The Carcinostatic Activity of Thiosemicarbazones of Formyl Heteroaromatic Compounds.[1] III. Primary Correlation

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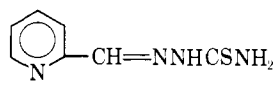
Thiosemicarbazones of 41 formyl heterocycles and two heterocyclic ketones were prepared and tested on four mouse tumor systems. The variables studied included: 16 ring systems, different positions of attachment of the formyl group relative to the ring heteroatoms, additional ring substituents, and terminal substituents in the thiosemicarbazone side chain. The minimum requirement for activity is formyl group attachment to a ring carbon α to an unencumbered ring nitrogen of heteroaromatic character. The π-electron density must be low at this ring carbon and fairly high at the ring nitrogen. All the active compounds are potent ligands for the transition metals from iron through zinc. The antitumor activities of the thiosemicarbazones of 1-formylisoquinoline and 3-hydroxy-2-formylpyridine are sufficiently good to merit extensive further study.

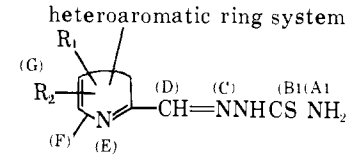
Brockman, *et al.*,[2] in 1956 made the singular observation of the mild but definite antileukemic effect of 2-formylpyridine thiosemicarbazone (I; Table I, **1**) in mice. This observation was verified in this laboratory.[3] Compound I is cumulatively toxic and activity is observed only at toxic levels. Miscellaneous, readily accessible aromatic and heteroaromatic aldehyde thiosemicarbazones were studied but no significant antitumor activity was found.[2-6]



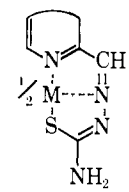
I

In spite of this unpromising background we reconsidered this area in detail in late 1963. Earlier we had postulated that I might be acting, at physiological pH values, as a tridentate N*—N*—S* ligand.[2] The formula for I may be rewritten in the SH form with double bonds in place of either single C–N bond shown in the conventional structure. Such canonical forms and their resonance hybrids would lead to electronic coupling throughout the side chain, into the ring nitrogen through the α-carbon, involving the entire heteroaromatic ring system. Thiosemicarbazones of this general type are, in fact, soluble in moderately strong alkali and these solutions are bright yellow. Furthermore, some of them form strong coordination compounds, often intensely colored, with divalent iron, cobalt, nickel, copper, zinc, and their higher atomic weight analogs but do not coordinate magnesium, calcium, etc.[7] This behavior can be quite selective and varies considerably with small changes in structure. These studies will be reported when complete.

The general formula II was postulated as the series model. Formula III illustrates the functioning of the ligand. The formation of two five-membered chelate rings of partially conjugate character would favor octahedral coordination of two ligands to one divalent



II



III

metal ion yielding an electrically neutral coordination compound. Depending on the particular metal ion and the pH, these compounds could also act as bidentate or unidentate ligands. An additional requirement is that the thiosemicarbazone is in the *syn* form. Several ancillary hypotheses, subjectable to test, were also entertained. Using Stuart–Briegleb model studies as a guide, it was postulated that replacement of hydrogens on the terminal amino group would be adverse due to steric interference with the SH group. Changing the SH to SR, the >NH to >NR or the -CH= to -CR= were formulated as adverse substitutions. Using pyridine as a model it was also considered that bulky substituents in the 6 position would be detrimental. These considerations were partly steric, partly electronic, and stemmed, in part, from the basic hypotheses, *i.e.*, retaining the integrity of the conjugate N*—N*—S* ligand system.

Considering the ring, the first assumption is that an unencumbered heteroaromatic nitrogen is required and that the carbonyl attachment must be α to this. The ring nitrogen must be a reasonably good donor to transition metals and hence a pyridine-like character is desirable. From the available data on π-electron densities, correlated with data on reactivity of groupings attached to α positions, it was postulated that the π-electron density at the point of attachment of the aldehyde moiety should be low. No attempt was made to predict the effect of additional substituents other than the notion that they should enhance or at least not interfere with the other desired factors.

(1) This investigation was supported by Grant CA-03287 from the National Cancer Institute.

