Coordination Chemistry of Vitamin C. Part I. Interaction of L-Ascorbic Acid with Alkaline Earth Metal Ions in the Crystalline Solid and Aqueous Solution

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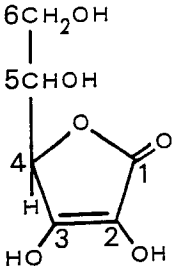
ABSTRACT

The interaction of L-ascorbic acid with alkaline earth metal ions has been investigated in aqueous solution at pH 6–7. The solid salts of the type Mg(L-ascorbate)$_2 \cdot 4H_2O$, Ca(L-ascorbate)$_2 \cdot 2H_2O$, Sr(L-ascorbate)$_2 \cdot 2H_2O$ and Ba(L-ascorbate)$_2 \cdot 2H_2O$ were isolated and characterized by means of ^{13}C NMR and FT-IR spectroscopy. Spectroscopic and other evidence suggested that in aqueous solution, the binding of the alkaline earth metal ions is through the O-3 atom of the ascorbate anion, while in the solid state the binding of the Mg(II) is different from those of the other alkaline earth metal ion salts. The Mg(II) ion binds to the O-3, O-1 atom of the two ascorbate anions and to two H_2O molecules, while the eight-coordination around the Ca(II), Sr(II), and Ba(II) ions would be completed by the coordination of three acid anions, through O-5, O-6 of the first, O-3, O-5, O-6 of the second and O-1 of the third anion as well as to two H_2O molecules. The structural properties of the alkaline earth metal-ascorbate salts are different in the solid and aqueous solution.

INTRODUCTION

The biological activity of vitamin C (Scheme 1) has been known for many years. The



L-Ascorbic acid

SCHEME 1

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recent findings of Hollis et al. [1], on the antitumor activities of several platinum diamine ascorbate compounds and Cardin and Roy [2], on the anticancer properties of some organometallic titanium and tin ascorbate complexes have added more attention to the biochemistry of this sugar molecule. On the other hand, the interaction of vitamin C with metal ions plays an important role in the reversible oxidation of ascorbic acid in living cells. Vitamin C has several donor atoms capable of metal complex formation and complexes of metal ascorbate are generally assumed to be of a chelate in the crystalline solid, but chelate formation was suggested to be weak in aqueous solution [3, 4]. However, it is not clear which of the acid donor atoms, O-1, O-2, O-3, O-4, O-5, and O-6 are normally involved in metal-ligand bondings. In a recent study by Kriss [4], the ability of each donor atom (O-1, O-2, O-3, and O-4) in metal-ascorbate complexation has been examined. In his study, the electronic absorption, the stability constants, and several other spectroscopic properties of some transition and non-transition metal-ascorbate complexes were analyzed [4]. It has been pointed out that chelate formation through O-2, O-3, and metal ion is difficult, even though all the three atoms lie in the same plane and ascorbate anion (deprotonated at O-3 group) behaves as an unidentate ligand in aqueous solution [4]. However, in the solid state, the coordination of the ascorbate anions via O-5, O-6 (chelation), O-3, O-5, O-6 (chelation), or O-1 (unidentately) has been found for several metal-ascorbate salts [5–7]. It is interesting to note that in the crystal structures of platinum diamine ascorbate complexes, chelation was uniquely found via C-2 and O-5 atoms [1], whereas in the organometallic titanium and tin complexes, the binding was through the O-3 (unidentately) or O-3 and O-2 (chelation), depending on the nature of metal cation and the pH of the solution [2]. Therefore, it is of interest to study the binding mode of L-ascorbic acid with the alkaline earth metal ions, both in the solid and aqueous solution.

In this work, the interaction of L-ascorbic acid with the Mg(II), Ca(II), Sr(II), and Ba(II) ions has been investigated in the solid and aqueous solution, using ^{13}C NMR and FT-IR spectroscopy, that have not been previously reported.

EXPERIMENTAL

Materials and Methods

L-ascorbic acid was purchased from Aldrich Chemical Co. and used as supplied. D_2O (99.90%) was from Cambridge Isotopic Laboratories. All other chemicals were reagent grade and were used without further purification.

