Reports

Probability Distribution of Enantiomorphous Forms in Spontaneous Generation of Optically Active Substances

Abstract. The crystallization of 1,1'-binaphthyl from its racemic melt is an example of spontaneous generation of optical activity. The distribution of specific rotations in 200 individual samples varied from $[\alpha]_D = -218$ degrees to $[\alpha]_D = +206$ degrees with a mean of +0.14 degree and standard deviation of 86.4 degrees. This resolution into enantiomers is determined by chance development, with equal probability, of right- or left-handed crystallites; it can be controlled and made stereospecific by addition of dissymmetric compounds at low concentrations.

The spontaneous generation of optically active material in a closed racemic system in the absence of any dissymmetric agent has never been demonstrated. We now describe briefly the genesis of optically active substances and present our results in the case of a racemic mixture that undergoes totally spontaneous development of optical activity. This example is the first systematic and statistically meaningful study of the probability distribution of enantiomorphs in the spontaneous generation of optically active substances. The system supplies a simple case of autocatalytic development of enantiomers according to a recent suggestion of Calvin (1).

The possibility of synthesizing optically active material without the direct action of living material (that is, dissymmetric compounds) or without use of dissymmetric compounds obtained from living material was at one time denied with considerable authority (2). However there have been many attempts and some well-established successes in producing optically active samples from racemic materials by dissymmetric agents in the absence of other dissymmetric compounds (3-6). Bias in the production or destruction of

one enantiomorph has been demonstrated under the influence of polarized light (7), catalysis by dissymmetric solid surfaces (3), selective decomposition with beta rays (8), and crystallization of racemic mixtures initiated by foreign dissymmetric seeds (3, 9). Such experiments can involve the transference of dissymmetry from an inanimate source to a single enantiomer of a natural substance and they provide models for the so-called "inorganic" genesis of optical activity in natural materials (4). However, these experiments all involve the action of an external dissymmetric influence and as such they are not examples of truly spontaneous genesis of optical activity. There are only a few experiments where generation of optical activity has occurred during purposeful exclusion of all external dissymmetric influences.

The experiments where no dissymmetric influences would seem to be present involve the spontaneous deposition of symmetric species into dissymetric crystals such as quartz or NaClO₄, the spontaneous crystallization of one of two rapidly interconverting enantiomers from solutions [that is, tri-o-thymotide (10) and methylethylallylanilinium iodide (11)], and the spontaneous resolution of various racemic mixtures (3) such as the uninitiated crystallization of part of a racemic D,L-tartrate solution (12). The latter example is the only one that has been looked at with more than a few individual samples in order to determine whether the optical activity produced was determined by chance. Surprisingly, it seems to be nearly impossible to eliminate trace sources of



Fig. 1 (left). Percentage of binaphthyl samples giving specific rotations between ± 240 . Two hundred samples were crystallized from the pure melt at 150°. The curve shows the Gaussian normal distribution calculated from the mean of ± 0.14 and standard deviation of 86.4. Fig. 2 (right). Ratio of samples having positive (+) rotations to the total number of samples of binaphthyl crystallized at 150°. The 200 samples are treated as two sets of 100 each. The curves are confidence limits calculated for a 0.99 degree of confidence and a probability of 50 percent positive (+) and 50 percent negative (-) for each sample.



SCIENCE, VOL. 174

1018

dissymmetry and obtain the conditions required for the perfectly symmetrical experiment.

In a racemic system it is expected that the crystallization of right- and left-handed enantiomorphs would be equally probable. Wald (4) has pointed out that the available data indicate that the elimination of obscure sources of dissymmetric influence is very difficult. Thus experiments (12) on the unseeded crystallization of racemic sodium ammonium tartrate solutions have led to a predominance of cases giving the dextrorotatory enantiomer even under controlled conditions (66 times out of 86). A recent series of experiments has given a set of ten runs with negative rotation in the precipitates (13). If all dissymmetric influences were truly eliminated, and the probabilities of each enantiomer precipitating were really equal, the chance that this set of ten minus rotations would occur is one in 256. It has been suggested (4, 6) that a reason for such results is contamination by omnipresent dissymmetric materials such as optically active dust. Harada (6) has reported that, even with precautions taken to avoid contamination by dust and with different sources of materials, spontaneous crystallization of a D,Laspartic acid copper complex led to only the dextrorotatory enantiomer in three different laboratories (in Japan and in Florida). Wald suggests that dissymmetric contamination may come from experimenters themselves, and that "in a world contaminated with life, dissymmetric materials may no longer be free to crystallize at random" (4). A small energy difference (of unspecified origin) between enantiomeric crystal structures has also been suggested (13). Possibly the world has an innate dissymmetry which might impose a weak bias in chemical reactions where no more direct dissymmetric influence is present (14). The spontaneous resolution of racemic 1,1'-binaphthyl (15) provides an opportunity to test, with a large number of samples, whether a bias in production of one enantiomorph is present.