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

(3) F. A. French and B. L. Freedlander, *ibid.*, **18**, 1290 (1958).

(4) F. A. French and B. L. Freedlander, *ibid.*, **20**, No. 7, Part 2, 505 (1960).

(5) F. A. French, B. L. Freedlander, and E. J. Blanz, Jr., *ibid.*, **21**, No. 8, Part 2, 349 (1961).

(6) F. A. French and E. J. Blanz, Jr., *ibid.*, **23**, No. 2, Part 2, 9 (1963).

(7) This laboratory, unpublished data.

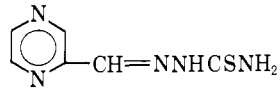
TABLE I
ANTITUMOR DATA

No.	Cmpd	Leukemia L1210		Sarcoma 180		Lewis lung carcinoma		Adenocarcinoma 755	
		Dose, mg/kg	% T/C	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C
1	2-Formylpyridine TSC[a,b]	10	130	10	60	10[c]	47	10[c]	71
2	2-Formylpyridine 4′-methyl-TSC[d]	150	111	100	82	100	106	100	120
3	2-Formylpyridine 4′-phenyl-TCS[e]	100	104	50	79	75	94	100	47[f]
4	Methyl 2-pyridyl ketone TSC[g]	10	101	10	87	5	79	4	65
5	Bis(2-pyridyl) ketone TSC	30	111	30	90	30	70	30	55
6	6-Methyl-2-formylpyridine TSC[b]	150	125	150	85	150	30	150	64
7	3-Hydroxy-2-formylpyridine TSC[h]	71	179	71	49	71	12	71	32
8	3-Ethoxy-2-formylpyridine TSC	100	141	100	47	75	42	75	50
9	3-Hydroxy-6-methyl-2-formylpyridine TSC	400	122	200	91	200	125	200	85
10	3-Hydroxy-2-formyl-1-methylpyridinium iodide TSC	200	101	150	86	100	87	150	145
11	3-Formylpyridine TSC[b]	106	104	75	87	75	98	75	80
12	4-Formylpyridine TSC[b]	283	99	200	90	200	104	200	86
13	2-Formylpyrazine TSC[i]	75	136	75	53	75	29	75	25
14	2-Formylpyrazine 4′-methyl-TSC	75	138	75	87	75	61	75	42
15	Bis(2-formylpyrazine) thiocarbohydrazone	100	130	50	78	75	44	75	81
16	2-Formylpyrrole TSC[b]	75	98	75	154	75	83	75	69
17	2-Formyl-1-methylpyrrole TSC[b]	75	95	75	127	75	73	50	89
18	3-Formylpyrazole TSC	200	116	200	98	300	80	300	132
19	4(5)-Formylimidazole TSC[b]	400	104	400	115	250	104	400→200[j]	121
20	4-Formyl-1H-1,2,3-triazole TSC	200	99	200	79	200	67	175	67
21	2-Formylthiophene TSC[g]	200	97	200	66	150	76	150	142
22	2-Formylfuran TSC[g]	100	103	200	74	100	60	100	72
23	5-Methyl-2-formylfuran TSC	250	96	250	98	250	81	250	113
24	2-Formylindole TSC[k]	50	113	50	89	50	47[f]	50	57
25	3-Formylindole TSC[k]	200	99	200	68	200	91	200	89
26	2-Formylquinoline TSC[l]	100	91	100	79	150	53	100	57
27	8-Formylquinoline TSC[m]	30	101	25	104	20	65	20	85
28	1-Formylisoquinoline TSC[n]	67	147	35	76	67	20	67	10
29	1-Formylisoquinoline 4′-methyl-TSC	50	97	50	73	75	71	63	56
30	1-Formylisoquinoline 4′-ethyl-TSC	40	96	30	77	40	74	40	54
31	Bis(1-formylisoquinoline) thiocarbohydrazone	18	109	18	81	18	46	18	82
32	3-Formylisoquinoline TSC[n]	150	113	100	90	100	72	75	101
33	3-Formylcinnoline TSC	100	101	100	81	100	87	100	101
34	4-Formylcinnoline TSC	300	99	300	120	300	92	300	71
35	2-Formylbenzothiazole TSC[o]	25	103	25	89	25	70	25	136
36	2-Formylquinoxaline TSC[g]	100	101	100	91	100	79	200	145
37	3-Hydroxy-2-formylquinoxaline TSC	200	95	200	98	100	114	100	106
38	3-Methoxy-2-formylquinoxaline TSC	250	109	250	74	100	90	100	105
39	4-Formylquinazoline TSC	28	144	28	57	40[c]	51	40[c]	47
40	2-Hydroxy-4-formylquinazoline TSC	200	100	200	65	150	69	150	114
41	4-Hydroxy-2-formylquinazoline TSC	200	100	200	79	200	73	200	113
42	2,3-Dimethyl-1-phenyl-4-formyl-3-pyrazolin-5-one TSC[b]	400	112	400	89	400	96	400	101
43	6-Formylpurine TSC[p]	35	135	30	91	30	55	30	73

