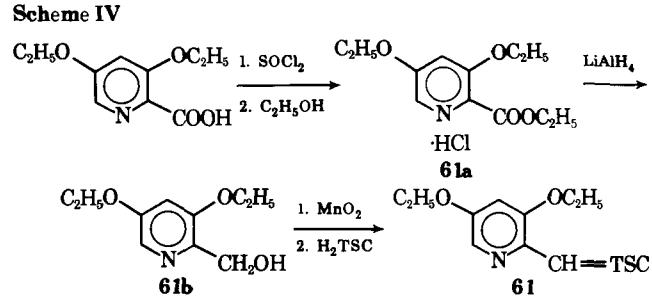
The methods used for the evaluation of antitumor activity in mice have been described elsewhere.[11]

**Enzyme Preparation.** Ribonucleoside diphosphate reductase was prepared from human epidermoid carcinoma cells (H.Ep.-2) grown in suspension culture. Cells were harvested by centrifugation, washed free of medium with cold physiological saline, and resuspended in cold 0.05 *M* Tris buffer, pH 7.5, to a concentration of 1 g of cells per 5 ml. The cells in suspension were sonically disrupted at 4° by means of a Branson sonifier (Model LS-75, Branson Instruments, Inc., Stamford, Conn.); three 15-sec pulses were spaced 1 min apart at maximum output (20 kcps, 150 W) of the apparatus. The broken cell suspension was centrifuged at 100,000g for 1 hr in the Spinco L-2 centrifuge and the supernatant solution was filtered through Schleicher and Schull membrane filters. The clear filtrate served as the starting point for ammonium sulfate fractionation of protein; maximum RDR activity was obtained in the protein fraction precipitating between 25 and 40% saturation with ammonium sulfate at 4°. This 40% ammonium sulfate precipitate was dissolved in 0.05 *M* Tris–HCl, pH 7.5, and dialyzed overnight against this buffer. The specific activity of RDR prepared in this manner is expressed as nanomoles of deoxyribonucleotide formed/mg of protein/30 min at 37°. Multiple preparations of enzyme were made during the course of this work; the most active preparation had a specific activity of 24.3

**Scheme III**




**Scheme IV**



and the least active preparation used had a specific activity of 7.6. Most preparations exhibited specific activities in the range of 10–15 nmol/mg of protein/30 min.

**Assay for Reductase Activity and Evaluation of Inhibitors.** The standard reaction mixture used for enzyme assays in this work was that described by Moore.[27] The composition of the reaction mixture and the concentration (m*M*) of components were as follows: potassium phosphate buffer, pH 7, 8.3; ATP, 4.4; magnesium acetate, 2.7; sodium fluoride, 8.3; ferrous chloride or ferrous ammonium sulfate, 0.06; dithioerythritol, 6.2; cytidine [2-$^{14}$C]diphosphate, 0.4 (0.1 $\mu$Ci). The final volume of the reaction mixture, including enzyme protein solution, was 0.2 ml. In the standard assay the reaction mixture was incubated at 37° for 30 min and then rapidly heated to boiling for 2 min; denatured protein was removed by centrifugation. Snake venom phosphodiesterase (1 mg, Sigma), *E. coli* alkaline phosphatase (0.2 unit, Worthington), and carrier deoxycytidine (50 $\mu$g) were added to the supernatant solution and incubated at 37° for 90 min. Such treatment converted all nucleotides to nucleosides. [2-$^{14}$C]Deoxycytidine thus derived from deoxycytidine diphosphate, which was formed by enzymatic reduction of cytidine diphosphate, was separated from [2-$^{14}$C]cytidine by descending paper chromatography. This method and the solvent for developing the chromatograms were based on that of Reichard;[69] namely, ethanol–5 *M* ammonium acetate (pH 9.5)–saturated sodium tetraborate–0.5 *M* EDTA (11:1:4:0.02). Radioactivity on the chromatograms was detected by exposing the chromatograms to X-ray film for 1 week; quantitative determination of radioactivity was accomplished by cutting out the radioactive spots and counting them in a liquid scintillation spectrometer (Packard Instrument Co.). For details of the procedure for quantitative determination of radioactivity see ref 70.

