Optically Active Co-ordination Compounds. Part 41.[1] Bis(dipeptidato)-cobalt(III) Complexes

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The preparation and separation of the meridional isomers of complexes of the type $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ is described [$H\alpha_1\text{-}H\alpha_2$ is a dipeptide, $H_2NCHR'CONHCHR^2CO_2H$, ranging from glycylglycine (gly-gly) to L-phenylalanyl-L-phenylalanine (L-phe-L-phe)]. With L-α_1-L-α_2, L-α_1-gly, or gly-L-α_2, each of the several preparative methods yields meridional $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ as a mixture of its two diastereoisomers which have been separated by ion-exchange chromatography. All the preparative methods give minor products which can be removed by ion-exchange chromatography. The meridional isomer of the complexes has been characterised by electronic, ^1H n.m.r., and circular dichroism (c.d.) spectroscopy. The c.d. spectra of the pairs of diastereoisomers are discussed. Detailed comparison of the ^1H n.m.r. spectrum of the pairs of diastereoisomers enabled determination of their absolute configuration unambiguously for $[Co(L\text{-phe-glyO})_2]^-$ and $[Co(gly\text{-}L\text{-pheO})_2]^-$ [L-phe-glyO = L-phenylalanylglycinate(2−)] and for the others by comparison. The protons in the C-terminal residue are activated to exchange in alkaline solution. Knoevenagel reactions have been performed with the bis(glycylglycinato)-complex.

THE relative stabilities of diastereoisomeric transition-metal complexes with optically active ligands are of interest for several reasons. We have been particularly concerned with attempting to understand the stereochemical factors responsible for the high stereoselectivity of reactions catalysed by metalloenzymes, and our efforts in so-called model systems have therefore been concentrated on such ligands as amino-acids, hydroxy-acids, and to a smaller extent on aminoalcohols, such as ephedrine, and on 1,2-diamines.[2] It is clearly of importance to establish the stereochemistries and relative stabilities of complexes of metal ions with peptides. In this paper we follow our earlier work on the isolation, resolution,[3] and crystal structure [4] of the bis(glycylglycinato)-complex of cobalt(III) with an account of the stereoselectivity and reactivity of the bis complexes of a number of dipeptides with Co^{III}. An earlier paper [5] described some results on oxygen uptake by the cobalt(II)–dipeptide systems to give the same cobalt(III) dipeptide complexes and no further attention is given here to that aspect of the research.

In order to compare the influence of the size and nature of the amino-acid side chain on the properties of the bis-(dipeptidato)cobalt(III) complexes, and especially on the n.m.r. and c.d. spectra, the complexes of the peptides containing glycine (gly), L-alanine (L-ala), L-leucine (L-leu), and L-phenylalanine (L-phe) were all studied in detail, as was also $[Co(L\text{-ala-}D\text{-alaO})_2]^-$ [L-ala-D-alaO = L-alanyl-D-alaninate(2−)].

RESULTS AND DISCUSSION

Some Comments on the Preparative Methods.—The complicated consecutive reactions which occur in the oxygenation of solutions containing Co^{II} and dipeptides have already been discussed [5] and presumably similar complications must occur in the other preparative methods which involve cobalt^{II}. Following the early experiments by Gilbert *et al.*,[6] most other workers [7–11] have used oxygenation of Co^{II} to prepare bis(dipeptidato)cobaltate(III) complexes, although Beck and Gorog [7] also synthesised $[NH_4][Co(gly\text{-}glyO)_2]$ [gly-glyO = glycylglycinate(2−)] by the reaction of glycylglycine with $[Co(NH_3)_6]Cl_3$ and prepared a solution of what was presumably the same complex by the reaction [12] of gly-gly with 'cobalt(III) hydroxide'. For the purpose of the present paper the important fact is that the final product of all the preparations is the magenta singly charged anion *mer*-bis(dipeptidato)cobaltate(III), in which the peptide groups are almost planar. This has been established for the glycylglycinato-complex [4,9] by two independent *X*-ray crystallographic studies, and the determination of the crystal structure of one diastereoisomer of the glycyl-L-alaninato-complex is at present under way.[13] However, we have found no evidence that at high pH a bis(dipeptidato)(hydroxo)cobaltate(III) complex $[Co(gly\text{-}glyO)_2(OH)]^{2-}$ is formed in significant quantity, although it has recently been suggested on the basis of rather inconclusive evidence that significant amounts of this must be formed.[14]

It is perhaps an interesting comment on the stability of bis(dipeptidato)cobaltate(III) ions that they are formed by so many different reactions: from Co^{II}; by displacement of various unidentate ligands from Co^{II} by a dipeptide; and by disproportionation of the triammine-monodipeptidatocobalt(III) or diethylenetriaminemono-dipeptidatocobalt(III) complexes.[15] These ions are also formed in non-aqueous solution from hexakis(urea)-cobalt(III) perchlorate. However, dipeptides failed to react with $(+)[Co(en)_3]^{3+}$ at 50 °C for 12 h even in the presence of charcoal, so this potentially stereoselective route to resolved $[Co(gly\text{-}glyO)_2]^-$ is not possible.

The N-terminal-substituted dipeptides we have studied give the two diastereoisomers in high yield. However, the diastereoisomers do not always separate completely on the QAE-Sephadex-A25 resin and it is necessary to collect fractions, and check the purity by the circular dichroism (c.d.) or ^1H n.m.r. spectrum. Diastereoisomeric complexes from C-terminal-substituted dipeptides can be separated more easily but, particularly when the side chain is large (*e.g.* with glycyl-L-phenylalanine), the preparations are much less clean, and the yields of

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mer-bis(dipeptidato)cobaltate(III) are lower. This is particularly so in the case of preparations commencing with aqueous Co^{II} and is a result of the slower rate of oxygenation and greater stability or inertness of the oxygen-bridged complexes when the peptide is C-terminal substituted. These dimeric peroxo-species are best removed by preliminary chromatography on Sephadex G-10, when they are eluted before the monomers.

The oxygen-bridged dimers are not the only by-products. With each combination of peptide and preparative method tried there are also minor quantities (up to *ca.* 10% of the total) of a number of other products. Some of these are doubtless complexes formed by degradation products of the peptide or by trace impurities in the peptide as supplied. These by-products will be discussed in a later paper. The yields of these minor products depend very much on the peptide (being larger with glycylglycine and with dipeptides of the type gly-L-α whereas only trace amounts are formed with peptides with substituents in the N-terminal residue) and on the preparative method. The methods starting from Co^{II} give larger amounts of the minor products while the use of sodium tris(carbonato)cobaltate(III) gives less, and cobalt(III) hydroxide oxide gives very little of these products. Thus for the present study the preferred preparation is that from cobalt(III) hydroxide oxide since this gives the highest yield of the monomeric *mer*-isomer, and reduces problems of purification. The major disadvantage is the long time (*ca.* 1 week for the less water-soluble peptides) required for complete reaction.

Since it was usually very difficult to isolate crystalline bis(dipeptidato)cobaltate(III) complexes, we had to develop methods which give a solution containing only the required complex. Chromatography * on the dextran gel Sephadex G-10 readily removes both the brown dimeric intermediates and (for most dipeptides) the free peptide and inorganic salts from bis(dipeptidato)-cobaltate(III). Notable exceptions to this are complexes of peptides containing aromatic substituents: these complexes are adsorbed more strongly on Sephadex G-10 and are less easily separated from inorganic salts. Partial separation of the diastereoisomers of $[Co(L\text{-ala-gly}O)_2]^-$ also occurs on Sephadex G-10, ' diastereoisomer b ' being slightly more strongly adsorbed than ' diastereoisomer a ' (see later for discussion of diastereoisomers). We have also used alumina to separate the diastereoisomers of $[Co(L\text{-ala-}L\text{-ala}O)_2]^-$; the use of alumina for the separation of $[Co(gly\text{-}L\text{-ala}O)_2]^-$ has been reported.[11]

The bis(dipeptidato)cobaltate(III) complexes often cannot be obtained crystalline and the solid samples may be very hygroscopic; thus the solution of the complexes has been considered pure if it was homogeneous when chromatographed on Sephadex G-10 and QAE-Sephadex, if it showed a constant electronic absorption spectrum (the ratio of the absorption coefficients for the higher- and lower-energy *d–d* transitions is a particularly useful check), and if the ^1H n.m.r. spectrum showed the absence of free peptide and of diastereoisomeric complexes. Evaporation of the pure solution to a glass and measurement of the ratio of the cobalt : carbon : nitrogen content was also useful.

Characterisation and Physical Properties.—Electrophoresis shows that all these bis(dipeptidato)-complexes are anionic at neutral pH, as has been well established for the bis(glycylglycinato)cobaltate(III) complex.[8,18] However, the use of large cations, *e.g. cis*-$[Co(en)_2(NO_2)_2]^+$ and $[Co(en)_3]^{3+}$ (en = ethylenediamine), to crystallise these anionic complexes was unsuccessful.

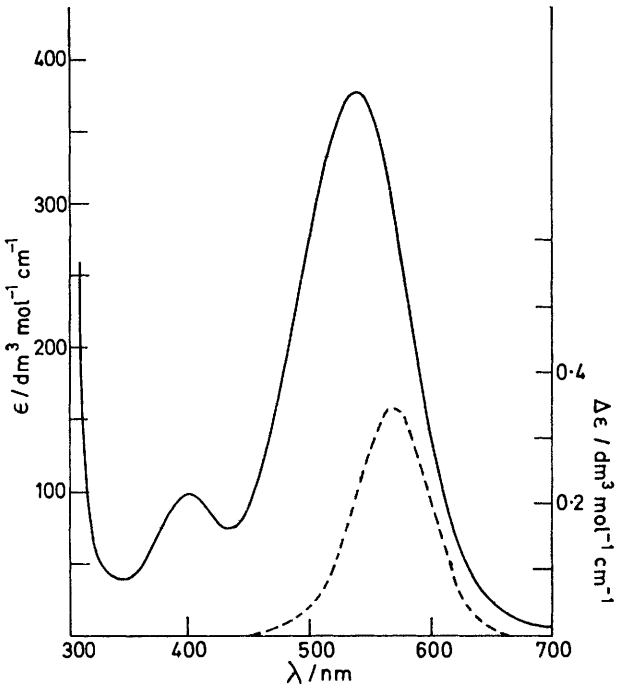

FIGURE 1 Electronic-absorption (—) and c.d. spectra (- - -) of $(+)[Co(gly\text{-}glyO)_2]^-$

Like the glycylglycinato-complex,[9] the complexes containing substituted peptides are cationic at pH 0.