Synthesis of the Alkaline Earth Metal-Ascorbate Salts

The metal-ascorbate salts were prepared by the addition of the hydrated alkaline earth metal carbonate 1 mmol in H_2O (10 ml) to a hot solution of ascorbic acid 2 mmol in H_2O (30 ml). The solution was heated at 70°C until all the metal carbonate was dissolved and then was cooled down to room temperature and decolorized by activated charcoal. The solution was filtered in acetone-ether mixture (50/50) (100 ml) and was kept refrigerated overnight to bring down the white precipitate. This was filtered off and washed with acetone-ether mixtures several times and dried over $CaCl_2$. The analytical results showed the composition of $M(L\text{-ascorbate})_2 \cdot nH_2O$, where M = Mg(II), Ca(II), Sr(II), and Ba(II) ions and n = 2 or 4. The alkaline earth metal-ascorbate salts are very hygroscopic and they should be kept in a desiccator over

$CaCl_2$. Upon contact with moisture, the white color turns dark yellow, which could be due to the acid oxidation. These metal-ascorbate salts are very soluble in water and not soluble in common organic solvents; they are recrystallized according to Merrill and Ruskin [8]. The Zn(II)-ascorbate salt was prepared for comparative purposes, in a similar fashion as mentioned for the alkaline earth metal-ascorbate salts. The deuterated samples were prepared for comparative purposes by dissolving the free acid or its metal ion salt in D_2O solution, followed by evaporation until dryness. This procedure was repeated several times to ensure maximum sample deuteration.

Physical Measurements

The ^{13}C NMR spectra were recorded on a Bruker WP-80 MHz instrument in D_2O solution containing dioxane as reference. The FT-IR spectra were recorded on a NICOLET-5DXB Fourier Transform infrared instrument equipped with DTGS detector and KBr beam-splitter. The spectra were taken as KBr pellets with resolution of 2–4 cm^{-1}. The pH of solutions were adjusted with a micro-electrode and an Orion Research Model 721 pH meter.

RESULTS AND DISCUSSION

^{13}C NMR Spectra

The ^{13}C NMR chemical shifts of L-ascorbic acid and its alkaline earth metal ions salts are compared with those of the Na-ascorbate and Zn-ascorbate salts in D_2O solution; the results are shown in Table 1. Our ^{13}C NMR results for the free acid and the sodium salt are similar to those of the literature reported [9]. Upon acid ionization, drastic changes were observed for the chemical shifts of the C-3 (20 ppm downfield), C-2 (5 ppm upfield), C-1 (5 ppm downfield), and C-4 (3 ppm downfield) (Table 1). The C-5 and C-6 chemical shifts were not affected significantly on salt formation (Table 1). The major downfield shift of the C-3 is due to the ionization of the O(3)-H group, which causes the lengthening of the C-2-C-3, C-3-C-4, C-1-O-1, and C-2-O-2 bonds and the shortening of the C-1-C-2 and C-3-O-3 bonds, in the monoanion formed in the crystal structure of the Na-ascorbate salt [5], and also related to the delocalization of the electron distribution throughout the endol-diol and carbonyl groups [5]. Such structural modifications are also responsible for the downfield shifts of the C-1, C-4, and the upfield shift of the C-2 chemical shifts (Table 1).

The ^{13}C NMR spectra of the alkaline earth metal-ascorbate salts were similar to that of the sodium salt, but different from that of the Zn-ascorbate compound (Table 1). However, the binding mode of the ascorbate anion is rather different in the solid structures of the Na-ascorbate and Ca-ascorbate salts [5, 6]. The sodium ion binds to four ascorbate anions, through O-6, O-6 of the first, O-1, O-2 of the second, O-3 of the third, and O-6 of the fourth anion [5], whereas calcium ion binds to three acid anions via O-5, O-6 of the first, O-3, O-5, O-6 of the second, and O-1 of the third anion [6]. The marked similarities observed between the ^{13}C NMR spectra of the alkaline earth metal-ascorbate and that of the sodium salt that indicative of the negligible amount of metal-ascorbate binding in aqueous solution. However, if there is some degree of the alkali or alkaline earth metal-ligand binding, it is through the negatively charged O-3 atom only. These findings are consistent with the low stability constants, which were observed for the alkaline earth metal-ascorbate complexes formed in aqueous

TABLE 1. ^{13}C NMR Chemical Shifts (ppm) for L-Ascorbic Acid and its Alkaline Earth and Zinc Metal Ion Salts in D_2O Solution*

