also included in Table I. The poor precision of the results plus the fact that the value of the distribution coefficient differed so markedly from that for uranyl ion leads to the hypothesis that a reaction other than the exchange is taking place. Despite the fact that Np(VI) is stable in molar perchloric acid with respect to reduction to Np(V) the obvious assumption to make is that the organic substrate, *i.e.*, either the hydrocarbon skeleton of the Dowex-50 and/or the nuclear sulfonic acid which acts as the exchange group, promotes this reduction. This conclusion is supported by the data included in Table I for the distribution of Np(V) between molar perchloric acid and the resin.[6]

The reduction of Np(VI) to Np(V) was then qualitatively demonstrated by equilibrating a solution of 6×10^{-3} *M* Np(VI) in 0.955 *M* perchloric acid with about 0.2 g. of the dry resin for a period of one hour. At the end of this time the solution was centrifuged and an absorption spectra of the aqueous layer was taken. The spectra showed the presence of Np(V). Within the experimental uncertainties reduction was complete. The slow increase in the distribution coefficient in the Np(V) solution indicates a further reduction to Np(IV) is occurring.

In the light of these observations the correlation between cation exchange column behavior and the degree of association between Np(VI) and chloride ion[7] is subject to reservations.

[6] Qualitative tests for the presence of Cl^- and iron in the wash liquid from the resin were negative. Resin that had been equilibrated for as long as 24 hours with molar perchloric acid showed some evidence of decomposition, namely, a positive test for sulfate in the aqueous phase.

[7] R. M. Diamond, K. Street, Jr., and G. T. Seaborg, THIS JOURNAL, **76**, 1461 (1954).

CHEMISTRY DIVISION
ARGONNE NATIONAL LABORATORY
LEMONT, ILLINOIS

Preparation of Palladium(II) Chloride–1,2,3-Benzotriazole Coördination Compounds[1]

BY RAY F. WILSON AND LOUBERTA E. WILSON

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In connection with a general study of the reactions between the platinum elements and 1,2,3-benzotriazole, two palladium coördination compounds have been prepared. 1,2,3-Benzotriazole was first suggested as a precipitant for silver by Remington and Moyer[2] and later by Tarasevich[3] and Cheng.[4] Curtis[5] has employed the same reagent for the determination of copper. These authors report that nickel(II), cobalt(II), iron(II), zinc and cadmium are also precipitated by 1,2,3-benzotriazole; however, a study of the interaction of palladium or other platinum elements with this reagent appears not to have been made previously. The reactions of previously reported ions with 1,2,3-benzotriazole, in contrast to the corresponding palladium reactions, involved the removal of an acidic hydrogen from the reagent. Similar coördination compounds of palladium[6,7] have been reported.

Experimental

Materials.—A weighed amount of palladium(II) chloride, obtained from Coleman and Bell Company, was dissolved in a small amount of concentrated hydrochloric acid and then diluted to volume with distilled water. The resulting solution, which was approximately 0.1 *M* in hydrochloric acid, was analyzed for palladium content both gravimetrically[8] and polarographically.[9]

Standard 1,2,3-benzotriazole solution in 50% acetic acid was prepared after recrystallizing this reagent, Eastman Kodak Chemical No. 2759, twice from chloroform; the melting point of the recrystallized reagent was 100.0°.

All other materials used were reagent grade chemicals.

Apparatus.—A mechanized, micro-combustion Sargent apparatus was employed for the determination of carbon, hydrogen and palladium.

Procedure.—To four beakers each containing 0.417 mmole of palladium were added 10 ml. of 2 *M* acetic acid–sodium acetate buffer, and a slight excess of 1,2,3-benzotriazole (dissolved in 50% acetic acid); one palladium ion in excess benzotriazole reacts with two molecules of the reagent. The white colored precipitates were digested for 10 minutes, and then filtered using weighed, medium porosity, sintered-glass crucibles. The precipitates were washed several times with dilute hydrochloric acid (1:100), and finally several times with distilled water. After each precipitate had been dried at 110° for 1 hour to constant weight, the following weights of the four precipitates were obtained: 174.1, 173.0, 173.4, 173.0 mg. *Anal.* Calcd. for the white colored precipitate: C, 34.66; H, 2.42; Cl, 17.06; N, 20.21; Pd, 25.66. Found: C, 34.68; H, 2.45; Cl, 17.05; N, 20.19; Pd, 25.64.

