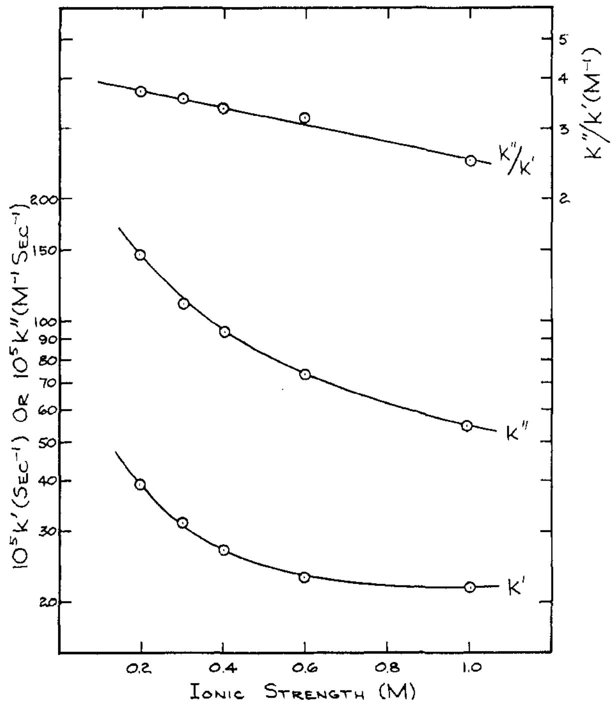

Figure 2.—Variation of k', k'', and k''/k' with ionic strength.

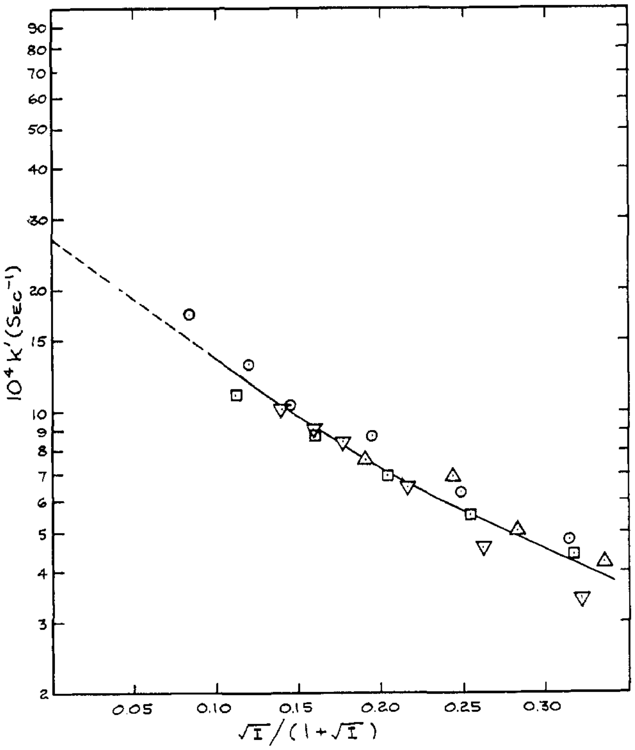

Figure 3.—Variation of k' at low ionic strength with $\sqrt{I}/(1 + \sqrt{I})$. Acidities are as follows: ⊙, 0.0025 *M*; ⊡, 0.01 *M*; ▽, 0.02 *M*; △, 0.05 *M*. The dotted portion of the curve has a slope of −3.

tion is that the dechelation process is far from complete, so that *K* in reality describes a double equilibrium

$$Cr(C_2O_4)_3^{3-} \rightleftarrows Cr(C_2O_4)_2OC_2O_3^{3-}, \qquad K' \quad (1A')$$

$$Cr(C_2O_4)_2OC_2O_3^{3-} + H_3O^+ \rightleftarrows Cr(C_2O_4)_2OC_2O_3H^{2-} + H_2O, \ K'' \quad (1B)$$

Then, since $K = K'K'' \sim 5 \times 10^{-2}$ and $K'' \sim 10^2$, K' must be no greater than 5×10^{-4}. With this low a value for the dechelation equilibrium, it seems unlikely that water is entering the sixth coordination position, as in our earlier suggestion (eq 1), since this would have a large stabilizing influence favoring the monodentate oxalato species. This revised view of the dechelation–protonation process is similar to the one supported by Bunton, *et al.*, differing only in the order of the events, a kind of question it is impossible to answer from kinetic data.

