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METAL ION COMPLEXING PROPERTIES OF CARCINOGEN METABOLITES*

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Abstract—The metal ion chelating properties of metabolic or other derivatives of certain carcinogens were determined by potentiometric titration in 70% aqueous dimethylsulfoxide, an eminently suitable solvent for this purpose, or by isolation of the complexes. 2-Amino-1-fluorenol, 2-amino-3-fluorenol, 3-amino-2-fluorenol, and 2-amino-4-phenylphenol were somewhat better chelating agents than 2-aminophenol with Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, and Co^{2+}. These ligands and also *N*-hydroxy-2-fluorenylacetamide formed 2:1 complexes with bivalent metal ions, while *N,N*-5-dimethylamino-2-phenylazophenol gave a 1:1 complex. The chelates exhibited a weak infrared absorption around 15 μ ascribed to metal-oxygen stretching or chelate ring vibration; other data in the infrared spectra are presented. 2-Amino-1-naphthol, 1-amino-2-naphthol, and adenine were poorer ligands. 2-Fluorenamine, 2,7-fluorenediamine, 7-amino-2-fluorenol, 2-(*N,N-p*-dimethylaminophenylazo)phenol, adenosine, and diphosphopyridine nucleotide had no complexing ability under our experimental conditions. 2-Fluorenamine, and 2-naphthylamine, however, yielded a mixed complex with Cd^{2+} and CrO_4^{2-}, but 3-fluorenamine did not. The chelating properties of the carcinogen metabolites studied may account in part for their toxic effects by virtue of competitive enzyme inhibition. However, it seems unlikely that this phenomenon plays a direct role in the carcinogenic process.

METAL ions play an essential role in living phenomena, and the study of their action at the molecular level has been useful in elucidating the intimate nature of their involvement. Many reactions mediated by enzymes require the presence of certain metal ions.[2–5] In addition, metal ions are reported to be part of the structure of nucleic acids.[6] The activity of some drugs may depend on their ability to complex with metal ions.[7, 8] While the exact nature of the participation of the metal in these structures is not known in all cases, it appears possible that it functions to bring together various components involved in a reaction (cf. Vallee and Coombs[9]; Felber *et al.*[10]). The metal ion may also serve to hold certain selected smaller molecules at a specific site on a macromolecule in a precise stereochemical relationship.[11] In these structures the substitution and intervention of unnatural materials, capable of complexing, would be expected to have important repercussions. Thus, certain metals or their compounds have been found carcinogenic under various conditions.[12–17] Furthermore, Boyland and Watson[18] raised the question of whether metal chelation, especially by *o*-aminophenols might be involved in chemical carcinogenesis with certain classes of compounds. Data on three compounds of this type were published by Sims.[19] Also, French[20] has indicated in a preliminary report that metal ions

* An abstract of this work has appeared.[1]

complexed with certain azo dye carcinogens and related compounds. Furst[21] has recently considered some aspects of this question.

In the present study, metabolites of aromatic amines or certain compounds derived from carcinogenic azo dyes were examined for their ability to complex with metal ions. Furthermore, the complexing ability of diphosphopyridine-nucleotide (NAD) and some related compounds was investigated to determine whether carcinogen metabolites might displace these cofactors from enzymes by virtue of a higher binding constant.[22, 23] Thus the complex formation constants of a series of compounds with five metal ions—namely copper, nickel, zinc, lead, and cobalt—were determined by standard titration techniques. In view of the low solubility of the complexes in ordinary solvents, the titrations were performed in 70% dimethylsulfoxide solution. This solvent was well adapted to this purpose. In addition, some insoluble complexes were isolated and their probable structure was derived from elemental analyses and spectroscopic observations.

EXPERIMENTAL PROCEDURES

Materials. Dimethylsulfoxide (Stepan Chemical Co., Chicago, Ill.) was purified by two vacuum distillations using a 30-cm Vigreux column giving a material of bp (5 mm) 60°. Doubly glass-distilled water was used to prepare 0·01 M solutions of the metal ions used in the form of the corresponding ACS reagent grade nitrates. The concentration of the metal ions in these solutions was checked by potentiometric titration in the presence of EDTA with standard methodology.

Carbonate-free 0·05 N sodium hydroxide in 70% dimethylsulfoxide was standardized with reagent grade potassium acid phthalate. Exactly 0·01 N nitric acid was prepared and the concentration determined by titration against the sodium hydroxide solution.

2-Aminophenol, 1-amino-2-naphthol, and 2-amino-4-phenylphenol were commercial compounds which were recrystallized to constant melting point in appropriate solvents prior to use. Adenine, adenosine, and diphosphopyridine nucleotide were purchased. Pure *N*-hydroxy-2-fluorenylacetamide was generously donated by Drs. James and Elizabeth Miller, University of Wisconsin. The remainder of the chemicals (see tables) were suitably purified specimens of compounds synthesized in our laboratory.

