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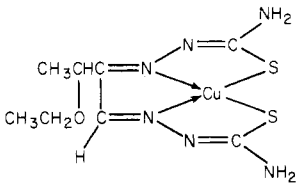
Comparative Analysis of the Cytotoxicity of Substituted [Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelates†

Eugene A. Coats,* Stanley R. Milstein, Gary Holbein, John Mc Donald, Ronald Reed, and Harold G. Petering

College of Pharmacy and Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45221. *Received May 5, 1975*

Seven para-substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates (**12–18**) have been designed, synthesized, and tested for their ability to inhibit the respiration of rat liver slices as a normal cell model and Ehrlich ascites cells as a tumor cell model. Relationships between chemical structure and respiratory inhibition are described on a quantitative basis using substituent constants (π, E_s, and σ_p) by computerized multiparameter regression analyses. The correlations indicate that changes in E_s have the largest effect on liver slice toxicity of chelates while π and σ_p account for most of the variation in toxicity to ascites cells. A comparative analysis strongly suggests that electron-donating substituents with greater water solubility should increase cytotoxicity to ascites cells at the expense of cytotoxicity to the rat liver cells. The predictions of the equations were checked by synthesizing and testing an additional derivative. The results strengthen the initial predictions.

The development of metal chelates and chelating agents as potential antitumor agents has led to the selection of a class of copper chelates of bis(thiosemicarbazones) for initial quantitative SAR studies. Many reports have appeared dealing with KTS [2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone), kethoxal bis(thiosemicarbazone)] and its copper(II) chelate **1** as antitumor agents.[1-9]

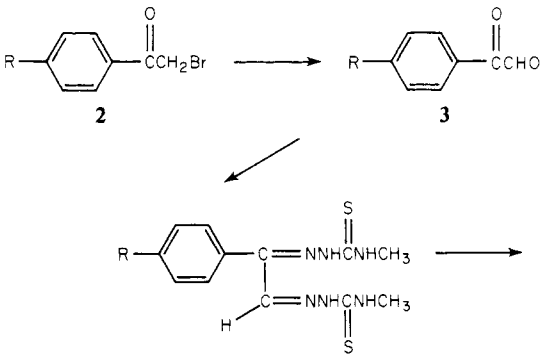


1

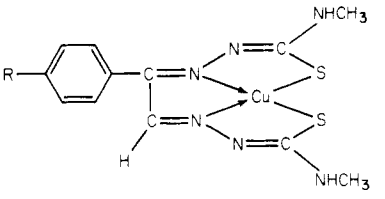
Considerable evidence has been presented in support of the view that **1** serves as a vehicle for transporting copper into the cell and that the cytotoxicity is actually due to the accumulation of copper within the cell and its effect on enzymes involved in DNA synthesis. More recent investigations[10] have indicated that an additional mechanism of cytotoxicity may reside in the ability of the copper chelate of KTS to uncouple oxidative phosphorylation in isolated rat liver mitochondria. These chelates are also capable of inhibiting the respiration of Ehrlich ascites cell suspensions and of rat liver slices.[10] In light of these findings a new series of bis(thiosemicarbazone)copper(II) chelates (**12–18**) was designed in an attempt to allow further delineation of the mechanism of action and to allow investigation of possible quantitative relationships between chelate structure and biological activity. The molecules examined in this stage of our investigation contain completely conjugated phenyl rings for the primary purpose of determining the electronic effect of substituents on biological activity. If the chelate is in fact a metal ion transport system, it seemed reasonable that the substituted phenyl rings would affect chelate stability and hence the biological effectiveness of the bis(thiosemicarbazone) chelate. The substituents were selected, therefore, to obtain the largest feasible variance in electron-donating and -withdrawing ability as measured by the Hammett σ constant.[11] In addition, the variance in steric[12] and hydrophobic[13] properties was considered with care being taken to select only those substituents for which accurate physicochemical parameters were readily available.

† This paper is dedicated to the memory of Professor Edward E. Smissman whose scientific insight, personal integrity, and constant warmth and concern continue to serve as an example to one young medicinal chemist (Eugene A. Coats).

