

# Tetrahedral Complexes of Cobalt(II) with L-Histidine, Histamine, Imidazole, and N-Acetyl-L-histidine<sup>1</sup>

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**Abstract:** In highly alkaline aqueous solutions, cobalt(II) forms violet, tetrahedral, bis complexes with L-histidine, histamine, and N-acetyl-L-histidine. Imidazole similarly forms a tetrakis complex. Generation of the tetrahedral complexes is dependent on deprotonation of the imidazole ring pyrrole nitrogen for the L-histidine, histamine, and imidazole complexes, and on two amide hydrogen ionizations for the 2:1 N-acetyl-L-histidine complex. The tetrahedral complexes all possess four nitrogen donor atoms, at least two of which are negatively charged. Approximate, average  $pK_a$  values for metal ion promoted pyrrole hydrogen ionizations from 2:1 complexes of L-histidine with cobalt(II) and copper(II) are 12.5 and 11.7, respectively. Minimization of steric crowding and reduction of charge repulsion appear to be the reasons for cobalt(II) preferring a tetrahedral environment to an octahedral one in these cases.

In a recent study of some oxygenation reactions, the properties of several cobalt(II) complexes of amino acids in oxygen-free solution were reported.<sup>2</sup> For cobalt(II) solutions containing L-histidine, raising the pH to about 12 produces a color change from pale pink-yellow to deep violet. This change has been ascribed to the conversion of octahedral 2:1 histidine-cobalt(II) complexes to a 2:1 tetrahedral complex accompanied by the deprotonation of the pyrrole nitrogen of histidine. The tetrahedral complex contains two bidentate histidines each carrying a 2- charge.<sup>3</sup>

Previous work in this laboratory indicated that the behavior of the violet cobalt(II) complex of histidine was complicated. In the present paper we have more fully investigated the conditions necessary for the formation of the violet histidine complex of cobalt(II). Compounds related to histidine have also been examined in an attempt to find the structural requirements for forming the violet color.

## Experimental Section

The equivalent weights of all the high quality ligands obtained from commercial sources were checked by titrations with standard base. All experiments involving cobalt(II) were carried out under scrupulously oxygen-free conditions, maintained by using tank nitrogen purified by passage through vanadium(II) chloride scrubbers to remove any oxygen.<sup>4</sup> Spectra were recorded on a Cary 14R spectrophotometer. Because of the extended concentration range needed in some of the experiments, molar absorbances greater than 2 (at about 560 nm) were often obtained, even in 1-mm cells. In such cases, the reference compartment contained a solution similar in composition to the test solution but of lower and known absorbance. The accuracy of the molar absorbances (at about 560 nm) recorded in this way was checked by comparing the molar absorbances at about 1100 nm, which were always less than 2. Circular dichroism was measured with a Durrum-Jasco ORD-UV-5 recording spectropolarimeter with a circular dichroism attachment. Values of pH were measured using a Radiometer Type TTT-1 titrator and associated GK 2021 B combined glass-calomel electrode and T 301 temperature compensator. All experiments were conducted at  $24 \pm 1^\circ$ .

(1) This research was supported by a grant from the National Science Foundation.

(2) M. S. Michailidis and R. B. Martin, *J. Amer. Chem. Soc.*, **91**, 4683 (1969).

(3) C. C. McDonald and W. D. Phillips, *ibid.*, **85**, 3736 (1963).

(4) L. Meites, "Polarographic Techniques," Interscience Publishers, New York, N. Y., 1955, p 34.

## Results

Aqueous solutions containing at least 2 mol of L-histidine per mol of cobalt(II) exhibit, at pH  $\sim 9$ , a pink-yellow color indicative of high-spin octahedral cobalt(II) complexes. At this pH the only important species is the bis cobalt(II) complex containing two tridentate histidine ligands each carrying one net negative charge due to the carboxylate group. The histidines are bound through their carboxylate oxygen,  $\alpha$ -amino nitrogen, and 1-imidazole (pyridine) nitrogen atoms.<sup>5</sup> Since the bulky imidazole groups are probably *trans* in solution, as in the crystal, the complex containing only the L-amino acid must possess *cis* carboxylate and *cis*  $\alpha$ -amino groups. Even in the presence of a moderate excess of histidine, only the bis complex is formed, there being little tendency to coordinate a third molecule of histidine.<sup>2</sup> The aqueous solution spectrum of the octahedral 2:1 L-histidine-cobalt(II) complex, at pH  $\sim 9$ , has absorption maxima at 482 nm ( $\epsilon$  20) and 1020 nm ( $\epsilon$  4) (Figure 1).

