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Optical Resolution by Direct Crystallization of Enantiomer Mixtures

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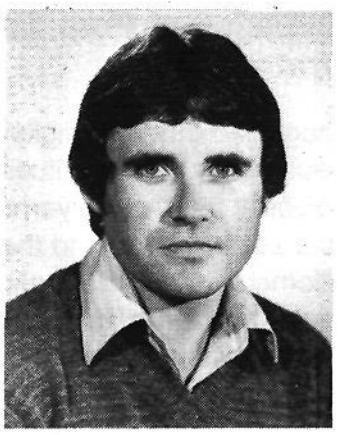

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I. Introduction

Of the two main types of crystalline racemates, racemic compounds and conglomerates,[1-3] the latter are of particular interest. Not only is their occurrence rarer but also they are far easier to resolve than are racemic compounds. The sep-

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aration of enantiomers forming a racemic compound necessarily requires the utilization of diastereomeric interactions,[4,5] including formation of diastereomers with a resolving agent, chromatography on an optically active adsorbent, or asymmetric destruction by biological or chemical processes. Contrariwise, the separation of two enantiomers forming a conglomerate does not require any optically active auxiliary agent since resolution has occurred spontaneously during the course of crystallization.

Although it is also possible to resolve a substance forming a conglomerate via diastereomeric interactions, taking advantage of this spontaneous resolution is actually the most straightforward and most economical route to the pure enantiomers when amounts ranging from several grams to tons of material are needed. Nevertheless, while most chemists are aware of the theoretical possibility of performing such resolutions, they generally are not aware of the fact that practical methods, analogous to the historic resolution of Pasteur (1848), have become available recently to carry out effective separations of spontaneously resolved compounds.

These methods belong to two categories: (i) those for which the two enantiomers are allowed to crystallize at the same time under conditions in which a *stable* solubility equilibrium is maintained; (ii) those processes in which one promotes the crystallization of a single enantiomer, a situation which involves *metastable* solubility equilibria in supersaturated solutions or in supercooled melts.

Since the appearance of the review by Secor[6] in 1963, a great deal of work has been done on the theory of these processes, and numerous practical developments have been reported. We believe that it is now possible to make clear, even to organic chemists who may not be really familiar with phase diagrams, all the available procedures that take advantage of the spontaneous resolution and more especially the important technique of resolution by entrainment.

In order to clarify some aspects of this discussion, it seems worthwhile to first examine those physical properties of conglomerates upon which the resolution methods described in this review are based. We emphasize that a large part of the following analysis concerning the solubility properties of conglomerates (sections II.B and II.C) has not previously been reported.

II. Melting Points and Solubilities of Conglomerates

A conglomerate is a mechanical mixture of crystals of the *pure* enantiomers. Such a system is equivalent to an ordinary mixture of two compounds A and B whose phase diagram shows a single eutectic. The only special feature of a conglomerate with respect to the general case is the symmetry of its phase diagram. This symmetry follows from the identical thermodynamic properties of the pure enantiomers, i.e., identical melting points and enthalpies of fusion. As a consequence of this symmetry, the eutectic of a conglomerate system must have a racemic composition.

We need to consider here the phase diagrams which correspond to *binary* mixtures of enantiomers (melting-point phase diagrams) and to *ternary* mixtures of enantiomers in the presence of a solvent (solubility diagrams). We will not analyze in any detail all of the elementary properties of these diagrams which are summarized in the phase rule;[7] we will focus attention only on those properties which are essential to the understanding of the resolution processes described below.

A. Calculation of Melting-Point Phase Diagrams

The most important property of a conglomerate system is probably that the racemate melts appreciably lower than do the pure enantiomers, this as a consequence of the shape of the

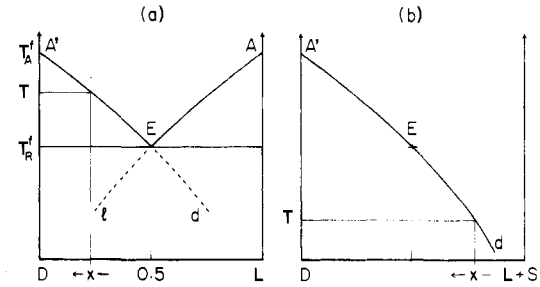


Figure 1. (a) Melting-point phase diagram for a conglomerate. (b) Liquidus curve of enantiomer D in the binary solvent L + S.

binary phase diagram (Figure 1). This lowering in melting point is of the order of typically 25–35 °C.

Such a binary system is amenable to a general thermodynamic treatment to which one may submit any mixture of two compounds which are fully miscible in the liquid state and fully immiscible in the crystalline state. The *liquidus* curve of D or L (that is, A'Ed or AEl, respectively, in Figure 1a) may be calculated by means of the Schröder–Van Laar equation[8–10] (eq 1). In this equation, x is the mole fraction of one enantiomer

$$\ln x = \frac{\Delta H_A^f}{R}\left(\frac{1}{T_A^f} - \frac{1}{T}\right) - \frac{C^l - C^s}{R}\left(\ln \frac{T_A^f}{T} + 1 - \frac{T_A^f}{T}\right) \quad (1)$$

in a mixture whose melting terminates at T (in Kelvin); $x \geq 0.5$ corresponds to part A'E (or AE) of the liquidus curve, i.e., to a *stable equilibrium*; $x < 0.5$ corresponds to part Ed (or El), which represents a *metastable equilibrium* in which one of the enantiomers remains supercooled. ΔH_A^f (cal mol^{-1}) and T_A^f (K) are the enthalpy of fusion and melting point of the pure enantiomers, respectively; C^l and C^s (cal mol^{-1} deg^{-1}) are the specific heats of the enantiomers in the liquid and solid states, while R is the gas constant (R = 1.9869 cal mol^{-1} deg^{-1}).

The second term of eq 1 containing the specific heats is generally negligible relative to the first term, and thus the Schröder–Van Laar equation is virtually always employed in its simplified form (eq 2).

$$\ln x = \frac{\Delta H_A^f}{R}\left(\frac{1}{T_A^f} - \frac{1}{T}\right) \quad (2)$$

In the case of conglomerates, the calculated and experimental liquidus curves are generally in close agreement; this is a consequence of the quasi-ideal behavior of enantiomer mixtures in the liquid state.[1] Note that eq 1 or 2 permits, inter alia, the calculation of the melting point of the racemate from the melting point and enthalpy of fusion of pure enantiomers.

B. Relative Solubilities of Pure Enantiomers and Their Conglomerate

It is generally recognized that in conglomerate systems the racemate is more soluble than the constituent enantiomers. The so-called "double solubility rule" of Meyerhoffer[11,12] states that a conglomerate has a solubility equal to the sum of that of the corresponding enantiomers. More precisely, the Schröder–Van Laar equation indicates that in an *ideal* solution, in equilibrium with crystals of a pure constituent at a given temperature, the mole fraction of this constituent in the liquid phase depends only on the enthalpy of fusion and melting point of the substance and not on the properties of the solvent, which may itself be a single substance or a mixture (in Figure 1b, the liquidus curve A'Ed does not change when L is replaced by L + solvent). Given x_R and x_A, the solubilities (as mole fraction) of a conglomerate and of one pure enantiomer, respectively, at the same temperature, from the foregoing, the mole fraction of enantiomer

TABLE I. Some Common Expressions of the Solubility Ratio α

A. Definitions

molecular weights of the solute and of the solvent
M, M_s

composition of the saturated solution
W_A, W_R: weight of enantiomer or racemate
W_s: weight of solvent
d_A, d_R: densities of the solutions

B. Solubility Expressions

x_A, x_R: mole fraction of the solute
γ_A, γ_R: grams of solute per 100 g of solution
M_A, M_R: moles of solute per liter of solution

C. Solubility Ratio α

$$\alpha_x = \frac{x_R}{x_A} = \frac{W_R}{W_A} \cdot \frac{W_A + W_s(M/M_s)}{W_R + W_s(M/M_s)}$$

$$\alpha_\gamma = \frac{\gamma_R}{\gamma_A} = \frac{W_R}{W_A} \cdot \frac{W_A + W_s}{W_R + W_s}$$

$$\alpha_M = \frac{M_R}{M_A} = \frac{\gamma_R}{\gamma_A} \cdot \frac{d_R}{d_A}$$

in dilute solution $\alpha_x \simeq \alpha_\gamma \simeq \alpha_M$

L (or D) in the racemic saturated solution, which is equal to $x_R/2$, must also be equal to x_A, hence $x_R = 2x_A$. This leads us to call attention to the various ways of expressing solubilities; the ratio of solubility of the racemate to that of the enantiomers, designated as α, depends upon the manner in which the concentrations are defined. Three ways of formulating this ratio are given in Table I. Rigorously speaking, the "double solubility rule" is only valid in the solubility relationship expressed as mole fraction ($\alpha_x = 2$). However, in dilute solution all of these expressions become equivalent.

The arguments just made are valid only if the species present in solution are the same as those present in the crystal, that is, provided that the dissolved molecules of the enantiomers are not dissociable (or at least not dissociated); this is actually the case for the majority of organic compounds which are not salts.

Let us now turn to the behavior of dissociable molecules AX and $\bar{A}X$ in which A and $\bar{A}$ represent enantiomeric ions (or molecules) and X is an achiral ion (or molecule). The solubility and dissociation equilibria may be written as shown in eq 3 and

$$AX\ \text{solid} \rightleftharpoons AX\ \text{solution} \qquad K_s = [AX] \tag{3}$$

$$AX\ \text{solution} \rightleftharpoons A + X \qquad K_d = [A][X]/[AX] \tag{4}$$

4 so as to define the respective constants K_s and K_d. Equation

$$K_s K_d = [A][X] \tag{5}$$

5 is then derived from eq 3 and 4.

In order to simplify the arguments which follow, let us equate activities to concentrations (ideal solutions). If S_A is the solubility of the enantiomer AX, we have

$$S_A = [AX] + [A] = K_s + [A]$$

$$[A] = S_A - K_s$$

Since $[A] = [X]$,

$$K_s K_d = (S_A - K_s)^2 \tag{6}$$

For the case in which AX + $\bar{A}X$ exists as a conglomerate, equilibria 3 and 4 still hold. If S_R is the solubility of the racemate, that of the enantiomer in this solution is $S_R/2$ and thus

$$S_R/2 = [AX] + [A] = K_s + [A]$$

$$[A] = (S_R/2) - K_s$$

Since in the racemate $[X] = 2[A]$, eq 5 gives eq 7.