It was established that the enzymatic reduction of CDP was linear with time for up to 60 min and was proportional to enzyme concentration over a sixfold range. The enzyme reaction required dithioerythritol and was inhibited by the iron-chelating agent deferoxamine. During the course of this study multiple preparations of RDR were made from H.Ep.-2 cells. In all experiments in which inhibitors were evaluated for their effect on the enzymatic reduction of [2-$^{14}$C]CDP adequate controls were included. Since many of the thiosemicarbazones are difficultly soluble in aqueous solution it was necessary to solubilize them in organic solvents such as DMSO or ethanol. DMSO at a final concentration of 5% or ethanol at a final concentration of 2.5% in the enzyme reaction mixture was found to be satisfactory. In all such cases DMSO or ethanol controls were included. In evaluating a new compound for inhibition of RDR it was standard practice to examine the effect of a range of concentration of inhibitor, usually from $10^{-3}$ to $10^{-6}$ *M* final concentration. From the results of such an initial experiment an estimate of the minimal concentration for inhibition of enzyme activity could be made. In subsequent experiments concentrations of inhibitor over a more limited range were examined to provide a 50% inhibitory concentration ($I_{50}$). From these data graphs were prepared in which molar concentration of inhibitor was plotted against enzyme activity relative to aqueous control or solvent control. It was deemed necessary to have at least three concentrations of inhibitor within the $I_{10}$–$I_{90}$ range in order to obtain a good value for $I_{50}$. Determinations of $I_{50}$ values (for reference compounds, such as 1 and **27**) were reproducible within limits of ±10% in independently prepared batches of enzyme. The specific activities of different enzyme preparations used in inhibition studies ranged between 10 and 20 mmol of deoxyribonucleotide formed/mg of protein/30 min.

It should be pointed out that RDR prepared from H.Ep.-2 cells also reduced UDP when ATP was present as activator. ADP was reduced in the presence of dGTP as activator and GDP was reduced in the presence of dTTP plus ATP. These results are in agreement with the findings of Moore.[27]

**Chemical Procedures.** Melting points are corrected and were measured on a Thomas-Hoover capillary melting point apparatus. Microanalyses were performed by Chemical Analytical Services, University of California, Berkeley, Calif. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

**3-Acetoxy-2-formylpyridine Thiosemicarbazone (19).** 3-Hydroxy-2-formylpyridine thiosemicarbazone[64] (1.96 g, 0.01 mol) was dissolved in 50 ml of 0.2 *N* NaOH. The solution was cooled to 5° and 2.1 g (0.021 mol) of $Ac_2O$ was added dropwise with rapid stirring. The temperature was kept between 5 and 10° by cooling with an ice bath. During the addition of the $Ac_2O$ the 3-acetoxy derivative precipitated. The precipitate was filtered, washed well with $H_2O$, and dried in a vacuum dessicator over $P_2O_5$ to yield 1.7 g (72%), mp 197° dec.

The 5-acetoxy isomer was prepared in the identical manner to give a yield of 86%, mp 204–205° dec. This isomer, prepared by another method,[12] had mp 200–201° dec. A mixture melting point of both isomers gave no melting point depression (mp 201–202° dec).

**5-Chloroacetoxy-2-formylpyridine (41a).** 5-Hydroxy-2-formylpyridine (4.4 g, 0.036 mol) was dissolved in 100 ml of absolute $C_2H_5OH$, which contained 850 mg of Na as the ethoxide. The solution was evaporated to dryness and the resulting Na salt dissolved in 100 ml of $H_2O$. After the aqueous solution was cooled to 0–5°, 4.1 g of chloroacetyl chloride (0.036 mol) dissolved in $CH_2Cl_2$ (100 ml) was added dropwise (0.5 hr) with vigorous stirring. The $CH_2Cl_2$ layer was washed with $KHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated to an oil which crystallized on standing. Recrystallization of the solution with petroleum ether (bp 60–110°) gave a product which had mp 67–69.5°. This material was sublimed at reduced pressure to yield 4.5 g (64%), mp 71.5–73°.

