anhydrous methanolic methylamine were added. The tube was sealed and heated at 50° for 65 hr in a rocking bomb.[28] The solvent was evaporated under a stream of nitrogen, and the residue was dried *in vacuo* over P_2O_5. The residue (15.9 mg) was taken up in water and run into a column (5 mm i.d. × 15 cm) containing 3 ml of Dowex 50W-X4 (H^+ form). Neutral, ultraviolet-absorbing materials were removed by washing with water, and then the product was eluted with 1.0 *N* NH_4OH. Evaporation *in vacuo* in a rotary evaporator in a water bath (25–50°) followed by drying *in vacuo* over P_2O_5 furnished 6.6 mg of crude product (ultraviolet spectra were qualitatively indistinguishable from the standard). The product was further purified on a column (5 mm i.d. × 22 cm) of silica gel (J. T. Baker, 3405) (2.5 g), packed in methylene chloride–methanol (97:3). The column was developed with the same solvent. Upon increasing the methanol concentration to 6%, the product was eluted in six 10-ml cuts. The four center cuts were combined on the basis of ultraviolet and counting data and also by radiopapergram analysis. Concentration *in vacuo* furnished 2.8 mg (0.12 mcurie, 11 mcuries/mmole) of amorphous product IX which was indistinguishable from MADU by ultraviolet analysis (optical density ratio comparisons) and homogeneous by radiopapergram analysis using unlabeled MADU as standard.

Radiopapergram Analysis.—Whatman No. 1 filter paper strips and 2-propanol–concentrated (38%) HCl–water (75:8:17) solution were used for descending-type papergram analysis. Sections of the paper were placed in phosphor solution and read directly in a liquid-scintillation counter.[24] MADU-6-H^3 was differentiated from possible interfering contaminants (see Table III).

TABLE III

Standards	R_f values
5-Methylaminouracil	0.22
MADU	0.32
Uracil	0.63
2-Deoxyuridine	0.73
5-Iodouracil	0.75
IDU	0.80

In Vitro **Cleavage Studies of Substituted 5-Amino-2′-deoxyuridines.**—The assay for determining the cleavage of nucleosides by human spleen thymidine phosphorylase was carried out by Dr. Morris Zimmerman of these laboratories essentially as described[29] for measuring the formation of 2′-deoxyribose 1-phosphate. The nucleoside was incubated with the enzyme in phosphate buffer. The cleavage rate of thymidine was taken as 100%. The rates with the 5-substituted 2′-deoxyuridines are as follows: 5-amino (II), 36%; 5-methylamino (I), 27%; 5-dimethylamino (III), 0%.

Acknowledgment.—The authors wish to thank Mr. Richard N. Boos for microanalytical data, Mr. Alec Kalowsky for ultraviolet measurements, Mr. Edward A. MacMullan for spectrophotometric analysis (p*K* and isosbestic point determinations), Dr. Charles Rosenblum for assistance with radioactivity measurements, and Dr. James J. Wittick for ORD analysis.

(28) IDU was completely destroyed using the same conditions, 80° for 18 hr, found successful for amination of BrDU. A series of 10-mg runs with unlabeled IDU was performed to determine optimum conditions.

(29) G. E. Boxer and C. E. Shonk, *J. Biol. Chem.*, **233**, 535 (1958).

Anticancer and Potential Antiviral Activity of Complex Inorganic Compounds

STANLEY KIRSCHNER, YUNG-KANG WEI, DAWN FRANCIS, AND JOHN G. BERGMAN

Department of Chemistry, Wayne State University, Detroit, Michigan

Received August 2, 1965

Recent studies indicate that viruses are associated with many types of cancerous tumors. The authors propose that complex inorganic compounds might be successfully used in the alteration of these viruses with the consequent destruction or diminution of their activity. Twenty-six complex inorganic compounds have been prepared in this study, and among them are five which are active against certain cancers, including complexes containing coordinating agents which are themselves carcinostatic, such as 6-mercaptopurine. Both the platinum(IV) and palladium(II) complexes of 6-mercaptopurine have been found to be extremely active against Adenocarcinoma 755 and Sarcoma 180.