TABLE II. Experimental Solubility Ratio α_x for Conglomerates

compd	solvent	T, °C	α_x	ref
A. Substances Which Are Not Dissociated in Solution				
2-(1-naphthoxy)propionamide	ethanol	25	2.01	12
anisylidenecamphor	methanol	25	2.19	12
N-acetyl-α-methylbenzylamine	water	35	2.26	12
2-(*p*-methoxyphenyl)propiophenone	hexane	25	2.48	12
N-acetylglutamic acid	water	25	2.08	17
dilactyldiamide	water	60	2.18	14
N-acetylproline monohydrate	water	20	2.78	15
N-chloroacetylproline	acetone	20	2.29	15
N-butyrylproline	water	20	2.64	15
threo-1-*p*-nitrophenyl-2-amino-1,3-propanediol	methanol	25	2.09	12
B. Ionized Substances in Aqueous Solution				
ammonium *N*-acetyltryptophanate		25	1.70	16
ammonium glutamate		25	1.40	16
glutamic acid hydrochloride		25	1.51	16
histidine monohydrochloride		45	1.59	12
lysine 3,5-dinitrobenzoate		30	2.02	29
alanine *p*-chlorobenzenesulfonate		45	1.97	17
Dopa naphthalenesulfonate, $1.5H_2O$		10	1.45	17
leucine benzenesulfonate		15	2.28	17
lysine *p*-aminobenzenesulfonate		45	1.41	17
serine *m*-xylenesulfonate		15	1.90	17
tryptophan benzenesulfonate		15	1.63	17
$[Co(ox)(en)_2]Cl, H_2O$		5	1.56	13
$[Co(ox)(en)_2]Br, H_2O$		5	1.56	13
cis-$[Co(NO_2)_2(en)_2]Cl$		5	1.58	13
cis-$[Co(NO_2)_2(en)_2]Br$		5	1.33	13
C. Free Amino Acids in Aqueous Solution				
asparagine		30	2.02	16
threonine		30	1.68	16
homocysteic acid		30	1.38	16

$$K_s K_d = 2[(S_R/2) - K_s]^2 \tag{7}$$

Combining eq 6 and 7, we have $(S_A - K_s)^2 = 2[(S_R/2) - K_s]^2$, from which we may obtain eq 8. This equation would be

$$S_R = \sqrt{2}(S_A - K_s) + 2K_s \tag{8}$$

rigorously valid for ideal solutions, when solubilities S_R and S_A are expressed as mole fractions. In practice, it is generally valid only for rather dilute solutions; under these conditions, we have seen above that the ways in which solubilities are expressed become equivalent.

Returning to eq 8, we see immediately that for dissociation tending toward zero, i.e., $K_s \rightarrow S_A$, one finds the "double solubility", $S_R = 2S_A$. On the other hand, in the case of total dissociation ($K_s \rightarrow 0$) we obtain eq 9. This is a relationship

$$S_R = \sqrt{2}S_A \tag{9}$$

which was arrived at by Yamanari et al.[13] Otherwise put, eq 8 shows that the solubility of a conglomerate is equal to twice that of the undissociated enantiomer fraction (K_s) plus $2^{1/2}$ times the dissociated fraction ($S_A - K_s$). More generally, for conglomerates of compounds AX_n, one has the rule given in eq 10.

$$S_R = \sqrt[n+1]{2}(S_A - K_s) + 2K_s \tag{10}$$

The preceding arguments may be applied as well to those important cases in which acid–base equilibria also intervene. Carboxylic acids in aqueous solution are only slightly dissociated; hence S_R is close to $2S_A$. This is certainly true, a fortiori, in organic solvents. Salts of strong acids and weak bases, or of weak acids and strong bases, are highly dissociated in water and the solubility of the racemate tends toward $2^{1/2}S_A$. Finally, salts formed from weak acids and bases lead, for a conglomerate, to a solubility between $2^{1/2}S_A$ and $2S_A$.

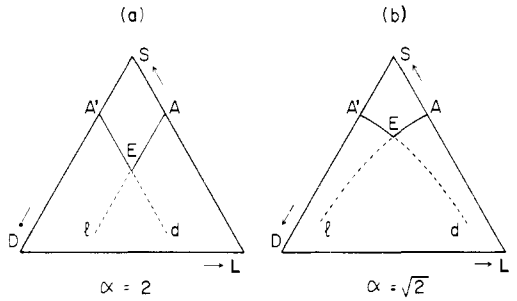


Figure 2. Shape of the solubility curves.

The experimental data available on the relative solubilities of enantiomers and their conglomerate are in very good agreement with the foregoing theoretical considerations.[12-17] These data are assembled in Table II.

C. Shape of the Solubility Curves in Ternary Phase Diagrams

As we will see later, the solubility ratio α and the form of the solubility curves of the enantiomers in the ternary phase diagram have an important bearing on the process of resolution by preferential crystallization.

Let us first examine the case of conglomerates of nondissociable compounds. The Schröder–Van Laar equation indicates that in an ideal solution the solubility of, say L, remains equal to x_A whatever be the concentration of D in the solvent S. This requires that the solubility curve for L in the triangular phase diagram D, L, S (Figure 2a) be a *straight line* parallel to side SD (just as the solubility curve for D must be the straight line parallel to SL corresponding to $x_D = x_L = x_A$). If concentrations are expressed other than as mole fractions, then straight lines are still obtained but these are no longer parallel to the sides of the triangle (except for dilute solutions).

Let us next consider dissociation. We will only analyze the case corresponding to a conglomerate of AX and $\bar{A}X$ which is *completely* dissociated in solution (eq 9). The solubility/dissociation equilibrium allows us to define solubility constant K_{sd} (eq 11). For a pure enantiomer whose solubility is S_A, we have

$$\text{AX solid} \rightleftharpoons \text{A solution} + \text{X solution} \qquad K_{sd} = [A][X] \tag{11}$$

$S_A = [A] = [X]$ and $K_{sd} = S_A^2$. Let us consider a saturated solution of enantiomeric purity p ($-1 < p \leq +1$) whose concentration is $S_p = [A] + [\bar{A}] = [X]$. The solubility of the enantiomer AX in this saturated solution is given by eq 12.

$$(S_A)_p = [A] = S_p \frac{1+p}{2} \tag{12}$$

From eq 11 and since $[X] = S_p$, it follows that

$$[A][X] = S_p^2 \frac{1+p}{2} = S_A^2$$

which in turn yields the solubility of the mixture of enantiomeric purity p as eq 13. For the racemate ($p = 0$) eq 13 reduces

$$S_p = S_A \sqrt{\frac{2}{1+p}} \tag{13}$$

to $S_R = 2^{1/2} S_A$ as required. The solubility curve is defined by the concentration of an enantiomer in the saturated solution. Combination of eq 12 and 13 leads finally to eq 14.

$$(S_A)_p = S_A \sqrt{\frac{1+p}{2}} \tag{14}$$

In Figure 2b we have plotted the solubility curves calculated by means of eq 14, taking $S_A = 0.3$ (mole fraction) for the solubility of a pure enantiomer. It is evident that, contrary to the case of conglomerates of undissociated enantiomers (Figure 2a), the solubility curves are no longer straight. Nevertheless, the curvature found is rather slight.

Note that in both Figures 2a and 2b parts AE and A'E of the solubility curves correspond to stable equilibria, while Ed and El describe metastable solubility equilibria which we will consider in section IV.

In a general way, the preceding conclusions remain valid even when weaker or less specific interactions are manifested between enantiomers in solution. In the case which we have just elaborated in detail the interactions are due to the existence of a common ion X. In the general case, the occurrence of intermolecular association, in particular with solvent, gives rise to equilibria which all lead to the same consequences: the solubility of each enantiomer is affected by the presence of the other, and the solubility curves are no longer straight.

Examples of experimental ternary diagrams which support these statements can be found in ref 12 and 13.

III. Separations Based upon Simultaneous Crystallization of Two Enantiomers

A. Manual Sorting of Conglomerates. Triage

Allusion has been made to the memorable experiments of Louis Pasteur who, during the month of May 1848 and under the watchful and incredulous eyes of Biot, separated the dextrorotatory and levorotatory crystals constituting the racemic double salt sodium ammonium tartrate.[16-20]

While these historical experiments retain considerable value as examples of simplicity and economy with respect both to concept and to resources, they do not nowadays serve as useful models of practical manipulations which are of general applicability. This Pasteurian separation is extremely laborious. It permits one to collect only crystals which are well-formed and which exhibit well-defined morphological characteristics (hemihedrism) which distinguish "left" from "right" crystals, a situation which does not always obtain even if relatively large crystals are available. Nevertheless the manual sorting of conglomerates of small and poorly formed crystals is feasible if one takes advantage of all the properties, which may be more or less evident, which allow one to differentiate the enantiomers.

We cite the experiments carried out by Jungfleisch on the resolution of the same sodium ammonium tartrate wherein he took advantage of the insolubility of the calcium racemate (i.e., racemic calcium tartrate) to recognize without dissolving them whether two crystals of tartrate are or are not of the same sign:[21] "It suffices to detach by means of a sharp needle a small portion of the crystal to be examined, and to deposit it along with a drop of water on a glass slide which itself rests on black paper. Once the crystal is dissolved, half of the solution is removed to another part of the slide by means of a stirring rod, and a drop of aqueous dextrorotatory calcium tartrate . . . is added to it. A drop of reactant . . . prepared from levorotatory calcium tartrate, is added to the other half of the original solution. The precipitate of calcium racemate appears immediately in that drop for which the reactant added possesses a rotatory power of opposite sense to that of the crystal examined. Since no reaction would be expected in the other drop, the test would thus be subject to control. Moreover, this test can be carried out rapidly for many crystals on the same glass slide."

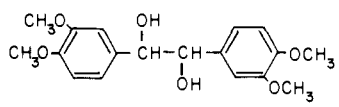
When one is dealing with substances having accessible melting points (which was not the case with the mineral tartrate salts studied by Pasteur), then an alternative possible analysis is to remove some fragments from an isolated crystal, for example, by means of a razor blade. These may be tested by

mixing them with a small sample derived from other crystals to see whether melting-point lowering does or does not take place.[22]

The sign of rotation can also be determined polarimetrically by dissolving a crystal in a given solvent without weighing it or measuring the volume, prior to assembling the various solutions having the same sign.[23] The sensitivity of modern polarimeters has significantly increased the utility of this technique.

