Reaction of the Antiproliferative and Antineoplastic Agent *trans*-Bis(salicylaldoximato)copper(II) and Related Chelates with Glutathione and Cysteine. Correlation between Reactivity and Biological Activity

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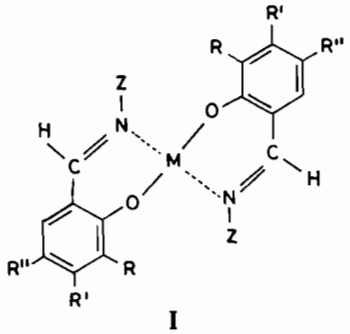
We recently reported the antiproliferative activity of *trans*-bis(salicylaldoximato)copper(II) ($CuSAO_2$; I, R = R′ = R″ = H, Z = OH, M = Cu) and related chelates. The parent compound and some derivatives were shown to totally arrest the proliferation of tumor cells *in vitro* in the concentration of about



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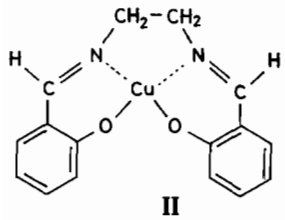

II

5 ppm [1, 2]. Further, $CuSAO_2$ was shown to have a powerful and in some cases even curative antitumor activity against Ehrlich ascites carcinoma *in vivo* [1]. Only the intact chelates have antiproliferative activity. The free ligand of $CuSAO_2$, salicylaldoxime, is totally inactive, and free copper ions have only a very weak activity. It was also found that fairly small modifications in the structure of the chelate may drastically weaken its activity. Thus, the nickel(II) analog ($NiSAO_2$) of $CuSAO_2$ has only a weak activity. The effect of the corresponding cobalt(II) compound is intermediate between that of $CuSAO_2$ and $NiSAO_2$. The addition of one hydroxyl group to each of the benzene rings does not appreciably affect the activity but the addition of two hydroxyls strongly weakens it. The resorcylaldoxime derivative $CuRES_2$ (I, R = R″ = H, R′ = Z = OH, M = Cu) is equally active with $CuSAO_2$ but the 2,3,4-trihydroxybenzaldehyde derivative $CuTRI_2$ (I, R = R′ = Z = OH, R″ = H, M = Cu) is only very weakly active. Compound **II**, in which the two ligands are connected to each other by a carbon chain, is totally devoid of activity [2]. The mechanism of action of $CuSAO_2$ and related chelates is so far unknown. On the basis of some indirect evidence, it was concluded that pyridoxal antagonism might be involved [2]. However, there is no direct evidence for this theory, and the drastically different antiproliferative activities of $CuSAO_2$, $CoSAO_2$ and $NiSAO_2$ are difficult to explain on the basis of it alone. The copper(II) chelates of various bis(thiosemicarbazones) that constitute a well-known group of metal-containing antitumor agents, are known to react chemically with thiols, e.g. glutathione (GSH). The reaction occurs also within tumor cells and drastically reduces their thiol content, and it is probably involved in the mechanism of action of the compounds. The copper(II) of the chelates is reduced to copper(I), and the intracellular copper(I) is believed to have a crucial role in the cytotoxicity of those chelates, possibly because of its catalytic activity [3–5]. Therefore, I have now studied the reactivity of $CuSAO_2$ and some of its derivatives and analogs with GSH and cysteine (CYS). For comparison, reactivity with ascorbic acid (ASC), another easily oxidizable cellular constituent, was also studied. A distinct correlation between antiproliferative activity and reactivity with the thiols, especially GSH, was found.

A series of test-tube reactions was performed. Each of the metal complexes to be tested was dissolved in dimethyl sulfoxide and varying amounts of GSH, CYS or ASC were added as aqueous solutions. The tubes were observed for changes in or disappearance of the typical color of the appropriate chelate (see Table I for details and a summary of results). The highly antiproliferative chelates $CuSAO_2$, $CuRES_2$ and **Ic** were found to react very rapidly with GSH. When the molar ratio chelate: GSH was 1:2 or 1:1, the color of each chelate immediately disappeared on mixing of the solutions. Also with CYS, a rapid reaction often ensued and, in most cases, a brown color developed. In contrast, compound **II** that is totally devoid of antiproliferative activity, retained its color on treatment with GSH, the first small changes being clearly observable first after a few hours. This result was confirmed with visible spectroscopy (data not shown). With CYS, compound **II** reacted more rapidly than with GSH but in any case much more slowly than did $CuSAO_2$ or $CuRES_2$. As is evident from Table I, the typical color of compounds with intermediate or weak anti-

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TABLE I. The Results of Test-tube Reactions