Despite considerable controversy in recent years regarding the possible role of viruses as cancer-producing agents, there is no longer any question that certain types of cancer are virus caused and some scientists[1] feel that most, if not all cancers, have one or more viruses associated with them. Since viruses contain proteins and nucleic acid units, some atoms of which are excellent coordinating agents for metal ions, it was decided to study the possibility of altering the virus *via* its coordination and/or chelation to metal ions with the expectation that this alteration will result in the elimination or diminution of its viral activity.

Discussion

Perhaps the most important aspect of this problem involves "appropriate" introduction of a metal ion into the vicinity of a virus so that complexation or coordination may occur. Mere introduction of simple or hydrated metal ions into living organisms will not suffice because of their almost certain coordination to one or more of the many nonviral proteins, amino acids, and other coordinating groups found in most parts of the animal system. Consequently, this investigation is concerned with the introduction of metal ions in the form of moderately stable complex inorganic compounds (coordination compounds), the ligands of which might possibly be displaced by viruses, thus forming new complexes with the metal ion, for example

$$m(\mathrm{Vi}) + [\mathrm{M}^{+x}(\mathrm{L}^{-y})_n]^{x-ny} = [\mathrm{M}^{+x}(\mathrm{Vi})_m]^{+x} + n\mathrm{L}^{-y} \quad (1)$$

where Vi represents a virus; M, a metal ion; L, a ligand; and where m and n represent the number of molecules of virus and ligand, and x and y represent the charges on the metal ion and ligand, respectively.

Another important aspect of this problem is finding and capitalizing upon some difference between viral systems which may cause abnormal growth and the many benign coordinating systems in animals, because

(1) S. E. Luria, *Cancer Res.*, **20**, 669 (1960).

the latter will compete for any metal ion introduced into a system in the form of a complex. Although the reasons for the marked anticancer activity of these complexes are not completely understood, further study of the effects of these coordination compounds may lead to a delineation of some of the differences, even though they may be slight. For example, if viral and nonviral systems tend to form *rings of different numbers of atoms* with metal ions, the former could have a much higher stability than the latter as part of a coordination compound (*e.g.*, six-membered *vs.* seven-membered rings). In this case it would almost certainly mean the difference between a reaction of the initial complex with the viral system and no reaction with the nonviral one.

Another slight difference in structure which might be detectable by the use of complexes is an atom difference. If the molecules of benevolent and malevolent coordinating ligands are similar in structure and in chelate ring size but differ in that they have a different coordinating atom in a particular location (*e.g.*, one contains a sulfur where the other has an oxygen or nitrogen), then the selection of the appropriate metal ion may help to utilize this difference to alter the physiological properties of the malevolent coordinating agent. Platinum ions, for example, form very stable complexes with sulfur as a coordinating atom of a ligand and relatively weak complexes with oxygen as a donor atom. The situation is practically reversed, however, with magnesium or beryllium ions.

Metal ions were chosen from those areas of the periodic table which would be representative of three major types of atom coordination. Platinum metals were chosen as examples of good sulfur coordinators, and complexes of these metal ions are reported here. Magnesium, beryllium, and aluminum metals are currently being studied as examples of good oxygen coordinators, as are iron and cobalt as examples of good nitrogen coordinators.

Because the exact nature of the difference between coordinating sites on cancer-producing viruses and noncarcinogenic proteins, nucleic acids, *etc.*, is not yet known, design of coordination compounds having ligands which are easily displaced by viruses but not by nonviral proteins or nucleic acids is extremely difficult. For example, proteins usually have many possible and different coordination sites[2,3] such as amino, imidazole, sulfhydryl, phenoxyl groups, *etc.*, and each reacts differently toward different metal ions. Several metal–protein complexes and metal–amino acid complexes have already been prepared by other investigators[4-13] and these have been studied with regard to other types of physiological activity.