Raising the pH of an aqueous solution of the above 2:1 L-histidine-cobalt(II) complex results in a color change from pink-yellow through red (pH  $\sim 11.5$ ) to violet. The violet color is fully developed in solutions 1 M in NaOH, and the spectrum is shown in Figure 1. Table I lists the absorption maxima. This violet

**Table I.** Approximate Ionization Constants and Absorption Spectra Data for Violet Cobalt(II) Complexes at  $24^\circ$

Ligand	$\sim pK_a$	Absorption spectra					
		nm	$\epsilon$	nm	$\epsilon$	$Dq$ , $cm^{-1}$	$B$ , $cm^{-1}$
L-Histidine	12.5	558	320	1115	80	528	738
Histamine	13	572	515	1110	115	532	700
Imidazole	12.5	569	660	1105	180	535	704
N-Acetyl-L-histidine	10.9	568	535	1150	130	512	731

solution shows a weak circular dichroism with  $\Delta\epsilon = -0.1$  at 545 nm. The violet color appears to arise from a single, monomeric, 2:1 L-histidine-cobalt(II) species. In solutions containing a concentration of NaOH  $\geq 1$

(5) M. M. Harding and H. A. Long, *J. Chem. Soc., A*, 2554 (1968).

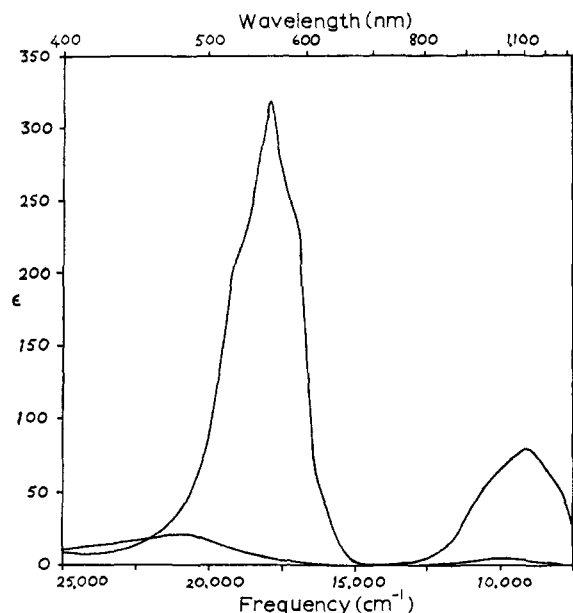


Figure 1. Absorption spectra of 2:1 L-histidine-cobalt(II) complexes in absence of oxygen in 1 *M* NaOH (upper curve) and pH 8.3 (lower curve). The upper and lower curves refer to tetrahedral and octahedral complexes of cobalt(II), respectively.

*M*, the molar absorptivities are independent of basicity, cobalt(II) concentration from 0.04 to 0.45 *M*, L-histidine concentration from 0.16 to 2.00 *M*, and the molar ratio of L-histidine to cobalt(II) from 4 to 20. Although the violet complex is obtained at an L-histidine to cobalt(II) molar ratio of 2, competition from hydroxide for the cobalt(II) results in a time-dependent spectrum and the slow appearance of a precipitate (presumably cobalt(II) hydroxide). Consequently, molar ratios greater than 2 were used in most of the work. The midpoint of the spectral changes occurs at about pH 12.5, corresponding to an approximate averaged  $pK_a$  value for the ionizations occurring in the 2:1 complex.

In contrast to the results in solutions 1 *M* or greater in NaOH, the spectral characteristics of solutions containing L-histidine and cobalt(II) in the intermediate pH range (*ca.* 11.5 to 13.5) are complicated. The "molar absorptivity" of the incompletely formed violet complex is dependent on the concentrations of both components. Both the magnetic susceptibility and pmr contact shifts reported for the violet complex were obtained in this intermediate pH region.<sup>3</sup> Thus the magnetic susceptibility of 4.5 BM obtained at pH 11.5 evidently represents a maximum value for the violet histidine complex.