[a] TSC = thiosemicarbazone. [b] R. E. Hagenbach and H. Gysin, *Experientia*, **8**, 184 (1952). [c] Drug given every other day. [d] See ref 15. [e] P. Hemmereich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, **41**, 2058 (1958). [f] Weight loss, result not significant. [g] F. E. Anderson, C. J. Duca, and J. V. Scudi, *J. Am. Chem. Soc.*, **73**, 4967 (1951). [h] See ref 14. [i] Aldehyde prepared by method of H. Rutner and P. E. Spoerri, *J. Org. Chem.*, **28**, 1898 (1963). [j] Dose dropped because of toxicity. [k] F. P. Doyle, W. Ferrier, D. O. Holland, M. D. Mehta, and J. H. C. Nayler, *J. Chem. Soc.*, 2853 (1956). [l] E. Hoggarth, A. R. Martin, N. L. Storey, and E. H. P. Young, *Brit. J. Pharmacol.*, **4**, 248 (1949). [m] F. Gialdi and R. Ponci, *Farmaco Sci. Tec.* (Pavia), **6**, 332 (1951). [n] See ref 10. [o] D. H. Jones, R. Slack, S. Squires, and K. R. H. Woolridge, *J. Med. Chem.*, **8**, 676 (1965). [p] V. M. Zubarovskii, *Dokl. Akad. Nauk SSSR*, **87**, 759 (1952). [q] L. Toldy, T. Nógrádi, L. Vargha, G. Ivánovics, and I. Koczka, *Acta Chim. Acad. Sci. Hung.*, **4**, 303 (1954). [r] A. Giner-Sorolla, I. Zimmerman, and A. Bendich, *J. Am. Chem. Soc.*, **81**, 2515 (1959).

Additionally, the usual design considerations apply. The thiosemicarbazone must not hydrolyze appreciably *in vivo* or monotonic convulsant activity will be produced. Absorbability and toxicity are complex phenomena and must be determined experimentally.

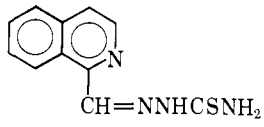
To test these hypotheses it was necessary to design two series of compounds. The first series would be active and conform to the stated requirements. The second series would be inactive. Each negative compound would contain, as nearly as possible, only one deviation from the hypothesis set. Before committing an extensive effort it was necessary to have evidence that additional active compounds could be produced and that the activity of I was not an isolated observation. 2-Formylpyrazine thiosemicarbazone (IV; Table I, **13**) was prepared and found more active and less



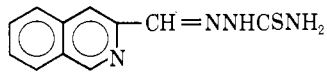
IV

toxic than I. Compound IV is also unique in its obvious *in vivo* chelation effects.[8]

To establish an additional activity the isoquinoline ring was chosen. Calculated π-electron densities[9] indicate that substituents in position 1 are activated as they are in the 2-pyridine derivatives and that position 3 is much less active and more nearly aromatic. Chemical evidence is in accord with this. Also both positions would yield configurations otherwise conforming to the positive specification. Both 1-formylisoquinoline thiosemicarbazone (V; Table I, **28**) and 3-formylisoquinoline thiosemicarbazone (VI; Table I, **32**) were prepared. It was predicted and found that V would be active[10] and better than IV and that VI would be inactive.

