substrate and positional selectivity of the He^3H^+ ion, comparable with those observed in the reactions of poorly solvated alkyl ions in aprotic solvents, can be explained on the grounds of the exceedingly high reactivity of the unsolvated, gaseous reagent.

Acknowledgments. The authors wish to thank Professor G. Giacomello for his interest in the present work and useful suggestions and criticisms. S. C. acknowledges a fellowship from the National Research Council (C.N.R.).

Stereospecificity in the Chelation of Aspartic Acid

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Abstract: It has been shown that the steric requirements of D- (or L-) aspartate, functioning as a tridentate ligand, dictate the formation of both geometric and optical isomers in the complex [Co(D- or L-aspartate)(diethylenetriamine)]$^+$. Consideration of the relationship between the optical activity (circular dichroism, CD) resulting from the presence of the asymmetric amino acid in the complex and the chirality described by the chelate rings permits a tentative assignment of configuration to the three isomers found. These assignments are in accord with chromatographic behavior and visible absorption data. It has been found that the CD attributable to chelated aspartic acid in these isomers is similar to that observed for aspartic acid in [Co(L-Asp)$(NH_3)_3$]$^+$. The differences, though, raise some interesting questions concerning the source of optical activity in chelate complexes.

The stereochemistry of complexes of a certain class of amino acids and polypeptides containing these amino acids is being investigated. These amino acids (aspartic acid, glutamic acid, cysteine, homocysteine, methionine, etc.) have three donor sites and are consequently potentially tridentate ligands. Although amino acids functioning as bidentates have been extensively investigated,[2] little work on tridentate amino acids has been reported and none with cobalt(III) complexes of these acids. Proton nmr contact shifts[3] and X-ray crystallography[4] were used to establish the tridentate nature of histidine complexes of certain dipositive metal ions, and infrared spectroscopy supported tridentate coordination of aspartic acid in the bisaspartatocopper(II) anion.[5]

These chelated tridentate amino acids are expected to have some interesting stereochemical properties not found for the bidentate amino acids. Consider the geometric and optical isomers of [Co(D-Asp)(dien)]$^+$ (D-Asp = D-aspartate and dien = diethylenetriamine) which might be expected to form (Figure 1) without taking into consideration the steric requirements of D-aspartate. Indeed, with a similar ligand, such as $^-OOCCH_2CH_2NHCH_2COO^-$, all the isomers shown in Figure 1 would be expected, and, for the symmetrical ligand iminodiacetic acid (IDA) in the analogous complex [Co(IDA)(dien)]$^+$, the three expected geometric isomers were found:[6] *trans* (I in Figure 1), s-*cis* (II), and u-*cis* (III and IV identical).

An examination of Hirschfelder molecular models shows that D- (or L-)aspartate can only span a face and furthermore will show absolute stereospecificity on coordination. For the isomer formed from D-aspartate shown in Figure 2, the six- and five-membered rings must be on the left and right, respectively. In the other configuration (six- and five-membered rings reversed), the rings are extremely strained. That is, only one optical isomer of II, III, and IV can form with a given antimere of the amino acid: D-aspartate[7] (Figure 2) should form II-A, III-A, and IV-B; and L-Asp, II-B, III-B, and IV-A. This report is concerned with the preparation, isolation, and study of the circular dichroism (CD) of these isomers and of the related complex [Co(L-Asp)$(NH_3)_3$]$^+$.