The electronic spectrum of $[Co(gly\text{-}glyO)_2]^-$ has already been reported;[19,20] it is typical of a cobalt(III) chromophore of the type CoN_4O_2 having two *d–d* transitions at 19 200 and 25 200 cm^{-1} (Figure 1). With most cobalt(III) chromophores, however, these two transitions have about equal intensity whereas for the bis-(dipeptidato)cobaltate(III) complexes, and also the bis(tripeptidato)cobaltate(III) complexes which we have also prepared,[21] the lower-energy transition has an enhanced intensity (ε 350—400 dm^3 mol^{-1} cm^{-1}) while the higher-energy transition has an intensity more typical of cobalt(III) complexes (ε *ca.* 80—100 dm^3 mol^{-1} cm^{-1}). The absorption maxima and the molar absorption coefficients for these complexes are given in Table 1. The ratio of the absorption coefficients for the lower- and higher-energy *d–d* bands of $[Co(gly\text{-}glyO)_2]^-$ (3.8) is

* We have found Sephadex G-10 to be invaluable for preliminary purification of a wide range of cobalt(III) and rhodium(III) complexes [16,17] with ligands such as ammonia, cyanide, amino-acids, peptides, diamines, phenanthroline, carboxylic acids, *etc.*

much higher than observed [15] for $[Co(NH_3)_3(gly\text{-}glyO)]^+$ (1.8) and higher than for $[Co(dien)(gly\text{-}glyO)]^+$ (2.9 : 1). The presence of impurities, particularly of free peptide or oxygen-bridged complexes, increases the absorption in the u.v. region, and tends to obscure the higher-energy *d–d* band; in pure samples this is clearly defined.

partially resolved this complex, and our present results differ in two respects. First, we obtained the dextrorotatory enantiomer (in an optical purity of *ca.* 35%) in the first fractions eluted from the starch column, whereas the earlier work [3] found this isomer to be more firmly adsorbed. This difference may arise from the use of a different solvent; a 30% ethanol–water mixture was used in the early work. However, we also find that the maximum of the c.d. spectrum is at 555 nm for both the early and late fractions whereas a maximum at rather higher wavelength (570 nm) was found before; * moreover, the o.r.d. curve has a more-intense positive component and a less-intense negative component in the lower-energy *d–d* band.

TABLE 1

Absorption and c.d. spectra of bis(dipeptidato)cobaltate(III) complexes

Peptide		Absorption maxima (λ/nm)		C.d. maxima (λ/nm)			Configurational contribution to c.d. at 570 nm	Absolute configuration	Evidence for absolute configuration [a]
gly-gly		520 (375)	394 (100)	555 (+0.35) [b]			+	$R(C_2)$	III
L-ala-gly	a	522 (370)	395 (70)		502 (−2.67)	396 (+0.70)	−	$S(C_2)$	II
	b	522 (370)	395 (105)	568 (+1.45)	499 (−3.57)	406 (+1.55)	+	$R(C_2)$	II
gly-L-ala	a	520 (365)	392 (86)		508 (−447)	406 (+0.56)	−	$S(C_2)$	III
	b	520 (361)	394 (115)	574 (+0.32)	502 (−2.09)	390 (−0.63)	+	$R(C_2)$	III
L-leu-gly	a	524 (413)	396 (91)		504 (−3.04)	402 (+0.89)	−	$S(C_2)$	II
	b	525 (388)	396 (101)	575 (+1.34)	502 (−4.33)	405 (+1.46)	+	$R(C_2)$	II
gly-L-leu	a	524 (393)	398 (94)		520 (−4.22)	418 (+0.16)	−	$S(C_2)$	III
	b	525 (357)	399 (103)		506 (−2.68)	392 (−0.77)	+	$R(C_2)$	III
L-phe-gly	a	525 (418)	398 (103)		509 (−1.71)	395 (+0.27)	−	$S(C_2)$	I,II
	b	527 (376)	400 (100)	575 (+1.86)	504 (−5.10)	408 (+1.56)	+	$R(C_2)$	I,II
gly-L-phe	a	525 (406)	397 (111)		512 (−5.11)	408 (+0.81)	−	$S(C_2)$	I
	b	525 (351)	400 (125)		508 (−4.60)	387 (−0.32)	+	$R(C_2)$	I
L-ala-L-ala	a	517 (423)	393 (96)		501 (−4.76)	392 (+0.85)	−	$S(C_2)$	II,III
	b	524 (385)	395 (136)	578 (+0.60)	500 (−5.03)	410 (+0.67)	+	$R(C_2)$	II,III
L-ala-D-ala	a	523 (386)	396 (71)	548 (+1.95)		386 (+1.27)	+	$R(C_2)$	II
	b	522 (377)	395 (92)	540 (−0.26)	490 (−0.28)	394 (+1.64)	−	$S(C_2)$	II
L-leu-L-leu	a	525 (433)	397 (106)		508 (−6.16)	408 (+0.29)	−	$S(C_2)$	II,III
	b	530 (387)	403 (167)	605 (+0.09)	508 (−6.60)	412 (+0.40)	+	$R(C_2)$	II,III

[a] I = Upfield shift in ^1H n.m.r. spectrum due to ring current of phenylalanine; II = effect of carboxylate on the ^1H n.m.r spectrum; III = comparison of configurational contribution to c.d. at 570 nm. (This is much less reliable than I or II.) [b] Incompletely resolved chromatographically on starch.

Resolution of Bis(glycylglycinato)cobaltate(III).— Although crystals of (+)-*cis*-$[Co(en)_2(NO_2)_2][Co(gly\text{-}glyO)_2]$ could be obtained and recrystallised, even after recrystallisation a solution of the crystals show no optical activity for the $[Co(gly\text{-}glyO)_2]^-$ anion. An attempt at resolution using the L-histidinium salt also failed, although both of these cations have proved useful for the $[Co(edta)]^-$ (edta = ethylenediaminetetra-acetate) anion.[22,23] Stereoselective synthesis from (+)-$[Co(en)_3]^{3+}$ failed, and no resolution could be obtained by attempted crystallisation of $(+)[Co(en)_3][Co(gly\text{-}glyO)_2]_3$. Although asymmetric reduction by the bacterium *Proteus vulgaris* has been successfully used [24] to destroy preferentially one enantiomer of a number of cobalt(III) complexes including $[Co(en)_2(phen)]^{3+}$ (phen = 1,10-phenanthroline), $[Co(glyO)_3]$, and $[Co(edta)]^-$, no reduction at all, and hence no resolution, was observed with $[Co(gly\text{-}glyO)_2]^-$.

Many cationic cobalt(III) complexes have been resolved chromatographically on cation-exchange Sephadex,[17,25,26] but no resolution of $[Co(gly\text{-}glyO)_2]^-$ was obtained by chromatography either on anion-exchange Sephadex (QAE) or on Sephadex itself (G-10). However, some resolution was obtained by chromatography of an aqueous solution on starch, which has long been known to be useful for the resolution of, for example, $[Co(glyO)_3]$.[27]

The c.d. and optical rotatory dispersion (o.r.d.) spectra of partially resolved $[Co(gly\text{-}glyO)_2]^-$ have been reported before.[3] Using $Li[Co(gly\text{-}glyO)_2]$, which had been carefully purified by chromatography on Sephadex G-10 and QAE-Sephadex, we have again

Diastereoisomers of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$, $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$, $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$, *and* $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$. When these complexes are synthesised by any of the methods, except by disproportionation of $[Co(NH_3)_3(gly\text{-}L\text{-}\alpha)]^+$ (in which case racemisation of the C-terminal amino-acid occurs), the bis(dipeptidato)cobaltate(III) formed always shows a large negative Cotton effect at *ca.* 510 nm; its magnitude is essentially independent of the method of synthesis. Moreover, no significant change in the optical rotation was observed during the purification of the complexes by chromatography on Sephadex G-10. These observations led to the idea that the formation was stereospecific, or at least highly stereoselective, although molecular models show no obvious reasons for stereoselectivity, and similar systems, *e.g.* $[Co(L\text{-}\alpha)_3]$, usually show little if any stereoselectivity in terms of optical isomerism.[28,29]

However, careful fractionation by chromatography on a long column (120 × 2.5 cm) of Sephadex G-10 or of starch gives fractions with slightly different c.d. spectra.

* Although changing the solvent from water to water–alcohol changes the intensity of the c.d. band somewhat, it does not affect its wavelength.

In addition, in the ^1H n.m.r. spectra of the bis(dipeptidato)cobaltate(III) complexes with L-alanyl dipeptides the methyl resonance clearly shows the presence of both diastereoisomers: indeed n.m.r. spectroscopy may conveniently be used to measure the ratio of the amounts of the two diastereoisomers.