Scheme I



2 **3** **4-10**



12-18

Chemistry. The chelates were prepared according to that shown in Scheme I, starting with the appropriately substituted phenacyl bromide (**2**).

Following procedures established by Kornblum, the known phenylglyoxals **3** were prepared by treating **2** with dimethyl sulfoxide at room temperature.[14] Conversion of **3** to the bis(4-methyl-3-thiosemicarbazones) **4–10** proceeded smoothly in the presence of excess 4-methyl-3-thiosemicarbazide and an acid catalyst.[15] Treatment of **4–10** with stoichiometric amounts of cupric acetate in hot

Table I. Physical Properties

No.	R	% yield	Mp, °C	λ max (95% EtOH), nm (ε)	Formula	Analyses[a]
			4-R-Phenylglyoxal Bis(4-methyl-3-thiosemicarbazone)			
4	H	52	223–225 dec[b]	337 (26,000)	$C_{12}H_{16}N_6S_2$	C, H, N
5	Br	88[c]	232–233 dec	337 (34,000)	$C_{12}H_{15}BrN_6S_2$	C, H, N
6	Cl	79[c]	227–229 dec	337 (26,000)[d]	$C_{12}H_{15}ClN_6S_2$	H, N; C[e]
7	OCH_3	27	215–216 dec	338 (30,000)	$C_{13}H_{18}N_6OS_2$	C, H, N, S
8	CH_3	44	217–218 dec	338 (26,000)	$C_{13}H_{18}N_6S_2$	C, H, N, S
9	NO_2	82[c]	238–239 dec	336 (22,000)[f]	$C_{12}H_{15}N_7O_2S_2$	C, H, N
10	C_6H_5	46	216–217 dec	337 (41,000)	$C_{18}H_{20}N_6S_2$	C, H, N, S
11	OH	49	235–236 dec	341 (35,000)	$C_{12}H_{16}N_6OS_2$	C, H, N
			[4-R-Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelate			
12	H	85	234–235 dec	306 (26,000), 500 (6,000)	$C_{12}H_{14}CuN_6S_2 \cdot 0.5H_2O$	C, H, S, Cu; N[g]
13	Br	75	240–241 dec	307 (24,000), 500 (4,500)	$C_{12}H_{13}BrCuN_6S_2$	C, H, N
14	Cl	95	239–240 dec	307 (20,000), 500 (8,000)	$C_{12}H_{13}ClCuN_6S_2$	C, H, N
15	OCH_3	68	221–222 dec	307 (25,000), 498 (5,400)	$C_{13}H_{16}CuN_6OS_2$	C, H, N
16	CH_3	84	237–238 dec	305 (22,000), 500 (5,000)	$C_{13}H_{16}CuN_6S_2$	C, H, N
17	NO_2	77	253–255 dec	307 (15,000), 463 (4,600)[f]	$C_{12}H_{13}CuN_7O_2S_2$	C, H, N, S
18	C_6H_5	86	242–243 dec	313 (16,500), 500 (3,400)	$C_{18}H_{18}CuN_6S_2$	C, H, N
19	OH	89	218–220 dec	307 (28,000), 505 (6,500)	$C_{12}H_{14}N_6OS_2Cu \cdot H_2O$	C, H, N

[a] Analyses within 0.4%. [b] Lit.[15] mp 224°. [c] Crude yield. [d] Taken in CH_3OH. [e] C: calcd, 42.04; found, 41.55. [f] Taken in 75% $CHCl_3$–25% CH_3OH. [g] N: calcd, 22.18; found, 21.53.

