# Metal Complexes of 3-Ethoxy-2-oxobutyraldehyde Bis(thiosemicarbazone) and Related Ligands as Antitumor Agents[1]

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3-Ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) or $H_2KTS$, a new antitumor agent which requires copper(II) for activity, has been shown to complex with at least sixteen different metals. Complexes of $H_2KTS$ with Fe(II), Fe(III), Ni(II), Cu(II), Zn(II), and Ag(I) have been isolated and formulas proposed. Copper(II) complexes of eight bis(thiosemicarbazones) related to $H_2KTS$ have also been isolated and shown to form structures similar to Cu(II)KTS. The bis(thiosemicarbazones) studied are quadridentate ligands which react with monovalent metals in a 1:1 or 1:2 ratio, with divalent metals in a 1:1 ratio, and with trivalent metals in a 3:2 ratio (ligand:metal). The absorption maxima of the complexes vary in wavelength and molar absorbancy depending upon the metal ion which is bound in the complex but are relatively unaffected by changes in the ligands. The results of some preliminary *in vitro* antitumor tests are reported.

3-Ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) or kethoxal bis(thiosemicarbazone) ($H_2KTS$)[3] has been shown by several investigators to have marked antitumor activity against a spectrum of rodent tumors.[4] $H_2KTS$ and 3-methoxy-2-oxobutyraldehyde bis(thiosemicarbazone) were found to be the most active agents of a series of related bis(thiosemicarbazones) which were studied.[4a,5] Further investigations revealed that the antitumor activity of $H_2KTS$, as well as its toxicity, are profoundly affected by the nutritional intake of the animals receiving the drug.[6] The greatest effects were due to variation in the intake of copper(II), zinc(II), and cobalt(II); copper(II) is required for antitumor activity, zinc(II) affects the host toxicity without altering the therapeutic effect, and cobalt(II) antagonizes the action of copper(II).[6a]

Because interaction of $H_2KTS$ with trace metals in the environment of the animals appeared to govern the biological activity of the drug and since bis(thiosemicarbazones) are known metal-complexing agents, we undertook the investigation of the interaction of $H_2KTS$ and some related bis(thiosemicarbazones) with a number of transition series metal ions. The compounds studied are variations of the general formula

$$\begin{array}{l} R_1C{=}NNHCSNR_2 \\ | \\ HC{=}NNHCSNR_2 \end{array}$$

where $R_1$ is $CH_3CH(OC_2H_5)$, $CH_3CH(OCH_3)$, $CH_3CH(OOCCH_3)$, $CH_3$, or $CH_2(OC_2H_5)$; and $R_2$ is $H_2$, $H(CH_3)$, or $(CH_3)_2$.

This report deals with the synthesis, probable formulas, and preliminary biological testing of these complexes. Other reports have shown the high *in vivo* antitumor activity of the cupric chelate of $H_2KTS$ [Cu(II)KTS][7] and have discussed the unique role of copper in the antitumor activity of $H_2KTS$.[7,8]

## Experimental Section

The synthesis and properties of the bis(thiosemicarbazone) ligands have been described in earlier reports.[4a,5] Published methods[9] for preparing complexes of thiosemicarbazones proved tedious and inconvenient; therefore, the methods described below were employed. The preparation of the cupric chelate of $H_2KTS$ and its dihydrochloride are given as typical for the preparation of the complexes studied. The elemental analyses, spectral data, and probable formulas of the metal chelates of $H_2KTS$ are given in Table I while similar data for the cupric chelates of the related bis(thiosemicarbazones) are given in Table II. Absorption spectra were determined in 95% ethanol unless otherwise indicated. Infrared spectra were determined in Nujol mulls.

