TABLE II—PEAK SERUM LEVEL AND AREA UNDER THE BLOOD LEVEL-TIME CURVE AFTER ORAL ADMINISTRATION OF SUSPENSIONS OF ANHYDROUS AMPICILLIN AND AMPICILLIN TRIHYDRATE

Form of Ampicillin	Test Species	Peak Serum Level, mcg./ml.	Peak Time, min.	Area Under Curve (mcg./ml. × hr.)
Anhydrous[a]	Dog	20.6	45	36.6
Trihydrate[b]	Dog	11.0	90	22.8
Anhydrous[a]	Human	2.2	60	6.9
Trihydrate[b]	Human	1.7	120	5.7

[a] Administered as Omnipen for oral suspension, Wyeth Laboratories, Inc., Radnor, Pa. [b] Administered as Polycillin for oral suspension, Bristol Laboratories, Syracuse, N. Y.

in distilled water have been determined over a temperature range of 7.5 to 50°. Below the transition temperature, 42°, the anhydrous form was found to be significantly more water soluble than the trihydrate. In addition, the solubility of the anhydrous crystal was shown to be inversely related to temperature.

The thermodynamic values have been calculated for the anhydrous-trihydrate ampicillin system. The greater thermodynamic activity of the anhydrous form correlates with the observed enhanced biological availability noted with this crystal form of the antibiotic.

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Keyphrases

Ampicillin, anhydrous, trihydrate—thermodynamic properties
Dissolution rate—ampicillin, anhydrous, trihydrate
Solubility—ampicillin, anhydrous, trihydrate
Blood serum levels—ampicillin, anhydrous, trihydrate
Iodometric titration—analysis

Potential Antitumor Agents III

Sodium Salts of α-[*N*]-Heterocyclic Carboxaldehyde Thiosemicarbazones

By KRISHNA C. AGRAWAL and ALAN C. SARTORELLI

Sodium salts of four of the most active antineoplastic agents in a series of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones have been prepared as a means of solubilizing for parenteral administration these extremely insoluble compounds. The sodium salt of 1-formylisoquinoline thiosemicarbazone (II) is soluble in nonaqueous vehicles for injection such as propylene glycol, whereas the sodium salts of 5-hydroxy-1-formylisoquinoline thiosemicarbazone (III), 3-hydroxy-2-formylpyridine thiosemicarbazone (IV), and 5-hydroxy-2-formylpyridine thiosemicarbazone (V) are readily soluble in water. Compounds III and IV, at the optimum effective dosage regimens, caused a greater prolongation of the survival time of mice bearing the L1210 lymphoma than did the parent derivatives, while II and V produced antineoplastic activity against sarcoma 180 and the L1210 lymphoma, respectively, equivalent to that of the parent compounds.

A VARIETY OF thiosemicarbazones of α-(*N*)-heterocyclic carboxaldehydes has been prepared and tested for antineoplastic activity (1–7). Several of these derivatives, especially 1-formylisoquinoline thiosemicarbazone (2, 3), its 5-hydroxy derivative (4), and both 3-hydroxy-2-formylpyridine thiosemicarbazone and 5-hydroxy-2-formylpyridine thiosemicarbazone (5, 6), have demonstrated pronounced antineoplastic activity when tested against a relatively wide spectrum of transplanted rodent tumors. To

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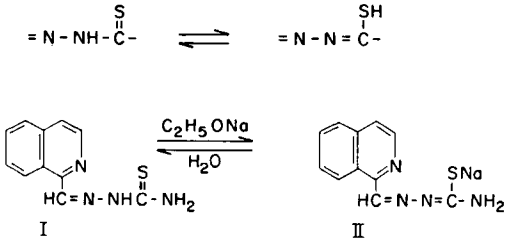
further elucidate the structural requirements for biological activity expounded by French and Blanz (2), the authors have synthesized and tested for tumor-inhibitory potency a series of 5-substituted 1-formylisoquinoline thiosemicarbazones (4), as well as a variety of derivatives with alterations in the side-chain of the molecule (7). These studies support the concept that an unsubstituted formylthiosemicarbazone group adjacent to a heteroaromatic ring nitrogen atom is required for optimum antineoplastic activity.