Table II. Cytotoxicity of [4-Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelates

		Liver			Ascites			pI_{50} (ascites) – pI_{50} (liver)	
No.	R	I_{50}[a]	pI_{50} (obsd)	pI_{50} (pred)[b]	I_{50}[c]	pI_{50} (obsd)	pI_{50} (pred)[d]	Obsd	Pred[e]
12	H	1.56 (0.54)	2.81	2.76	0.32 (0.24)	5.49	5.05	2.69	2.42
13	Br	2.00 (0.85)	2.70	2.60	8.92 (1.60)	4.05	4.23	1.35	1.71
14	Cl	1.84 (0.75)	2.73	2.63	4.23 (1.60)	4.37	4.33	1.64	1.80
15	OCH_3	3.14 (0.66)	2.50	2.68	0.48 (0.24)	5.32	5.37	2.82	2.71
16	CH_3	2.87 (1.55)	2.54	2.59	3.00 (0.81)	4.52	4.87	1.98	2.29
17	NO_2	3.67 (1.26)	2.43	2.42	4.83 (1.38)	4.32	4.37	1.89	1.76
18	C_6H_5	5.90 (2.05)	2.24	2.25	11.80 (3.79)	3.93	3.77	1.69	1.37
19	OH	1.36 (0.58)	2.87[f]	2.68	0.09 (0.01)	6.03[f]	5.91	3.16[f]	3.17

[a] I_{50} values are in millimoles per milliliter of test solution per liver slice (100 mg av wt) × 10^3; numbers in parentheses are standard deviations. [b] Predicted by eq 3. [c] I_{50} values are in millimoles per milliliter of test solution per milligram of ascites cells × 10^5; numbers in parentheses are standard deviations. [d] Predicted by eq 7. [e] Predicted by eq 8. [f] This data point was not available when eq 3, 7, and 8 were derived.

solution followed by very slow cooling afforded the chelates **12–18** in relatively high yield.[16] The chelates could be readily identified by the characteristic shift in ultraviolet absorption maxima from 337 nm for **4–10** to 307 nm for **12–18**. This shift was accompanied by the appearance of a new band in the visible region. Pertinent chemical and spectral properties of all new compounds are summarized in Table I.

Biological Results. Compounds **12–18** were evaluated for their ability to inhibit the respiration of Ehrlich ascites cell suspensions as a tumor cell model and these results compared to the inhibition of rat liver slice respiration as a normal cell model. Ehrlich ascites tumor cells have been extensively utilized as a system for study of various biochemical properties of intact cells.[17–19] Rat liver slices have been demonstrated as one of the few ways of measuring effects on totally intact liver cells as physical or chemical methods of obtaining liver cell suspensions often appear to damage the cell membranes.[20] These two in vitro systems were selected for a comparative analysis because of their relative ease in handling and their potential for allowing quantitative measurement of respiratory inhibition. The respiratory rates were followed potentiometrically by oxygen electrode[21] and the percent inhibition of oxygen uptake correlated with chelate concentration by the method of least squares to obtain values for 50% inhibition (I_{50}). The observed and predicted biological activities are summarized in Table II.

The differences in potency against liver slice vs. ascites cell respiration are not as great as the values in Table II might indicate since the liver slice values were calculated per slice in 5 ml while the ascites values were calculated per milligram per milliliter of ascites cells. The actual differences are in the range of 10–20-fold in favor of ascites.

Table III. Substituent Constants

No.	R	π[a]	E_s[b,c]	MR[a]	σ_p[a]
12	H	0.00	1.24	1.03	0.00
13	Br	0.86	0.08	8.88	0.23
14	Cl	0.71	0.27	6.03	0.23
15	OCH_3	−0.02	0.69	7.87	−0.27
16	CH_3	0.56	0.00	5.65	−0.17
17	NO_2	−0.28	−1.28	7.36	0.78
18	C_6H_5	1.96	−2.58	25.36	−0.01
19	OH	−0.67	0.69	2.85	−0.37

[a] Reference 22. [b] M. S. Tute, *Adv. Drug Res.*, **6**, 72 (1971). [c] E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).

Table IV. Correlation Coefficients (r^2) between Regression Parameters for Chelates **12–18**

	π	E_s	MR	σ_p
π	1.00	0.38	0.69	0.06
E_s		1.00	0.75	0.11
MR			1.00	0.01
σ_p				1.00

Analyses and Discussion. The biological data for compounds **12–18** were subjected to computerized multiparameter regression analyses in an effort to delineate structural effects on respiratory toxicity. The values for the substituent constants utilized in the regression analyses reported below are presented in Table III. Table IV is the covariance matrix for these substituent parameters. In all cases, every potentially meaningful combination of

linear and squared parameters was investigated. The best single parameter correlations with liver slice respiratory inhibition were eq 1–3 involving π, the hydrophobic substituent constant,[13] MR, molar refraction,[22] and E_s, the Taft steric parameter,[12] respectively.