**Method A.**—$H_2KTS$ (20 mmoles 5.52 g) was dissolved in 130 ml of boiling MeOH. To this solution was added 20 mmoles (4.0 g) of $Cu(OAc)_2 \cdot H_2O$ dissolved in 74 ml of hot water (80°) with stirring. The temperature was held at 80° throughout the addition of the $Cu^{2+}$ solution and for 5 min after the addition was completed. The flask was slowly cooled to room temperature over a period of 24 hr to allow crystallization. After 3 days at room temperature, the crystals were collected by filtration, washed with MeOH–$H_2O$ (1:1), and air dried. A yield of 5.3 g (78%) was obtained.

**Method B.**—$H_2KTS$ (20 mmoles 5.52 g) was dissolved in 180 ml of boiling MeOH. To this solution was added 20 mmoles (3.41 g) of $CuCl_2 \cdot 2H_2O$ dissolved in 20 ml of MeOH. The solution immediately turned a dark green color. It was cooled under tap water and centrifuged to remove a small amount (70 mg) of yellow-colored precipitate which formed. The amount of this material forming during the reaction is regulated by both acidity and temperature of the reaction mixture. The amount formed under the conditions described is minimal. To the supernatant (210 ml) was added 4.0 ml of concentrated HCl and 840 ml (4 vol) of ether. A deep green crystalline precipitate formed. After standing at 4° overnight, the precipitate was collected by centrifugation, washed with 200 ml of ether, and air dried. A yield of 8.10 g (98%) was obtained as dihydrochloride.

**Method C.**—The reaction mixture of A was concentrated to a small volume from which crystals formed.

**Method D.**—The reaction mixture of A was evaporated to dryness.

**Method E.**—The reaction mixture of A was diluted with 2 vol of $H_2O$ and extracted ($Et_2O$). The ether solution was dried ($Na_2SO_4$) and evaporated to dryness.

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(3) The free ligand KTS is written in this manner to designate the replaceable hydrogens lost during chelation.

(4) (a) H. G. Petering, H. H. Buskirk, and G. E. Underwood, *Cancer Res.*, **24**, 367 (1964); (b) F. A. French and B. L. Freedlander, *Cancer Res. Suppl. VII* **20**, 505 (1960); (c) E. Mihich and C. A. Nichol, *Proc. Am. Assoc. Cancer Res.*, **4**, 44 (1963).

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(7) J. A. Crim and H. G. Petering, *ibid.*, **27**, 1278 (1967).

(8) H. G. Petering and G. J. Van Giessen, Proceedings of the International Symposium on Biochemistry of Copper, Academic Press Inc., New York, N. Y., 1966, pp 197–208.

(9) (a) G. Bähr, *Z. Anorg. Allg. Chem.*, **268**, 351 (1952); (b) *ibid.*, **273**, 325 (1953); (c) G. Bähr and E. Schleitzer, *ibid.*, **278**, 136 (1955).

