

## Optically Active Co-ordination Compounds. Part XXVI.<sup>1</sup> Determination of the Optical Purity of Cobalt Complexes by the Method of Isotope Dilution

By R. D. Gillard \* and J. R. Lyons, Chemical Laboratory, University of Kent at Canterbury

The method of isotope dilution has been used to determine the optical purities of samples of partially resolved salicylatobis(ethylenediamine)cobalt(III) nitrate and of oxalatobis(ethylenediamine)cobalt(III) chloride and hence to obtain values of  $\Delta\epsilon_{\max}$  for peaks in the circular dichroism spectra of the fully resolved complex ions.

ASSESSMENT of the completeness of an optical resolution often rests solely on the purification of one enantiomer or of a derived diastereoisomer until constancy in a convenient physical property, such as optical rotation or m.p., is achieved. However, these are not absolute criteria, though greater reliance may be placed on them if samples obtained by independent procedures have identical properties or if both enantiomers are separated and shown to have specific rotations of equal magnitude. It is also possible to estimate the optical purity of one compound by chemical conversion into another whose optical purity is independently known. These widely

<sup>1</sup> Part XXV, R. D. Gillard and R. Maskill, *J. Chem. Soc. (A)*, preceding paper.

used approaches to the problem and the disadvantages associated with them have been discussed at length by Raban and Mislow<sup>2</sup> in a review of the modern methods available for the determination of optical purity. These methods are based variously upon isotope dilution, kinetic resolution (including the use of stereoselective enzymes), total resolution by g.l.c., n.m.r., and calorimetry.

We are interested in the first of these techniques, which we have applied to salicylatobis(ethylenediamine)-cobalt(III) nitrate and to oxalatobis(ethylenediamine)-cobalt(III) chloride. This is the first report of a study

<sup>2</sup> M. Raban and K. Mislow, *Topics in Stereochemistry*, 1967, **2**, 199.

of the optical purity of resolved co-ordination compounds by the method of isotope dilution. The historical development of this technique, which is important as it gives an absolute result, and its application to organic molecules have been fully reviewed<sup>2</sup> and we here give only an outline of the procedure suitable for our present purpose.

The principle is simple. The optically active test sample, of natural isotopic composition, is mixed in solution in known proportion with isotopically labelled racemate. As the two enantiomers were present in the original sample in unequal amounts, then in the solution they will contain different concentrations of isotopic label. A material with optical activity different from that of the solution must then be separated (generally by crystallisation, it being assumed that no isotope effects operate). Knowledge of the isotope content and optical activity of the test sample, of the labelled racemate, and of the recovered material provides sufficient data to determine the optical purity of the test sample.

## RESULTS AND DISCUSSION

The salicylatobis(ethylenediamine)cobalt(III) nitrate trihydrate was labelled isotopically with <sup>14</sup>C at the 1-position in the salicylate ligand and the oxalatobis(ethylenediamine)cobalt(III) chloride monohydrate with [<sup>14</sup>C]oxalate. Scintillation counting was used to determine the concentration of the label.

The extinction coefficients for the absorption bands in the electronic spectrum of the racemic salicylate complex were measured in neutral and in acidic aqueous solution. The values obtained supersede those reported earlier<sup>3,4</sup> (see Experimental section).

The results of the isotope dilution experiment on the salicylate complex were calculated as follows; the nomenclature has been chosen to conform with that of Berson and Ben-Efraim,<sup>5</sup> and (D) is an abbreviation for one of the enantiomers. Labelled racemate (*a* g; *c*<sub>0</sub> counts min<sup>-1</sup> g<sup>-1</sup>) is mixed with unlabelled optically active material [*b* g; containing *r* g of racemate and the remainder (D)]. From this mixture is isolated a material containing a fraction *x* of (D) and which has a count rate (*c*) given by equation (1). In order to

