Optically Active Co-ordination Compounds. Part XXXIII.[1] Bacterial Resolution of Bis(ethylenediamine)1,10-phenanthrolinecobalt(III) Chloride, 2,2'-Bipyridylbis(ethylenediamine)cobalt(III) Chloride, and Potassium (ethylenediaminetetra-acetato)cobaltate(III)

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A number of co-ordination compounds of cobalt(III) have been screened as potential nitrogen sources for bacterial growth under aerobic conditions. A strain of *Pseudomonas stutzeri* has been isolated and its use as a ' biological resolving agent ' to bring about stereoselective metabolism of racemic $[Co(en)_2(phen)]Cl_3$ and of $[Co(en)_2(bipy)]Cl_3$ (en = ethylenediamine, phen = 1,10-phenanthroline, and bipy = 2,2'-bipyridyl), when either complex is supplied to the organism as the sole nitrogen source, is reported. The organism will also reduce, stereoselectively, racemic K[Co(edta)] (edta = ethylenediaminetetra-acetato) in the presence of NH_4Cl (0·1% w/v) but it is unable to utilize this complex as a nitrogen source. The cobalt species produced during these various degradations have been characterized. There is no reduction when racemates of $[Co(en)_3]Cl_3$, $[Co(en)_2(sal)]Cl$, $[Co(en)_2(pic)]Cl_2$, or *abd*-$[Co(gly)_3]$ [sal = salicylate(2−), pic = α-picolinato, and gly = glycinato-*O*] are supplied to the organism either as the sole sources of nitrogen or together with NH_4Cl (0·1% w/v).

THE technique of asymmetric destruction by micro-organisms was first discovered by Pasteur [2] when he observed stereospecific metabolism of the natural (R)-(+) half of racemic ammonium tartrate by *Penicillium glaucum*. This technique has since been considerably refined and enzymes, the active agents,[3] are now used to resolve a variety of compounds, and in particular amino-acids.[4] In the light of present knowledge of the high stereospecificity of enzymes with their substrates, the asymmetric destruction of such substrates in the presence of their enantiomers is not surprising. However, the use of micro-organisms to resolve such non-physiological compounds as synthetic transition-metal complexes has received comparatively little attention. Bailar *et al.*[5] reported the growth of *Pseudomonas aeruginosa* on the complex D-$[Co(en)_3]Cl_3$ (en = ethylenediamine) when it was supplied as the sole nitrogen source, although the L-form was inhibitory and growth on the racemate was not observed, so that resolution was not achieved. More recently stereoselective reduction, under anaerobic conditions, of *abd*-$[Co(gly)_3]$ (gly = glycinato-*O*) by *Enterobacter cloacae*, *Aerobacter aerogenes*, *Proteus vulgaris*, and *Escherichia anaerogenes* has been studied in this laboratory,[6-8] although another organism, *Serratia marcescens*, showed no similar activity. D-*abd*-$[Co(gly)_3]$, shown to be optically pure by the method of isotope dilution, was obtained by anaerobic incubation of the racemate with *P. vulgaris* followed by recovery and fractional crystallization of the remaining unattacked complex.[8]

A number of cobalt(III) complexes have been screened as potential nitrogen sources for bacterial growth under aerobic conditions. This paper reports the isolation of a strain of *Pseudomonas stutzeri* [9,10] and its use as a ' biological resolving agent ' to bring about stereoselective reduction, both of racemic $[Co(en)_2(phen)]Cl_3$ (phen = 1,10-phenanthroline) and of $[Co(en)_2(bipy)]Cl_3$ (bipy = 2,2'-bipyridyl), when either complex is supplied to the organism as the sole source of nitrogen. The organism will also reduce stereoselectively racemic K[Co(edta)] (edta = ethylenediaminetetra-acetato) in the presence of NH_4Cl (0·1% w/v) but it is unable to utilize this complex as a nitrogen source. The cobalt species produced during these various degradations have been characterized.