TABLE I

ANALYTICAL DATA AND PROBABLE COMPOSITION OF SOME METAL COMPLEXES OF $H_2KTS$

| Metal ion | Method[a] | % yield | Probable formula | Calcd. % | | | | | | Found, % | | | | | | Ultraviolet[c] | | Infrared, $cm^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S | Metal | Other | C | H | N | S | Metal[b] | Other | $\lambda_{max}$, mμ | ε | |
| Iron(II) | D | 60 | FeKTS·2HCl | 23.8 | 4.0 | 20.9 | 15.9 | 13.9 | Cl, 17.6 | 23.7 | 3.7 | 20.5 | 14.9 | 13.6 | Cl, 16.8 | 351 | 15,450 | 3420, 3320, 3164, 1602, 1497, 1203, |
| | | | | | | | | | | | | | | | | 420 | 6,850 | 1150, 1104 |
| Iron(III) | C[d] | 61 | $Fe_2(KTS)_3 \cdot 2H_2O$ | 29.7 | 4.8 | 26.0 | 19.8 | 11.5 | $H_2O$, 3.7 | 30.9 | 4.8 | 26.8 | 19.3 | 7.9[e] | $H_2O$, 3.8 | 350 | 15,700[f] | 3280, 3160, 1675, 1610, 1505, 1315, |
| | | | | | | | | | | | | | | | | 420 | 5,800[f] | 1204, 1105 |
| Nickel(II) | E | 93 | NiKTS | 28.8 | 4.2 | 25.2 | 19.3 | 17.6 | | 29.6 | 4.4 | 24.6 | 18.5 | 17.3 | | 263 | 32,700 | 3440, 3300, 3140, 1675, 1630, 1570, |
| | | | | | | | | | | | | | | | | 402 | 12,000 | 1530, 1240, 1170, 1095, 1055 |
| Copper(II) | A | 78 | CuKTS | 28.4 | 4.2 | 24.9 | 18.9 | 18.8 | | 29.1 | 4.2 | 24.5 | 19.2 | 19.1 | | 314 | 22,400 | 3480, 3310, 3100, 1645, 1605, 1575, |
| | | | | | | | | | | | | | | | | 489 | 6,500 | 1525, 1210, 1180, 1075 |
| | B | 98 | CuKTS·2HCl | 23.4 | 3.9 | 20.5 | 15.6 | 15.5 | Cl, 17.3 | 23.9 | 4.6 | 20.1 | 15.3 | 15.5 | Cl, 17.1 | 313 | 21,950 | 3850, 3570, 3240, 2660, 1615, 1550, |
| | | | | | | | | | | | | | | | | 488 | 6,200 | 1210, 1100, 1060 |
| Zinc(II) | F | 70 | $ZnKTS \cdot H_2O$ | 26.9 | 4.5 | 23.5 | 17.9 | 18.3 | $H_2O$, 5.0 | 27.1 | 4.6 | 23.6 | 18.4 | 18.0 | $H_2O$, 5.3 | 256 | 12,800 | 3420, 3390, 3280, 3170, 3100, 1640, |
| | | | | | | | | | | | | | | | | 309 | 11,250 | 1600, 1530, 1175, 1095 |
| | | | | | | | | | | | | | | | | 432 | 10,850 | |
| | G | 69 | $ZnKTS \cdot 3HOOCCH_3$ | 32.2 | 5.0 | 16.2 | 12.3 | 12.6 | | 31.8 | 5.2 | 16.6 | 11.9 | 13.1 | | 256 | 12,450 | 3280, 3150, 1590, 1100 |
| | | | | | | | | | | | | | | | | 309 | 10,500 | |
| | | | | | | | | | | | | | | | | 429 | 10,000 | |
| Silver(I) | A | 94 | $Ag(HKTS) \cdot 0.5HNO_3$ | 23.2 | 3.8 | 22.0 | 15.5 | 26.0 | | 22.3 | 4.3 | 21.6 | 14.5 | 26.8 | | 294[g] | 13,950 | 3400, 3250, 3160, 1600, 1490, 1325, |
| | | | | | | | | | | | | | | | | 349[g] | 10,050 | 1275, 1095, 1060, 1030 |
| | A[h] | 75 | $Ag_2KTS \cdot HNO_3$ | 17.4 | 2.7 | 17.7 | 11.6 | 39.0 | | 17.5 | 3.4 | 18.1 | 11.3 | 38.8 | | 293[g] | 14,200 | 3400, 3270, 3160, 1600, 1485, 1310, |
| | | | | | | | | | | | | | | | | 355[g] | 9,270 | 1095, 1060, 1030 |

[a] See Experimental Section. Initial reactants combined in 1:1 ratio except as noted. [b] All metal determinations were made by ashing except copper and zinc which were determined by atomic absorption. [c] Only major absorbances given; in most cases other minor maxima and shoulders were present. [d] Combined 2 mmoles of $H_2KTS$ with 1 mmole of ferric tartrate. [e] Difficulties were experienced in obtaining consistent analyses for iron; however, since the other values were consistent, this compound was included. [f] ε values were calculated using a molecular weight equivalent to one bis(thiosemicarbazone), in this case one-third of the actual molecular weight. [g] Samples dissolved in 5 ml of DMSO and diluted to 100 ml with 95% ethanol. The spectra are directly comparable with those in 95% EtOH. [h] Combined 1 mmole of $H_2KTS$ with 2 mmoles of $AgNO_3$.

