Biomolecules Bearing the S- or $SeAsMe_2$ Function: Amino Acid and Steroid Derivatives

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A series of molecules of the type $GXAsMe_2$ have been synthesized in which X is S or Se and G is a moiety such as an amino acid, a di- or tripeptide, or a lipid. The compounds have been characterized by NMR, mass spectroscopy, and elemental analysis. Cysteine was found to react directly with dimethylarsinic acid to yield cystine and *S*-dimethylarsinocysteine (1). This reaction occurs also with other biomolecules containing thiol groups and raises serious questions concerning the use of cacodylate buffers in the study of enzyme kinetics and in sample preparation for electron microscopy. In the presence of dimethylchloroarsine and diethylamine, homocysteine thiolactone reacts to form both the dipeptide and the S–$AsMe_2$ bond. Results of carcinostatic, bacteriostatic, and fungicidal testing of these compounds are reported. A hypothesis is advanced to explain the observed carcinostatic action of the dimethylarsino group.

In 1973[1b] the synthesis of the *S*-dimethylarsinous acid ester of 1-thio-β-D-glucose was described. When it was found that this compound displays in vitro activity against nasopharyngeal epidermoid carcinoma cell cultures, a number of other sugar derivatives (e.g., C_1- and C_6-β-D-glucose and antimonous and phosphinous acid derivatives) were prepared and a number of arsenic derivatives were found to display carcinostatic activity, in vivo, in mouse leukemia screening tests.[2,3] Derivatives of thio- and selenogalactose have been described.[4,5] Biochemical testing of these compounds as carcinostatic agents has yielded encouraging results. Hence, it was decided to prepare derivatives in which the biochemical moiety is something other than a sugar molecule. This paper describes the synthesis of dimethylarsinous acid esters of some thio and seleno amino acids, di- and tripeptides, a purine, and cholesterol. Results of biochemical testing are presented and possible interpretations of the carcinostatic activity of these arsenicals are discussed.

Biochemical Testing. Tumor Screening Tests. It has been mentioned in the introductory portion of this paper that a number of compounds of the type $GSAsMe_2$ or $GSeAsMe_2$, where G is a monosaccharide moiety, display carcinostatic activity, in vivo, against mouse leukemias (P388 test system). Such activity has been confirmed in derivatives of both glucose and galactose.[2,4,5] As a natural consequence of these observations, the compounds which are the subject of this study were prepared in order to determined whether a biological moiety, G, other than a monosaccharide would yield derivatives which also display biological activity. The results of screening tests are summarized in Table I. Also shown are the results of several additional tests (L1210 test system).

It is to be noted that the synthesized compounds are not highly toxic. Using the established NIH criterion for carcinostatic activity (%T/C ≥ 125), it can be seen that of eight compounds tested six satisfy such a criterion. It is also to be noted that two compounds, the glutathione and lipoic acid derivatives, demonstrate activity in both test systems.

The *S*-dimethylarsino-6-mercaptopurine (3) showed good activity at a relatively low dose level. It is interesting to note that 6-mercaptopurine was tested concurrently with compound 3 and showed a T/C = 124 at a slightly higher dose level. The enhancement of the activity of this compound due to the presence of the dimethylarsino group is indicated. Failure to reproduce the initial activity may have resulted from decomposition as a result of hydrolysis during the test procedures.

Bacteriostatic and Fungicidal Testing. Two of the compounds, 1 and 4, were chosen for bacteriostatic and fungicidal testing because of their water solubility.[5] Both compounds 1 and 4 were tested in their pure form against Gram-positive bacteria (*Staph. aureus* 209) and found to inhibit growth for 15 and 20 mm, respectively, around the area of sample spotting. The growth of the bacteria in a nutrient broth was totally inhibited by 1 and 4. Further tests with the peptide 4 showed activity against Gram-negative (*K. pneumoniae*) as well as Gram-positive bacteria.