We can now consider in some detail the total equilibrium system defined by reactions 1A, 2A, and 2B. Utilizing the known k' values in the present paper and the previously determined[1] k_wK_1 values, one can tabulate the magnitude of K_t at 50° and various ionic strengths,[19] as shown in Table I. At low ionic strength, where theory is applicable, it has already been noted (Figure 3) that the plot of log k_1 *vs.* $\sqrt{I}/(1 + \sqrt{I})$ has the expected limiting slope of −3. The dependence on *I* becomes negligible at the higher ionic strengths where, apparently, the cancellation of competitive activity effects is complete. Unfortunately, theory does not allow us quantitative interpretation of such phenomena.

Table I

Equilibrium Data for the Aquation–Anation System

$$Cr(C_2O_4)_3^{3-} + H_3O^- + H_2O \rightleftarrows Cr(C_2O_4)_2(H_2O)_2^- + HC_2O_4^-$$

Ionic strength, *M*	10^5k_1K, M^{-1} sec^{-1}	$10^4k_wK_1$, M^{-1} sec^{-1}	K_t
0.00	270	5	5.4
0.10	60	11	0.55
0.20	39	15	0.26
0.50	25	21	0.12
1.00	22	31	0.07

Acknowledgment.—Support of this research through Contract No. AT(30-1)-1578 with the U. S. Atomic Energy Commission is gratefully acknowledged.

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[19] K_t is essentially independent of temperature since the enthalpies of activation of k' and k_wK are identical within experimental error.

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Optical Rotatory Dispersion Study on the Iron Complex with L-Cysteine and Its Reaction with Carbon Monoxide and Nitric Oxide

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The reversible reaction between bis(cysteinato)iron(II) and carbon monoxide was discovered by Cremer.[1] Bis(cysteinato)dicarbonyliron(II), isolated later by

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[1] W. Cremer, *Biochem. Z.*, **206**, 228 (1929).

Schubert,[2] dissociates in the presence of light, and the carbonyl complex is regenerated in the absence of light. This reversible reaction is similar to that of an oxygen-carrying complex such as hemoglobin. The corresponding nitrosyl compound has not been isolated to date.

One purpose of this work was to determine the optical rotatory dispersion (ORD) curves of ferrous and ferric complexes with L-cysteine at several pH values. Another was to investigate the new type of optically active complex containing carbonyl or nitrosyl and optically active L-cysteine. Lastly, the reversible reaction mentioned above was studied by ORD methods.

Experimental Section

Materials.—Reagent grade ferrous ammonium sulfate and ferric ammonium sulfate were used as the Fe(II) and Fe(III) salts, respectively. L-Cysteine hydrochloride monohydrate was reagent grade, the purity of which was assayed to be 96% by ferricyanide oxidation; it was used without further purification. Carbon monoxide (Takachiho Co., 99%) was passed through potassium hydroxide solution and sodium hydrosulfite solution. Nitric oxide was prepared and purified according to an accepted procedure.[3]

ORD and Absorption Spectra of the Ferrous Complex with Cysteine.—All operations were carried out under nitrogen atmosphere. The calculated amount of L-cysteine hydrochloride was added to 20 ml of water in a special flask, which had a 1-cm quartz cell for spectral analysis, a side compartment for ferrous salt, a nitrogen inlet and outlet, and a buret containing 10 ml of potassium hydroxide solution. The dissolved oxygen in the solution was displaced by purified nitrogen which was previously saturated with water. After sufficient bubbling of nitrogen, ferrous salt was introduced into the main compartment. The dissolution and mixing were accelerated by nitrogen bubbling. Different amounts of potassium hydroxide solution, which was previously deoxygenated and standardized, were added and ORD and absorption spectra were recorded.

