Potential Antitumor Agents. IV. 4-Substituted 1-Formylisoquinoline Thiosemicarbazones[1]

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Received November 12, 1969

To ascertain the structural requirements for the biological activity of 1-formylisoquinoline thiosemicarbazone (I), a potent antineoplastic agent, substituents such as OH and OAc, two groups that increased therapeutic potency when substituted at the 5 position of I, have been introduced at the 4 position. Syntheses were accomplished by rearrangement of 1-methylisoquinoline *N*-oxide (II) with Ac_2O; acid hydrolysis resulted in two major products, 4-hydroxy-1-methylisoquinoline (III) and 1-hydroxymethylisoquinoline (IV). A third compound was isolated in relatively small yield and was characterized by nmr and mass spectroscopy to be α,α'-bis(1-isoquinolyl)ethylene glycol (IX). Oxidation of 4-acetoxy-1-methylisoquinoline (VIII) with SeO_2 produced the corresponding 1-carboxaldehyde, which on acid hydrolysis yielded the 4-hydroxy derivative. Treatment of heterocyclic aldehydes with thiosemicarbazide produced the desired thiosemicarbazones. The 4-substituted derivatives were less effective than I as antineoplastic agents against Sarcoma 180 ascites cells in mice; however, the Na salt of the 4-OH derivative was considerably more efficacious than I on the L1210 lymphoma. The findings in this system compared with those previously reported indicate that the Na salt of 4-hydroxy-1-formylisoquinoline thiosemicarbazone (XIII) possessed the highest therapeutic index of the active antineoplastic agents in the isoquinoline series.

α-(*N*)-Heterocyclic carboxaldehyde thiosemicarbazones have considerable potential as antineoplastic agents.[3] One of the compounds in this class, 1-formylisoquinoline thiosemicarbazone (I), has been shown[4] to cause pronounced inhibition of the growth of a relatively wide spectrum of transplanted rodent tumors. The biochemical basis for the carcinostatic activity of I has been studied in our laboratory;[5] this agent caused marked inhibition of the synthesis of DNA by preventing the conversion of ribonucleotides into deoxyribonucleotide forms, and lesser blockade of the formation of RNA and protein.

As part of a program designed (a) to develop antineoplastic agents with greater therapeutic efficacy and (b) to define the structural requirements of this new class of compounds for inhibition of growth, I has been subjected to systematic structural modification. The initial approach employed was the synthesis of a series of 5-substituted derivatives of I.[6] The overall dimensions of I in this position could be modified with the retention of high activity, since substituents such as 5-OH and 5-OAc were found to confer therapeutic indices to the resultant derivatives that were higher than for the parent compound. A number of alterations were also made in the formyl thiosemicarbazone side chain;[7] modifications made at the various positions of the side chain resulted in either a decrease or a complete loss of antineoplastic activity indicating that the structure of this portion of the molecule is critical.

It was deemed desirable to elaborate further on the effects of substituent groups at different positions of the isoquinoline ring. Position 4, being *para* to position 1, was considered to be of prime importance with regard to the exertion of electronic effects by the substituent group on the thiosemicarbazone side chain. The two groups, OH and OAc, that produced increased therapeutic potency when substituted at the 5 position were chosen for introduction at position 4.

Chemistry.—The synthesis of 4-substituted 1-methylisoquinolines was carried out by utilizing the procedure[8] for the rearrangement of isoquinoline *N*-oxide and 3-methylisoquinoline *N*-oxide. Both of these compounds on refluxing with Ac_2O were shown to rearrange mainly to derivatives of the corresponding isocarbostyrils; small amounts of the corresponding 4-hydroxyisoquinoline derivatives were also formed. No 3-OH or 3-CH_2OH derivatives were isolated from the reactions presumably because attack at the 3 position would disrupt the aromaticity of the benzene ring. When 1-methylisoquinoline *N*-oxide (II) was treated with refluxing Ac_2O and subsequently distilled, a mixture of esters was obtained which could not be separated effectively by either fractional distillation or chromatography on columns of alumina, charcoal, or silica gel. No further attempt was made to isolate the easily hydrolyzable acetyl derivatives; instead, the mixture was subjected directly to acid hydrolysis (Scheme I). Two major products, 4-hydroxy-1-methylisoquinoline (III) and 1-hydroxymethylisoquinoline (IV), were isolated. These derivatives were readily separated by their differences in solubility in NaOH. When II was treated with $POCl_3$ in $CHCl_3$, only one product, 1-chloromethylisoquinoline (V), was obtained. The identity of V was demonstrated by mixture melting point and by comparison of the ir spectrum with that