Compounds	C-1	C-3	C-2	C-4	C-5	C-6
L-ascorbic acid	173.79	156.07	118.51	76.87	69.66	62.80
Na-ascorbate	178.09	176.34	113.85	79.28	70.46	63.46
$Mg(ascorbate)_2 \cdot 4H_2O$	178.39	175.85	114.64	79.30	70.52	63.49
$Ca(ascorbate)_2 \cdot 2H_2O$	178.19	175.85	114.45	79.30	70.52	63.30
$Sr(ascorbate)_2 \cdot 2H_2O$	178.19	176.20	113.93	79.21	70.46	63.38
$Ba(ascorbate)_2 \cdot 2H_2O$	177.90	175.17	114.21	79.21	70.39	63.32
$Zn(ascorbate)_2 \cdot 2H_2O$	177.80	174.37	122.45	78.77	70.60	63.24

*Dioxane was used as reference (67.40 ppm).

solution [3]. It is worth mentioning that solution studies have previously indicated that in acidic pH, the interaction of L-ascorbic acid and the Ca(II) ion occurs through O-3, O-5, and O-6 groups, in the 1:1 Ca-ascorbato complexes formed [10, 11]. On the basis of our ^{13}C NMR results (Table 1) such chelation through O-3, O-5, and O-6 atoms did not occur, for the Ca(II) and ascorbate anion in aqueous solution at neutral pH. Evidence for chelate formation comes from the ^{13}C NMR results of the Zn-ascorbate compound in D_2O solution, which showed considerable downfield shift (9 ppm) for the C-2 chemical shift (compared with that of the Na-ascorbate) (Table 1). This is indicative of the ascorbate anion chelation through the O-2 and O-3 atoms. In a recent study [12] on the interaction of cobalt-ammine cations with ascorbate anion in aqueous solution, it was suggested that the ascorbate anion binding is unidentately in the cobalt-pentammine ascorbate compound and bidentately in the cobalt-tetrammine complexes [12]. Evidence for this comes from the major downfield shifts of the C-3 atom chemical shift, in the cobalt-pentammine ascorbate and the C-3 and C-4 chemical shifts (compared with the Na-salt) in the cobalt-tetrammine ascorbate complexes [12].

However, if chelate formation by monoanionic form of the ascorbate anion (deprotonated at O(3)-H group at pH 6–7) is difficult, dianionic ascorbate anion (deprotonated at O(3)-H and O(2)-H groups at pH 11–12) can easily form metal chelate complexes [2].

FT-IR Spectra

The FT-IR spectra of the free L-ascorbic acid and its alkali and alkaline earth metal ion salts (and deuterated samples) have been studied in the region of 4000–500 cm^{-1} and the results of the spectral analysis are discussed below.

L-Ascorbic Acid OH Stretching Vibrations and Metal Ion Binding. The x-ray and neutron diffraction analysis of the free L-ascorbic acid showed that each unit cell contains eight distinct intermolecular hydrogen bonded OH groups [13]. Based on the intermolecular O . . . O and O . . . H distances, the eight sharp absorption bands with medium intensities observed in the region of 3550–2700 cm^{-1} and the corresponding dueterated bands in the region of 2700–2000 cm^{-1} are assigned to the free acid OH and OD stretching vibrations, as summarized below: A sharp band at 3525 cm^{-1} (deuterated at 2607 cm^{-1}) is assigned to the O(6)-H(O . . . O = 2.93 Å), a band at 3410 cm^{-1} (deuterated at 2533 cm^{-1}) is related to the O(5)-H (O . . . O = 2.78 Å), a sharp band at 3314 cm^{-1} (deuterated at 2469 cm^{-1}) is due to the O(6′)-H (O . . . O =