$$c = \frac{a\{(a + 2b - r) - 2x(b - r)\}c_0}{(a + 2b - r)(a + r)} \text{ counts min}^{-1} \text{ g}^{-1} \quad (1)$$

calculate *r* (and hence evaluate the optical purity of the original sample) *x* must be determined, all the other parameters being measurable experimentally. This has been carried out by using circular dichroism measurements. If, at a specific wavelength, Δε<sub>L,r</sub> = *d*<sub>1</sub> for the optically active sample under test, *d*<sub>2</sub> for the recovered material, and *d*<sub>3</sub> for the pure (D) form then we obtain equations (2) and (3), and thence (4). Therefore we obtain equation (5). Application of the standard

$$d_1 = \frac{(b - r)}{b} d_3 \quad (2)$$

$$d_2 = (2x - 1)d_3 \quad (3)$$

$$x = \frac{b + (b - r)(d_2/d_1)}{2b} \quad (4)$$

$$c = \frac{a\{b(a + b) - (b - r)^2(d_2/d_1)\}c_0}{b(a + 2b - r)(a + r)} \text{ counts min}^{-1} \text{ g}^{-1} \quad (5)$$

methods for the solution of quadratic equations will therefore, in general, give *r*. If the recovered material is racemic then *d*<sub>2</sub> = 0 and the expression for *c* simplifies to the form (6) derived by Berson and Ben-Efraim.<sup>5</sup>

$$c = \frac{a(a + b)c_0}{(a + 2b - r)(a + r)} \quad (6)$$

No evaluation of optical purity is possible if the recovered material has the same optical and isotopic composition as the solution, because in this event *d*<sub>2</sub>/*d*<sub>1</sub> = *b*/(*a* + *b*) and hence *c* = *ac*<sub>0</sub>/(*a* + *b*), viz., independent of *r*. Under all other conditions the optical purity can be determined, though for experimental convenience it is preferable that the difference in optical composition between the recovered material and the bulk solution should be large. In practice this means that, wherever possible, the recovered material should be racemic.

Certain other factors also influence the accuracy of the experiment. Wise choice of the relative magnitudes of *a* and *b* will increase the accuracy of the final results. Equation (5) shows that if *a* ≫ *b*, then *c* → *c*<sub>0</sub>. Thus it is better to use a small amount of labelled racemate mixed with a large amount of optically active material, i.e., *b* > *a*. If *b* is made very much greater than *a*, however, then the concentration of radioactive-label in the recovered sample may be so low as to prohibit precise analysis. The relative merit of different ratios of *a* to *b* is illustrated by the numerical examples given in the Table, where for simplicity it has been

Values of *c*/*c*<sub>0</sub> calculated for various ratios of *a* and *b* from equation (6) for test samples of different optical purities

Optical purity of test sample (%)	<i>a</i> / <i>b</i>				
	0.1	0.33	1.0	3.0	10.0
100	0.524	0.571	0.667	0.800	0.917
75	0.170	0.364	0.582	0.777	0.913
50	0.115	0.291	0.533	0.762	0.911
25	0.096	0.257	0.508	0.753	0.910
0	0.091	0.250	0.500	0.750	0.909

assumed that the recovered material is racemic and therefore equation (6) applies.

These data also show that when the test sample is close to 100% optical purity, the variation in the ratio *c*/*c*<sub>0</sub> (governing the accuracy of the determination of that optical purity) is at its most sensitive. Thus the method is most usefully applied when the test sample contains pure or almost pure enantiomer.

<sup>3</sup> K. Garbett and R. D. Gillard, *J. Chem. Soc. (A)*, 1968, 979.

<sup>4</sup> A. G. Beaumont and R. D. Gillard, *J. Chem. Soc. (A)*, 1970, 1757.

<sup>5</sup> J. A. Berson and D. A. Ben-Efraim, *J. Amer. Chem. Soc.*, 1959, **81**, 4083.