	n	s	r	
$pI_{50} = -0.11\ (\pm 0.27)\ \pi + 2.62\ (\pm 0.24)$	7	0.196	0.420	(1)
$pI_{50} = -0.02\ (\pm 0.02)\ MR + 2.75\ (\pm 0.20)$	7	0.126	0.811	(2)
$pI_{50} = +0.13\ (\pm 0.09)\ E_s + 2.59\ (\pm 0.11)$	7	0.109	0.864	(3)

In these equations the numbers in parentheses are the 95% confidence intervals for the coefficients, n is the number of data points, s is the standard deviation, and r is the correlation coefficient. As can be seen, in eq 1, π is not very effective in accounting for changes in biological activity. MR and especially E_s do a much better job of explaining structural effects on liver slice respiration with both eq 2 and 3 indicating that small substituents are most effective. These results are somewhat surprising as one would expect lipophilicity to play a more significant role in the transport into liver cells. Two parameter equations in π and MR, σ_p and E_s, and in σ_p^+ and E_s exhibited decreased standard deviations and increased correlation coefficients over eq 1–3; however, the improvements were not statistically significant as indicated by the F test. As indicated in Table IV, there is a considerable degree of covariance between π, E_s, and MR, which clearly has a deleterious effect on our attempts to separate and identify the influence of these parameters. The difficulty encountered in deriving meaningful correlations here is undoubtedly also a reflection of the relatively small variance in biological activity obtained as well as the limited size of the data set.

Correlation of Ehrlich ascites respiratory inhibition afforded eq 4–6 in π, MR, and E_s, respectively. The effect of covariance between π, E_s, and MR is even more obvious here as eq 4, 5, and 6 are essentially identical. Addition of an electronic term to eq 5 and 6 does not afford substantial improvement; however, eq 7 is clearly an improvement over eq 4 ($F_{1,4} = 8.88$; $F_{1,4;\,\alpha\,0.05} = 7.71$). While eq 7 is based upon a relatively small number of data points, considerable significance can be attached to it since the substituents were selected specifically to examine hydrophobic and electronic effects. Consequently, these two parameters are virtually orthogonal (Table IV, $r^2 = 0.06$) with a variance of over one log unit in each, conditions which according to Unger[23] can often allow identification of parameters affecting biological response based upon relatively few, well-spread, data points. The resonance

	n	s	r	
$pI_{50} = -0.53\ (\pm 0.69)\ \pi + 4.86\ (\pm 0.61)$	7	0.496	0.663	(4)
$pI_{50} = -0.05\ (\pm 0.07)\ MR + 5.01\ (\pm 0.81)$	7	0.517	0.626	(5)
$pI_{50} = 0.35\ (\pm 0.35)\ E_s + 4.65\ (\pm 0.43)$	7	0.434	0.756	(6)
$pI_{50} = -0.66\ (\pm 0.48)\ \pi - 1.12\ (\pm 1.04)\ \sigma_p + 5.05\ (\pm 0.45)$	7	0.309	0.909	(7)

terms, σ_p^+ and σ_p^-, were also investigated in combination with π and found to be inferior to σ_p. These relationships indicate that more hydrophilic, electron donating substituents should increase inhibition of ascites cell respiration.