EXPERIMENTAL

Optical densities were measured with a Unicam SP 600 spectrophotometer, electronic spectra with a Unicam SP 800 spectrophotometer, and circular dichroism (c.d.) spectra with a Roussel–Jouan model B Dichrographe. C, H, and N anslyses were carried out by Mr. G. H. J. Powell of this laboratory, using a Hewlett–Packard model 185 C, H, N analyser. The cation exchanger SP-Sephadex C-25 was used for chromatography, with NaCl (1·2M) solution as the eluant. The u.v. absorption (254 nm) of the eluate was recorded continuously with a Uvicord 1 LKB 4700 instrument and fractions subsequently collected with an UltroRac LKB 7000 fraction collector.

Preparation of Complexes.—Bis(ethylenediamine)(1,10-phenanthroline)cobalt(III) bromide. The complex $[Co(en)_2(phen)]Br_3$ was prepared by a known method [11] (Found: C, 32·1; H, 4·1; N, 14·0. Calc. for $C_{16}H_{24}Br_3CoN_6$: C, 32·0; H, 4·0; N, 14·0%).

(2,2'-Bipyridyl)bis(ethylenediamine)cobalt(III) bromide diperchlorate. The mixed salt $[Co(en)_2(bipy)]Br(ClO_4)_2$ was prepared according to our recent method [12] (Found: C,

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[1] Part XXXII is ref. 12.
[2] L. Pasteur, *Compt. rend.*, 1858, **46**, 615.
[3] L. Rosenthaler, *Biochem. Z.*, 1908, **14**, 238.
[4] V. E. Price and J. P. Greenstein, *J. Biol. Chem.*, 1948, **175**, 969.
[5] J. C. Bailar, H. Itatani, M. J. S. Crespi, and J. F. Geldard, *Advances in Chemistry*, 1967, **62**, 103.
[6] R. D. Gillard and C. Thorpe, *Chem. Comm.*, 1970, 997.
[7] C. Thorpe, Ph.D. Thesis, University of Kent at Canterbury, 1972.
[8] R. D. Gillard, J. R. Lyons, and C. Thorpe, *J.C.S. Dalton*, 1972, 1584.
[9] R. Y. Stanier, N. J. Pallerone, and M. Doudoroff, *J. Gen. Microbiol.*, 1966, **43**, 254.
[10] C. B. van Niel and M. B. Allen, *J. Bacteriol.*, 1952, **64**, 413.
[11] R. D. Gillard, R. E. E. Hill, and R. Maskill, *J. Chem. Soc. (A)*, 1970, 1447.
[12] L. S. Dollimore and R. D. Gillard, *J.C.S. Dalton*, 1973, 933.

27·5; H, 4·1; N, 13·6. Calc. for $C_{14}H_{24}BrCl_2CoN_6O_8$: C, 27·4; H, 4·0; N, 13·7%).

Potassium (ethylenediaminetetra-acetato)cobaltate(III) *dihydrate.* The complex K[Co(edta)],$2H_2O$ was prepared by the method of Kirschner [13] (Found: C, 28·5; H, 2·9; N, 6·9. Calc. for $C_{10}H_{16}CoKN_2O_{10}$: C, 28·5; H, 3·8; N, 6·6%).*

Tris(1,10-*phenanthroline*)*cobalt*(II) *chloride hexahydrate.* A solution of 1,10-phenanthroline hydrate (5·0 g) was added to a deoxygenated (N_2 bubbler) solution of $CoCl_2$,$6H_2O$ (2·0 g) in water (20 cm³) and heated on a steam-bath for 15 min. On cooling, fine yellow crystals of the complex $[Co(phen)_3]Cl_2,6H_2O$ formed (Found: C, 55·0; H, 4·50; N, 10·9. Calc. for $C_{36}H_{36}Cl_2N_6O_6$: C, 55·4; H, 4·65; N, 10·8%).

Tris(2,2′-*bipyridyl*)*cobalt*(II) *perchlorate.* The complex $[Co(bipy)_3](ClO_4)_2$ was prepared by a method similar to that given for $[Co(phen)_3]Cl_2,6H_2O$, but an excess of $NaClO_4$ solution was added to the cooled reaction mixture. Fine ochre crystals of the perchlorate were precipitated (Found: C, 49·9; H, 3·25; N, 11·7. Calc. for $C_{30}H_{24}Cl_2CoN_6O_8$: C, 49·6; H, 3·30; N, 11·6%).