1,1-Binaphthyl is a dissymmetric molecule by virtue of its twisted conformation of two adjoining naphthalene rings. In the melted phase (above 159° C) the equilibration of (+) and (-) enantiomers is very rapid (halflife less than 0.5 second). The binaphthyl melt may be supercooled indefinitely to temperatures as low as 120° C,



but when a sealed ampule containing this metastable melt is held momentarily against a piece of Dry Ice and then replaced in a constant temperature bath, crystallization is induced. The binaphthyl can thus be seeded by suddenly cooling a small portion of the bulk of the melt. There are no dissymmetric agents involved in this crystallization, only the chance formation of crystals of one or the other enantiomer. Weighed samples of 20 mg each from many different batches of racemic binaphthyl were sealed in individual glass ampules, were melted at 170° to 185°C, and then were equilibrated at 150°C. Crystallization was initiated in each ampule, and after 3 hours at 150°C the samples were dissolved in 3 ml of benzene and analyzed for optical activity (16). The material obtained was almost always optically active, although excessive application of the cooling Dry Ice resulted in no activity. These few cases of specific rotations less than ± 2 degrees were not considered in the following statistical analysis.

The distribution of the specific rotation, $[\alpha]_D$, for 200 separate samples, is shown in Fig. 1, which is a presentation of percentage of observations falling within various specific rotations. The experimental extremes of optical rotation were -218 and +206 degrees -optically pure binaphthyl has $[\alpha]_{\rm D} =$ ± 245 degrees (15); however, as shown, samples of low rotation of ± 2 to ± 48 degrees were most common. The observed rotations fit a Gaussian distribution with a mean of $[\alpha]_{\rm D} = +0.14$ degree and standard deviation of 86.4. From this distribution it is apparent that obtaining binaphthyl with an optical purity above 90 percent by this method would be a very exceptional event (observable about once in 150 trys).

Individual crystals of the high melting form (15) of binaphthyl are composed of either (+) or (-) enantiomers. The predominate nucleation and growth of an enantiomorph, supplied by the rapid interconversion of (+) \rightleftharpoons (-)

in the melt, results in optically active samples. This is an example of autocatalytic selection of an enantiomer from a pool of racemic material (1). Although an individual sample of binaphthyl may reach quite a high optical purity, a low optical activity is more probable. As far as overall results are concerned, in contrast to the spontaneous crystallization of tartrates or aspartic acid complexes mentioned above, the nucleation of many samples of binaphthyl does not show predominant formation of one enantiomer. Nucleation occurs in a random manner and a symmetrical distribution of both (+) and (-) optical activities, with a mean close to $[\alpha]_D = 0.0$, is produced.

The symmetry of the distribution is also shown in Fig. 2 by the ratio of number of (+) samples to total number of samples plotted for two runs of 100 samples each. As the total number of samples increases, the ratio tends toward 0.5 and it falls at all points within the 99 percent confidence limits calculated for a probability of 50 percent (+) and 50 percent (-) samples. After two hundred crystallizations in individual sealed vials, the number of dextrorotatory samples obtained (95) is insignificantly different from the number of levorotatory samples obtained (105). Although we cannot eliminate, even with many more samples, the possibility of a very small bias in production of one enantiomorph, this experiment seems to be the first to illustrate an essentially equal probability for spontaneous generation of enantiomorphs. Generation of optical activity in a racemic melt of binaphthyl proceeds without an external dissymmetric agent; the molecular dissymmetry of binaphthyl is itself sufficient to cause the production with equal probability of either (+) or (-) crystallites which then grow autocatalytically. An individual sample of racemic binaphthyl can develop a high optical rotation: the symmetrical distribution with many samples proves that no external agent contributes to this spontaneous generation of optical activity.

From these results it is clear that the spontaneous resolution of binaphthyl is not highly sensitive to trace contaminants such as dust, or to any less well-defined dissymmetric forces. However, dissymmetric compounds do greatly effect the distribution of optical activities. The presence of 5 percent by weight of *d*-mandelic acid in experiments at 130° C similar to those de-

scribed above produced binaphthyl with excess (+) rotation in 18 out of 19 samples. On the other hand, 5 percent *l*-mandelic acid in binaphthyl gave samples with (-) rotations (after correction for the small rotation arising from mandelic acid) in 17 trys out of 17. These distributions fall well outside those expected for a 50-50 probability and they indicate that the configuration of mandelic acid essentially completely controls the configuration of binaphthyl obtained. Such specificity in the case of binaphthyl, combined with the largely stereospecific results with tartrates (12, 13) and aspartates (6), lends support to the suggestion of Wald that contamination by the ubiquitous dissymmetric compounds of life may prevent other dissymmetric materials from crystallizing at random. Binaphthyl is a relatively unnatural compound compared to tartaric and aspartic acids and its nucleation and crystallization may therefore be less sensitive to dissymmetric impurities.