Apparatus. The titration vessel was essentially that described by Freiser *et al.*,[24] but had smaller dimensions (2·856 cm). A Greiner microburet, a nitrogen inlet tube, and the electrodes of a Beckman pH meter, model G, were inserted through the cover of the vessel. The pH meter was standardized before and after each determination. All titrations were performed at 36° for reasons of solubility, and also because the data obtained might approximate more closely the phenomena existing *in vivo*. The solutions were stirred by a magnetic stirrer.

Titration procedure for soluble complexes. The ligand ($2{\cdot}5 \times 10^{-4}$ M) was dissolved in 14 ml of redistilled dimethylsulfoxide. Upon addition of 5 ml of 0·01 N nitric acid, 0·5 ml of 0·01 M metal ion, and 0·5 ml of water, the solution was titrated with 0·05 N sodium hydroxide in 70% dimethylsulfoxide. Each experiment was performed in

duplicate. The complex formation constants* were calculated as described by Freiser *et al.*[24]

Procedure for complexes insoluble in 70% dimethylsulfoxide. In some cases the complexes were insoluble in the solvent used. For this reason the formation constants could not be evaluated by titration, but advantage was taken of the insolubility of the complexes to isolate them. With efficient stirring, 0·98 mmole of sodium hydroxide was added to a solution of 1·0 mmole of compound and 0·495 mmole of the metal nitrate in aqueous ethanol, dioxane, or dimethylsulfoxide. The resulting precipitate was extracted repeatedly with solvent and dried *in vacuo*. The materials so obtained were subjected to elemental analysis. The infrared spectra were recorded on a Perkin-Elmer spectrophotometer as solids (about 1·3 mg) in potassium bromide disks.

By following the procedures of Genchev and Dimov[25] unusual complexes derived from 2-naphthylamine (0·5 g) and 2-fluorenamine (0·1 g) were prepared. Briefly, to a mixture of 3 (0·6) g of $Cd(NO_3)_2 \cdot 4H_2O$ in 10 ml of water and 50 (10) ml of 1% solution of K_2CrO_4 was added a solution of the amine in 10 (2) ml of acetone and 10 (2) ml of ethanol. The precipitate was extracted with benzene. Both 2-naphthylamine and 2-fluorenamine gave yellow crystals weighing 626 and 89 mg, respectively, mp 340° dec. The structure of the complexes corresponded to that postulated by Genchev and Dimov, namely $[(amine)_3 \cdot Cd^{2+} \cdot Cr_2O_7^{=} \cdot Cd^{2+} \cdot (amine)_3]^{2+}\ CrO_4^{2-}$.

It may be noted that 3-fluorenamine failed to form a complex and was recovered unchanged under identical reaction conditions as used for the 2-isomer.

RESULTS

Comparison of data obtained under two different experimental conditions

In their studies on the chelate formation constants of *o*-aminophenol, Charles and Freiser[26] used 100-fold excess of ligands, a temperature of 25° and 50% dioxane as solvent. In our studies we used only a 50-fold excess of ligand because of the limited availability of certain of our compounds. In view of their lower solubility, we employed and increased temperature of 36° and 70% dimethylsulfoxide as solvent. Owing to the

* We are greatly indebted and grateful to Dr. D. L. Leussing, National Bureau of Standards, Washington, D.C., for developing the following mode of calculating and evaluating the complex formation constants for *N*-hydroxy-2-fluorenylacetamide where $pK_a = 11{\cdot}0$, but where the acetyl-substituted nitrogen is of low basicity and does not give an enolizable hydrogen to the keto group.

Let T_{RNOH} = total concentration of ligand RNOH, T_{OH} = base, T_M = metal ion,

$$T_{RNOH} = RNOH + RNO^- + M(RNO)^+ + 2M(RNO)_2 \quad (1)$$

$$T_{OH} = [OH^-] - [H^+] + RNO^- + M(RNO)^+ + 2M(RNO)_2 \quad (2)$$

$$T_{RNOH} - T_{OH} = RNOH \quad [OH^-] + [H^+] \quad (1)-(2)$$

Or $RNOH = T_{RNOH} - T_{OH} + [OH^-] - [H^+]$;

also $RNOH = RNO^- + H^+ \therefore ([RNO^-][H^+])/[RNOH] = K_a$;

from which $RNO^- = K_aRNOH/H^+$. From equation (1), $M(RNO)^+ + 2M(RNO)_2 = T_{RNOH} - RNOH - RNO^-$, and division by T_M gives:

$$n = \frac{M(RNO)^+ + 2M(RNO)_2}{T_M} = \frac{T_{RNOH} - RNOH - RNO^-}{T_M}$$

A plot of n versus $p\ [RNO^-]$ gives approximations to pK_1 and pK_2 at $n = 0{\cdot}5$ and $n = 1{\cdot}5$, respectively.

excellent solubilizing power of dimethylsulfoxide, titrations could be performed which would have been impossible in other mixtures owing to precipitation of reactants. Furthermore, this solvent possesses a fairly high dielectric constant (45), an advantage not shared by many other organic solvents. This property may lead to data *in vitro* which can conceivably be extrapolated to situations *in vivo*.