Characteristic spectroscopic maxima (nm) of certain cobalt complexes in NaCl (1·2M)

Species								
$[Co(en)_2(phen)]^{3+}$	349	334(sh)	319(sh)	305(sh)			273	
$[Co(phen)_3]^{2+}$	345	329(sh)			293(sh)			268
$[Co(phen)_3]^{3+}$	348	334(sh)		303		280	274	
$[Co(en)(phen)_2]^{3+}$	349	334(sh)	317(sh)	304(sh)		280(sh)	273	
$[Co(en)_2(bipy)]^{3+}$	319(sh)	309	299(sh)					
$[Co(bipy)_3]^{2+}$		305	295					
$[Co(bipy)_3]^{3+}$	318	306						

Tris(1,10-*phenanthroline*)*cobalt*(III) *perchlorate trihydrate and tris*(2,2′-*bipyridyl*)*cobalt*(III) *perchlorate trihydrate.* The preparation, analyses, and electronic spectra in water of these complexes have been described.[12] The spectra of the same samples in NaCl (1·2M) showed no differences in peak positions and only marginal differences in relative intensities.

Ethylenediaminebis(1,10-*phenanthroline*)*cobalt*(III) *perchlorate.* The complex $[Co(en)(phen)_2]Cl_3,H_2O$ was prepared by Palade's method.[14] The yellow crystals were redissolved in water and the salt $[Co(en)(phen)_2](ClO_4)_3$,$2H_2O$, was obtained by addition of an excess of $NaClO_4$ solution (Found: C, 39·0; H, 3·10; N, 10·5. Calc. for $C_{26}H_{28}Cl_3CoN_6O_{12}$: C, 38·4; H, 3·55; N, 10·8%).

Electronic spectral characteristics for the above cobalt complexes, in NaCl (1·2M), are given in the Table. The other complexes tested as potential nitrogen sources, *i.e.* $[Co(en)_3]Cl_3$,[15] $[Co(en)_2(sal)]Cl$,[16] $[Co(en)_2(pic)]Cl_2$,[17] and *abd*-$[Co(gly)_3]$ [18] were prepared by literature methods. Before the bacterial experiments the complexes $[Co(en)_2(phen)]Br_3$ and $[Co(en)_2(bipy)]Br(ClO_4)_2$ were converted to their chlorides by anion exchange ('Deacidite' FF-IP resin, Cl^- form).

Isolation and Growth of Organism.—An enrichment culture was prepared by incubating a sample of sewage aerobically at 30 °C in a minimal salts solution (see below), containing the complex $[Co(en)_2(phen)]Br_3$ (5×10^{-3}M). Sub-culturing of the supernatant on nutrient agar (Oxoid Ltd.) resulted in the isolation of an organism, since identified by the National Collection of Industrial Bacteria (Torry Research Station, Aberdeen) as a strain of *Pseudomonas stutzeri*.

The minimal salts solution contained KH_2PO_4 (2·8 g), Na_2HPO_4 (4·2 g), $MgSO_4,7H_2O$ (0·2 g), $FeCl_3$ (1·0 cm³, $6{\cdot}18 \times 10^{-2}$M-solution), and trace-element solution (1·0 cm³) ($CaCl_2,2H_2O$ $4{\cdot}5 \times 10^{-3}$M, $ZnSO_4,7H_2O$ $6{\cdot}25 \times 10^{-4}$M, $CuSO_4,5H_2O$ $6{\cdot}4 \times 10^{-4}$M, $MnSO_4,4H_2O$ $6{\cdot}7 \times 10^{-4}$M, $CoCl_2,6H_2O$ $7{\cdot}55 \times 10^{-4}$M, H_3BO_3 $1{\cdot}62 \times 10^{-3}$M, and $Na_2MoO_4,2H_2O$ $1{\cdot}24 \times 10^{-3}$M) per litre. Growth of the organism was stimulated by the presence of thiamine hydrochloride (3×10^{-6}M) and glucose (0·2%, w/v) was used as a carbon source.

