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Notes

Elucidation of the Structure of the Antineoplastic Agents, 2-Formylpyridine and 1-Formylisoquinoline Thiosemicarbazones

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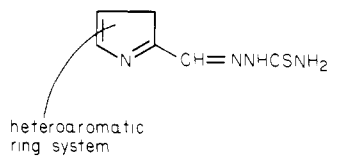
The geometrical isomers of the antineoplastic agents 2-formylpyridine and 1-formylisoquinoline thiosemicarbazones were synthesized and their structure was studied by spectroscopic methods. It was found that the compounds previously described in the literature and tested for carcinostatic activity were isomers with *E* configuration which probably contained minor amounts of *Z* isomers.

α-*N*-Formyl heterocyclic thiosemicarbazones are potential antineoplastic agents, particularly thiosemicarbazones of 1-formylisoquinoline and 2-formylpyridine, and a multitude of their derivatives have been examined for activity against a variety of transplanted animal tumors.[1,2] These compounds primarily block DNA synthesis in mammalian cells by inhibiting the enzyme ribonucleoside-diphosphate reductase, presumably either via chelation with an iron ion required by the enzyme or because a preformed metal chelate of the inhibitor interacts with the target enzyme.[3-7] French and Freedlander[8] proposed a sterically noncommittal ligand model for these compounds. Then French and Blanz[9] postulated the general formula 1 as a model for this series of antineoplastic agents.



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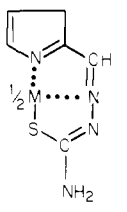

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This formula can be rewritten in the S–H tautomeric form. Formula **2** illustrates the functioning of the α-*N*-formyl heterocyclic thiosemicarbazones as tridentate ligands. A requirement for this was, according to French and Blanz, that the thiosemicarbazone is in the *Z* (syn) form. This statement was rectified by Mathew and Palenik[10] who performed a x-ray diffraction study and a complete structural formulation on bis(isoquinoline-1-carboxaldehyde thiosemicarbazanato)nickel(II) monohydrate. They clearly established that in the transition metal chelates the α-*N*-formyl heterocyclic thiosemicarbazones act as tridentate ligands in the thiol form. Besides, their work ascertained that, in the chelate form, the aldimine bond is *E* and that the geometry of the other double bond (pseudothiourea) is *Z*.

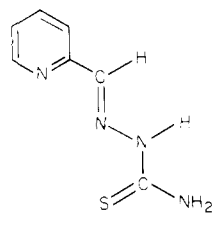
In order to verify if the ligand also has a structure similar to that of the chelate, we synthesized the geometrical isomers of the 2-formylpyridine and 1-formylisoquinoline thiosemicarbazones, two known tumor-inhibitory agents,[4,11,12] studying their structure by spectroscopic methods.

Chemistry and Spectral Studies. 2-Formylpyridine and 1-formylisoquinoline thiosemicarbazones (PT and IQ-1) were synthesized from 2-formylpyridine or 1-formylisoquinoline and thiosemicarbazide in hot ethanol according to the methods described in the literature.[13-15] In both cases, the reaction between the aldehyde and thiosemicarbazide gave pale yellow crystals which consist of a mixture of geometrical isomers [(*E*)- and (*Z*)-PT, (*E*)- and (*Z*)-IQ-1], as was checked by TLC, in which the slower eluted isomers largely prevail. When these crystals were purified by crystallization from EtOH, the slower eluted pure isomers of PT and IQ-1 were obtained. The faster eluted isomers were separated by column chromatography on silica gel. As the yields of faster eluted isomers were very low, we experimented with several isomerization methods with the aim of isomerizing the slowly eluted isomers. We found that the most convenient method consists of heating the slowly eluted isomers with SiO_2 in methanol. In this way the faster eluted isomers in about 30% yields were obtained. In solution, in several protic and aprotic solvents, they are labile and within some time are converted to a mixture of the geometrical isomers; however, in the dry crystalline state they are stable for several days.

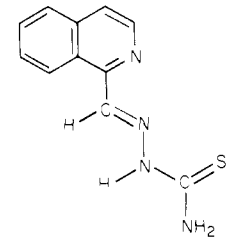
Qualitative tests in methanol show that with iron(II) the slowly eluted isomers of PT and IQ-1 form intensely colored complexes (red and dark green, respectively); the faster eluted isomer of PT gives a light green color which on standing changes into red, whereas the faster eluted isomer of IQ-1 gives no coloring within 10 min; after this time a green color appears. We did not verify if this transition metal acts as a catalyst in the isomerization process of the faster eluted isomers or if only the solvent is responsible for that.