In our experiment on the salicylatocobalt(III) complex ion, the parameters gave values of  $a = 0.2983$  g,  $b = 0.9200$  g,  $c_0 = 47.07$  counts  $\text{min}^{-1} \text{mg}^{-1}$ , and  $d_1 = 0.482$  (measured at 506 nm) for the starting materials and  $c = 12.63$  counts  $\text{min}^{-1} \text{mg}^{-1}$  and  $d_2 = +0.085$  for the recovered material. Calculation gave  $r = 0.5137$  g, showing that the test sample was 44% optically pure [given by  $(b - r)100/b$ ]. The pure enantiomer therefore has  $\Delta\epsilon_{506} = +1.09$  ( $\pm 0.1$ ).

The best available value of  $\Delta\epsilon_{506}$  for the salicylatobis(ethylenediamine)cobalt(III) ion is  $\Delta\epsilon_{506} = -1.05$ .<sup>6</sup> This refers to the enantiomer not used in the present experiment [prepared by fractional crystallisation of the  $\alpha$ -bromo-(D)-(+)-camphorsulphonate salt]. It agrees closely in magnitude with the value obtained here.

Our experimental conditions were chosen so that  $a/b$  had a value of 0.32. However our sample was only 44% optically pure, which is far from the ideal described above. This was primarily due to the difficulty encountered in isolating a sufficiently large quantity of highly optically active material. No racemic material could be separated from the mixture of labelled and optically active complex and therefore the sample isolated from this solution which has the weakest optical activity was used as our 'recovered material'. This entailed little reduction in accuracy; under the conditions used the  $(b - r)^2 d_2/d_1$  term in the numerator of equation (5), which would be zero for reisolated racemate, has a magnitude of less than 3% of the other dominant, term in the numerator.

A slightly different procedure was adopted for oxalatobis(ethylenediamine)cobalt(III) chloride, which is known to undergo spontaneous resolution when crystallised at room temperature.<sup>7</sup> Advantage of this phenomenon was taken to obtain a maximal difference in enantiomeric and radiochemical composition between two samples isolated from a single mixture. This circumvents the problem of lowered sensitivity of the method should the original test sample not be almost 100% optically pure. Unlabelled and incompletely resolved complex was mixed with labelled racemate as above. Fractional crystallisation yielded a highly optically active first crop and, intermediate fractions being discarded, a final crop, which was racemic. The count rate for the first crop ( $c_1$ ) is given by equation (5) and that for the final crop ( $c_2$ ) by equation (6). Each equation can be rearranged to give an expression for  $(a + 2b - r)(a + r)/c_0$  and equating these gives equation (7) and thence (8) which can be solved for  $r$ .

$$\frac{a\{b(a + b) - (b - r)^2(d_2/d_1)\}}{bc_1} = \frac{a(a + b)}{c_2} \quad (7)$$

$$\therefore (b - r)^2 = \frac{(a + b)(c_2 - c_1)bd_1}{c_2 d_2} \quad (8)$$

<sup>6</sup> K. Garbett and R. D. Gillard, unpublished result.

<sup>7</sup> K. Yamasaki, H. Igarashi, Y. Yoshikawa, and H. Kuroya, *Inorg. Nuclear Chem. Letters*, 1968, **4**, 491.

<sup>8</sup> F. P. Dwyer, I. K. Reid, and F. L. Garvan, *J. Amer. Chem. Soc.*, 1961, **83**, 1285.

In our experiment  $a = 0.0595$  g,  $b = 0.5454$  g,  $c_1 = 1297$  counts  $\text{min}^{-1} \text{mg}^{-1}$ ,  $c_2 = 1741$  counts  $\text{min}^{-1} \text{mg}^{-1}$ ,  $d_1 = 0.795$ , and  $d_2 = 1.985$  (measured at 520 nm) thus giving  $r = 0.3625$  g in the original incompletely resolved sample, which was therefore 33.7% optically pure. Consequently  $\Delta\epsilon_{520} = 2.36 \pm 0.07$  for the pure enantiomer.