Other stratagems for distinguishing between crystals of opposite signs may be envisaged, such as that which consists of exposing a conglomerate to the vapor of an optically active reagent so as to form a visible coating which differs for (+) and (-) crystals of substrate.[24]

Although this procedure of manual sorting of crystals is only rarely of preparative value, the possibility is nonetheless of considerable interest. After all, the initial resolution of sodium ammonium tartrate was a crucial experiment in the subsequent development of stereochemistry as a whole. Sometimes, the demonstration that a substance is resolvable may directly provide stereochemical information about its structure. Thus, the observation that spontaneous resolution takes place allowed an immediate distinction to be made between the meso and DL forms of hydroveratroin[25] (**1**) and of *o*-hexaphenylene[26] (**2**) to cite only two examples.



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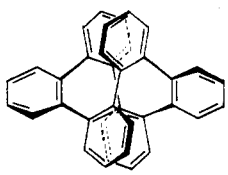

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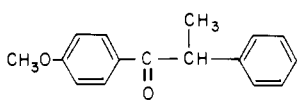
Most often manual sorting is utilized to collect the first crystals of enantiomer required to apply the technique of resolution by entrainment which we will describe below (section IV). This is particularly useful when resolution by formation of diastereomers is impossible, unsuitable, or simply susceptible to complication as, for example, in the case of enolizable ketones such as **3**.[22,27]



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B. Localization of Crystallization of the Enantiomers

A more attractive variant of the process which we have just described consists in the *localization* of crystallization of individual enantiomers on suitably disposed seeds within a racemic supersaturated solution. This process was conceived by Jungfleish,[21] again in connection with the resolution of sodium ammonium tartrate. A racemic solution is prepared such that its supersaturation is ca. 150–160 g/L at the temperature of crystallization. "One operates in crystallizing dishes containing one to two liters of liquid and whose ground edges permit an air-tight enclosure to be maintained by covering the dishes with flat glass plates. The saturated solution is placed in the vessel while still warm. Upon condensation, the water vapor emitted by the warm solution wets the edges of the dish as well as the glass cover by capillary action thus yielding a hydraulic closure which allows the solution to cool completely while remaining supersaturated . . . The solution having attained room temperature, one carefully wets the hands and places a small fragment of dextrorotatory sodium ammonium tartrate between the fingers; one washes the crystal by exposing it momentarily to the stream of a washbottle and . . . allows it to fall in the right hand side of the crystallizing dish. One does the same with a crystal of levorotatory salt which is allowed to fall in the left side, and then immediately replaces the glass cover . . . The introduction of crystalline dust must be carefully avoided which would otherwise rapidly lead to a mixed crystallization. The wetted crystals do not yield this phenomenon: they momentarily dilute the liquid layer which surrounds them, enlarge themselves slowly and attain their maximal size only after two to three days by which time the solution has ceased to be supersaturated. They remain perfectly isolated . . . and the dextrorotatory crystal has been enlarged only by dextrorotatory tartrate, while the levorotatory crystal is formed exclusively with levorotatory salt. It is easy to obtain well formed and isolated crystals in this way each weighing 180 to 200 g."

TABLE III. Resolution of (±)-Lysine 3,5-Dinitrobenzoate in a Fluidized Bed System[a]

run	racemate concentration, g/100 g of H_2O	T, °C	time, min	yield, g (-)	yield, g (+)	optical purity, % (-)	optical purity, % (+)
A	23.0	35	45	2.2	2.4	78.5	72.2
B	23.0	35	160	3.8	3.4	58.1	59.5
C	36.0	55	125	2.8	2.8	95.1	95.1
D	36.0	55	200	3.2	3.2	92.2	93.1
E	36.0	55	300	4.4	4.2	89.7	92.5

[a] Flow rate 250 mL/min; seed crystals 1.0 g, 60–115 mesh; adapted from ref 29.

This experimental procedure has been perfected to obtain separations which are industrially useful. Zaugg[28] has described an apparatus which allows the simultaneous crystallization of the enantiomers of methadone: 50 g of racemic substance is dissolved in 145 mL of petroleum ether (bp 63–68 °C). Through slow evaporation at 40 °C over a period of 125 h the solution loses about one quarter of its volume. Two crystals of (+)-methadone weighing 13.0 g and two crystals of (-)-methadone weighing 13.1 g develop from seeds deposited in the solution at the onset.

Another variant of this procedure consists in allowing the supersaturated solution of the racemate to circulate over the suitably arranged (+)- and (-)-seeds (fluidized bed system). The apparatus employed for the resolution of lysine 3,5-dinitrobenzoate is constructed from two columns each separable into halves to permit loading of seeds and collection of crystals.[29] Columns of dimensions 8 × 2 cm are fitted with fritted-glass plates at each end. They are loaded each with 1 g of seeds (one enantiomer to each column). The supersaturated solution of racemate is then allowed to circulate through the two columns which are arranged in parallel. After a given time, the crystals which have developed are removed from each compartment. Table III gives an idea of the results obtained.

Along the same lines, Brugidou et al.[30] have described an apparatus which allows one to obtain monocrystals of one of the enantiomers starting from a conglomerate. This device (Figure 3) is made up of two jacketed tubes A and B which are maintained at different temperatures by circulation of appropriate thermostatted fluids through the jackets. A minipump is interpolated in D to provide for the slow circulation of the solution from the warm and toward the cold enclosure. The cycle is completed by means of tube C, the return line. A seed of one of the enantiomers suspended from a wire is introduced in cold tube B. The crystal of the corresponding enantiomer grows from the seed with the solution originating in the warm tube providing the "nourishment". The crux of the process is, of course, that the warm solution becomes supersaturated upon arrival in the cold tube.

This apparatus was used in the resolution of (±)-hydrobenzoin. Ethyl acetate was employed as solvent and the temperature of the warm tube was maintained at 22–24 °C while that of the

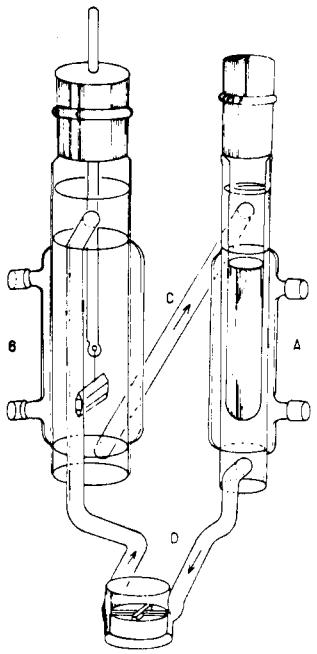


Figure 3. Reproduced with permission from ref 30. Copyright 1974 Société Chimique de France.

cold tube was kept at 14–15 °C. Monocrystals weighing between 1.0 and 2.9 g and having an optical purity of 98–100% were obtained.

A device which combines the key elements of the Sato device and the Brugidou device has been successfully employed in the resolution of 3-fluoro-DL-alanine *2-d*-benzenesulfonate on a scale as large as 13 kg.[31]

C. Differentiated Crystallization of the Enantiomers

Watanabe and Noyori[16] have described an ingenious resolution procedure through seeding which avoids some of the difficulties which are inherent to resolutions by entrainment and which depend on the maintenance of supersaturation with respect to one of the enantiomers. The process consists in the seeding of a racemic supersaturated solution with relatively large seeds of one enantiomer whose growth will give rise to even larger crystals. At the same time, the spontaneous crystallization of the other enantiomer will produce small crystals which may be separated from the larger enantiomeric crystals by sifting.

The experimental protocol is as follows: racemic acetylglutamic acid (30 g) is dissolved in 150 g of hot water and the solution is cooled to 43 °C; (−)-acetylglutamic acid crystals (10 g) of size greater than 30 mesh are added and stirring is carried out at 43 °C for 30 min. The crystals which deposit are filtered, washed, and dried; the solid is sifted through a 30-mesh sieve whereupon 13.6 g of practically pure product $[\alpha]^{17}_{D}$ −16.1° (*c* 2, water) remains in the sieve while 2.6 g of acid $[\alpha]^{17}_{D}$ +10.8° passes through. A refinement of this protocol consists in seeding with the (−)-enantiomer, as above, but simultaneously with 1 g of (+)-enantiomer of crystal size less than 200 mesh. Sifting yields 13.7 g of acid $[\alpha]^{17}_{D}$ −15.0° and 4.8 g of acid $[\alpha]^{17}_{D}$ +13.6°. As is evident from this balance sheet, one is dealing with a resolution in which equilibrium between the solution and the two enantiomers is practically undisturbed (taking into account the weight of seeds, 3.7 g of one and 3.8 g of the other enantiomer are actually obtained).

A very similar process has been applied with success by Brienne and Jacques to the separation of a mixture of two diastereomers forming an eutectic.[32]

IV. Resolution by Entrainment

Except for the direct isolation of natural products such as sucrose, the largest amounts of optically active substances produced industrially are obtained in two ways: fermentation processes, which are limited to the synthesis of several amino acids of natural configuration, and the technique to which Amiard gave the name "resolution by entrainment"[33] (dédoublement par entraînement) and which is often called resolution by *preferential crystallization*. As an example of the scale in which the latter process has been employed, 13 000 tons of L-glutamic acid was derived annually in the period 1963 to 1973 from resolution by entrainment of the synthetic acid prepared from acrylonitrile.[34a] There are currently at least two commercially important compounds which are resolved by direct crystallization of enantiomers, these being L-α-MeDopa[34b,99] and *l*-menthol[34c,57] (the latter via menthyl benzoate).

We do not wish to imply by this that this technique is only of interest to industrial-scale resolutions. Resolution by entrainment on a laboratory scale sometimes constitutes the method of choice when several grams or tens of grams of two enantiomers of an optically active substance are required.

A. History and First Examples

The first observation which showed the way to resolution by entrainment is due to Gernez who was a student of Pasteur. The discovery was announced in all of 12 lines in a letter[35] addressed to Pasteur in 1866: "I have observed that a supersaturated solution of levorotatory double salt sodium ammonium tartrate does not crystallize in the presence of a fragment of this salt which is hemihedric in the dextrorotatory sense; and vice-versa, the supersaturated solution of the dextrorotatory salt yields no crystals when seeded with levorotatory salt.

This fact led me to study the inactive solution of the double salt sodium ammonium racemate. I prepared a supersaturated solution of this salt from the racemic acid . . . When seeded by a particle of dextrorotatory salt, it yielded only dextrorotatory crystals. A portion of the same liquid in contact of a levorotatory crystal produced a deposit of levorotatory salt. Here then is a simple means for separating at will one or the other of the two salts which constitute the double salt sodium ammonium racemate".

In a paper dated 1882, Jungfleish, while confirming the observations of Gernez, also cited the disadvantages of the method and recognized the role of supersaturation:[21] "if the solution is not strongly supersaturated only a low yield is obtained in each operation. Contrariwise, it is difficult to prevent the crystallization of the non-seeded salt from a strongly supersaturated solution".

