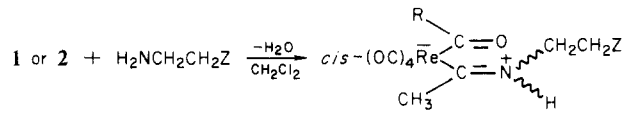
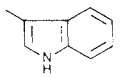
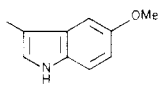
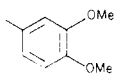
Chart I



R	Z	isomer	compd
CH_3	-Cl	intra + inter	6
CH_3	$(-S)_2$ (dimer)	intra + inter	7
CH_3		intra	8
CH_3		intra	9
CH_3		intra	10
CH_3		intra	11
CH_3	Ph	intra	12
i-Pr	Ph	intra	13

Hz), 3.24 (m, 1, CH, J = 8 Hz), 3.77 (quartet, 2, CH_2N, J = 8 Hz), 7.25 ("s", 5, C_6H_5), 13.27 (br s, 1, NH). Anal. ($C_{18}H_{20}NO_5Re$) C, H, N.

Results and Discussion

Complex **1** or **2** condenses with 2-chloroethylamine, cystamine, histamine, tryptamine, *O*-methylserotonin, *O*,*O*-dimethyldopamine, and 2-phenethylamine to afford, respectively, the corresponding rhena β-keto imine complexes **6–13** as shown in Chart I.

Proton NMR spectra of crude reaction residues indicate that these condensation reactions proceed in high yield. The low yields reported reflect material loss when separating the products from unreacted amine and solvent. These products are yellow oils except for complexes **6**, **9**, and **10**, which are pale yellow solids. Complexes **9** and **10** are the first intra isomers known to exist as solids at room temperature. IR spectra of these complexes are consistent with rhena β-keto imine formation.[1,2,4]

Complexes **8–11** exhibited an unusual pattern of relative chemical shifts for the two methyl groups within the rhena moiety. For rhena β-keto imine derivatives of *N*-alkyl primary amines, the ^{1}H NMR spectra of the intra isomers show a sharp singlet at ca. δ 2.62 for the acetyl methyl group and a broader singlet at ca. δ 2.73 ppm for the iminium methyl group.[2] This pattern is observed for complexes **6** and **7**, also. However, complexes **8–11** exhibit a reversed pattern for these two singlets. In these intra isomers, the sharp acetyl methyl resonance appears at the normal chemical shift of δ 2.64 ± 0.02, but the more broad iminium methyl resonance now appears, *in each case*, at *higher* field than the acetyl methyl resonance. These iminium methyl resonances appear in the range of δ 2.29–2.60 and represent an upfield shift of from 13 to 44 Hz relative to the "normal" chemical shift of an iminium methyl group in *N*-alkyl rhena β-keto imines.

This upfield shift of the iminium methyl resonance is attributed to a through-space interaction between this methyl group and the π system of an aromatic substituent attached to the carbon atom which is β to N. Crude molecular models confirm the proximity of these moieties in several of the various conformations of intra isomers containing such *N*-alkyl substituents. Complexes **12** and **13** were prepared as the most simple *N*-alkyl derivative containing a phenyl group β to N. For both complexes, the iminium methyl resonance appears at high field (δ 2.42 ± 0.02). In complex **12**, this resonance is 21 Hz to higher field than the acetyl methyl resonance. Thus, the position of the iminium methyl resonance is greatly affected by the presence of an aromatic substituent on the β-carbon atom of the *N*-alkyl group.[7,8]

The preparation of rhena derivatives of other biologically important amines which are representative members of important classes of pharmaceutical drugs is being pursued with appropriate selectivity.

Acknowledgment. C.M.L. thanks the National Science Foundation (Grant Nos. CHE-7907557 and CHE-8106140) and the University Research Council of Vanderbilt University for support of this research. C.M.L. acknowledges support from the Alfred P. Sloan Foundation as a Research Fellow.

Registry No. **1**, 59299-78-4; **2**, 66808-98-8; **6** (intra isomer), 80374-42-1; **6** (inter isomer), 80327-16-8; **7** (intra isomer), 80374-41-0; **7** (inter isomer), 80327-15-7; **8** (intra isomer), 80327-14-6; **9** (intra isomer), 80327-13-5; **10** (intra isomer), 80327-32-8; **11** (intra isomer), 80327-31-7; **12** (intra isomer), 80327-30-6; **13** (intra isomer), 80339-93-1; 2-chloroethylamine, 689-98-5; cystamine, 51-85-4; histamine, 51-45-6; tryptamine, 61-54-1; *O*-methylserotonin, 608-07-1; *O*,*O*-dimethyldopamine, 120-20-7; 2-phenethylamine, 64-04-0.