The biochemical basis for the growth-inhibitory activity of 1-formylisoquinoline thiosemicarbazone has also been studied in this laboratory (8–10); this agent caused marked inhibition of the synthesis of DNA by preventing the conversion of ribonucleotides to deoxyribonucleotide forms. Blockade of the formation of RNA and protein also occurred, but these were considerably less sensitive to drug-induced inhibition. A similar mechanism of action appeared to be operative with both 3-hydroxy-2-formylpyridine thiosemicarbazone and 5-hydroxy-2-formylpyridine thiosemicarbazone (11).

Although 1-formylisoquinoline thiosemicarbazone has been shown to possess activity against transplanted tumors in mice when administered orally (3), the expectation of variable gastrointestinal absorption of this relatively insoluble agent and related heterocyclic carboxaldehyde thiosemicarbazones would appear to preclude oral usage as the ideal route of administration. Thus, the ultimate utility of these heterocyclic carboxaldehyde thiosemicarbazones as antineoplastic agents in man appeared to be limited by an inability to formulate the compounds for parenteral administration because of their great insolubility. A number of solvents, both polar and nonpolar, have been investigated in an effort to solubilize these drugs; only dimethyl sulfoxide appeared to be capable of achieving the degree of solubilization necessary for concentrations required in tests of antineoplastic potency. However, the use of dimethyl sulfoxide in man at present is questionable. Neither salt formation, such as the hydrochloride of 5-amino-1-formylisoquinoline thiosemicarbazone (4), nor quaternization of the heterocyclic nitrogen atom as in 3-hydroxy-2-formyl-1-methylpyridinium iodide thiosemicarbazone (2) resulted in the attainment of effective tumor-inhibitory compounds with adequate solubility. Furthermore, initial efforts to synthesize water-soluble derivatives, such as 1-formylisoquinoline thiosemicarbazone-5-sulfonic acid, resulted in agents with decreased therapeutic potency (4).

DISCUSSION

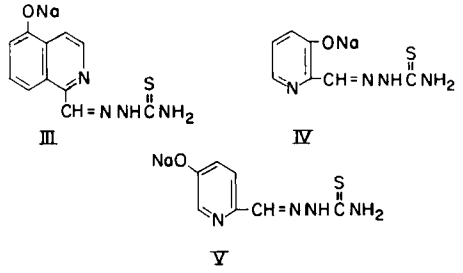
This report describes the preparation of sodium salts of four of the most active antitumor agents in this series. 1-Formylisoquinoline thiosemicarbazone (I), when treated with sodium ethoxide in alcoholic solution, reacts to form the monosodium derivative (II) of the resonance-hybrid anion as shown in Scheme I. Compound II



Scheme I

(sodium 1-amino-4-isoquinolyl-2,3-diazabuta-1,3-diene-1-thiol) is essentially a butadiene derivative in which the hydrogen atom of the —NH— group of the formyl thiosemicarbazone side-chain is apparently displaced in its lactim form by sodium. Compound II is, however, quantitatively hydrolyzed in the presence of water to give the original thiosemicarbazone. Compound I is very sparingly soluble in alcohols and glycols, whereas Compound II is very soluble in these solvents. Thus, solutions of Compound II in propylene glycol were found to be suitable for parenteral administration and were employed in biological tests for carcinostatic activity.

The sodium salts of the hydroxy derivatives shown in Scheme II were fabricated by dissolving



Scheme II

the thiosemicarbazones in equimolar aqueous NaOH solutions; excess water was removed under vacuum employing absolute ethanol. These compounds (III, IV, and V) are readily soluble in water. In accordance with the previous findings that the substitution of electron-donating groups increased antitumor activity, while electron-withdrawing groups lessened antineoplastic activity (4); it was anticipated that

TABLE I—EFFECT OF SODIUM SALTS OF HYDROXY DERIVATIVES OF α-(*N*)-HETEROCYCLIC CARBOXALDEHYDE THIOSEMICARBAZONES ON THE SURVIVAL TIME OF MICE BEARING L1210 LYMPHOMA

