[CONTRIBUTED FROM THE NOYES LABORATORY OF CHEMISTRY, UNIVERSITY OF ILLINOIS]

The Stereochemistry of Complex Inorganic Compounds. XV.[1] A Partial Resolution of Racemic Mixtures of Organic Acids by Means of Preferential Coördination[2]

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When an aqueous solution of carbonato-bis-levo-propylenediamine cobalt(III) ion, $[\mathrm{Co}(l\text{-pn})_2(\mathrm{CO}_3)]^+$, was treated with a 100% excess of a racemic mixture of an organic acid and evaporated to dryness, in all of the cases studied the dextro form of the acid appeared to enter the coördination sphere of the complex more readily than the levo form. This resulted in a partial resolution of the racemic mixture since the coördinated and uncoördinated optically active acid fractions could be recovered separately. This paper presents the experimental results obtained for the partial resolutions of rac-tartaric acid, rac-α-chloropropionic acid and rac-lactic acid by this method.

Introduction

In a previous article[1] the investigations of earlier workers in this field were discussed. It was noted that the work of Hürliman,[3] Jaeger[4] and Smirnoff[5] provided a basis for the development of the investigations begun in this Laboratory.[6] The results of the above experiments indicate that if a complex contains two optically active bidentate groups, a third optically active group seems to coördinate more firmly if it has the same configuration as those already present. This principle was employed in the present investigation as well as in that previously reported.

When a racemic mixture of an organic acid reacts with an equimolecular amount of a complex such as carbonato-bis-levo-propylenediamine cobalt(III) chloride, two isomers are generated, one containing the levo form and one the dextro form of the acid anion. These isomers have been shown to have different stabilities.[1] Investigating the resolution of *rac*-tartaric acid, Hamilton[7] was able to achieve a partial resolution by treating a mixture of the tartrato-bis-levo-propylenediamine cobalt(III) complexes with an additional equimolecular amount of *rac*-tartaric acid. Upon heating the solution, the coördinated isomer which was less firmly held (the levo-tartrate ion) was displaced by the isomer which probably has a configuration like that of the levo-propylenediamine in order to form the more stable complex. This resulted in a partial resolution of the racemic acid, since Hamilton succeeded in recovering both the coördinated and uncoördinated acid fractions.

From Hamilton's results it appeared that a resolution of an organic acid might be obtained more readily if a 100% excess of the racemic acid were allowed to react with carbonato-bis-levo-propylenediamine cobalt(III) chloride. One enantiomorph of the acid should enter the coördination sphere of the complex ion more readily than the other, the form preferentially coördinating presumably having the same configuration as the levo-propylenediamine present within the coördination sphere. The isomer having the smaller tendency to coördinate should remain in the anionic form. This should result in a resolution of the racemic acid since the coördinated and uncoördinated acid fractions may be removed separately. This procedure was successfully employed in the partial resolutions of tartaric acid, α-chloropropionic acid and lactic acid.

Experimental

Preparation of Materials.—*Rac*-propylenediamine was resolved as previously described,[7] and *cis*-dichloro-bis-levo-propylenediamine cobalt(III) chloride was prepared by the method of Bailar and co-workers as modified by Sister Mary Martinette.[8] The naturally occurring dextro-tartaric acid was racemized by the method of Campbell, Slotin and Johnston.[9]

The Partial Resolution of *rac*-Tartaric Acid.—An aqueous solution of one-hundredth of a mole of carbonato-bis-levo-propylenediamine cobalt(III) ion was treated with one-hundredth of a mole of sodium carbonate and two-hundredths of a mole of *rac*-tartaric acid (100% excess). The sodium carbonate was added to effect complete neutralization of the tartaric acid, thus permitting preferential coördination in the form of the tartrate ion.

The tartrate solution was evaporated to dryness on the steam-bath and then oven-dried at 85–90° for 96 hours. The resulting somewhat powdery mass was dissolved in 50 ml. of water and a 150% excess of a saturated aqueous solution of barium hydroxide was added. The initial barium tartrate precipitate was immediately filtered from the solution, digested with hot 3 *N* acetic acid, washed with water, oven-dried at 85° and weighed. The original filtrate, containing the excess barium hydroxide and the tartrato complex, was set aside for four days to allow the tartrate fraction which had preferentially coördinated to precipitate from the solution as barium tartrate. This precipitate was treated in the same manner as the initial one.