(7) The ^{1}H NMR spectrum of the rhena β-keto imine derivatives of ethyl L-phenylalaninate shows the same "reversed" pattern of rhena–methyl group resonances for the intra isomer. However, the inter isomer exhibits a "normal" pattern for the two methyl resonances. Molecular models reveal that an iminium methyl–aromatic interaction is not possible for the inter isomer, which is consistent with these spectral data reported here: Lukehart, C. M.; Afzal, D., unpublished results.[9]

(8) Note that the *N*-benzylrhenaacetylacetonimine complex reported in ref 2 does *not* exhibit this "reversed" order of the two rhena–methyl resonances in the intra isomer. In this complex, the aromatic group is a substituent on the carbon atom α to N.

(9) When **9** or **10** are prepared at 1:1 stoichiometry with a reaction time of 16 h, both intra and inter isomers are observed. The chemical shifts of the acetyl- and iminium-methyl resonances of the inter isomers *also* follow the "normal" pattern.

Contribution from the Department of Biophysics, Michigan State University, East Lansing, Michigan 48824

High-Performance Liquid Chromatography Studies on Platinum Thymine Blue

J. Derek Woollins* and Barnett Rosenberg

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Platinum pyrimidine blues (PPBs) are currently of great interest because of their unusual color and their antitumor properties.[1] Apart from an extended X-ray absorption fine structure study on platinum uridine blue[2] and a powder X-ray diffraction study on platinum acetamide blue,[3] the majority of our knowledge of these species is based upon comparison with platinum α-pyridone blue, whose structure has been solved by single-crystal X-ray studies.[4] PPBs have proved to be very difficult to prepare reproducibly; for example, the EPR spectra vary considerably from batch to batch.[5] Their visible spectra do not obey Beer's law, with the absorptions also showing a

(1) Davidson, J. P.; Faber, P. J.; Fischer, R. G., Jr.; Mansy, S.; Peresie, H. J.; Rosenberg, B.; Van Camp, L. *Cancer Chemother. Rep.* **1975**, *59*, 287.
(2) Teo, B. K.; Kijima, K.; Bau, R. *J. Am. Chem. Soc.* **1978**, *100*, 621.
(3) Laurent, M. P.; Biscoe, J.; Patterson, H. H. *J. Am. Chem. Soc.* **1980**, *102*, 6575.
(4) Barton, J. K.; Szalda, D. J.; Rabinowitz, H. N.; Waszczak, J. V.; Lippard, S. J. *J. Am. Chem. Soc.* **1979**, *101*, 1434.
(5) Lippert, B. *J. Clin. Hemat. Oncol.* **1977**, *7*, 26.

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time dependence;[5] gel chromatography and mass spectral studies[6] on platinum thymine blue (PTB) indicate that this compound contains a mixture of molecular weights ranging from 3000 down to 1000. The above results, together with precipitation studies and electrophoresis experiments, have led to the proposition that the PPBs consist of mixtures of different chain length, mixed-valence, pyrimidine-bridged cations.[4,5] It is the purpose of this paper to report high-performance liquid chromatography (HPLC) studies on PTB that reveal that typical preparations of this compound contain several colorless platinum compounds ("whites") as well as a number of blue species.

Experimental Section

All reactants and solvents were of at least reagent grade. Thymine was purchased from Sigma Chemical Co. and *cis*-$Pt(NH_3)_2Cl_2$ was from Johnson Matthey or Engelhard Industries.

Eluents for HPLC were prepared by using HPLC-grade water from Burdick & Jackson Labs., Inc., or from distilled and deionized water (ASTM Class 1). All eluents were filtered through 0.47-μm filters (Millipore) prior to use and thoroughly degassed. Tetrabutylammonium nitrate solutions were prepared by addition of nitric acid (Mallinckrodt, Inc., analytical reagent) to tetrabutylammonium hydroxide (MCB, supplied as 25% solution in methanol, or Aldrich 40% solution in water). Samples for HPLC were prepared immediately prior to injection (unless stated otherwise) and filtered through 0.22-μm filters; pH adjustments on sample solutions were carried out with 10^{-3} M nitric acid or 10^{-3} M potassium hydroxide. For time-dependence studies samples were stored in vials, with no special exclusion of air, in the dark at room temperature. No studies were carried out on high pH (>7) solutions of PTB since basic conditions discharge the blue color although even quite strongly acidic solution (0.1–1 M HNO_3) have no effect.

