Optically Active Co-ordination Compounds. Part XXIX.[1] Resolution of 1,2,4-Triglycinatocobalt(III) by a Bacterial Method and Determination of its Optical Purity by Isotope Dilution

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1,2,4-[Co(gly-$\bar{O}$)$_3$]† has been partially resolved by stereoselective decomposition using *Proteus vulgaris*. Further resolution was achieved by fractional crystallisation from water. This sample was shown to be 99·8 ± 1·5% optically pure by the method of isotope dilution. The pure enantiomer therefore has $\Delta\varepsilon_{535}$ = 2·75 ± 0·05.

IN this laboratory we are studying the stereoselective metabolism of 1,2,4-[Co(gly-$\bar{O}$)$_3$] by whole cells and by extracts of *Enterobacter cloacae*.[2] In order to calculate the absolute stereoselectivities of these processes it has been necessary to determine the optical activity of the pure enantiomer. We now report both a method of resolution, in which the complex is stereoselectively reduced by *Proteus vulgaris*, and the subsequent determination of the optical purity of a resolved sample by the isotope dilution technique.[3]

RESULTS

The bacterium *Proteus vulgaris* strain NCIB 4175, grown under anaerobic conditions, was harvested and the cells were suspended in phosphate buffer at 30° containing racemic 1,2,4-[Co(gly-$\bar{O}$)$_3$] and glucose as an energy source. Under these conditions the L-enantiomer is preferentially reduced.‡ Incubation for 48 h yielded a solution containing 62% of the original complex, ($\Delta\varepsilon_{535}$ = +1·63). The cells were removed by centrifugation. Cobalt(II) and bacterial products were precipitated by the addition of three volumes of acetone. Controlled addition of acetone to the filtrate gave a purple solid, containing 40% by weight of complex, which was further enriched in the D-enantiomer: $\Delta\varepsilon_{535}$ = +2·46. Crystallisation of this solid from water at room temperature gave a first crop of chemically pure crystals with $\Delta\varepsilon_{535}$ = +2·74.

The optical purity of this sample was established by the method of isotope dilution using added racemic complex containing [^{14}C] glycine. Fractional crystallisation of the mixed solution gave crops of widely differing optical composition ($\Delta\varepsilon_{535}$ = +2·79, +2·01, +0·70). This enabled three independent determinations of the optical purity: 98·6, 100·3, 100·5%; average 99·8 ± 1·5%. The $\Delta\varepsilon$ values for the maxima in the circular dichroism spectrum of the pure D-enantiomer are therefore: +2·75 ± 0·05 at 535 nm and −0·64 ± 0·02 at 372 nm. The maximum value of $\Delta\varepsilon$ at 535 nm observed in this laboratory is +2·79 ±

† gly-$\bar{O}$ = $NH_2{\cdot}CH_2{\cdot}COO^-$.

‡ For the absolute configuration of the resolved complex see M. G. B. Drew, J. H. Dunlop, R. D. Gillard, and D. Rogers, *Chem. Comm.*, 1966, 42.

[1] Part XXVIII, R. D. Gillard, P. R. Mitchell, and M. G. Price, *J.C.S. Dalton*, 1972, 1211.

[2] R. D. Gillard and C. Thorpe, *Chem. Comm.*, 1970, 997; and unpublished observations.

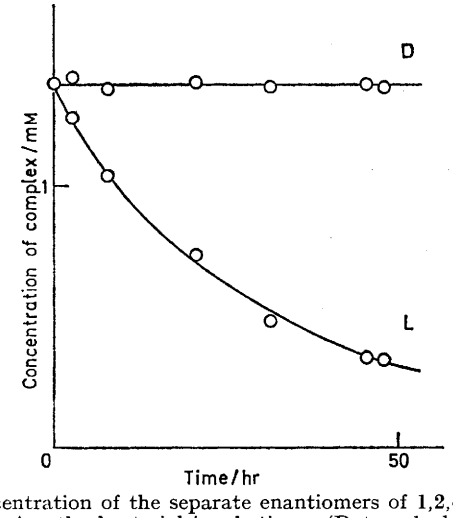
[3] R. D. Gillard and J. R. Lyons, *J. Chem. Soc.* (*A*), 1971, 2817.

0·05; we know of no literature reports in which substantially complete resolutions have been achieved. We have also determined the extinction coefficient at the maximum of the lowest energy *d–d* band: $\varepsilon_{542} = 96{\cdot}5 \pm 1{\cdot}5$; values of 93 (ref. 4), 100 (ref. 5), and 108 (ref. 6) have been reported.

The solubility in water of samples of 99·8% optically pure complex and of racemate were measured at 21° and 43°: enantiomer, 5·8 and 10·5 g/l; racemate, 8·8 and 16·2 g/l.