The method of Gernez was forgotten for a long period of time, no doubt due to the fact that so few spontaneous resolutions were known. It was not until 1914 that Werner rediscovered the same phenomenon though in a somewhat different form.[36] Werner had observed a large difference in solubility of the optically active and racemic (oxalato)bis(ethylenediamine)cobalt bromides, $[Co(ox)(en)_2]Br$. He tried to recover the less soluble optically active salt by precipitation with alcohol from an aqueous solution of this complex partially enriched with the dextrorotatory enantiomer. The attempt succeeded, but, much more remarkably, examination of the residual mother liquors showed that they had changed sign and that they now contained an excess of levorotatory salt. A further addition of alcohol furnished a crop of levorotatory crystals along with new and now dextrorotatory mother liquors, etc. This observation was subsequently confirmed with similar success on the dinitrobis(ethylenediamine)cobalt chloride, $[Co(NO_2)_2(en)_2]Cl$, whose optically active form is also less soluble than the racemic form.

Just as was true of the work of Gernez a half-century earlier, the results of Werner were also forgotten for quite some time. It was only some 20 years later, through a demonstration of

TABLE IV. Resolution of (±)-Hydrobenzoin by Entrainment[a]

run	hydrobenzoin added, g: racemic	hydrobenzoin added, g: (−)	yield of resolved hydrobenzoin,[b] g: (−)	yield of resolved hydrobenzoin,[b] g: (+)
1	11.0	0.37	0.87	
2	0.9			0.9
3	0.9		0.8	
4	0.8			0.75
5	0.7		0.7	
6	0.7			0.75
7	0.75		0.8	
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
overall 15 runs	23.5	0.37	6.5	5.7
	11 g recovered		12.2 g	

[a] The experimental conditions are given in the text. [b] Enantiomers having ca. 97% optical purity.

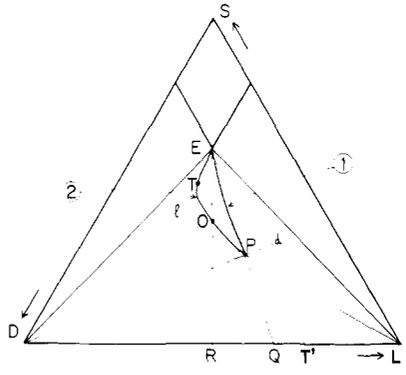


Figure 4.

the efficiency of the resolution by entrainment of histidine monohydrochloride by Duschinsky,[37] that the method really began to interest chemists, in particular those in industry.

B. Description of the Process of Resolution by Entrainment

The use of resolution by entrainment such as it is practiced nowadays may be exemplified by the case of hydrobenzoin.[38] Racemic hydrobenzoin (11 g) is dissolved along with 0.37 g of (−)-hydrobenzoin in 85 g of 95% ethanol, and the solution is cooled to 15 °C. Seeds of (−)-hydrobenzoin (10 mg) are added and the stirred solution is allowed to crystallize for 20 min. The weight of (−)-hydrobenzoin recovered after filtration (0.87 g) is roughly double that of the (−) enantiomer introduced in excess at the beginning of the experiment. Racemic hydrobenzoin is then added to the remaining solution in an amount equal to that of the (−) crystals collected. The solution is heated until the solid is completely dissolved. It is then cooled to 15 °C and crystallized as above after seeding with 10 mg of (+) enantiomer to yield a weight of (+)-hydrobenzoin nearly equal to that of the (−) isomer earlier collected. The same cycle of operations, i.e., loading with racemic hydrobenzoin and collection of (+) and (−) crystals, is carried out 15 times, yielding 6.5 g of (−) and 5.7 g of (+) enantiomer each having ca. 97% optical purity. Table IV gives a summary of the process.

Even though individual cases differ somewhat from one another and thus require specific procedures, most resolutions by entrainment which have been described in the literature, including patents, require this sequence of alternate crystallization of the two enantiomers. The initial system (or its "mirror image") is reconstituted between each cycle through addition of a quantity of racemate equal, or approximately equal, to that of the enantiomer just isolated (see, for other examples of this process, ref 27, 33, and 39).

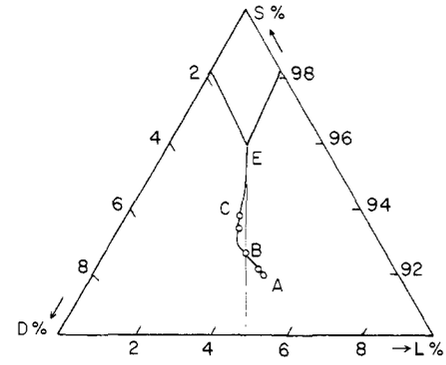


Figure 5. Crystallization curve of *N*-acetylleucine in acetone at 25 °C. Initial optical purity 11% (A); the solution was seeded with a very small amount of pure L crystals. Point B (change of sign of the solution) occurs at the end of ca. 20 min, and point C is reached at the end of 40 min. Only the upper part of the ternary diagram is shown (see the scales at the sides of the triangle).

C. Interpretation Based upon Solubility Diagrams

A resolution by entrainment can take place only if the enantiomers can crystallize separately. In other words, *the solubility diagram of the racemate must be that of a conglomerate*. We will return to this essential requirement which has given rise to much confusion in the literature in section IV.D.

Consider for a moment the triangular diagram of Figure 4 and observe first of all what takes place when a mixture of composition P is crystallized. This mixture is supersaturated with respect to the two enantiomers but the extent of supersaturation of L is greater than that of D. We know[4,7] that once the solubility equilibrium is attained the mother liquor will have composition E (racemic) and that of the solid will have composition Q. What matters now is to understand *how* this equilibrium will be attained. If enantiomer L crystallized by itself, then the composition of the mother liquor would be displaced along line Pl. By the same token, if D crystallized by itself, then the composition of the mother liquor would be displaced along Pd. With D and L crystallizing simultaneously, the composition of the mother liquor is displaced along a resultant curve which finally terminates at point E. Since the appearance of this curve depends upon the ratio of the crystallization rates of the two enantiomers, when these rates are comparable a curve of type 1 would obtain. If, on the other hand, L crystallizes more rapidly than D at first, one might find a curve of type 2. In the latter case, the rotation of the mother liquor would change sign during the crystallization. Initially it would be (−) at point P; it would vanish at O and be (+) at point T, for example. From this fact, the solid which would deposit just then (and which would have composition T′) necessarily would contain more of enantiomer L than there was initially in excess (P).

It must be clear that the best resolution conditions will be those for which curve POT will remain close to line Pl for the longest possible time, corresponding to a large difference in crystallization rates between the enantiomers. In the extreme, this is equivalent to the precipitation of a single enantiomer, namely, L. These ideas are reinforced by Figure 5 which shows an experimental curve obtained from a supersaturated solution of *N*-acetylleucine in acetone. Even though crystallization was carried out under routine conditions, the change of sign of the mother liquor was quite evident. An example of an optimized crystallization curve, corresponding to the resolution by entrainment of hydrobenzoin, is shown in Figure 6.

We are now ready to interpret a resolution by entrainment by means of a ternary diagram such as that described above. The initial solution M (Figure 7) contains the racemate and a slight excess of enantiomer L. It is prepared at an elevated temperature and then cooled to T_0, a temperature for which the

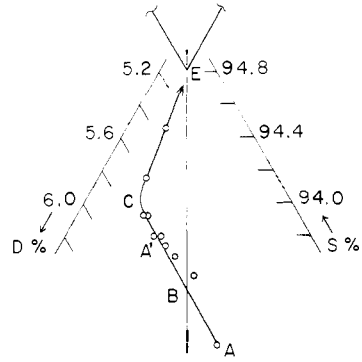


Figure 6. Crystallization curve optimized for the resolution by entrainment of hydrobenzoin in ethanol at 15 °C (enlargement of the part of the ternary diagram located in the vicinity of the eutectic point E). Initial conditions (A): concentration 11.7 g/100 g of solution, optical purity 2.6%; seeding with 10 mg of finely ground (−)-crystals; stirring speed 215 rpm. The change of sign (B) takes place after 25 min, with the maximum rotation (C) obtained at the end of 180 min. The point A′, symmetrical with respect to A, is attained in 85 min.

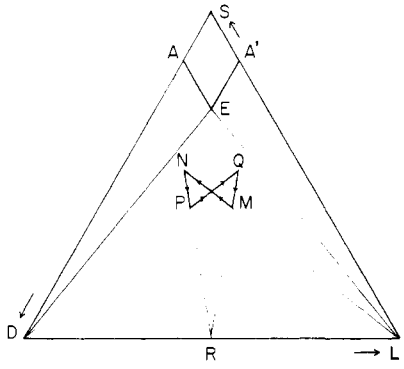


Figure 7. Resolution by entrainment: cyclic alternate crystallization of the two enantiomers.

solubility curves are AEA′. The solution is then supersaturated with respect to *both* enantiomers, but the extent of supersaturation is greater for L. Upon seeding, L is induced to crystallize alone and the point representing the mother liquor composition is displaced along the line LMN and toward N. For convenience, the duration of the crystallization is adjusted so as to yield a quantity of crystals of L which is double that taken initially in excess. The composition of the mother liquor is then given by N and its rotation attains a value effectively equal and of opposite sign to the starting value.[40] After the crystals of L are removed, an equivalent weight of racemate is added to the now dextrorotatory mother liquor so as to yield system P which is symmetric with M. This mixture is heated to dissolve the solid, cooled to T_0, and seeded with enantiomer D which then crystallizes. The composition of the mother liquor varies from P to Q, after which the solid is collected. Racemate is added once again to return to M and the cycle may then be repeated.

In practice, points M_i, N_i . . . corresponding to successive cycles need not be rigorously superimposable. Figure 8 gives a bird's-eye view of the dispersion of compositions observed in the case of hydrobenzoin under conditions approximating those shown in Figure 6. Provided that the mode of seeding and all the conditions of crystallization be standardized, then the duration of the process M → N or P → Q may be considered as a constant which may be established during preliminary experiments.

D. Racemates Resolvable by Entrainment

Let us now return to the requirement, which we stated earlier, regarding the nature of the racemate and how this affects its ability to be resolved by entrainment.