A high-performance liquid chromatograph (Waters Assoc.) consisting of a Model 6000A pump, U6K injector, and 440 dual wavelength absorbance detector (operating at 254 and 658 nm), and a 3.9 × 300-mm μBondapak C_{18} column was used for all of the separations shown here. The white components in PTB were separated with pH 3.0 nitric acid as the eluent. For the separation of the blue species, the column was conditioned at the start of each day for 20 min (flow rate 2 mL/min) with pH 2.0, 0.05 M *n*-Bu_4NNO_3. A step gradient with pH 4.5 nitric acid as the initial eluent and the tetrabutylammonium nitrate solution as the second eluent, 6 min after injection, enabled several blue fractions to be resolved. After each gradient was completed, 18–20 min of washing with the nitric acid solution was required before the next injection. The above conditions were chosen after a variety of gradients involving nitric acid and tetrabutylammonium nitrate were investigated. Two different Waters μBondapak C_{18} columns and a Whatman ODS-3 column were used for the separations to test the reproducibility. Agreement between the three columns was very good, with retention times varying by no more than 5%. The concentration, pH, and time dependence studies on the blue components were carried out three times, with similar results being obtained upon each occasion.

Atomic absorption (AA) measurements were carried out by Ray Sculley in this laboratory on a Varian AA-175 spectrophotometer equipped with a CRA-90 graphite furnace. Digestions using nitric acid–perchloric acid (6:4) followed by HCl and extraction into 0.05 M tri-*N*-octylamine in xylol from a 25% solution of stannous chloride in 3 M HCl were carried out on a number of samples. We noted no significant difference in the results between untreated and digested samples.

Platinum thymine blue was prepared by the reported procedure.[1]

$Pt(NH_3)_2Cl(TH–H)$ (TH–H = Thymine Monoanion). Thymine (2.52 g, 20 mmol) was dissolved with stirring and heating in water (200 mL), and *cis*-$Pt(NH_3)_2Cl_2$ (3.0 g, 10 mmol) was added. The solution was stirred at 75 °C for 1 h and then cooled in an ice bath. Unreacted thymine was filtered off, and the filtrate was reduced in volume to approximately 50 mL and treated with ethanol and ether to precipitate the desired product. The precipitate was filtered off, washed with DMF and ethanol, and dried in vacuo; yield, 1.6 g (45%).

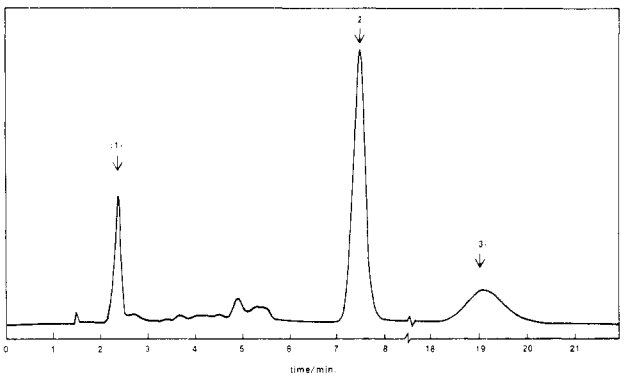

Figure 1. Chromatogram of the white components in PTB. Conditions: column, Waters μBondapak C_{18} (3.9 × 300 mm); eluent, pH 3.0 HNO_3, 2 mL/min; detection, 258 nm; injection, 20 μL 1 mg/mL solution.

Anal. Calcd (Canadian Microanalytical Services, Vancouver, B.C.): C, 15.41; H, 2.84; N, 14.38; Cl, 9.10. Found: C, 15.67; H, 2.62; N, 13.83; Cl 8.47.

Results and Discussion

(I) White Components. A typical chromatogram of the white components in PTB is shown in Figure 1. Five different commercial batches (Johnson Matthey, England) as well as two samples prepared in this laboratory were measured with two different Waters μBondapak C_{18} columns and a Whatman ODS-3 column. In all of these samples we observed peaks 1, 2, and 3 as the major components, with no blue compounds being eluted under the conditions of Figure 1.