Experimental Section

Silver D-Aspartate. Ag_2D-Asp (D- and L-aspartic acid from K & K Laboratories and Aldrich Chemical Co., respectively) was prepared using a method previously described for the preparation of silver salts of aminodicarboxylic acids.[6] The salt was kept shielded from the light to prevent darkening.

s-*cis*-D-Aspartato(diethylenetriamine)cobalt(III) Nitrate. During the following operations, the solution was constantly stirred between 65 and 67°. [$CoCl_3$(dien)],[8] 8.06 g (0.03 mole), was dissolved in 150 ml of water, and 3 g of decolorizing charcoal was added. After 10 min Ag_2D-Asp, 10.4 g (0.03 mole), was added in small portions (5 to 10 min), and the solution was stirred 10–15 min. A solution of 5.30 g (0.0312 mole) of silver nitrate in 30 ml of water was added dropwise (5 min). After 5 min the stirring was stopped, and the solution was allowed to cool for 2 hr. The silver chloride and charcoal were removed by filtration (medium fritted disk), and the solution was evaporated in air to about 20 ml. The red-orange

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(2) See, for example, D. A. Buckingham, J. P. Collman, D. A. R. Happer, and L. G. Marzilli, *J. Am. Chem. Soc.*, **89**, 1082 (1967); L. G. Marzilli and D. A. Buckingham, *Inorg. Chem.*, **6**, 1042 (1967); R. G. Asperger and C. F. Liu, *ibid.*, **6**, 796 (1967); J. I. Legg, D. W. Cooke, and B. E. Douglas, *ibid.*, **6**, 700 (1967); M. D. Alexander and D. H. Busch, *ibid.*, **5**, 1590 (1966); R. G. Denning and T. S. Piper, *ibid.*, **5**, 1056 (1966); J. H. Dunlop and R. D. Gillard, *Advan. Inorg. Chem., Radiochem.*, **9**, 204 (1966), and references therein.

(3) C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc.*, **85**, 3736 (1963).

(4) K. A. Fraser, H. A. Long, R. Candlin, and M. M. Harding, *Chem. Commun.*, 344 (1965).

(5) S. Kirschner, *J. Am. Chem. Soc.*, **78**, 2372 (1956).

(6) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **5**, 594 (1966).

(7) For determination of absolute configurations of amino acids, see J. C. Speakman, *Progr. Stereochem.*, **2**, 19 (1958).

(8) P. H. Crayton, *Inorg. Syn.*, **7**, 207 (1963).

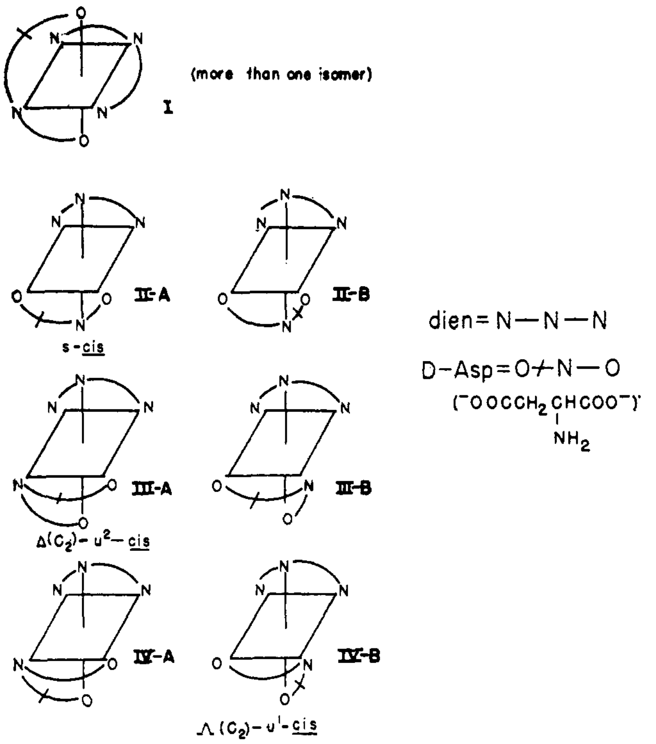

Figure 1. Geometric isomers expected for $[Co(D\text{-}Asp)(dien)]^+$ without considering steric requirements of D-aspartic acid and those found (II-A, III-A, and IV-B).

precipitate which formed was stirred, filtered, and washed with 5 ml of ice water, 5 ml of 50% ethanol, ethanol, and acetone. The air-dried yield was 2.3 g. The isomer was purified by dissolving it in 12 ml of water at 50° and reprecipitating by the dropwise addition of 25 ml of ethanol. The s-*cis*-$[Co(D\text{-}Asp)(dien)]NO_3$ was filtered and washed as before to give 0.9 g. *Anal.* Calcd for $[CoC_8H_{18}N_4O_4]NO_3 \cdot H_2O$: C, 25.74; H, 5.40; N, 18.77. Found: C, 25.75; H, 5.45; N, 18.92.