Comparison of the NMR spectra in dimethyl-d_6 sulfoxide at 60 MHz revealed that in the PT and IQ-1 slower eluted isomers, the proton H_a linked to the carbon of the

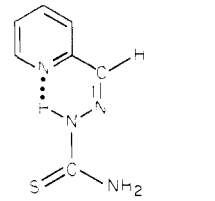
thiosemicarbazone group gave a signal downfield relative to that of the corresponding proton of the faster eluted isomers (see Table I). These chemical shift differences allow the assignment of the *Z* (syn) configuration to the thiosemicarbazone group of the faster eluted isomers and the *E* (anti) configuration to the others.[16]



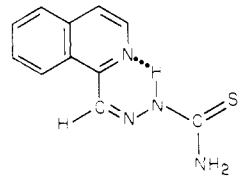
(*E*)-PT



(*E*)-IQ-1

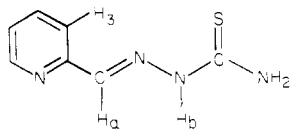


(*Z*)-PT



(*Z*)-IQ-1

Deshielding is observed to be marked mostly for the imino proton H_b in the *Z* isomers and the very large downfield shifts are typical of a proton involved in intramolecular hydrogen bonding.[17] Moreover, we observed that three of the ring protons of (*Z*)-PT (H_4, H_5, and H_6) and the two ring protons of the pyridine ring of (*Z*)-IQ-1 (H_3 and H_4) are deshielded relative to the same protons of the *E* isomers. This deshielding is consistent with a withdrawal of electron density from the pyridine ring via the nitrogen atom which is involved in hydrogen bonding. The fact that H_3 is more shielded in (*Z*)-PT than in (*E*)-PT may be rationalized by considering that in the rotational conformation 3 of (*E*)-PT H_3 can experience deshielding due to the magnetic anisotropy of the >C=N- group and that of the sp^2-hybridized exocyclic nitrogen atom; the chemical shifts for H_3, δ (*Z*–*E*), are in accord with those noted for 2-formylpyridine 2′-pyridylhydrazone.[17]



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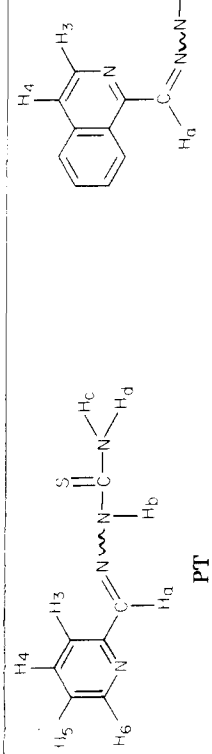
The presence of intramolecular hydrogen bonding in (*Z*)-PT and (*Z*)-IQ-1 was confirmed by comparison of the UV spectra of the *E* and *Z* isomers; *Z* isomers, in fact, have an absorption maximum at longer wavelength than *E* isomers (see Table I).[18]

The infrared spectra of the geometric isomers in Nujol show ν (N–H) between 3420 and 3120 cm^{-1}, but no ν (S–H) at 2570 cm^{-1}, indicating that in the solid state the isomers exist in the form 1. However, in solution and in presence of some metal ions the *E* isomers probably exist in equilibrium with the tautomeric S–H form 4 which could act as a singly charged tridentate ligand by coordinating through the mercapto sulfur and the asterisked nitrogens.

In conclusion, it may be affirmed that the 2-formylpyridine and 1-formylisoquinoline thiosemicarbazones, prepared according to the methods described in the literature, are mixtures of geometric isomers in which the isomer with an *E* (anti) configuration, similar to that of the nickel(II) chelate of IQ-1, predominates; in the solid

Table I. Physical Properties and Spectral Parameters for (*E*)-PT, (*Z*)-PT, (*E*)-IQ-1, and (*Z*)-IQ-1

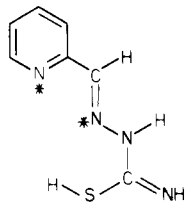


PT

IQ-1

Compd	R_f[a]	Mp, °C	Formula[b]	NMR chemical shifts (δ)							IR max, cm^{-1}[c]	UV max, nm (ϵ)
				H_a	H_b	H_c	H_d	H_3	H_4	H_5	H_6	
(*E*)-PT	0.48	207–208	$C_7H_8N_4S$	8.13	11.60	8.30	8.15	8.28	7.74	7.40	8.60	3420, 3245, 3150	323 (35 220)
(*Z*)-PT[d,e]	0.84		$C_7H_8N_4S$	7.49	14.15	8.62	8.29	7.85	8.20	7.66	8.80	3370, 3255, 3170	332 (24 310)
(*E*)-IQ-1	0.49	234–235	$C_{11}H_{10}N_4S$	9.23	12.18	9.02	8.30–8.80	9.14	8.45			3370, 3260, 3120	312 (11 430), 357 (23 100)
(*Z*)-IQ-1[d,e]	0.88		$C_{11}H_{10}N_4S$	9.05	15.60	9.40	9.05	9.36	8.70			3370, 3260, 3120	268 (7 700), 277 (s) (6 200), 305 (6 307), 318 (6 700), 357 (6 860), 372 (6 700)

[a] TLC, SiO_2–EtOAc. [b] The compounds were analyzed for C, H, N, and S. [c] Only ν N–H are shown. [d] Not crystallizable. [e] It is impossible to determine the melting points of the *Z* isomers because they undergo a complete thermic isomerization before melting.