It was desirable to compare the two experimental conditions in order to have a reference point to the earlier studies. Table 1 shows that the complexing constants of

TABLE 1. COMPARISON OF CHELATE FORMATION CONSTANTS OF *o*-AMINOPHENOL UNDER CONDITIONS OF THIS WORK (W.) AND THOSE OF CHARLES AND FREISER[26] (C. & F.)

Solvent Temperature Excess ligand	C. & F. 50% Dioxane 25° 100-fold	W. 70% Dimethylsulfoxide 36° 50-fold
Metal ion	Log K_1	Log K_2
Cu	9·25	10·90
Pb	6·25	7·80
Ni	6·10	7·28
Zn	5·99	6·98
Co	5·81	7·01

o-aminophenol with copper, lead, nickel, zinc, and cobalt ions were in general higher by at least one unit over those obtained under the conditions of Charles and Freiser. The increased dielectric constant of the solvent as well as the higher temperature may be responsible for the differences observed.

Complex formation constants of polynuclear o-*aminophenols*

Table 2 lists the compounds and metal ions studied in the order of decreasing complexing abilityfrom top to bottom and from left to right. 2-Amino-3-fluorenol exhibited the largest stability constant, K_1. The value with 2-amino-1-fluorenol was slightly lower, but no data could be determined for 3-amino-2-fluorenol with copper ion because of precipitation of the complex. On the basis of the constants obtained with this compound and the other metal ions studied, however, it appears that this compound exhibited a slightly lower complexing ability than the aforementioned compounds. The hydroxamic acid type *N*-hydroxy-2-fluorenylacetamide gave lower binding constants with copper and nickel, but solid chelates were readily isolated (see below). The substituted biphenyl, 2-amino-4-phenylphenol, in turn was a poorer ligand than the fluorene derivatives, although it had a slightly higher complexing ability than the monocyclic 2-aminophenol. In the naphthalene series 2-amino-1-naphthol and especially the isomeric 1-amino-2-naphthol seemed to have very low complexing constants. It may be noted, however, that during the titration runs the naphthalene derivatives yielded brownish solutions with all of the metal ions studied. The characteristic blue, green, and reddish solutions noted with other ligands and copper, nickel, and cobalt, respectively were absent. Thus it could be that the naphthalene derivatives underwent oxidative decomposition despite the pains that were taken to maintain a nitrogen atomosphere during the titrations. The complex formation

TABLE 2. COMPLEX FORMATION CONSTANTS

Compound	Cu^{2+}		Ni^{2+}		Zn^{2+}		Pb^{2+}		Co^{2+}			
	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1*	pK_2†
2-Amino-3-fluorenol	12·2	7·5	8·6	5·4	8·0	6·4	7·4	6·2	7·2	6·0	4·8	13·0
2-Amino-1-fluorenol	12·1	10·1	8·5	6·4	7·9	6·2	7·8	5·9	7·3	5·6		
N-Hydroxy-2-fluorenylacetamide	6·5	4·6	8·7	8·4								11·0
3-Amino-2-fluorenol			8·2	4·8	7·7	5·5	8·9	6·4	7·3	5·7	4·80	12·8
2-Amino-4-phenylphenol	11·3	9·0	7·5	5·6	7·7	6·2			7·0	5·7	4·26	12·4
2-Aminophenol	10·9	9·2	7·3	6·0	7·0	6·0	7·8	6·0	7·0	6·3	4·68	12·7
2-Amino-1-naphthol	7·4	5·9	5·9	4·6	6·1	5·1	6·2	5·1	5·8	4·9	4·31	11·9
1-Amino-2-naphthol	4·9	4·5	4·6	4·4	4·7	4·6					4·26	11·7
Adenine	7·8	7·2									4·32	10·4

* Ionization constant of $RNH_3^+ \rightleftharpoons RNH_2 + H^+$.
† Ionization constant of $ROH \rightleftharpoons RO^- + H^+$.

TABLE 3. METAL ION COMPLEXES ISOLATED

Compound	Metal ion	Color	Melting pt, complex	Formula, complex	Analyses‡ Calculated C	H	N	Me^{2+}	Found C	H	N	Me^{2+}
3-Amino-2-fluorenol	Ni^{2+}	P-GY	>320°	$C_{26}H_{24}O_4N_2Ni$	64·10	4·93	5·75		63·01	5·05	5·64	
N-Hydroxy-2-fluorenylacetamide	Cu^{2+}	Y	228–230°	$C_{30}H_{24}O_4N_2Cu$	66·67	4·48		11·77	65·94	4·82		11·48
2-Amino-4-phenylphenol	Cu^{2+}	GR-GY	>320°	$C_{24}H_{24}O_4N_2Cu$	61·59	5·17	5·99	13·58	63·83	5·02	5·62	13·76
2-Amino-4-phenylphenol	Ni^{2+}	W	>320°	$C_{24}H_{24}O_4N_2Ni$	62·24	5·22	6·05		62·05	5·29	6·10	
2-Amino-4-phenylphenol	Zn^{2+}	W	>320°	$C_{24}H_{24}O_4N_2Zn$	61·36	5·11	5·96	13·92	63·21	5·03	6·14	14·11
2-Amino-4-phenylphenol	Co^{2+}	P	>320°	$C_{24}H_{24}O_4N_2Co$	62·24	5·18	6·05		62·34	5·17	5·90	
N,*N*-5-dimethylamino-2-phenylazophenol†	Ni^{2+}	P-B	>320°	$C_{14}H_{15}O_2N_3Ni$	53·21	4·79	13·30		53·74	5·03	12·78	
2-Naphthylamine	Cd^{2+}, CrO_4^-	Y	>340° dec.	$C_{60}H_{54}O_{11}Cd_2Cr_3N_6$	50·89	3·84	5·94	11·02	51·89	4·28	5·45	11·70
2-Fluorenamine	Cd^{2+}, CrO_4^-	Y	>340° dec.	$C_{78}H_{66}O_{11}Cd_2Cr_3N_6$	56·97	4·05	5·11	9·49	56·24	4·90	4·96	9·39