ORD and Absorption Spectra of the Ferric Complex with Cysteine.—ORD and absorption spectra of the ferric complex in the visible region were determined in a borate buffer solution saturated with oxygen. Since cysteine bound to ferric ion is rapidly oxidized to cystine, cysteine was added in excess (cysteine/iron = 100) to maintain a constant concentration of ferric complex with cysteine. The rotation and absorption at many fixed wavenumbers were observed continuously, and the rotation and absorption values were found by extrapolation to zero time.

Preparation of the Complexes.—Bis(cysteinato)dicarbonyliron(II) was prepared according to Schubert.[2] *Anal.* Calcd for $FeC_8H_{10}N_2O_6S_2$: Fe, 15.42; C, 26.53; H, 3.34; N, 7.73. Found: Fe, 15.68; C, 26.85; H, 3.36; N, 7.64. The infrared spectrum (as potassium bromide pellet) of this complex showed sharp bands at 2020 and 1972 cm^{-1}, assignable to carbonyl stretching vibrations.

An attempt to prepare bis(cysteinato)dinitrosyliron(II) was unsuccessful, only cystinatodinitrosyliron(II) dihydrate being obtained according to the following procedure. Into a solution of bis(cysteinato)iron(II) (0.02 mole in 40 ml of water) of pH about 6.5, nitric oxide was bubbled for 2 hr. A fine, brown precipitate was filtered and washed with a large amount of water until the infrared absorption bands of contaminated potassium sulfate disappeared. Then it was dried at room temperature *in vacuo* for 4 days. *Anal.* Calcd for $FeC_6H_{14}N_4O_8S_2$: Fe, 14.31; C, 18.47; H, 3.62; N, 14.36. Found: Fe, 14.60; C, 18.60; H, 3.20; N, 14.60. The infrared spectrum (as potassium bromide pellet) seemed to be the superposition of that of cystine and the band at 1776 cm^{-1}, which is the N–O stretching band of the nitrosium ion, NO$^+$, coordinated to a metal ion.[4]

ORD Spectra of Carbonyl and Nitrosyl Complexes.—ORD, circular dichroism (CD), and absorption curves of the carbonyl complex were measured in neutral aqueous solution, and those of the nitrosyl complex, in 0.1 *N* hydrochloric acid solution. After nitrogen, carbon monoxide, or nitric oxide gas was passed through these solutions for a determined time at 77° or at 27°, the changed ORD curve was recorded at 27°.

Apparatus.—ORD and CD curves were recorded on a Japan Spectroscopic ORD/UV-5 spectropolarimeter. The other instruments used were a Hitachi EPS-2 spectrophotometer and a Japan Spectroscopic DS-301 infrared spectrometer.

Results and Discussion

Ferrous Complex.—L-Cysteine was titrated by KOH solution in the absence or presence of ferrous ion. The relationships between the equivalents of KOH and H$_3$L$^+$, pH values, and color are summarized in Table I, where H$_3$L$^+$ denotes $(HSCH_2CHNH_3COOH)^+$.

TABLE I

RELATIONSHIPS AMONG THE EQUIVALENTS OF KOH, pH VALUES OF THE SOLUTION, AND THE COLOR OF COMPLEX ($H_3L^+/Fe = 2.0$)

KOH/ H_3L^+	pH	Formula	Color of system
0.0	1.8	$Fe^{2+} + 2(H_3L)^+$	Pale green
1.0	3.5–5.0	$[Fe(H_2L)_2]^{2+}$	Pale yellow
1.5	6.5	FeL	Yellow (low solubility)
2.0	6.9	$Fe(HL)_2$	Orange
3.0	8.6	$[FeL_2]^{2-}$	Brownish yellow
4.0	10.4	$Fe(OH)_2 + 2L^{2-}$	Green precipitate