All solutions containing complex were sterilized by Millipore filtration (HA, 0·45 μm); other solutions and apparatus were sterilized by autoclaving (15 p.s.i., 15 min). The organism was maintained by subculture on slopes of nutrient agar and stored at 4 °C.

Resolution of the Ethylenediamine Complexes.—*Pseudomonas stutzeri* was grown aerobically at 30 °C, overnight, in minimal salts solution containing ethylenediamine (0·05%, w/v) as the sole nitrogen source. This culture was then used to inoculate a minimal salts solution containing glucose and complex (5×10^{-3}M). The cultures were incubated at 30 °C and subjected to vigorous aeration on a Gallenkamp orbital shaker. Aliquot portions (10 cm³) were taken at regular intervals and the turbidity (O.D. at 700 nm) recorded. The aliquot portions were then centrifuged (38 000 *g*, 10 min) and a sample of the supernatant (5·0 cm³) was subjected to chromatography on a Sephadex cation-exchange column. The electronic spectra of the fractions were examined, the fractions containing unchanged complex combined and then made up to 25 cm³ with NaCl (1·2M). The magnitude of the absorbance of the lowest energy *d–d* band (463 nm) was measured, together with any corresponding c.d. reflection (483 nm). Figures 1 and 2 summarize the results.

Resolution of K[Co(edta)] *under Growth Conditions.*—An inoculum was prepared under similar conditions to the preceding experiments using NH_4Cl (0·1%, w/v) as the sole source of nitrogen. Culture and sampling conditions were as described above. Spectroscopic measurements were made directly on the supernatant liquor of centrifuged samples. The magnitude of the absorbance of the lowest energy *d–d* band (538 nm) was measured, together with any c.d. deflection at 582 nm. Figure 3 summarizes the results.

Reduction of K[Co(edta)] *by Cell Suspension.*—*Pseudomonas stutzeri* was grown overnight in glucose–minimal

* Low H values have been obtained with our C, H, N analyser, for compounds with loosely held water of crystallization.[12]

[13] S. Kirschner, *Inorg. Synth.*, 1957, **5**, 186.
[14] D. M. Palade, *Russ. J. Inorg. Chem.*, 1967, **12**, 520.
[15] J. B. Work, *Inorg. Synth.*, 1957, **5**, 186.
[16] K. Garbett and R. D. Gillard, *J. Chem. Soc.* (*A*), 1968, 979.
[17] C. T. Liu and B. E. Douglas, *Inorg. Chem.*, 1964, **3**, 1356.
[18] M. Mori, M. Shibata, E. Kyono, and M. Kanaya, *Bull. Chem. Soc. Japan*, 1961, **34**, 1837.

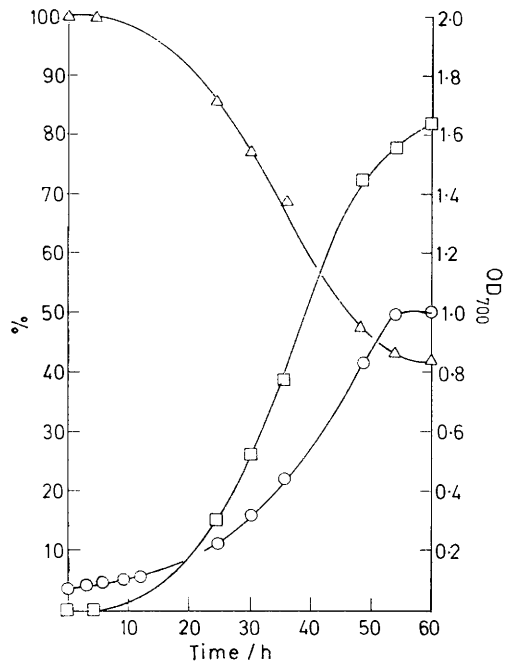


FIGURE 1 Growth of *P. stutzeri* with the complex $[Co(en)_2(phen)]Cl_3$ (5×10^{-3}M) as sole nitrogen source: growth curve (OD_{700}) (○); complex remaining (%) (△); and optical purity (%) (□)

salts solution (8 l), containing ethylenediamine (0·2%, w/v) as the sole nitrogen source. The cells were harvested by centrifugation at 4 °C (23 000 *g*, 15 min) and washed twice with cold KH_2PO_4–Na_2HPO_4 buffer (0·05M, pH 7·0). The cells (30 g wet weight) were stored at −20 °C. Cells (4 g wet weight) were suspended in 200 cm³

