was added 3.65 g (0.028 mole) of sodium fluoropyruvate.[13] On cooling overnight at 0° the desired compound crystallized, was removed by filtration, and washed with cold water, yielding 3.55 g (76%) of I. The product turned brown at *ca.* 185°, melting at 206–207° dec; lit.[6] turns brown at 198–200° and decomposes at 205°.

5-Fluoromethyl-6-azauracil (II).—A mixture of 1.0 g (6 mmoles) of I, 50 ml of $SOCl_2$, and 1 drop of dry pyridine was refluxed 1 hr (clear solution), allowed to stand overnight at room temperature, and evaporated to 15 ml *in vacuo*. On cooling II crystallized and was removed by filtration through sintered glass and washed with dry ether, yielding 550 mg (63%) after drying at 80° *in vacuo*, mp 127–128°. A portion was recrystallized for analysis from chlorobenzene and dried at 80° *in vacuo*; needles, mp 127.5–129°; $\lambda_{max}^{95\%\ EtOH}$ 265 mμ (log ε 3.78); $\lambda_{min}^{95\%\ EtOH}$ 224 mμ; λ_{max}^{Nujol} 3.05, 3.12, 5.82–6.00, 7.75, 8.17, 9.81, 10.06, 11.53, 12.65, 13.00, 13.50, 13.82 μ.

Anal. Calcd for $C_4H_4FN_3O_2$: C, 33.11; H, 2.78; F, 13.10. Found: C, 33.16; H, 2.89; F, 13.29

Fluoropyruvic Acid 3-Thiosemicarbazone (III).—A solution of 915 mg (10 mmoles) of thiosemicarbazide, 11 ml of 1 *N* HCl, and 640 mg (5 mmoles) of sodium fluoropyruvate[13] was allowed to stand at room temperature for 1 min and then cooled in an ice bath. On cooling 600 mg (67%) of III crystallized as needles, mp 186–189° dec. Recrystallization from water (prolonged heating and standing at room temperature being avoided) gave needles; the product turned orange-red at 150–160° and melted at 185–190° dec; $\lambda_{max}^{95\%\ EtOH}$ 230 mμ (log ε 3.84), 301 mμ (log ε 4.13); $\lambda_{min}^{95\%\ EtOH}$ 217, 240 mμ; λ_{max}^{Nujol} 2.92, 3.05, 3.10, 4.01, 6.00, 6.21, 6.32, 6.92, 7.15, 7.90, 8.02, 8.38, 8.81, 9.55, 9.97, 10.31, 11.75, 12.40, 12.72 μ.

Anal. Calcd for $C_4H_6FN_3O_2S$: C, 26.81; H, 3.38; N, 23.45; S, 17.90. Found: C, 26.70; H, 3.40; N, 23.20, 23.43; S, 17.76.

Fluoropyruvic Acid 3-Methylisothiosemicarbazone (IV).—A solution of 4.66 g (20 mmoles) of 3-S-methylisothiosemicarbazide hydriodide[14] and 1.28 g (10 mmoles) of sodium fluoropyruvate[13] in 30 ml of water was allowed to stand at room temperature for 10 min and then at 2° for 2 days, yielding IV which was dried *in vacuo* at 60°; yield 1.35 g (70%), transition to needles at 140–150°, mp 182–184° dec. Recrystallization from ethyl acetate followed by drying *in vacuo* at 70° did not change the melting point; $\lambda_{max}^{95\%\ EtOH}$ 262 mμ (log ε 4.16); λ_{max}^{Nujol} 3.11, 3.21, 6.01, 6.60, 7.24, 8.25, 8.90, 9.71, 10.20, 11.35, 11.72, 13.15, 13.75, 14.08 μ.

Anal. Calcd for $C_5H_8FN_3O_2S$: C, 31.08; H, 4.17; N, 21.75; S, 16.60. Found: C, 30.95; H, 4.39; N, 21.55; S, 16.35.

3-(Methylthio)-6-fluoromethyl-*as*-triazin-5(2H)-one (V).—A mixture of 320 mg (1.51 mmoles) of IV and 5 ml of water was heated to boiling and cooled (prolonged heating being avoided). On cooling 150 mg (57%) of V was obtained as needles, mp 180–187° dec. Recrystallization of a portion of V from water followed by drying at 70° *in vacuo* raised the melting point to 185–187° dec; $\lambda_{max}^{95\%\ EtOH}$ 235 mμ (log ε 4.26); λ_{max}^{Nujol} 3.15, 3.75, 6.25, 6.31, 6.60, 7.40, 7.61, 7.90, 8.08, 8.88, 9.70, 10.02, 10.40, 10.80, 12.58, 12.86, 13.85 μ.

Anal. Calcd for $C_5H_6FN_3OS$: C, 34.28; H, 3.45; N, 23.99; S, 18.31. Found: C, 34.54; H, 3.26; N, 24.08; S, 18.35.