(2) S. W. Putnam, *Proteins*, **1B**, 893 (1953).
(3) W. L. Koltun, *J. Am. Chem. Soc.*, **79**, 6681 (1957).
(4) G. Felsenfeld and M. P. Printz, *ibid.*, **81**, 6259 (1959).
(5) B. L. Vallee, *Proc. 4th Intern. Congr. Biochem., Vienna*, **8**, 138 (1960).
(6) G. S. Shields, H. Markowitz, G. E. Cartwright, and M. Wintrobe, "Metal Binding in Medicine," M. J. Seven and L. A. Johnson, Ed., J. B. Lippincott Co., Montreal, 1960, p 259.
(7) F. R. N. Gurd and D. S. Goodman, *J. Am. Chem. Soc.*, **74**, 670 (1952).
(8) C. Tanford, *ibid.*, **74**, 211 (1952).
(9) T. Peters, Jr., *Biochim. Biophys. Acta*, **39**, 546 (1960).
(10) I. M. Klotz and H. G. Curme, *J. Am. Chem. Soc.*, **70**, 939 (1948).
(11) I. M. Klotz, J. M. Urquhart, T. A. Klotz, and A. Ayers, *ibid.*, **77**, 1919 (1955).
(12) E. Breslow and F. R. N. Gurd, *J. Biol. Chem.*, **238**, 1332 (1963).
(13) F. R. N. Guard and P. E. Wilcox, *Advan. Protein Chem.*, **11**, 311 (1956).

TABLE I
METAL COMPLEX COMPOUNDS FOUND TO BE INACTIVE AS ANTICANCER AGENTS[a]

Compd[b]	Formula	Test system[c]
$[Cu(gg)Cl]\cdot H_2O$	$C_4H_7ClCuN_2O_3\cdot H_2O$	S180
		L1210
		Ca755
$[Co(gg)Cl]\cdot H_2O$	$C_4H_7ClCoN_2O_3\cdot H_2O$	S180
		L1210
$Na_2[Zn(gg)_2Cl_2]\cdot H_2O$	$C_8H_{14}N_4Na_2O_8Zn\cdot H_2O$	S180
		L1210
		Ca755
$Na[Ni(gg)(H_2O)Cl_2]$	$C_4H_9Cl_2N_2NaNiO_4$	S180
		L1210
		Ca755
Cu(hi)Cl·HCl	$C_6H_9ClCuN_3O_2\cdot HCl$	S180
$[Ni(hi)_2Cl_2]$	$C_{12}H_{18}Cl_2N_6NiO_4$	S180
		L1210
		Ca755
Zn(hi)Cl$_2$	$C_6H_9Cl_2N_3O_2Zn$	S180
		L1210
$[Co(im)_2(H_2O)Cl_2]$	$C_6H_{10}Cl_2CoN_4O$	S180
		L1210[d]
		Ca755[d]
$Ni(im)_6Cl_2$	$C_{18}H_{24}Cl_2N_{12}Ni$	S180
		L1210
		Ca755
$Co(im)_6Cl_2$	$C_{18}H_{24}Cl_2CoN_{12}$	S180
		L1210
		Ca755
$Zn(im)_4Cl_2\cdot H_2O$	$C_{12}H_{16}Cl_2N_8Zn$	S180
		L1210
		Ca755
$Cu(mc)_2\cdot HCl$	$C_{10}H_{22}CuN_2O_4S_2\cdot HCl$	S180
		L1210
		Ca755
$Co(bz)_2Cl_2$	$C_{14}H_{12}Cl_2CoN_4$	S180
		L1210
		Ca755
$Zn(bz)_2Cl_2$	$C_{14}H_{12}Cl_2N_4Zn$	S180
		L1210
$Pt(bz)_2Cl_4$	$C_{14}H_{12}Cl_4N_4Pt$	S180
		L1210
		Ca755
$Ni(bz)_2Cl_2$	$C_{14}H_{12}Cl_2N_4Ni$	S180
		L1210
		Ca755
$[Pt(dar)_2Cl_2]Cl_2\cdot 4H_2O$	$C_{24}H_{22}Cl_6N_4Pt\cdot 4H_2O$	S180
		Ca755
$[Pd(da)_2Cl_2]$	$C_{24}H_{22}Cl_4N_4Pd$	S180
		L1210
$[Zn(O)(da)_2]_2$	$C_{48}H_{44}Cl_4N_8O_2Zn_2$	S180
$[Pd(dcda)Cl_2]_2$	$C_{21}H_{20}Cl_8N_4Pd_2$	L1210
		LL
$[Zn(dcda)_2(OH)_2]\cdot H_2O$	$C_{12}H_{12}Cl_2N_2O_2Zn\cdot H_2O$	LL
		L1210