TABLE II

ANALYTICAL DATA OF SOME CUPRIC CHELATES OF BIS(THIOSEMICARBAZONES) RELATED TO $H_2KTS$

| Ligand[a] | | | | | | Calcd, % | | | | | | Found, % | | | | | | Ultraviolet[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | $R_1$ | $R_2$ | Salt | Method[b] | % yield | C | H | Cl | Cu | N | S | C | H | Cl | Cu | N | S | $\lambda_{max}$, mμ | ε | Infrared, $cm^{-1}$ |
| 1 | $C_2H_5OCH(CH_3)$ | $H(CH_3)$ | | A | 97 | 32.8 | 4.9 | | 17.4 | 23.0 | 17.5 | 33.2 | 5.3 | | 17.4 | 23.0 | 18.4 | 318 | 20,750 | 3360, 3210, 3100, 1600, 1570, 1525, |
| | | | | | | | | | | | | | | | | | | 489 | 7,500 | 1235, 1190, 1105 |
| | $C_2H_5OCH(CH_3)$ | $H(CH_3)$ | 2HCl | B | 96 | 27.4 | 4.6 | 16.2 | 14.5 | 19.2 | 14.6 | 27.4 | 5.0 | 15.8 | 14.7 | 18.8 | 14.7 | 318 | 14,850 | 3440, 3190, 3100, 2700, 1585, 1535 |
| | | | | | | | | | | | | | | | | | | 488 | 4,550 | 1505, 1275, 1220, 1110, 1035 |
| 2 | $C_2H_5OCH(CH_3)$ | $(CH_3)_2$ | | A[d] | 80 | 36.6 | 5.6 | | 16.1 | 21.3 | 16.3 | 36.6 | 5.5 | | 16.1 | 20.5 | 16.1 | 322 | 18,800 | 1510, 1505, 1255, 1130, 1105 |
| | | | | | | | | | | | | | | | | | | 500 | 8,700 | |
| 3 | $CH_3OCH(CH_3)$ | $H_2$ | | A | 79 | 26.0 | 3.7 | | 19.6 | 26.0 | 19.8 | 25.9 | 3.6 | | 20.2 | 25.7 | 20.1 | 314 | 22,050 | 3440, 3400, 3280, 3180, 3060, 1630, |
| | | | | | | | | | | | | | | | | | | 488 | 5,350 | 1615, 1575, 1525, 1310, 1205, 1180, |
| | | | | | | | | | | | | | | | | | | | | 1090, 1070 |
| | $CH_3OCH(CH_3)$ | $H_2$ | 2HCl | B[e] | 95[e] | 21.2 | 3.6 | 17.9 | 16.0 | 21.2 | 16.2 | 21.9 | 4.0 | 16.7 | 16.3 | 20.8 | 15.7 | 313 | 20,900 | 3240, 3060, 2680, 1620, 1550, 1315, |
| | | | | | | | | | | | | | | | | | | 488 | 5,600 | 1210, 1110 |
| 4 | $CH_3CO_2CH(CH_3)$ | $H_2$ | | D[f] | 78 | 27.3 | 3.4 | | 18.1 | 23.9 | 18.2 | 27.5 | 3.7 | | 18.6 | 23.6 | 18.5 | 316 | 18,550 | 3410, 3330, 3160, 1725, 1610, 1595, |
| | | | | | | | | | | | | | | | | | | 488 | 4,650 | 1585, 1525, 1240, 1055 |
| 5 | $HOCH(CH_3)$ | $H_2$ | 2HCl | H[f] | 60 | 18.8 | 3.2 | 18.5 | 16.6 | 22.0 | 16.8 | 19.4 | 3.7 | 17.9 | 16.0 | 21.3 | 16.2 | 312 | 19,950 | 3260, 3100, 2700, 1620, 1550, 1215, |
| | | | | | | | | | | | | | | | | | | 486 | 5,200 | 1120, 1070 |
| 6 | $CH_3$ | $H_2$ | | I | 66 | 21.5 | 2.9 | | 22.7 | 30.0 | 22.9 | 22.1 | 3.2 | | 20.6 | 29.7 | 22.3 | 310 | 21,950 | 3390, 3280, 3100, 1630, 1590, 1535, |
| | | | | | | | | | | | | | | | | | | 484 | 5,400 | 1500, 1200, 1175 |
| 7 | $CH_3$ | $H(CH_3)$ | | J | 45 | 27.3 | 3.9 | | 20.7 | 27.3 | 20.9 | 27.3 | 3.8 | | 20.9 | 27.0 | 20.5 | 314 | 21,200 | 3380, 3320, 1555, 1530, 1510, 1240, |
| | | | | | | | | | | | | | | | | | | 485 | 7,250 | 1195, 1180 |
| 8 | $C_2H_5OCH_2$ | $H(CH_3)$ | 2HCl | B | 89 | 25.4 | 4.3 | 16.7 | 15.0 | 19.8 | 15.1 | 25.7 | 4.5 | 16.1 | 14.7 | 19.5 | 15.2 | 317 | 20,600 | 3400, 3200, 3100, 2640, 1620, 1575, |
| | | | | | | | | | | | | | | | | | | 489 | 7,300 | 1505, 1275, 1235, 1115, 1040 |