* P, purple; GY, gray; Y, yellow; GR, green; W, white; B, brown.
† 2-Hydroxy-4-dimethylaminoazobenzene.
‡ Analyses by the Microanalytical Laboratory of the National Institutes of Health.

constant of adenine with copper ions, already studied by Harkins and Freiser[27] and Cheney *et al.*,[28] was also found to be fairly low under our different experimental conditions.

Copper was the ion most strongly chelated by the ligands studied, exhibiting an affinity 3 to 4 log units higher than that of the next best metal. The constants with nickel, zinc, lead, and cobalt were, on the other hand, rather similar, with values around 7 to 8. The order of decreasing affinities for the ligands was nickel, zinc, lead and cobalt, except for a few inversions which may be related to the higher stability of ligand itself under the conditions of the experiment rather than to a lower affinity of the metal ion for the ligand.

The second stability constants showed trends similar to those described for log K_1. In general, copper ion exhibited higher constants than the other four ions studied with all the ligands investigated. However, 2-amino-3-fluorenol had a considerably lower log K_2 value than 2-amino-1-fluorenol, whereas their log K_1 values were similar. Log K_2 was about 5 to 6 for nickel, zinc, lead, and cobalt ions, with most of the compounds examined.

The titration curves of the compounds listed below were similar to those of the metal ion hydrolysis curves; thus it must be concluded that they were not complexing agents under our conditions: 2-fluorenamine; 2,7-fluorenediamine; 7-amino-2-fluorenol; 2-(*N*,*N*-*p*-dimethylaminophenylazo)phenol; adenosine; diphosphopyridine nucleotide (NAD).

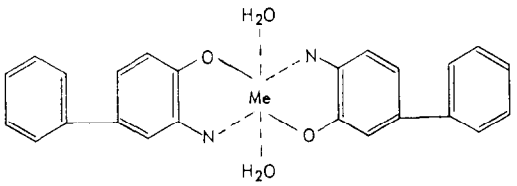
Isolation of insoluble metal ion complexes

During the titration procedures it was noted that a number of the ligands readily gave precipitates with certain of the metal ions. It was therefore decided to attempt to isolate some of these complexes on a larger scale by the procedure described earlier. Table 3 lists the properties of the complexes obtained. The *o*-aminophenols (3-amino-2-fluorenol and 2-amino-4-phenylphenol) gave rise to neutral complexes composed of two molecules of ligands per metal ion. In addition, two equivalents of of water were linked to the metal ion giving a hexacovalent structure, typified by formula I. The chelates had the characteristic colors described in the table. Their melting points were all higher than 320°, as determined on a Kofler micro-melting-point apparatus.

The important carcinogenic metabolite of 2-fluorenylacetamide, *N*-hydroxy-2-fluorenylacetamide,[29] is not, strictly speaking, an *o*-aminophenol but is more typical of a hydroxamic acid. The nitrogen bears the hydroxy group and in addition it is substituted by the electron-withdrawing acetyl substituent. Thus the electron density of the nitrogen atom would be expected to be considerably lower than in an ordinary amino group. This compound readily formed a stable copper complex, which had a melting point of 228 to 230°. The chelate has recently been used to immobilize the *N*-hydroxy-2-fluorenylacetamide at the site of injection into rats and thus render it more effective over a longer period of time. Consequently, low dosages of the copper chelate of this compound sufficed for tumor induction.[30] It also served to isolate this metabolite directly from the urine of animals given *N*-2-fluorenylacetamide.[31]

The azo dye 5-*N*,*N*-dimethylamino-2-phenylazophenol yielded a 1:1 complex with nickel ions which was derived from a basic nickel salt, formula II. The elemental analyses for these complexes in respect to hydrogen, nitrogen, and metal usually

checked the calculated values reasonably well. In a few instances, however, the data for carbon were somewhat high; the complex between nickel ion and 3-amino-2-fluorenol showed a low carbon value. It is believed that these discrepancies are ascribable to difficulties in the analytical method rather than to an impurity in the chelates.