Platinum determination on the eluent from the HPLC by atomic absorption shows that peaks 1 and 3 account for the majority of the platinum being eluted, while peak 2 contains no platinum. Comparison of the retention times observed with PTB against known platinum thymine complexes[7] enables the identification of fraction 2 as thymine and fraction 3 as *cis*-$Pt(NH_3)_2(TH–H)_2$ (with one thymine monoanion coordinating through N_1, and the other through N_3). The situation is less clear for fraction 1. Both *cis*-$Pt(NH_3)_2Cl(TH–H)$ and *cis*-$Pt(NH_3)_2(NO_3)(TH–H)$ (obtained in solution by treatment of the chloride with $AgNO_3$) have identical retention times to this peak. The retention time of this band is unaltered by the use of a new eluent, pH 3.0 ethanesulfonic acid. Since this eluent normally forms ion pairs with cations and increases their retention times on reverse-phase columns, the above is strong evidence that fraction 1 is nonionic. Two possibilities exist to explain these observations. First, the nitrato and the chloro compounds may not be resolvable under the conditions used here. Second, the two compounds may exist in solution as the same species.[8]

Further evidence of the nonionic nature of the "white" in Figure 1 was obtained by open column chromatography with Amberlite CG-120 strong cation-exchange resin. In a typical experiment a Pasteur pipet was packed with resin and loaded with 50 μL of a 5 mg/mL solution of PTB. Elution with water and subsequent injection of aliquots (100 μL) of the eluent into the HPLC showed that all of the white components seen in the HPLC of the blue solution were washed off the CG-120 column within five of its void volumes. The blue components could not be removed from the CG-120 resin even with solutions of high ionicity (1 M $LiNO_3$, 5 M NaCl).

The use of either HPLC or the ion-exchange method described above enabled us to assay for the proportion of white compounds (except thymine) in PTB by simply collecting the

(6) MacFarlan, R. D.; Torgerson, D. F. *Science (Washington, D.C.)* **1976**, *191*, 920.

(7) Courtesy of R. Pfab, Anorganisch-Chemisches Institut der Technischen Universität München.

(8) Lippert[5] has discussed the species that may exist in solutions of $Pt(NH_3)_2(NO_3)(TH–H)$.

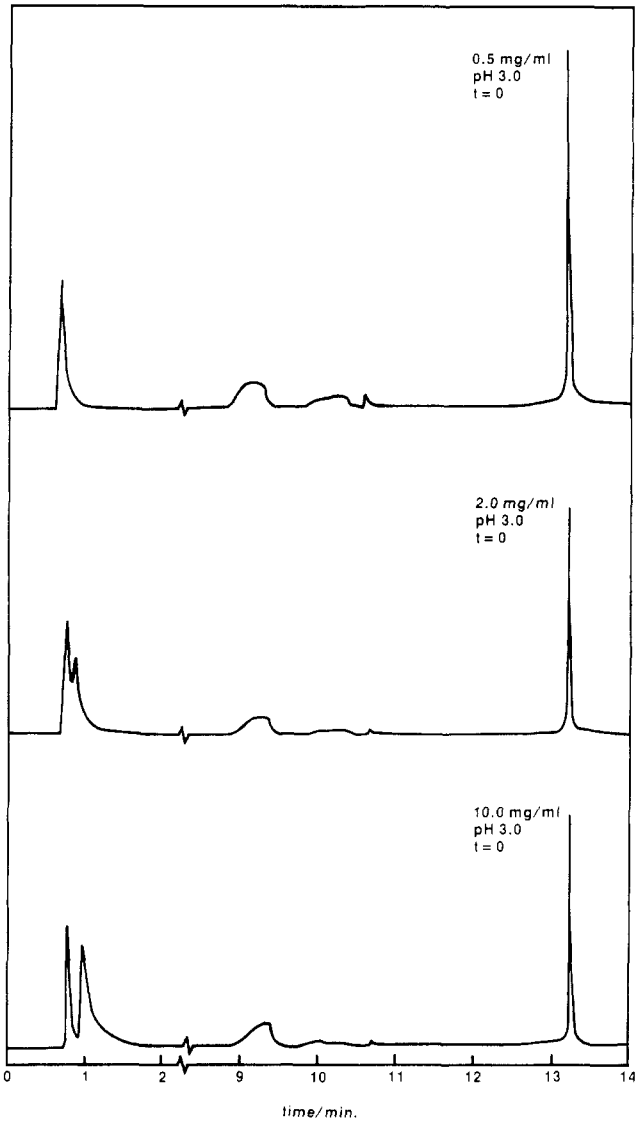

Figure 2. Concentration dependence of the blue components in PTB. Conditions: column, Waters μBondapak C_{18} (3.9 × 300 mm); eluent, solution A, pH 4.5 HNO_3, solution B, pH 2.0, 0.05 M n-Bu_4NNO_3, 2 mL/min; step gradient from A to B after 6 min; detection, 658 nm; injection, 10 μL.

eluent from either column and determining its platinum content from AA. Comparison with "spiked" eluents gave the percentage of platinum present as white compounds. The results were in good agreement for the two techniques, being in the range 10–30% depending upon the batch of PTB used. The percentage of white material present did not appear to be dependent upon the concentration of the PTB solution, but we did note a slow time dependence of the chromatogram with a gradual increase in the intensity of peak 1.