Werner[36] stipulated as a necessary condition the simple fact

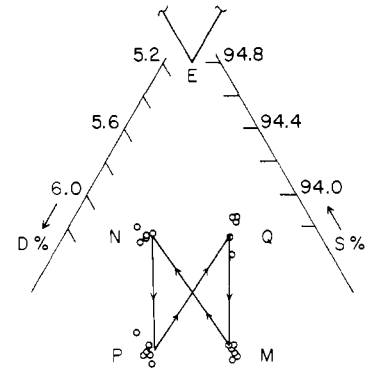
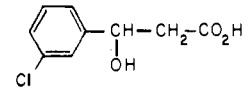


Figure 8. Successive cycles of resolution of hydrobenzoin by entrainment (same scale and conditions as in Figure 6).

that the enantiomer be less soluble than the racemate, without specifying the nature of the latter. We are aware that this conclusion was based upon the results obtained by him in the resolution of several complexes which later were recognized to be conglomerates. Other authors[41,42] have generalized the statement of Werner to mean that such resolutions may also be applied to racemic compounds, provided that these be more soluble than either pure enantiomer. However, Duschinsky[37] had actually earlier been the first to correctly identify the following requirement, which follows from the properties of the phase diagram *as a whole* and not only from those individual solubilities of *pure* racemate and enantiomer: a necessary condition for resolution by entrainment to be successful is that the racemic compound, if it exists at all, not crystallize during the operation. This may correspond to several possibilities: (i) The conglomerate is the stable crystalline form, and no racemic compound exists. The above necessary condition is then always fulfilled. (ii) Even when the conglomerate is the stable form, the existence of a metastable racemic compound is likely to make the entrainment difficult if not impracticable. For example, though the thermodynamically stable form of acid (±)-**4** is a conglomerate,[1]



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its crystallization in a solvent always yields a metastable racemic compound whose solubility is around seven times greater than that of the enantiomers. All attempts at resolving this compound by entrainment have been in vain.[43] (iii) A substance may exist as a racemic compound or as a conglomerate according to the temperature. In this case, the resolution is generally feasible in the region of stability of the conglomerate. The best known example of this possibility is that of sodium ammonium tartrate which may be resolved by entrainment below 27 °C. Sometimes, such resolution is possible also in the region of stability of the racemic compound; the conglomerate is then metastable, a situation which may introduce difficulties. The case of histidine hydrochloride[37] illustrates this possibility. (iv) The stable racemic form is a racemic compound under all conditions. As a matter of fact, this is the most common case; here entrainment is virtually unrealizable even if the racemic compound is more soluble than either enantiomer.

E. Search for Conditions Favoring Entrainment. The Method of Amiard

The use of a resolution by entrainment procedure requires the mastery of those factors which influence the *rate of crystallization*. Some of these factors are in turn largely dependent upon the properties of the ternary diagram itself which, in particular, limit the concentration ranges which are useful.

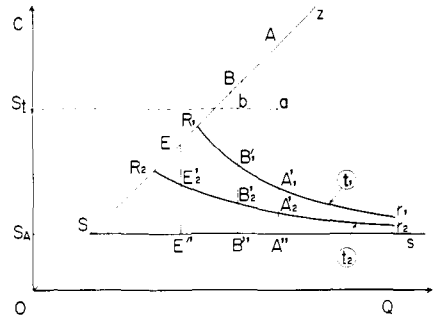


Figure 9. Residual supersaturation curves according to Amiard's experiments; after time t_1, R_1r_1; after time t_2, R_2r_2.

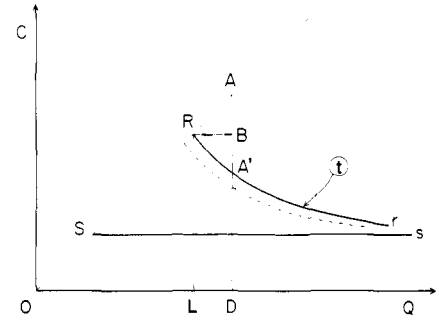


Figure 10. Alternative view of a resolution by entrainment showing the use of a residual supersaturation curve.

Others which are more experimental in character, such as the rate of stirring or the mode of seeding, lend themselves to the possibility of relatively greater variation.

The first detailed analysis of the mechanism of this type of resolution, carried out by Duschinsky, deals with the case of histidine monohydrochloride which is complicated by a polymorphism associated with solvation of crystals. More recently, in connection with their work on the resolution of threonine and of *threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol,[44] Amiard and his collaborators[33,45-47] applied themselves to a systematic search for conditions permitting the maintenance of supersaturation of the nonseeded enantiomer during the course of the crystallization of the other. Their contribution rests on two ideas, that of *residual supersaturation* (supersaturation rémanente) and that of the *entrainment* of crystallization, from which the name of the method was coined.

Consider the isothermal solubility diagram of a pure enantiomer shown in Figure 9. The quantity of dissolved substance C is shown as a function of the total quantity present (Q) in a given volume of solvent. The straight line Ss corresponds to the solubility S_A of the compound at the temperature of the experiment. The unsaturated solutions are described by segment OS while the supersaturated solutions prior to crystallization are given by line Sz. During the course of the crystallization of a solution represented initially by A, the point representing the state of the system is displaced vertically until the solubility equilibrium is finally attained at A''. In reality, this type of equilibrium occasionally takes a very long time to establish. Thus, in our example (Figure 9), the solution of initial composition A may be represented at time t_1 after seeding by A'_1, at time t_2 by A'_2, etc. These points correspond to the residual supersaturations, σ_{t_1}, σ_{t_2}, ... of solution A at the times specified. If the values of the residual supersaturations are determined as a function of the quantity of compound initially dissolved (Q), under specified conditions for crystallization, one obtains curves such as R_1r_1 (for a time t_1) or R_2r_2 for t_2. The shape of these curves shows that the residual supersaturation σ varies, after a while, in a sense inverse to that of the extent of the initial supersaturation ($\sigma_{t_1} = A'_1A''$ for A, B'_1B'' for B). It is this phenomenon which reveals the second idea of entrainment of crystallization: after a given time, the larger the initial concentration of the system, the closer it is to solubility equilibrium.

We may now make an important inference from our analysis of Figure 9. At time t_2, a supersaturated solution E will have attained composition E'_2. However, the fact that E is found below the residual supersaturation curve R_1r_1 also means that after a time t_1 this solution will have shown no evidence of crystallization in the presence of seeds. This last criterion clearly distinguishes *residual* supersaturation from the *metastable* supersaturation of Ostwald and Miers (vide infra). The latter defines a concentration limit below which crystallization of a supersaturated solution never takes place spontaneously but is triggered upon the introduction of seeds, while residual supersaturation can only be defined with respect to a *given time* of crystallization. It is important that these two states of supersaturation not be confused with one another.

The notion of residual supersaturation allows one to specify, in a rather simple way, some conditions under which resolution by entrainment may be achieved. In the first instance, the residual supersaturation curve of one enantiomer is determined for a given set of conditions in a racemic supersaturated solution. A curve (Rr, Figure 10) whose coordinates may be slightly different from those for the pure enantiomer (dotted line) is thus obtained. The separation between the two curves reflects the influence of the presence of one enantiomer on the behavior of the other. On the basis of this curve, a supersaturated solution of racemate is prepared such that the concentration of L and D each is equal to OL (Figure 10). The point which represents enantiomer L prior to crystallization is thus R. An additional quantity of enantiomer D (equal to LD) is then dissolved. Thus, the total quantity of D is OD while the solution is represented by A. If crystallization is now induced under the conditions in which curve Rr was obtained, it follows from the preceding considerations that compound L remains residually supersaturated, while D is deposited until the point A' on curve Rr is attained. The quantity of D thus precipitated, which is given by AA', is necessarily larger than that originally invested (corresponding to LD = AB). What has to be done, with the aid of the diagram, is to choose the initial excess LD in such a way that AB = BA', so as to obtain, after time t, a doubled quantity of enantiomer. After these crystals have been separated, reconstitution of conditions inverse to those which obtained previously (R representing the concentration of enantiomer D and A that of enantiomer L) requires only the addition of a weight of racemate equal to AA'.

In summary, the method consists of the crystallization of a supersaturated solution *in a time period and under a given set of conditions* in which one of the enantiomers is at the upper limit of its residual supersaturation while the other is present in an excess determined by the first.

This analysis actually corresponds more to the description of a process than to a theory. That it allows one to *adjust* the initial concentrations of the two enantiomers optimally is due to the prior empirical determination of experimental conditions. One can actually attempt the mastery of resolution by entrainment by considering, in a different way, two series of factors on which it depends: (i) those which derive in a direct way from the ternary diagram, and (ii) those which are more concerned with kinetic factors in the crystallization. Let us not, however, lose sight of the fact that in reality these two groups of factors are never completely independent of one another.

F. Derivation of Favorable Conditions from the Ternary Phase Diagram

Ternary diagrams allow us to locate the region of concentration in which resolution by entrainment is possible. They also provide information on the course of the resolution by showing

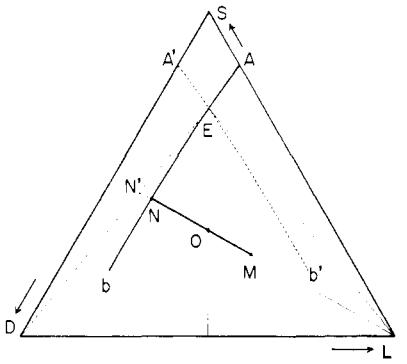


Figure 11. Solubility equilibrium of enantiomer L in solvent D + S.

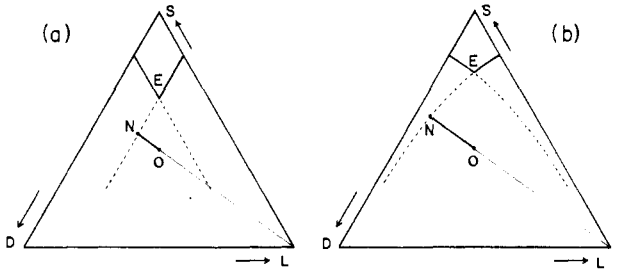


Figure 12. Variation in the area of the region of the phase diagram usable for the resolution by entrainment as a function of the solubility ratio $\alpha = S_R/S_A$; (a) $\alpha = 2$; (b) $\alpha = 2^{1/2}$. The solubilities of the pure enantiomers and the coordinates of point O are identical in both diagrams.

the effect of the crystallization of one enantiomer on the supersaturation of the other.

The solubility diagram of a conglomerate is shown in Figure 11. Let us consider the isotherm at T_0 under circumstances such that only one of the enantiomers, say L, crystallizes. Those parts of the diagram which correspond to the solubility equilibrium of enantiomer D (the solubility curve A'E and the tie line ED) no longer are involved in the behavior of the system (in other terms, this diagram describes the solubility of L in the binary solvent S, D). Thus, the crystallization of L will continue up to its solubility curve, which is no longer limited to segment AE but extends all the way to b. Point E, which here does not signify a eutectic, nonetheless has a physical meaning: it delimits the curves of *stable* (AE) and of *metastable* solubility equilibrium (Eb). Only the latter equilibrium interests us inasmuch as entrainment is not possible in the region AEL.