Chromatography of the Solution of Isomers. After removal of some of the s-*cis* isomer as described above, 15 mmoles (half the original amount of complexed cobalt(III) present) of the filtrate diluted to 400 ml was added (1 ml/min) to a strong acid ion-exchange column (diameter = 4 cm, height of resin = 55 cm) containing 1600 mequiv of Dowex 50W-X8, 100–200 mesh, resin in the sodium form which had been uniformly packed by backwashing.[9] The adsorbed complex formed an even (this is very important) 1-cm band at the top of the column. A violet substance was eluted with water. The band was then eluted with 0.35 *M* $NaClO_4$ (0.3–0.4 ml/min) over a period of 35 days. The band separated into a red-orange band which eluted well before two closely spaced but completely separated red bands. A brown-orange band remained at the top of the column. The three eluted bands were collected in fractions, and circular dichroism spectra showed that each band consisted of only one isomer.

u¹-*cis*-, u²-*cis*-, and s-*cis*-D-Aspartato(diethylenetriamine)cobalt(III) Perchlorates. Evaporation of the more concentrated fractions with simultaneous removal of some of the $NaClO_4$ yielded the perchlorate salts of the isomers in some cases. Further extraction of the fractions with ethanol as previously described for the isolation of *cis*-$[Co(EDDA)(en)]ClO_4$[10] yielded more product. The yields of the various fractions were combined; each isomer was dissolved in a minimum amount of water and reprecipitated with an excess of ethanol. The yields of the perchlorate salts after filtering and washing with ethanol and acetone were u¹-*cis*, 0.11 g; u²-*cis*, 0.5 g; and s-*cis* 0.4 g. *Anal.* Calcd for u¹-*cis*-$[CoC_8H_{18}N_4O_4]ClO_4 \cdot {}^3/_4H_2O$: C, 23.66; H, 4.84; N, 13.80. Found: C, 23.72; H, 4.82; N, 13.81. Calcd for u²-*cis*- and s-*cis*-$[CoC_8H_{18}N_4O_4]ClO_4$: C, 24.47; H, 4.62; N, 14.27. Found for u²-*cis*: C, 24.16; H, 4.69; N, 14.37. Found for s-*cis*: C, 24.13; H, 4.59; N, 14.40.

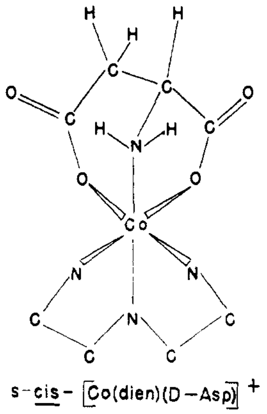

Figure 2. Perspective of chelated aspartic acid.

L-Aspartato(diethylenetriamine)cobalt(III) Isomers. The procedure was repeated as a check using L-aspartic acid. The solution was chromatographed on analytical grade resin, AG 50W-X8 (Bio-Rad), 200–400 mesh, with 0.2 *M* $NaClO_4$ over a period of 66 days. Some improvement in the sharpness of the bands was noted, and the three isomers found had circular dichroism spectra which were mirror images of the spectra obtained for the D-aspartato analogs.