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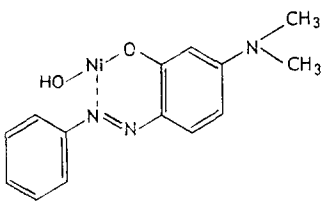


II

Infrared spectra of metabolites and their metal chelates

Relatively few observations have been published on the infrared spectra of chelates of *o*-hydroxyamines or *o*-hydroxyazo dyes. Le Fevre *et al.*[32] have reported on the spectrum of the copper complex of benzeneazo-β-naphthol while Cohen and Zand[33] recently published the spectra of cuprous chloride complexes of two bridged azo compounds.

The bands of the metal chelates prepared in the present investigation are given in Table 4; therefore only the more outstanding features will be discussed.

TABLE 4. INFRARED ABSORPTION BANDS OF COMPLEXES ISOLATED*

Ni^{2+} complex of 3-amino-2-fluorenol

3·03 m, 3·07 m, 3·33 m, 6·19 m, 6·27 m, 6·42 w, 6·73 m, 6·88 m, 7·04 m, 7·38 m, 7·64 m, 7·76 m, 8·25 m, 8·40 w, 8·64 w, 8·92 m, 9·14 w, 9·60 m, 9·84 w, 10·19 w, 10·59 w, 11·54 m, 11·78 w, 11·98 w, 13·23 m, 13·80–13·98 mb, 14·97 m.

3-amino-2-fluorenol

2·99 s, 3·06 s, 3·33–3·47 mb, 3·72 mb, 3·95 mb, 6·18 m, 6·33 mb, 6·70 s, 6·74 s, 6·89 s, 6·95 s, 7·15 s, 7·40 s, 7·57 m, 7·66 s, 7·79 s, 8·05 m, 8·26 m, 8·35 m, 8·44 m, 8·58 m, 8·91 m, 9·15 w, 9·86 m, 10·22 m, 10·57 m, 10·90 sb, 11·35 s, 11·65 m, 11·97 m, 12·48 mb, 13·18 s, 13·73 m, 13·96 s, 14·23 mb, 15·04 wb.

Cu^{2+} complex of *N*-hydroxy-2-fluorenylacetamide

3·10 w, 3·32 w, 5·13 w, 5·57 w, 6·10 w, 6·16 w, 6·33 m, 6·37 m, 6·40 m, 6·88 m, 7·04 m, 7·15 m, 7·31 m, 7·47 w, 7·71 w, 7·83 w, 7·94 w, 8·20 w, 8·35 w, 8·47 m, 8·72 m, 9·23 m, 9·73 m, 9·80 m, 9·92 w, 10·34 m, 10·55 m, 10·85 m, 11·44 m, 11·73 m, 11·98 m, 12·23 w, 12·99 s, 13·37 m, 13·65 s, 14·25 m, 15·15 m.

TABLE 4—*continued*

N-hydroxy-2-fluorenylacetamide

3·23 mb, 3·50 mb, 6·00 s, 6·08 s, 6·18 sb, 6·73 s, 6·88 s, 7·12 s, 7·45 m, 7·77 m, 7·99 m, 8·23 m, 8·36 m, 8·48 m, 8·76 m, 9·17 m, 9·73 m, 9·90 m, 9·99 m, 10·28–10·32 wb, 10·48–10·53 mb, 10·58 m, 10·83–10·86 m, 11·50 m, 11·73 m, 12·04 s, 13·07 s, 13·71 s.

Cu^{2+} complex of 2-amino-4-phenylphenol

5·86 w, 6·21 m, 6·46 w, 6·60 m, 6·73 s, 6·88 w, 7·11 w, 7·65 s, 7·93 m, 8·40 w, 8·68 w, 8·87 m, 9·20 w, 9·26 w, 9·83 w, 11·11 m, 11·30 m, 12·13 m, 12·48 w, 13·20 s, 14·50 m, 15·03 m.

Ni^{2+} complex of 2-amino-4-phenylphenol

3·02 m, 3·16 w, 3·32 w, 6·19 m, 6·47 w, 6·64 m, 6·73 s, 6·91 w, 7·14 w, 7·65 s, 7·77 s, 7·96 m, 8·50 w, 8·57 w, 8·68 w, 8·89 m, 9·27 w, 9·34 w, 9·56 m, 9·85 w, 11·12 w, 11·47 m, 12·33 m, 12·71 w, 13·23 m, 13·76 w, 14·05 w, 14·35 m, 14·97 w.

Zn^{2+} complex of 2-amino-4-phenylphenol

3·09 m, 3·31 w, 3·45 w, 4·24 w, 5·13 w, 5·33 w, 6·22 m, 6·47 w, 6·60 m, 6·73 s, 6·90 w, 7·12 m, 7·64 s, 7·94 m, 8·40 w, 8·68 w, 8·86 m, 9·47 m, 9·83 w, 10·68 w, 11·11 m, 11·29 m, 12·18 m, 12·67 m, 13·21 s, 13·68 m, 14·48 m, 15·03 w.