Let us now return to Figure 11. During the course of the crystallization of enantiomer L beginning with a system of composition M, the point which describes the solution moves along line LM beyond M up to the solubility curve at N (and not at N' on line ED as is sometimes indicated erroneously[42]). Solubility curve Eb is therefore a limit in resolution by entrainment. One sees immediately that the extent of the usable region of the diagram is directly influenced by the form of the solubility curves and, in particular, by the value of the solubility ratio $\alpha = S_R/S_A$ (where S_R and S_A represent the solubilities of the conglomerate and the pure enantiomers, respectively). As we can see from Figure 12, and contrary to what we might intuitively have imagined, it is the cases for which the racemate is *much more soluble* than the enantiomers ($\alpha > 2$) which correspond to the *least favorable* situation for entrainment. On the other hand, a ratio $\alpha < 2$ substantially enlarges the usable part of the diagram (Figure 12b).

There is another important piece of information which may be extracted from these diagrams and which has a bearing on the alteration of the degree of supersaturation of the nonseeded enantiomer during the course of the crystallization of the other. Our reasoning is summarized in Figure 13. During the crystallization of L, the composition of the solution may, for example, vary from M to N. The degree of supersaturation of D, which must remain in solution, corresponds to the distance, along the tie line from D, between the point which describes the solution and the extension of the solubility curve A'E. Thus, at M the supersaturation in enantiomer D is represented by MF, and at N by NG.

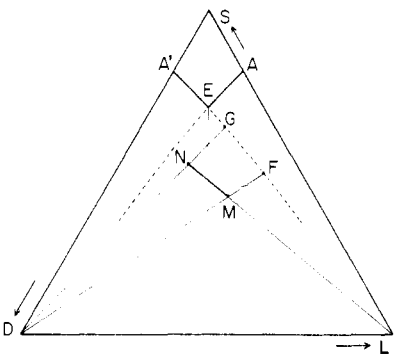


Figure 13. Extent of supersaturation of the nonprecipitating enantiomer during resolution by entrainment.

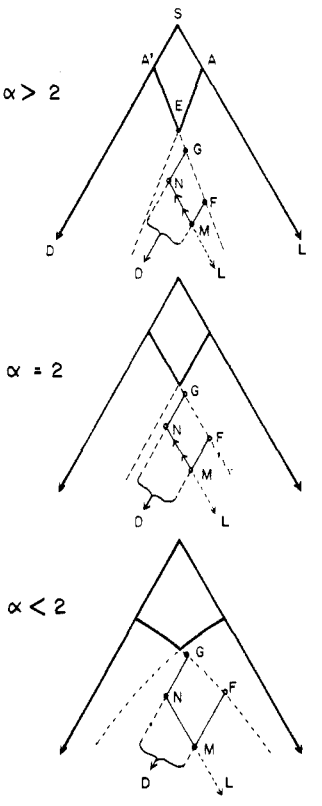


Figure 14.

The alteration of this degree of supersaturation during the process of entrainment may be qualitatively probed for dilute solutions as a function of the solubility ratio α. Since in dilute solutions the region of interest is pushed back toward vertex S of the triangle, line LMN on one hand and lines DMF and DNG on the other become nearly parallel to sides LS and DS, respectively. The conclusions are sketched out in Figure 14. For $\alpha > 2$, the degree of supersaturation of the undesired enantiomer D increases during the crystallization of the other (MF < NG). If $\alpha = 2$ it does not vary, and, finally, it decreases when $\alpha < 2$ (MF > NG). In more concrete terms, with $\alpha > 2$ the solution tends to destabilize during the course of the resolution process, with the consequence that the risk of spontaneous nucleation of the undesired enantiomer is increased. Contrariwise, when $\alpha < 2$ the solution becomes increasingly stable

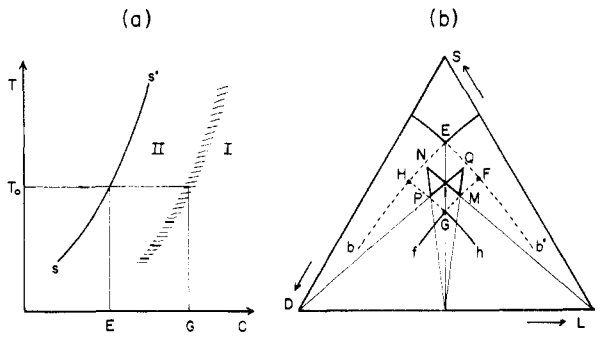


Figure 15. (a) Regions of supersaturation of a solution, e.g., racemic: ss', solubility curve as a function of temperature; E, solubility of the racemate at T_0; G, maximum concentration of a solution which does not spontaneously crystallize at T_0 within a stated time interval (see text). (b) Determination of the region of the phase diagram which is useful for resolution by entrainment.

during the development of the process. It should then be possible to obtain purer crystals with a large extent of resolution since it is also when $\alpha < 2$ that the area in which entrainment may occur is largest.

Watanabe and Noyori[18] have studied this problem in connection with the industrial-scale resolution of glutamic acid and its derivatives. Though their analysis is somewhat different from ours, they arrive at similar conclusions which may be illustrated by several examples. For the case of glutamic acid hydrochloride (α = 1.5) or of ammonium glutamate (α = 1.4), it is shown that one easily crystallizes virtually all of the supersaturated fraction of the seeded enantiomer in an optically pure state. On the other hand, with free glutamic acid (α = 2.35) or with *N*-acetylglutamic acid (α = 2.18), one observes that the other enantiomer crystallizes before maximal resolution has been attained. As a matter of fact, it is precisely in order to overcome this problem that these authors recommend in this case the simultaneous seeding of the solution with seeds of the two enantiomers having different sizes (section III.C).

Finally, we may deduce from the foregoing analysis that resolution by entrainment must generally be easier to carry out with salts than with undissociable organic compounds. It is for such salts that there is the greater possibility of finding a ratio α which is substantially smaller than 2 (see section II.B). This is what has led to systematic searches for *dissociable derivatives* which may exist as conglomerates by combining the substrate to be resolved with a variety of achiral reagents.[17,39]

G. Control of Crystallization Rate

There are two opposing requirements associated with attempts to control the rate of resolution by entrainment. It is necessary to reconcile the relatively rapid growth of the crystals of the desired enantiomer with the lowest possible rate of crystallization of the other.

Let us first examine the second of these requirements. The spontaneous crystallization of a supersaturated solution comprises two steps: (a) the appearance of crystalline seeds (nucleation), and (b) the development of crystals from these seeds. Ostwald and Miers have defined two ranges of supersaturated states. One is a region of "labile supersaturation", I (Figure 15a), found at high concentration in which spontaneous nucleation is possible. The other is a region of "metastable supersaturation", II, found between the former region and the solubility curve, in which the spontaneous generation of seeds would seem not possible. In reality, there is no sharp demarcation line between these two regions;[48] nevertheless, this analysis is useful in practice and it turns out to be justified by theory (see below).

While the appearance of crystalline seeds depends upon numerous factors which are not all easily controllable,[49] it may nevertheless be possible to determine empirically if not the limit

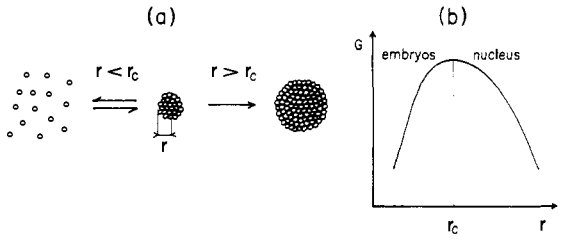


Figure 16. The process of nucleation.

of metastable supersaturation at least a concentration limit beyond which crystallization may not be prevented. Moreover, in the context of the method of Amiard, it is logical to take time into account. In practice, we will determine the maximum concentration G of a racemic solution, at a temperature T_0, in which no crystallization will have taken place during a given period of time (e.g., 1 h), under specified conditions of stirring and without seeding. The region of the phase diagram (Figure 15b) in which resolution by entrainment is favorable would approximately be given by drawing lines parallel to the solubility curves such as FGf and HGh. Within these limits (EFGH), we may draw a crystallization cycle MNPQ from which the conditions of resolution may be further refined if required.

The practical problem remaining is related to the fact that the limit of labile supersaturation of the undesired enantiomer is not really a well-defined line. This is particularly true in the presence of growing crystals of the seeded enantiomer which might well catalyze the nucleation of the other. While theories seeking to describe the rather complex mechanism of nucleation are as yet imperfectly supported by experiment in the case of solutions, some of their implications may nevertheless be useful. Consequently it may be worthwhile to present them here briefly.[50]

These theories propose that solute molecules first undergo collision to form very small aggregates of several molecules called "embryos" through a reversible process sketched in Figure 16a. The change in free energy accompanying the formation of these aggregates increases with their size, passes through a maximum at a critical size r_c, and then diminishes (Figure 16b). This means that aggregates smaller than r_c have a greater tendency to fall apart than to grow while those which have exceeded the critical size and are called "nuclei" may develop. The mean size r of the aggregates which are in equilibrium with the solution increases with the free energy of the solution itself, that is, with its supersaturation. There is a critical concentration for which equilibrium corresponds to aggregates of size r_c; this is how theory interprets the "metastable supersaturation" limit. The physical reality of this critical size r_c has been experimentally brought to light by Ostwald whose results indicate that the minimum size of aggregates able to grow corresponds to a sphere of several microns in diameter containing 10^{13} molecules. Theoretical calculations, which are generally considered more realistic, give a considerably smaller size, that is, several dozen Å and several hundred molecules. All of this boils down to the proposition that the minimum size for efficient nucleation is for the most part smaller than the limits of visual observation. Consequently, it will not suffice to obtain a clear supersaturated solution to be quite certain that one has eliminated seeds of the enantiomer whose crystallization one wants to avoid.

In order to obtain reproducible results it is essential to rid the system of aggregates of size greater than r_c. This requirement may be met by prolonged heating of the solution at a temperature at which it is no longer supersaturated, that is, a temperature which is distinctly higher than that at which crystallization is to take place. The duration of this preheating may range from a few minutes to tens of minutes, depending upon the substrate and the solvent used. This simple "sterilization" procedure generally guarantees reproducible results.[51,52] Undissolved seeds may also be eliminated along with foreign particles by filtration,

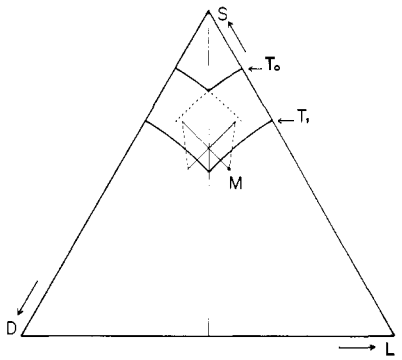


Figure 17.