[a] All compounds were nontoxic except were noted otherwise. [b] gg = glycylglycinato anion, hi = histidine, im = imidazole, mc = methionate anion, bz = benzimidazole, da = daraprim [2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine], dcda = dichlorodaraprim[2,4-diamino-5-(3,4-dichlorophenyl)-6-ethylpyrimidine]. [c] S180 = Sarcoma 180, L1210 = L1210 lymphoid leukemia, Ca755 = Adenocarcinoma 755, LL = Lewis lung carcinoma. [d] Toxic.

Furst[14] has studied the possible role of metals and has concluded that they play a significant and important role in cancer.

Anticancer Ligands.—Another aspect of this research on the physiological activity of complex inorganic com-

(14) A. Furst, ref 6, Chapter 46, p 339.

TABLE II

ACTIVITY OF METAL COMPLEX COMPOUNDS FOUND TO BE ACTIVE AS ANTITUMOR AGENTS

				Animal tumor activity					
								Specificity test	
Compd	Test system	Daily dose, mg/kg	Survivors	Animal wt dif (T − C), g	Tumor wt (T/C), mg	Cures[a]	% tumor wt decrease	Confidence	Index
I	S180[b]	150	6/6	−3.2	92/1176		7		
		100	6/6	−2.0	122/1176		10		
		63.6	6/6	−0.8	292/1176		24		
		44.4	6/6	−1.7	349/1176		29		
								99.7	2.1
	Ca755[c]	28.0	10/10	−3.7	23/930		2		
		28.0	10/10	−2.5	0/984		0		
		28.0	9/10	−6.6	0/1439		0		
		28.0	9/10	−3.5	0/1544		0		
		28.0	9/10	−5.3	22/1629		1		
		28.0	9/10	−2.3	4/1436		0		
	Ca755[c]	32.0	10/10	−5.0	18/1699		1		
		16.0	10/10	−2.6	0/1699		0		
		8.0	10/10	−3.4	71/1699		4		
		4.0	10/10	−2.5	89/1699		5		
								99.7	4.9
II	S180[b]	100	5/6	−1.2	150/1134		13		
		100	4/6	−0.3	119/714		16		
		100	4/6	−1.6	106/901		11		
		100	5/6	−3.2	318/1740		18		
								99.7	2.6
	Ca755[c]	9.00	10/10	−0.9	68/1427		4		
		9.00	10/10	−1.6	247/1557		15		
		9.00	9/10	−1.5	569/1706		33		
		9.00	8/10	−4.1	84/1106		7		
		9.00	10/10	−4.4	197/1684		11		
		9.00	7/10	−2.8	68/1503		4		
		36.00	10/10	−5.3	24/1150		2		
		18.00	9/10	−4.0	2/1150		0		
		9.00	10/10	−3.1	122/1150		10		
								99.7	3.9
III	S180[b]	112	6/6	−3.7	170/1028		16		
		56	6/6	−2.8	249/1028		24		
		28	6/6	−0.9	358/1028		34		
		14	6/6	−1.2	750/1028		72		
								95.0	2.4
	Ca755[c]	46	7/10	−3.6	21/1379	5			
		23	10/10	−1.9	0/1379	10			
		11	10/10	−2.3	35/1379	7			
		46	9/10	−4.6	17/1803	6	0		
		23	10/10	−3.5	5/1803	9	0		
		11	10/10	−1.9	57/1803	4	3		
		10	10/10	−1.0	0/485	10			
		8	10/10	−1.2	5/485	9			
		6	10/10	−1.6	0/485	10			
		4	10/10	−1.6	10/485	8			
		2	10/10	−0.3	108/485				
		10	10/10	−1.7	17/1021		1		
		8	10/10	−0.9	16/1021	8	1		
		6	10/10	−1.2	63/1021	8	6		
		4	10/10	−1.9	35/1021	6	3		
		2	10/10	−0.1	429/1021	5	42		
		1	10/10	−1.1	406/1021		39		
		0.5	10/10	+0.1	619/1021		60		
								99.7	7.8
IV	Ca755[c]	240	10/10	−0.1	12/1042		1		
		120	10/10	−1/3	14/1042		1		
		60	10/10	−2.7	43/1042		4		
		30	10/10	1.3	497/1042		47		
		15	10/10	0.8	416/1042		39		
		30	10/10	−2.8	162/842		19		
		15	9/9	−0.9	241/842		28		
		7.5	10/10	−1.2	718/842		85		
		3.25	10/10	−0.7	817/842		97		
		1000	10/10	−1.8	33/706		4		