In experiment I, the two barium tartrate precipitates were dissolved in dilute hydrochloric acid and the solutions diluted to 25 ml. The rotations obtained for the solutions are recorded in the following table, the weight of tartaric acid having been calculated from the weight of the barium tartrate precipitates.

Experiment I	Wt. tartaric acid, g.	Obsd. α	[α]D
1, Initial precipitate	1.337	−0.044°	−0.412°
2, Final precipitate	1.762[a]	+0.033°	+0.234°

[a] The barium tartrate precipitate from which this weight of tartaric acid was calculated is believed to have contained a considerable amount of barium carbonate because of effervescence noted upon the addition of acid. Barium carbonate would precipitate if the solution absorbed carbon dioxide from the air.

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[1] For the previous article in the series, see Bailar, Jonassen, and Gott, THIS JOURNAL, **74**, 3131 (1952).

[2] Most of the work reported in this article was taken from the doctorate thesis of Allan D. Gott, 1952.

[3] Hans Hürliman, Thesis, University of Zurich, Reviewed in Jaeger, "Optical Activity and High Temperature Measurements," McGraw-Hill Book Co., Inc., New York, N. Y., 1930, pp. 167–168.

[4] F. M. Jaeger and H. B. Blumendal, *Z. anorg. allgem. Chem.*, **175**, 161 (1928).

[5] A. P. Smirnoff, *Helv. Chim. Acta*, **3**, 177 (1920).

[6] John C. Bailar, Jr., C. A. Stiegman, J. H. Balthis and E. H. Huffman, THIS JOURNAL, **61**, 2402 (1939).

[7] N. H. Hamilton, Thesis, University of Illinois (1947).

[8] Sr. Mary Martinette B.V.M., Thesis, University of Illinois, 1949.

[9] A. N. Campbell, L. Slotin and S. A. Johnston, THIS JOURNAL, **55**, 2604 (1933).

In order to determine the extent of resolution achieved, a known weight of barium dextro-tartrate, prepared from commercial dextro-tartaric acid, was dissolved in dilute hydrochloric acid, diluted to 25 ml. and the rotation determined. The specific rotation of this solution, +3.74°, indicated that about an 8% resolution had been obtained by the new resolution procedure.

An investigation of some of the factors which might affect the rotation of a solution of optically active tartaric acid showed that the rotation of the acid was considerably decreased by the presence of barium ions, calcium ions and concentrated sulfuric acid. This suggested removal of the barium ions from the optically active tartaric acid solutions. It was found that barium chloride has a low solubility in concentrated hydrochloric acid whereas tartaric acid is very nearly as soluble in this medium as in water. By treating the barium tartrate precipitates with concentrated hydrochloric acid and removing the precipitated barium chloride by filtration, the barium ion content was reduced to such an extent that it produced no detectable decrease in the rotations of the optically active tartaric acid solutions.

In experiment II, the same procedure was used as in experiment I, except that most of the barium ions were removed by the hydrochloric acid treatment. The weights of tartaric acid were again calculated from the weights of the barium tartrate precipitates.

Experiment II	Wt. tartaric acid, g.	Obsd. α	$[\alpha]_D$
1, Initial precipitate	1.370	−0.075°	−0.685°
2, Final precipitate	1.115	+0.057°	+0.640°

Preparation of the Di-α-chloropropionato- and Dilactato-bis-levo-propylenediamine Cobalt(III) Complexes.—These complexes were prepared exactly as the tartrato complexes except that two-hundredths of a mole of the racemic acids was added to the aqueous solution of the carbonato complex. Since these coördinating agents are both monodentate, two acid anions enter each coördination sphere.

The Resolution of Racemic α-Chloropropionic Acid by Recrystallization of the Cinchonine Salt.—In order to have a sample of optically active α-chloropropionic acid for comparison, the acid was resolved by a standard method. The cinchonine salt was prepared by adding 14.7 g. (one-half the amount of alkaloid necessary for complete salt formation) to a hot aqueous solution of 21.7 g. of the acid. Enough boiling water was then added to produce a clear solution. On cooling, cinchonine α-chloropropionate crystallized from the solution and, after standing overnight, was removed by filtration. An analysis of this salt showed the presence of two molecules of acid for each molecule of alkaloid. *Anal.* Calcd. for cinchonine di-α-chloropropionate: C, 58.6; H, 6.29; N, 5 48. Found: C, 58.17; H, 6.29; N, 5.43.