ORD curves of the Fe^{2+}–H_3L^+–KOH system are shown in Figure 1. For curve A, which shows that of H$_3$L$^+$ in the absence of ferrous ion, the [M] values are shown as twice the actual values owing to the convenience of the comparison with the other curves where [M] values are plotted relative to iron concentration ($H_3L^+/Fe^{2+} = 2.0$). This curve shows a positive Cotton effect, whose maximum is at 45,000 cm^{-1}, as reported by many workers.[5] This Cotton effect is due to the n–π^* transition of the carbonyl group. When ferrous salt was added to this solution at a pH value of 1.6, there appeared no essential change in the ORD curve (curve B). Upon addition of an equimolar amount of KOH relative to cysteine (*i.e.*, $Fe^{2+}:H_3L^+:KOH = 1:2:2$), the rotation in the visible region became negative, but the Cotton effect at ~49,000 cm^{-1} remained and the [M] value at the maximum decreased a little (curve C). The magnitude of this peak was little different from those in curves A and B, even though the carboxylate anion of cysteine was considered to coordinate to the ferrous ion under the condition corresponding to curve C. Upon further addition of KOH, Cotton effects at about 36,000 cm^{-1} and in the visible region appeared. The former Cotton effect is attributed to the n–σ^* transition in the thiol group.[6] The appearance of this Cotton effect may be due to the coordination of sulfur atom in this stage (KOH/H$_3$L$^+$

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(2) M. P. Schubert, *J. Am. Chem. Soc.*, **55**, 4563 (1933).

(3) A. A. Blanchard, *Inorg. Syn.*, **2**, 126 (1946).

(4) J. Lewis, R. J. Irving, and G. Wilkinson, *J. Inorg. Nucl. Chem.*, **7**, 32 (1958).

(5) See, for example, I. P. Dirkx and F. L. J. Sixma, *Rec. Trav. Chim.*, **83**, 522 (1964).

(6) D. W. Urry, D. Miles, D. J. Caldwell, and H. Eyring, *J. Phys. Chem.*, **69**, 1603 (1965).

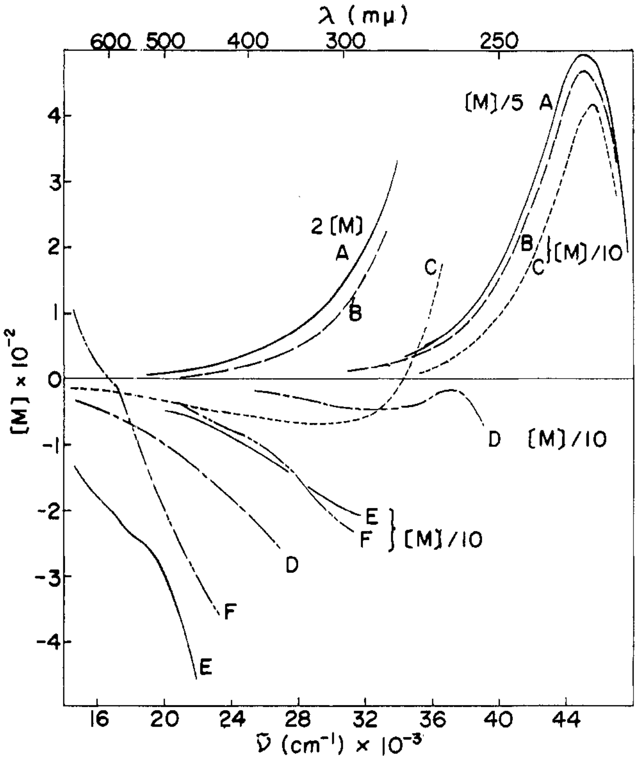

Figure 1.—ORD curves for the Fe^{2+}–$2H_3L^+$–KOH system plotted relative to Fe^{2+} concentration. Mole ratios (Fe^{2+}: H_3L^+:KOH) are: (A) 0:1:0; (B) 1:2:0; (C) 1:2:2; (D) 1:2:3; (E) 1:2:4; (F) 1:2:6.