TABLE II (*Continued*)

				Animal tumor activity				
Compd	Test system	Daily dose, mg/kg	Survivors	Animal wt dif (T − C), g	Tumor wt (T/C), mg	Cures[a]	% tumor wt decrease	Specificity test: Confidence	Index
IV	Ca755[c]	250	10/10	−0.7	360/706		50		
		62	10/10	+0.2	535/706		75		
		15	10/10	+0.8	506/706		71		
								99.7	9.8
V	Ca755[c]	10	7/10	−5.8	6/1042		0		
		5	10/10	−4.9	4/1042		0		

[a] National Cancer Institute specification. [b] Host: Swiss mice. [c] Host: BDF_1 mice.

pounds is the study of metal complexes containing ligands which themselves have biological (*e.g.*, carcinostatic) activity. With such complexes, the coordination of virus or other proteins to the metal ion would result in the release of a biologically active ligand (see eq 1), which would further inhibit undesirable biological activity (*e.g.*, abnormal growth). An important carcinostatic agent (6-mercaptopurine) was chosen as a ligand in the initial group of complexes prepared, and, because of success with this complexing agent, several other carcinostatic materials are also under study as complexing agents.

Results

Table I lists 21 metal complex compounds which have been synthesized, tested for anticancer activity, and found to be inactive in this regard against the test systems listed when administered in dosages ranging from 1.5 to 500 mg/kg per day. Table II lists five metal complex compounds which have been synthesized and found to have marked anticancer activity against the test systems listed. The procedures for synthesizing these complexes are given in the Experimental Section (*vide infra*).

The active compounds listed all contain ligands which have anticancer activity in their own right, but an initial comparison of the activities of ligands compared to the metal complexes containing these ligands indicates that the complexes may exhibit a somewhat greater anticancer activity than does the free ligand. Studies are now in progress to determine the extent of enhancement of anticancer activity of the complexes over their corresponding ligands. In some other cases studied, complex formation diminished certain anticancer activity (*e.g.*, with respect to L1210 lymphoid leukemia) of the free ligand.

Under study at this time is the relationship between metal–ligand bond type and carcinostatic activity. In addition, the possibilities of utilizing X-rays for the determination of the extent of uptake of a metal by the tumor systems, as well as of enhancing the activity of these complexes by making the metal and/or coordinating agents radioactive are also under current study.

Experimental Section

Syntheses of Active Complexes. $Na_2[Pt(mp)_2Cl_4]\cdot 2H_2O$ (I).—A solution of 1.35 g of reagent grade platinum(IV) chloride in 10 ml of water was added to 1.4 g of 6-mercaptopurine (mp) in 10 ml of 1 *N* aqueous NaOH and 5 ml of dioxane. The resulting solution was heated on a water bath at 50° for 1 hr with constant stirring. The orange-brown precipitate which formed after the solution was cooled was filtered, washed with water–dioxane, then ethanol, and dried under vacuum.