The cinchonine α-chloropropionate was recrystallized five times or until the specific rotation of a portion of the salt remained constant at +146.1° for several recrystallizations. Since the common salts of α-chloropropionic acid are either quite soluble or tend to decompose in water, an aqueous solution of the acid is most easily obtained from the cinchonine salt by dissolving the salt in boiling water and precipitating the alkaloid by the addition of sodium hydroxide. To determine the specific rotation of the acid a portion of the recrystallized cinchonine salt was oven-dried at 80° for an hour, weighed and decomposed by the addition of sodium hydroxide to the hot aqueous salt solution. The cinchonine was removed by filtration and the filtrate acidified with dilute hydrochloric acid. The solution was diluted to a definite volume and the rotation determined. The specific rotation at room temperature and the D line of sodium varied from −3.36 to −3.44° with an average of −3.40°. In order to prove that this rotation was not affected by incomplete removal of the cinchonine, sodium hydroxide was added to a hot saturated aqueous solution of the alkaloid and after cooling to room temperature, the excess alkaloid was filtered from the solution. The rotation of this filtrate was found to be +0.001° which is well within experimental error, and indicated that cinchonine had been effectively removed.

The Partial Resolution of *rac*-α-Chloropropionic Acid and *rac*-Lactic Acid.—The resolutions of *rac*-α-chloropropionic acid and *rac*-lactic acid were carried out in a manner very similar to that used for the resolution of *rac*-tartaric acid, with two notable exceptions. First, these two acids are monodentate in nature so that four-hundredths of a mole was added to one-hundredth of a mole of a carbonate solution of the carbonato complex. Second, since the common salts of these acids are either quite soluble or tend to decompose in water, the acid fractions were recovered by extraction with ether. It was found that ten extractions with ether removed virtually all of the uncoördinated acid. A few drops of dilute sulfuric acid were added to the solution prior to the ether extraction to be sure that none of the acid remained in the solution as a salt. In order to recover the fraction of the acid which had preferentially coördinated, the complex was decomposed by the addition of excess sodium sulfide which precipitated the cobalt as cobalt sulfide. The precipitate was filtered from the solution; the filtrate was acidified and extracted with ether. Aqueous solutions of these fractions were prepared by evaporating the ether on a steam-bath and subsequently diluting with water to 25 ml. The concentration of acid in the aqueous solutions was determined by titration with standard sodium hydroxide solution using phenolphthalein as the indicator. From these early experiments it is apparent that some of the optically active acid vaporized during the evaporation of the ether. In experiment III the filtrate from the removal of cobalt sulfide was acidified with dilute hydrochloric acid. In experiment IV the method was somewhat improved by acidification with dilute sulfuric acid. The results of these experiments are recorded below:

Experiment III	Wt. α-chloropropionic acid, g.	Obsd. α	$[\alpha]_D$
1, Initial extract	0.562	+0.051°	+1.133°
2, Final extract	1.040	− .048°	−0.577°
Experiment IV			
1, Initial extract	0.575	+ .050°	+1.088°
2, Final extract	.400	− .058°	−1.812°

The results of the above experiments and others of the same sort indicate that a somewhat greater than 30% resolution of the *rac*-α-chloropropionic acid was achieved. It is significant that the levorotatory isomer of the acid preferentially entered the coördination sphere of the complex while the dextrorotatory isomer remained uncoördinated.

The resolution of *rac*-lactic acid was attempted in order to determine the configuration of the levorotatory form of the α-chloropropionic acid which had preferentially coördinated. Since the structures of these two molecules are similar it was assumed that the steric configuration of the optically active acids would be similar. Dextro-lactic acid is reported to be levorotatory with a specific rotation of 3.80°,[10] though this value is known to vary, depending upon the lactic acid concentration.

The resolution of lactic acid was effected in exactly the same manner as the resolution of the α-chloropropionic acid. In this case, however, it was not deemed necessary to recover the fraction of the acid which had coördinated preferentially since the primary interest in this resolution was to relate the sign of rotation and sterical configuration of the optically active α-chloropropionic acid. The specific rotation of the recovered uncoördinated lactic acid fraction is recorded below

Experiment V	Wt. of acid, g.	Obsd. α	$[\alpha]_D$
1, Initial extract	0.578	+0.059°	+1.275°

This again represents approximately a 30% resolution of the *rac*-acid.