(13) Commercially available.

(14) M. Freund and Th. Paradies, *Chem. Ber.*, **34**, 3110 (1901).

The Synthesis and Antitumor Activity of Several Thiosemicarbazones Related to Kethoxal Bis(thiosemicarbazone)[1]

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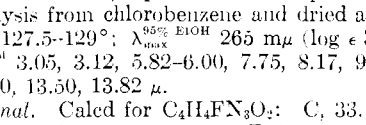
A recent report from our laboratory[2] described the synthesis, physical properties, and antitumor activity of a series of α-ketoaldehyde bis(thiosemicarbazones). The most active compounds were shown to be 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone)[3] (I) and 2-keto-3-ethoxybutyraldehyde bis(N^4-methylthiosemicarbazone)[3] (II). Both compounds were found to have high antitumor activity *in vivo* and *in vitro*. Several closely related compounds including 2-keto-3-ethoxybutyraldehyde bis(N^4-dimethylthiosemicarbazone) showed little or no *in vivo* activity.

We have now prepared several additional compounds closely related to I and II in order to explore further the effects of structure on antitumor activity, several of which do have high biological activity. We wish to report here the synthesis of these compounds and some preliminary biological data.



I, R_1 = $C_2H_5OCH(CH_3)$–; R_2 = H_2
II, R_1 = $C_2H_5OCH(CH_3)$–; R_2 = $H(CH_3)$
III, R_1 = $CH_3OCH(CH_3)$–; R_2 = H_2
IV, R_1 = $CH_3OC_2H_4OCH(CH_3)$–; R_2 = H_2
V, R_1 = $CH_3CO_2CH(CH_3)$–; R_2 = H_2
VI, R_1 = CH_3; R_2 = $(CH_3)_2$

Chemistry.—The new compounds which were prepared are listed in Table I together with their abbreviations, formulas, and physical and chemical properties. The compounds were prepared by treating the appropriate α-ketoaldehyde with thiosemicarbazide or N^4-dimethylthiosemicarbazide in 2–5% acetic acid solutions. The reactions were straightforward and no complications were experienced.

Three of the new compounds, namely, III, IV, and V, involve modifications of the 3-ethoxy group of the highly active kethoxal bis(thiosemicarbazone) (I). Compound VI was prepared to complete the series of pyruvaldehyde derivatives previously reported.[2]

Biological Results.—The antitumor activity of III, IV, and V is compared with that of I in Table II. The compounds were tested against a nitrogen mustard resistant variant of the Walker 256 carcinosarcoma implanted in Sprague–Dawley male rats, under conditions previously described.[2] VI was not available in sufficient amounts to conduct animal studies on its activity, but *in vitro* tests[4] indicated that this compound was considerably less active than any of the other compounds described here.

Experiment A of Table II shows that 2-keto-3-acetoxybutyraldehyde bis(thiosemicarbazone) (V) when given orally to rats had the least activity of any of the compounds tested by this route, although its toxicity was as great as any of the others. However, when V was given intraperitoneally (expt B) much higher activity was obtained. The low oral activity of this compound may be due to its poor absorption from the intestinal tract or to the splitting of the ester group.

The most active compound of this series was 2-keto-3-methoxybutyraldehyde bis(thiosemicarbazone) (III) as indicated in expt C and D. Because of its high oral activity III was also tested by the intraperitoneal route.

(1) Reported in part at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 5–9, 1965, Abstracts, p 13N.

(2) H. G. Petering, H. H. Buskirk, and G. E. Underwood, *Cancer Res.*, **24**, 367 (1964).

(3) 2-Keto-3-ethoxybutyraldehyde has been designated kethoxal. Kethoxal bis(thiosemicarbazone) is generally abbreviated as KTS.

(4) These studies were made using a modification of the method described by T. Arai and M. Suzuki [*J. Antibiot.* (Tokyo), **A9**, 169 (1956)]. Further details are now being prepared for publication.

TABLE I: ANALYTICAL DATA AND PHYSICAL PROPERTIES OF THIOSEMICARBAZONES SYNTHESIZED

Compd[a]	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % S	Found, % C	Found, % H	Found, % N	Found, % S	$\lambda_{max}^{95\%EtOH}$, mμ	ε
2-Keto-3-methoxybutyraldehyde bis(thiosemicarbazone) (III)	208–212	$C_7H_{14}N_6OS_2$	32.1	5.4	32.1	24.4	32.8	5.5	31.4	24.1	233	9,100
											271	7,350
											348	47,450
2-Keto-3-methoxyethoxybutyraldehyde bis(thiosemicarbazone) (IV)	213–216	$C_9H_{18}N_6O_2S_2$	35.3	5.9	27.4	20.9	35.4	6.1	26.9	20.9	237	9,250
											267	7,500
											347	48,200
2-Keto-3-acetoxybutyraldehyde bis(thiosemicarbazone)[b] (V)	210–212	$C_8H_{14}N_6O_2S_2$	33.1	4.9	29.0	22.1	33.3	4.9	28.2	22.2	247	14,200
											346	28,500
Pyruvaldehyde bis(N^4-dimethylthiosemicarbazone) (VI)	115 dec	$C_9H_{18}N_6S_2$	39.4	6.6	30.6	23.4	39.1	6.7	30.3	22.7	232	15,400
											328	24,300
											410	3,320