Anal. Calcd for $C_{10}H_8Cl_4N_8Na_2PtS_2\cdot 2H_2O$: C, 16.67; H, 1.39; Cl, 19.70; N, 15.54; S, 8.90. Found: C, 17.27; H, 1.31; Cl, 19.90; N, 14.96; S, 8.58.

$Na_2[Pd(mp)_2Cl_2]\cdot H_2O$ (II).—A solution of 1.8 g of reagent grade palladium(II) chloride in 10 ml of water was added to 3.2 g of 6-mercaptopurine in 20 ml of 1 *N* aqueous NaOH and 5 ml of dioxane. The resulting solution was heated on a water bath at 50° for 1 hr with continuous stirring. The brown precipitate which formed after the solution was left at room temperature for several hours was filtered, washed with water–dioxane, and dried under vacuum.

Anal. Calcd for $C_{10}H_8Cl_2N_8Na_2PdS_2\cdot H_2O$: C, 22.10; H, 1.47; N, 20.60; S, 11.80. Found: C, 22.67; H, 1.45; N, 20.29; S, 11.93.

$Na_2[Bi(O)(mp)_4]\cdot 3H_2O$ (III).—A solution of 1.17 g of reagent grade bismuth(III) nitrate pentahydrate in 5 ml of concentrated HNO_3 and diluted to pH 1 with water was added to 1.28 g of 6-mercaptopurine in 25 ml of 0.34 *N* aqueous NaOH. After the two solutions were mixed, 30 ml of 1 *N* NaOH was added. The bright yellow precipitate formed during this reaction was filtered, washed consecutively with water, ethanol, and ether, and dried *in vacuo* over P_2O_5.

Anal. Calcd for $C_{15}H_9BiN_{12}Na_2OS_4\cdot 3H_2O$: C, 23.14; H, 1.94; N, 21.59; S, 12.36. Found: C, 23.44; H, 1.82; N, 21.49; S, 12.14.

[Pd(butp)$_3$Cl]Cl (IV).—To 1.5 g of reagent grade palladium(II) chloride in 150 ml of water containing 5 ml of concentrated HCl was added slowly and with stirring, a 3.5 g of butylthiopurine (butp) in 20 ml of aqueous 1 *N* NaOH. The light yellow precipitate was filtered, washed with water, and then successively dried over $CaCl_2$ and P_2O_5 under vacuum.

Anal. Calcd for $C_{27}H_{36}Cl_2N_{12}PdS_3$: C, 40.42; H, 4.52; N, 20.95; S, 11.99. Found: C, 41.23; H, 3.88; N, 19.49; S, 11.10.

[Bi(tgn)$_3$(H$_2$O)]$\cdot$3.5H$_2$O. (V).—A solution of 1.0 g of bismuth(III) nitrate pentahydrate in 5 ml of concentrated HNO_3, diluted with water to pH 1 was added to 1.2 g of thioguanine (tgn) in 7.2 ml of 1 *N* aqueous NaOH which was diluted to 25 ml with water after dissolution. The red precipitate was filtered, washed consecutively with water, ethanol, and ether, then dried over P_2O_5 under vacuum.

Anal. Calcd for $C_{15}H_{14}BiN_{15}OS_3\cdot 3.5H_2O$: C, 22.84; H, 2.68; N, 26.64. Found: C, 22.80; H, 2.63; N, 27.72.

Acknowledgments.—The authors wish to express their sincere appreciation to the following organizations, without whose assistance this work would not have been possible: the U. S. Public Health Service for a research grant (CY-5561) from the National Institutes of Health, and for its assistance in testing the compounds for carcinostatic activity; to the American Cancer Society for a research grant; and to the Sloan–Kettering Institute for its testing for bactericidal and carcinostatic activity.