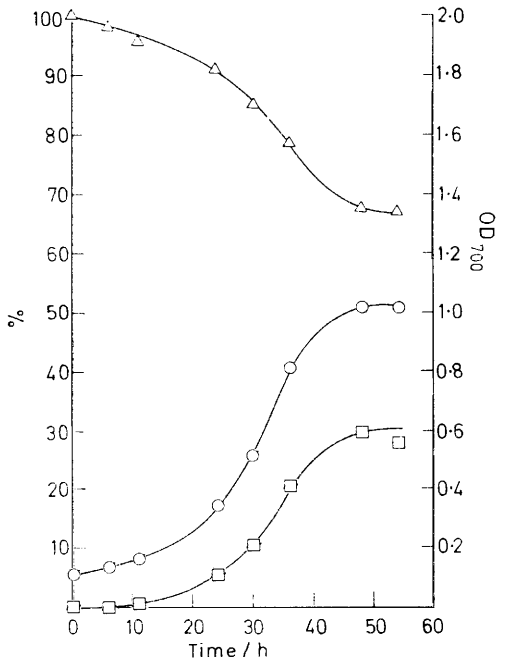


FIGURE 2 Growth of *P. stutzeri* with the complex $[Co(en)_2(bipy)]Cl_3$ (5×10^{-3}M) as sole nitrogen source. For key see Figure 1

phosphate buffer (0·05M, pH 7·0) containing glucose (0·4%, w/v) and incubated at 30 °C overnight. The complex

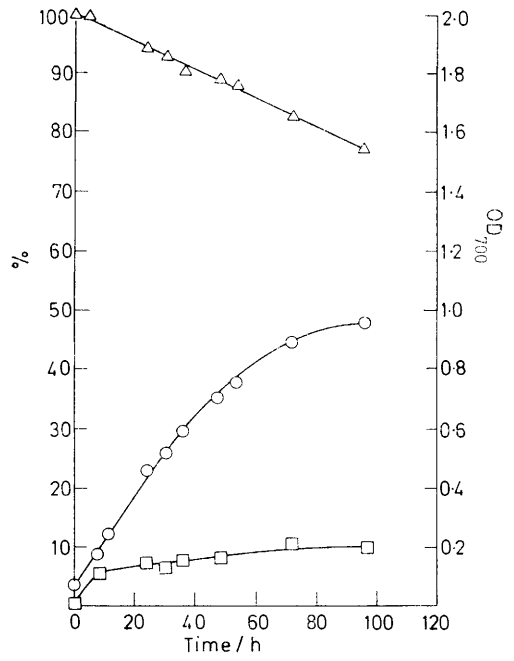


FIGURE 3 Growth of *P. stutzeri*, using NH_4Cl (0·1% w/v) as nitrogen source, in the presence of the complex K[Co(edta)] (2×10^{-3}M). For key see Figure 1

K[Co(edta)] (2×10^{-3}M, final concentration) was then added to the washed cell suspension and the cells incubated

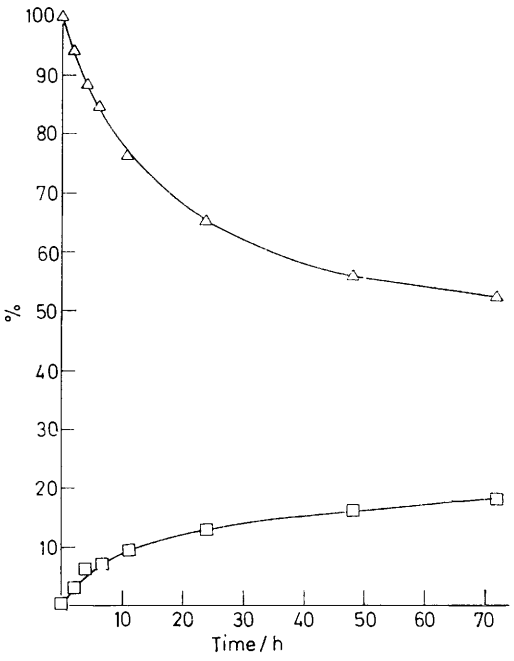


FIGURE 4 Incubation of *P. stutzeri* in the presence of the complex K[Co(edta)] (2×10^{-3}M). For key see Figure 1