In order to prevent precipitation of cobalt(II) hydroxide, at least a 100-fold excess of histamine to cobalt(II) is required, at a cobalt(II) concentration of 0.01 *M* and an NaOH concentration of  $\geq 1$  *M*. The yellow solution containing a molar ratio of histamine to cobalt(II) of 100, at pH  $\sim 9$ , presumably contains mainly the 3:1 histamine-cobalt(II) complex. As the pH is raised, a violet color appears and is fully developed in solutions 2 *M* in NaOH. The absorption maxima are reported in Table I. These spectral characteristics are independent of cobalt(II) concentration and the molar ratio of histamine to cobalt(II), indicating that only a single, monomeric, species is responsible for

the violet color. The midpoint of the color transition occurs at about pH 13.

A 1000-fold excess of imidazole was needed to prevent precipitation at high pH in solutions containing a cobalt(II) concentration of about 0.01 *M*. Raising the pH produces a color change from pink to violet. The change is complete when the concentration of NaOH has reached 1 *M*, and the spectral maxima of the resulting solution are given in Table I. These molar absorptivities are independent of both the cobalt(II) concentration and the amount of excess imidazole. The midpoint in the growth of both spectral peaks is about pH 12.5.

In titrating solutions containing cobalt(II) and N-acetyl-L-histidine, a cobalt(II) concentration  $\geq 0.035$  *M* and a 20-fold excess of ligand were necessary to prevent precipitation. Titration showed an end point at pH  $\sim 9$  corresponding to the addition of one equivalent of base per mole of N-acetyl-L-histidine and to deprotonation of the imidazolium group. Addition of further base produced a color change from pink to violet followed by an intensification of this violet color and a second end point at pH  $\sim 11.5$ . This second portion of the titration curve corresponds to the addition of a further two equivalents of base per mole of cobalt(II), *i.e.*, to the removal of one proton from each of two N-acetyl-L-histidine ligands now coordinated to each cobalt(II). The pH midpoint of the second buffer region is 10.9. Development of the violet color was complete at 1 *M* NaOH and the solution shows the absorption maxima given in Table I. These are independent of both the cobalt(II) concentration and the amount of excess N-acetyl-L-histidine.

Any hydrolysis of N-acetyl-L-histidine to L-histidine cannot account for the observed spectral results. Table I shows that the approximate ionization constants, molar absorptivities, and positions of the absorption maxima are significantly different for the two cobalt(II) complexes. Also, exposure of the violet L-histidine complex to air rapidly produces a yellow-brown solution of the oxygenated binuclear complex,<sup>2</sup> while in the N-acetyl-L-histidine case the violet solution changes color only over a period of days. Of the four cobalt(II) complexes given in Table I, the first two oxygenate rapidly and the last two only slowly.

Attempts to produce deep violet colors by the addition of base to deoxygenated solutions of cobalt(II) and the following ligands were unsuccessful: L-3-methylhistidine, L-1-methylhistidine, 1-methylimidazole, D,L-2,3-diaminopropionic acid, ethylenediamine, glycine, glycinamide, tetraglycine, biuret, glycyl-L-histidine, and EDTA. The last two ligands, at ligand to cobalt(II) molar ratios of 2 and 1, respectively (cobalt(II) concentration about 0.1 *M*), formed wine red solutions at about pH 13. The absorption maxima were shifted to longer wavelengths compared to solutions at lower pH, but the molar absorptivities were unchanged. Evidently, hydroxide ion is replacing some nitrogen donor atoms in these high-pH solutions. No amide hydrogen ionizations appear to take place in any of the above systems. The behavior of the cobalt(II)-glycylglycine system, where amide hydrogen ionization does occur, has been described previously.<sup>2</sup> The interpretation of the results for the addition of base to solutions containing cobalt(II) and one of

L-histidine methyl ester, L-histidinamide, L-histidylglycine, histidylhistidine, or glycyglycinamide was complicated by hydrolysis of the ligand.