Co^{2+} complex of 2-amino-4-phenylphenol

3·00 m, 3·05 w, 3·28 w, 6·19 m, 6·45 m, 6·64 m, 6·73 s, 6·90 w, 7·14 w, 7·24 w, 7·66 s, 7·77 s, 7·96 m, 8·49 w, 8·57 w, 8·66 w, 8·88 m, 9·33 w, 9·60 w, 9·93 m, 11·10 m, 11·46 m, 12·33 m, 13·23 s, 14·36 m 14·98 w.

2-amino-4-phenylphenol

2·96 m, 3·03 s, 3·82 mb, 6·18–6·23 mb, 6·32 m, 6·55 s, 6·70 s, 6·81 s, 6·97 m, 7·02 m, 7·14 m, 7·63 s, 7·75 s, 7·99 s, 8·40 m, 8·65 m, 9·00 m, 9·28 w, 9·61 m, 9·77 w, 10·84 mb, 11·32 s, 12·23 sb, 12·82 m, 13·18 s, 14·20 m, 14·44 s, 14·80 w, 15·00 w.

Ni^{2+} complex of *N*,*N*-5-dimethylamino-2-phenylazophenol

2·88–2·96 wb, 3·44 w, 6·20 s, 6·30 w, 6·54 m, 6·63 m, 6·78 m, 6·83 m, 6·94 m, 7·23 m, 7·36–7·45 s, 7·59 m, 7·73 m, 7·88 w, 7·98 m, 8·38 m, 8·55 mb, 8·74 m, 9·28 m, 9·75 w, 10·27 w, 10·50 m, 10·74 w, 11·01 m, 11·50 w, 12·26 m, 12·75 m, 13·11 m, 13·80 w, 13·98 m, 14·38 m, 14·86 w.

N,*N*-5-dimethylamino-2-phenylazophenol

2·90 wb, 3·48 wb, 6·17 s, 6·30 m, 6·45 w, 6·54 m, 6·79 m, 6·93 m, 7·14 m, 7·26 m, 7·42 m, 7·56 s, 7·68 s, 7·85 m, 8·14 m, 8·37 m, 8·67 m, 8·81 s, 10·37 wb, 10·72 w, 10·96 m, 11·32 m, 11·63 wb, 12·20 m, 12·58 m, 13·09 m, 13·90 w, 14·12 m, 14·55 m, 15·35 w.

Cd^{2+} and chromate complex of 2-naphthylamine

3·13 mb, 3·33 m, 3·65 mb, 3·95–4·0 mb, 6·12 m, 6·23 m, 6·39 m, 6·61 s, 6·79 m, 7·27–7·30 m, 7·90 m, 8·20 m, 8·43 m, 8·84 m, 9·82 s, 10·47 m, 10·83 s, 11·13 s, 11·29–11·41 sb, 11·78 s, 12·06–12·43 sb, 13·63 s, 14·05 m, 15·00 w.

Cd^{2+} and chromate complex of 2-fluorenamine

3·06 m, 3·23–3·27 mb, 3·60 mb, 3·95 mb, 5·18–5·23 wb, 6·20 s, 6·28 s, 6·42 m, 6·58 m, 6·72 m, 6·88 s, 6·94 m, 6·96 m, 7·17 m, 7·44 wb, 7·61 m, 7·95 m, 8·22 m, 8·39 m, 8·52 m, 8·67 mb, 8·80 m, 8·91 m, 9·13 m, 9·53 s, 9·79 m, 10·55 m, 10·82 s, 10·98 s, 11·34 sb, 11·48 s, 11·75 s, 11·97 sb, 12·45 sb, 13·3 s, 13·70 s, 13·77 s, 14·38 wb, 15·05 wb.

* The numbers are expressed in wave length (μ) of bands and indication of relative intensity as w = weak, m = medium, s = strong, b = broad. The KBr disks usually contained 1·3 mg of compound and 130 mg of KBr. The spectra were recorded on a Perkin–Elmer spectrophotometer, model 21.

In the metal chelates of the *o*-hydroxyamines a new weak but sometimes quite broad band appeared at 14·65 to 15·15 μ, which may be due to the metal ion[34] or to the chelate ring containing the metal ion.

In *N*-hydroxy-2-fluorenylacetamide the C=O stretching band was at 6·0 to 6·18 μ. In the copper complex this band was shifted to 6·33 to 6·40 μ with a double peak. Such shifting has been attributed to a weakened carbonyl due to resonance between the C—O—Me and C=O - - - - - Me linkage.[35] The parent compound also showed very broad bands at 3·23 and 3·50 μ, indicating that there is hydrogen bonding between the N—OH and the C=O groups. In the chelate these bands were shifted to 3·10 and 3·32 μ and had become quite weak. The band due to copper in the chelate appeared at 15·15 μ and was of low intensity but fairly broad.

With 2-hydroxy-4-dimethylaminoazobenzene we confirmed the report of Dolinsky and Jones[36] who found no appreciable absorption that could be assigned to a hydroxy or NH group in the 3-μ region in any of 13 *o*-hydroxyaminoazo compounds. However, in the nickel complex of this material weak but broad bands appeared at 2·88 to 2·96 μ and at 3·44 μ. The most striking feature of the spectrum of the chelate was the strengthening of the band at 6·20 μ due to the aromatic C=C stretching. The band at 14·86 μ ascribed to the nickel in the chelate was also weak but broad.