The two diastereoisomers can be completely separated by anion-exchange chromatography of the preparative mixture from any of the synthetic routes on QAE-Sephadex A-25. The separation is easier for complexes of the type $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ than for $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ complexes. Although crystallisation from a purified mixture of the diastereoisomers was not usually possible, once the diastereoisomers had separated chromatographically, crystallisation was usually much easier: indeed for complexes containing L-phenylalanine one diastereoisomer is much less soluble than the other. However, crystals *were* obtained from a purified mixture of the diastereoisomers of $[Co(L\text{-}leu\text{-}L\text{-}leuO)_2]^-$ and the c.d. spectrum of a single untwinned crystal showed that it contained both diastereoisomers in equal amounts, *i.e.* it was a quasi-racemate. Formation of quasi-racemates among complex compounds is rather uncommon, although DL-α-$[Co(L\text{-}valO)_3]$ (valO=valinate) also gives a quasi-racemate.[30]

The two diastereoisomers are naturally very similar, but certain unifying features are found. The isomer (a) which is less strongly adsorbed both on Sephadex G-10 and on anion-exchange DEAE- and QAE-Sephadex has a higher * absorption coefficient † for the lower-energy *d–d* band, ε_{522}, and a larger ratio of the absorption coefficients for the two *d–d* bands $\varepsilon_{522}:\varepsilon_{394}$ than the more strongly adsorbed isomer (b); there are also differences in the inequivalence of the two protons of the CH_2 groups in the ^1H n.m.r. spectrum (see later). In all the synthetic routes from Co^{II}, two to three times as much

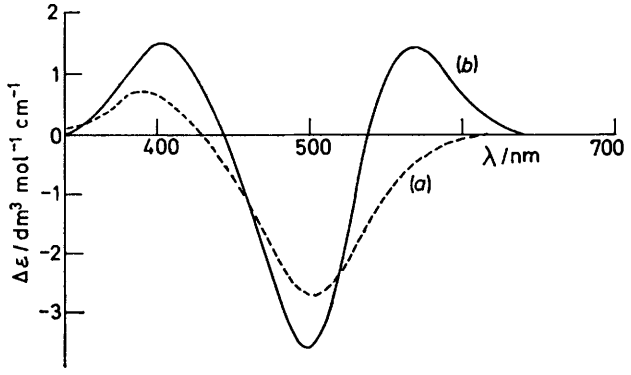

FIGURE 2 C.d. spectra of the diastereoisomers of $[Co(L\text{-}ala\text{-}glyO)_2]^-$: (*a*) less strongly adsorbed isomer on QAE-Sephadex, $S(C_2)$; (*b*) more strongly adsorbed isomer, $R(C_2)$. Similar spectra are obtained for the diastereoisomers of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ and of $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$

isomer a is formed as isomer b; however, when activated charcoal was added to a synthesis of $[Co(gly\text{-}L\text{-}leuO)_2]^-$ nearly equal amounts of the isomers were formed. Syntheses from $[Co(CO_3)_3]^{3-}$ yielded much more isomer b.

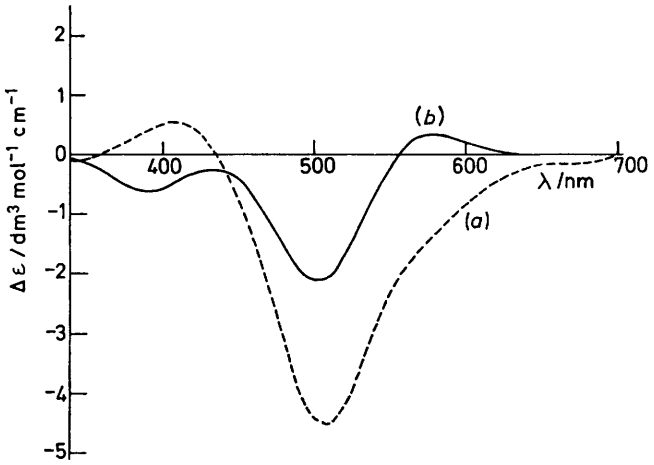

FIGURE 3 C.d. spectra of the diastereoisomers of $[Co(gly\text{-}L\text{-}alaO)_2]^-$. Details as in Figure 1. Similar spectra are obtained for the diastereoisomers of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$

C.D. Spectra.—For all the L-peptides we have studied, both diastereoisomers show strong negative Cotton effects associated with the lowest-energy *d–d* band; indeed, the c.d. spectra of the pairs of isomers are often very similar, particularly for peptides of the type L-α-L-α. However, four types of c.d. spectra can be identified. The spectra of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ are typified by those of the isomers of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ (Figure 2) and the spectra of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ by those of the isomers of $[Co(gly\text{-}L\text{-}alaO)_2]^-$ (Figure 3).

The main band, particularly for isomers a of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ and isomers b of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ has an extremely high c.d. absorption coefficient (Table 1) of 4—5 $dm^3\ mol^{-1}\ cm^{-1}$, corresponding to the anomalously high absorption coefficient for the lower-energy *d–d* band. The asymmetry factor $g(=\Delta\varepsilon/\varepsilon)$ is normal, $\approx 10^{-2}$. On the other hand, for most optically active d^3 and d^6 octahedral metal complexes, for the higher-energy *d–d* band, *g* is much lower,[31] whereas for bis(dipeptidato)-cobaltate(III) complexes it is also *ca.* 10^{-2}, and for certain complexes, *e.g.* isomer a of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ and for both isomers of $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$, it is actually *higher* than for the lower-energy *d–d* band, which is most unusual. Only isomers b of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ have a negative Cotton effect associated with the higher-energy *d–d* band. For $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$, isomer b shows a strong positive Cotton effect at a lower energy than the main band (*ca.* 570 nm), whereas isomer b of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ shows a much weaker Cotton effect which is barely visible for the L-leucyl- and L-phenyl-alanyl peptides; for L-peptides, isomer a never shows a Cotton effect in

* The diastereoisomers of $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$ are anomalous in this respect.

† Because in many cases the crystalline diastereoisomers could not be isolated, the absorption coefficient was determined by measuring the cobalt content of the solution used for absorbance and the c.d. measured colorimetrically after decomposition to Co^{2+} by peroxodisulphate (see Experimental section). The absorption coefficients are therefore subject to a rather higher error than is usual.

this region. An earlier attempt [32] to estimate the c.d. spectra of the pure diastereoisomers from the c.d. spectrum of the preparative mixture obtained by oxygenation of a cobalt(II) solution, together with the determination of the ratio of diastereoisomers by ^{1}H n.m.r. spectroscopy, gave reasonable values for one of the isomers, b (isomer I in ref. 32), but no agreement at all for the other isomer, a (isomer II in ref. 32).

For $[Co(gly\text{-}glyO)_2]^-$, in which only a configurational contribution is possible, the c.d. spectrum is centred at 555 nm. It seems that for complexes of L-amino-acids the isomers b, with a positive c.d. in the 550—580 nm region, have a positive configurational contribution in this region, and isomers a have a negative configurational contribution.

Not surprisingly, the diastereoisomers of $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$ are anomalous: isomer b is remarkable in that it has a comparatively large Cotton effect in the higher-energy *d–d* band, and almost no activity in the lower-energy *d–d* band. We therefore suspect that vicinal effects dominate in the lower-energy *d–d* bands and that for $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$ the two opposing vicinal effects partly cancel; this will be discussed in a subsequent publication.

The vexing question of the absolute configuration of the complex remains open. Although, for complexes of bidentate ligands, empirical rules [33,34] have been found useful in determining the absolute configuration, such rules are unreliable for complexes of polydentate ligands.[35] The high stereoselectivity of formation of complexes such as $S(C_2)\text{-}(-)_{546}[Co\{(+)\text{-pdta}\}]^-$ (ref. 36) and $\Delta(-)[Co\{(-)\text{-pn}\}_3]^{3+}$ (ref. 37) enabled assignment of absolute configuration, but for the bis(dipeptidato)-cobaltate(III) complexes, which have essentially planar chelate rings,[4,9] there is no obvious cause of the observed stereoselectivity, which anyway is strongly dependent on the synthetic route; indeed, the addition of activated charcoal to the synthesis from Co^{2+}, which yields more nearly equal amounts of the two diastereoisomers, strongly suggests that the observed stereoselectivity has a kinetic rather than a thermodynamic origin. Thus, no certain conclusion about the absolute configuration can be deduced solely from the c.d. spectra of the diastereoisomers. The ^{1}H n.m.r. spectra do help in this respect.

Proton N.M.R. Spectra.—The ^{1}H n.m.r. spectrum of $[Co(gly\text{-}glyO)_2]^-$ in slightly acidic D_2O has three resonances, a sharp signal at δ 4.35 and broad peaks at *ca.* 4 and at 3.34 p.p.m. The rapid exchange of the peak at *ca.* 4 p.p.m. in neutral D_2O (pD *ca.* 7) confirms that it arises from the NH_2 group. As the NH_2 is exchanged to ND_2 the peak at 3.34 p.p.m. sharpens to a well defined AB pattern indicating that it arises from the N-terminal CH_2 group (Figure 4); this is confirmed by comparison with the spectra of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ and of $[Co(gly\text{-}L\text{-}alaO)_2]^-$ (Table 2). This assignment agrees with that previously reported.[9,38,39] In contrast to one earlier report,[39] the resonance of the C-terminal CH_2 is a sharp singlet at all frequencies (60, 100, and 220 MHz) which we could use, giving no hint of the expected inequivalence, whereas the N-terminal CH_2 shows a clear AB pattern even at 60 MHz. Inequivalence at the C-terminal CH_2 group can, however, be observed at 220 MHz for $[Co(L\text{-}ala\text{-}glyO)_2]^-$, but only for isomer b; like $[Co(gly\text{-}glyO)_2]$,$^-$ isomer a shows no hint of broadening even at this high frequency.