It has been noted that oxygen-free solutions of cobalt(II) and gelatin (and other proteins) turn violet at high pH.<sup>6</sup> For the reported gelatin solution in 1 *M* KOH, the spectrum shows a maximum at 560 nm with  $\epsilon$  170. We repeated these experiments using solutions 1 or 2.5% in commercial gelatin,  $10^{-3}$ – $10^{-2}$  *M* in cobalt(II), and 1 *M* in NaOH. Violet solutions are formed, the spectra of which show three maxima of the following wavelengths and approximate molar absorptivities (based on cobalt(II) concentration): 500 nm ( $\epsilon$  130), 560 (165), and 1100 (25). These  $\epsilon$  values are increased by raising the base concentration. Although of lower intensity and with an additional peak at 500 nm, this spectrum is similar to those obtained for the complexes in Table I. This similarity suggests a possible cobalt(II) interaction of the histidyl residues of gelatin. However, because the histidine content of gelatin is low, cobalt(II) is present in excess in the above experiments. Since the  $\epsilon$  value is constant over a tenfold concentration range of cobalt(II), it appears that cobalt(II) also interacts at other sites in the protein. We were unable to prepare stable high-pH solutions of cobalt(II) with other proteins and such systems are not considered further. The spectra reported in Table I and that given above for gelatin bear similarities to those reported for the cobalt(II)-substituted zinc metalloenzyme carbonic anhydrase in the presence of inhibitors.<sup>7</sup>

Titration of a solution containing a 2:1 molar ratio of L-histidine to copper(II), at a copper(II) concentration of 0.05 *M*, shows only one end point. This occurs at two equivalents of NaOH added per mole of copper(II). At the endpoint the pH rapidly rises to about 9.5, then slowly rises as further base is added. It requires a total of about four equivalents of NaOH, per mole of copper(II), to raise the pH to 12. The color of the solution changes from blue to a darker blue as the pH is raised from 7 to 12. This is reflected in a change in the spectral maximum from 637 nm ( $\epsilon$  90) to 625 nm ( $\epsilon$  109). From the titration curve, the average  $pK_a = 11.7$  for ionizations from both ligands in the 2:1 complex.

The analogous L-3-methylhistidine-copper(II) system behaves identically with the L-histidine one up to pH 7. However, at this end point the pH rapidly rises to about 10.5. A pH of 12 is reached after the addition of a total of only about 2.4 equivalents of NaOH per mole of copper(II). No color change occurs between pH 7 and 12, and the spectral characteristics remain unchanged with a maximum at 640 nm ( $\epsilon$  90).

Solutions containing molar ratios of L-histidine or L-3-methylhistidine to nickel(II) of 2:1, at a nickel(II) concentration of 0.05 *M*, behave very similarly to the above copper(II)-L-3-methylhistidine solution. Titration shows only one end point (at two equivalents of NaOH per mole of nickel(II)). Addition of further base rapidly raises the pH to 12. No apparent color or spectral changes occur in the pale blue solution between pH 7 and 12. The addition of excess base yields no intensification of color.

(6) J. Bello and H. R. Bello, *Nature*, **192**, 1184 (1964).

(7) S. Lindskog, *J. Biol. Chem.*, **238**, 945 (1963).

## Discussion

The relatively high intensities and the wavelengths of the absorption maxima listed in Table I are most reasonably assigned to tetrahedral cobalt(II) complexes possessing four nitrogen donor atoms. Even grossly distorted octahedral complexes should not yield, for these ligands, molar absorptivities greater than 50. Hydroxide-ion binding did not increase the spectral intensity of the octahedral cobalt(II) complexes of EDTA and glycylyhistidine. Most complexes of cobalt(II), which have been assigned a five-coordinate structure, display three distinct absorption bands that occur throughout the spectrum between 400 and 1000 nm.<sup>8,9</sup> On the other hand one medium intensity band, with structure in the visible from 600 to 750 nm, and another weaker band in the near-infrared region at >1000 nm have been observed for numerous tetrahedral cobalt(II) complexes.<sup>9</sup>