L-Aspartatotriamminecobalt(III) Nitrate. The preparation of the solution containing the complex was the same as that used for s-*cis*-$[Co(D\text{-}Asp)(dien)]NO_3$ (the quantities of reagents were scaled down to one-third) except that $[CoCl_2(H_2O)(NH_3)_3]Cl$[11] (2.34 g, 0.010 mole) was used in place of $[CoCl_3(dien)]$ and 2.5 g of urea was added prior to the addition of Ag_2L-Asp. Evaporation yielded an oil, but the desired product was isolated chromatographically. The reaction solution was added to a strong acid (50W-X8, 100–200 mesh, Na^+ form) column (diameter = 4 cm, height of resin = 15 cm). A large volume of purple solution eluted with water. The adsorbed band was then eluted with 0.3 *M* $NaClO_4$ and separated into a major red band containing the desired complex, several small bands, and a brown-orange band retained by the resin. Circular dichroism spectra of the fractions obtained for the red band showed that one complex was present. The impure perchlorate salt which precipitated on evaporation of the combined fractions was purified by passing it through a strong base (Dowex 1-X8) column in the nitrate form. The eluent was evaporated to 3 ml, and the precipitated nitrate salt was filtered and washed with 50% ethanol, ethanol, and acetone and air-dried. The $[Co(L\text{-}Asp)(NH_3)_3]NO_3$ was further dried in a vacuum desiccator over calcium sulfate, yield 0.4 g. *Anal.* Calcd for $[CoC_4H_{14}N_4O_4]NO_3$: C, 15.83; H, 4.66; N, 23.15. Found: C, 15.86, 15.91; H, 4.80, 4.72; N, 23.01, 23.05. It was estimated that the complex constituted about 15% of the original reaction mixture.

Spectra. The visible absorption and circular dichroism spectra were recorded on a Cary Model 14 spectrophotometer and a JASCO Model ORD/UV-5 with CD attachment, respectively. Aqueous solutions (5.53×10^{-3} *M*) of the $[Co(Asp)(dien)]ClO_4$ isomers and $[Co(NH_3)_3(Asp)]NO_3$ were used for both measurements.

Results and Discussion

The nomenclature adopted for the isomers of $[Co(IDA)(dien)]^+$ has been used for the aspartic acid analogs (Figure 1). The superscripts on the u-*cis* isomers, u¹ and u², are used to distinguish between geometric isomers III and IV, where the numbers refer to the order of chromatographic elution. These isomers represent the first reported examples of optically active bistridentate chelate complexes of cobalt(III).

Assignment of Configuration to the Isomers. Consideration of the three isomers of $[Co(D\text{-}Asp)(dien)]^+$ (Figure 1) would predict that III-A and IV-B should be quite similar, in fact, pseudo-mirror-image isomers (*vide infra*). This suggests that the s-*cis* isomer was the first to elute, followed by the closely spaced

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[9] "Dowex-Ion Exchange," The Dow Chemical Co., Midland, Mich., 1964.

[10] J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **4**, 1576 (1965).

[11] G. Schlessinger, *Inorg. Syn.*, **6**, 180 (1960).

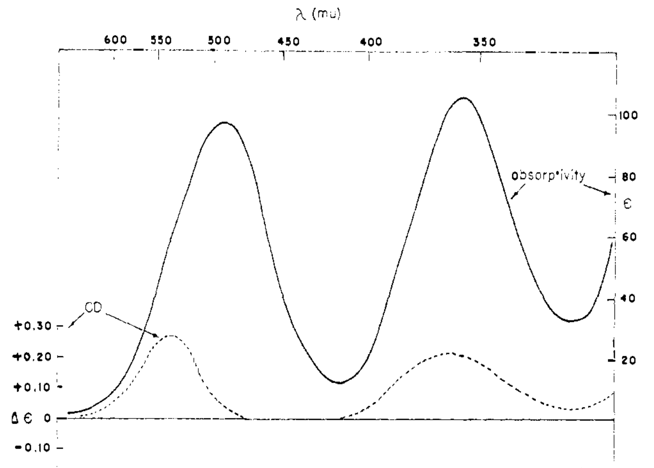

Figure 3. Visible and circular dichroism spectra of $[Co(\text{L-Asp})(NH_3)_3]^+$.