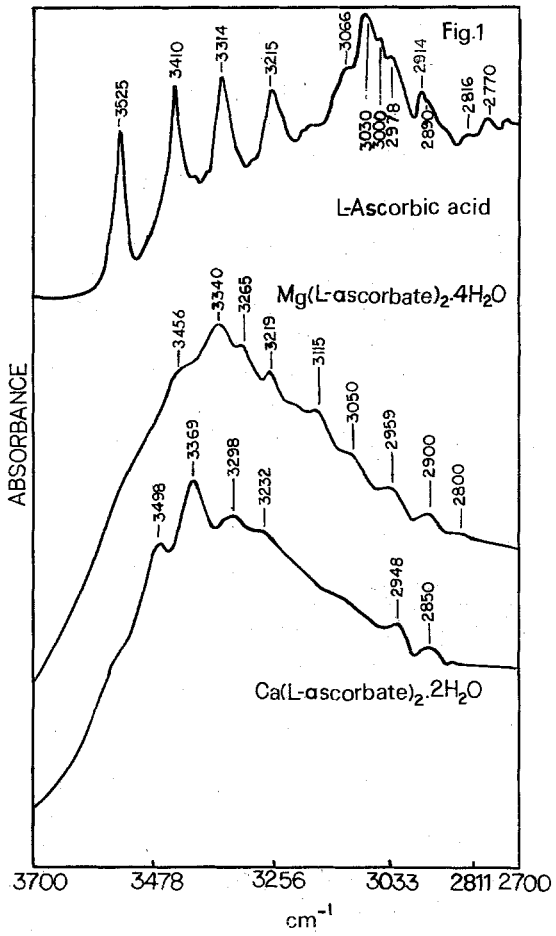

FIGURE 1. FT-IR spectra of L-ascorbic acid and its alkaline earth metal complexes in the region of 3700–2700 cm^{-1}.

2.76 Å), a band at 3215 cm^{-1} (deuterated at 2400 cm^{-1}) is attributed to the O(5′)-H (O . . . O = 2.70 Å), a shoulder band at 3066 cm^{-1} (deuterated at 2261 cm^{-1}) is assigned to the O(3′)-H (O . . . O = 2.66 Å), a broad band at 3030 cm^{-1} (deuterated at 2070 cm^{-1}) is due to the O(3)-H (O . . . O = 2.65 Å), a shoulder band at 3000 cm^{-1} (not observed in deuterated sample) is due to the O(2′)-H (O . . . O = 2.64 Å), and a medium intensity band at 2770 cm^{-1} (not observed in deuterated sample) is attributed to the strongly hydrogen bonded O(2)-H (O . . . O = 2.61 Å) stretching vibrations (Fig. 1). Our present assignments are consistent with the literature reported [14].

Upon acid ionization, there are four strong absorption bands at 3370, 3330, 3260, and 3200 cm^{-1}, in the IR spectrum of the Na-ascorbate salt. The observed spectral changes are related to the ionization of the acid O(3)-H group, which perturbs the electron distribution within the ring system and alters the sugar vibrational frequencies. It is worth mentioning that the interaction of the Na(I) ion with the acid anion OH groups is also responsible for the observed spectral changes in the Na-salt. The Na(I) ion binds to four ascorbate anions through O-5, O-6 (first anion), O-3, O-2

(second), O-3 (third), and O-6 (fourth), in the crystal structure of the Na-ascorbate salt [5].

Upon alkaline earth metal ion interaction, a strong and broad band was observed at about 3350 cm^{-1}, with several other weak bands in this region (Fig. 1). The broadening of the acid OH stretching vibrations upon alkaline earth metal complexation, would be related to the participation of the acid anion OH groups in metal-ascorbate bindings. This is consistent with the structural information of the Ca(L-ascorbate)$_2 \cdot 2H_2O$ salt, which showed the direct involvement of the sugar OH groups, in the metal-complex formation [6]. The Ca(II) ion binds to three acid anions via O-5, O-6 (first anion), O-3, O-5, O-6 (second) and O-1 (third) as well as to two H_2O in the crystal structure of the Ca(L-ascorbate)$_2 \cdot 2H_2O$ salt [6]. However, extensive hydrogen bonding systems with coordinated H_2O molecule and the ascorbate anion OH groups are present in the crystal structures of the alkaline earth metal-ascorbate salts, which are responsible for the holding of the acid anions together and stabilizing the crystal structure [6].

