Metal Binding in Tetracyclines. Cobalt(II) and Nickel(II) Complexes[1]

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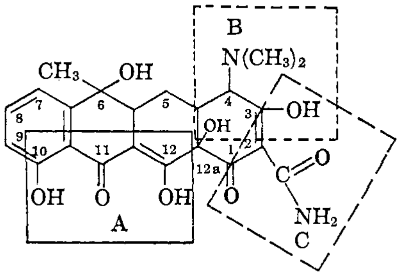
Abstract: Cobalt(II) and nickel(II) complexes of three tetracyclines have been prepared and isolated and their spectral and magnetic properties studied. The complexes are octahedral and contain two tetracycline anions and two water molecules per metal ion. The electronic spectra indicate that the molecules coordinate through oxygen, probably in the 1,2,3-tricarbonylmethane system.

Tetracycline (I) and its derivatives have been known for several years for their antibacterial activity.[2]

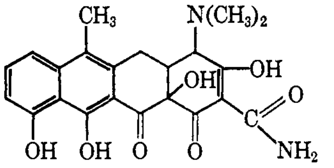


I

The actual mechanism of this effect has not been definitely established; however, it appears to be linked to the ability of the molecule to form complexes with a large variety of metal ions,[3,4] and therefore the behavior of tetracycline and its analogs toward metal ions has been the subject of numerous investigations.[5-7] The question of which specific group the tetracycline uses to bind to the metal has not, however, been established with certainty. Based on a comparison of the effects of metal ions on the absorption spectrum, specifically the band at 370 mμ of oxytetracycline with the corresponding effect of the ions on model compounds, Conover[5] concluded that the binding group is the enolized β-diketone group at C_{11} and C_{12} (group A of I).

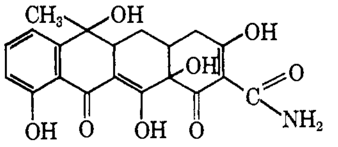
On the other hand, Doluisio and Martin[4] have concluded on the basis of potentiometric titrations of tetracycline and some of its analogs in the presence and absence of certain metal ions that the binding group, at least for Cu(II), Ni(II), and Zn(II), is the dimethylamino at C_4 and the hydroxyl at either C_3 or C_{12a} (group B of I). Their conclusions were based on the assignment of the acidity constants made by Stephens, *et al.*,[8] and recently several different groups[9-11] have questioned these assignments. If the assignments of these latter workers can be considered correct, then the conclusions of Doluisio and Martin are invalid.

We have decided to attack the problem of the coordination site through the study of the d–d spectra of transition metal complexes of tetracycline and certain of its analogs. Specifically, we have studied the Co(II) and Ni(II) complexes of tetracycline, anhydrotetracycline (II), and dedimethylaminotetracycline (III).[12]



II

Since little has been done in the way of isolating and characterizing complexes of the tetracyclines, we have chosen to concentrate on this aspect rather than work with solutions of complexes which have never been isolated and therefore are of indefinite composition.



III

Experimental Section

Tetracycline and Analogs. Tetracycline hydrochloride was generously furnished by Bristol Laboratories. Anhydrotetracycline was obtained from Bristol Laboratories and Lederle Laboratories. Dedimethylaminotetracycline was supplied by the Pfizer Research Laboratories.

Bis(tetracycline)diaquonickel(II). $NiCl_2 \cdot 6H_2O$ was dissolved in methanol, and tetracycline hydrochloride was added in a 2.10:1 ligand-to-metal ratio. The pH was raised to 7.4 with triethylamine and stirred for 1 hr. The pH was raised to 7.55 and the slurry placed in a flask and magnetically stirred under positive N_2 pressure for 48 hr. The resulting precipitate was twice slurried, filtered, and washed with methanol. The resulting green-yellow precipitate was vacuum dried at 45°.

Anal. Calcd for $C_{44}H_{50}N_4O_{18}Ni$: C, 53.82; H, 5.14; N, 5.71; Ni, 5.98; water, 3.64. Found: C, 53.76; H, 5.05; N, 5.46; Ni, 6.18; water, 3.85.