There are a number of data in the literature with which to compare this value. Some of this information is in the form of  $[\alpha]_D$  rather than  $\Delta\epsilon_{520}$ , and has been brought to a common basis by use of the observation in this laboratory that a solution of  $(-)-[\text{Co}(\text{en})_2(\text{C}_2\text{O}_4)]\text{Br} \cdot \text{H}_2\text{O}$  with  $[\alpha]_D = -820^\circ$  had  $\Delta\epsilon_{520} = 2.16$ . Literature values for  $\Delta\epsilon_{520}$  are therefore: for the chloride salt, 2.23,<sup>7</sup> 2.45;<sup>8</sup> oxalate salt, 2.26;<sup>7</sup> bromide salt, 2.16,<sup>8</sup> 2.50,<sup>9</sup> 2.14;<sup>10</sup> and for the perchlorate salt, 2.6.<sup>11</sup>

The oxidative degradation of  $(-)$ -salicylatobis(ethylenediamine)cobalt(III) ion yielding oxalatobis(ethylenediamine)cobalt(III) ion has been investigated in this laboratory.<sup>3,4,12,13</sup> Using concentrated nitric acid as oxidant gave a racemic product, but the oxalato-complex, even if formed optically active, would racemise under the reaction conditions employed.<sup>13</sup> On the other hand degradation of salicylato-complex ( $\Delta\epsilon_{\text{max.}} \approx -0.9$ ) with acid permanganate gave optically active oxalato-complex ( $\Delta\epsilon_{\text{max.}} \approx -2.4$ ).<sup>13</sup> The reaction with the latter oxidant must therefore occur without significant racemisation.

#### EXPERIMENTAL

**Salicylatobis(ethylenediamine)cobalt(III) Salts.**—The complex was prepared as the chloride salt and converted into the nitrate form by anion exchange.<sup>3</sup> The radio active-labelled complex contained  $[1\text{-}^{14}\text{C}]$ salicylic acid, whose synthesis is reported elsewhere.<sup>12</sup> Chromatography on Sephadex G-10 and recrystallisation from water were used for purification. The complex ion was resolved by fractional crystallisation of the  $\alpha$ -bromo-D-(+)-camphorsulphonate salt,<sup>3</sup> the most soluble fraction being taken. Attempted resolution by chromatography on ion-exchange Sephadex<sup>14</sup> SP C-25 was unsuccessful. Both racemic and partially resolved complex nitrate analysed as the trihydrate (Found for radioactive-labelled complex: C, 30.5; H, 6.1; N, 16.6; found for optically active complex: C, 30.6; N, 16.5. Calc. for  $\text{C}_{11}\text{H}_{26}\text{CoN}_5\text{O}_9$ : C, 30.6; H, 6.0; N, 16.3%).

**Oxalatobis(ethylenediamine)cobalt(III) Chloride Monohydrate.**—The complex was prepared<sup>4</sup> and resolved<sup>8</sup> by literature methods. The radioactive-labelled complex contained  $[^{14}\text{C}]$ oxalic acid (supplied by the Radiochemical Centre, Amersham). The complex chloride crystallises (with spontaneous resolution) as the tetrahydrate, which gradually loses three molecules of water of crystallisation

<sup>9</sup> M. E. Farago and C. F. V. Mason, *J. Chem. Soc. (A)*, 1970, 3100.

<sup>10</sup> J. A. Broomhead, *Nature*, 1966, **211**, 741.

<sup>11</sup> A. J. McCaffrey, S. F. Mason, and B. J. Norman, *J. Chem. Soc.*, 1965, 5094.

<sup>12</sup> A. G. Beaumont, R. D. Gillard, and J. R. Lyons, *J. Chem. Soc. (A)*, 1971, 1361.

