both enantiomers S' and R' have been found in the S crystal. However, after dissolution of the first layers, the R' impurity is completely removed, while S' is still present in the bulk of the crystal.

In the dissolution experiments, the magnitude of the effect has been found to be strongly dependent on the size of the crystals and maximal when the impurity is preadsorbed on them, excluding the possibility that stereoselective solution interactions be responsible for resolution. Futhermore, these experiments indicate that the major role of the impurity is on crystal growth (or dissolution) rather than on the preferential nucleation of one of the enantiomorphs. Further investigation is, however, needed to clarify the exact contribution of nucleation to the overall resolution.[12]

The above information suggests that it should be possible, by exploiting this mechanism, to improve not only the resolution of enantiomers crystallizing in the form of conglomerates but also the separation in any process involving competition in the crystallization of two phases, such as diastereoisomers or polymorphic forms (reacemate against conglomerate[13] and the like).

This process is not competitive with seeding but can be efficiently coupled with it, by using the seeds of the wanted enantiomer while inhibiting the growth of the unwanted one by impurity. Further, there may be a connection between the process here and other processes involving chiral recognition by adsorption, such as chromatographic separation on chiral phases.[14] Studies in this direction are under investigation.

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[12] Nucleation of both enantiomers is in general delayed in the presence of impurity. We do not know, however, whether this influence is stereoselective. In several systems we found similar resolutions in the presence of seeds R,S as without seeds.

[13] Sakata, Y. *Agr. Biol. Chem.* **1961**, *25*, 829.

[14] (a) Mikes, F.; Boshart, G.; Gil-Av, E. *J. Chromatogr.* **1976**, *122*, 205. (b) Weinstein, S.; Leiserowitz, L.; and Gil-Av, E. *J. Am. Chem. Soc.* **1980**, *102*, 27.

Useful Impurites for Optical Resolutions. 3. An Improved Pasteur-Type Resolution of Conglomerates and a New Empirical Method for Assignment of Absolute Configuration

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In 1848 Pasteur separated for the first time the two antipodes from a racemic mixture by mechanically sorting enantiomorphous crystals of sodium ammonium tartrate tetrahydrate under a microscope.[1] This process was made possible by the fact that the enantiomorphous crystals developed appropriate hemihedral faces, which permitted their visual identification. This feature is very rare and introduces additional limitations to the already severe requirement that to allow resolution by this method the racemate must crystallize in the form of a conglomerate.[2,3]