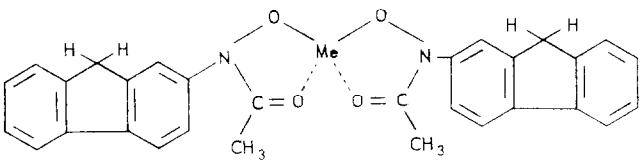
In the series of spectra of chelates from 3-amino-2-fluorenol and the nickel, copper, and cobalt ions, the peaks due to the metal appeared in the 14·65 to 15·10μ region. In the copper complex a small band appeared at 5·92 μ in the carbonyl region which may have been caused by partial oxidation of the hydroxyamine to a quinone or quinone imine during preparation of the complex.

In the complexes derived from copper, zinc, nickel, cobalt, and 2-amino-4-phenylphenol, the spectrum of the copper complex showed a medium weak band at 5·86 μ in the carbonyl region, possibly due to partial oxidation of the amine during formation of the complex. In these complexes no new absorption attributed to the metal ion could be discovered. Such a band might conceivably be located in the far infrared region.

DISCUSSION

By the application of standard techniques we have demonstrated that certain metabolites derived from the carcinogenic compounds 2-fluorenamine and 2-naphthylamine, from certain other aromatic amines, and also from a carcinogenic azo dye, readily undergo complex formation with metal ions. It was shown that the structural feature required, which confers on a compound this property, is generally a phenolic group proximate to a nitrogen atom. Thus, in most of the compounds investigated, there was an amino group *ortho* to a phenolic hydroxy group. This type of structure upon chelation leads to the classic type of a stable five-membered ring. The stability would be enhanced even more by addition of another ligand and consequent formation of a 2:1 complex. The complexes that were isolated and analyzed were of that sort. With *N*-hydroxy-2-fluorenylacetamide, the elemental analyses and infrared spectrum also established a dimeric chelate constitution. The nature of this complex compound is probably different from that observed with a classical *o*-aminophenol. In the first instance, the copper complex isolated had a relatively low melting point. Second, the complex stability constants with copper and nickel ions were of a low order, certainly

much lower than with the *o*-aminophenols. Third, the only five-membered ring structure that can be written for this compound involves an ionic bond between the oxygen attached to the nitrogen atom and a coordinate covalent bond between the keto function of the acetyl group. Of course, secondary valence forces between the metal ion and the nitrogen atom may also participate (formula III).



III

2-Fluorenamine and 2-naphthylamine failed to form chelates under the experimental conditions where *o*- or *N*-hydroxylated derivatives did. However, the amines themselves were induced to complex with cadmium and chromate, yielding a stable structure which could be isolated.

Whereas the aminophenols gave rise to stable, neutral 2:1 complexes, the phenol derived from the azo dye, *N,N*-5-dimethylamino-2-phenylazophenol, formed a basic 1:1 complex with nickel ion. It is noteworthy that the isomeric phenol, a symmetrical structure in which the hydroxy group is attached to the 2′-position, did not form a complex and gave the same titration curve in the presence and in the absence of metal ions. Spatially and sterically both of these compounds could form stable six-membered chelate rings. It may be the electronic structure and configuration of the compound,[37] owing to the presence of the dimethylamino group, that permit the formation of one but not the other of the chelates.

Dimethylsulfoxide was found to be a very useful solvent in this series of experiments for reasons mentioned earlier. Although this compound itself can enter into complex formation with several metal ions in anhydrous media,[38] it is believed that this particular property did not play any substantial role under our conditions.

The question arises as to the biochemical significance of the demonstrated ability of certain metabolites of the aromatic amines and azo dyes reported herein to chelate to metals. Some of these compounds exert toxic effects in rats upon chronic oral administration. It is conceivable that such effects are mediated via a removal of essential trace metals from their normal surroundings. In order to investigate the reality of such an explanation, the complexing ability of adenine, adenosine, and NAD was investigated and was found considerably weaker than that of the *o*-aminophenols. In fact, under our conditions, adenosine and NAD exhibited no chelate formation. The nature of the bond between cofactors, such as NAD and the corresponding enzyme-substrate complex, is not well understood. If a metal ion were involved in this association, it is easy to visualize a displacement of the cofactor as a result of the presence of a carcinogen metabolite. The reaction controlled by this fraudulent system would thus be inhibited, yielding consequently a picture of toxicity. This type of combination could also be representative of the so-called protein-bound

carcinogens. This possibility of a protein-metal-carcinogen metabolite combination requires, however, further investigation. It is conceivable, though, that a small amount of the protein-bound carcinogen might be accounted for by the metal ion complex of certain of the carcinogen metabolites which are highly insoluble and high-melting solids.[39]