Further comparison of the n.m.r. spectra of the pairs of diastereoisomers led to some interesting general observations which may be of use in the assignment of the absolute configuration. In isomers a there is no detectable inequivalence for the C-terminal CH_2 and only

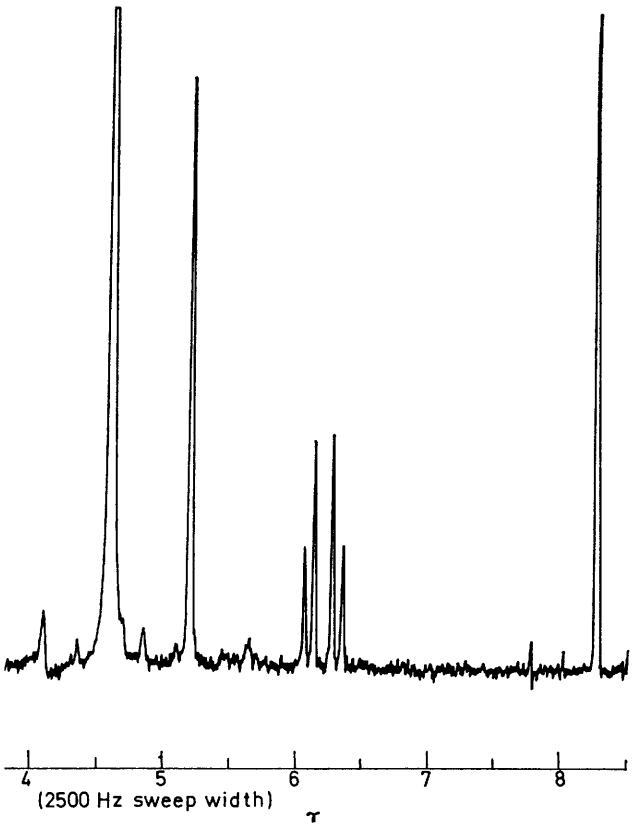

FIGURE 4 Hydrogen-1 n.m.r. spectrum of $[Co(gly\text{-}glyO)_2]^-$ at 220 MHz

slight inequivalence for the N-terminal CH_2 at 100 MHz. In $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ the N-terminal CH_2 groups of isomer b are always inequivalent, $\delta(\nu_{AB}) \geqslant 0.25$ p.p.m. However, for isomers b of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$, inequivalence of the C-terminal CH_2 group is just detectable at 100 MHz for $[Co(L\text{-}ala\text{-}glyO)_2]^-$ and $[Co(L\text{-}leu\text{-}glyO)_2]^-$, whereas for $[Co(L\text{-}phe\text{-}glyO)_2]^-$ the C-terminal CH_2 group can be clearly seen to be inequivalent even in spectra run at 60 MHz [$\delta(\nu_{AB})$ 0.21 p.p.m.].

There are also striking differences in the chemical shifts of the resonances of the pairs of diastereoisomers. An N-terminal CH group of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ or of $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$ is at *ca.* 0.2 p.p.m. to lower field for isomer a than b, whereas the reverse is true for $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$. The positions of the N-terminal CH

resonances in the two diastereoisomers of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ correspond well with those of the inequivalent CH_2 group in $[Co(gly\text{-}\alpha)_2]^-$. The same is true in general for the C-terminal amino-acid, although here very little inequivalence of the CH_2 group is observed and the shift between the resonances of the CH protons in the two diastereoisomers is very small (0.01—0.05 p.p.m.).

The Absolute Configuration.—Striking exceptions to these generalisations about the ^1H n.m.r. spectra are found with the diastereoisomers of $[Co(L\text{-phe-glyO})_2]^-$ and $[Co(gly\text{-}L\text{-pheO})_2]^-$. The shifts observed, which may be attributed to the upfield shift due to the aromatic ring current,[40] may be used to assign the absolute configuration of the bis(dipeptidato)cobaltate(III) complexes.[41]

caused by an aromatic ring) as 0.2—0.5 p.p.m. depending on the rotation of the benzyl group of the phenylalanine. The model of the $S(C_2)$ isomer indicates that both of the methylene protons will be influenced by the phenylalanine ring to a similar but smaller extent. Thus it may be deduced that for $[Co(L\text{-phe-glyO})_2]^-$ the isomer which is more strongly adsorbed on anion-exchange Sephadex, isomer b, can be assigned the $R(C_2)$ absolute configuration (Figure 7) and isomer a the $S(C_2)$ configuration.

There is, moreover, further evidence in favour of this assignment. For isomer b there is only a small difference between the chemical shifts of the two protons of the phenylalanyl CH_2 group ($\Delta\delta$ 0.098 p.p.m.) whereas for isomer a the difference is much larger ($\Delta\delta$ 0.37 p.p.m.), one proton being shifted 0.13 p.p.m. upfield and the

TABLE 2

Chemical shifts (p.p.m.) of the diastereoisomers a and b of bis(dipeptidato)cobaltate(III) complexes

		N-terminal (α_1)					C-terminal (α_2)				
		methylene protons		CH_3	$\delta(\nu_{CH_2})$	$J(CH_2)$ (Hz)	$J(CHCH_3)$ (Hz)	methylene protons		CH_3	$\delta(\nu_{CH_2})$	$J(CH_2)$ (Hz)	$J(CHCH_3)$ (Hz)
α_1—α_2													
gly-gly		3.432	3.241		0.190	16.5		4.352					
L-ala-L-gly	a	3.540		1.320			7.2	4.334					
	b		3.351	1.347			7.08	4.445	4.292		0.15	19.6	
	a—b	0.187		0.027									
gly-L-ala	a	3.394	3.223		0.14	16.8		4.441		1.631			6.98
	b	3.444	3.158		0.28	16.6			4.430	1.603			7.11
	a—b	−0.050	0.065					0.011		0.028			
L-ala-L-ala	a	3.480		1.322			7.1		4.475	1.602			7.0
	b		3.314	1.352			7.0	4.519		1.610			7.5
	a—b	0.166		−0.030				−0.044		−0.008			
L-ala-D-ala	a		3.447	1.327			7.2	4.544		1.699			7.2
	b	3.603		1.320			7.2	−4.517		1.638			7.2
	a—b	−0.156		0.007				0.027		0.061			
L-leu-gly	a		0.95					4.408					
	b		3.39	0.95				4.43	4.43				
	a—b	0.14		0.01				*ca.* −0.02					
gly-L-leu	a	3.305								0.950			
	b	3.423	3.167		0.26	16.4		4.460		1.010			
gly-L-leu	a—b							−0.057		−0.060			
L-leu-L-leu	a	3.468						4.646					
	b		3.291					4.697					
	a—b	0.197						−0.051					
L-phe-gly	a	3.65						4.24					
	b		3.4					4.39	4.18		0.21	18.7	

Isomer a of $[Co(L\text{-phe-glyO})_2]^-$ has the typical sharp singlet for the glycyl CH_2 group, although shifted *ca.* 0.15 p.p.m. upfield from the usual position, whereas for isomer b the resonance of one of the two glycyl methylene protons is in the average position found for a C-terminal CH_2 group in $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, 4.4 p.p.m., and the other is shifted 0.2 p.p.m. upfield: this results in a striking inequivalence for this CH_2 resonance ($\Delta\delta$ 0.21 p.p.m.). Examination of molecular models for the pair of diastereoisomers indicates that in the $R(C_2)$ isomer * only one of the methylene protons can be significantly influenced by the ring current of the phenylalanine ring. From the approximate distance in the model, the upfield shift for this proton can be estimated (by comparison with values in the literature [40] for the upfield shifts other 0.23 p.p.m. downfield, compared with the average shift for isomer b. Models indicate that in the $R(C_2)$ isomer neither phenylalanine methylene proton can approach a carboxylate group, whereas in the $S(C_2)$ isomer one proton is close to a carboxylate group and the other further away.

This assignment of configuration for $[Co(L\text{-phe-glyO})_2]^-$ can be extended to the diastereoisomers of $[Co(L\text{-}\alpha\text{-glyO})_2]^-$, $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$, and $[Co(L\text{-}\alpha\text{-}D\text{-}\alpha)_2]^-$. It is clear from models that in the $R(C_2)$ isomer the N-terminal CH groups are near the carboxylate groups whereas in the $S(C_2)$ isomer they are remote from the carboxylate groups. Thus it was suggested earlier [32] that, subject to the assumption that the carboxylate group causes an upfield shift, the absolute configurations could be tentatively assigned. We have observed that there is considerable inequivalence of the N-terminal CH_2, but not of the C-terminal CH_2, and that likewise there is a significant difference between the chemical

* We designate the isomer by the helicity of the two-fold axis, because the I.U.P.A.C. nomenclature [42] using pairs of skew lines fails to define an unambiguous configurational label for complexes containing two identical planar tridentate ligands, such as $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, without a further convention.

shifts of the N-terminal CH resonances of the diastereoisomers: the resonance for isomer b is shifted 0.2 p.p.m. upfield from that of isomer a for $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ and $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$; the reverse is true for $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$. It is thus clear that, whatever the effect of more strongly adsorbed isomer, b, has a positive configurational effect in the c.d. spectrum in the 550—580 nm region. Thus, both the chromatographic behaviour and the configurational c.d. imply that for all of the complexes with L-peptides the absolute configurations

TABLE 3

Summary of the differences between the diastereoisomers

Property	Isomer a	Isomer b
Adsorption on Sephadex G-10	less strongly adsorbed	more strongly adsorbed
on QAE- and DEAE-Sephadex	less strongly adsorbed	more strongly adsorbed
on starch	more strongly adsorbed [a]	less strongly adsorbed
Adsorption spectrum	higher ε_{520}	
	higher $\varepsilon_{520} : \varepsilon_{400}$	
C.d. spectrum [b]	for L-α-gly higher $\lvert\Delta\varepsilon_{500}\rvert$	
	for gly-L-α lower $\lvert\Delta\varepsilon_{500}\rvert$	
	no maximum at *ca.* 580 nm	for L-α-gly $\Delta\varepsilon_{580}$ is positive
		for gly-L-α $\Delta\varepsilon_{580}$ is slightly positive
	$\Delta\varepsilon_{400}$ positive	for L-α-gly Δ_{400} is positive
		for gly-L-α $\Delta\varepsilon_{400}$ is negative
Configurational contribution to c.d. at 580 nm [c]	—	+
Absolute configuration [c]	$S(C_2)$	$R(C_2)$
^{1}H n.m.r. spectrum	C-terminal and N-terminal CH_2 resonance is singlet	C-terminal and N-terminal CH_2 give AB pattern
	N-terminal CH in L-α is shifted downfield by *ca.* 0.2 p.p.m.	