Table I. Carcinostatic Activity of Synthesized Arsino Compounds

compd	T/C:[a,b] dose, mg/kg	toxicity: dose, mg/kg	% ILS:[c,d] dose, mg/kg	anal.[e]
1	110 (50)	200	0	C, H
2	117 (25)	50	2 (50)	C, H, S
2·HCl	128[e] (50)	100	3 (25)	
3	144 (100)	>200		C, H, S
4	164 (100)	400	33 (500)	C, H, S
5	128 (50)	100	33 (100)	C, H, S
6	144[f] (25)	50	7 (25)	C, H, S
7	NA[g]	NA[g]		C, H, S
9	129 (200)	>200		

[a] Ratio of survival of test to control animals expressed as a percent. [b] Tested by NCl using P388 lymphocytic leukemia. [c] ILS = increased life span. [d] Tested by Wadley Institutes of Molecular Medicine using L1210 lymphoid leukemia. [e] Analyses for the elements indicated were within ±0.4% of the theoretical values required. [f] Results not reproducible. [g] Not yet available.

The fungicidal activity was determined by the use of a mixed fungal spore suspension and was found to be marginal.

Possible Modes of Carcinostatic Action of Arsenicals. As of this writing, 21 compounds in this series have been shown to demonstrate carcinostatic activity. There appears to exist a statistically meaningful activity which is associated with the R_2As group. In molecules of the type $GXMR_2$, where G is a biological moiety, where X is S or Se, and when M is a group V element, we have made several observations.

For the biomolecules, G, those that are water insoluble appear to yield compounds which are more active than those from biomolecules which are water soluble. This trend was found in the comparison of amino acids vs. peptides and previously with blocked vs. free sugars. Sulfur analogues tend to be more active than the selenium analogues. This may be due to the instability of the selenium compounds. Of all the group V elements tested, only organoarsenic compounds are active. Of all R groups used, only methyl, ethyl, and hydroxyethyl groups give

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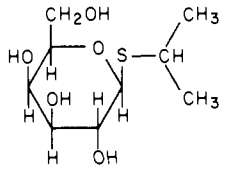
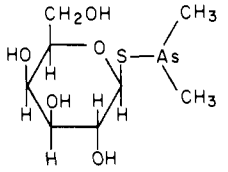
compounds with more than marginal activity.

The biological mode of action for these compounds has not been elucidated. Several arsenicals have been shown to be effective mitotic poisons. Since the SH/S–S ratio becomes elevated during mitosis,[7,8] it is possible that the $SAsR_2$-containing molecule reacts directly or indirectly with the sulfhydryl groups.[4] In a recent report, Gillard and Nelson found that 1-[S-(dimethylarsino)thio]-β-D-glucose is an irreversible active-site inhibitor of the rabbit muscle debranching enzyme amyl-1,6-glucosidase 4-α-glucanotransferase.[9] This suggests an alternate mode of action.

Finally, it is of interest to observe the similarity in structure between molecules A and B. Molecule A is the



A

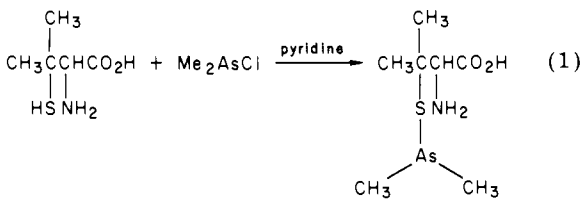


B

repressor isolated by Gilbert and Müller-Hill[10] that is responsible for repressing β-galactosidase activity. Molecule B is the inhibitor of glucanotransferase and displays carcinostatic activity.

The action of the molecules in this study can be explained in terms of a chemical reaction between enzyme-SH groups and AsR_2 groups, which results in enzyme deactivation via enzyme–S–AsR_2 bond formation. Fundamental studies which definitively establish their mode of interaction with cellular constituents would be well justified.