Employing the labels of the tetrahedral symmetry point group the longer wavelength band in the near-infrared may be assigned to the  ${}^4A_2 \rightarrow {}^4T_1(F)$  transition and the shorter wavelength band in the visible to  ${}^4A_2 \rightarrow {}^4T_1(P)$ . The latter transition is magnetic dipole forbidden in  $T_d$ , which is consistent with the weak circular dichroism in the visible for the violet L-histidine complex of cobalt(II). Using these assignments the ligand field splitting parameter,  $Dq$ , and the Racah interelectron repulsion parameter,  $B$ , were calculated<sup>10</sup> and the results are listed in Table I. The  $Dq$  values for the first three ligands in Table I may be the highest values known for tetrahedral cobalt(II) complexes. The next highest values appear to occur for the related ligand benzimidazole, which has been shown<sup>11</sup> to form tetrahedral cobalt(II) complexes that give  $Dq = 510 \text{ cm}^{-1}$ . For all of the ligands in Table I, the average value of  $B$  is  $718 \text{ cm}^{-1}$ , which is identical with that calculated<sup>10</sup> for  $\text{CoCl}_4^{2-}$ . In contrast, for the pink-yellow octahedral 2:1 L-histidine-cobalt(II) complex, we calculate the high  $Dq$  of  $1100 \text{ cm}^{-1}$  ( $4/9$  of which is  $490 \text{ cm}^{-1}$ ) and  $B = 800 \text{ cm}^{-1}$ .

Because most of the transitions to violet colors occur at high pH, only in the case of N-acetyl-L-histidine was it possible to establish that two equivalents of protons is removed per cobalt(II). This, together with the result that the violet color is produced when the L-histidine to cobalt(II) molar ratio is only 2, suggests that one proton is removed from each of two histidine-type ligands bound in the violet 2:1 complexes of L-histidine, histamine, and N-acetyl-L-histidine with cobalt(II). The violet imidazole-cobalt(II) complex is apparently a 4:1 species since its absorption maxima are similar to those of the above complexes, each of which has four nitrogen donors. However, it is impossible to determine how many of the bound imidazoles undergo deprotonation on raising the concentration of NaOH to 1 *M*. The approximate  $pK_a$  values of 12.5–13 for the first three complexes in Table I can reasonably be assigned to deprotonation of the pyrrole nitrogen of the

(8) D. W. Herlocker, *Inorg. Chem.*, **8**, 2037 (1969); M. Ciampolini and N. Nardi, *ibid.*, **5**, 41 (1966); P. Bamfield, R. Price, and R. G. J. Miller, *J. Chem. Soc., A*, 1447 (1969).

(9) A. B. P. Lever, "Inorganic Electronic Spectroscopy," Elsevier Publishing Co., Amsterdam, 1968.

(10) A. B. P. Lever, *J. Chem. Educ.*, **45**, 711 (1968).

(11) M. Goodgame and F. A. Cotton, *J. Amer. Chem. Soc.*, **84**, 1543 (1962).

imidazole ring common to the ligands. In free imidazole and histidine this ionization exhibits  $pK_a$  values of 14.2 and 14.4, respectively.<sup>12</sup> There is some ambiguity as to which proton the  $pK_a$  of 10.9 for N-acetyl-L-histidine refers. Since it is nearly 2  $pK_a$  units less than for the first three complexes of Table I, we suggest that it refers to the ionization of the hydrogen attached to the amide nitrogen. Amide hydrogen ionization occurs near pH 10 in the cobalt(II) complex of glycylglycine.<sup>2</sup> After this ionization, the amide nitrogen becomes the donor atom so that the 2:1 N-acetyl-L-histidine-cobalt(II) tetrahedral complex possesses four nitrogen donor atoms.

The four tetrahedral cobalt(II) complexes of Table I display similar characteristics, although N-acetyl-L-histidine undergoes amide nitrogen, rather than pyrrole nitrogen, deprotonation. All of the complexes possess four nitrogen donors including a minimum of two aromatic imidazole groups. Both the L-histidine and histamine complexes have two primary amino and two negatively charged pyrrole nitrogen donor atoms. In the N-acetyl-L-histidine complex two six-membered chelate rings also occur, but coordination is *via* two pyridine-type nitrogen atoms and two negatively charged amide nitrogen atoms. The remote carboxylate side chains of L-histidine and N-acetyl-L-histidine tetrahedral complexes appear to be unimportant. For the imidazole complex, only two of the four ligands need to have undergone ionization at the pyrrole nitrogen to conform with the pattern of two negative and two neutral nitrogen donor atoms. Blocking of either imidazole nitrogen position in L-histidine with methyl groups prevents formation of a tetrahedral complex. Similarly, 1-methylimidazole fails to form a violet complex. D,L-2,3-Diaminopropionic acid also forms only octahedral complexes with cobalt(II). The inability of glycinamide to yield a tetrahedral complex suggests that potential amide hydrogen ionization alone is insufficient. Hence, participation of an aromatic imidazole group possessing an ionizable pyrrole hydrogen and a free pyridine nitrogen is crucial to formation of these tetrahedral cobalt(II) complexes.