u^1-*cis* and u^2-*cis* isomers. This is in accord with the elution pattern found for the analogous *cis* isomers of $[Co(IDA)(dien)]^+$ where the isomers could be identified through their nmr spectra.[6] Comparison of the visible spectra between the Asp and IDA isomers supports this observation. As would be anticipated the spectra of the three isomers of $[Co(\text{D-Asp})(dien)]^+$ and $[Co(\text{L-Asp})(NH_3)_3]^+$ are quite similar and are exemplified by the spectrum of the latter (Figure 3). However, there are some systematic differences (Table I). The low-energy band (related to T_{1g} in O_h symmetry), $\bar{\nu}_1$, of the u-*cis* isomers, where the secondary amines are *cis*, occurs at lower frequency than $\bar{\nu}_1$ for the s-*cis* isomers, secondary amines *trans*. As expected $\bar{\nu}_1$ for $[Co(\text{L-Asp})(NH_3)_3]^+$ occurs at lower frequency than for the diethylenetriamine analog reflecting the weaker crystal field expected for the three ammines. Although the relative intensities of the absorption bands of the IDA isomers are consistent with the removal of the plane of symmetry in going from the s-*cis* to the u-*cis* isomer,[6] no such correlation is found or expected for the aspartate isomers since all are asymmetric.

Table I. Visible Absorption Spectra

Isomer	$\bar{\nu}_1{}^a$ (ϵ_1)	$\bar{\nu}_2$ (ϵ_2)
$[Co(Asp)(NH_3)_3]^+$	20.2 (102)	28.1 (122)
s-*cis*-$[Co(Asp)(dien)]^+$	20.8 (100)	28.4 (81.8)
u^1-*cis*-$[Co(Asp)(dien)]^+$	20.3 (94.6)	28.4 (91.7)
u^2-*cis*-$[Co(Asp)(dien)]^+$	20.3 (101)	28.4 (109.0)
s-*cis*-$[Co(IDA)(dien)]^+$	20.6 (72)	28.6 (85)
u-*cis*-$[Co(IDA)(dien)]^+$	19.5 (105)	28.0 (124)

[a] $\bar{\nu}$ in $cm^{-1} \times 10^{-3}$.

Of greater importance, though, is the relative behavior of the circular dichroism (CD) spectra of the three isomers of $[Co(\text{D-Asp})(dien)]^+$. In addition to substantiating the above assignments, CD offers a means by which the two u-*cis* isomers can be distinguished and offers insight into the factors which contribute to optical activity in octahedral complexes.

Whereas s-*cis*-$[Co(IDA)(dien)]^+$ is optically inactive, the substitution of D- (or L-) aspartate for IDA renders this isomer active. Using the terminology adopted by Liu and Douglas,[12] aspartate is said to exhibit a vicinal (effect) contribution to the observed optical activity as compared to a configurational contribution resulting from an asymmetric distribution of chelate rings, *e.g.*, as in the optical isomers of $[Co(en)_3]^{3+}$. It should be noted that in determining the configurational contribution in a complex only the relative distribution and not the nature of the chelate rings is being considered.[13] If a particular ring is asymmetric as for a chelated *l*-propylenediamine ring, then this asymmetry is associated with the vicinal contribution.[14] Liu and Douglas postulated that to a first approximation the vicinal and configurational effects, if both occur in an isomer, should be additive.[12] The validity of such an assumption has been demonstrated by several examples.[12,14-16]

The two u-*cis* isomers (III-A and IV-B in Figure 1) are then mirror images of each other if only the configurational contribution is considered. The difference arises from the presence of D-aspartate in both isomers. That is, the two isomers should have opposite configurational contributions but approximately equal vicinal contributions. The following relationship between the optical activity of the three isomers might then be expected.