L-Ascorbic Acid Carbonyl Stretching and Ring Vibrational Modes. The free acid carbonyl (C-1 = O) stretching vibration appeared as a band with medium intensity at 1753 cm^{-1} and shifted towards a lower frequency at about 1725 cm^{-1}, upon alkaline earth metal ion interaction (Fig. 2). Similarly, the strong bands at 1670 and 1650 cm^{-1} in the free acid, which are attributed to the C = O and C = C stretching vibrations [14], exhibited shifting towards lower frequencies (Fig. 2). The shifting of the carbonyl stretching vibrations towards lower frequencies are related to the participation of the C-1 = O group in metal ascorbate bindings. It should be noted that in the spectrum of the sodium-ascorbate salt, a larger shift of the carbonyl stretching from 1753 (free acid) to 1700 cm^{-1} (Na-salt) ($\Delta\bar{\nu}$ = 53 cm^{-1}) was observed, compared with those of the alkaline earth metal-ascorbate salts ($\Delta\bar{\nu}$ = 30 cm^{-1}). The larger shift of the carbonyl stretching in the spectrum of the sodium salt is related to the formation of the intramolecular O(1)...H(2)O system, found in the solution structure of the acid anion [4], which causes the lowering of the carbonyl stretching vibration. However, a smaller displacement of the C = O stretching in the spectra of the alkaline earth metal ion salts (compared with Na-salt), would be related to the break down of the intramolecular H-bonding as a result of metal ion coordination.

Due to the overlappings of the water OH stretching vibrations with those of the sugar OH stretchings in the region of 3500–3000 cm^{-1} and the bending of the H_2O with the strong C = O and C = C stretchings in the region of 1700–1600 cm^{-1}, in the spectra of the alkaline earth metal-ascorbate salts, it was not possible to locate any absorption band related to the metal bonded H_2O molecules (Figs. 1 and 2).

Several absorption bands in the region of 1500–1200 cm^{-1} of the free acid spectrum, which were assigned to the strongly coupled C-O-H, CH_2, and OH bending modes [14] exhibited major spectral changes and were overlapped by the strong and broad C-3-O^- stretching vibration [15] at about 1400 cm^{-1} in the spectra of the alkaline earth metal-ascorbate complexes (Fig. 2). The observed spectral modifications are related to the acid ionization and the participation of the anion OH groups in metal-ligand bondings. The broadening of the C-O^- band at about 1400 cm^{-1} is related to the participation of the C-3-O^- group, in the alkaline earth metal complex formation (Fig. 2). Such broadening of the C-O^- stretching band was not observed in the spectrum of the Na-ascorbate salt. It should be noted that the absorption bands

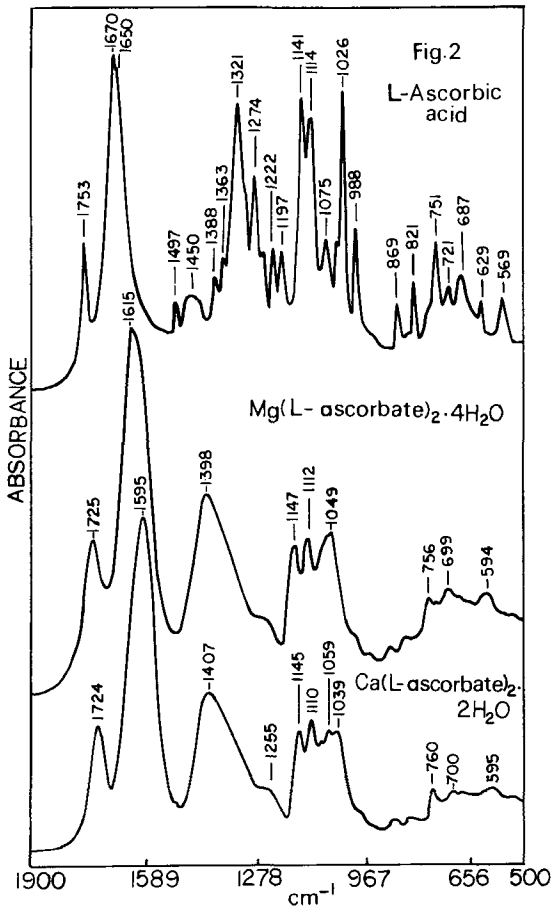

FIGURE 2. FT-IR spectra of L-ascorbic acid and its alkaline earth metal complexes in the region of 1900–500 cm^{-1}.

related to the C-O-H and OH bending modes in the region of 1500–1200 cm^{-1} showed marked shiftings towards lower frequencies upon acid deuteration. Several other sharp absorption bands at 1141, 1114, 1075, 1026, and 988 cm^{-1} of the free acid, which are attributed to the sugar ring C-O and C-C stretching vibrations [14], exhibited considerable intensity changes and shiftings upon metal ion interaction (Fig. 2). These observed spectral alterations are attributed to the acid ionization and the participation of the sugar C-3-O^- group, in metal-ascorbate bindings which are consistent with the structural properties of the Ca-ascorbate compound [6]. The skeletal C-O-C and C-C-C deformations of the free acid [14] in the region of 900–500 cm^{-1} lost intensities and shifted towards lower frequencies on metal ion complexation (Fig. 2). The ionization of the O(3)-H group will drastically alter the electron distribution within the ring system, where the vibrations are mostly localized and finally causes the ring distortion.