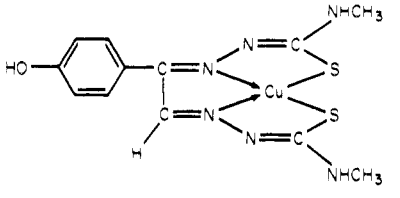
In view of the striking differences in the correlations of liver slices and ascites respiratory inhibition, a quantitative comparative analysis was conducted by correlating the differences in pI_{50} for the two systems. By subtracting the pI_{50} for liver slice from that for ascites for each derivative, it is possible to ascertain structural features which may be responsible for selectivity. For all practical purposes, the ratio of ascites to liver pI_{50} represents a kind of therapeutic index, and correlations with this ratio as dependent variable may indicate ways to increase ascites toxicity while minimizing liver toxicity. A similar procedure was employed in the derivation of the Taft σ^* parameter[24,25] by Kutter[26] to compare intravenous with intraventricular activities of analgetics and in a quantitative comparison of thrombin and trypsin inhibition.[27] This treatment is in effect a subtraction of regression equations, which is here conducted by computer in order to obtain 95% confidence intervals for coefficients as well as standard deviations and correlation coefficients. As in the derivation of eq 1–7, all potentially meaningful relationships were examined, with eq 8 involving the linear combination of π and σ_p emerging as the most significant (vs. equation in π only; $F_{1,4} = 6.37$; $F_{1,4;\,\alpha\,0.10} = 4.54$). In this relationship the negative coefficient associated with π indicates that more hydrophilic substituents should

	n	s	r	
pI_{50} (ascites) − pI_{50} (liver) = $-0.54\ (\pm 0.52)\ \pi - 1.04\ (\pm 1.14)\ \sigma_p + 2.42\ (\pm 0.49)$	7	0.339	0.863	(8)

increase the difference in pI_{50} and the negative coefficient associated with σ_p indicates that electron-donating substituents should also increase this difference.

Equations 1–8 serve to emphasize several differences in the two test systems. The apparent high dependence on steric properties observed only in the rat liver slice correlations can be attributed to the tissue matrix surrounding the liver cells which might be expected to retard the penetration of the compounds into the cells. Equation 8 does serve to point out, in quantitative terms, the most important differences in the two systems. Electron donation and thus increased chelate stability should increase ascites cell toxicity at the expense of liver slice toxicity. More hydrophilic substituents are predicted to have a similar effect. These results appear to provide additional evidence suggesting that tumor cell membranes may be more polar than normal cell membranes and thus more permeable to hydrophilic molecules.[28,29]

An additional check on the validity of the correlations described above was then provided by designing a new derivative with a *p*-hydroxy substituent, **19**, which fulfills the criteria of electron donation through the aromatic ring and an increase in water solubility for increased ascites cell toxicity as indicated by eq 8.



19

This derivative was predicted by eq 3 to exhibit a pI_{50} of 2.68 against liver slice respiration and was predicted by eq 7 to exhibit a pI_{50} of 5.91 against Ehrlich ascites respiration. Equation 8 predicts a pI_{50} difference of 3.17. Subsequent synthesis of **19** was carried out in a manner similar to the first seven chelates except that the 4-hydroxyphenylglyoxal was obtained by selenium dioxide oxidation of the corresponding 4-hydroxyacetophenone[30] as well as by the Kornblum oxidation of 4-hydroxyphenacyl bromide.[31] Biological testing afforded observed pI_{50}'s of 2.87 against liver slice respiration, of 6.03 against Ehrlich ascites respiration, and a corresponding pI_{50} difference of 3.16.

In view of the limited number of data points used in deriving the initial correlations, these observed activities are in excellent agreement with the predicted values and consequently provide support for the indicated influence of electronic character and hydrophobic character on biological response in both test systems. As a final step in this first stage of our investigations, all correlations were rerun including the *p*-hydroxy derivative **19** in the data set. The resulting equations (9–11) corresponding to eq 3, 7, and 8, respectively, are given below. In eq 10 and 11, improvements in 95% confidence intervals and correlation coefficients are evident while no changes in the parameters which apparently influence activity have occurred.

	n	s	r	
pI_{50} (liver) = 0.14 (±0.09) E_s + 2.62 (±0.10)	8	0.120	0.852	(9)
pI_{50} (ascites) = −0.69 (±0.33) π − 1.17 (±0.74) σ_p + 5.08 (±0.29)	8	0.279	0.951	(10)
pI_{50} (ascites) − pI_{50} (liver) = −0.54 (±0.36) π − 1.03 (±0.81) σ_p + 2.42 (±0.31)	8	0.303	0.919	(11)

Conclusions

This stage in the application of QSAR techniques to the design of potential antineoplastic agents is significant from several viewpoints. QSAR by its nature requires that reasonably accurate quantitative biological data be available. Previously established techniques for monitoring changes in cell respiration in vitro have been adapted to allow a quantitative comparative analysis of inhibitor effects on a normal cell model vs. a tumor cell model. A small set of chelates has been designed to gain insight into the physicochemical properties which may be related to respiratory inhibition. The substituent variations chosen have allowed identification of the hydrophobic and electronic effects on ascites cell respiration but were poorly chosen for separation of apparent steric effects on liver cell respiration, which are qualitatively and quantitatively different from those on tumor cells. The predictive capacity of the correlations has been tested by evaluation of a new derivative and found to be surprisingly good.