**5-Dimethylaminoacetoxy-2-formylpyridine Thiosemicarbazone Hydrochloride (41).** The above aldehyde (**41a**, 3.5 g, 0.0175 mol) dissolved in 35 ml of $C_2H_5OH$ was added to 350 ml of boiling $C_2H_5OH$ containing 1.5 g (0.0164 mol) of thiosemicarbazide and 1 ml of AcOH. After the solution was refluxed for 5 min, gaseous dimethylamine was bubbled through the solution for 45 min and the solution was concentrated to 100-ml volume. The hot solution was filtered to remove some solid present. The solution was then concentrated to a syrup in a flash evaporator, redissolved in 100 ml of EtOH, and refiltered again to remove a small amount of solid material. The resulting cold EtOH solution was treated with a small amount of concentrated HCl and a pale yellow material precipitated immediately. The precipitate was filtered, washed with a small amount of cold EtOH and $Et_2O$, and dried. The thiosemicarbazone was crystallized from a minimum amount of boiling $C_2H_5OH$: yield 2.1 g (40%); mp 231.5–232.5° dec.

**Ethyl 1-Methyl-5-nitronicotinate.** Sodium nitromalonaldehyde monohydrate (39.0 g, 0.252 mol) and 32.7 g (0.253 mol) of ethyl 3-aminocrotonate (commercial) were added to 250 ml of $Ac_2O$ and 150 ml of pyridine. The reaction mixture was stirred at room temperature. After a short period of time the reaction mixture became mildly exothermic and the temperature rose to 85°. As the temperature rose to 60° the reaction medium became a slurry. Between 60 and 80° the slurry went back into solution. The solution was allowed to cool to room temperature and was flash evaporated *in vacuo* to a semisolid phase. The product was added to 1 l. of $H_2O$ and stirred for 15 min. The crude ester was filtered and recrystallized from 600 ml of 50% EtOH. The yield was 45 g (85%), mp 64–65°.

**Ethyl 3,5-Diethoxypicolinate Hydrochloride (61a).** 3,5-Diethoxypicolinic acid (5.0 g, 0.024 mol) was allowed to react with 20 ml of $SOCl_2$ at room temperature. The reaction mixture was refluxed for 0.5 hr and then the excess $SOCl_2$ flash evaporated. The acid chloride hydrochloride was added to 250 ml of absolute EtOH and evaporated to dryness and allowed to crystallize. The resultant crystals were washed well with $Et_2O$ and dried, yielding 5.3 g (82%) of product, mp 134–136°.

**3,5-Diethoxy-2-hydroxymethylpyridine (61b).** Five g (0.018 mol) of **61a** dissolved in 10 ml of $H_2O$ was neutralized with $NaHCO_3$ and extracted with 75 ml of $Et_2O$. The $Et_2O$ extract was dried over anhydrous $MgSO_4$ for 24 hr and added dropwise (40 min) to 50 ml of $Et_2O$ containing 1.0 g of 95% $LiAlH_4$. The temperature was kept at −10–0° during the reaction. After the ester was added, the reaction was stirred for an additional 15 min and then 3.0 ml of $H_2O$ added dropwise to decompose the excess $LiAlH_4$ and complex. The $Et_2O$ phase was filtered and the residue washed well with 50 ml of boiling $Et_2O$. The extracts were dried over anhydrous $MgSO_4$ and evaporated to give the crude carbinol, which was crystallized from petroleum ether (bp 60–110°) to yield 3.5 g (74%) of white needles, mp 67.5–68°. The picrate was prepared and crystallized from EtOH, mp 133.5–134.5°. *Anal.* ($C_{16}H_{18}N_4O_{10}$) C, H, N.

**3,5-Diethoxy-2-formylpyridine (61c).** 3,5-Diethoxy-2-hydroxymethylpyridine (**61b**, 3.5 g, 0.018 mol), dissolved in 50 ml of $CHCl_3$, was treated with 13 g of active $MnO_2$. The reaction mixture was allowed to stir and reflux for 2 hr and then filtered, and the $MnO_2$ cake washed well with two 100-ml portions of $CHCl_3$. The $CHCl_3$ extracts were evaporated to a solid aldehyde which was crystallized from petroleum ether (bp 60–110°): yield 3.0 g (87%); mp 105–105.5°.

**Acknowledgments.** We thank Douglas French for the preparation of many of these compounds and Arvia Hosking and June French for the *in vivo* antitumor evaluations.