= 1.5). The small Cotton effect in the visible region is explicitly observed in curves E and F, where the complexes are considered to be chelate compounds as shown in Table I. This Cotton effect is associated with a very weak absorption at about 18,900 cm^{-1} (sh), which corresponds to the spin-forbidden d–d transition in the ferrous ion.[7] These results are consistent with the fact that chelation of the optically active ligand, rather than simple coordination, is required for the vicinal effect on the d–d transition to be observed.[8] This small Cotton effect is not due to the presence of the ferric complex as impurity, since the Cotton effect of the ferric complex is quite different as shown below.

Ferric Complex.—The reaction product between ferrous ion and cysteine was quite easily oxidized at a higher pH region. In order to determine if the reactions were complicated by small amounts of oxygen, the reaction between ferric ion and cysteine was carried out in an atmosphere of oxygen, instead of nitrogen. Figure 2 represents ORD and absorption plots at t = 0, 3, and 6, where t denotes the time from mixing of reagents in minutes. As the rate of oxygen bubbling was not constant and cysteine was added in excess, this figure gives only rough spectra. The effect of cystine (originating from oxidation) on these curves would be much more serious than that of cysteine. However, in the case of curves A and A′, this effect could be neglected through the procedure of extrapolation to zero time, when no oxidation of cysteine occurred. The effect of excess cysteine is relatively small in this region. Plots A and A′ correspond to the spectra of tris(cysteinato)iron(III).[9] The appearance of the shoulder at 25,000 cm^{-1} in curve B′ may indicate the presence of an unknown intermediate complex in this reaction. It can be seen from curve C that cysteine has been completely oxidized to cystine after 6 min. This curve is nearly identical with that of free cystine.[5] This ferric complex was found to be very stable at lower temperatures. A detailed account of the ORD behavior of this system will be the subject of a later publication.[9]

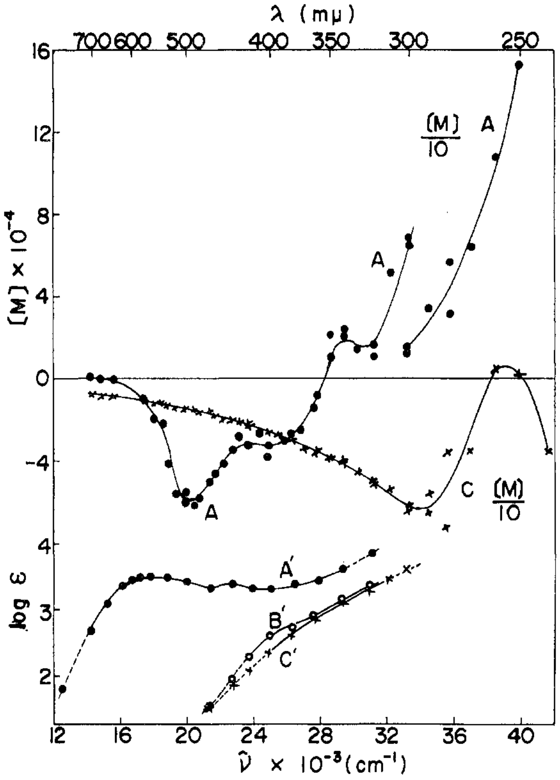

Figure 2.—ORD (A, C) and absorption (A′, B′, C′) plots of the Fe^{3+}–excess H_3L^+ system plotted relative to Fe^{3+} concentration. Fe^{3+} was 0.25 mM and H_3L^+ 25 mM in potassium borate buffer. Times from the mixing of reagents are: A and A′, 0; B′, 3 min; C and C′, 6 min.