(1) Presented in part before the Division of Medicinal Chemistry at the 156th National Meeting of the American Chemical Society, Atlantic City, N. J. Sept 1968; This work was supported by Grant CA-02817 from the National Cancer Institute, U. S. Public Health Service, and Grant T-23 from the American Cancer Society and the National Institutes of Health (HE 03558 and FR 00356) and the National Aeronautics and Space Administration (NAS 9-8072 and NGL 07-004-008) (Section of Physical Sciences).

(2) Section of Physical Sciences.

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

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

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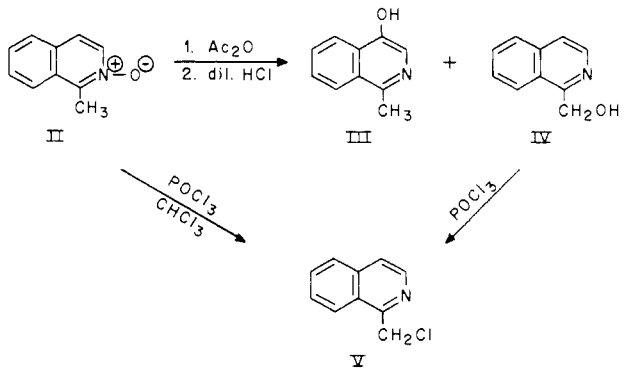
(6) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, **11**, 700 (1968).

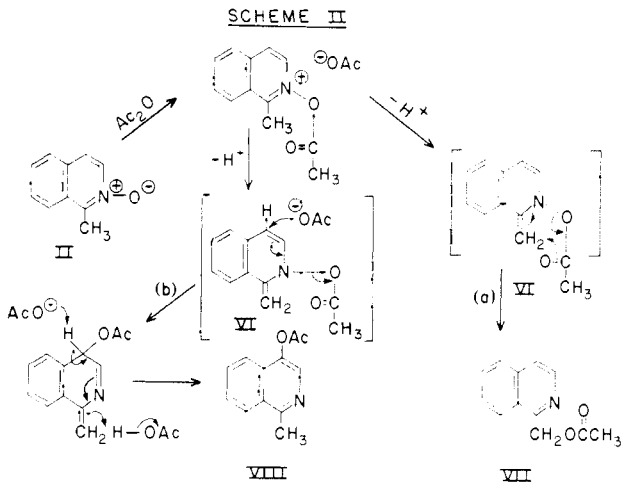
(7) K. C. Agrawal and A. C. Sartorelli, *ibid.*, **12**, 771 (1969).

(8) (a) M. M. Robison and B. L. Robison, *J. Org. Chem.*, **21**, 1337 (1957); (b) *J. Amer. Chem. Soc.*, **80**, 3443 (1958).

SCHEME I



SCHEME II

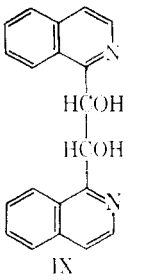


of the compound prepared from the reaction of $POCl_3$ and IV.

Several possible mechanisms including ionic[9] and free radical[10] formation have been proposed for the analogous rearrangement of 2-picoline *N*-oxide. In the case of the isoquinoline series, where no rearrangement products at position 3 have been isolated, an intermediate (VI, Scheme II) resulting from abstraction of an active H from the 1-Me by AcO^- would be reasonably expected to predominate in the transformations. Such an intermediate cannot be formed from 3-methylisoquinoline, since it would result in the disruption of the aromaticity of the benzene ring. Conversion of VI to the 1-methylacetate derivative (VII, Scheme II, pathway a) may result from an intramolecular rearrangement analogous to that suggested for 2-picoline *N*-oxide.[11] Rearrangement to the 4 position of the isoquinoline ring most probably occurs by a series of addition and elimination reactions (Scheme II, pathway b) to produce 1-methyl-4-acetoxyisoquinoline (VIII).