[a] The number refers to the uncomplexed ligand; abbreviations in common use for these ligands are: **1**, KTSM; **2**, $KTSM_2$; **3**, KMTS; **4**, KATS; **5**, KHTS; **6**, PTS; **7**, PTSM; **8**, EPTSM. [b] See Experimental Section. [c] See footnote *c*, Table I. [d] This compound does not form a hydrochloride under these conditions (method B). [e] Recrystallized by precipitation from MeOH–HCl with ether as in B. Over-all yield is indicated. [f] Initial reactants dissolved in 50% aqueous EtOH.

**Method F.**—The reactants were finely ground together and suspended in 200 ml of $H_2O$ which was heated to 90° and held there for 10 min. $ZnKTS \cdot H_2O$ was obtained by hot filtration.

**Method G.**—$ZnKTS \cdot H_2O$ was dissolved in AcOH and poured into 4 vol of $Et_2O$ with constant agitation. The sticky precipitate was triturated with diethyl ether and Skellysolve B until a crystalline product resulted.

**Method H.**—The 2HCl of the cupric chelate of **5** was obtained when attempts to prepare the 2HCl of the cupric chelate of **4** using method B resulted in hydrolysis of the acetoxy group. The reaction mixture was evaporated to dryness, extracted with warm MeOH (50°), and crystallized by adding 10 vol of $Et_2O$.

**Method I.**—Compound **6** was suspended in boiling MeOH and a hot aqueous solution of $Cu(OAc)_2 \cdot H_2O$ was added. The mixture was refluxed for 20 min and the precipitate was isolated by filtering the hot mixture.

**Method J.**—The reactants were ground together and extracted with hot MeOH–$Me_2CO$ from which the complex was crystallized by cooling to −10°.

**Determination of Metal to Ligand Ratio.**—Separate stock solutions of metal salt and ligand were prepared at $2 \times 10^{-3}$ *M* in 95% EtOH. The desired aliquot of metal ion was added to 1.0 ml of ligand solution, mixed, and diluted to 10.0 ml. After standing for 2–3 min to allow complete reaction, a further 1:10 dilution was made with 95% EtOH and the absorption spectrum was run. Completeness of the reaction was checked by again running the spectrum after a 10–15-min waiting period to see whether any further changes occurred. With all metals studied except Ni(II), the reaction was complete within 2–3 min. Changes were observed in the Ni(II)KTS spectrum even after 24 hr. The absorption spectra were determined on a Beckman DB spectrophotometer equipped with a Sargent SRL recorder. The final concentration of bis(thiosemicarbazone) was $2 \times 10^{-5}$ *M* in all cases.