Compound	Structure					Observations[a] with									Antiproliferative activity against tumor cells *in vitro*[c]
	M	R	R′	R″	Z	GSH			CYS			ASC			
						1:2[b]	1:1	2:1	1:2	1:1	2:1	1:2	1:1	2:1	
Ia ($CuSAO_2$)	Cu	H	H	H	OH	+++	+++[d]	+, P	CC	CC	C	P	–	–	+++
Ib ($CuRES_2$)	Cu	H	OH	H	OH	+++	+++[d]	+	+++	+, C	+, C	–	–	–	+++
Ic	Cu	H	H	OH	OH	+++	+++[d]	+	+	CC	C	+	+	–	+++
Id	Cu	H	H	H	H	+, C	CC	C	CC	CC	C	C	–	–	++(+)
Ie ($CoSAO_2$)	Co	H	H	H	OH	–	–	–	–	–	–	(+)	(+)	–	++
If ($NiSAO_2$)	Ni	H	H	H	OH	+, C	+, C	+, C	CC	CC	CC	+	+	±	+(+)
Ig ($CuTRI_2$)	Cu	OH	OH	H	OH	+	+	CC	+	+	CC	–	(C)	(C)	+
Ih	Cu	H	H	H	$(CH_2)_2OH$	C	C	C	(C)	–	–	–	–	–	+
II						–	–	–	(C)	(C)	(C)	–	–	–	–

The chelates were dissolved as 3 mM solutions in dimethyl sulfoxide (Merck, UVASOL grade). To 2.0 ml aliquots of each chelate solution, 0.5 ml aliquots of 24 mM, 12 mM or 6 mM aqueous solutions of L-GSH, L-CYS (both from Merck, biochemical grade) or L-ASC (European Pharmacopea grade) were added at room temperature, and the tubes were shaken manually for a few seconds to mix the solutions. Thus, the mole ratios chelate: (GSH, CYS or ASC) were 1:2, 1:1 and 2:1, respectively. In all cases, mixtures of 2.0 ml of the appropriate chelate solution plus 0.5 ml of water were used as controls.

[a]+++ = immediately total disappearance of the color of the chelate, ++ = total disappearance of color within 30 min, + = color was distinctly weakened immediately, (+) = color was distinctly weakened within 30 minutes, CC = color change immediately, C = color slightly changed immediately, (C) = color slightly changed within 30 min, P = prevented the partial precipitation of the sparingly water-soluble chelate that occurred in the corresponding control; no other apparent difference from the control, – = no distinct difference from control observed during 30 min. [b]Mole ratios chelate: (GSH or CYS or ASC). [c]Measured against leukemia L1210 cells. +++ = highly active, ++ = moderate activity, + = weak activity, – = inactive. Data taken from references 1, 2. [d]A very faint violet-blue color developed within a few minutes after disappearance of the intensive green color of the chelate solution.

proliferative activity was in most cases altered or weakened on treatment with GSH or CYS, but in no case totally disappeared within 30 min. It is also evident from Table I that among the $CuSAO_2$ type compounds studied, a distinct correlation exists between antiproliferative activity and changes of color on treatment with CYS and, especially, GSH. The only apparent exception to the correlation is formed by $CoSAO_2$. In contrast, treatment with ASC in most cases had no effect on the color of the chelates, and no correlation was found between antiproliferative activity and reactivity with ASC.

On the basis of the above results, reaction with cellular thiols, especially GSH, a resultant destruction of the chelate and possibly formation of a catalytically active species appears as a noteworthy mechanistic possibility for $CuSAO_2$ type compounds. The only apparent exception, the cobalt chelate $CoSAO_2$, might also act by a related mechanism if it has catalytic activity *per se*. Thus, it is possible that the mechanism of action of the present group of antineoplastic metal complexes is essentially similar to that of the well-known bis(thiosemicarbazonato) complexes. It can even be speculated that the majority of, if not all, antineoplastic copper(II) complexes may ultimately share a common mechanism of action since there is evidence suggesting that many types of them react with the thiols of tumor cells [3]. The rapidity of the reaction with GSH is also capable of explaining the finding (H. Elo *et al.*, unpublished results) that, when treated with $CuRES_2$, tumor cells very rapidly (in less than one hour) lose their ability to divide and become non-transplantable, just as typically occurs on treatment with various thiol blocking agents such as iodoacetate, iodoacetamide and *N*-ethylmaleimide [6–8]. Destruction of the chelate in the reaction with GSH could also explain our unpublished finding that in spite of its very low solubility in water and fat solvents, solid $CuRES_2$ is rapidly absorbed from the rat peritoneal cavity.

Further work is underway to elucidate details of the reactions observed and to identify the reaction products. In addition, it would be important to study, whether reaction with GSH and other thiols occurs also in tumor cells, and whether test-tube reactivity tests with GSH could be used as prescreens for the selection of copper(II) chelates for antitumor testing.

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