Bis(tetracycline)diaquocobalt(II). $CoCl_2 \cdot 6H_2O$ was dissolved in methanol, and tetracycline hydrochloride was added in a 3.0:1 ligand-to-metal ratio. The pH was raised to 7.4 by titration with

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[1] This work was supported by the National Institutes of Health under Grant No. AI05409.

[2] A. C. Finlay, *Science*, **111**, 85 (1950).

[3] A. Albert, *Nature*, **172**, 201 (1953).

[4] J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6**, 16 (1963).

[5] L. H. Conover, Special Publication No. 5, The Chemical Society, London, 1956, p 48.

[6] N. Zasshi, *J. Pharm. Soc. Japan*, **79**, 1381 (1959).

[7] M. Ishidate and T. Sakaguchi, *Pharm. Bull.* (Tokyo), **3**, 147 (1955).

[8] C. R. Stephens, K. Murai, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **78**, 4155 (1956).

[9] L. J. Leeson, J. E. Kinger, and A. Nach, *Tetrahedron Letters*, No. **18**, 1155 (1963).

[10] K. K. Kalnin'sh and B. G. Belen'kii, *Dokl. Akad. Nauk SSSR*, **157**, 619 (1964).

[11] N. E. Rigler, S. P. Bag, D. E. Leyden, J. L. Sudmeier, and C. N. Reilley, *Anal. Chem.*, **37**, 872 (1965).

[12] For simplicity, the following abbreviations will be used: tetracyline, Tc; anhydrotetracycline, ATc; dedimethylaminotetracycline, DTc.

triethylamine and the slurry placed in a flask and stirred under positive N_2 pressure for 72 hr. The precipitate was filtered and washed with methanol. The filter cake was slurried, filtered, and washed with methanol. The yellow-brown precipitate was vacuum dried at 45°.

Anal. Calcd for $C_{44}H_{50}N_4O_{18}Co$: C, 53.82; H, 5.14; N, 5.71; Co, 5.94. Found: C, 54.06; H, 5.26; N, 5.62; Co, 5.94.

Bis(anhydrotetracycline)diaquonickel(II). $NiCl_2 \cdot 6H_2O$ was dissolved in methanol, and anhydrotetracycline was added in a 2.20:1 ligand-to-metal ratio. The pH was raised to 7.4 by titration with triethylamine and the slurry placed in a flask and stirred under positive N_2 pressure for 48 hr. The precipitate was filtered and washed with methanol. The filter cake was slurried, filtered, and washed with methanol. The resulting brown-yellow precipitate was vacuum dried at 45°.

Anal. Calcd for $C_{44}H_{46}N_4O_{16}Ni$: C, 55.87; H, 4.91; N, 5.93; Ni, 6.21. Found: C, 55.75; H, 5.04; N, 6.14; Ni, 6.52.

Bis(anhydrotetracycline)diaquocobalt(II). The same procedure was followed as in the preparation of the nickel analog, with the exception that the pH was raised to 8.0. The resulting complex was brown in color.

Anal. Calcd for $C_{44}H_{46}N_4O_{16}Co$: C, 55.87; H, 4.91; N, 5.93; Co, 6.23. Found: C, 56.14; H, 5.06; N, 6.15; Co, 6.40.

Bis(dedimethylaminotetracycline)diaquonickel(II). $NiCl_2 \cdot 6H_2O$ was dissolved in methanol, and dedimethylaminotetracycline was added in a 2.70:1 ligand-to-metal ratio. The pH was raised to 5.9 by titration with triethylamine and the slurry placed in a flask and stirred for 360 hr under positive N_2 pressure. The precipitate was filtered and washed with methanol. The filter cake was slurried with methanol, cooled to 0°, filtered, and washed with methanol. The resulting green-yellow precipitate was vacuum dried at 45°.

Anal. Calcd for $C_{40}H_{40}N_2O_{18}Ni$: C, 53.63; H, 4.50; N, 3.12; Ni, 6.56. Found: C, 50.55; H, 5.11; N, 3.54; Ni, 6.99.

It was never possible to obtain a completely satisfactory analysis for the product of this reaction. The analyses always indicated the presence of triethylamine. This will be considered further in the discussion.

Bis(dedimethylaminotetracycline)diaquocobalt(II). The same procedure was used as in the case of the nickel analog, except that the pH was raised to 7.07 and run for 240 hr. The resulting complex was brown-yellow in color.