The next stage in the investigation will involve utilization of the equations derived and application of the recently published techniques of cluster analysis[32] in the design of congeners, which will reduce the covariance between π, E_s, and MR and which will allow further refinement in the reported correlations. Since the biological test systems are whole cell systems, it is also anticipated that appropriate selection of additional derivatives may allow delineation of a parabolic relationship in π. Such a relationship often exists when multiple partitionings are experienced by a molecule en route to its site of action.[33]

The molecules reported in this study are clearly too lipophilic for in vivo activity, and those tested in the L1210 leukemia screen were inactive. It is envisioned, however, that the necessary link between the in vitro model and the in vivo screen can be established with the preparation of the more hydrophilic chelates currently scheduled for synthesis.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Elemental analyses were carried out by Midwest Microlab, Ltd., Indianapolis, Ind., and were within 0.4% unless otherwise noted. Ir, NMR, and uv data were consistent with the assigned structures. Uv spectra were recorded on a Beckman Acta V spectrophotometer. Ir spectra were recorded on a Beckman IR-33 spectrophotometer as neat oils, KBr pellets, or Nujol mulls. NMR spectra were determined on a Hitachi Perkin-Elmer R-24 high-resolution NMR spectrometer as 20% solutions in $CDCl_3$, acetone-d_6, or Me_2SO-d_6, with Me_4Si as an internal standard. 4-Methyl-3-thiosemicarbazide was obtained from the Aldrich Chemical Co. Representative synthetic procedures are presented below for the molecules described in Table I.

4-Methoxyphenylglyoxal Bis(4-methyl-3-thiosemicarbazone) (7). A solution of 2.8 g (0.015 mol) of 4-methoxyphenylglyoxal (from 4-methoxyphenacyl bromide by the method of Kornblum[14]) in 50 ml of 95% EtOH was added dropwise to a refluxing solution of 3.9 g (0.037 mol) of 4-methyl-3-thiosemicarbazide and 8 ml of concentrated HCl in 35 ml of H_2O and 25 ml of 95% EtOH. Heating was continued for 5 min after the addition and the hot solution was filtered under suction to give a yellow solid. Recrystallization from methanol afforded 1.4 g (27%) of crystals: mp 215–216° dec; λ max 338 nm (ε 30,000). Anal. ($C_{13}H_{18}N_6OS_2$) C, H, N, S.

[4-Methoxyphenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelate (15). Following the method of Petering[16] a solution of 0.59 g (0.0029 mol) of cupric acetate in 20–30 ml of hot water (70–80°) was added dropwise to a refluxing solution of 1.0 g (0.0029 mol) of **7** in 120 ml of MeOH. Heating was continued for 10 min; the reaction mixture was allowed to cool slowly over a period of 5 hr and stirred at room temperature for 4 days. The product in the form of a bronze colored precipitate was isolated by vacuum filtration and dried to give 0.8 g (68%) of **15**: mp 221–222° dec; λ max 307, 498 nm (ε 25,000, 5400). Anal. ($C_{13}H_{16}CuN_6OS_2$) C, H, N.

4-Hydroxyphenylglyoxal. Method A. 4-Hydroxyacetophenone was oxidized by SeO_2 in dioxane according to the procedure of Fodor and Kovacs.[30] The crude glyoxal, obtained as a viscous amber oil (80%), was used without further purification in the preparation of **11**. The oil was converted for identification purposes to its hydrate by crystallization from H_2O: mp 110° (lit.[30] mp 111°).