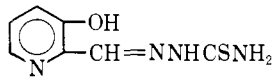


V



VI

Very recently in this study 3-hydroxy-2-formylpyridine thiosemicarbazone (VII; Table I, **7**) was prepared and found to be as active as V and perhaps better in some respects. The detailed data on the antitumor activity of this compound will be reported elsewhere.[11]



VII

Biological Activity

Antitumor Tests.—The methods used in this laboratory for the evaluation of antitumor activity in mice have been described elsewhere.[10] The finely ground drugs were suspended in a solution of sterile distilled water with a drop of Tween 80. Doses were determined by preliminary toxicity tests. The drugs were given daily, intraperitoneally, at approximately the maximum tolerated dose starting 24 hr after tumor inoculation. The index of evaluation in Table I is per cent treated/control. In leukemia L1210, % T/C = (mean survival time of treated mice/mean survival time of control mice)100. A T/C value of $\geqq$125 is considered positive In Sarcoma 180, Lewis lung carcinoma, and Adenocarcinoma 755, % T/C (mean tumor weight of treated mice/mean tumor weight of control mice)100. A % T/C value of $\leqq$50 is considered positive.

Results.—The level of toxicity and side effects varied greatly in this group. The following single dose (intraperitoneal) LD_{50} values were observed: **1**, 40 mg/kg; **13**, ~400 mg/kg; **28**, ~800 mg/kg; and **7**, 1000 mg/kg. Convulsant effects at toxic levels occur with **1**, **4**, **5**, **15**, **17**, **22**, and **35**. Compound **2** produces paralysis, while **14** is somewhat hypnotic and **21** is markedly so. Compound **25** induces a peculiar postmortem twitching.

Typical screening data are presented in Table I. These data, in the case of positive compounds, are not chosen as the best vaues but they are representative from a large amount of data. It may be noted that in no case where the full unencumbered N*—N*—S* ligand is not present was significant activity demonstrated. This is illustrated by **11**, **12**, **21–23**, **25**, **35**, and **42**. Referring to the general formula II we may systematically examine the results of modifying structural details at points (A) through (G). Substitution of methyl, ethyl, or phenyl for a hydrogen on the terminal nitrogen (A) was tried with **2**, **3**, **14**, **29**, and **30**. In each case activity was reduced or lost. Substitution at points (B) and (C) has not yet been tested in this series but was found adverse in a previous series.[4] Replacement of the aldehyde hydrogen in position (D) by methyl or 2-pyridyl (**4** and **5** relative to **1**) resulted in a loss of activity. Position (E) of the active compound **7** was quaternized to yield **10** which was completely inactive. In the pyridine derivatives, changing **1** to **6** by introducing a methyl group in position 6 (F) caused little change in therapeutic effect although potency per unit weight was reduced over tenfold. The same substitution, changing **7** to **9**, led to a complete loss of activity.

The effect of introducing a second aromatic ring, equivalent in the case of pyridine to closure at the 5 and 6 (F) positions, was examined in the case of **26**, **33**, and **36–38**. While none of these was active, information on this structural modification must be considered equivocal since none of these compounds was well absorbed. Derivatives with better solubility–distribution characteistics are required. Compound **35**, an isostere of **26**, is also inactive and fairly toxic. Where the position of formyl group attachment is changed so that the molecule cannot form the tridentate ligand. illustrated in III, no activity has been found. This modification is illustrated by **11**, **12**, **25**, and **34**. In **27** the N*—N* geometry is changed and a 6,5 ring chelate would be formed instead of the 5,5 system. Compound **27** is toxic and inactive.

A beneficial effect of the introduction of a second ring is illustrated by **28**. In this case there is no steric impedence to the chelation configuration caused by the second ring. Similar remarks apply to the 4-quinazoline derivative, **39**. Unfortunately, this compound turned out to be rather toxic. One would also expect the 6-formylpurine derivative (**43**) to be active. It is active but severely nephrotoxic.

The adverse effect of a high (aromatic) π-electron density at the aldehyde carbon is illustrated in **32** and **33**. This point cannot be assumed as certain, however, because significant drug residua were found at autopsy. In all cases examined to date, a hydrogen fixed to a ring nitrogen has an adverse effect. This feature is present in the case of **16**, **18–20**, and **24**. It may also be argued in the case of **40** and **41** that the potentially ligand ring nitrogen is largely in the NH form and hence

(8) F. A. French, A. E. Lewis, E. J. Blanz, Jr., and A. H. Sheena, *Federation Proc.*, **24**, 402 (1965).