Anal. Calcd for $C_{40}H_4N_2O_{18}Co$: C, 53.63; H, 4.50; N, 3.12; Co, 6.58. Found: C, 53.33; H, 4.78; N, 3.40; Co, 6.34.

Oxidized Bis(dedimethylaminotetracycline)diaquoiron(II). $FeCl_2 \cdot 6H_2O$ was dissolved in methanol, and dedimethylaminotetracycline was added in a 2.20:1 ligand-to-metal ratio. The slurry was titrated with triethylamine to pH 8.0 and transferred to a flask under positive N_2 pressure. It was stirred for 48 hr while maintaining positive N_2 pressure. The green complex was filtered and washed with methanol. The filter cake which had begun to turn brown was reslurried, filtered, and washed with methanol. The now brown complex was vacuum dried at 45°. Analysis of the product indicates a likely formula of the complex $Fe^{III}(DTc)_2(H_2O)OH$.

Anal. Calcd for $C_{40}H_{39}N_2O_{18}Fe$: C, 53.87; H, 4.41; N, 3.14; Fe, 6.27. Found: C, 53.46; H, 4.39; N, 3.30; Fe, 6.60.

Bis(tetracycline)diaquoiron(II) and Oxidized Bis(tetracycline)diaquoiron(II). $FeCl_2 \cdot H_2O$ was dissolved in methanol, and tetracycline hydrochloride was added in a 2.20:1 ligand-to-metal ratio. The solution was rapidly titrated to pH 8.1 and immediately transferred to a flask under positive N_2 pressure. The slurry thus formed was stirred for 48 hr under positive N_2 pressure. The slurry was filtered directly from the flask by means of a glass-fritted filtering assembly built into it. The residue was washed with methanol, during which time a large volume of N_2 was passed over the precipitate to prevent oxidation. The large volume of N_2 also served to remove the methanol in the precipitate. The entire flask was sealed off and placed in the drybox under an atmosphere of dry argon gas. One portion of the material was placed in a Gouy tube and the second wrapped in Saran Wrap. Magnetic measurements were made on the material contained in the Gouy tube, while the reflectance spectrum was obtained from the portion in Saran Wrap. The Gouy tube was then opened to the air. Oxidation of the material was indicated by a color change from green to brown. Magnetic and spectral measurements were again made on the oxidized material. It was then analyzed for carbon, hydrogen, nitrogen, and iron. The analysis suggested its formula to be $Fe^{III}(TC)_2(H_2O)OH$.

Anal. Calcd for $C_{44}H_{49}N_4O_{18}Fe$: C, 54.04; H, 5.06; N, 5.73; Fe, 5.71. Found: C, 53.58; H, 4.85; N, 5.78; Fe, 5.69.

Samples stored in the drybox for periods of up to 1 month showed no change in spectra or magnetic properties.

Magnetic Measurement. Magnetic susceptibilities were obtained on a standard Gouy balance which utilized a Varian Association 4-in. electromagnet and a Mettler Model H-16 balance. The sample tube was calibrated with $Hg[Co(NCS)_4]$. All measurements were made at three field strengths and were found to be independent of field. Diamagnetic corrections for tetracycline and its analogs were obtained from measured susceptibilities of the pure metals. Moments reported are accurate to about ±0.05 BM.

Spectra. Solution spectra were obtained in methanol on a Perkin-Elmer Model 4000A Spectrocord. Reflectance spectra of the cobalt and nickel complexes were obtained using a Beckman Model DK-2 which uses an integrating sphere and omits the specular beam. Reflectance spectra of the iron complexes were obtained on a Beckman DU equipped with the standard attachment for reflectance.

Analyses. Analyses for C, H, and N were performed by Schwarzkopf Microanalytical Laboratories and Galbraith Laboratories, Inc. Water analyses were performed by Karl Fisher titrations.

Results and Discussion

Magnetic Data. The magnetic moments for the Co(II) and Ni(II) complexes are given in Table I. The moments given have been corrected for diamagnetism and temperature-independent paramagnetism.