aerobically at 30 °C. Aliquot portions (10 cm³) were taken at regular intervals and centrifuged (38 000 *g*, 10 min). The extinction at 538 nm and the c.d. deflection

at 582 nm were measured on the supernatant liquor as described above. Figure 4 summarizes the results.

RESULTS

Bis(ethylenediamine)(1,10-phenanthroline)cobalt(III) *Chloride.*—When our strain of *Pseudomonas stutzeri* was grown in glucose–minimal salts solution with racemic $[Co(en)_2(phen)]Cl_3$ (5×10^{-3}M), as the sole nitrogen source, the intensity of the lowest energy *d–d* band (463 nm) showed a decrease which is associated with the simultaneous appearance of a Cotton effect. Growth of the organism was slow, in comparison with similar cultures containing ethylenediamine as the sole nitrogen source; after 60 h, 42% of the original complex remained (Figure 1). The system is highly stereoselective, favouring removal of the complex D-$[Co(en)_2(phen)]^{3+}$. After 60 h the remaining $[Co(en)_2(phen)]^{3+}$ had an optical purity of 82% * (Figure 1). This optical purity exceeds the majority of the values obtained by conventional resolutions.[11,20,21]

Chromatographic separation and spectroscopic examination of the culture supernatants, during the course of the experiment, showed the formation of three 1,10-phenanthroline-containing species. Comparison of the position and relative magnitudes of the phen absorption bands with those of authentic samples (Table) showed two of the complexes to be $[Co(phen)_3]^{2+}$ and its aerobic oxidation product $[Co(phen)_3]^{3+}$. The third species (which is present at a much lower concentration than the other components) appears to contain cobalt(II) and has a maximum at 272 nm and a shoulder at 298 nm. Bubbling oxygen through a solution of this species caused no change in its u.v. spectrum but oxidation with chlorine produced a spectrum identical to that of the complex $[Co(en)_2(phen)]^{3+}$, indicating that this minor component is probably $[Co(en)_2(phen)]^{2+}$. None of these separated components showed any optical activity and no racemization of the partially resolved complex L-$[Co(en)_2(phen)]^{3+}$ was detected over the period of the experiment. A precipitate of pink cobalt(II) phosphate accumulated in the culture flask during the course of the experiment, indicating a deficiency of chelating agent in the system.

(2,2′-Bipyridyl)bis(ethylenediamine)cobalt(II) *Chloride.*—The results for the complex $[Co(en)_2(bipy)]Cl_3$ (5×10^{-3}M) are qualitatively similar to those for $[Co(en)_2(phen)]Cl_3$. Although there is no significant difference between the two growth rates, there is a marked difference in the rate of disappearance of the complex (Figure 2). After the cultures had reached an optical density (700 nm) of 1·0, 68·0% of the 2,2′-bipyridyl complex remained, compared with 43·3% of the analogous phen complex (Figure 1). The stereoselectivity is low; the optical purity, at an optical density (700 nm) of 1·0, was only *ca.* 30% (Figure 2). Removal of the D-isomer is again favoured.

Chromatographic separation of culture supernatants during the experiment showed the formation of two 2,2′-bipyridyl-containing species. These species were identified, by comparison of their u.v. spectra, with those of authentic samples (Table), as being the complex $[Co(bipy)_3]^{2+}$ and its aerobic oxidation product $[Co(bipy)_3]^{3+}$. As in the previous experiments, pink crystals of cobalt(II) phosphate accumulated in the culture medium during the growth of the organism. Neither of the identified cobalt species showed any optical activity and no racemization of partially resolved samples of the complex L-$[Co(en)_2(bipy)]Cl_3$ was detected over the period of the experiment.