4-Hydroxyphenylglyoxal. Method B. 4-Hydroxyacetophenone was brominated using $CuBr_2$ in $CHCl_3$–EtOAc according to the procedure of King and Ostrum.[31] Recrystallization from C_6H_6 followed by several recrystallizations from $CHCl_3$ (Norit A) gave colorless needles of 4-hydroxyphenacyl bromide (30%): mp 130° (lit.[31] mp 124–126°). Kornblum oxidation[14] afforded 4-hydroxyphenylglyoxal (46%), identical in its properties with that obtained via method A.

Biological Testing. A. Liver Slice. The liver from a 150–200-g rat was quickly removed and placed in ice-cold phosphate–Ringer buffer of pH 7.4. Thin slices of 75–125 mg each were obtained using a Harvard Apparatus Tissue Slicer Model 140 and were weighed before respiratory measurement. The slice respiration was followed using 0.11 *M* sodium succinate as substrate in a phosphate–Ringer buffer, pH 7.4, at 37° with a Yellowsprings Model 53 oxygraph. The effect of compounds on the respiration rate was determined by adding a dimethyl sulfoxide solution of the compound to the slice in buffer and incubating for 30 min at 37° before measuring respiration rates. Inhibition was determined by comparing treated respiration rates to control values from slices incubated with identical volumes (0.1–0.3 ml)

of dimethyl sulfoxide without compound. Sufficient buffer was used to give a total volume of 5.0 ml in each case.

B. Ascites. The Ehrlich ascites tumor cells were maintained by weekly transfer of 0.1–0.2 ml of the cell-rich ascites fluid each from Swice white mice with 7–10-day tumors. For respiration studies, approximately 4 ml of the ascites fluid was withdrawn from one mouse following cervical dislocation. Normally, at least 500×10^6 cells (2–3 g wet) could be obtained. The cells were washed in isotonic saline before use and were suspended in 10 ml of saline and maintained in ice until used. From 0.2 to 0.4 ml of this suspension was sufficient for individual respiration rate determinations. In measurements of respiratory inhibition the cells were incubated with pure dimethyl sulfoxide (0.1–0.3 ml) as a control or with dimethyl sulfoxide plus chelate (0.1–0.3 ml) as treated in sufficient glucose-free phosphate–Ringer to give a total volume of 3.0 ml. As with the liver slice measurements, the incubation period was 30 min at 37° at which time the respiration rate was recorded using a Yellowsprings Model 53 oxygraph.

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Copper Chelates as Possible Active Forms of the Antiarthritic Agents†

John R. J. Sorenson*

G. D. Searle and Co., Chicago, Illinois 60680, and Kettering Laboratory, University of Cincinnati Medical Center, Cincinnati, Ohio 45267. *Received May 20, 1975*

Copper complexes, a unique class of potentially more therapeutically useful antiarthritic agents having both antiinflammatory and antiulcer activities, are presented. Points of interest with regard to their relatively low toxicities and mechanisms of action are discussed.

Cupric acetate was observed to be more active than hydrocortisone in the carrageenan foot edema model of inflammation.[1] To account for this observation, it was suggested that the administration of copper (Cu) in the form of cupric acetate resulted in the formation of Cu chelates in vivo and that these chelates were responsible for the observed antiinflammatory activity as illustrated in Figure 1. Similarly, the observed antiinflammatory activity of the clinically used antiarthritic agents could be attributed to the formation of a Cu chelate or complex in blood since it is well known that serum Cu levels increase markedly in arthritic disease.[2] As a result, it seemed plausible to suggest that a Cu chelate could be formed in vivo and provide the intermediate required for the observation that both Cu and chelating compounds have antiinflammatory or antiarthritic activity.

A search of the literature with regard to the biologic requirements and toxicity of Cu led to the following conclusions. Copper is an essential element and is required for normal metabolism in man.[2-4] Copper, like the essential fats, amino acids, and enzyme cofactors, is required for normal metabolism of all tissues. Since coordinated forms of Cu are always more stable forms, compared to

† This manuscript is dedicated to the memory of a scholar, major professor, and friend, Edward E. Smissman.

*Address correspondence to this author at Kettering Laboratory.