Table I. Magnetic Moments of Iron, Cobalt, and Nickel Complexes

Complex	Magnetic moment (BM)	
	Room temp	105°K
$Ni(Tc)_2(H_2O)_2$	3.37	3.39
$Ni(ATc)_2(H_2O)_2$	3.39	3.43
$Ni(DTc)_2(H_2O)_2$	3.28	3.32
$Co(Tc)_2(H_2O)_2$	4.89	4.70
$Co(Atc)_2(H_2O)_2$	5.25	5.07
$Co(DTc)_2(H_2O)_2$	4.79	4.63
$Fe(Tc)_2(H_2O)_2$(?)	5.41	5.35
$Fe(Tc)_2(H_2O)(OH)$(?)	5.85	5.82
$Fe(DTc)_2(H_2O)(OH)$(?)	6.00	5.91

There are two conclusions to be drawn from these data. First, all of the values fall into the range expected for octahedral complexes.[13] The slight drop at lower temperatures for the cobalt complexes as well as the temperature independence of the moments of nickel and iron(III) are also compatible with an octahedral array of ligands.[13] We do not believe that the somewhat higher value obtained for $[Co(ATc)_2(H_2O)_2]$ is significant, but we believe that it represents a slight excess of metal in the product. Such an excess is indicated in all of the analyses on this material. Thus the formulas indicated by the analyses, *i.e.*, two tetracyclines and two waters per metal ion which give an octahedral structure, are supported by these data. This assumes, of course, the tetracycline to be a bidentate group.

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(13) B. N. Figgin and J. Lewis, *Progr. Inorg. Chem.*, 6, 37 (1964).

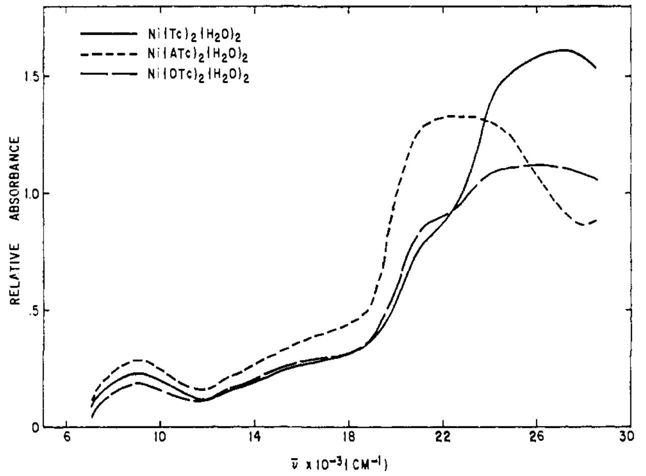

Figure 1. Reflectance spectra of Ni(II) complexes.

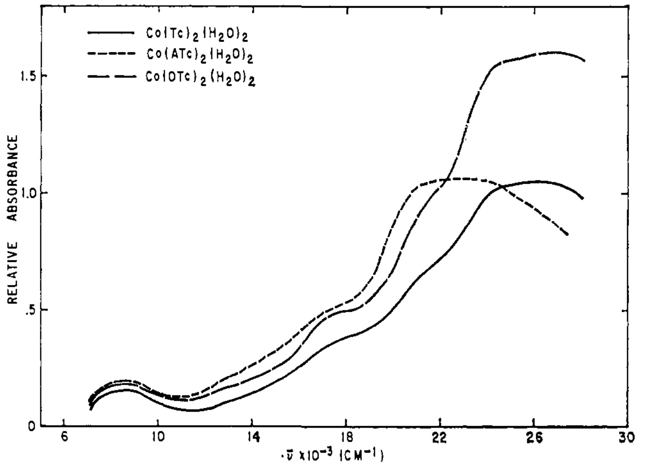

Figure 2. Reflectance spectra of Co(II) complexes.

The second point to be made is that the data support the assumption that the original green iron complex obtained (see Experimental Section) is Fe(II), but upon exposure to air it is oxidized rapidly to Fe(III). The formulas given for the complexes are the ones we feel are most probable on the basis of the analyses and the magnetic data.