*In Vitro* **Antitumor Activity.**—The assay used is a modification of the method described by Arai and Suzuki[10] and involves incubation of the drug with freshly isolated tumor cells in agar. Inhibition of cell respiration due to the drug is determined visually by the increased time necessary for the cells to reduce the methylene blue indicator in the agar compared with untreated control cell suspension.[11]

## Results and Discussion

**Metals Complexed by $H_2KTS$ and Related Ligands.**—In surveying the various metal ions readily available, it was found that $H_2KTS$ as well as **1**–**4**[12] reacted with many transition metal ions including most of the biologically important metals. Metals were tested for reactivity by adding several drops of a dilute aqueous metal salt solution to a dilute alcoholic solution of the ligand. Accepted as evidence for reaction was either a change in color or a distinct deepening in intensity of the same color.

Although some slight variations in reactivity were observed, it was found that the above ligands readily coordinated with the following metal ions: Ag(I), Au(III), Bi(III), Cd(II), Co(II), Cu(I,II), Fe(II,III), Hg(II), Ni(II), Pb(II), Pd(II), Pt(IV), and Sn(II). The series of pyruvaldehyde derivatives **6**–**8** reacted similarly although they were not all tested with all of the metals listed.

**Synthesis of Complexes.**—Since $H_2KTS$ was of greatest clinical interest as an antitumor agent, a number of $H_2KTS$ complexes were prepared of those metals having greatest biological importance. In addition, a number of copper(II) complexes and/or their hydrochlorides were prepared of the compounds related to $H_2KTS$ and pyruvaldehyde bis(thiosemicarbazone). In most cases, the initial reaction products were of sufficient purity to be used for biological studies without further purification.

The complexes of $H_2KTS$ which were prepared in acceptable analytic purity are shown in Table I, together with elemental and spectral data and probable formulas. Table II lists the cupric complexes which were prepared of the related bis(thiosemicarbazones). Analytical data indicate that the cupric chelates of Table II have the same type of structure as the cupric chelate of $H_2KTS$. The cupric chelate of $H_2KTS$ in its triclinic form has been shown by Taylor, *et al.*,[13] by X-ray crystallography to have a distorted planar structure with a high degree of resonance.

In addition to the above compounds, the $H_2KTS$ complexes of Co(II), Cu(I), Au(III), and Hg(II) have been prepared and isolated; however, on the basis of elemental analysis a probable formula could not be assigned. This is due primarily to the presence of solvent ligands, such as acid and water, which were included in the coordination complex, making the analyses difficult to interpret. This was especially true for the acid salts since these were all hygroscopic, and sometimes the final traces of water were difficult to remove without also removing the acid salt. Similar difficulties were reported by Gingras, *et al.*,[14] who were able to prepare the copper(II) chelates of several bis(thiosemicarbazones), but were unable to identify the products of similar reactions with other metal ions.

All of the complexes were colored, some highly colored, ranging from yellow (Ag), to gold (Au, Zn), brown (Fe), dark green (Ni), and black (Co). Copper(II) formed red-brown solid complexes with all of the ligands studied which changed to varying shades of green upon formation of the dihydrochloride addition salt. The cupric chelates of all of the ligands in Table II readily formed dihydrochlorides except **2** and **4**. In **2** this was attributed to the completely substituted amine groups which result in a much different electronic configuration and prevent formation of the acid salt in the usual manner. All attempts to prepare the dihydrochloride of the cupric chelate of **4** resulted in the loss of the 3-acetoxy group and the formation of the dihydrochloride of the cupric chelate of the 3-hydroxy compound (**5**).