From this result it is apparent that the levorotatory isomer of the lactic acid entered the coördination sphere preferentially. Since this form possesses a dextro configuration it is assumed that the levorotatory isomer of the α-chloropropionate ion which preferentially coördinated also possesses a dextro configuration whereas dextro-tartaric acid is dextrorotatory. If this assumption is correct then there is complete agreement in the resolutions of *rac*-tartaric acid, *rac*-α-chloropropionic acid, and *rac*-lactic acid, since the dextro-isomers of each of these acids preferentially entered the coördination sphere of the complex.

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[10] P. Karrer, "Organic Chemistry," Third Ed., Elsevier Publishing Co., Inc., New York, N. Y., 1947, p. 253.

In order to prove that no decomposition of α-chloropropionic acid and lactic acid took place during this resolution, anilide derivatives of the recovered acid fractions were prepared according to standard methods.[11] The melting points of these derivatives indicated that no decomposition had occurred.

Discussion

The mechanism of the above reaction is not completely understood but it is probably more complex than has been indicated here. This is shown, for example, by the fact that when carbonato-bis-levo-propylenediamine cobalt chloride is evaporated with an excess of tartaric acid and then taken back into solution, the excess tartrate is not completely precipitated by the addition of the calculated quantity of barium hydroxide. Moreover, it is difficult to understand why the resolution of α-chloropropionic acid and lactic acid both proceeded to the extent of approximately 30% whereas the resolution of tartaric acid was much less. These problems will be studied further.

The supposition that α-chloropropionic acid acts primarily as a monodentate coördinating agent was supported by precipitation of the uncoördinated α-chloropropionate fraction by silver ions at a *p*H of about 6. The result is only approximate, for the silver salt of the acid is fairly soluble and was found to decompose slowly into silver chloride and lactic acid on standing in solution. However, the results were sufficiently exact to indicate that one-half of the acid originally added was not readily precipitated by silver ion, so that two acid anions must have entered each coördination sphere.

Since it appears that the dextro form of the acid enters the coördination sphere of the carbonato-bis-levo-propylenediamine cobalt(III) complex preferentially, this reaction could be used as a method of determining the configuration of any optically active organic acid which would coördinate either as a monodentate or a bidentate group. If the molecule or anion readily entered the coördination sphere of the complex it should have a dextro configuration regardless of the sign of rotation. Comparison of this result with a resolution of a racemic mixture of the acid by this method should yield a clear indication of steric configuration.

It should be mentioned that this procedure has also been extended to the resolution of *rac*-α-bromopropionic acid, though the results in this case are purely qualitative. The resolution proceeds in exactly the same manner, with the levorotatory isomer coördinating preferentially.

Though a resolution of 30% is only partial it is significant. The resolution of *rac*-lactic acid, especially, proceeds much more readily by this method than by the usual methods of recrystallization of alkaloid salts of the acid. The procedure as described also appears to be of a general nature and should be adaptable to the resolution of many other organic acids.

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(11) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," Third Ed., John Wiley and Sons, Inc., New York, N. Y., 1948, pp. 158, 222–223.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Acid–Base Titration, Viscosity and Density of α-, β- and γ-Casein

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Titration curves, viscosities and densities of α-, β- and γ-casein show that these proteins differ and that the variation in properties is related to their amino acid compositions. Results obtained on preparations of these proteins separated by alcohol or urea were the same as those obtained by the milder procedure of isoelectric precipitation at 2°, indicating that no change in structure was produced by alcohol or urea.

Numerous studies have been reported on the acid- and base-combining capacity of casein,[2–4] its viscosity and its density.[5–8] The demonstration by Mellander[9] that casein is a mixture of at least three components, and the separation of these components in our laboratory make it possible for the first time to determine the properties of these pure caseins.

Since the components of casein are difficult to separate, it might be expected that they would have only small differences in properties. The present study showed, however, that their titration curves, viscosities and densities are markedly different.

Materials and Methods

Caseins.—α-, β- and γ-casein were prepared by several methods, namely, isoelectric precipitation from water at 2°, differential solubility in 50% aqueous alcohol with change in *p*H and temperature, and differential solubility in various concentrations of aqueous urea. These methods have been described in detail by Warner[10] and Hipp, *et al.*[11,12] The unfractionated casein was prepared from unpasteurized bovine skim milk by acid precipitation, washed several times and purified by two isoelectric reprecipitations in the manner previously described.[11]

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(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

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