Chemistry. The preparation of compounds **2**, **4-7**, and **9** was straightforward. Thus, the reaction of penicillamine with dimethylchloroarsine in 1,2-dimethoxyethane in the presence of pyridine yields the *S*-(dimethylarsino) derivatives (eq 1). This procedure was first reported by



(1)

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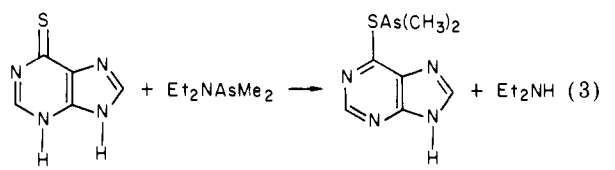
Zingaro and Thompson.[1b] The synthesis of 6-[*S*-(dimethylarsino)mercapto]purine (**6**) was carried out by first preparing diethylaminodimethylarsine in situ[11] (eq 2).

$$Me_2AsCl + 2Et_2NH \xrightarrow{Et_2O} Me_2AsNEt_2 + Et_2NH_2^+Cl^- \quad (2)$$

The mercaptopurine in chloroform then undergoes reaction with this reagent to form the desired compound (eq 3).



(3)

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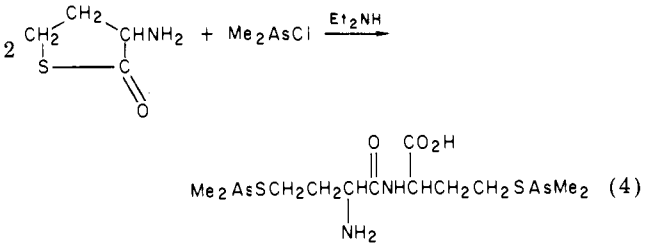
The structure of 6-mercaptopurine in solution has been shown to be the thione, though it must react with the arsine in the form of the thiol.

The synthesis of compound **3** involved a base (diethylamine) assisted opening of the thiolactone ring, formation of the sulfur–arsenic bond, and peptide bond formation (eq 4). This rather complex series of reactions



(4)

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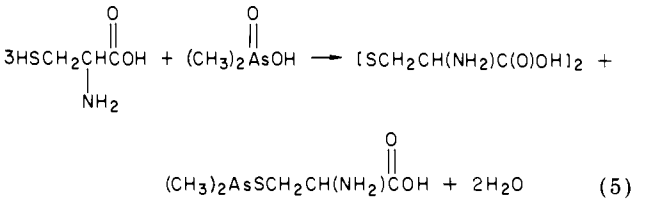
is accomplished in reasonable yield without the isolation of any intermediates. The first step probably involves an opening of the thiolactone ring. Whether the next step involves formation of the *S*-(dimethylarsino) derivative, which is followed by dipeptide bond formation or the reverse occurs, cannot be concluded from these experiments. A mechanistic study was not attempted.

Homocysteine was allowed to react with dimethylchloroarsine to give the dimethylarsinohomocysteine, but the product could not be purified. Therefore, no further work was done with the system.

It was found that compound **1** could be prepared by the direct reaction between aqueous solutions of dimethylarsinic acid and cysteine (eq 5).[12] The stoichiometry



(5)

1

shown in eq 5 has recently been demonstrated in our laboratories. This synthetic method was also used successfully for the preparation of compound **4**. The fact that dimethylarsinic acid is able to undergo an oxidation–reduction reaction with thio amino acids suggests that it may react in a similar manner with sulfhydryl groups on proteins. This observation strongly corroborates the suggestion of Jacobson and Murphy[13] that cacodylate buffers should be avoided in the study of enzymes or of enzyme kinetics. Their use as buffers in the preparation of samples for electron microscopy should also be questioned.

Thiocholesterol was prepared by a literature procedure in good yield.[14] Cholesterol diselenide (**8**) was prepared by the tosylation of cholesterol followed by nucleophilic displacement of the tosylate group by selenocyanate. Reduction of the selenocyanate by sodium borohydride in pyridine, under nitrogen, gives the recrystallized product in 55% yield. Because of its inherent instability, the *Se*-(dimethylarsino)selenocholesterol was never isolated in pure form. In comparison, the S–As analogue was quite stable. This again illustrates the differences in the nature of the two bonds. On standing, the *Se*-(dimethylarsino)selenocholesterol converted to the diselenide.

Experimental Section

General Methods. Melting points were determined using a Büchi SMP-20 apparatus and are uncorrected. NMR spectra were obtained using a Varian Model T-60 spectrophotometer with Me_4Si as the internal standard when possible. Column chromatography was accomplished using Brinkmann silica gel 60. Purity of compounds was checked by TLC with Baker-flex silica gel 1B and developed by exposure to I_2 vapor. Elemental analyses were determined by Galbraith Laboratories, Inc.