Aliquots of the standard solution of palladium were transferred to three beakers, and 10 ml. of buffer added to each beaker. Then standard 1,2,3-benzotriazole solution was added in amounts such that the molar concentration of palladium remained in slight excess; in each case 0.500 mmole of reagent was added. After standing at room temperature for 12 hours, the precipitates were filtered using fine porosity, sintered-glass crucibles and were washed, dried and weighed in the usual manner. The three reddish-brown precipitates gave the following weights: 148.4, 147.6 and 148.1 mg. *Anal.* Calcd. for the reddish-brown colored precipitate: C, 24.28; H, 1.70; Cl, 23.90; N, 14.16; Pd, 35.96. Found: C, 24.29; H, 1.70; Cl, 23.89; N, 14.14; Pd, 35.96.

Quantitative analysis for nitrogen was carried out using micro Kjeldahl procedure[10] for chlorine by the Carius method[10]; and for carbon, hydrogen and palladium by modifying slightly the procedure described by Steyermark.[10] A Sargent carbon–hydrogen apparatus was employed for the micro-determination of carbon and hydrogen. Following the determination, the carbon dioxide and the water absorption tubes were removed from the apparatus, and the palladium was ignited again in an atmosphere of carbon dioxide to make sure that all the palladium and/or palladium residue had been converted to the metal.

Discussion

Palladium(II) chloride reacts with 1,2,3-benzotriazole to form two different coördination compounds, depending on which is in excess, palladium or 1,2,3-benzotriazole. Both the white and the reddish-brown colored precipitates were very insoluble in most organic solvents and in most concentrated inorganic acids. These precipitates appeared to be sparingly soluble in warm sulfuric acid;

[1] This work was supported by a grant from the National Science Foundation.

[2] W. J. Remington and H. V. Moyer, "Dissertation Abstract," Ohio State Univ. Press, Columbus, Ohio, 1937, p. 24.

[3] N. I. Tarasevich, *Vestnik Moskov. Univ.*, **3**, No. 10, 161 (1948).

[4] K. L. Cheng, *Anal. Chem.*, **26**, 1038 (1954).

[5] J. A. Curtis, *Ind. Eng. Chem., Anal. Ed.*, **13**, 349 (1941).

[6] J. H. Yoe and L. G. Overholser, THIS JOURNAL, **61**, 2059 (1939).

[7] J. R. Hayes and G. C. Chandler, *Ind. Eng. Chem., Anal. Ed.*, **14**, 491 (1942).

[8] W. F. Hillebrand, G. E. F. Lundell, H. A. Bright and J. I. Hoffman, "Applied Inorganic Analysis," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1953, p. 379.

[9] R. F. Wilson and R. C. Daniels, *Anal. Chem.*, **27**, 904 (1955).

[10] A. Steyermark, "Quantitative Organic Microanalysis," The Blakiston Company, Philadelphia, Pa., 1951, pp. 82–191.

the dried precipitates were stable up to temperatures of at least 300°.

Weight relations under "Experimental" and the elemental analyses gave convincing evidence for the formation of two coördination type compounds in the interaction of palladium(II) chloride and 1,2,3-benzotriazole. On the basis of the palladium content, the observed formulas are $Pd(C_6H_4NHN_2)_2Cl_2$ and $Pd(C_6H_4NHN_2)Cl_2$, and their formula weights are 415.86 and 296.74, respectively.