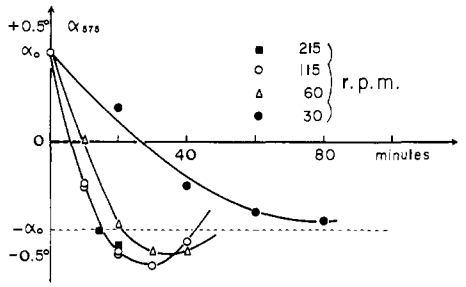


Figure 18. Resolution by entrainment of hydrobenzoin as a function of the stirring rate. Initial conditions: 11.8 g/100 g of solution; optical purity 3.75%.

ultrafiltration, or centrifugation of the solution employed.[49]

In principle, we know some of the factors tending to slow down the crystallization of the enantiomer which must remain in solution if our resolution is to succeed. Let us now examine what we need to do to favor the crystallization of the other. From the experimental point of view, the rate of growth of a crystal under a given set of conditions depends on the extent of supersaturation of the solution, $(S_t - S_A)$, and of the area $\mathcal{A}$ of the crystals exposed to the growth. An equation such as

$$-\frac{dS_t}{dt} = k\mathcal{A}(S_t - S_A)^n$$

is in most cases compatible with the kinetics observed.[53] In this equation, S_t represents the concentration of the dissolved enantiomer at time t and S_A its equilibrium solubility at the temperature considered; n is the order of the reaction and k a constant which incorporates, inter alia, the stirring rate. The equation makes it clear that the extent of supersaturation of the desired enantiomer should be as high as possible at the onset. In order to optimally carry out a resolution by entrainment it is necessary to begin with a substance which is already partially resolved. In practice, the usable ranges of initial optical purity and supersaturation are both closely linked to the dimensions of the usable part of the phase diagram (points M or P, Figure 15b).

The area $\mathcal{A}$ of the growing crystals depends upon their shape, their number N, and their total weight W. Since the weight of a spherical crystal of density d is $w = {}^4/_3\pi r^3 d$, the number of crystals is $N = W/w$, and their total area is $\mathcal{A} = 4\pi r^2 N$, it follows that $\mathcal{A} = 3W/rd$. In a very general way, the total area of a group of crystals of any shape may be estimated by an expression such as $\mathcal{A} = fW/ld$, in which f is a "geometric" constant (e.g., $f = 3$ for a sphere), and l is an average linear dimension for the crystals. From this, one can deduce the requirement that in order to attain a large initial area $\mathcal{A}_0$ for a given overall weight, the seeds should have a particle size as small as possible. In order to have reproducible kinetics over several successive cycles of crystallization, it is important to carefully systematize the conditions of seeding. This requires the accumulation of a sample of seeds for each enantiomer which is enantiomerically pure and uniformly ground and sifted.

TABLE V. Duration of the Resolution of Hydrobenzoin as a Function of Stirring Rate

stirring rate, rpm	30	60	115	215
t_1 ($\alpha = 0$), min	28	10	7	7
t_2 ($\alpha = -\alpha_0$), min		21	15	15

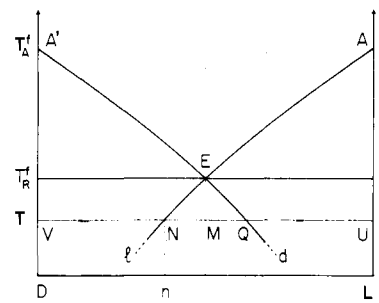


Figure 19. Resolution by entrainment in a supercooled melt.

The industrial-scale resolution of glutamic acid hydrochloride has involved the development of a special seeding technique. Formation of a very large number of optically pure microcrystalline seeds is induced by ultrasonic irradiation (10 to 100 kHz). The resulting crystallization occurs very rapidly, with the entire resolution requiring less than 15 min.[54]

A more classical procedure consists in the following: the solution is heated to T_1 which is a temperature slightly higher than that at which resolution is to take place. At this temperature the solution is supersaturated only with respect to the enantiomer present in excess. In this situation seeding or spontaneous crystallization generates crystals which are necessarily optically pure and which will serve as seeds when the solution is cooled to temperature T_0 at which the resolution is to take place (see Figure 17). The resolution of threonine[33] illustrates this technique.

Finally let us consider the rate of stirring of the solution in which the crystallization is taking place. This is also an important variable. Schematically, the growth of a crystal in solution may be broken down into a number of steps: (i) diffusion of solute molecules to the crystal–solvent interface, (ii) adsorption of the molecules to the surface of the crystal, and (iii) incorporation of these molecules into the growing crystal layer. At the same time, the inverse of this series of steps also takes place (dissolution). In an unstirred solution, it is often the diffusion rate (step i) which is the limiting factor in the crystallization rate since steps ii and iii are rapid. The effect of stirring would then be to accelerate diffusion and, consequently, the growth of the crystals.

When the stirring rate is gradually increased, an increase in crystal growth is observed at first which is usually followed by a horizontal step. The latter arises when the diffusion rate exceeds those of the following steps (steps ii or iii) which then determine the rate. The range of stirring speeds which are useful in controlling the crystallization rate is thus limited. The resolution of hydrobenzoin illustrates this in a particularly clear way. Figure 18 shows the change in rotation of identical solutions in the course of a resolution for different rates of stirring. The crystallization rate of the (+) enantiomer rapidly increases as the stirring rate is increased from 30 to 60 and then 115 rpm. Higher stirring rates no longer influence the crystallization: the curve corresponding to a stirring rate of 215 rpm is identical with that of 115 rpm. Also note that the stirring rate has but a small effect on the maximum rotation between 60 and 215 rpm. Table V gives the times required for the rotation of the solution to drop to zero ($\alpha = 0$ at t_1) and those required to reach a value equal but opposite in sign to the starting value ($\alpha = -\alpha_0$ at t_2) which determine the optimal duration of the resolution.[55]

TABLE VI. Theoretical Maximum Resolution Yield as a Function of Degree of Supercooling, $\Delta T = (T_R^f - T)$[a,b]

		isolated crystals						residue
		X_s^1	X_s^2	X_s^3	X_s^4	X_s^5	X_s^6	X_l^6
ΔT	ρ	L	D	L	D	L	D	
1	0.031	0.016	0.031	0.030	0.029	0.029	0.028	0.837
2	0.073	0.037	0.070	0.065	0.060	0.056	0.052	0.660
3	0.113	0.057	0.107	0.095	0.084	0.074	0.061	0.518
4	0.152	0.076	0.140	0.119	0.101	0.086	0.073	0.405
5	0.219	0.110	0.195	0.152	0.119	0.093	0.073	0.259

[a] The liquidus curve of the binary phase diagram was calculated with the Schröder-Van Laar equation by using the following data: $T_A^f = 60$ °C, $\Delta H_A^f = 4$ kcal mol^{-1}; the calculated melting point of the conglomerate is $T_R^f = 26$ °C (see section II.A). [b] ΔT is in °C; X_s and X_l are in mol.

An additional remark is that too great a stirring rate increases the risk of spontaneous nucleation of the other enantiomer by generating vibrations and shocks within the solution. It is pointless, therefore, if not detrimental, to increase the stirring rate beyond that which determines the maximum rate of crystallization (115 rpm in the example described).

V. Resolution by Entrainment in a Supercooled Melt

A. Theory

Just as it is true for resolution by entrainment from solutions, so it is the case that entrainment in the molten state, i.e., for a supercooled conglomerate, is an entirely valid alternative for the resolution of enantiomers. This type of resolution has been carried out in only a few instances. The theory of this process may easily be deduced from the binary diagram in Figure 19.

The liquidus curves AE and A'E, which correspond to stable equilibria, retain their meaning beyond point E. Point N, on AEI, indicates the composition of the liquid at equilibrium with crystals of L at temperature T. Of course, N describes a metastable equilibrium which may subsist only as long as crystals of D are absent. In practice, this condition is almost always met if the extent of supercooling ($T_R^f - T$) is not too large. In a conglomerate which has been supercooled to temperature T (point M), seeding with enantiomer L, for example, will induce the crystallization of the latter. The point describing the composition of the liquid is displaced from M toward the equilibrium curve at N. From 1 mol of racemate, one obtains X_s^1 = MN/NU mol of pure crystals of L and X_l^1 = MU/NU mol of liquid enriched with respect to D (of composition n). If the X_l^1 mole of supercooled liquid N is seeded with D after separation of the crystals of L, D crystallizes right up to the point where the liquid attains the composition Q on the liquidus curve A'Ed. The amount of crystals equals $X_s^2 = X_l^1$ NQ/VQ and that of liquid (now enriched in L) equals $X_l^2 = X_l^1$ NV/VQ. Seeding of this X_l^2 mole of liquid Q by enantiomer L would lead to crystals of L in liquid N, and so on. Alternate crystallization of L and D would lead, in principle, to the exhaustion of all of the initial racemate.

The yield of each crystallization depends upon the ratio ρ = NQ/VQ which increases with the extent of supercooling ($T_R^f - T$). This is a problem which is similar to that observed in the case of resolution in solution: as the degree of supercooling is increased, that is, as the temperature T is lowered, the risk of spontaneous nucleation by the nonseeded enantiomer is increased. What is required is to find optimal conditions of temperature and stirring which allow the maximum harvest of pure crystals in the briefest possible time and without disturbing the metastable equilibrium upon which the entire process depends.

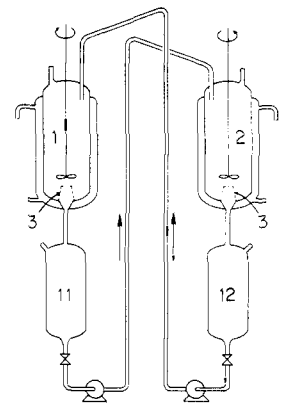
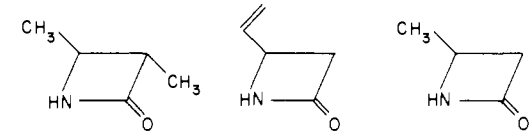


Figure 20. Apparatus for resolution by entrainment in a supercooled melt; reproduced by courtesy of H. Jensen (ref 56).