(9) H. C. Longuet-Higgins and C. A. Coulson, *Trans. Faraday Soc.*, **43**, 87 (1947).

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

(11) F. A. French and E. J. Blanz, Jr., in preparation.

TABLE II

No.	Compd	Formula	Mp, °C	Calcd, %				Found, %			
				C	H	N	S	C	H	N	S
5	Bis(2-pyridyl) ketone TSC[a]	$C_{12}H_{11}N_5S$	194–196	56.01	4.31	27.22	12.46	56.06	4.89		12.32
8	3-Ethoxy-2-formylpyridine TSC	$C_9H_{12}N_4OS$	222–223 dec	48.19	5.39	24.98	14.30	47.84	5.46	24.42	14.49
9	3-Hydroxy-6-methyl-2-formylpyridine TSC	$C_8H_{10}N_4OS$	237 dec	45.20	4.74	26.65	15.25	45.30	4.93	26.39	15.32
10	3-Hydroxy-2-formyl-1-methylpyridinium iodide TSC	$C_8H_{11}IN_4OS$	218–219	28.41	3.28	16.57	9.48	28.40	3.55	16.65	9.52
14	2-Formylpyrazine 4′-methyl-TSC	$C_7H_9N_5S$	231–233	43.06	4.65	35.87	16.42	42.99	4.83	35.23	15.51
15	Bis(2-formylpyrazine) thiocarbohydrazone	$C_{11}H_{10}N_8S$	227–228	46.14	3.52	39.14	11.20	45.91	3.80	39.04	11.26
18	3-Formylpyrazole TSC[b]	$C_5H_7N_5S$	199–200	35.49	4.17	41.39	18.95	35.69	3.85	40.94	18.73
20	4-Formyl-1H-1,2,3-triazole TSC $\cdot 0.67H_2O$[c]	$C_4H_6N_6S\cdot 0.67H_2O$	201–203 dec	26.37	4.06	46.13	17.60	26.60	4.41	45.53	17.58
23	5-Methyl-2-formylfuran TSC[d]	$C_7H_9N_3OS$	160–161	45.88	4.95	22.93	17.50	46.05	5.12	22.64	17.58
29	1-Formylisoquinoline 4′-methyl-TSC[e]	$C_{12}H_{12}N_4S$	183.5–184.5	58.99	4.95	22.93	13.12	58.91	4.97	22.67	13.27
30	1-Formylisoquinoline 4′-ethyl-TSC $\cdot 0.75H_2O$[e]	$C_{13}H_{14}N_4S\cdot 0.75H_2O$	189–192	57.45	5.75	20.61	11.79	57.58	5.96	20.06	12.06
31	Bis(1-formylisoquinoline) thiocarbohydrazone monohydrate[e]	$C_{21}H_{16}N_6S\cdot H_2O$	196–197 dec	62.67	4.51	20.88	7.97	62.62	4.47	20.69	7.65
33	3-Formylcinnoline TSC[f]	$C_{10}H_9N_5S$	263 dec	51.94	3.92	30.28	13.86	51.74	4.06		13.81
34	4-Formylcinnoline TSC[g]	$C_{10}H_9N_5S$	260–261 dec	51.94	3.92	30.28	13.86	51.15	3.85		13.61
37	3-Hydroxy-2-formylquinoxaline TSC[h]	$C_{10}H_9N_5OS$	262–263 dec	48.57	3.67	28.32	12.97	49.02	3.76	28.88	13.29
38	3-Methoxy-2-formylquinoxaline TSC[i]	$C_{11}H_{11}N_5OS$	253–254 dec	50.55	4.24	26.80	12.27	50.61	4.39	27.00	12.14
39	4-Formylquinazoline TSC	$C_{10}H_9N_5S$	230–230.5 dec	51.94	3.92	30.28	13.86	52.08	4.10	30.16	13.66
40	2-Hydroxy-4-formylquinazoline TSC	$C_{10}H_9N_5OS$	244–244.5 dec	48.57	3.67	28.32	12.97	48.52	3.40		12.61
41	4-Hydroxy-2-formylquinazoline TSC	$C_{10}H_9N_5OS$	280–281 dec	48.57	3.67	28.32	12.97	48.32	3.79		12.66