DISCUSSION

The resolution of 1,2,4-[Co(gly-$\bar{O}$)$_3$] into its optical antipodes is less easily achieved than that of the majority of cobalt(III) complexes. The widely used method of diastereoisomer formation is inapplicable since at the present time no suitable ionic derivative is known. Although partial resolutions have been achieved by



The concentration of the separate enantiomers of 1,2,4-[Co(gly-$\bar{O}$)$_3$] during the bacterial incubation. (Data calculated from optical density and circular dichroism measurements using the determined value of $\Delta\varepsilon_{535}$)

chromatography on starch [7] the method is convenient only on a small scale.

The resolution of organic racemates by preferential metabolism of one enantiomer by micro-organisms was first reported by Pasteur [8] and has been widely applied to organic substrates.[9] This paper describes the extension of the method to inorganic complexes. Using the experimentally determined value of $\Delta\varepsilon_{535}$, the concentration of each enantiomer of 1,2,4-[Co(gly-$\bar{O}$)$_3$] during the incubation period was calculated (see Figure). The bacterial reduction is stereospecific: L-enantiomer is removed in a process which follows first-order kinetics over at least two half-lives. This technique may provide a useful method for the resolution of other cobalt(III) complexes.

Repeated observations show that the optical purity of partially resolved 1,2,4-[Co(gly-$\bar{O}$)$_3$] is improved both by fractional precipitation with acetone and by recrystallisation from water. The material obtained, which within our experimental error is pure enantiomer, is in the form of needle-shaped crystals of the monohydrate. In contrast racemic solutions yield plates of the dihydrate; we have observed no spontaneous resolution on crystallisation of racemic solutions (at 0—25°). The solubility behaviour of this ternary system (D-enantiomer/L-enantiomer/solvent) fits the general case described by Secor and illustrated in diagram 4 of his review.[10] In such a system, at equilibrium, spontaneous resolution is observed only if the solubility of racemate exceeds twice that of the enantiomer. This condition is not met in our system. The observation that the total concentration of complex (both D- and L-forms) in a saturated racemic solution is greater than the concentration of a saturated solution of enantiomer is not in itself significant.

The procedure adopted for the isotope dilution experiment is the same as that reported previously,[3] with the improvement that small quantities of complex were measured not by weight but by the determination of the optical density of an aqueous solution of known volume. This enabled the scale of the determination to be reduced without impairing the accuracy; only 0·1 g of resolved complex was taken and provided a substantial excess over requirements. Furthermore optical density provides a direct measure of the molarity of the solution and so complications arising from the different degrees of hydration of the crystalline enantiomer and racemate are avoided. Finally since the resolved test-sample was almost optically pure the conditions for the experiment were at an optimum.[3]

EXPERIMENTAL

Instruments used for the measurement of absorption spectra and circular dichroism spectra have been described [3] and were appropriately calibrated.

Preparation of (±)1,2,4-[Co(gly-$\bar{O}$)$_3$],2H$_2$O.—The method of Mori *et al* [4] was used; the product was recrystallised from water and dried over silica gel at room temperature. [^{14}C]Racemate was similarly prepared using [1-^{14}C]glycine (supplied by the Radiochemical Centre, Amersham) and was additionally purified by chromatography on Sephadex G10. (Found for unlabelled racemate: C, 22·95; H, 4·95; N, 13·15; for [^{14}C]racemate: C, 22·55; H, 4·65; N, 13·3%. $C_6H_{16}CoN_3O_8$ requires: C, 22·7; H, 5·1; N, 13·25%).

Growth of Organism and Resolution of Complex.—Proteus vulgaris NCIB 4175 was maintained by sub-culture on slopes of Oxoid nutrient agar. The organism was grown in

[4] M. Mori, M. Shibata, E. Kyuno, and M. Kanaya, *Bull. Chem. Soc. Japan* 1961, **34**, 1837.
[5] F. Basolo, C. J. Ballhausen, and J. Bjerrum, *Acta Chem. Scand.*, 1955, **9**, 810.
[6] R. G. Neville and G. Gorin, *J. Amer. Chem. Soc.*, 1956, **78**, 4895.
[7] H. Krebs and R. Rasche, *Z. anorg. Chem.*, 1954, **276**, 236; B. E. Douglas and S. Yamada, *Inorg. Chem.*, 1965, **4**, 1561; J. H. Dunlop, Ph.D. Thesis (University of Sheffield), 1965; also unpublished experiments in this laboratory.
[8] L. Pasteur, *Compt. rend.*, 1858, **46**, 615.
[9] See, for example, R. M. Dodson, N. Newman, and H. M. Tsuchiya, *J. Org. Chem.*, 1962, **27**, 2707; T. W. Reid, T. P. Stein, and D. Fahrney, *J. Amer. Chem. Soc.*, 1967, **89**, 7125; J. P. Greenstein and M. Winitz, Chemistry of the Amino Acids, vol. 1, p. 728, Wiley, New York, 1961.
[10] R. M. Secor, *Chem. Rev.*, 1963, **63**, 297.