[a] Only $[Co(gly\text{-}L\text{-}ala\text{-}o)_2]^-$ was studied. [b] L-ala-D-ala is different. [c] Reverse is true for L-ala-D-ala.

the carboxyl group, the upfield shift of one N-terminal CH can be used to correlate the absolute configuration of all of the complexes with an N-terminal L-amino-acid. This leads to the assignment of isomer b of $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$, isomer b of $[Co(L\text{-}\alpha\text{-}L\text{-}\alpha)_2]^-$, and isomer a of $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$ to the $R(C_2)$ structure. It may also be concluded that the effect of the carboxyl group is in fact an upfield shift.

There is an even more striking difference between the chemical shifts of the N-terminal CH_2 in the isomers of $[Co(gly\text{-}L\text{-}pheO)_2]^-$: for isomer b, both protons are shifted considerably upfield (0.4 and 0.7 p.p.m.) from the position observed for other $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ complexes, including isomer a of $[Co(gly\text{-}L\text{-}pheO)_2]^-$. Models indicate that, in the $R(C_2)$ isomer, the aromatic ring can influence one or both N-terminal methylene protons on both peptides, depending on the conformation, whereas in the $S(C_2)$ isomer the only possible influence is on one of the protons in the same peptide, and this only with an improbable eclipsed conformation of the C–CH_2Ph bond. Thus isomer b of $[Co(gly\text{-}L\text{-}pheO)_2]^-$ can be assigned the $R(C_2)$ configuration.

Models indicate that the C-terminal CH_2 or CH group is more nearly in the same plane as the carboxylate and the resulting very small shift does not permit extension of this assignment of $[Co(gly\text{-}L\text{-}pheO)_2]^-$ to $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ as was possible for complexes of N-terminal-substituted dipeptides. However, there are two empirical methods which allow a comparison of the absolute configurations of complexes of the type $[Co(gly\text{-}L\text{-}\alpha)_2]^-$, but not unambiguously.

Since a chromatographic separation of diastereoisomers depends on a stereoselective interaction between the adsorbed species and the column, the more strongly adsorbed isomers may well have the same configuration.[43] With the exception of $[Co(L\text{-}ala\text{-}D\text{-}alaO)_2]^-$, the of the more strongly adsorbed isomers are the same, *i.e.* $R(C_2)$.

Last, $(+)[Co(gly\text{-}glyO)_2]^-$ has presumably the $R(C_2)$ configuration since both it and $R(C_2)$-$[Co(gly\text{-}L\text{-}alaO)_2]^-$ are eluted first in chromatography on starch in aqueous solution, and both have a clear positive configurational contribution to the c.d. in the 550—580 nm region.

The absolute configurations we assign to the diastereoisomers of the various bis(dipeptidato)cobaltate(III) complexes are summarised, with the c.d. results, in Table 1. Of all the methods available[35] for the determination of configuration of a metal complex by comparison with the configuration of an organic ligand, n.m.r. spectroscopy has not been used so widely although ^{1}H n.m.r. combined with the conformational preference of a propane-1,2-diamine chelate ring was used[44] to assign the absolute configurations of tris(bidentate ligand)cobalt(III) complexes. There appears to be no previous report of the use of the upfield shift in the n.m.r. spectrum caused by an aromatic ring system to determine the absolute configuration of metal complexes, although this method has been very useful in investigating other features of ternary metal complexes.[45]

Carbon-13 N.M.R. Spectra.—The ^{13}C resonances of $[Co(gly\text{-}glyO)_2]^-$ are shifted downfield from those of free glycylglycine (Table 4); such downfield shifts on coordination have been observed for ^{13}C spectra of a number of cobalt(III) complexes.[46] On acidification, two of the resonances, which may be presumed to be those of the N-terminal glycine, shift significantly back upfield: the resonance of the C-terminal methylene carbon does not shift at all, whereas a small shift is observed for the resonance which must be due to the C-terminal carboxylate carbon; the reason for this small shift is not known.

Chemical Properties and Reactions.—Protonation. It has long been known that the absorption and c.d.

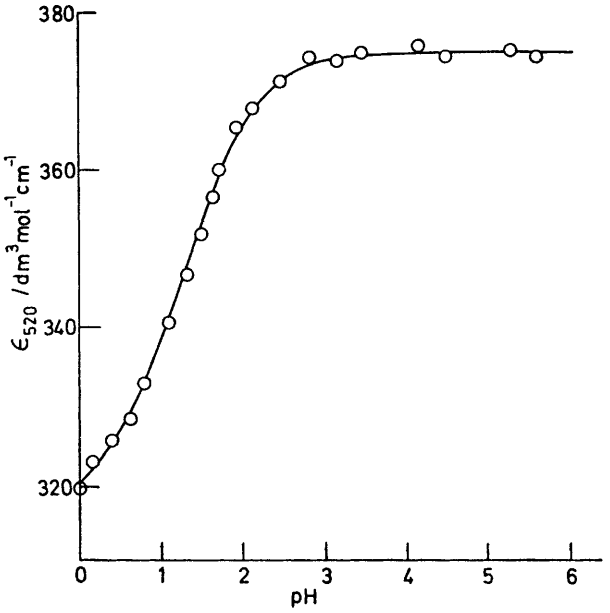

FIGURE 5 Variation of the absorption coefficient of $[Co(gly\text{-}glyO)_2]^-$ with pH: the curve is the least-squares computer fit of the points

spectra of bis(dipeptidato)cobaltate(III) complexes change in strongly acid solution, pH $\leqslant$ 2. The electronic absorption shifts from 520 to *ca.* 500 nm in 10 mol dm^{-3} H^+ (*cf.* ref. 8) and the absorption coefficient decreases; from the variation of ε_{520} with pH (pH 0—6) the pK_a for the first protonation of $[Co(gly\text{-}glyO)_2]^-$ (Figure 5) and of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ (Figure 6) can be computed * as 1.3 and 0.96 respectively. The c.d. absorption coefficients for $[Co(L\text{-}ala\text{-}glyO)_2]^-$ (80% diastereoisomer a) at 500 and 400 nm decrease dramatically in acid solution: this change is reversible indicating that it is not due to decomposition or a change in diastereoisomer ratio; the variation of $\Delta\varepsilon$ with pH (Figure 6) likewise gives pK_a as 0.91. It was not possible to calculate precisely the pK_a for the protonation of the second peptide group from these measurements which were carried out down to pH 0, but the calculations suggest that pK_{a2} is *ca.* −0.2 for $[Co(L\text{-}ala\text{-}glyO)_2]^-$ and probably even lower for $[Co(gly\text{-}glyO)_2]^-$.

The ^{1}H n.m.r. resonance of the N-terminal CH_2 of $[Co(gly\text{-}glyO)_2]^-$ shifts downfield strikingly (0.6 p.p.m.) on acidification (pH 0), whereas the resonance of the C-terminal CH_2 shifts downfield much less (*ca.* 0.2 p.p.m.); for this protonation pK_{a1} = 1.34. The relative shift of the two methylenes confirms that the protonation is not, as had earlier been suggested,[3,8] on the peptide nitrogen, but rather is on the peptide oxygen. This is true for all the $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ complexes which we report in this paper. The effect that co-ordination to cobalt has on the pK of the peptide nitrogen is remarkable: in free glycylglycine the pK is 15.[47] In these peptidatocobalt(III) complexes the pK of the peptide nitrogen is decreased by more than 15 log units; indeed the peptide oxygen, and the co-ordinated carboxylate oxygen of $[Co(dien)(\alpha_1\text{-}\alpha_2)]^+$ and $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$, is protonated in preference to the planar trigonal peptide nitrogen.

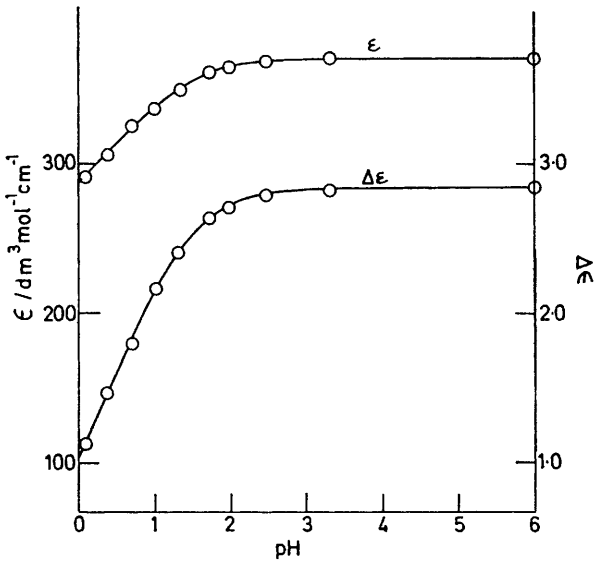

FIGURE 6 Variation of the absorption coefficient and the c.d. absorption coefficient of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ with pH; the curves are the least-squares computer fits of the points

These changes in acidic solutions of $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ parallel (in many ways) the first protonation observed [15] in the absorption, c.d., and ^{1}H n.m.r. spectra of $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$ and $[Co(dien)(\alpha_1\text{-}\alpha_2)]^+$. However, no evidence could be found for further protonation, at the co-ordinated carboxylate group, observed for both these other types of cobalt(III) dipeptide complexes nor for displacement of the carboxylate group by co-ordination of a ligand such as Cl^- or Br^- in strongly acid

TABLE 4

Carbon-13 n.m.r. spectra [a]

	C=O		CH_2		
	N-terminal	C-terminal	N-terminal	C-terminal	Ref.
gly-gly	167.9	177.1	44.2	41.5	*b*
$[Co(gly\text{-}glyO)_2]^-$	187.8, 180.7		50.5, 50.0		This work
$[Co(Hgly\text{-}glyO)_2]^+$	175.9, 163.7		42.0	50.6	This work

[a] In p.p.m. downfield from external $SiMe_4$. [b] L. F. Johnson and W. C. Jankowski, 'Carbon-13 NMR Spectra,' Wiley, New York, 1972, no. 72.