Drug	Dose, mg./kg.[a]	×	No. of Daily Doses	Av. Δ wt., %[b]	Av. Survival, days ± *S.E.*
None	—		—	+16.3	9.7 ± 1.6 (58)[c]
5-Hydroxy-1-formylisoquinoline thiosemicarbazone	30	×	2	+5.4	13.9 ± 0.9 (15)
	40	×	2	+10.2	15.5 ± 1.0 (15)
	50	×	2	+18.3	10.0 ± 0.9 (4)
	60	×	1	+8.9	13.6 ± 1.1 (10)
	80	×	1	+3.2	15.1 ± 1.9 (10)
	100	×	1	+18.7	12.4 ± 1.4 (5)
Sodium 1-formylisoquinoline-5-ol thiosemicarbazone (Compound III)	32.5	×	2	−7.4	24.7 ± 2.1 (13)
	43.5	×	2	−10.8	24.3 ± 1.7 (10)
	65	×	1	−16.0	13.5 ± 0.6 (10)
	87	×	1	−14.4	16.4 ± 1.6 (10)
	109	×	1	−16.7	9.6 ± 2.2 (5)
3-Hydroxy-2-formylpyridine thiosemicarbazone	10	×	4	−4.0	18.1 ± 1.2 (15)
	20	×	4	−5.3	19.2 ± 1.3 (5)
	20	×	2	−1.6	22.2 ± 1.9 (10)
	30	×	2	−2.7	21.1 ± 2.1 (10)
	40	×	1	−2.4	17.1 ± 0.7 (10)
	60	×	1	0.0	17.2 ± 0.7 (10)
	80	×	1	−2.4	16.8 ± 0.3 (10)
Sodium 2-formylpyridine-3-ol thiosemicarbazone (Compound IV)	11	×	4	−5.0	23.9 ± 1.6 (14)
	22	×	4	−10.0	22.2 ± 7.0 (5)
	22	×	2	−4.5	28.8 ± 2.9 (10)
	33	×	2	−6.9	21.6 ± 1.8 (9)
	44	×	1	+5.4	10.5 ± 0.2 (10)
	67	×	1	+6.2	10.8 ± 0.4 (10)
	89	×	1	0.0	13.9 ± 0.6 (10)
5-Hydroxy-2-formylpyridine thiosemicarbazone	10	×	2	+0.9	24.6 ± 2.0 (10)
	20	×	2	+1.0	21.5 ± 1.1 (10)
	30	×	2	0.0	27.6 ± 3.0 (10)
	40	×	2	−5.5	24.2 ± 2.1 (10)
Sodium 2-formylpyridine-5-ol thiosemicarbazone (Compound V)	11	×	2	+4.5	21.0 ± 1.1 (10)
	22	×	2	+4.0	23.4 ± 2.8 (10)
	33	×	2	−2.0	24.6 ± 1.8 (10)
	44	×	2	−8.1	21.2 ± 1.1 (10)

[a] Administered as indicated daily for 4 consecutive days, beginning 24 hr. after tumor implantation. [b] Average weight change from onset to termination of drug treatment. [c] The figure in parentheses indicates the number of mice employed.

the stronger electron-donating properties of the phenoxide anion of Compounds III, IV, and V might possibly serve to increase biological activity over the parent compounds.

Biological Tests—Experiments were performed on 21- to 24-g. male C57BL X DBA F_1 mice (Cumberland View Farms, Cumberland, Tenn.). Transplantation of lymphoma L1210 ascites cells was carried out by withdrawing peritoneal fluid from a donor mouse bearing a 7-day tumor growth. The suspension was centrifuged for 2 min. (1600×*g*), the supernatant peritoneal fluid was decanted, a 10-fold dilution with isotonic saline was made, and 0.1 ml. of the resulting cell suspension (approximately 8×10^6 cells) was injected intraperitoneally into each animal. For any one experiment, mice were distributed into groups of five to ten animals of comparable weight and maintained throughout the course of the experiment on Purina laboratory chow pellets and water *ad libitum*. Experiments with more than five mice per group were performed at least two times.

Drugs were administered by intraperitoneal injection as indicated for 4 consecutive days beginning 24 hr. after tumor implantation. The hydroxy derivatives of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones were given in fine suspension following homogenization in absolute ethanol (adjusted so that the final concentration of the drug solution was 5% with respect to ethanol) and 2–3 drops of a 20% aqueous solution of polysorbate 80[1] and then made up to volume with isotonic saline. Compounds III, IV, and V were injected in solution in distilled water. Control tumor-bearing animals given injections of comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiment, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

The effects of molar equivalent doses of these agents on the survival time of mice bearing lymphoma L1210 was used as a measure of therapeutic efficacy; the results obtained are shown in Table I. In most instances the daily frequency of drug administration appeared to be of importance, with treatment twice daily being

[1] Tween 80, Atlas Chemical Co., Wilmington, Del.