Mass spectra were obtained from the mass spectroscopic laboratory of the Department of Biochemistry and Biophysics, Texas A&M University, using a CEC 21-110B high-resolution mass spectrometer.

All starting materials were commercially obtained and recrystallized before use. All solvents were dried and distilled before use. Dimethylchloroarsine was prepared by the method of Van der Kelen.[14] Diethylaminodimethylarsine was prepared by the method of Sarquis and Zingaro.[11]

***S*-(Dimethylarsino)-DL-cysteine (1).** The preparation of **1** follows that of King and Ludford.[12] To a solution of DL-cysteine hydrochloride (2.36 g, 15 mmol) in 5 mL of H_2O, dimethylarsinic acid (0.69 g, 5 mmol) in 5 mL of H_2O was added. Within 10 min a white precipitate formed (cystine). At this time, 15 mL of 1 N NaOH was added with cooling. The solution then was saturated with CO_2, sealed, and allowed to react overnight. The disulfide was removed by filtration and the product, **1**, was obtained upon evaporation of the solvent. Recrystallization from EtOH–HOH afforded 1 g (89% yield) of **1** as its monohydrate: mp 219–220 °C, lit.[12] 219–220 °C; NMR (D_2O) δ 1.5 (s, 6 H), 3.1 (s, 1 H); ^{13}C NMR (dioxane reference) δ 182.86 (C, s), 79.90 (CH_2, t), 65.21 (CH_3, q), 49.8 (CH, d); mass spectrum (*m/e*) 242, 210, 195, 180, 137, 105.

***S*-(Dimethylarsino)-DL-penicillamine (2).** To a slurry of DL-penicillamine (5.0 g, 29.9 mmol) in glyme, dimethylchloroarsine (4.2 g, 29.9 mmol) was added slowly. Pyridine (4.6 g, 59.8 mmol) was then added dropwise, and the mixture was heated to reflux, immediately cooled, and finally stirred for an additional 4 h. At the end of this time, the solid (**2**) and unreacted thiol were removed by filtration. Recrystallization from EtOH gave **2** in 62% yield (5.3 g): mp 189–190 °C; NMR (D_2O) δ 1.3–1.37 (2 d, 12 H), 3.8 (s, 1 H); mass spectrum (*m/e*) for **2**·HCl 275, 259, 242, 212, 179, 137, 105, 103, 89.

Bis[*S*-(dimethylarsino)homocysteine] (3). To a slurry of DL-homocysteine thiolactone hydrochloride (5.6 g, 36 mmol) in $CHCl_3$ was added dimethylchloroarsine (5.0 g, 36 mmol) and diethylamine (5.3 g, 72 mmol). The thiolactone reacted rapidly with evolution of heat and the resultant product went into solution. After 1 h, the solution was filtered and then washed with water (3 × 25 mL). The solution was dried over Na_2SO_4 and upon evaporation of the solvent afforded **3**. The product was triturated with ether and recrystallized from acetone to give pure **3** (2.8 g, 34% yield): mp 179–180 °C; NMR ($CDCl_3$) δ 1.4 (s, 12 H), 1.9–2.3 (m's 12 H), 2.7–2.9 (m, 10 H), 4.10–4.13 (m's, 4 H); mass spectrum (*m/e*) 442, 427, 323, 279, 224, 178, 137, 105.

***S*-(Dimethylarsino)glutathione (4).** The procedure shown in the preparation of **2** was used for **4**. Glutathione (14.0 g, 45.6 mmol) was stirred rapidly in glyme while dimethylchloroarsine (6.5 g, 45.6 mmol) was added dropwise. Pyridine (6.9 g, 91.2 mmol) was then added to the slurry and the mixture was subsequently heated to reflux. The heat was removed immediately and the mixture stirred at room temperature for 4 h. Isolation of the resultant insoluble solid and recrystallization from ethanol afforded **4** as the pyridinium hydrochloride (75% yield): mp 115–118 °C; NMR (D_2O) δ 1.35 (s, 6 H), 1.9–4.1 (m's, 10 H), 7.8–9.0 (m, 5 H); mass spectrum (*m/e*) 140, 125, 110, 105, 79, 52, 45, 36.