Potassium (Ethylenediaminetetra-acetato)cobaltate(III).—Bacterial growth in the presence of the complex K[Co(edta)] (2×10^{-3}M) is very slow and is associated with a similarly slow reduction. After the culture had reached an optical density (700 nm) of 0·95, only 23·4% of the complex had been reduced. The stereoselectivity of the system, which favours reduction of the complex R(C_2)-$(+)_{546}[Co(edta)]^-$, although initially high, rapidly decreased; the final optical purity obtained was only *ca.* 10% (Figure 3).

Using a thick-cell suspension, reduction of the complex was far more rapid; after 72 h, 47·3% of the complex had been reduced (Figure 4). Stereoselectivity is still very poor and the final optical purity was 18·2% after 72 h (Figure 4). No chromatographic separation of the culture supernatant liquors was required as the only other species detected was $[Co(edta)]^{2-}$. The absorption coefficient of this complex in the region of the lowest energy *d–d* band of the cobalt(III) species is insignificant and does not interfere with any chiroptical measurements. No racemization of the partially resolved samples was detected over the period of the experiment.

Other Complexes.—No reduction was observed when the complexes $[Co(en)_3]Cl_3$, $[Co(en)_2(sal)]Cl$, $[Co(en)_2(pic)]Cl_2$, or *abd*-$[Co(gly)_3]$ (previously resolved [6–8]) were supplied either as the sole sources of nitrogen or together with NH_4Cl (0·1%, w/v). Thick-cell suspensions were similarly inactive.

DISCUSSION

Evidence has been presented for stereoselective reduction of the complexes $[Co(en)_2(phen)]Cl_3$, $[Co(en)_2(bipy)]Cl_3$, and K[Co(edta)] by *Pseudomonas stutzeri*. The nature of this activity appears to be different from the reduction of the complex *abd*-$[Co(gly)_3]$ by the facultative anaerobes *Enterobacter cloacae* and *Proteus vulgaris*. With these latter organisms reduction of the complex *abd*-$[Co(gly)_3]$ only occurs during incubation under anaerobic conditions, with anaerobically grown cells. This contrasts with *Pseudomonas stutzeri* which stereoselectively reduced certain cobalt complexes when both grown and incubated under aerobic conditions.

The first product of the reduction of the complexes DL-$[Co(en)_2(AA)]^{3+}$ (where AA = 1,10 phenanthroline or 2,2′-bipyridyl) by *Pseudomonas stutzeri* appears to be D-$[Co(en)_2(AA)]^{2+}$. In the absence of bacterial cells the complex $[Co(en)_2(AA)]^{2+}$ will form an equilibrium mixture with the species $[Co(en)_3]^{3+}$ and possibly $[Co(AA)_2(en)]^{3+}$ and $[Co(AA)_3]^{3+}$. Attempts to oxidize the species believed to be $[Co(en)_2(phen)]^{2+}$ with oxygen were unsuccessful.

In the presence of bacterial cells capable of utilizing ethylenediamine the system is greatly simplified. Complete utilization of ethylenediamine will lead to

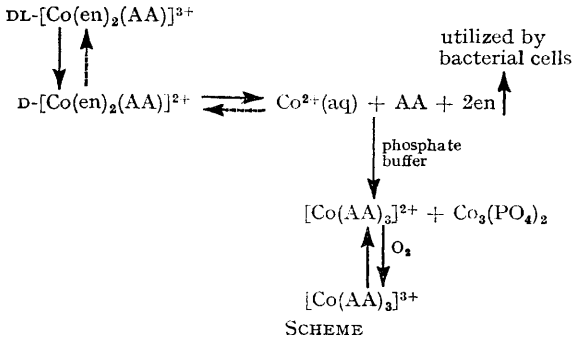
* All optical purity figures are based on chiroptical values for the optically pure species obtained previously.[12,19]

[19] R. D. Gillard, *Spectrochim. Acta*, 1964, **20**, 1431.

[20] F. M. Jaeger, *Proc. Roy. Acad. Sci. Amsterdam*, 1926, **29**, 559.