[a] W. Mathes and W. Sauermilch, *Ber.*, **86,** 109 (1953). TSC = thiosemicarbazone. R. Huttel, *ibid.*, **74,** 1680 (1941). [b] J. C. Sheehan and C. A. Robinson, *J. Am. Chem. Soc.*, **71,** 1436 (1949). [d] Aldehyde available from L. Light and Co., Ltd., Coinbrook, England. [e] R. S. Barrows and H. G. Lindwell, *J. Am. Chem. Soc.*, **64,** 2430 (1942). [f] H. J. Haas and A. Secliger, *Ber.*, **96,** 2427 (1963). [g] R. N. Castle and M. Onda, *J. Org. Chem.*, **26,** 4465 (1961). [h] H. Olde, *Ber.*, **76,** 624 (1943). [i] Aldehyde prepared by the oxidation of 2-methoxy-3-(*d-arabino*-tetrahydroxybutyl)quinoxaline with sodium periodate.

the potentially favorable electronic configuration is largely eliminated.

The only instances of substituent effects clearly demonstrated are in the simple pyridine derivatives. Hydroxylation of **1** to yield **7** yields a very significant increase in depth and breadth of activity and at the same time reduces toxicity 25-fold. This hydroxyl group is essentially phenolic. Formation of the ethyl ether of **7** (**8**) diminishes activity.

In conclusion, there are many available ring systems, not yet explored, that meet the basic criteria established so far. Additionally, it would appear desirable to study substituent effects systematically.

Experimental Section[12,13]

The carbonyl thiosemicarbazones were, in general, prepared from the corresponding aldehyde or ketone and the thiosemicarbazides (Tables I and II). The carbonyl hydrazones in Table II are not reported in the literature but most of the carbonyl components and all of the thiosemicarbazides have been described. The references to the syntheses of these carbonyl compounds are referred to in Table II. The aldehydes for **8** and **9** have not been reported previously and were prepared by the oxidation of the appropriate 2-hydroxymethylpyridines with MnO_2.[14] 3-Hydroxy 2-formyl-1-pyridinium iodide was prepared from 3-hydroxy-2-formylpyridine and methyl iodide in acetone. The formylquinazolines were prepared *via* the nitrone method. The nitrones were not isolated in pure form but were hydrolyzed directly to the aldehydes and allowed to react with thiosemicarbazide.

2-Formylpyridine 4′-Methylthiosemicarbazone (2).—To 100 ml of hot ethanol was added 21.0 g (0.20 mole) of 4-methylthiosemicarbazide. After the compound dissolved, 21.4 g (0.20 mole) of freshly distilled 2-formylpyridine was added and heated at gentle reflux for 40 min. The solution was refrigerated overnight. The resultant crystals were filtered and washed with 200 ml of water, 50 ml of absolute ethanol, and dried, yielding 35.0 g (90.2%) of product, mp 250–251° dec.[15]

2-Formyl-3-hydroxy-6-methylpyridine.—5-Hydroxy-6-hydroxymethyl-2-picoline (13.9 g, 0.10 mole) and amorphous MnO_2 (8.7 g, 0.10 mole), prepared by heating manganous carbonate for 12 hr at 300–350°, were suspended in 200 ml of ethanol and heated with stirring to reflux temperature, and 10.2 g (0.10 mole) of 96% H_2SO_4 in 50 ml of ethanol was added over a period of 30 min. After additional heating under reflux for 2 hr, the black solid turned brown and the pH rose to 6. The reaction mixture was cooled to 40° and filtered. The dark yellow solution was evaporated *in vacuo* to a thick syrup and 200 ml of saturated $KHCO_3$ solution was added. The mixture was extracted with four 200-ml portions of ether. The extracts were filtered, dried ($MgSO_4$), and evaporated to a solid. After crystallizing from petroleum ether (bp 60–110°), 6.0 g (43.8%) of product was obtained, mp 105–106°.

Anal. Calcd for $C_7H_7NO_2$: C, 61.31; H, 5.15; N, 10.21. Found: C, 61.49; H, 5.12; N, 10.16.