Bis[*S*-(dimethylarsino)-α-dihydrolipoic acid] (5). For the preparation of **5**, α-dihydrolipoic acid was used as the starting compound. This dithiol was formed in 85% yield by $NaBH_4$ reduction of the disulfide, lipoic acid.[15] α-Dihydrolipoic acid (6.0 g, 28.7 mmol) was dissolved in glyme, and the resultant solution was stirred rapidly while dimethylchloroarsine (8.1 g, 57.4 mmol) was added. Pyridine (9.05 g, 114.8 mmol) was then added dropwise to the mixture. The solution was allowed to react for 4 h at room temperature. The mixture was then poured into H_2O and the product extracted with $CHCl_3$. The $CHCl_3$ solution was dried and the solvent removed to give 9.0 g of **5** (75% yield) as an oil. Crystallization attempts were unsuccessful. The oil was repeatedly chromatographed to remove unreacted dithiol using ethyl acetate as the eluent. Pure **5** was obtained in 68% yield: NMR ($CDCl_3$) δ 1.4 (s, 12 H), 1.6–3.2 (m's, 13 H); mass spectrum (*m/e*) 415, 401, 311, 279, 210, 206, 137.

***S*-(Dimethylarsino)-6-mercaptopurine (6).** The procedure used was that described by Sarquis and Zingaro.[11] To a slurry of 6-mercaptopurine (0.8 g, 5.3 mmol) in $CHCl_3$ was added freshly prepared diethylaminodimethylarsine (0.94 g, 5.3 mmol). The reaction mixture was stirred for 2 h, and the unreacted mercaptopurine was removed by filtration. The solution was evaporated to dryness and the crude product was recrystallized from $CHCl_3$ to give 0.5 g of the arsine **6** (37% yield): mp 178–179 °C dec; NMR ($CHCl_3$) δ 1.6 (s, 6 H), 7.2–7.6 (d, 2 H); mass spectrum (*m/e*) 256, 241, 226, 119, 107, 89.

Dimethylarsinothiocholesterol (7). The thiocholesterol, used in the preparation of **7**, was synthesized from cholesterol using the procedure of King et al.[16] The sodium salt of thiocholesterol (0.5 g, 1.24 mmol) was dissolved in anhydrous ether and the solution was stirred as dimethylchloroarsine (0.18 g, 1.24 mmol) was added dropwise. The resulting solution was allowed to react for 4 h. Subsequent evaporation of the solvent afforded crude **7** (0.3 g, 48% yield). Column chromatography gave pure **7**: mp 73–74.5 °C; NMR ($CDCl_3$) δ 0.6–2.4 (44 H), 1.3 (s, 6 H), 5.2 (m, 1 H); mass spectrum (*m/e*) 506, 491, 475, 402, 368, 326, 256, 105, 90, 29.

Cholesterol Diselenide (8). The diselenide **8** was prepared from cholesterol selenocyanate, which was in turn prepared from cholesterol tosylate. The cholesterol tosylate (27 g, 50 mmol), prepared by standard methods,[17] was dissolved in 70 mL of DMF, and potassium selenocyanate (7.2 g, 50 mmol) was then added. The solution was heated to 100 °C for 36 h. After cooling to room temperature the dark mixture was poured over ice. The precipitate was isolated and recrystallized from $CHCl_3$–acetone. The selenocyanate was obtained in 47% yield, mp 207–208 °C, and used directly. Cholesterol selenocyanate (5.0 g, 1 mmol) was dissolved in 100 mL of pyridine and stirred under N_2. To this solution, $NaBH_4$ (0.4 g, 1 mmol) was added slowly, and the subsequent viscous solution was allowed to react for 72 h. At the end of this time, the N_2 flow was discontinued and air was passed through the solution for 12 h. The mixture then was poured onto ice and the resultant precipitate was separated. Crude **8** was recrystallized from $CHCl_3$–EtOH to yield 2.8 g (55%) of purified diselenide: mp 145–150 °C; NMR ($CDCl_3$) δ 0.5–2.9 (88 H), 5.4 (m, 2 H); mass spectrum (*m/e*) 450, 368, 353, 327, 275, 255, 213, 145, 81.