[21] J. Hidaka and B. E. Douglas, *Inorg. Chem.*, 1964, **3**, 1180.

formation of only $[Co(AA)_3]^{2+}$ and its oxidized form $[Co(AA)_3]^{3+}$ (Scheme). Experimentally with the complex $[Co(en)_2(bipy)]^{3+}$ only $[Co(bipy)_3]^{2+}$ and $[Co(bipy)_3]^{3+}$ were found. The complex $[Co(en)_2(phen)]^{3+}$



SCHEME

is more readily reduced (58%) compared with the analogous bipy complex (32·5%), although the same amount of growth occurs in both systems. This implies that an excess of ethylenediamine is present in the case of $[Co(en)_2(phen)]^{3+}$, which could explain the presence of the extra cobalt species in this system. In both systems, a deficiency of chelating agent in the presence of phosphate buffer results in the precipitation of pink cobalt(II) phosphate.

Reduction of the complex K[Co(edta)] results in a mixture of the initial complex and the cobalt(II) species in culture supernatant liquors. This cobalt(II) species cannot be reoxidized to cobalt(III) under the conditions of the experiment.

The bacterial resolution of inorganic metal complexes has several immediate applications. The most obvious of these is the use of suitable organisms as biological * resolving agents when conventional techniques have proved unsuccessful, *e.g.* stereospecific resolution of *abd*-$[Co(gly)_3]$ by *Proteus vulgaris*.[8] With this neutral complex, techniques involving formation of diastereoisomers are inappropriate and only partial resolution has been achieved using starch chromatography.[23] The resolution of charged species is usually a simpler procedure. There have been many reports of the resolution of the complexes $[Co(edta)]^-$ (refs. 24—26) and $[Co(en)_2(phen)]^{3+}$ (refs. 11, 12, 20, and 21), although before complete resolution [12] of the analogous $[Co(en)_2(bipy)]^{3+}$ only one report of partial resolution had been published.[27]

A second possible application is the use of microorganisms for assignment of absolute configuration. Assuming interaction at the same site, enantiomers of closely related complexes with the same configuration will be preferentially reduced. In the case of the complexes $[Co(en)_2(phen)]^{3+}$ and $[Co(en)_2bipy)]^{3+}$ the enantiomers more readily reduced by *Pseudomonas stutzeri* have related absolute stereochemistries. The importance of such evidence is difficult to assess, however, as the biochemical mechanism for this type of reduction is at present unknown. The value of such experiments will be greatly increased, following isolation and purification of the system or systems involved.

We wish to thank Shell Research Limited and Dr. R. J. Watkinson of Milstead Laboratory of Chemical Enzymology for assistance and advice, Dr. C. Thorpe, who pioneered the early stages of this bacterial resolution project, and the M.R.C. for financial support of this and related work.

[2/2354 *Received*, 16*th October*, 1972]

* In its strictest sense the term resolution means separation of the two enantiomers of a racemate. Thus Pasteur's method of obtaining (S)-(−)-tartaric acid from the ammonium salt of the racemate using *Penicillium glaucum* has been termed an asymmetric destruction.[22] Preferential destruction of one enantiomer of a racemic transition-metal complex should therefore be termed kinetic asymmetric destruction, since, unlike Pasteur's method, both enantiomers are degraded but at different rates.

[22] E. L. Eliel, in 'Stereochemistry of Carbon Compounds,' McGraw-Hill, 1962, p. 77.
[23] H. Krebs and R. Rasche, *Z. anorg. Chem.*, 1954, **276**, 236; B. E. Douglas and S. Yamada, *Inorg. Chem.*, 1965, **4**, 1561.
[24] D. H. Busch and J. C. Bailar, *J. Amer. Chem. Soc.*, 1953, **75**, 4574.
[25] F. P. Dwyer, E. C. Gyarfas, and D. P. Mellor, *J. Phys. Chem.*, 1955, **59**, 296.
[26] F. P. Dwyer and F. L. Garvan, *Inorg. Synth.*, 1960, **6**, 192.
[27] A. J. McCaffery, S. F. Mason, and B. J. Norman, *J. Chem. Soc.*, 1965, 5094.