It is worth mentioning that the IR spectra of the alkaline earth metal ascorbate salts were markedly different from that of the Na-ascorbate, both in the OH stretching and the fingerprint regions. The observed spectral disimilarities can be attributed to

the different metal ion binding modes and the H-bonding networks, which have been found in the crystal structures of the alkali and alkaline earth metal-ascorbate salts [5, 6]. However, the stronger type of metal-ligand bondings (covalent bonding) of the alkaline earth metal-ascorbate complexes with respect to those of the alkali metal interaction (largely ionic), can contribute also to the spectral differences observed for the alkali and alkaline earth metal-ascorbate salts.

Solution Studies

The IR spectra of the double bond region 1800–1400 cm^{-1} of the free ascorbic acid and its alkaline earth metal salts were examined in D_2O solution. There were marked spectral similarities between the solid and solution spectra in this region. This is indicative of the acid ionization and metal ion complexation in aqueous solution. The broadening of the C-3-O^- band at 1400 cm^{-1} observed in solution was indicative of the metal ion binding via the negatively charged O-3 atom. Such broadening of the C-3-O^- stretching band was not observed in the spectrum of the Na-salt. The binding of these metal ions through the O-3 atom in aqueous solution is consistent with our ^{13}C NMR results discussed here (see ^{13}C NMR discussion).

In conclusion, the binding of the alkaline earth metal ions is through the O-3 atom of the ascorbate anion in aqueous solution, whereas, in the solid state, chelate formation was observed through O-5, O-6 and O-3, O-5, O-6 for the Ca(II), Sr(II), and Ba(II) ions and via O-1, O-3 for the Mg(II) ascorbate salts. The binding mode of the ascorbate anion is different for the alkali and alkaline earth metal ions, both in the crystalline solid and the aqueous solution.

REFERENCES

1. L. S. Hollis, A. R. Amundsen, and E. W. Stern, *J. Am. Chem. Soc.* **107**, 274 (1985).
2. C. J. Cardin and A. Roy, *Inorg. Chim. Acta* **107**, L37–L39 (1985).
3. A. E. Martell, in *Ascorbic Acid Chemistry, Metabolism and Uses*, P. A. Seib and B. M. Tolbert, Eds., American Chemical Society, Washington, DC, 1982, pp. 153–173.
4. E. E. Kriss, *Russ. J. Inorg. Chem.* (Engl. Transl.) **23**, 1004 (1978).
5. J. Hvoslef, *Acta Crystallogr.* **B25**, 2214 (1969).
6. J. Hvoslef and K. E. Kjellevold, *Acta Crystallogr.* **B30**, 2711 (1974); R. A. Hearn and C. E. Bugg, *Acta Crystallogr.* **B30**, 2705 (1974).
7. D. L. Hughes, *J. Chem. Soc. Dalton Trans.* 2209 (1973).
8. A. T. Merrill and S. L. Ruskin, *Science* 505 (1949).
9. J. V. Paukstelis, D. D. Mueller, P. A. Seib, and D. W. Lillard, in *Ascorbic Acid Chemistry, Metabolism and Uses*, P. A. Seib and B. M. Tolbert, Eds., American Chemical Society, Washington, DC, 1982, pp. 125–151.
10. O. Forsberg, K. Johanssén, P. Ulmgren, and O. Wahlberg, *Chem. Scripta* **3**, 153 (1973).
11. P. Ulmgren and O. Wahlberg, *Chem. Scripta* **3**, 159, 193 (1973).
12. H. A. Tajmir-Riahi, *Biophys. Chem.* **25**, 37 (1986).
13. J. Hvoslef, *Acta Crystallogr.* **B24**, 23, 1431 (1968).
14. J. Hvoslef and P. Klaeboe, *Acta Chem. Scand.* **25**, 3043 (1971).
15. N. P. Evtushenko, K. B. Yatsimirskii, E. E. Kriss, and G. T. Kurbatovs, *Russ. J. Inorg. Chem.* (Engl. Transl.) **22**, 839 (1977).

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