* Using a Hewlett–Packard model 9821 desk computer attached to a model 9825 plotter.

solution, as observed [15] for $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$. Unfortunately, such ligands are also reducing agents and decomposition of $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ occurs, with formation of Co^{II}.

Exchange of C-terminal CH_2 *and* CH *groups.*[38] In strongly alkaline solution (pD > 13) the C-terminal CH_2 resonances in $[Co(gly\text{-}glyO)_2]^-$ and in $[Co(L\text{-}ala\text{-}glyO)_2]^-$ and the C-terminal CH resonance in $[Co(gly\text{-}L\text{-}alaO)_2]^-$ exchange readily. No exchange of N-terminal CH_2 or CH resonances in these three complexes has been observed even in extremely strong alkali (10 mol dm^{-3}) on standing or on heating in alkaline solution

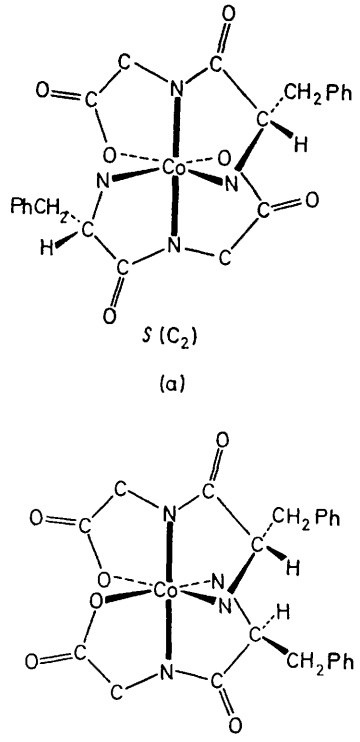

FIGURE 7 Absolute configurations of the diastereoisomers b and a of $[Co(L\text{-}phe\text{-}glyO)_2]^-$: the c.d. spectrum of isomer b resembles Figure 2(*b*)

until the complexes start to decompose. Nor has any exchange of CH_2 or CH groups been observed for any of the three peptides themselves even under conditions sufficiently alkaline to cause peptide hydrolysis.

It is interesting that this exchange is specific for the C-terminal CH_2; in nickel(II) and cobalt(III) complexes of Schiff bases (*e.g.* sal-gly-gly) derived from salicylaldehyde and dipeptides at pD *ca.* 11 exchange takes place exclusively at the N-terminal CH_2 (or CH) group.[48] This was said to occur because ' the electron-withdrawing effect of the metal ion can reach the α-carbon atom through the azomethine nitrogen atom, whose electronic structure should differ entirely from that of the simple amino nitrogen in glycylglycine. This will probably enhance the reactivity of the N-terminal methylene group in the glycylglycine moiety '. These workers also observed that, for the nickel(II) complexes only, in much more alkaline solution (pD 13.6, 40 °C, 23 d) some exchange of the C-terminal methylene group of $[Ni(sal\text{-}gly\text{-}gly)]^-$ occurred; the cobalt complex $[Co(NO_2)_2(sal\text{-}gly\text{-}gly)]^-$ decomposed under these conditions and no exchange could be observed. Selective exchange of the N-terminal CH_2 group in nickel(II) complexes of Schiff-base derivatives of tripeptides has also been observed,[49] and in these complexes the central CH_2 is also activated under more alkaline conditions (pD 13.1, 40 °C, 12 d) but no exchange of the C-terminal CH_2 group could be observed at all. Thus the bis(dipeptidato)cobaltate(III) complexes and the related $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$ and $[Co(dien)(\alpha_1\text{-}\alpha_2)]^+$ complexes [15] are the only ones in which exchange occurs exclusively at the C-terminal CH_2.

Moreover, proton exchange at the C-terminal CHR in $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ occurs much faster than does racemisation of the optically active amino-acid. Our original observation,[38] that during the time required for exchange in $[Co(gly\text{-}L\text{-}alaO)_2]^-$ no change occurs in the c.d. spectrum, was made for a mixture of diastereoisomers (70% a and 30% b) since we had not then separated the diastereoisomers in any quantity. However, if racemisation had in fact occurred at the carbon during those experiments, then not only would the c.d. decrease dramatically, even for the mixture of diastereoisomers, since the vicinal effect is the main contribution to the c.d. for this complex,[21] but the n.m.r. spectrum would show the formation of the other diastereoisomer (unless simultaneous racemisation occurred at the cobalt); neither effect was observed. Our more recent study [50] on the racemisation of bis(dipeptidato)cobaltate(III) complexes was also carried out with a mixture of diastereoisomers.

These experiments have now been repeated using the pure diastereoisomers of $[Co(gly\text{-}L\text{-}alaO)_2]^-$, $[Co(gly\text{-}L\text{-}leuO)_2]^-$, and $[Co(gly\text{-}L\text{-}pheO)_2]^-$, and our preliminary observations confirmed. For example, for $[Co(gly\text{-}L\text{-}pheO)_2]^-$, isomer b, exchange is complete in 0.5 mol dm^{-3} Na[OH] within 6 h at 35 °C, whereas the c.d. of a more dilute solution in 0.5 mol dm^{-3} Na[OH] decreases by only 9% in 169 h, and the c.d. of a sample in which the proton was exchanged for a deuteron, in the n.m.r. experiment, and then the deuteron was re-exchanged for a proton and the solution neutralised, had a c.d. identical with that of the starting material.

In general, rather forcing conditions (pH *ca.* 13) are required for exchange of $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, and indeed also for $[Ni(sal\text{-}gly\text{-}gly)]^-$ and $[Co(NO_2)_2(sal\text{-}gly\text{-}gly)]^-$. Exchange of the uncharged $[Co(glyO)_3]$,[51] and the cationic $[Co(NH_3)_3(gly\text{-}glyO)]^+$ and $[Co(dien)(gly\text{-}glyO)]^+$ complexes [15] takes place more readily (pH *ca.* 11); $[Co(en)_2(glyO)]^{2+}$ exchanges [52] even at pH 8.

We also investigated the possibility of activation of exchange by u.v. irradiation. No exchange was observed at pD *ca.* 7, even under forcing conditions such that some decomposition of the complex occurred.

Reactions of the activated C-terminal methylene. Several

groups of workers have synthesised amino-acids by a Knoevenagel reaction of aldehydes or ketones with glycine activated by copper;[53] under the conditions used for the Knoevenagel reaction, exchange with the deuterons of D_2O by the protons of bis(glycinato)-copper(II) occurs.[50] Exchange occurs even more readily in aqua(*N*-salicylideneamino-acidato)copper(II) complexes,[54] and these also react with aldehydes [55] and alkyl halides [56] to give substituted amino-acids. In view of our observation of the exchange of the C-terminal methylene of $[Co(gly\text{-}glyO)_2]^-$ in alkaline solution, we have investigated the reaction of aldehydes with it in alkaline solution. Unfortunately, under the very basic conditions required for exchange of $[Co(gly\text{-}glyO)_2]^-$ considerable quantities of organic by-products are formed by self condensation of the aldehyde.

The reaction with formaldehyde was poor: however, repeated chromatography on Sephadex G-10 permitted separation of the organic by-products, unchanged $[Co(gly\text{-}glyO)_2]^-$, and another bis(dipeptidato)cobaltate(III) complex which could not be crystallised. Analyses of the sodium and ammonium salts, which were obtained as glassy solids, confirmed that glycine had been converted into serine. However, insufficient material was obtained for ^1H n.m.r. verification that reaction had occurred at the C-terminal residue. The presence of serine was also confirmed by breakdown of the complex with sulphide followed by paper chromatographic identification of the amino-acid.

The reaction of acetaldehyde was better; just enough of the bis(dipeptidato)cobaltate(III) product was obtained for a ^1H n.m.r. spectrum in which the methyl resonance of threonine and/or allothreonine and the N-terminal CH_2 resonance were observed. The unchanged N-terminal CH_2 indicates that, as expected from the exchange studies, reaction had taken place exclusively at the C-terminal glycine. In this product, and in that from the analogous reaction with benzaldehyde, the amino-acids were again identified by paper chromatography.

There remains the interesting question of the stereoselectivity of the reaction of aldehyde with complexes such as $[Co(L\text{-}ala\text{-}glyO)_2]^-$. A mixture of the two diastereoisomers of this reacted with aldehydes to give bis[L-alanyl-DL(?)-threoninato]cobaltate(III). However, the separation of the diastereoisomers of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ in quantity is particularly difficult, and thus it has not yet been possible to carry out the reaction using a pure isolated diastereoisomer. At present this interesting question remains unanswered.

Slight stereoselectivity in the reaction with aldehydes has been noticed for $(+)[Co(en)_2(glyO)]^{2+}$,[57,58] $(+)[Co(en)(glyO)_2]^+$,[58] and $(+)[Co(sal\text{-}gly)_2]^-$,[59] in which the only asymmetry is that of the cobalt: in $(+)_{530}[Co(sal\text{-}L\text{-}val)(sal\text{-}gly)]^-$ the two asymmetric centres have a mutual effect on the stereoselectivity.[60]

Conclusions.—Bis(dipeptidato)cobalt(III) complexes are readily prepared by many unrelated synthetic routes. With optically active peptides the formation is stereoselective, but not stereospecific as had earlier been suspected. The origin of this stereoselectivity cannot readily be explained. These peptide complexes are activated to exchange and to reaction, but exclusively at the C-terminal residue. *No* exchange and *no* reaction at the N-terminal residue could be observed even under most vigorous conditions. This contrasts sharply with the stereospecific exchange of the N-terminal peptide residue in copper(II) complexes of Schiff-base adducts of dipeptides. The stereoselectivity of the exchange at such activated methylene groups clearly merits further attention.