***Se*-(Dimethylarsino)selenocholesterol (9).** Cholesterol diselenide (**8**, 2.2 g, 2.5 mmol) was dissolved in 40 mL of $CHCl_3$, and the flask was filled with N_2. To this solution, $NaBH_4$ (0.4 g, 10 mmol) in 50 mL of DMF was added slowly and the reaction mixture was stirred for 18 h, always under N_2. At the end of this time, 2 mL of dry MeOH was added and the solution was stirred for an additional 1 h. Dimethylchloroarsine (1 mL, 10 mmol) was then added dropwise. The resultant mixture was stirred for 24 h and the $CHCl_3$ removed under reduced pressure. The resultant DMF suspension was refrigerated overnight, and **9** was crystallized from the solution. The crude product was rubbed with dry MeOH and air-dried to give 2.4 g of **9** (89% yield): NMR ($CDCl_3$) δ 0.5–2.9 (44 H), 1.6 (s, 6 H), 5.3 (m, 1 H); mass spectrum (*m/e*) 558, 464, 450, 369, 353, 247, 213, 185, 105, 81.

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Platinum and Palladium Derivatives for Chemotherapy Studies[1]

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Analogues of *cis*-dichlorodiammineplatinum(II) were prepared in which substituted pyridines (A), 1-(4-aminobenzylidene)indene (B), or DL-3,5,3′5′-tetraoxo-1,2-dipiperazinopropane (ICRF-159) was used in place of ammonia, and in some cases platinum(IV) or palladium(II) was used in place of the platinum(II). Both platinum complexes with ICRF-159 were active against leukemia 1210, but none of the others produced significant life extension following a single ip dose of 400 mg/kg. Attempts to prepare complexes of ICRF-159 with Zn(II), Mn(II), and Cr(III) were unsuccessful, but there were indications of complex formation with $CuCl_2$ and with $NiCl_2$.

The announcement by Rosenberg et al.[2] that *cis*-dichlorodiammineplatinum(II) had antitumor activity encouraged us to prepare analogues containing other nitrogen compounds and submit them for testing. The first samples we submitted were *cis*-dichlorobis(substituted pyridine)platinum(II) compounds whose preparation and physical properties had been reported by Patterson and Bull.[3] None of these was effective against L-1210 leukemia at a single dose of 400 mg/kg, but some were toxic (see Table I). None of them has been tested by the methods used by Gale, Howle, and Walker,[4] who found *cis*-dichlorobis(pyridine)platinum(II) effective in several in vitro systems and against Ehrlich ascites carcinoma in vivo.

1-(4-Aminobenzylidene)indene (1) has been found to exert a strong effect against the growth of Walker 256 tumors in rats,[5] but not against L-1210 in mice. It formed palladium and platinum complexes which were inactive against L-1210 at 400 mg/kg and did not kill any of the animals at that dose level.

DL-3,5,3′,5′-Tetraoxo-1,2-dipiperazinopropane (ICRF-159) has attracted attention because of activity against several tumors. Woodman et al.[6] reported a marked synergism when ICRF-159 and *cis*-dichlorodiammineplatinum(II) were administered to BDF_1 mice after ic innoculation with L-1210. It seemed possible that the administration of a compound containing both ICRF-159 and platinum(II) might be more effective than separate administration of two drugs. Complexes of ICRF-159 with divalent and tetravalanet platinum and divalent palladium were prepared.

Creighton[7] found that the (ICRF-159)$PtCl_2$ complex inhibited [^{3}H]thymidine uptake in vitro to about the same extent as ICRF-159 itself, on a molar basis (see Table II). In tests against L-1210 under the auspices of the National Cancer Institute it produced life extension: 9 × 200 mg/kg doses gave T/C = 1.21 and 9 × 400 mg/kg gave T/C = 1.60. The tetrachloro(ICRF-159)platinum(IV) complex was active against L-1210 at a lower dose level than the platinum(II) complex: 3 × 100 mg/kg gave T/C = 1.43 and 9 × 12.5 mg/kg gave T/C = 1.33.