Bis(cysteinato)dicarbonyliron(II).—ORD, CD, and absorption curves of this complex in neutral aqueous solution are shown in Figure 3. They have absorption maxima at 20,500 (ε 152), 27,800 (sh), and 35,700 cm^{-1} (ε 10,700), and CD maxima at 21,000 ($\Delta\epsilon$ = +1.6), 28,500 ($\Delta\epsilon$ = −0.6), 34,000 ($\Delta\epsilon$ = −0.5), and 41,000 cm^{-1} ($\Delta\epsilon$ = −3.6). The ORD curve corresponds reasonably to these CD maxima. The absorption band at 35,700 cm^{-1} must be due to the overlap of the n–σ^* transition of the thiol group[6] and the charge transfer from iron to the carbonyl group.[10] The shoulder at 28,000 cm^{-1} can be assigned to the charge transfer from cysteine to iron in comparison with the assignment for the cobalt complex with cysteine.[6] The band of low-

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(7) C. J. Ballhausen, "Introduction to Ligand Field Theory," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 251.

(8) D. C. Bhatnagar and S. Kirschner, *Inorg. Chem.*, **3**, 1256 (1964).

(9) A. Tomita, H. Hirai, and S. Makishima, paper presented at the 10th International Conference on Coordination Chemistry, Nikko, Japan, 1967.

(10) R. T. Lundquist and M. Cais, *J. Org. Chem.*, **27**, 1167 (1962).

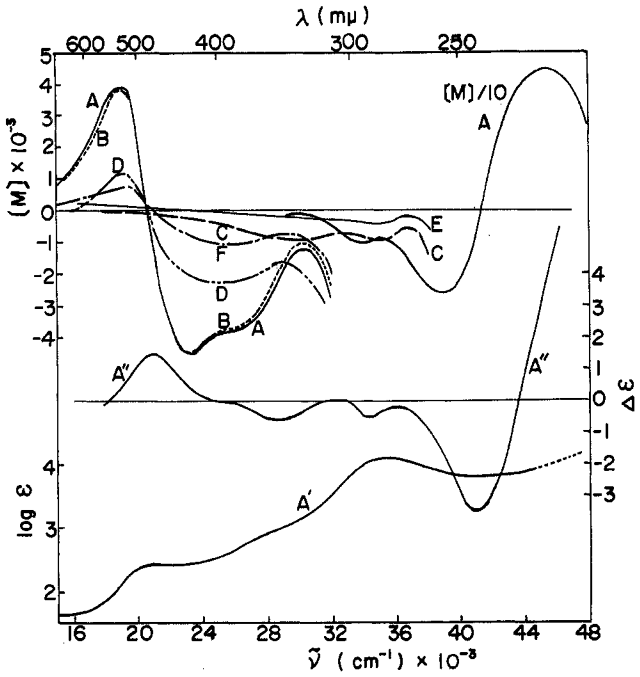

Figure 3.—ORD (A), CD (A''), and absorption (A') curves of the carbonyl complex; ORD curve obtained after bubbling N_2 through solution A for 60 min at 27° (B); N_2 through B for 3 min at 77° (C); CO through C for 60 min at 27° (D); N_2 through D for 3 min at 77° (E); CO through E for 30 min at 27° (F).

est frequency has a relatively larger extinction coefficient than that of the ferrous complex with cysteine and also corresponds to a d–d transition. The positive Cotton effect due to this band exhibits a large molecular amplitude of 8500. The present data are too meager to elucidate completely the origin of this optical activity, but it may be postulated to be due solely to the vicinal effect of cysteine. Supposing this complex had configurational isomers, they should be diastereoisomers because the ligand is optically active. The selective precipitation of either of them was quite probable in the isolation step. If this was the case and the racemization of this isomer did not occur upon dissolution, the CD curve of the solution of isolated complex (curve A'') would be considerably different from that of the solution before precipitation. These curves were, however, nearly identical. Therefore, the possibility that the complex itself is configurationally dissymmetrical may be almost excluded.