## References


(1) R. W. Brockman, J. R. Thompson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, **16**, 167 (1956).
(2) F. A. French and B. L. Freedlander, *ibid.*, **18**, 1290 (1958).
(3) F. A. French, A. E. Lewis, E. J. Blanz, Jr., and A. H. Sheena, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **24**, 402 (1965).
(4) F. A. French, A. E. Lewis, A. H. Sheena, and E. J. Blanz, Jr., *ibid.*, **25**, 235 (1966).
(5) This laboratory, unpublished data.
(6) M. Mathew and G. J. Palenik, *J. Amer. Chem. Soc.*, **91**, 6310 (1969).
(7) F. A. French and E. J. Blanz, Jr., *Cancer Res.*, **25**, 1454 (1965).
(8) F. A. French and E. J. Blanz, Jr., *ibid.*, **26**, 1638 (1966).
(9) F. A. French and E. J. Blanz, Jr., *J. Med. Chem.*, **9**, 585 (1966).
(10) E. J. Blanz, Jr., and F. A. French, *Cancer Res.*, **28**, 2419 (1968).
(11) F. A. French, E. J. Blanz, Jr., J. R. DoAmaral, and D. A. French, *J. Med. Chem.*, **13**, 1117 (1970).
(12) E. J. Blanz, Jr., F. A. French, J. R. DoAmaral, and D. A. French, *ibid.*, **13**, 1124 (1970).
(13) F. A. French and E. J. Blanz, Jr., *Cancer Chemotherapy Rep.*, **2** (1), 199 (1971).
(14) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, 11, 700 (1968).
(15) K. C. Agrawal and A. C. Sartorelli, *J. Pharm. Sci.*, **57**, 1948 (1968).
(16) K. C. Agrawal, R. J. Cushley, W. J. McMurray, and A. C. Sartorelli, *J. Med. Chem.*, **13**, 431 (1970).
(17) K. C. Agrawal, R. J. Cushley, S. R. Lipsky, J. R. Wheaton, and A. C. Sartorelli, *ibid.*, **15**, 192 (1972).
(18) K. C. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, *ibid.*, **15**, 1154 (1972).
(19) A. J. Lin, K. C. Agrawal, and A. C. Sartorelli, *ibid.*, **15**, 615 (1972).
(20) K. C. Agrawal, B. A. Booth, J. R. Wheaton, E. C. Moore, and A. C. Sartorelli, Abstracts, 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 1972, MEDI 45.
(21) P. D. Mooney, B. A. Booth, E. C. Moore, K. C. Agrawal, and A. C. Sartorelli, ref 20, MEDI 46.
(22) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, **16**, 715 (1973).
(23) A. C. Sartorelli, *Biochem. Biophys. Res. Commun.*, **27**, 26 (1967).
(24) A. C. Sartorelli, *Pharmacologist*, **9**, 192 (1967).
(25) A. C. Sartorelli, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, No. C-133.
(26) E. C. Moore and P. Reichard, *J. Biol. Chem.*, **239**, 3453 (1964).
(27) E. C. Moore in "Methods in Enzymology," Vol. 12, S. P. Colowick and N. D. Kaplan, Ed., Academic Press, New York, N. Y., 1967, pp 155–174.
(28) P. Reichard, "The Biosynthesis of Deoxyribose," Wiley, New York, N. Y., 1967, p 48, ref 46; E. C. Moore, private communication.
(29) N. C. Brown, R. Eliasson, P. Reichard, and L. Thelander, *Eur. J. Biochem.*, **9**, 512 (1969).
(30) E. C. Herrmann and E. C. Moore, Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, BIOL-190.
(31) S. Hopper, ref 30, BIOL-191.
(32) E. C. Moore and R. B. Hurlbert, *J. Biol. Chem.*, **241**, 4802 (1966).
(33) H. I. Hochman and A. C. Sartorelli, Abstracts, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, BIOL-35.
(34) E. C. Moore, *Cancer Res.*, **29**, 291 (1969).