Experimental Section

Dichlorobis[1-(4-aminobenzylidene)indene]platinum(II). To a stirred solution of 220 mg (1.0 mmol) of 1 in 15 mL of tetrahydrofuran (THF) and 5 mL of H_2O was added 209 mg (0.5 mmol) of K_2PtCl_4. The solution was held at 60 °C in a H_2O bath for 1 h and then allowed to stand overnight. The yellow precipitate which began to appear soon after the mixing was washed with H_2O and THF, yield 169 mg (48%). Anal. ($C_{32}H_{26}N_2PtCl_2$) C, H.

Dichlorobis[1-(4-aminobenzylidene)indene]palladium(II). A solution of 219 mg (1.0 mmol) of 1 in 10 mL of THF and 5 mL of H_2O was added slowly to a solution of 164 mg (0.5 mmol) of K_2PdCl_4 in 12.5 mL of H_2O and 12.5 mL of THF. After 2 h, the orange precipitate was washed successively with H_2O, MeOH, C_6H_6, and Et_2O and then dried, yield 297 mg (97%). Anal. ($C_{32}H_{26}N_2PdCl_2$) C, H, N.

Dichloro(ICRF-159)palladium(II). A solution of 4.5 mmol of ICRF-159 in 450 mL of H_2O was added, during 2–3 min, to a solution of 4.8 mmol of K_2PdCl_4 in 20 mL of H_2O. After stirring the mixture 5 h, it was filtered. The yellow precipitate was washed with two 3-mL portions of MeOH and then dried, yield 87%. Anal. ($C_{11}H_{16}N_4O_4PdCl_2$) C, H, Pd. In another run, the ICRF was dissolved in THF and the K_2PdCl_4 in 80:20 THF–H_2O before mixing. A small amount of black precipitate was removed by filtration, and then the filtrate was evaporated under reduced pressure, producing a yellow precipitate.

Dichloro(ICRF-159)platinum(II). Approximately 9.0 mmol of Na_2PtCl_4 dissolved in 1 L of acetone was added during 20 min to 4.5 mmol of ICRF-159 dissolved in 1.2 L of boiling acetone. After the solution was left standing overnight at room temperature, the tan precipitate was recovered. More was obtained by evaporation of the acetone under reduced pressure. The product was washed twice with 2-mL portions of H_2O and twice with 1-mL portions of MeOH and then dried: yield 75%; mp 275–289 °C. Anal. ($C_{11}H_{16}N_4O_4PtCl_2$) C; H: calcd, 24.73; found, 24.30. Another sample prepared in H_2O solution gave C: calcd, 24.94.

Tetrachloro(ICRF-159)platinum(IV). A solution of 3.5 mmol of ICRF-159 in 800 mL of acetone was added to a solution of 3.3 mmol of $Na_2PtCl_6·6H_2O$ in 185 mL of acetone at room temperature during 15 min. After 2 days a white precipitate was filtered off and discarded. The solution was evaporated under vacuum to dryness. The resulting yellow precipitate was washed with 50 mL of THF and three times with 2–3 mL of MeOH and then dried: yield 98%; mp 240–250 °C dec. Anal. ($C_{11}H_{16}N_4O_4PtCl_4$) C, H, Pt.

Attempts to prepare complexes of ICRF-159 with Zn(II), Mn(II), and Cr(III) did not meet with any success, but what appeared to be an 80% yield of an impure green complex of $CuCl_2$ with ICRF-159 was obtained when a solution of 270 mg of ICRF-159 in 150 mL of hot absolute EtOH was added to a solution of 170 mg of $CuCl_2·2H_2O$ in 25 mL of absolute EtOH. The mixture was stirred for 1 h, cooled, and filtered. Water appeared to decompose the substance, and no satisfactory solvent for recrystallization was found. Likewise, a green solid was obtained by mixing 140 mg of ICRF-159 dissolved in 50 mL of DMF with a solution of 120 mg of $NiCl_2·6H_2O$ in 50 mL of DMF, allowing the mixture to stand overnight, removing DMF under vacuum,

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