TABLE 5. CARCINOGENICITY OF COMPOUNDS

Compound	Mode of administration	Species	Organ affected	Reference
2-Amino-1-naphthol hydrochloride	Paraffin pellet in bladder	Mouse	Bladder	43
2-Amino-1-naphthol hydrochloride	Paraffin pellet in bladder	Mouse	Bladder	44
2-Amino-1-naphthol hydrochloride	Subcutaneous injection	Mouse	Sarcomas at injection site	44
2-Amino-1-naphthol hydrochloride	Subcutaneous injection	Rat	Sarcomas at injection site with metastases and invasion of underlying muscle	44
2-Amino-1-naphthol hydrochloride 1-Amino-2-naphthol hydrochloride	Paraffin pellets in bladder	Mouse	Bladder	45
2-Amino-1-naphthol hydrochloride	Cholesterol pellet in bladder	Mouse	Bladder	18
3,3′-Dihydroxybenzidine	Oral	Rat	Liver, colon, stomach, bladder, sebaceous glands	46
3,3′-Dihydroxybenzidine	Subcutaneous injection	Mouse	Lymphoma	47
3,3′-Dihydroxybenzidine	Subcutaneous injection Oral Painting	Mouse	Mammary gland, liver, hemopoietic system, lung	48
3,3′-Dihydroxybenzidine	Oral Oral + injection Injection	Rat	Mammary gland, liver, hemopoietic system, lung	48
N-(1-Hydroxy-2-fluorenyl)acetamide *N*-(3-Hydroxy-2-fluorenyl)acetamide	Oral	Rat		49, 29
2-Naphthylhydroxylamine	Intraperitoneal injection	Rat	Abdomen (sarcomas)	50
N-Hydroxy-2-fluorenylacetamide	Oral	Rat	Liver, mammary gland, intestine, ear duct, forestomach	29
	Intraperitoneal injection	Rat	Liver, mammary gland, ear duct, intestine, peritoneum	29
2-Hydroxy-4-dimethylaminoazobenzene	Oral	Rat		51

The fact that metal ions play a role in certain aspects of chemical carcinogenesis has been established by a number of investigators. Certain metals or their inorganic or organic derivatives possess demonstrated carcinogenicity. The mode of action of the metal in this connection is not understood. A reasonable assumption would be that an imbalance in a crucial process, caused by the presence of large amounts of ions capable of being chelated, is involved. The carcinogenicity of the iron dextran complex[16] may be a special case, since the structure of this material may be of the nature of a clathrate (Van der Waals bonds) rather than that of a true chelate.

It has also been observed that copper, cobalt, and nickel salts inhibit carcinogenesis by azo dyes.[40] This was explained by the partial metal ion-catalyzed destruction of carcinogen in the diet during storage.[41] However, a diet containing copper acetate, in which the level of azo dye was apparently not reduced, decreased the tumor incidence as compared with controls on dye alone.[42] Table 5 presents a survey of the carcinogenicity of certain *o*-hydroxy and *N*-hydroxy derivatives of naphthylamine, benzidine, and fluorenamine. 2-Amino-1-naphthol and 1-amino-2-naphthol have produced tumors in the bladders of mice when the compounds were inserted as a paraffin or cholesterol pellet into that organ. Subcutaneous injection of 2-amino-1-naphthol led to sarcomas at the point of injection in mice and rats. 3,3′-Dihydroxybenzidine also was somewhat carcinogenic in rats and mice after various modes of administration. In view of problems associated with the instability of these *o*-aminohydroxy compounds, however, the question arises whether the tumorigenic power is actually inherent in the molecule administered. The carcinogenicity might be due to some decomposition product, perhaps analogous to that noted in the present study. Where the structure of this class of chemicals is stabilized by acetylation of the amino group, as for example in the hydroxyfluorenylacetamides, the compounds were inactive. However, reversible biochemical deactylation would make the free *o*-aminohydroxy compounds available *in vivo*.

The *N*-hydroxy derivatives of 2-naphthylamine and *N*-2-fluorenylacetamide are powerful carcinogens, and it is now believed that these compounds are proximate agents derived by metabolic transformation of the amines. As is apparent from the present work, the fluorene and presumably also the naphthalene derivative can complex with a variety of metal ions. 2-Hydroxy-4-dimethylaminoazobenzene is not carcinogenic under standard conditions of oral intake. This compound has chelating properties probably involving the β-nitrogen of the azo bond.

The sketchy results outlined in the table provide only a meager correlation between carcinogenicity and chelating properties of certain molecules. However, as noted above, the possible involvement of metal ions in chemical carcinogenesis has been raised by Boyland and Watson and by Furst. The existing body of facts and the data reported in this paper do not necessarily substantiate such an implication. Some additional doubt is attached to this concept by the fact that 1-hydroxy-2-dimethylaminonaphthalene, the structure of which exhibits all the requirements for a good chelating agent, is not carcinogenic, whereas the closely related 1-methoxy-2-naphthylamine, which presumably cannot chelate metals owing to the alkylation of the hydroxy group, is quite carcinogenic. It must be concluded, therefore, that the role of metal ions in the biochemistry of chemical carcinogenesis requires further elucidation.

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