$$\text{s-}cis\text{(II-A)} \approx [\text{u}^1\text{-}cis\text{(IV-B)} + \text{u}^2\text{-}cis\text{(III-A)}]/2$$

The experimental circular dichroism spectra obtained for the three isomers are shown in Figure 4. The curves of the two u-*cis* isomers have been added together and the resulting curve divided by two. Comparison of this curve with that obtained for the s-*cis* isomer shows that although they are not quantitatively the same, they are quite similar, having the same general shape and magnitude, the same sign (negative in all regions for D-aspartate), and maxima in the first (T_{1g}) and second (T_{2g}) absorption band regions.

The relationship between the CD curves confirms the assignments suggested by the chromatographic elution pattern and visible spectra. Although the u-*cis* isomers lack any elements of symmetry, a tentative assignment of absolute configuration can be made if the symmetry is approximated by C_{2v} (removal of chelate rings to give *cis*-$[CoA_4B_2]$). Mason and co-workers[17] have shown that for *cis*-$[CoA_4B_2]$ complexes where A > B in the spectrochemical series that the electronic transition to the A_2 (C_{2v}) state which has the same orbital construction as the dominant E_a (D_3) transition in $[Co(en)_3]^{3+}$ (for which the absolute configuration is known; E_a positive for $\Delta(C_2)$-$[Co(en)_3]^{3+}$)[18] occurs at higher energy in the T_{1g} (O_h) region (*i.e.*, closer to the parent compound, $[Co(A)_6]$, transition) than the B_1 (C_{2v}) transition. Using the octant sign[19] or the ring-pairing method to relate the configurations,[13] the tentative assignments of absolute configuration are $\Lambda(C_2)$ to the u^1-*cis* isomer (the first eluted of the two u-*cis* isomers) with a negative CD

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(12) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964).

(13) J. I. Legg and B. E. Douglas, *J. Am. Chem. Soc.*, **88**, 2697 (1966).

(14) B. E. Douglas, *Inorg. Chem.*, **4**, 1813 (1965).

(15) D. A. Buckingham, S. F. Mason, A. M. Sargeson, and K. R. Turnbull, *ibid.*, **5**, 1649 (1966); B. E. Douglas and S. Yamada, *ibid.*, **4**, 1561 (1965).

(16) J. I. Legg, D. W. Cooke, and B. E. Douglas, *ibid.*, **6**, 700 (1967).

(17) A. J. McCaffery, S. F. Mason, and B. J. Norman, *J. Chem. Soc.*, 5094 (1965).

(18) A discussion of the terminology, $\Delta(C_2)$, $\Lambda(C_3)$, etc., is given in ref 13 and 17.

(19) C. J. Hawkins and E. Larsen, *Acta Chem. Scand.*, **19**, 185 (1965).

component at 20,700 cm^{-1} (Figure 4) and $\Delta(C_2)$ to the u^2-*cis* isomer (second eluted) with a positive CD component at 21,100 cm^{-1}. The absolute configuration of s-*cis*-[Co(dien)(D-Asp)]$^+$ is automatically determined. These assignments are shown in Figure 1.

Optical Activity Associated with Chelated Aspartic Acid. For [Co(L-Asp)(NH$_3$)$_3$]$^+$, a CD spectrum quite similar to that of the diethylenetriamine analog is anticipated. Although the signs of the components (keeping in mind that different antimeres of aspartic acid are present in each isomer) and the general appearance of these spectra are the same (Figures 3 and 4) a shift of the T_{1g}-related CD component to lower energies is observed for the triammine complex (Table II). In fact the CD spectrum of this complex in the T_{1g} region[20] is closer to the vicinal curve obtained by averaging the u-*cis* isomer curves (Figure 4 and Table II).