We have attempted to remove the white compounds by precipitation of the PTB with $NaBPh_4$, dissolution of the blue tetraphenylboron salt in acetone, and reprecipitation with lithium nitrate solution. This treatment reduced the level of "whites" appreciably (from 30% to less than 5% when performed twice) and completely removed the thymine. The platinum white remaining was present mostly as band 3.

(II) Blue Components. The step gradient between nitric acid and tetrabutylammonium nitrate described above resolves several blue fractions in PTB and elutes around 95% of the injected platinum. Separations of PTB (previously freed of whites) were carried out by using samples at five concentrations at three pHs (10, 5, 2, 1, 0.5 mg/mL; pH 7.0, 5.0, 3.0). It is apparent from Figure 2 that the relative proportion of

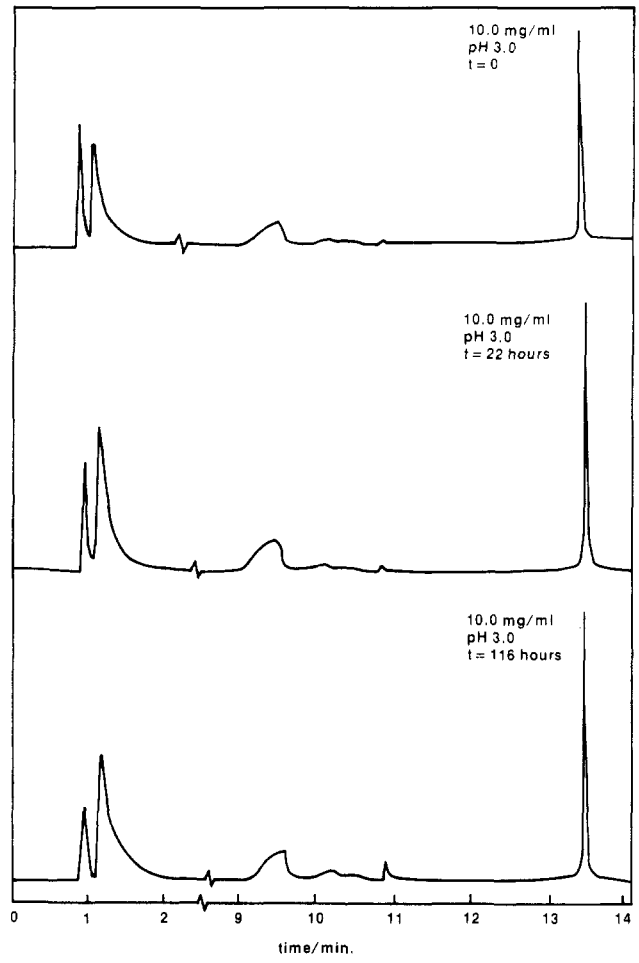

Figure 3. Time dependence of the blue components in PTB. Conditions as in Figure 2; pH values refer to $t = 0$; no adjustments were made during the experiment.

the different blue species in PTB is concentration dependent, with the earliest band (retention time 0.9 min, band 2A) being more intense in dilute solutions at the expense of the second band (retention time 1.1 min, band 2B). Changing the pH also has a marked effect, with a high pH favoring band 2A. For example, a freshly prepared 2 mg/mL solution at pH 7.0 shows almost none of band 2B, in contrast to the pH 3.0 solution in Figure 2. The composition of the PTB solution is also time dependent (for all concentrations and pHs), with band 2A losing intensity while band 2B strengthens; the bands at around 9 min in the chromatogram also increase in intensity very slightly with time; see Figure 3. Finally, we note that the chromatogram was quite variable depending upon the preparative batch of PTB used.

The eluent required to elute the blues off the column indicates their ionic nature. As cations they probably bind to the nonderivatized Si–OH sites in the packing and may be removed by some kind of ion-exchange mechanism by the tetrabutylammonium nitrate. Assuming the mechanism described above, one would suppose that the later bands in the chromatogram (e.g., 9.5 and 13.2 min) contain the more ionic materials.

This work is, to our knowledge, the first time that the various blue components in any PTB have been separated. The above observations, in particular the concentration dependence, lends support to the proposed oligomeric nature of PTB. We are currently investigating preparative-scale separations of PTB.

Acknowledgment. This work was supported by grants from the Engelhard Corp. and the International Nickel Co.

Registry No. *cis*-$Pt(NH_3)_2Cl_2$, 15663-27-1; thymine, 65-71-4.