(35) A. E. Martell, "Chemistry of the Metal Chelate Compounds," Prentice Hall, New York, N. Y., 1952, pp 370, 388, 407, 412.
(36) D. D. Ulmer and B. L. Vallee, *Advan. Chem. Ser.*, **No. 100**, 187 (1971).
(37) B. L. Vallee in "Newer Trace Elements in Nutrition," W. Mertz and W. E. Cornatzer, Ed., Marcel Dekker, New York, N. Y., 1971, pp 33–50.
(38) E. C. Moore, N. S. Zedeck, K. C. Agrawal, and A. C. Sartorelli, *Biochemistry*, **9**, 4492 (1970).
(39) B. A. Booth, E. C. Moore, and A. C. Sartorelli, *Cancer Res.*, **31**, 228 (1971).
(40) E. C. Moore, B. A. Booth, and A. C. Sartorelli, *ibid.*, **31**, 235 (1971).
(41) A. C. Sartorelli, K. C. Agrawal, and E. C. Moore, *Biochem. Pharmacol.*, **20**, 3119 (1971).
(42) A. C. Sartorelli and B. A. Booth, *Proc. Amer. Ass. Cancer Res.*, **6**, 61 (1968).
(43) E. L. Eliel, *J. Chem. Educ.*, **48**, 163 (1971).
(44) L. P. Hammett, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1970.
(45) H. H. Jaffee, *Chem Rev.*, **53**, 191 (1953).
(46) C. Hansch in "Drug Design," Vol. 1, E. J. Ariëns, Ed., Academic Press, New York, N. Y., 1970, pp 271–342.
(47) A. Albert and J. N. Phillips, *J. Chem. Soc.*, 1294 (1956).
(48) S. F. Mason, *ibid.*, 674 (1958).
(49) S. F. Mason, *ibid.*, 4874 (1957).
(50) S. F. Mason, *ibid.*, 5010 (1957).
(51) D. E. Metzler and E. E. Snell, *J. Amer. Chem. Soc.*, **77**, 2431 (1955).
(52) R. E. Banks, J. E. Burgess, W. M. Cheng, and R. N. Hazeldine, *J. Chem. Soc.*, 575 (1965).
(53) R. D. Chambers, J. Hutchinson, and W. K. R. Musgrave, *ibid.*, 5634 (1964).
(54) A. R. Katritzky, J. D. Rowe, and S. K. Roy, *ibid.*, 758 (1967).
(55) K. C. Agrawal, P. D. Mooney, B. A. Booth, E. C. Moore, and A. C. Sartorelli, *Proc. Amer. Ass. Cancer Res.*, 14, 79 (1973).
(56) J. H. Burchenal and M. Dowling, *ibid.*, 11, 13 (1970).
(57) G. B. Grindey, E. Mihich, and C. A. Nichol, *Cancer Res.*, **32**, 522 (1972).
(58) W. R. Laster, Jr., and R. W. Brockman, *Proc. Amer. Ass. Cancer Res.*, 14, 18 (1973).
(59) R. W. Brockman, R. W. Sidwell, G. Arnett, and S. Shaddix, *Proc. Soc. Exp. Biol. Med.*, **133**, 609 (1970).
(60) H. E. Skipper, F. M. Schabel, Jr., L. B. Mellett, J. A. Montgomery, L. J. Wilkoff, H. H. Lloyd, and R. W. Brockman, *Cancer Chemother. Rep.*, **54**, 431 (1970).
(61) E. Etcubanas, C. Tan, N. Wollner, V. Bethune, I. Krakoff, and J. Burchenal, *Proc. Amer. Ass. Cancer Res.*, 12, 38 (1971).
(62) R. C. Conti, B. R. Toftness, K. C. Agrawal, R. Tomchick, J. A. R. Mead, J. R. Bertino, A. C. Sartorelli, and W. A. Creasey, *Cancer Res.*, **32**, 1455 (1972).
(63) S. Ginsberg and I. B. Wilson, *J. Amer. Chem. Soc.*, **79**, 481 (1957).
(64) D. Heinert and A. E. Martell, *Tetrahedron*, **3**, 49 (1958).
(65) V. S. Traynelis and R. F. Martello, *J. Amer. Chem. Soc.*, **82**, 2744 (1960).
(66) H. J. den Hertz and B. Mulder, *Recl. Trav. Chim. Pays-Bas*, **67**, 957 (1948).
(67) A. F. Thomas and A. Marxer, *Helv. Chim. Acta*, **43**, 469 (1960).
(68) P. E. Fanta, *J. Amer. Chem. Soc.*, **75**, 737 (1935).
(69) P. Reichard, *J. Biol. Chem.*, **236**, 2511 (1961).
(70) R. W. Brockman, S. Shaddix, W. R. Laster, Jr., and F. M. Schabel, Jr., *Cancer Res.*, **30**, 2358 (1970).