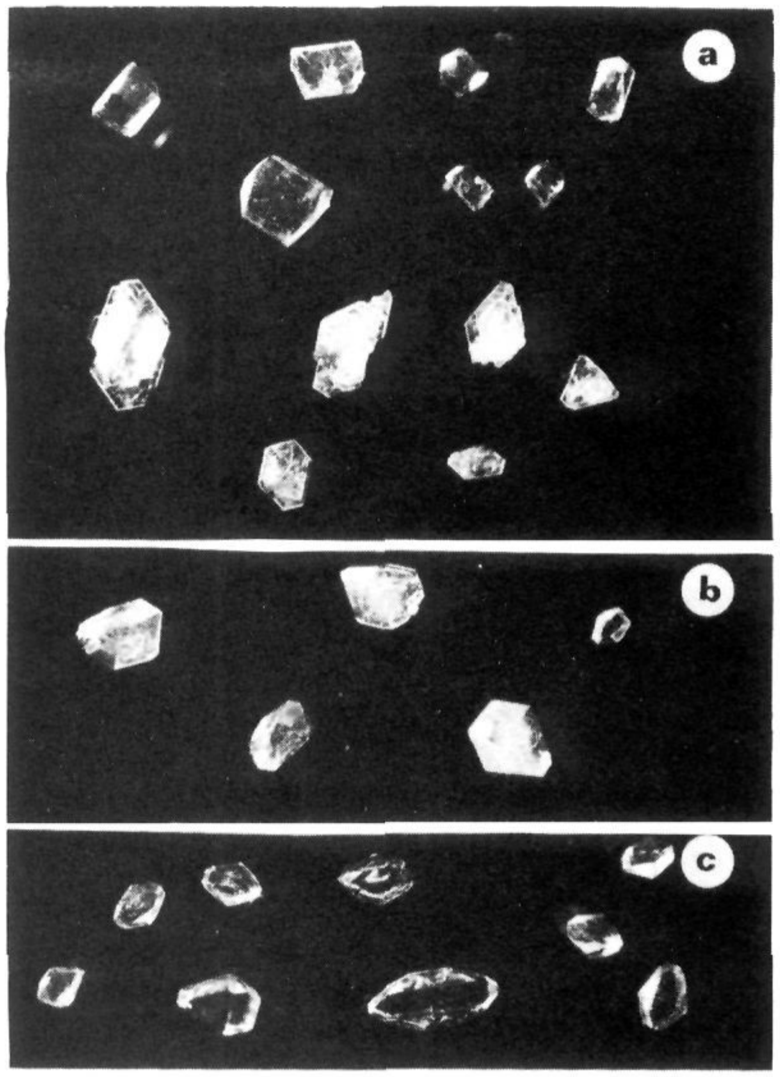

Figure 1. Crystal morphology of (R,S)-asparagine crystallized in the presence of S impurity: (a) (R,S)-Asn + (S)-Asp; manual separation: upper, unaffected R crystals; lower, S crystals; (b) (R,S)-Asn + (S)-Glu, S crystals; (c) (R,S)-Asn + (S)-Gln, S crystals; in (b) and (c) R crystals appear as in (a) upper.

Moreover, the correlation between the external morphology of the crystal and the absolute configuration of the enantiomer composing it is not straightforward.[4,5] We shall describe here a new method for visual separation of conglomerates and a new empirical method for assignment of absolute configuration of enantiomers.

In our studies[6,7] on the crystallization of conglomerates in the presence of chiral impurities, we have presented evidence for a mechanism involving preferential adsorption of the impurity at one or more of the faces of the stereochemically related crystal. Since the overall morphology of the crystal is determined by the rates of growth of the various faces, preferred adsorption on some of these, associated with changes of their relative growth rates, will result in an overall morphological change of the enantiomorph where adsorption took place,[7,8] while the antipode will be almost unmodified. We describe three examples of this phenomenon: asparagine·H_2O, threonine, and (R,R)- and (S,S)-ammonium hydrogen tartrate.

(R,S)-Asparagine (Asn) crystallizes from water at room temperature in the form of a conglomerate of space group $P2_12_12_1$.[9]

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[1] Pasteur, L. *Ann. Chim. Phys.* **1848**, *24*, 442.

[2] Fieser and Fieser [Fieser, L.; Fieser, M. "Advanced Organic Chemistry"; Reinhold: New York, 1961; p 71] report that only nine other examples of this kind have been found.

[3] Collet, A.; Brienne, M. J.; Jacques, J. *Bull. Soc. Chim. Fr.* **1972**, 127.

[4] An assignment of the absolute configuration of tartaric acid from its morphology [Waser, J. *J. Chem. Phys.* **1949**, *17*, 498] turned out to be incorrect: Bijvoet, J. M.; Peerdeman, A. F.; Van Bommel, J. A. *Nature* (*London*) **1951**, *168*, 271.

[5] For a description of other methods for identification of chirality of crystals, see: Lin, C. T.; Curtin, D. Y.; Paul, I. C. *J. Am. Chem. Soc.* **1974**, *96*, 6199.

[6] van Mil, J.; Gati, E.; Addadi, L.; Lahav, M. *J. Am. Chem. Soc.*, first of three papers in this issue.

[7] Addadi, L.; van Mil, J.; Lahav, M. *J. Am. Chem. Soc.*, preceding paper in this issue.

[8] On the effect of impurities on crystal morphology, see: Miles, F. D. *Proc. R. Soc. London, Ser. A* **1931**, *132*, 266. Buckley, H. E. "Crystal Growth"; Wiley: New York, 1951.

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Table I. "Morphological Separation" of Enantiomers of (*R,S*)-Asn Crystallized in the Presence of Additives

additive	morphology	weight, mg	absolute config	purity	$[\alpha]_D$, deg	resolution without separation, %
S-Asp	unchanged	59	*R*	94%	−28.5	64
	changed	27	*S*	87%	+26.5	
R-Asp	changed	3	*R*	78%	−23.6	79
	unchanged	45	*S*	92%	+28	
S-Glu	unchanged	30	*R*	67%	−20.5	49
	changed	8	*S*	79%	+24	
R-Glu	changed	22	*R*	84%	−23.5	24
	unchanged	35	*S*	91%	+27.6	
S-Gln	unchanged	53	*R*	98%	−29.7	12
	changed	44	*S*	92%	+28	