Table VI gives the theoretical maximum yield X_s^i of crystalline L and D obtainable from 1 mol of racemate subjected to six successive crystallizations as a function of the degree of supercooling, $\Delta T = T_R^f - T$. The calculations were carried out for a specific case; however, the import of the results is general (see section II.A). The last column of Table VI gives the amount of liquid remaining after six crystallizations (X_l^6). Examination of the data shows that the yield increases very rapidly as a function of the degree of supercooling. Thus, 1 °C below T_R^f, the total amounts of L and D collected during six crystallizations are, respectively, 0.075 and 0.088 mol and there remains 0.837 mol of liquid. At a temperature 3 °C below T_R^f, these amounts become 0.226 and 0.252 mol, respectively, of L and D and 0.518 mol of residual liquid. Otherwise stated, with 3 °C of supercooling, *half* of the racemate taken could theoretically be resolved in only six steps.

B. Application of the Method

The first examples of the application of resolution by entrainment in a supercooled melt are apparently those described by Jensen[56] in 1970. The publication is a patent dealing with resolution of β-lactam derivatives **5**–**7**. These compounds form



	5	6	7
T_A^f	60.6°	26.8°	26.7°
T_R^f	23.7°	-1°	-12.4°

conglomerates having low melting points which easily can be maintained in a supercooled state.

The apparatus employed allows the resolution to be carried out semiautomatically. The supercooled conglomerate is placed in equal parts in vessels 1 and 2 (Figure 20) which are fitted with stirrers and thermostatted at a temperature slightly lower than T_R^f. At first, seeds of enantiomers L are placed in vessel 1 and seeds of D in vessel 2. After a certain time during which crystallization takes place, the liquid phases in each compartment are separated by filtration through filters 3 from the crystals formed and collected in vessels 11 and 12. The liquid in 11, containing a slight excess of enantiomer D, is transferred to vessel 2 in which the first formed crystals of D remain, while the liquid in 12 is simultaneously transferred to vessel 1 where it comes in contact with crystals of L. After another period of time to allow further crystallization, the same operations (filtration and transfer of the liquids) are carried out again, and so on.

TABLE VII. Resolution by Entrainment of Amino Acids

compd	ref
alanine	
alanine monomaleate	107
alanine *p*-chlorobenzenesulfonate	17, 108
alanine benzenesulfonate	108, 109
serine	
anhydride	110
N-tosylserine	111
serine lithium oxalate complex	63
serine lithium chloride complex	63
serine lithium iodide complex	63
serine lithium nitrate complex	63
serine 2,4-dimethylbenzenesulfonate	64
N-benzoylserine, ammonium salt	65
cysteine	
no example found	
homocysteine	
homocysteine ammonium salt	66
2-amino-4-sulfobutyric acid	66
threonine	
threonine	33, 46, 67
aspartic acid	
asparagine	68
methionine	
N-acetylmethionyldimethylamide	69
valine	
valine hydrochloride	70, 71
proline	
N-chloroacetylproline	15, 72
N-acetylproline	15
N-butyrylproline	15, 72
N-isobutyrylproline	15, 72
3,4-dehydroproline anhydride	73
2-oxopyrrolidine-5-carboxylic acid lithium salt	74
2-oxopyrrolidine-5-carboxylic acid zinc salt	75
glutamic acid	
glutamic acid	6
N-acetylglutamic acid	16, 76
glutamic acid ammonium salt	5, 16, 77
glutamic acid magnesium salt	78
glutamic acid zinc salt	79
glutamic acid hydrochloride	16, 80
leucine	
leucine benzenesulfonate	17, 108
N-acetylleucine	81, 82
isoleucine	
N-acetylisoleucine	81
lysine	
N-benzoyllysine	83
lysine *p*-aminobenzenesulfonate	17, 84, 108
lysine 3,5-dinitrobenzoate	29
lysine 1-chloro-4-naphthalenesulfonate	29
lysine anthraquinone-2-sulfonate	29
α-amino-ε-caprolactam hydrobromide	85
α-amino-ε-caprolactam nickel chloride complex	86, 87
α-amino-ε-caprolactam 2-amino-1-naphthalene sulfonate	88
α-amino-ε-caprolactam 2-naphthalenesulfonate	88
histidine	
histidine monohydrochloride	37
arginine	
no example found	
phenylglycine	
phenylglycine	89
N-(carbethoxy)phenylglycine	90
N-benzoylphenylglycine	91
phenylglycine benzenesulfonate	92
phenylglycine camphorsulfonate (DL)	93
N-acetylphenylglycine ammonium salt	94
p-hydroxyphenylglycine *p*-toluenesulfonate	95
p-hydroxyphenylglycine *m*-xylenesulfonate	95
phenylalanine	
phenylalanine methyl ester hydrogen sulfate	96
tyrosine	
no example found for *p*-tyrosine	
m-tyrosine	97
Dopa	
3-(3,4-dihydroxyphenyl)alanine 2-naphthol-6-sulfonate	17, 108
3-(3,4-methylenedioxyphenyl)-2-methylalanine *p*-hydroxybenzenesulfonate	17, 108
N-acetyl-3-(3,4-methylenedioxyphenyl)alanine ammonium salt	39, 103
N-acetyl-3-(3,4-methylenedioxyphenyl)alanine di-*n*-butylammonium salt	39
N-acetyl-3-(3,4-methylenedioxyphenyl)-2-methylalanine hydrazinium salt	39
3-(3,4-dihydroxyphenyl)alanine (Dopa)	98
3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyl-Dopa)	99
3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid	100
3-(3,4-dimethoxyphenyl)alanine	101
N-acetyl-3-(3,4-methylenedioxyphenyl)alanine	101
2-(acetylamino)-3-(4-hydroxy-3-methoxyphenyl)propionitrile	102
tryptophan	
tryptophan benzenesulfonate	17, 108
tryptophan *p*-hydroxybenzenesulfonate	104
N-formyltryptophan ammonium salt	105
N-acetyltryptophan ammonium salt	105, 106
N-propionyltryptophan ammonium salt	105

Enantiomer L will eventually be collected from compartment 1 and enantiomer D will be collected from compartment 2.

In the resolution of β-lactam **5**, 250 g of racemate was divided between vessels 1 and 2 and supercooled to 20 °C (corresponding to 3.7 °C below T_R'). After seven stages of crystallization, each lasting 30 min, about 50 g of each enantiomer having optical purities around 85% were collected. There remained 150 g of nonresolved substance. The yield of enantiomers was estimated to be ca. 80 g L^{-1} of racemate h^{-1}.

Fleisher et al. have described[57] the resolution of supercooled menthyl benzoate by entrainment (T_A' 54.5 °C; T_R' 24.5 °C). The technique which they employed differs from the preceding one, being more closely related to that of resolution by entrainment in solution.

VI. Entrainment Combined with Simultaneous Racemization of the Substrate

By definition, the theoretical maximum yield in a resolution cannot exceed 50% for each enantiomer. This limit no longer applies if the undesired enantiomer can be racemized and routed back to the beginning of the resolution process. In most of the cases which are relevant to the preceding discussion (sections III–V), enantiomers are normally (and fortunately) optically stable under both the conditions of their resolution and subsequent utilization. The racemization of the substrate byproduct, which

should be included in any resolution leading to material of economic importance, thus represents an additional step which may be more or less difficult to carry out.

On the other hand, examples are known of enantiomers which are readily interconvertible in solution or in molten state. Some of them form conglomerates in the crystalline state. Such systems submitted to a rapid crystallization (with respect to the rate of racemization in the liquid phase) can yield but one product which is itself racemic. However, slow crystallization of one of the enantiomers, whether spontaneous or induced by seeding, may lead to the exclusive isolation of the latter. This special type of "second-order" asymmetric transformation[56] is rather rare as it cannot occur when the substance forms a racemic compound upon crystallization.

Earlier illustrations of this phenomenon are the "total spontaneous resolution" of *N*-methyl-*N*-ethyl-*N*-allylanilinium iodide[59] and that of tri-*o*-thymotide.[60] Both are obtained in optically active form upon slow crystallization within a solution which remains virtually racemic. One of the very rare examples of a fully inorganic optically active compound, $(NH_4)_2Pt(S_5)_3$, was recently obtained through such an asymmetric transformation.[61] 1,1′-Binaphthyl has been studied by Pincock et al. in considerable detail.[62] Conditions were found which permit a virtually total transformation of the molten racemate into one enantiomer.

A recent application of this process to the resolution of a substrate which is *not* optically labile under ordinary conditions demonstrates the utility of this optical-activation route and indicates the interest for further investigation in this field.[67] Racemic α-amino-ϵ-caprolactam (ACL), an important precursor of L-lysine, combines with nickel(II) chloride to form a mixture of two enantiomeric complexes expressed as $(L\text{-ACL})_3NiCl_2$ and $(D\text{-ACL})_3NiCl_2$. These compounds are easily interconvertible in ethanol solution in the presence of ethoxide ions as a catalyst. When a supersaturated solution $[(D\text{-ACL})_3NiCl_2 \rightleftharpoons (L\text{-ACL})_3NiCl_2]$ is seeded with crystals of $(L\text{-ACL})_3NiCl_2$, a nearly complete asymmetric transformation occurs. The isolated crystalline complex may be subsequently decomposed instantly into optically pure L-ACL through reaction with hydrogen chloride in methanol solution.

VII. Present Scope and Future Developments

Only some 30 cases of spontaneously resolved compounds were mentioned in the review of Secor[6] some 15 years ago. We are now aware of the existence of ca. 250 conglomerates, most of these being cited in ref 2 and 3. It should be clear that the comparatively small number of conglomerates relative to racemic compounds represents the main limitation to the possibility of resolution by entrainment or by the other techniques described in this review. This point calls for two comments, however.

First, it has by now been experimentally demonstrated that it is nearly always possible to find a conglomerate among derivatives of a given compound to be resolved. This statement can be illustrated for the amino acids, only a few of which are spontaneously resolved, e.g., glutamic acid, threonine, and asparagine. However, most of them have at least one derivative or precursor (acyl derivative, salt, etc.) which is resolvable by preferential crystallization. All of the important amino acids can therefore be resolved in this way. The amino acids and their derivatives susceptible to resolution by entrainment, which have actually been subjected to such resolutions, are listed in Table VII. The only amino acids known to date (1979) not to be resolvable in this manner are cysteine, arginine, and *p*-tyrosine.

The second comment bears on theory. Progress has been made in recent past years concerning the understanding of the alternative racemic compound vs. conglomerate. Some of the factors which are responsible for the large preference for racemic compound formation in the case of chiral covalent organic substances may be deduced from thermodynamics[1] and from an analysis of the crystal structures of a fair number of representative compounds.[112] We have reasons to believe that in the near future these ideas will lead to new and practical developments in the field of spontaneous resolution.

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