During the recrystallization of IV, an unknown compound was isolated in relatively small yield; this was characterized by nmr and mass spectra to be α,α'-bis(1-isoquinolyl)ethylene glycol (IX). Formation of

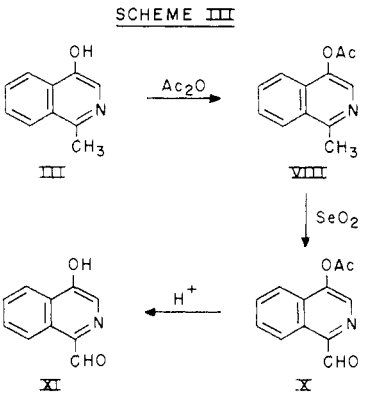
(9) I. J. Pachter, *J. Amer. Chem. Soc.*, **75**, 3926 (1953).
(10) V. Boekelheide and D. L. Harrington, *Chem. Ind.* (London) 1423 (1955).
(11) V. J. Traynelis and R. F. Martello, *J. Amer. Chem. Soc.*, **80**, 6590 (1958).



IX would not be expected during the rearrangement reaction; instead, when IV was exposed to air slow oxidative condensation occurred to form IX.

Acetylation of III produced VIII (Scheme III), which

SCHEME III



was then oxidized with a molar equivalent of SeO_2 in dioxane to give 4-acetoxyisoquinoline-1-carboxaldehyde (X). On acid hydrolysis X produced 4-hydroxyisoquinoline-1-carboxaldehyde (XI). Direct oxidation of III to yield XI resulted in poor yields. Both X and XI were reacted with thiosemicarbazide to produce the desired thiosemicarbazones.

Nmr Studies.—The nmr parameters for all of the compounds are consistent with the proposed structures. Resonance signals due to replaceable H^+ (OH, $=NH$) were verified by addition of strong acid or base to the nmr sample tubes.

The nmr spectrum of 1-methylisoquinoline contained an aromatic doublet at δ 8.40 which was assigned to H-3 since it was found at lowest field ($J_{3,4} = 5.7$ Hz). In II, field and diamagnetic anisotropy effects due to $N\rightarrow O$ caused H-3 to be shifted upfield by 0.22 ppm and the 1-CH_3 resonance signal to be shifted upfield by 0.16 ppm. In the case of III and VIII, singlets at δ 8.00 and 8.20, respectively, were assigned to H-3. The upfield shift of H-3 in these instances was due to the adjacent O. The H-3 singlet in both III and VIII was superimposed upon other aromatic H^+; however, assigment of the signal was readily made. The nmr spectrum of IX showed the presence of 6 aromatic H^+, an AB quartet centered at δ 5.66 ($J_{AB} \sim 4.2$ Hz), and no Me or CH_2 resonance signals. Addition of trifluoroacetic acid to the DMSO-d_6 solution of IX caused the disappearance of the upfield doublet of the AB quartet at δ 5.41, and the collapse of the low-field doublet at 5.90 to a singlet (6.23). The upfield doublet was assigned to an OH resonance signal, while the low-field doublet was assigned to CH. Thus, nmr data indicated the dimeric structure proposed for IX. The nmr spec-

trum of IX changed after being dissolved in the DMSO-d_6 solution for a relatively long period of time; this effect was most likely due to slow oxidation of the vicinal diol moiety by the solvent.

The nmr spectrum of XII gave a singlet at δ 8.49 which was assigned to H-3, and a three-proton singlet at 2.53 which was assigned to the OAc group of XII.

Mass Spectrometry.—The mass spectrum of IX was characterized by a weak molecular ion (<1%) at *m/e* 316 which on the basis of high resolution mass measurement corresponded to $C_{20}H_{16}N_2O_2$ (obsd, 316.118; calcd, 316.121). The mass spectrum was dominated by two groups of intense peaks. The first set occurred at *m/e* 157 (55%), 158 (69%), and 159 (63%) and had the elemental composition $C_{10}H_7NO$, $C_{10}H_8NO$, and $C_{10}H_9NO$, respectively. The second group was present at *m/e* 128 (63%), 129 (100%), and 130 (90%) and possessed the elemental composition C_9H_6N, C_9H_7N, and C_9H_8N, respectively. The molecular weight plus the similarity of the lower mass portion of the mass spectrum with 1-substituted isoquinolines suggested the glycol IX.