Spectra

Because of the fact that the complexes, once isolated, were insoluble in all solvents tried, their spectra were obtained by reflectance techniques. These are shown in Figures 1 and 2 and those of the free ligands in Figure 3. For each of the complexes there are two bands that can unambiguously be assigned to essentially d–d transitions localized on the metal. These are listed in Table II along with the band assignments. It is obvious that for each metal, the d–d spectra are identical. The slight variation for the second band is well within the accuracy of the determination of the band maxima. Furthermore, the spectra are typical of those found for octahedral Ni(II) and Co(II).[14] Implications of these spectra will be discussed later.

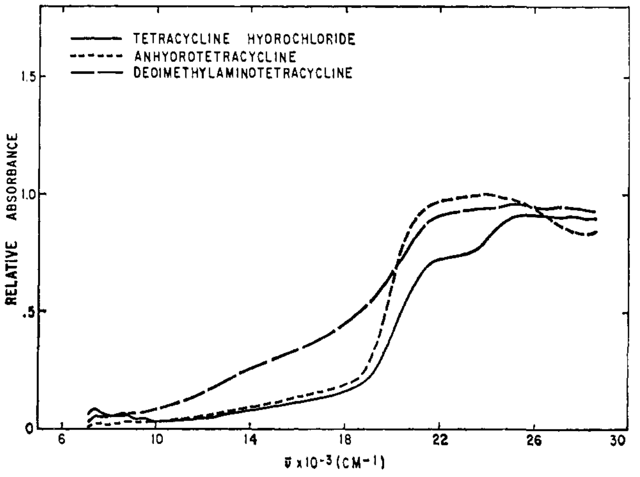

Figure 3. Reflectance spectra of tetracycline hydrochloride, anhydrotetracycline, and dedimethylaminotetracycline.

The spectrum of the Fe(II) complex prepared in a nitrogen atmosphere was also obtained as previously indicated. The main feature was a broad band centered at about 12,000 cm^{-1}. Upon exposure of the sample to the atmosphere, the intensity of the band rapidly decreased and after a short period of time, no d–d bands could be observed between 10,000 and 30,000 cm^{-1}. The 12,000-cm^{-1} band is typical for octahedral Fe(II) and the absence of bands after exposure to oxygen confirms the proposed oxidation to Fe(III) since the ^6A ground state of high-spin Fe(III) has only spin-forbidden bands of very low intensity.[14]

Table II

Complex	Energy, cm^{-1} Band I	Energy, cm^{-1} Band II
$Ni(Tc)_2(H_2O)_2$	9100 ($^3A_{2g} \rightarrow {}^3T_{2g}$)	15,400 ($^3A_{2g} \rightarrow {}^3T_{1g}$)
$Ni(ATc)_2(H_2O)_2$	9100	15,600
$Ni(DTc)_2(H_2O)_2$	9100	15,300
$Co(Tc)_2(H_2O)_2$	8700 ($^4T_{1g} \rightarrow {}^4T_{2g}$)	17,500 ($^4T_{1g} \rightarrow {}^4A_{2g}$)
$Co(ATc)_2(H_2O)_2$	8700	17,000
$Co(DTc)_2(H_2O)_2$	8700	18,200

Other spectra of interest are shown in Figure 4. These spectra show clearly that there is appreciable complex formation upon the addition of Tc·HCl to $NiCl_2 \cdot 6H_2O$, and that subsequent addition of triethylamine increases the amount of complex formed, but the position of the band around 9000 cm^{-1} does not change up to the point of the onset of precipitation (at slightly over 2.0 equiv of base). In fact, the band maximum is exactly the same as that found in the reflectance spectra of the precipitated complexes, indicating that the coordination site is the same in solution as in the solid.

Conclusion Regarding the Site of Tetracycline Complexation. On the basis of the data presented, some conclusions can be drawn regarding the site of complexation. The fact that the spectra of the complexes of each metal are identical indicates that the site of bonding is the same for each of the tetracyclines. This rules out involvement of the dimethylamino group since it is not present in dedimethylaminotetracycline. If the β-diketone group at C_{11}–C_{12} were the bonding site, one

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[14] C. K. Jorgensen, "Absorption Spectra and Chemical Bonding in Complexes," Addison-Wesley Publishing Co., Inc., Reading, Mass., 1962.

might expect differences between the d–d spectra of the tetracycline and anhydrotetracycline complexes, although it is not clear exactly how much.