The acid addition salts could be prepared either from the preformed Cu(II) ligands, or directly by treating the ligand with cupric chloride in anhydrous methanol and precipitating with 4–5 vol of ether. Preparation of these salts greatly increased their water solubility. In dilute aqueous solution, the dihydrochlorides tend to dissociate to form the unprotonated complexes which have a reddish color. The green color is retained in concentrated aqueous solution or in hydrochloric acid solution stronger than 1 *N*.

**Ligand:Metal Ratio.**—By calculating the decrease in the major bis(thiosemicarbazone) maxima in the 340–350-mμ region and the corresponding increase in visible maxima, the rate of complex formation was followed and the ratio of metal to ligand was determined.

(10) T. Arai and M. Suzuki, *J. Antibiot.* (Tokyo), **A9**, 169 (1956).
(11) Further details of this method are now being prepared for publication.
(12) See Table II.
(13) M. R. Taylor, E. J. Gabe, J. P. Glusker, J. A. Minkin, and A. L. Patterson, *J. Amer. Chem. Soc.*, **88**, 1845 (1966).
(14) B. A. Gingras, T. Suprunchuk, and C. H. Bayley, *Can. J. Chem.*, **40**, 1053 (1962).

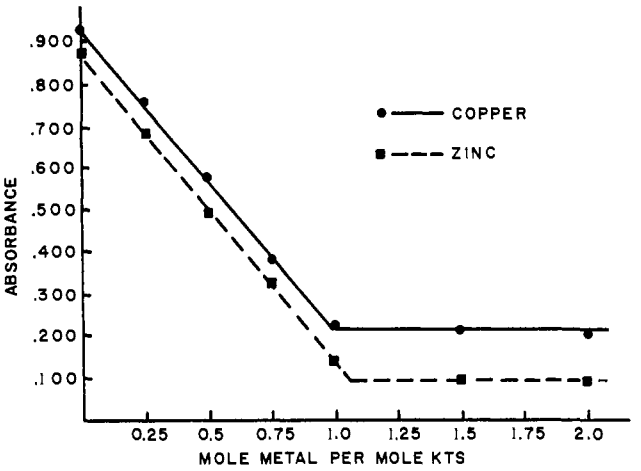


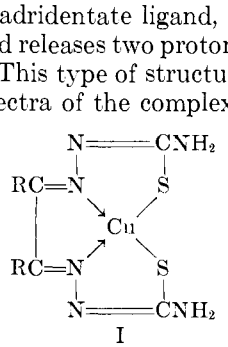

Figure 1.—The effect of copper(II) and zinc(II) on the 347-mμ absorption maximum of $H_2KTS$.

Figure 1 shows the effect of copper(II) and zinc(II) on the 347-mμ maxima of $H_2KTS$. The curves indicate that $H_2KTS$ reacts only in a 1:1 ratio with these metals. The linear decrease in absorbance with increasing amounts of metal, up to a 1:1 ratio, indicates that no discernible intermediates were formed. A lack of linearity was found when cobalt(II) was investigated which did indicate the possibility of such an intermediate.

Compound **1** reacted similarly with copper(II), zinc(II), and cobalt(II). Compound **2**, however, reacted quite differently, the curves indicating a probable stepwise reaction with copper(II) and possibly other metals.[15] The possibility of a stepwise formation mechanism is supported by the fact that silver(I) forms both 1:1 and 1:2 complexes with $H_2KTS$.

All of the divalent metals studied complex with $H_2KTS$ in a 1:1 ratio. Iron(III), the only trivalent metal which formed a stable complex which could be isolated and characterized, reacted in the ratio Fe:KTS = 2:3. The elemental analyses of the Au(III) complex of $H_2KTS$, although not sufficient to completely define its formula, also showed the ratio of Au:KTS = 2:3.[16] These data support the type of structure proposed by Bähr[9a] for the copper(II) and nickel(II) chelates of similar bis(thiosemicarbazones) as shown by formula I. In addition, the compound obtained with Fe(III) may be schematically represented by II to show the possible bonding arrangements. In each example, $H_2KTS$, being a quadridentate ligand, supplies four bonds to the metal and releases two protons.