<sup>13</sup> A. G. Beaumont and R. D. Gillard, *Chem. Comm.*, 1969, 438.

<sup>14</sup> Y. Yoshikawa and K. Yamasaki, *Inorg. Nuclear Chem. Letters*, 1970, **6**, 523.

on standing in air.<sup>7</sup> All samples were stored *in vacuo* over silica gel for several days to ensure that this change was complete (Found for unlabelled complex: C, 22.5; H, 5.8; N, 17.6; found for [<sup>14</sup>C] complex: C, 23.0; H, 5.7; N, 17.9. Calc. for C<sub>6</sub>H<sub>18</sub>ClCoN<sub>4</sub>O<sub>5</sub>: C, 22.5; H, 5.7; N, 17.5%).

**Absorption Spectra and Circular Dichroism Spectra.**—Absorption spectra were recorded on a Unicam SP 800 spectrophotometer and accurate extinction coefficients in the visible region determined on a Unicam SP 600 instrument. Circular dichroism spectra were obtained on a Roussel-Jouan model B Dichrograph.

The wavelength maxima and corresponding extinction coefficients in the electronic spectrum of the salicylatobis(ethylenediamine)cobalt(III) ion in aqueous solution are: 516 nm,  $\epsilon = 212$  (this is the average of three determinations for the chloride salt and two for the nitrate salt) and 330 nm,  $\epsilon = 2800$ . In 2N-sulphuric acid the maxima were at 508 nm,  $\epsilon = 219$  and 350 nm,  $\epsilon = 2860$ .

Neutral aqueous solutions of the complex ions under investigation were stable towards racemisation at room temperature. Thus a solution of salicylatobis(ethylenediamine)cobalt(III) nitrate ( $\Delta\epsilon_{508} = -0.37$ ) was unchanged after 55 days. Similarly no quantitative change was observed after 25 days in the circular dichroism spectrum of a solution of oxalatobis(ethylenediamine)cobalt(III) bromide ( $\Delta\epsilon_{520} = -2.16$ ); this is consistent with previous observations.<sup>15</sup>

**Scintillation Counting.**—(1) *Experiments on [<sup>14</sup>C]salicylatobis(ethylenediamine)cobalt(III) nitrate.* A known weight of complex dissolved in 2 ml of distilled water was added to 10 ml of a scintillator medium with composition: 1.75 g butyl PBD and 12.5 g naphthalene in 250 ml AnalaR

dioxan. This was counted in an Intertechnique SL20 spectrometer for 500 min (100 min for the samples in the calibration experiment). Samples for background counts contained the same weight of non-radioactive complex.

To confirm the absence of quenching problems, samples containing a constant weight of complex ( $5.7 \pm 0.2$  mg) but with varying proportions of radioactive-labelled material were examined. The results showed a linear relationship between the count rate and the weight of radioactive complex used.

(2) *Experiments on [<sup>14</sup>C]oxalatobis(ethylenediamine)cobalt(III) chloride.* The insolubility of this complex in all the scintillation media investigated precluded the use of the above technique. Instead the samples were burnt and the resultant [<sup>14</sup>C]carbon dioxide was trapped in 2-aminoethanol before counting; this procedure has been fully reported.<sup>12</sup> The liquid scintillation samples were counted for a minimum of 100 min.

**Isotope Dilution Experiments.**—Known weights of resolved complex and radioactive-labelled racemate were dissolved in the minimum of water and the solution fractionally crystallised at room temperature. Appropriate crops were selected, dried, and powdered to ensure homogeneity, before measurement of their circular dichroism spectrum and concentration of radioactive label. The results of these measurements (typically the average of at least three determinations) and the factors dictating the choice of crystal fraction have been given in the text.

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<sup>15</sup> E. Bushra and C. H. Johnson, *J. Chem. Soc.*, 1939, 1937; S. T. Spees and A. W. Adamson, *Inorg. Chem.*, 1962, **1**, 531.