EXPERIMENTAL

Peptides were obtained commercially from B.D.H., Fluka, Sigma, Nutritional Biochemical Co., and Cyclo Chemicals and were used without further purification. Spectra were recorded on the following instruments: Unicam SP 800B and SP 600 spectrophotometers (electronic); Roussel-Jouan 'Dichrographe' spectrometer, model B, calibrated * with a solution of isoandrosterone in purified dioxan as recommended by the manufacturers or a Cary 61 (c.d.); Bendix N.P.L. Polarmatic 62 spectropolarimeter calibrated with an aqueous solution of sucrose (o.r.d.); Perkin-Elmer 457 grating spectrometer (i.r., as Nujol mulls); Perkin-Elmer model R10, Varian Anaspect EM-360, Bruker WH-90FT, JEOL PS100, and Varian MR220 spectrometers (^1H n.m.r. at 60, 90, 100, and 220 MHz respectively). Hydrogen-1 n.m.r. spectra were calibrated with internal Bu^tOH [62] as reference at 1.27 p.p.m.; samples were dissolved in D_2O (*ca.* 1 cm^3) to allow exchange of labile protons, and then freeze-dried two or three times to reduce the size of the peak due to residual HOD which in many cases otherwise obscured solute resonances. pH Measurements were carried out on a Radiometer pH meter (type pH 4) using a semimicroelectrode with which it is possible to measure the pH of 0.3—0.5 cm^3 samples. Molar absorption coefficients were determined by cobalt analysis on solutions of known optical density. Complexes were broken down for cobalt analysis either by prolonged (several days) heating at 100 °C with *aqua regia* or by heating with 2% aqueous ammonium peroxodisulphate for 1 h;[63] the latter procedure has been found [64] to give the most consistent results for cobalt(III) peptide complexes, although with some samples it gave a black insoluble precipitate which dissolved in *aqua regia* only with difficulty. The Co^{II} in the resulting solution was determined colorimetrically [65] at 635 nm as $[Co(CNS)_4]^{2-}$ in 50% aqueous acetone. In some cases, complexes were also analysed for cobalt using atomic-absorption spectroscopy, but unless the complexes were first broken down with *aqua regia* or peroxodisulphate the results were unsatisfactory.

Sodium tris(carbonato)cobaltate(III),[66] hexakis(urea)-cobalt(III) perchlorate,[67] L-alanylglycinato(triammine)cobalt(III) perchlorate,[15] and hexa-amminecobalt(III) chloride [68] were prepared by standard methods.

Preparation and Purification of Bis(dipeptidato)cobaltate(III) Complexes.—Seven methods have been used to prepare bis(dipeptidato)cobaltate(III) complexes and examples

* However, isoandrosterone is very sensitive to impurities in the dioxan and unless carefully purified dioxan is used erroneous results are obtained. A more stable, convenient, and cheaper standard is a solution of camphor in ethanol; we have found [61] that $\Delta\varepsilon_{296} = 1.53$.

of the experimental procedures used are given below. However, the routes from CoO(OH) and $Co[CO_3]$ were found to be the most widely useful; both were used to prepare the complexes from a number of dipeptides.

(*i*) *By oxygenation of cobalt*(II) *solutions*. The peptide (0.02 mol) was dissolved in water (10 cm^3) and the pH adjusted to 10 with sodium hydroxide. The salt $CoCl_2 \cdot 6H_2O$ (2.38 g, 0.01 mol) in water (5 cm^3) was added and dioxygen bubbled rapidly through the solution for 12 h. With the dipeptides gly-L-leu and gly-L-phe, the peroxide-bridged intermediates are rather stable and the formation of the final bis(dipeptidato)cobaltate(III) complexes is slow unless activated charcoal is added. The preparation may be modified by oxidising with 30% hydrogen peroxide (15 cm^3) added dropwise whilst keeping the solution in an ice-bath. The complex anion was purified by ion exchange (permutit Zeo-Karb 225 in the sodium form) and then by chromatography on Sephadex G-10. The bands were separated; the first (brown) was rejected and only the second red-purple fraction was collected. The solution was then concentrated on a rotatory evaporator and attempts made to isolate crystalline products. In one or two cases, semi-crystalline solids were obtained but these were mostly deliquescent and rapidly reverted to a syrup. It was possible to obtain solids by evaporation to dryness of the purified solution on the rotatory evaporator. Analysis of these products suggest that they contain only pure complexes as shown by the results presented below: sodium bis(L-alanyl-L-alaninato)cobaltate(III) heptahydrate (Found: C, 27.8; H, 5.20; N, 10.6. $C_{12}H_{24}CoN_4NaO_{13}$ requires C, 27.3; H, 6.4; N, 10.6%).

(*ii*) *From cobalt*(II) *carbonate*. Cobalt(II) carbonate (60 mg, 0.5 mmol) was suspended in a solution of L-alanylglycine (146 mg, 1 mmol) in water (20 cm^3) and warmed to 60 °C with stirring. Hydrogen peroxide (30%, 4 cm^3) was added dropwise, allowing the vigorous effervescence to subside between additions. The mixture was cooled, and a little unchanged cobalt carbonate was removed. Cationic species remaining in solution were removed on Zeocarb 225 (lithium form), and, after concentration, inorganic salts and unchanged peptide were removed on a Sephadex G-10 column (2 × 50 cm). The diastereoisomers were then separated from each other and from the minor products on a QAE-Sephadex A-25 column (1 × 50 cm) using 0.1 mol dm^{-3} $Li[ClO_4]$ as eluant, and desalted on Sephadex G-10.

Crystalline complexes were not obtained from optically active dipeptides although crystalline lithium bis(glycylglycinato)cobaltate(III) hexahydrate was obtained (Found: C, 21.8; H, 4.7; Co, 13.0; N, 12.6. Calc. for $C_8H_{28}CoLiN_4O_{14}$: C, 22.2; H, 5.5; Co, 13.6; N, 12.9%).

This method yields relatively large amounts of the minor products especially with glycylglycine and with dipeptides of the type gly-L-α: only trace amounts of the minor products are formed with dipeptides of the type L-α-gly or L-α-L-α. The proportion of the diastereoisomer a formed by this method is higher than that formed in syntheses starting from cobalt(III) complexes (*ca.* 70%).

(iii) *From sodium tris(carbonato)cobaltate*(III). (*a*) Glycyl-L-alanine (110 mg, 0.75 mmol) and $Na_3[Co(CO_3)_3]$ (124 mg, 0.34 mmol) were mixed in water (20 cm^3), and stirred for 1 week at room temperature. Unchanged $Na_3[Co(CO_3)_3]$ was filtered off and the solution concentrated to 2 cm^3 using a rotary evaporator. Cobalt(II) was removed on Zeocarb 225 (potassium form) and, after concentration, KCl and unchanged peptide on a Sephadex G-10 column (2 × 90 cm). The diastereoisomers were then separated from one another and a small amount of minor products on a DEAE-Sephadex A-25 column (1 × 50 cm) using 0.1 mol dm^{-3} $Li[ClO_4]$ as eluant. The diastereoisomers were individually freed from $Li[ClO_4]$ on Sephadex G-10. This method gave the diastereoisomers in a ratio of *ca.* 1 : 2, the more strongly adsorbed diastereoisomer (b) being in excess. The lithium salt of the first eluted diastereoisomer crystallises as square plates; preliminary *X*-ray crystallographic results [13] indicate that the formula is $Li[Co(gly\text{-}L\text{-}alaO)_2] \cdot 4H_2O$. It has not yet been possible to crystallise the other diastereoisomer.

(*b*) Glycyl-L-leucine (3.76 g, 20 mmol) was dissolved in water (10 cm^3) and kept with $Na_3[Co(CO_3)_3]$ (3.6 g, 8.6 mmol) and activated charcoal (*ca.* 0.5 g) at 60 °C overnight. The solution was filtered, passed through a cation-exchange column (Zeo-Karb 225 in the sodium form), and evaporated to *ca.* 5 cm^3 on a rotatory evaporator. Acetone was added until the mixture was just cloudy, and then sufficient water to remove the turbidity. The solution was allowed to stand at 5 °C for several days, yielding on one occasion, a few magenta crystals of sodium bis(glycyl-L-leucinato)-cobaltate(III) hexahydrate (Found: C, 33.8; H, 6.7; N, 9.2. $C_{16}H_{40}CoN_4NaO_{12}$ requires C, 34.2; H, 7.1; N, 10.0%). On other occasions and with other peptides this procedure gave only purple gums.

(*iv*) *From hexa-amminecobalt*(III) *chloride*. An aqueous solution of the dipeptide (20 mmol) and $[Co(NH_3)_6]Cl_3$ (2.67 g, 10 mmol) was heated with activated charcoal (10 mg) on a steam-bath. Ammonia was evolved and after a few hours the orange-red solution had turned bright purple. Cationic complexes were removed on Zeo-Karb 225 (ammonia form) and the complex anion was purified by chromatography on Sephadex G-10. No crystalline products were isolated using this route, but evaporation to dryness of a carefully purified solution gave a reddish glass of ammonium bis(glycyl-L-alaninato)cobaltate(III) 13-hydrate (Found: C, 19.6; H, 8.2; N, 11.4: $C_{10}H_{46}CoN_5O_{19}$ requires C, 20.0; H, 7.7; N, 11.7%).

(*v*) *From hexakis(urea)cobalt*(III) *perchlorate*. Glycylglycine (100 mg, 0.76 mmol) and hexakis(urea)cobalt(III) perchlorate (272 mg, 0.38 mmol) were dissolved in dry acetonitrile, and an excess of triethylamine was added with stirring. A dark brown precipitate formed overnight which was filtered off leaving a yellow solution. The dark brown precipitate dissolved readily in water and the aqueous solution was chromatographed on QAE-Sephadex A-25 giving a red-brown band which was eluted easily. The remaining bands were very similar, both in appearance and in ease of elution, to the bands obtained from other preparations of $[Co(gly\text{-}glyO)_2]^-$. The major band was identified by its visible spectrum and ^1H n.m.r. spectrum as $[Co(gly\text{-}glyO)_2]^-$.