liquid culture at 30° in medium containing, K_2HPO_4 (7·0 g), KH_2PO_4 (3·0 g), NaCl (4·7 g), KCl (1·5 g), $MgCl_2$,-$6H_2O$ (0·2 g), $(NH_4)_2SO_4$ (2·6 g), trace elements suspension (0·1 ml) ($CaCl_2$ 2×10^{-3}M; $ZnCl_2$ 4×10^{-6}M; $FeCl_3$ 2×10^{-6}M), glucose (10 g) and Yeast Extract (Oxoid L21) (4·0 g) per litre. The glucose was sterilised separately and added aseptically. An inoculum culture (0·5 l) was grown in an unshaken conical flask (1 l) for 12 h. The contents were used to start 4·5 l of medium maintained under anaerobic conditions by passing a slow stream of nitrogen through the liquid. Stationary phase cells (20 h) were harvested by centrifugation at 4° in an M.S.E. High Speed 18 centrifuge (20,000 *g*, 15 min) and washed once in cold pH 7·0 buffer (7 g K_2HPO_4 and 3 g KH_2PO_4 per litre). The cells (10 g wet weight) were suspended in cold buffer (100 ml) and added to a flask containing buffer solution (900 ml) with (±)-1,2,4-[Co(gly-$\bar{O})_3$] (0·9 g) and glucose (9·0 g). Argon was bubbled through the solution maintained at 30°. The progress of the anaerobic incubation was followed by measurement of optical density and circular dichroism. For this purpose, samples (15 ml) were withdrawn by syringe through a side-arm sealed with a serum cap and centrifuged (38,000 *g*, 15 min). After 48 h the cells were removed leaving a solution containing 0·56 g complex ($\Delta\varepsilon_{535} = +1·63$).

Purification of Resolved Complex.—Acetone (2·4 l) was slowly added with stirring to the crude solution (0·8 l) at room temperature yielding a pale lilac precipitate. After filtration, acetone (0·7 l) was added to the stirred solution during 24 h giving a first crop of purple solid which darkened with time. This precipitate was separated by decantation and centrifugation (M.S.E. bench centrifuge), washed with acetone–water (4 : 1 by volume) and with acetone and dried *in vacuo* over silica gel. This solid (0·41 g containing 0·18 g complex with $\Delta\varepsilon_{535} = +2·46$) was fractionally crystallised from water at room temperature. The first crop of purple needles was washed with cold water and dried *in vacuo* (yield 0·11 g), $\Delta\varepsilon_{535} = +2·74$ (Found: C, 23·95; H, 4·75; N, 13·9%. Calc. for $C_6H_{14}CoN_3O_7$: C, 24·1; H, 4·7; N, 14·05%). This sample was used for the isotope dilution experiment. Subsequent crops were of lower optical purity and contaminated with colourless crystals.

Scintillation Counting.—The concentration of ^{14}C-label in the radioactive samples was determined by liquid scintillation counting in an Intertechnique SL20 spectrometer. Each sample contained 15 ml of scintillation medium (6·5 g butyl PBD and 50 g naphthalene in 1 l AnalaR dioxan) and 3 ml aqueous solution of complex (whose concentration was determined by measurement of the optical density at 542 nm). Precipitation of complex was avoided by using concentrations less than 0·75 mg/ml and by always adding the dioxan to the aqueous solution. Determinations were carried out in triplicate; typical counting times were 150 min. It was confirmed that, within the range used in our experiments, the count rate was proportional to the concentration of label and independent of the total concentration of complex.

Isotope Dilution Experiment (+)-1,2,4-[Co(gly-$\bar{O})_3$], H_2O (101·1 mg; $\Delta\varepsilon_{535} = +2·74$) and (±)-1,2,4-[Co([^{14}C]gly-$\bar{O})_3$],-$2H_2O$ (16·67 mg; $7·037 \times 10^8$ counts/min/mol) were dissolved in water (*ca.* 80 ml). The solution was fractionally crystallised by evaporation under a controlled air jet at 2°. The initial crop, an intermediate crop and the final mother liquor were selected, having optical activities ($\Delta\varepsilon_{535} = +2·79$, $+2·01$, $+0·70$) substantially different from the original mixed solution ($\Delta\varepsilon_{535} = +2·36$). Their specific count rates were 0·497, 1·391 and $3·011 \times 10^8$ counts/min/mol). The numbers for each of these recovered samples were inserted into the equation: [3]

$$c = \frac{a\{b(a + b) - (b - r)^2\{d_2/d_1)\}c_0}{b(a + 2b - r)(a + r)}$$

where a = number of moles of labelled racemate with count rate c_0 counts/min/mol; b = number of moles of optically active starting complex ($\Delta\varepsilon_{535} = d_1$) of which an unknown number of moles r are racemate; c and d_2 are respectively the count rate and $\Delta\varepsilon_{535}$ value for each recovered sample. Three independent values of r and hence of the optical purity ($(b - r) \cdot 100/b$) were obtained. On completion of the experiment no racemisation of a control sample of the original solution was detected.

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