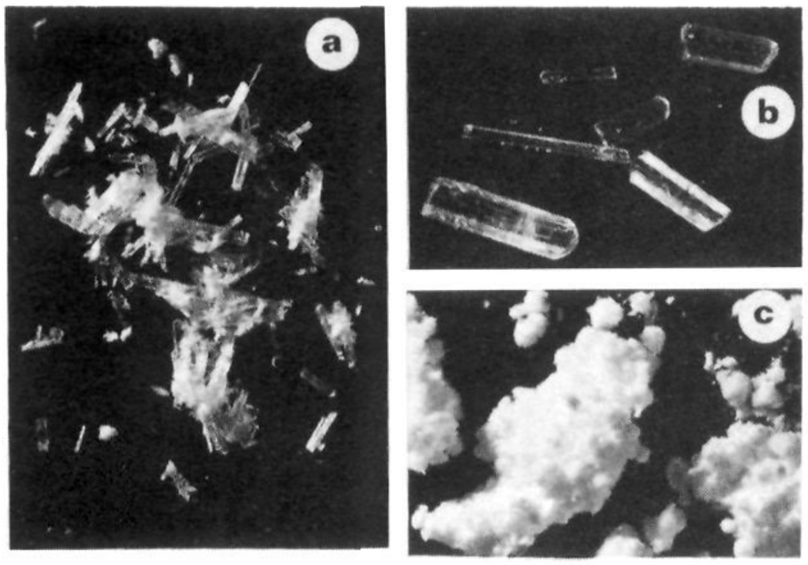

Figure 2. Batches of threonine crystallized in the presence of impurity: (a) (*R,S*)-Thr + (*S*)-Glu; (b) (*S*)-Thr + (*R*)-Glu; (c) (*S*)-Thr + (*S*)-Glu.

The normal habit of the crystal is shown in Figure 1. Glutamic acid (Glu), aspartic acid (Asp), and glutamine (Gln) are particularly effective in the kinetic resolution of the racemic amino acid;[7] these impurities have therefore been selected for the morphological studies as well.

Various batches of 100% supersaturated solutions (300 mg/3 cm^3) of (*R,S*)-Asn in water were prepared by cooling to room temperature hot filtered solutions to which resolved Glu, Asp, or Gln (12–50 mg) had been added. After 1–2 days of standing without agitation, spontaneous deposition of crystals started; these were separated by decantation after 1–2 additional days, dried, and examined. In each case the crystals could be separated manually into two groups of different morphologies but identical structure (powder diffraction and cell dimensions) (Figure 1). In Table I are reported some typical results on resolution of the batches separated in this way.

It can be seen from Table I and the figures that in each case the affected crystals, which appear in smaller amounts, have the same chirality as that of the additive. The optical purity of the separated batches or crystals reach, in some cases, ~100%. When it is lower, it is due only to difficulties in the physical separation of the crystals of different habits.

Racemic threonine (Thr) also crystallizes from water in a structure of space group $P2_12_12_1$. When supersaturated solutions of (*R,S*)-Thr in water were crystallized in the presence of (*S*)-Glu or (*S*)-Asn, as described for (*R,S*)-Asn, they appeared in the form of a mixture of big barlike crystals, coated with thin powder (Figure 2a). When (*S*)-Thr was crystallized in the presence of (*R*)-Glu or (*R*)-Asn, only big crystals were obtained (Figure 2b), whereas in the presence of (*S*)-Glu or (*S*)-Asn, under identical conditions, only fine powder precipitated (Figure 2c). Analysis of the material of Figure 2a revealed consistently that, while the powder contains mainly (*S*)-Thr, the big crystals are richer in (*R*)-Thr.[10]