[a] Abbreviations in common use for these compounds are: III, KMTS; IV, MKTS; V, KATS; and VI, $PTSM_2$. [b] Original sample prepared by B. D. Aspergren.

TABLE II: THE ANTITUMOR ACTIVITY[a] OF SEVERAL THIOSEMICARBAZONES COMPARED TO KETHOXAL BIS(THIOSEMICARBAZONE)

Expt	Drug	Dose, mg/kg	Dosing period, days[b]	Survival	Δ body wt, g	Δ tumor size, mm	% inhib[c]
A	...	Control	.... (17)	9/10	70.0	33.9	...
	I	25 oral	4–17	5/5	61.4	−1.8	>100
	V	25 oral	4–17	4/5	51.5	33.3	2
B	...	Control	.... (17)	9/10	45.2	32.5	...
	I	25 ip	5–15	5/5	18.0	0.7	98
	V	25 ip	5–15	5/5	8.2	7.0	79
C	...	Control	.... (18)	9/9	56.2	29.8	...
	I	25 oral	6–17	10/10	47.2	5.3	82
	III	25 oral	6–17	10/10	41.5	0.6	98
D	...	Control	.... (20)	9/9	66.8	38.0	...
	I	25 ip	6–19	5/5	29.2	2.2	94
	III	25 ip	6–19	4/4	22.8	−1.9	105
E	...	Control	.... (17)	4/4	88.8	42.3	...
	I	25 oral	4–15	5/5	45.8	3.0	93
	IV	25 oral	4–15	5/5	42.0	14.6	65

[a] Against a nitrogen mustard resistant variant of Walker 256 carcinosarcoma in Sprague–Dawley rats. [b] Days after tumor implantation. Dosed daily except Sundays. Parentheses indicate the day on which final measurements were made. [c] % inhibition = [change in average diameter (control tumors − treated tumors) × 100]/[change in average diameter (control tumors)].

It was found that this agent was equally active by either route of administration and that it was equal to or slightly more active than I, the parent compound, while the toxicities of the two drugs were similar.

In expt E we found that 2-keto-3-methoxyethoxybutyraldehyde bis(thiosemicarbazone) (IV) had less antitumor activity but that its toxicity to rats was essentially the same as that of I.

These data and those previously reported[2] show that certain modifications of the ketoaldehyde portion of I can be made without great loss of activity but that such changes are limited. Substitution of a methoxy group for the ethoxy group of I seemed to cause an increase in antitumor activity (*cf.* III) but the substitution of an acetoxy or methoxyethoxy group for the ethoxy radical caused a loss of biological activity (*cf.* IV and V). Previous work had indicated that pyruvaldehyde bis(thiosemicarbazone) and ethoxypyruvaldehyde bis(thiosemicarbazone) were considerably less active than I, which of course suggests that the nature of the ketoaldehyde is important in determining the antitumor activity of bis(thiosemicarbazones).

Experimental Section

Source of α-Ketoaldehydes.—Pyruvaldehyde was obtained as a 30% solution from Aldrich Chemical Co. 2-Keto-3-methoxybutyraldehyde and 2-keto-3-methoxyethoxybutyraldehyde were obtained from B. D. Tiffany, and 2-keto-3-acetoxybutyraldehyde from B. D. Aspergren, both of The Upjohn Co. laboratories.

Preparation of Thiosemicarbazones.—All bis(thiosemicarbazones) were prepared by small variations of the general method described earlier.[2] Recrystallization was accomplished from hot ethanol (III–V) by adding an equal volume of water or directly from absolute methanol (VI).

Testing of Drugs.—Drugs were prepared for testing and tested in the manner described earlier.[2] All other experimental conditions were also the same as previously reported.

Acknowledgment.—We wish to express our appreciation to E. E. Smith for his assistance with the animal studies.

Synthesis of Potential Antineoplastic Agents. XIII. 1-{*p*-[Bis(2-chloroethyl)amino]benzyl}-pyridinium *p*-Toluenesulfonate and Related Compounds[1,2]

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As part of our program directed toward the synthesis of various nitrogen mustard derivatives as potential antineoplastic agents it was desirable to have the

(1) Part XII: F. D. Popp and D. W. Alwani, *Can. J. Chem.*, **42**, 1506 (1964).

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