The intense fragments noted are readily explained by direct homolytic cleavage of the glycol C–C bond to yield the fragment at *m/e* 158. H transfer accompanies this reaction, producing the fragments at *m/e* 157 and 159, corresponding to isoquinoline-1-carboxaldehyde and III. Such fragmentation was observed for 1-(2-furanyl)-2-phenylethan-1,2-diol, and the H transferred was determined to be the OH. However, with the unsymmetrical glycol the fragment corresponding to the aldehyde was not observed.[12]

Additional evidence for the structure of IX was obtained by treatment with bistrimethylsilylacetamide in MeCN. The mass spectrum of the product showed an intense molecular ion at *m/e* 460, which resulted from the addition of two trimethylsilyl groups, a finding compatible with the proposed structure. The mass spectrum also showed a single strong fragment (base peak) at *m/e* 230 derived from the expected homolytic cleavage of the C–C glycol bond. The fragment derived from the H transfer reaction was suppressed by removal of the transferable hydrogen by the trimethylsilyl groups.[12]

The mass spectrum of the product of periodate oxidation (1.4 equiv) of IX possessed intense fragments at *m/e* 157 (57%), 129 (100%), and 128 (35%) and was identical with the mass spectrum of authentic isoquinoline-1-carboxaldehyde. This observation confirmed the presence of the glycol group in IX.

Biological Results and Discussion.—The effects of 4-substituted derivatives of I on the survival time of Sarcoma 180 tumor-bearing mice are shown in Table I. The results indicated that both XII and XIII were active antitumor agents. Maximum average survival times of 30.6 and 23.5 days were obtained under these conditions with XII and XIII, respectively, as compared with untreated control tumor-bearing mice, which survived for only 12.5 days. Neither of the compounds demonstrated toxicity for the host as measured by drug-induced loss in body weight. The finding that the 4- OAc compound is relatively nontoxic is in contrast to that observed for the 5-OAc derivative, which caused a 14.5% loss in body weight at the daily dose of 120 mg/kg.[6]

TABLE I
EFFECT OF 4-SUBSTITUTED 1-FORMYLISOQUINOLINE THIOSEMICARBAZONES ON THE SURVIVAL TIME OF MICE BEARING SARCOMA 180 ASCITES CELLS

Compound	Daily dosage (mg/kg)[a]	Av Δ wt (%)[b]	Av survival (days) ± SE
Control	None	+17.3	12.5 ± 0.29
4-Acetoxy-1-formylisoquinoline thiosemicarbazone (XII)	20	+12.2	12.4 ± 0.29
	40	+6.8	19.2 ± 0.74
	80	0.0	23.6 ± 2.18
	120	+5.0	30.6 ± 3.60 (20)[c]
	160	+0.7	25.4 ± 3.48 (20)
4-Hydroxy-1-formylisoquinoline thiosemicarbazone (XIII)	20	+24.6	14.2 ± 0.89
	40	+23.9	17.9 ± 0.63
	80	+1.9	23.2 ± 0.72
	120	+3.8	23.5 ± 0.63
	160	+5.3	22.8 ± 0.62

[a] Administered once daily for 6 consecutive days, beginning 24 hr after tumor transplantation. [b] Average weight change from onset to termination of drug treatment. [c] Percentage of mice surviving over 50 days. These were calculated as 50-day survivors in determination of the average survival time; 5-15 animals were employed in each group.

TABLE II
EFFECT OF 1-FORMYLISOQUINOLINE THIOSEMICARBAZONE AND ITS 4-HYDROXY DERIVATIVE ON THE SURVIVAL TIME OF MICE BEARING THE L1210 LYMPHOMA

Compound	Daily dose (mg/kg)[a]	Av Δ wt (%)[b]	Av survival (days) ± SE
Control	None	−5.2	8.4 ± 0.07
1-Formylisoquinoline thiosemicarbazone (I)	20	+10.5	11.8 ± 0.33
	40	+2.4	16.6 ± 0.91
4-Hydroxy-1-formylisoquinoline thiosemicarbazone (XIII)	30	−2.8	11.4 ± 0.46
	40	−1.0	13.4 ± 0.23
	60	−1.8	12.1 ± 0.22
Sodium salt of XIII	32.7	−2.9	25.4 ± 1.13 (10)[c]
	43.6	−1.9	26.7 ± 0.72 (13)
	65.4	−1.4	26.2 ± 1.05

[a] Administered twice daily for 4 consecutive days at 12-hr intervals beginning 24 hr after tumor transplantation. [b] Average weight change from onset to termination of drug treatment. [c] Percentage of mice surviving over 50 days. These were calculated as 50-day survivors in determination of the average survival time; 10–15 animals were employed in each group.