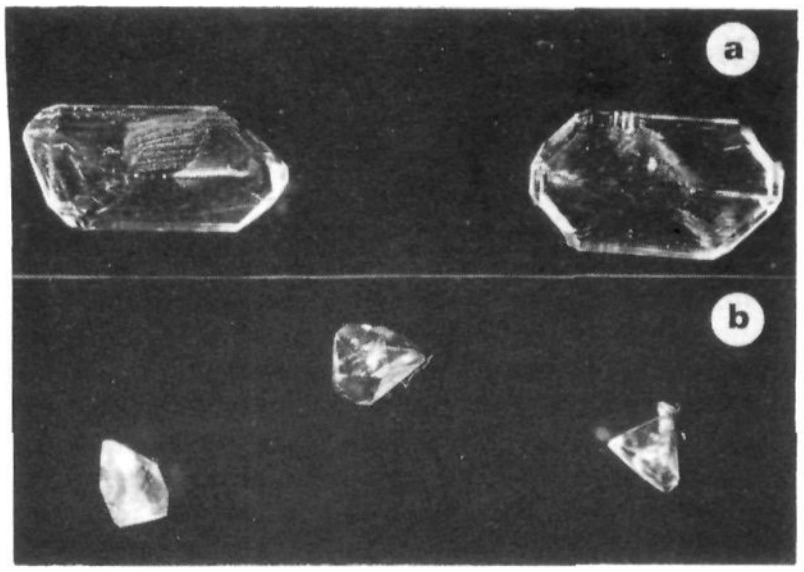

Figure 3. (*S,S*)-Ammonium hydrogen tartrate crystallized in the presence of (a) (*R*)-malic acid; (b) (*S*)-malic acid; in the absence of impurity the crystals have a morphology as in (a).

The third system investigated was ammonium hydrogen tartrate, which had been reported by McKenzie[11] to undergo preferential crystallization of one enantiomer in the presence of resolved malic acid. In our hands, under the conditions reported, the mixture crystallized only in the form of a racemic compound. Nevertheless, when the two resolved enantiomers were crystallized separately in the presence of (*S*)-malic acid, crystals of different morphologies were obtained (Figure 3). In the absence of (*S*)-malic acid the crystal's morphology is as in Figure 3a.

The striking morphological effects observed, together with the mechanistic studies reported previously,[6,7] supply a convincing proof for the correctness of the postulated mechanism.

This approach provides us with a new empirical principle for determination of absolute configuration by the independent methods of crystallization, preferential adsorption, and morphological differentiation. These methods should be applicable not only to materials undergoing spontaneous resolution but to any resolved compound forming chiral crystals. Once its generality is established,[12] it should be particularly useful in the assignment of absolute configuration of chiral crystals made of nonchiral molecules, such as glycine and benzyl. Moreover, from identification of the affected faces and a systematic study on the morphological changes as a function of stereochemistry of the impurity, one may deduce fundamental information on the energetics of binding of chiral molecules on surfaces with defined geometries. Studies along these lines are in progress.[13]

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(9) Piutti, A. *C. R. Hebd. Seances. Acad. Sci.* **1886**, *103*, 134. It has been reported by Ostromisslenky [Ostromisslenky, L. *Chem. Ber.* **1908**, *41*, 3035] that the introduction of crystals of glycine as seeds into a water solution of (*R,S*)-Asn caused preferential precipitation of either of the two enantiomers of Asn. After Iitaka [Iitaka, Y. [*Acta Crystallogr.* **1958**, **11**, 225] found a chiral structure of glycine, this effect was granted credibility and was repeatedly quoted in the literature (e.g.; Eliel, E. "Stereochemistry of Carbon Compounds"; Wiley: New York, 1962; p 48; Lin et al.;[5] Harada K. *Naturwissenscahften* **1970**, *57*, 114). We have observed during routine crystallizations that (*R,S*)-Asn can display autocatalytic seeding effects resulting in a small excess of enantiomers of either chirality. We feel therefore that it is not necessary to invoke chiral seeding by glycine as an explanation for the effect. For other studies on spontaneous asymmetric crystallization of (*R,S*)-Asn, see: Thiemann, J. *J. Mol. Evol.* **1974**, *4*, 85.

(10) The crystals of Thr were found to appear readily in the form of twins. This makes a complete separation between them impossible and gives ground to speculation on the efficiency of seeding techniques for the resolution of conglomerates whose crystals are subject to twinning.

(11) McKenzie, A. *J. Chem. Soc.* **1915**, *107*, 440. McKenzie, A.; Walker, N. *Ibid.* **1922**, *121*, 349. McKenzie, A.; Planderleith, H. J.; Walker, N. *Ibid.* **1923**, *123*, 2875.

(12) About 20 systems which have been investigated to the present time fulfill this rule, without exception. It is clear, however, that, like any new empirical method, this one requires further extensive confirmation as well.

(13) Addadi, L.; Berkovitch-Yellin, Z.; Domb, N.; Gati, E.; Lahav, M.; Leiserowitz, L., work in progress.