This type of structure is supported by the absorption spectra of the complexes of Table I which show that a




I

KTS≡Fe═KTS═Fe≡KTS

II

(15) Further studies with this ligand will be reported later.

(16) *Anal.* Calcd for $C_{24}H_{52}Au_2Cl_2N_{18}O_7S_6$ [$Au_2(KTS)_3 \cdot 2HCl \cdot 4H_2O$]: C, 21.2; H, 3.9; Au, 28.9; Cl, 5.2; N, 18.5; O, 8.2; S, 14.1. Found: C, 21.7; H, 2.5; Au, 30.8 (Ash); Cl, 5.7; N, 18.7; O, 6.2; S, 14.5.

TABLE III

*In Vitro* ANTITUMOR ACTIVITY *vs.* WALKER 256 TUMOR CELLS

| Compd | Cytotoxic level, μg/ml[a] Alone | +Cu(II) |
|---|---|---|
| $H_2KTS$ | >800 | 0.8 |
| 1 | 800 | 1.6 |
| 2 | 800 | >400 |
| 3 | 1600 | 1.6 |
| 4 | 800 | 3.2 |
| 6 | 1600 | 0.4 |
| 7 | 1600 | 0.4 |
| Cu(II)KTS | 1.6 | ... |
| Zn(II)KTS·$H_2O$ | 25 | ... |
| Fe(II)KTS·2HCl | 100 | ... |
| $[Fe(III)]_2(KTS)_3 \cdot 2H_2O$ | 100 | ... |
| Ni(II)KTS | 200 | ... |
| $[Ag(I)]_2KTS \cdot HNO_3$ | 100 | ... |

[a] Minimum level of drug which causes a 20% reduction in the respiratory activity of the tumor cell suspension using methylene blue as indicator.

distinct change occurs in the structure of $H_2KTS$ upon chelation. The spectra also suggest that the electronic configuration of the complex is primarily dependent upon the metal ion present and relatively independent of the rest of the molecule. This holds true for all of the bis(thiosemicarbazones) and bis($N^4$-methylthiosemicarbazones) studied, as well as for **2**, a bis($N^4$-dimethylthiosemicarbazone), which as an uncomplexed ligand exhibits a much different ultraviolet spectrum than do the former two types.[4a]

**Biological Activity.**—Preliminary *in vitro* antitumor activity was determined against freshly isolated Walker 256 rat tumor cells using the modified Arai–Suzuki test.[10,11] In some cases, the preformed chelates were tested and in others the chelates were formed *in situ* by treating the free ligand with cupric ion. The results of these tests are summarized in Table III.

The free ligands of Table II were all inactive when tested alone as was $H_2KTS$, since all had a cytotoxicity of >100 μg/ml.[17] However, when the free ligands were treated with $Cu^{2+}$, highly active chelates resulted with every compound except **2**. In each case, the $Cu^{2+}$ was present at a level of 0.4 μg/ml, a level which in combination with $H_2KTS$ was found to show maximum activity, and which itself was not toxic to the cells.[18]

When the preformed chelates of $H_2KTS$ (Table I) were tested, only the cupric and zinc chelates had appreciable activity and only Cu(II)KTS had high activity.

The cupric and zinc chelates of $H_2KTS$ have also been tested for antitumor activity in rats. The results correlate well with the above *in vitro* data since the cupric chelate was highly active and the zinc chelate, while not active alone, did enhance the activity of Cu(II)KTS under certain conditions.[7]

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(17) Values ≤1.6 μg/ml are considered highly active; 3.2–12.5 μg/ml, moderately active; and 25–50 μg/ml, slightly active. Anything ≤100 μg/ml is inactive.

(18) Unpublished data from this laboratory.