It has been reported[13] that the Na salt of the 5-OH derivative of I caused a greater prolongation of the survival time of mice bearing the L1210 lymphoma than did the parent derivative. Therefore, the Na salt of XIII was synthesized and tested in the same system; the results are shown in Table II. The parent compound I was also evaluated under these conditions. Untreated control tumor-bearing animals had an average survival time of 8.4 days. Compounds I and XIII at a daily dose of 40 mg/kg increased the survival time to 16.6 and 13.4 days, respectively, whereas the Na salt of XIII caused a pronounced increase in the life-span of the tumor-bearing mice; with a maximum effective level (43.6 mg/kg per day) this soluble derivative

(12) J. Kossanyi, J. P. Morizur, B. Furth, J. Wiemann, A. M. Duffield, and C. Djerassi, *Org. Mass Spectrom.*, **1**, 777 (1968).

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

caused a prolongation of survival time to 26.7 days. Since little toxicity, as measured by loss in body weight, was encountered, it would appear that the Na salt of XIII has the highest therapeutic index observed to date of the active compounds in the isoquinoline series, and is therefore the prime candidate of this series for clinical evaluation for antineoplastic activity in man. Since the Na salt would be expected to yield XIII *in vivo* it is difficult to explain readily the relatively high potency of this derivative.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses[14] were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, New York. Nmr spectra were performed on a Bruker HFX-3 spectrometer operating at 90 MHz. Chemical shifts (δ) are given in parts per million downfield from TMS in DMSO-d_6 (d, doublet; s, singlet; dd, doublet of doublets; etc.). Mass spectra were obtained on an Associated Electrical Industries (Manchester, England) MS-9 mass spectrometer. The low resolution spectra ($M/\Delta M \sim 1500$) were obtained at 70 eV and 100 μA ionizing current, while the mass measurements were obtained at 70 eV and 500 μA ionizing current and at an instrument resolution of 10,000. The source temperature varied between 150 and 200°. All samples were introduced *via* the direct insertion probe.

Biological Methods.—Compounds were tested for antineoplastic activity in mice bearing either Sarcoma 180 ascites cells or the L1210 lymphoma. Complete details of the biological methods have been described earlier.[6] Transplantation of the neoplasms was accomplished by inoculating mice intraperitoneally with approximately 4-8 $\times$ 10^6 ascites cells. Drugs were administered by intraperitoneal injection beginning 24 hr later either as fine suspensions or as a solution in distilled H_2O (Na salt of XIII); such therapy was continued once daily for 6 consecutive days in the case of mice bearing Sarcoma 180 ascites cells. Mice bearing the L1210 lymphoma were treated twice daily at 12-hr intervals for 4 consecutive days. Determination of the sensitivity of the tumors to these agents was based upon the prolongation of survival time afforded by the drug treatments.

Chemical Methods. 1-Methylisoquinoline *N*-Oxide (II).—The procedure employed by Robison and Robison[8a] for the fabrication of isoquinoline *N*-oxide was utilized to prepare II in 72% yield. Recrystallization (hexane) gave white needles, which after drying ($CaCl_2$) under vacuum had a mp 88-89° (reported[15] 82-83°); nmr as expected. *Anal.* ($C_{10}H_9NO$) C, H, N; loss in weight after drying at 50° for 2 hr, 1.17%.

Rearrangement of II. (A) By Ac_2O.—Compound II (5.0 g) was refluxed in 50 ml of Ac_2O for 2.5 hr. The excess Ac_2O was removed by flash evaporation; the resulting dark oily residue was then distilled under vacuum at 160-162° (1 mm) to yield 4.7 g (75%) of a mixture of acetates as a pale yellow oil. This oil was taken up in 10% HCl (50 ml) and heated for 1 hr at 100°. The solution, which turned red during heating, was treated with Norit-A and filtered. The filtrate was made strongly alkaline with a solution of 20% NaOH. An oily compound resulted which was extracted with Et_2O. The Et_2O extracts were washed (H_2O), dried (Na_2SO_4), and removed by flash evaporation to give an oil which crystallized on cooling to a pale yellow compound (IV), 1.5 g (30%) mp 72-74°. Recrystallization (hexane) gave colorless crystals: mp 78-79°; nmr δ 5.15 (s, 2 H, CH_2), 5.40 [s (broad), 1 H, OH]. *Anal.* ($C_{10}H_9NO$) C, H, N.