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state the geometric isomers of these thiosemicarbazones exist as thione tautomers.

Experimental Section

Melting points were determined on a Kofler micro hot bench and are uncorrected. NMR spectra were obtained with a JEOL JNM C60-HL spectrometer [sodium 3-(trimethylsilyl)propanesulfonate as internal standard]. The IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. TLC was carried out on TLC plates prepared with silica gel GF_{254} Merck (EtOAc as eluent). Spots were detected with an UV lamp (λ 254 nm). For column chromatography silica gel 60 Merck was used.

(*E*)- and (*Z*)-2-Formylpyridine and 1-Formylisoquinoline Thiosemicarbazones [(*E*)- and (*Z*)-PT, (*E*)- and (*Z*)-IQ-1]. The geometric isomers were prepared by heating for 45 min the corresponding aldehydes (0.03 mol) in 100 ml of EtOH with an equimolecular amount of thiosemicarbazide. The products, which separated as pale yellow crystals by cooling the reaction mixture, consist of *E* isomers contaminated by a small amount of *Z* isomers (as was checked by NMR and TLC); they were collected by filtration and purified by crystallization from EtOH: yields 75% for (*E*)-PT and 70% for (*E*)-IQ-1. The evaporation of the filtrate gave a residue which was dissolved with a mixture of MeOH–EtOAc and chromatographed on a silica gel column eluting with EtOAc. Removal of the solvent from the portion of the eluate containing the faster eluted isomers gave (*Z*)-PT and (*Z*)-IQ-1: yields 3 and 5%, respectively.

***Z* Isomers by Isomerization of *E* Isomers.** A suspension of 1 g of *E* isomer and 5 g of silica gel (0.08 mm) Merck in 50 ml of MeOH was heated for 60 min. Evaporation of the solvent gave a residue which was extracted with an hot mixture of MeOH–EtOAc. The solution was chromatographed on a silica gel column with EtOAc as eluent. Removal of the solvent from the portion of the eluate containing the faster eluted isomers gave (*Z*)-PT and (*Z*)-IQ-1: yields 25 and 30%, respectively.

Acknowledgment. This work was supported by the Italian National Research Council.

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Synthesis and Antihypertensive Activity of 1-Amino-3,4-dihydroisoquinolines

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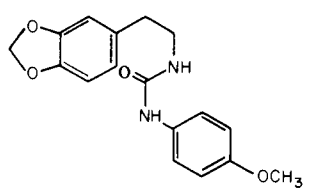
A series of 1-substituted 3,4-dihydroisoquinolines has been synthesized and screened for antihypertensive activity in the renal hypertensive rat. The 1-hydrazino homologue **6** and the corresponding acetaldehyde **25** and acetone **23** hydrazones exhibited good activity but were less effective than hydralazine (**7**).

The wide variety of pharmacological activity associated with amidines has been well documented[1] and includes such diverse types as antibacterial, hypoglycemic, and antihypertensive activities. 1-Amino-3,4-dihydroisoquinolines (**3**) represent a class of cyclic amidines, some of which have been reported to exhibit cardiovascular and pressor activity[2] as well as antitussive and antifibrillatory activity.[3] We wish to describe the synthesis and antihypertensive activity of several homologues in this series.

Chemistry. The synthetic sequence chosen to obtain the dihydroisoquinolines is outlined in Scheme I. This procedure was not applicable to the aromatic homologues (R_2 or R_3 = aryl).

The imino esters **2**, obtained from **1**[4] via Meerwein reagent,[5] were condensed with the appropriate amine producing the cyclic amidines[6] **3** which were isolated in most cases as their sulfate salt.

Aromatic amines did not react with the imino esters **2**, even under extreme conditions. The *N*-arylamidines **3a** (R_2 = H; R_3 = Ar) could be prepared in fair yield by cyclization of the appropriate urea. For example, **4** provided amidine **5** when treated with a mixture of phosphorus oxychloride and phosphorus pentoxide.



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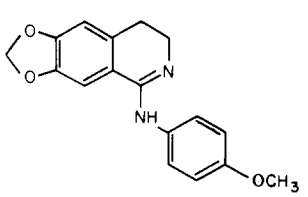

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The hydrazino homologue **6** was synthesized because of its similarity to hydralazine **7**, a useful antihypertensive