Cremer has reported that this complex releases carbon monoxide on illumination with light and picks up carbon monoxide in the dark reversibly.[1] In the present study, similar reversible reactions were also observed in two other ways. When an aqueous solution of this complex in a sealed tube was warmed up to 90°, the solution lost its orange color immediately, but the color was reproduced when the tube was set on ice. The other process was the alternate bubbling of nitrogen and carbon monoxide. Although curve A in Figure 3 was not affected by nitrogen bubbling at 27° for 2 hr (curve B), curve C, where nitrogen was passed at 77°, had no Cotton effect except at about 36,000 cm^{-1}, and this curve was very similar to curve

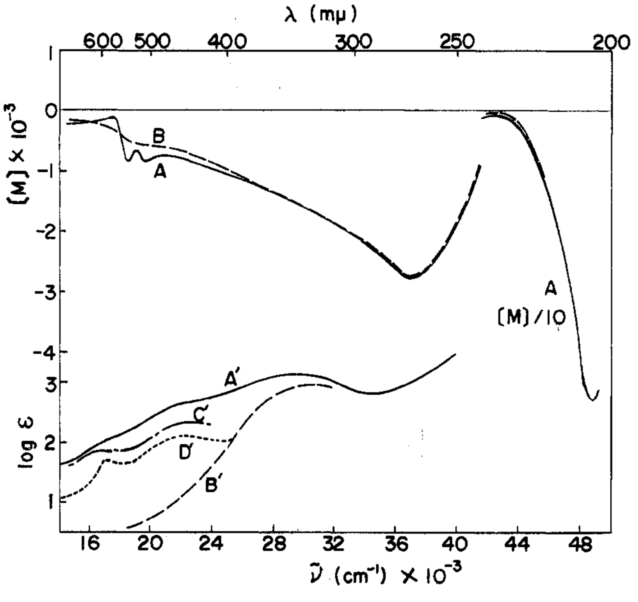

Figure 4.—ORD (A) and absorption (A') curves of nitrosyl complex; curves obtained after bubbling N_2 through solution A for 10 min at 77° (B, B'); absorption curve obtained after bubbling NO through solution B for 20 min at 27° (C'); absorption curve of the Fe(II)–NO complex in the absence of cysteine (D').

D in Figure 1. This indicated that the ferrous complex with cysteine was obtained by the above treatment as was expected. This fading solution became orange again by the introduction of carbon monoxide gas. Thus the reversible reaction between bis(cysteinato)iron(II) and carbon monoxide was clearly demonstrated by ORD measurement.

Cystinatodinitrosyliron(II) Dihydrate.—The ORD curve of the nitrosyl complex shows Cotton effects centering at 18,200 and 19,700 cm^{-1} and a large negative Cotton effect in the ultraviolet region, which is nearly identical with that of cystine. This result, coupled with the conclusion from the infrared spectrum, implies the ligand in this complex is not cysteine but cystine derived from the oxidation of cysteine. Nitric oxide is, in fact, known to act as an oxidizing agent in neutral aqueous solution.[11] The Cotton effects in the visible region are associated with the absorption band at 17,000 cm^{-1} which is attributed to the charge-transfer band in the Fe(II)–NO system.[11] Thus the charge-transfer band (from NO to Fe) can also be optically active owing to the vicinal effect of an intact optically active ligand (cystine in this case). In contrast with the carbonyl complex, the reaction between bis(cysteinato)iron(II) and nitric oxide was irreversible. Once the nitrosyl complex released nitric oxide at 77°, it decomposed into free ferrous ion and cystine. Upon repeated introduction of nitric oxide, the ORD curve was unchanged and absorption bands at 17,100 and 21,500 cm^{-1} (curve C') appeared again, which were identical with those obtained in the absence of cysteine (curve D'); these bands are due to the formation of pentaaquomononitrosyliron[11] from free ferrous ion and nitric oxide.

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(11) W. P. Griffith, J. Lewis, and C. Wilkinson, *J. Chem. Soc.*, 3993 (1958).