Like cobalt(II), copper(II) also promotes the deprotonation of a pyrrole-type hydrogen in L-histidine, but at about 2.7  $pK_a$  units lower than in the free ligand. This accounts for the observed shift in the spectral maximum to shorter wavelength on raising the pH from 7 to 12, while no such shift occurs in the L-3-methylhistidine complex. The depression of the titration curve, at pH above 9.5, for the L-histidine-copper(II) 2:1 system relative to the L-3-methylhistidine system also indicates additional ionizations within the L-histidine complex. Nickel(II) fails to promote ionization of the pyrrole hydrogen in L-histidine below pH 12. However, on the addition of sufficient excess base so that L-histidine dianions exist in solution, no strongly absorbing tetrahedral complex appears. This result con-

(12) G. Yagil, *Tetrahedron*, **23**, 2855 (1967).

forms with the greater relative stability of octahedral over tetrahedral complexes in nickel(II) as compared to cobalt(II).

The driving force for the octahedral to tetrahedral conversion appears to be reduction of charge repulsion of cobalt(II) and among the ligands and minimization of steric crowding. Ignoring any remote carboxylate groups, which appear unessential for tetrahedral complex formation, the net charge on the tetrahedral cobalt(II) complexes of L-histidine, histamine, and N-acetyl-L-histidine is zero. If these ligands were to maintain octahedral structures after pyrrole or amide hydrogen ionizations, then the cobalt(II) and its six nitrogen donor atoms would carry a net single negative charge. This assumes L-histidine and N-acetyl-L-histidine to be bidentate; if they were tridentate, then there would be a net 2- charge on the cobalt(II) and its neighboring four nitrogen and two oxygen atoms. However, few negatively charged octahedral cobalt(II) complexes are known; they are typically tetrahedral.

The imidazole group is bulky. Molecular models reveal that the observed slow oxygenation of the 4:1 imidazole and the 2:1 N-acetyl-L-histidine tetrahedral complexes of cobalt(II) may be simply a steric effect of ligand crowding about the cobalt(II) ion, limiting access of O<sub>2</sub>. Easier access of O<sub>2</sub> to the metal ion is apparent in models of the 2:1 L-histidine and histamine tetrahedral complexes. Formation of a tetrahedral complex relieves the severe steric crowding that would have been present if octahedral complexes with six nitrogen donors had been formed.

Oxygenation of 2:1 tetrahedral and octahedral complexes of L-histidine and cobalt(II) yields two different products.<sup>13</sup> The tetrahedral complex forms a species in 1 M KOH exhibiting a single strong absorption maximum at 366 nm, while the oxygenated octahedral cobalt(II) complex prepared at pH 9.3 displays bands at 385 and 329 nm. Previous results from this laboratory reported absorption bands at about 380 and 325 nm for the dimeric oxygenated complexes of L-histidine, L-histidinamide, and L-diaminopropionic acid prepared near pH 9.<sup>2</sup> In contrast all three of the ligands, ethylenediamine, glycinamide, and histamine, for which hydroxo as well as oxygen bridges are indicated in the oxygenated complexes prepared near pH 10, yield only a single absorption maxima at 350, 340, and 375 nm, respectively. Therefore the appearance of a single absorption maxima in the oxygenated complex of L-histidine prepared in 1 M KOH is suggestive of occurrence of an hydroxo bridge, which is also favored by vacant coordination positions in the originally tetrahedral cobalt(II) complex.<sup>14</sup>

(13) S. Bagger, *Acta Chem. Scand.*, **23**, 975 (1969).

(14) NOTE ADDED IN PROOF. Since submission of the manuscript we have determined that a solution containing a 40:1 ratio of L-histidinol to cobalt(II) yields in 1 M NaOH a deep violet color with absorption maxima at 567 nm ( $\epsilon$  325) and 1100 nm ( $\epsilon$  80). Thus a fifth ligand yielding a tetrahedral cobalt(II) complex is added to the other four of this paper.