Table II. Comparison of CD Spectra

Isomer	$\bar{\nu}_1{}^a$ ($\Delta\epsilon_1$)	$\bar{\nu}_2$ ($\Delta\epsilon_2$)
s-*cis*-[Co(D-Asp)(dien)]$^+$	20.4 (−0.75)	27.7 (−0.15)
(u^1-*cis* + u^2-*cis*)/2	19.6 (−0.46)	29.2 (−0.09)
s-*cis*-[Co(L-Asp)(NH$_3$)$_3$]$^+$	18.6 (0.28)	27.8 (0.22)

^{a} $\bar{\nu}$ in cm^{-1} × 10^{-3}.

It would appear then that there is a vicinal contribution associated with the chelated diethylenetriamine which is partially cancelled when the CD curves for the u-*cis* isomers are averaged, leaving primarily the contribution associated with chelated aspartate. Models show that the two rings of diethylenetriamine can assume a variety of conformations (kk, kk′, or k′k′ in the limit),[21] and it is expected that in these isomers there is some preferred asymmetric conformation for this molecule. It has been suggested that ring conformations make an important contribution to optical activity in chelate complexes,[22] and evidence for this has been obtained.[23]

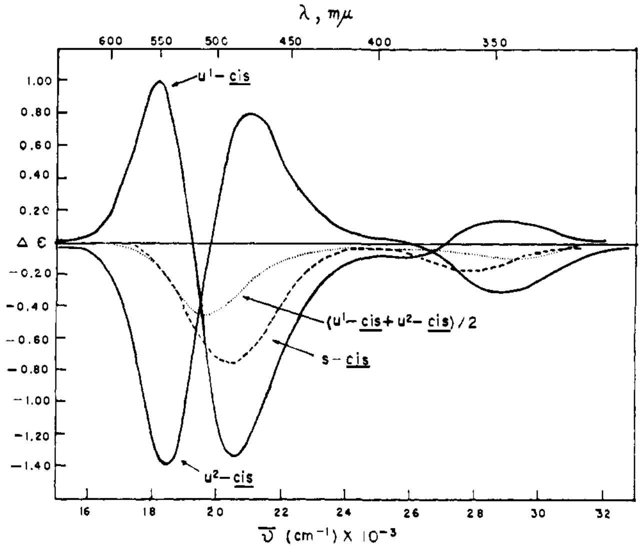

Figure 4. CD spectra of [Co(D-Asp)(dien)]$^+$ isomers.

On the other hand, aspartic acid is quite rigidly held when functioning as a tridentate chelate and forms less puckered rings than diethylenetriamine. It might then be expected that chelated aspartate makes a relatively constant contribution to the optical activity in these isomers. Investigation now in progress of the isomers of [Co(L-Asp)$_2$]$^-$ shows that the *trans* isomer for which only a vicinal contribution is expected (analogous to II-A in Figure 1) has a CD spectrum opposite in sign and similar in appearance (but shifted to lower energy due to a decrease in the over-all crystal field) to the vicinal contribution found for aspartate in the complexes discussed in this report.

Further study of complexes containing aspartic acid and analogous tridentate amino acid ligands should help to establish whether there is a systematic contribution to optical activity by these ligands in chelate systems.

Acknowledgments. This investigation was supported in part by funds provided for biological and medical research by State of Washington Initiative Measure No. 171.

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[20] The CD components in the T_{2g} region are not expected to be related to configuration; see discussion in ref 17.

[21] E. J. Corey and J. C. Bailar, Jr., *J. Am. Chem. Soc.*, **81**, 2620 (1959).

[22] F. Woldbye, *Proc. Roy. Soc.* (London), **A297**, 79 (1967); S. F. Mason, A. M. Sargeson, R. Larsson, B. J. Norman, A. J. McCaffery, and G. H. Searle, Proceedings of the Ninth International Conference on Coordination Chemistry, St. Moritz, 1966, p 509.

[23] D. A. Buckingham, P. A. Marzilli, A. M. Sargeson, S. F. Mason, and P. G. Beddoe, *Chem. Commun.*, 433 (1967).