2-Formyl-3-hydroxy-1-methylpyridinium Iodide Thiosemicarbazone (10).—3-Hydroxy-2-hydroxymethylpyridine hydrochloride (16.2 g, 0.10 mole) and amorphous MnO_2 (8.7 g, 0.10 mole) were suspended in 200 ml of ethanol and heated with stirring to reflux temperature, and 10.2 g (0.10 mole) of 96% H_2SO_4 in 50 ml of ethanol was added over a period of 30 min. After additional heating under reflux for 1 hr the black solid turned brown and the pH rose to 6. The work-up was the same as that of the previous compound with the exception that the crude aldehyde was not recrystallized but used in the crude form. The crude aldehyde was allowed to reflux with 28.4 g (0.20 mole) of methyl iodide in 50 ml of dry acetone for 3 hr. After the reaction mixture cooled it was evaporated to yield a solid. Attempts to crystallize this from various solvents gave only oils. Compound **10** was found to be very hygroscopic and further attempts to purify the product were abandoned. A total of 9.2 g of crude aldehyde was obtained; of this, 5.3 g, dissolved in 50 ml of water, was added to 50 ml of water containing 1.9 g (0.021 mole) of thiosemicarbazide. The solution was heated to reflux for 10 min and the solution was allowed to cool overnight. The thiosemicarbazone (4.3 g) (22% based on 3-hydroxy-2-hydroxymethylpyridine) separated as brilliant yellow crystals. The product was washed well with alcohol and ether and dried, mp 218–219° dec.

(4-Hydroxy-2-quinazolinoyl)methylpyridinium Iodide.—To 250 ml of dry pyridine was added 16.0 g (0.10 mole) of 2-methyl-4-hydroxyquinazoline and 25.4 g (0.20 g atom) of iodine. The dark solution was heated to reflux for 15 min and cooled, and 800 ml of dry benzene was added with stirring. The dark brown solid was filtered and washed with 50 ml of cold water, 50 ml of ethanol, and a final 50-ml ether wash. The crude product (25 g) had mp 238–239.5°. After the crude pyridinium iodide was treated with Norit A in 800 ml of boiling water and allowed to crystallize, 20.5 g (56%) of product was obtained, mp 245–246°.

Anal. Calcd for $C_{14}H_{12}IN_3O$: C, 46.04; H, 3.31; I, 34.75; N, 11.51. Found: C, 46.20; H, 3.36; I, 34.87; N, 11.39.

2-Formyl-4-hydroxyquinazoline Thiosemicarbazone (41).—To a mixture of 250 ml of chloroform and 250 ml of methanol was added 17.5 g (0.048 mole) of (4-hydroxy-2-quinazolinoyl)methylpyridinium iodide, 7.2 g (0.048 mole) of N,N-dimethyl-4-nitrosoaniline, and 20.8 g of 25% sodium methoxide–methanol solution. The solution was heated to boiling for 30 min and then evaporated *in vacuo* to a thick dark red oil. All attempts to isolate the pure nitrone failed. The crude nitrone was treated with 400 ml of 3.7% HCl and heated for 10 min at 60°. The resulting red solution was treated with 3 g of Norit A for 10 min and filtered. The aldehyde solution was then added to 200 ml of boiling water containing 5 g (0.055 mole) of thiosemicarbazide. An orange precipitate formed when the pH was adjusted to 5 with sodium acetate solution. The solution was allowed to cool to about 40° and filtered. The precipitate was washed successively with 100 ml of boiling water, three 100-ml portions of boiling alcohol, and 100 ml of ether and dried. The yield of the thiosemicarbazone was 8.2 g (66.7%), mp 280–281° dec.

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(12) Melting points are corrected and were measured on a Thomas–Hoover capillary melting point apparatus.

(13) Microanalyses were performed by the Berkeley Analytical Laboratory, Berkeley, Calif., and by Micro-Analysis, Inc., Wilmington, Del.

(14) D. Heinert and A. E. Martell, *Tetrahedron*, **3**, 49 (1958).

(15) F. Fujikawa, K. Hirai, M. Naito, and S. Tsukuma, *Yagugaku Zasshi*, **79**, 1231 (1959), reported a melting point of 224–226°.