The spectra of the nickel complexes also indicate that only oxygens are coordinating to the metal. A survey of the spectra of a large number of octahedrally coordinated complexes having both oxygens and nitrogens coordinated shows that whenever as many as one nitrogen is bound to the nickel, the first band occurs at an energy of at least 9500 cm^{-1} and for two nitrogens at least 9800 cm^{-1}. All of this suggests that the bonding takes place through two oxygens of the 1,2,3-tricarbonylmethane system (labeled C of I), the amide oxygen at C_2, and the hydroxyl at C_1 or C_3.

It is not possible to determine which oxygen, the C_1 or C_3, is coordinated or even if only one is involved. We did notice, however, that in preparing the dedimethylaminotetracycline complexes, the amount of triethylamine used was very critical. If even a slight excess was present, elemental analyses indicated some triethylamine in the resulting complexes. This was not true for the tetracycline complexes. Study of Fisher–Hirschfelder–Taylor models indicate that if the oxygen at C_3 were coordinated, it is sterically impossible for triethylamine to coordinate to the metal in the tetracycline complexes but quite possible in the dedimethylaminotetracycline complexes.

It has been shown by means of X-ray data that a considerable amount of intramolecular H-bonding involving the C_{10}–C_{11}–C_{12}–C_1 positions exists in solid tetracycline hydrochloride,[15] and infrared data indicate the same thing in solution.[10] We therefore believe that the shift in the 370-mμ band observed[5] to occur upon complex formation could result from a change in the extent of H-bonding and/or degree of protonation that would accompany ionization of the 1,2,3-tricarbonylmethane group and subsequent binding of a metal ion. That complexation in this position might affect the 370-mμ band is indicated by the work of Rigler, *et al.*,[11] who found that the degree of protonation, and thus the acid strength, of group C (or A) was quite dependent upon the degree of protonation of group A (or C). Thus there is a certain amount of electronic interaction between the two positions, whatever its source.

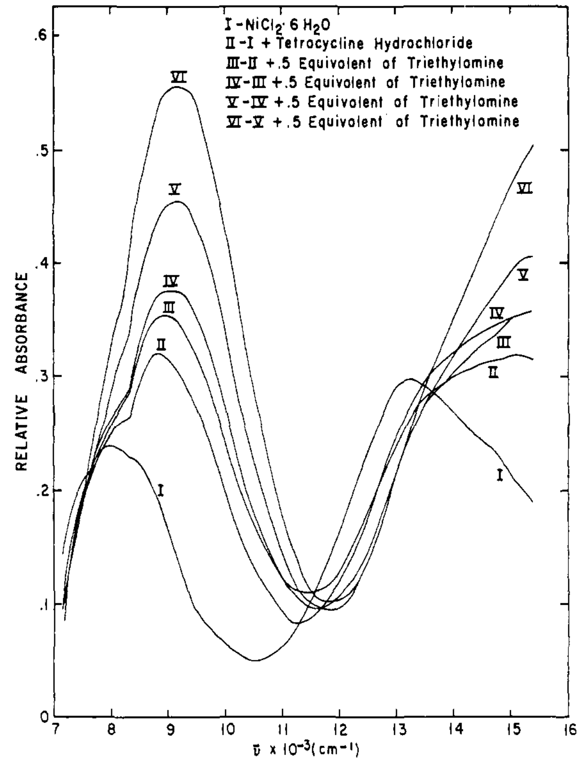

Figure 4. Solution spectra of $NiCl_2 \cdot 6H_2O$, and after addition of tetracycline hydrochloride and varying amounts of triethylamine.

We believe this work definitely excludes the possibility of involvement of the dimethylamino group in coordination to Ni(II) and Co(II). While it does not clearly distinguish between position C and position A, we believe it does show that group A cannot be excluded from consideration. It is possible that more than one site is coordinated, both in the solid and in solution. Obviously more work must be done on this very complicated system before positive statements are possible.

Acknowledgments. The authors wish to express their appreciation to Pfizer Research Laboratories, Lederle Laboratories, and particularly Bristol Laboratories for supplying some of the materials used in this work.

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(15) J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Am. Chem. Soc.*, **85**, 851 (1963).