The alkaline solution from the above experiment was neutralized to pH 7.0 with 20% HCl. A light brown precipitate formed which was filtered, washed (H_2O), and dried to yield III: 1.15 g (23%); mp 204-207° dec. Crystallization (C_6H_6) gave colorless crystals: mp 210-212° dec; nmr δ 2.80 (s, 3 H, Me), 10.23 [s (broad), 1 H, OH]; strong band in ir at 3430 cm^{-1} (OH). *Anal.* ($C_{10}H_9NO$) C, H, N.

(B) By $POCl_3$.—Compound II (2.0 g) was dissolved in 100 ml of $CHCl_3$ and cooled in ice. $POCl_3$ (10 ml) was added dropwise with stirring. The reaction mixture was then refluxed at 60-70° for 8 hr. During the heating a precipitate was formed that was decomposed in 100 g of ice and made alkaline with Na_2CO_3. After standing overnight, the mixture was extracted with $CHCl_3$, the $CHCl_3$ extracts were dried (Na_2SO_4), and the $CHCl_3$ was removed to give an oil that crystallized on cooling to yield 1.6 g (73%) of V, mp 58-60°. Recrystallization from petroleum ether gave colorless crystals: mp 65-66°; nmr as expected. *Anal.* ($C_{10}H_8NCl$) C, H, N.

1-Acetoxymethylisoquinoline (VII).—Acetylation of IV with Ac_2O as described for VIII produced colorless crystals from hexane, yield 86%, mp 47°. *Anal.* ($C_{12}H_{11}NO_2$) C, H, N.

4-Acetoxy-1-methylisoquinoline (VIII).—Compound III (1.59 g) was refluxed with 10 ml of Ac_2O for 30 min; excess Ac_2O was removed and the remaining oil was crystallized (hexane) in prisms to yield 1.9 g (90%): mp 74-75°; nmr as expected; ir, 1740 cm^{-1}. *Anal.* ($C_{12}H_{11}NO_2$) C, H, N.

α,α'-Bis(1-isoquinolyl)ethylene Glycol (IX).—Recrystallization of IV, obtained from Et_2O extracts in experiment II (A), from hexane gave a hexane-insoluble compound that was then recrystallized several times from abs EtOH to yield white crystals, mp 180-181° dec. *Anal.* ($C_{20}H_{16}N_2O_2$) C, H, N.

4-Acetoxyisoquinoline-1-carboxaldehyde (X).—Compound VIII (1.0 g) was oxidized with 0.55 g of SeO_2 in 25 ml of dioxane. The procedure was followed as described earlier,[6] to give 0.53 g (50%) of the corresponding aldehyde. Recrystallization from hexane (Norit-A) gave fine, long, colorless needles, mp 117-118°; ir as expected. *Anal.* ($C_{12}H_9NO_3$) C, H, N.

The thiosemicarbazone derivative (XII) had mp 223-224° dec. *Anal.* ($C_{13}H_{12}N_4O_2S$) C, H, N, S.

4-Hydroxy-1-formylisoquinoline Thiosemicarbazone (XIII).—Compound X (0.5 g) was heated with 5 ml of 10% HCl at 100° for 30 min. The red colored solution was treated with Norit-A and the clear filtrate was treated directly with thiosemicarbazide, followed by neutralization with NaOAc; the yellow precipitate that formed was filtered, washed (H_2O), and dried, mp 183-184° dec. *Anal.* ($C_{11}H_{10}N_4OS$) C, H, N, S.

Na salt of XIII was synthesized as described earlier.[13]

Acknowledgment.—The authors wish to thank Miss Andrea F. Gorske and Mr. Karim A. Alkadhi for their excellent assistance.

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

(15) E. J. Moriconi and F. A. Spano, *J. Amer. Chem. Soc.*, **86**, 38 (1964).