(*vi*) *From cobalt*(III) *hydroxide oxide*. Cobalt(III) hydroxide oxide, CoO(OH), prepared from cobalt(II) chloride hexahydrate (0.24 g, 1 mmol), an excess of potassium hydroxide, and 30% hydrogen peroxide, was washed by centrifugation until the supernatant was neutral. L-Alanylglycine (100 mg, 0.7 mmol) was added and the mixture was magnetically stirred at room temperature (*ca.* 20 °C) until no further increase in optical density at 520 nm was observed (*ca.* 1 week). The dark red-purple solution resulting was filtered and the filtrate passed through a cation-exchange column (Zeo-Karb 225 in the sodium form) to remove any Co^{II} present, and then, after concentration at reduced pressure and low temperature (*ca.* 30 °C), was

passed through a column (80 × 2.5 cm) of Sephadex G-10 dextran gel, which removes free peptide and any inorganic salts. The solution now contains only neutral and anionic cobalt(III) complexes, which were separated on Sephadex anion-exchange * dextran gel QAE-A-25 using lithium perchlorate (0.05—0.1 mol dm^{-3}) as eluant. The diastereoisomers were not separated in this preparation. Lithium perchlorate was removed by chromatography twice on a Sephadex G-10 column (2 × 100 cm). The eluant was concentrated at low temperatures, and finally slow evaporation at room temperature gave sodium bis(L-alanylglycinato)cobaltate(III) tetrahydrate (Found: C, 26.8; H, 5.9; N, 11.7. $C_{10}H_{24}CoN_4NaO_{10}$ requires C, 26.7; H, 5.4; N, 12.4%) as dark magenta crystals. A similar preparation using L-leucyl-L-leucine gave a few dark magenta crystals of lithium bis(L-leucyl-L-leucinato)cobaltate(III). The c.d. of a solution of a single untwinned crystal indicated that it contained both diastereoisomers in equal quantities, *i.e.* that it is a quasi-racemate.

(*vii*) *From triammine(glycylglycinato)cobalt*(III) *chloride.* The complex (0.5 g, 1.6 mmol) was dissolved in water (10 cm^3) and sufficient 10% sodium carbonate solution was added to bring the pH to 11. The solution was heated on a steam-bath for 1 h. The solution changed from red to purple, ammonia was evolved, and a brown precipitate of cobalt(III) hydroxide oxide was formed. The solution was filtered and the filtrate chromatographed on a column (80 × 2.5 cm) of Sephadex G-10. The major band was shown by paper electrophoresis to be anionic and identified as the bis(glycylglycinato)cobaltate(III) ion by its electronic (λ 520 nm) and ^1H n.m.r. spectra.

This method is suitable only for the preparation of bis-(glycylglycinato)cobaltate(III) and complexes of dipeptides with a substituted N-terminal residue: optically active dipeptides with a substituted C-terminal residue racemise under the alkaline conditions used owing to proton exchange on the methine group of the C-terminal residue. Diethylenetriamine(glycylglycinato)cobalt(III) chloride disproportionates similarly, but more slowly and only at higher pH to a mixture of $[Co(dien)_2]^{3+}$ and $[Co(gly\text{-}glyO)_2]^-$, which can be separated on Sephadex G-10. However, considerable decomposition of the $[Co(gly\text{-}glyO)_2]^-$ occurs under the vigorous conditions required and this synthesis is of little use.

Separation of Diastereoisomers.—Diastereoisomers of $[Co(gly\text{-}L\text{-}\alpha)_2]^-$ were readily separated by chromatography on QAE-Sephadex A-25 (120 × 2.5 cm column). Two completely separated bands were usually observed on elution with 0.05—0.1 mol dm^{-3} $Li[ClO_4]$. For $[Co(L\text{-}\alpha\text{-}glyO)_2]^-$ the two bands were usually not completely separated, and it was necessary to collect fractions and rechromatograph. In some cases, *e.g.* $[Co(L\text{-}ala\text{-}L\text{-}alaO)_2]^-$, complete separation into diastereoisomers can be achieved by chromatography on alumina. Sephadex G-10 is not effective for the complete separation of the diastereoisomers although detailed comparison of the c.d. spectrum of fractions of $[Co(L\text{-}ala\text{-}glyO)_2]^-$ from Sephadex G-10 indicates that fractionation occurs. Likewise, some fractionation of $[Co(gly\text{-}L\text{-}alaO)_2]^-$ was observed when it was chromatographed on starch.

Resolution of Bis(glycylglycinato)cobaltate(III).—No resolution of $[Co(gly\text{-}glyO)_2]^-$ was observed on QAE-Sephadex A-25. However, a concentrated solution of $[Co(gly\text{-}glyO)_2]^-$ was chromatographed on a starch column (60 × 2.5 cm), and eluted with water (containing a trace amount of toluene to inhibit bacterial action). Fractions were filtered through an asbestos pad supported on a no. 4 sintered-glass funnel to remove fine starch particles; if this caution was omitted the baseline of the c.d. spectrum was displaced. The first and last fractions were optically active; the first fractions had a negative c.d. spectrum at 555 nm, $\Delta\varepsilon = 0.35$.

Reactions of Bis(dipeptidato)cobaltate(III) *Complexes.*—*Sodium bis(glycyl-*DL*-threoninato)cobaltate*(III) *trihydrate.* Sodium bis(glycylglycinato)cobaltate(III) (1.2 g, 0.003 mol) was dissolved in water (10 cm^3) and sufficient 10% (w/v) sodium carbonate solution added to bring the pH to 11. Acetaldehyde (3 cm^3, 0.052 mol) was added and the solution was allowed to stand for 2 d at 37 °C. (Alternatively, the solution could be heated under reflux for 2 h, but this leads to a higher yield of by-products.) The solution was then neutralised with 0.1 mol dm^{-3} hydrochloric acid and evaporated to dryness on a rotatory evaporator. The solid was extracted twice with 2-cm^3 portions of water and the combined portions were then chromatographed on Sephadex G-10. Three fractions were obtained, first a brown one, then the required purple product, and finally a yellow fraction which adhered strongly to the top of the column and which was presumed to be the aldehyde polymer. The purple fraction was concentrated to *ca.* 1 cm^3 on the rotatory evaporator and rechromatographed, any remaining brown product being removed at this stage. The product was then passed down a column of cation-exchange resin in the sodium form. It was possible to obtain a minute amount of the sodium salt as a crystalline solid (Found: C, 32.1; H, 5.5; N, 12.3. $C_{12}H_{26}CoN_4NaO_9$ requires C, 31.9; H, 5.8; N, 12.4%).

Identification of the amino-acids. The product of the last synthesis was dissolved in water (10 cm^3) and adjusted to pH 8.5 with 0.1 mol dm^{-3} sodium hydroxide. Hydrogen sulphide was passed for 15 min and then the solution was adjusted to pH 3.5 with 0.1 mol dm^{-3} HCl. The cobalt sulphide was filtered off, then the whole process was repeated. The resulting solution was evaporated to dryness on a rotary evaporator and then extracted with methyl cellosolve (2-methoxyethanol) (2 × 2 cm^3). The combined portions were evaporated to dryness and the solid redissolved in HCl (5 mol dm^{-3}, 1 cm^3). This solution was heated to 110 °C in a sealed glass tube for 24 h. The resulting hydrolysate was cautiously evaporated to dryness, taken up in water (1 cm^3), and re-evaporated to dryness to remove excess of HCl. Dissolution in water and evaporation was then repeated once. The resulting solid was dissolved in water (1 cm^3), and chromatographed on Whatman 3 MM paper using butanol–acetone–water (2 : 2 : 1 v/v) as solvent. Two spots were obtained on development with ninhydrin and by comparison with standards run under the same conditions these spots were shown to be glycine and threonine.

Sodium bis(glycylserinato)cobaltate(III). This was prepared from $Na[Co(gly\text{-}glyO)_2]$ by the same method using formaldehyde in place of acetaldehyde and carrying out the reaction in a closed vessel to prevent loss of formaldehyde. No crystalline sample of this product could be isolated, but conversion into the sodium and ammonium salts and evaporation to dryness gave glassy solids.

* DEAE Sephadex A-25 gives less well resolved bands than QAE Sephadex A-25; in particular, the diastereoisomers of *mer*-bis(dipeptidato)cobaltate(III) are not as well separated on DEAE Sephadex A-25.

(Sodium salt decahydrate. Found: C, 20.8; H, 6.4; N, 9.6. $C_{10}H_{36}CoN_4NaO_{18}$ requires C, 20.6; H, 6.20; N, 9.60. Ammonium salt octahydrate. Found: C, 22.0; H, 5.2; N, 12.7. $C_{10}H_{36}CoN_5O_{16}$ requires C, 22.2; H, 6.65; N, 12.95%).

The constituent amino-acids were identified, after hydrolysis and paper chromatography, as glycine and serine by the same method as used for the glycylthreonine complex.

Sodium bis(glycyl-β-phenylserinato)cobaltate(III). This complex could be prepared by the same method using benzaldehyde in place of acetaldehyde, with the following modification. Since benzaldehyde is immiscible with water, the condensation was carried out in methyl cellosolve–water (9 : 1 v/v). No crystalline product could be isolated but the two amino-acids, glycine and β-phenylserine, were identified by paper chromatography after removal of the cobalt and hydrolysis of the peptide as described previously.

Sodium bis(L-*alanylthreoninato*)*cobaltate*(III) *trihydrate*. This complex was prepared by the method previously described for the analogous glycylthreoninato-complex. A small amount of solid product was obtained and analysed well as the trihydrate (Found: C, 34.6; H, 6.2; N, 12.0. $C_{14}H_{30}CoN_4NaO_9$ requires C, 35.0; H, 6.3; N, 11.7%). Again the constituent amino-acids were identified by paper chromatography.

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