optimum. The sodium salts of 3-hydroxy-2-formylpyridine thiosemicarbazone and 5-hydroxy-1-formylisoquinoline thiosemicarbazone, at the optimum effective dosage regimens, appeared to prolong the life-span of tumor-bearing animals to a greater extent than did the parent compounds; the most dramatic difference was obtained with the more insoluble compound 5-hydroxy-1-formylisoquinoline thiosemicarbazone, suggesting that the sodium salt of this agent was able to distribute itself more advantageously *in vivo*. The administration of the sodium salt of the most soluble of these α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, 5-hydroxy-2-formylpyridine thiosemicarbazone, did not increase the tumor-inhibitory potency of this derivative; thus, only the advantage of complete solubilization was attained by formation of the sodium salt of this agent. Preliminary results indicate that the sodium salt of 1-formylisoquinoline thiosemicarbazone (Compound II) administered in propylene glycol solution to mice bearing sarcoma 180 ascites cells was essentially equal in growth-inhibitory activity to the parent thiosemicarbazone (Compound I) employed in suspension. The sodium salts of the hydroxy derivatives appeared to cause, in general, a greater loss in host body weight than did the parent compounds; however, no other observable toxicity, such as gross tissue damage at the injection site was produced by these compounds. It would appear, therefore, that the solubility and in some instances the increased antineoplastic activity of the sodium salts of these heterocyclic aldehyde thiosemicarbazones would make the sodium salts the drug form of choice in initial clinical trials of these agents.

EXPERIMENTAL

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, New York, N.Y. The analytical data are listed in Table II.

Sodium Salt of 1-Formylisoquinoline Thiosemicarbazone—1-Formylisoquinoline thiosemicarbazone (2.3 g., 0.01 mole) was added in small portions with stirring to a solution of sodium ethoxide (prepared by dissolving 0.01 mole of sodium in 25 ml. of absolute ethanol). The thiosemicarbazone slowly dissolved and the solution turned yellow. The reaction mixture was stirred for another 5 min. at room temperature and then excess solvent was removed under vacuum at 25° to leave an orange-colored thick syrup. This was triturated with 100 ml. of anhydrous ether to yield a crystalline yellow precipitate, which was filtered, washed with anhydrous ether, and dried at 80° to give 2.4 g. (95.2%), m.p. 214–216° dec. Compound II is sensitive to moisture and is best stored over anhydrous $CaCl_2$.

TABLE II—SODIUM SALTS OF α-(*N*)-HETEROCYCLIC CARBOXALDEHYDE THIOSEMICARBAZONES

Compound	Formula	Anal. Calcd.	Anal. Found
II	$C_{11}H_9N_4NaS$	C, 52.38 H, 3.57 S, 12.70	C, 52.03 H, 3.90 S, 12.92
III	$C_{11}H_9N_4NaOS \cdot H_2O$	C, 46.15 H, 3.85 S, 11.19	C, 46.00 H, 4.24 S, 10.71
IV	$C_7H_7N_4NaOS \cdot H_2O$	C, 35.59 H, 3.81 S, 13.56	C, 35.71 H, 4.17 S, 13.14
V	$C_7H_7N_4NaOS \cdot H_2O$	C, 35.59 H, 3.81 S, 13.56	C, 36.62 H, 4.20 S, 13.20

General Procedure for Sodium Salts of Hydroxy Derivatives of α-(N)-heterocyclic Carboxaldehyde Thiosemicarbazones—The hydroxy derivative of an α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazone (0.01 mole) was dissolved in a solution of sodium hydroxide (0.4 g., 0.01 mole) in 10 ml. of water. The mixture was filtered to remove suspended material and the water from the filtrate was removed under vacuum at 25° using absolute ethanol. The resultant syrupy liquid was dissolved in 5 ml. of absolute ethanol; addition of 100 ml. of anhydrous ether resulted in the precipitation of the sodium salt which was collected by filtration, washed with anhydrous ether, and dried under vacuum at 80° over anhydrous calcium chloride. These compounds have 1 mole of water of crystallization and are hygroscopic; therefore, they should be stored in closed containers over $CaCl_2$. Compound III melts at 213–216° with decomposition. Compounds IV and V initially collapse with frothing at 182–185° and 192–194°, respectively, and then decompose at 208–212° and 220–222°, respectively.

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Keyphrases

Antitumor agents
α-(*N*)-Heterocyclic carboxaldehyde thiosemicarbazones Na salts—synthesis
Antitumor activity—mice