DEPARTMENT OF CHEMISTRY
TEXAS SOUTHERN UNIVERSITY
HOUSTON 4, TEXAS

On the Mechanism of Urease Action

BY JUI H. WANG AND DONALD A. TARR

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There are three proposed mechanisms for the hydrolysis of urea by urease in slightly acid solutions.[1] First we have the *carbonic acid mechanism* in which urea is assumed to be hydrolyzed first to carbamic acid, then to carbonic acid, and finally to carbon dioxide and water. Secondly we have the *carbamic acid mechanism* in which urea is first hydrolyzed to carbamic acid, and then the latter decomposes directly to carbon dioxide and ammonia without going through the carbonic acid stage. The weight of indirect experimental evidences in the literature seems to favor this latter mechanism. However, Sumner and Somers[1] recently proposed a third mechanism in which urea is directly hydrolyzed to carbon dioxide and ammonia without even going through the carbamic acid stage. These authors believe that the formation of ammonium carbamate in the absence of buffers is due to the recombination of the primary products CO_2 and NH_3. But it is by no means apparent just how urea can be hydrolyzed to CO_2 and NH_3 without going through the carbamic acid or carbamate stage. One possible mode of action is the reaction between a water molecule and a urea molecule under the influence of urease such that the two amide groups are simultaneously broken off and replaced by a single oxygen atom from the water molecule. But such a mechanism would require the simultaneous rupture of four bonds (two $N \overset{\cdots}{-} C$ bonds of urea and two H–O bonds of water) with the concurrent formation of three new ones (two H–N bonds of two ammonia molecules and one C=O bond of the carbon dioxide molecule), and is hence extremely unlikely. Another possible mechanism for the direct production of CO_2 is the replacement of the oxygen in urea by an unknown basic group on the urease molecule, the two amide groups are then hydrolyzed off one after the other, and finally the CO_2 is detached from the enzyme. In this way urea would indeed be converted to carbon dioxide and ammonia without going through the carbamate stage. Although there is no direct evidence to support this last mechanism, it would be risky to exclude it from our consideration in view of Sumner and Somers' suggestion. In the following discussion, this last mechanism will be referred to as the *carbon dioxide mechanism*.

It is well-known that at room temperature there is no detectable O^{18}-exchange between urea and water,[2] and that the O^{18}-exchange between carbon dioxide and water is also slow in the absence of carbonic anhydrase.[3] Consequently if ordinary urea is rapidly hydrolyzed by urease in slightly acid, H_2O^{18}-enriched water solution and the carbon dioxide liberated is immediately separated from the liquid mixture for mass-spectrometric analysis, the result should enable us to decide which one of the three mechanisms described above is correct.

For convenience of the subsequent discussion, let us introduce the new term "enrichment ratio" defined below.

$$\text{Enrichment ratio} = \frac{\text{atom \% excess of } O^{18} \text{ in } CO_2 \text{ just liberated from the urea soln.}}{\text{atom \% excess of } O^{18} \text{ in } CO_2 \text{ equilibrated with the urea soln.}}$$

The quantity in the denominator of the above ratio is determined by keeping the liberated CO_2 in contact with the liquid mixture for more than 5 hr., then separating it from the liquid and analyzing for O^{18}-content. A moment's consideration should convince us that the three mechanisms of urease action described above predict different enrichment ratios. The *carbonic acid mechanism* predicts an enrichment ratio of $^2/_3$, the *carbamic acid mechanism* predicts a ratio of $^1/_2$, and the *carbon dioxide mechanism* predicts a ratio of unity.

Water containing about 1.5 atom % of O^{18} was used as tracer in this work. The results are summarized in Table I. Three kinds of measurements were carried out. First dry sodium bicarbonate powder was mixed with excess of H_2O^{18}-enriched soln. of urea in citrate buffer (*p*H 5.5) in an evacuated vessel for $^1/_2$ minute at 0°. The mixture was then chilled in a Dry Ice–acetone bath, and the liberated CO_2 was sampled out for mass spectrometric analysis. The results of two such measurements listed in Table I (expts. 8 and 9) give an average O^{18} atom % of 0.22. Since the natural abundance of O^{18} is about 0.20 atom % and the water in the buffer soln. contained about 1.5 atom % in O^{18}, it is clear that under these experimental conditions the rate of O^{18}-exchange between liberated CO_2 and water is relatively slow and may be considered as negligible in all the other $^1/_2$-minute experiments at 0° described below.

In the second group of measurements the H_2O^{18}-enriched soln. of urea in citrate buffer was mixed with equal volume of H_2O^{18}-enriched soln. of urease in the same buffer for $^1/_2$ min. at 0°. The liberated CO_2 was separated and analyzed as before. The results of three determinations listed in Table I (expts. 1, 2 and 3) give an average O^{18} atom % of 0.863. In the last group of measurements, the same urea and urease soln. were mixed as before, but the liberated CO_2 was left in contact with the H_2O^{18}-enriched soln. for several hr. to reach exchange equilibrium before mass spectrometric analysis. The average O^{18} atom % in the equilibrated CO_2 obtained from four determinations (expts. 4, 5, 6 and 7 in Table I) is 1.47. Thus the

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[1] For references to the literature see J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, Inc., New York, N. Y., 1953, pp. 157–158.

[2] M. Cohn and H. C. Urey, THIS JOURNAL, **60**, 679 (1938).

[3] H. C. Urey and L. J. Greiff, *ibid.*, **57**, 321 (1935).