> **RICHARD E. PINCOCK** ROBERT R. PERKINS ALAN S. MA KEITH R. WILSON

Department of Chemistry, University of British Columbia, Vancouver 8, Canada

References and Notes

- 1. M. Calvin, Chemical Evolution (Oxford Uni-
- versity Press, London, 1969), p. 150. 2. F. R. Japp, *Nature* 58, 452 (1898). Examples of absolute asymmetric synthesis and some historical background are reviewed in references 3-6 below.
- 3. A. P. Terent'ev and E. I. Klabunovskii in The Origin of Life on the Earth, A. I. Oparin et al., Eds. (Pergamon Press, London, 1959), pp. 95 and 158.
- 4. G. Wald, Ann. N.Y. Acad. Science, 69, 352 (1957).
- A. Dauvillier, The Photochemical Origin of Life (Academic Press, New York, 1965), pp. 107-115
- 6. K. Harada, Naturwissenschaften 57, 114 (1970).
- 7. See (3) and a recent example of A. Moradpour, J. F. Nicoud, G. Balavoine, H. Kagan, G. Tsoucaris, J. Amer. Chem. Soc. 93, 2353 (1971).
- 8. A. S. Garay, Nature, 219, 338 (1968).
- 9. R. M. Secor, Chem. Rev. 63, 297 (1963).
- 10. A. C. D. Newman and H. M. Powell, J. Chem. Soc. London 1952, 3747 (1952). 11. E. Havinga, Biochim. Biophys. Acta 13, 171
- 12. F. S. Kipping and W. J. Pope, J. Chem. Soc. London 95, 103 (1909).
- 13. W. Thiemann and K. Wagener, Angew. Chem. Int. Ed. 9, 740 (1970).
- 14. Y. Yamagata, J. Theoret. Biol. 11, 495 (1966);
 T. L. V. Ulbricht, Quart. Rev. 13, 48 (1959).
 15. R. E. Pincock and K. R. Wilson, J. Amer. Chem. Soc. 93, 1291 (1971).
- 16. Observed rotations, α_{ob} , at 5893 A were measured with a Bendix automatic polarimeter type 143 A in a 1-cm cell; $[\alpha]_D = \alpha_{ob}(3)(10^4)$ per milligram of sample).
- 17. Supported by the National Research Council of Canada.
- 20 August 1971
 - 1020

Crystal Structure of a Naturally Occurring Dinucleoside Monophosphate: Uridylyl (3',5') Adenosine Hemihydrate

Abstract. The crystal structure of uridylyl (3',5') adenosine hemihydrate has been analyzed by x-ray diffraction. The two independent molecules found in the asymmetric unit exhibit conformations that differ significantly from those found in double-helical RNA. The conformational information obtained from this analysis provides considerable insight into the possible conformations of nonhelical "loop" regions of transfer RNA, as well as single-stranded regions of nucleic acids in general.

X-ray crystallographic determinations of the structures of nucleic acid constituents have formed the basis for the construction of molecular models of nucleic acids and polynucleotides. Many of the solid-state conformations have been confirmed by spectroscopic studies of nucleic acid constituents in solution. Until the present time, crystallographic data on the constituents of nucleic acids have been derived mainly from mononucleoside and mononucleotide structures (1). The present determination of the structure of a naturally occurring dinucleoside monophosphate, uridylyl (3', 5') adenosine hemihydrate (UpA), provides the first direct information on possible conformations about the 3',5' phosphodiester bonds (2).

The compound UpA crystallized readily from aqueous methanol solutions (pH 3.5) and formed large monoclinic crystals of space group $P2_1$ and unit cell dimensions a = 16.96 Å, b =12.35 Å, and c = 11.24 Å; $\beta = 95.9^{\circ}$. Comparison of the observed (1.625 g cm^{-3}) and calculated (1.651 g cm^{-3}) crystal densities indicated the presence of four molecules of UpA $(C_{19}H_{24}N_7O_{12}P)$ in the unit cell; the structure analysis revealed also the presence of two water molecules. Thus, there are two independent molecules of UpA and one molecule of water in the asymmetric unit of the structure; this gives a total of 79 nonhydrogen atoms and 50 hydrogen atoms. X-ray intensity data were collected on an automated fourcircle diffractometer; the θ -2 θ scan technique and CuK α radiation ($\lambda =$ 1.5418 Å) were used. Lorentz and polarization corrections were applied to the data, and a total of 3635 observed reflections were used in the structure determination.

The heavy atom method in combination with phase refinement by the tangent formula (3) provided the solution to the crystal structure. The positions of the phosphorus atoms were de-

termined by Patterson methods. Tangent refinement of the phases of 511 reflections based on the phosphorus atoms indicated the positions of the two independent adenine bases in the structure. Repeated cycles of structure factor, Fourier, and block-diagonal least squares calculations revealed the entire structure of the two independent UpA molecules, as well as the position of the water of crystallization. The agreement index R (4) after isotropic refinement is 0.12.

"backbone" The sugar-phosphate conformations (Fig. 1) observed for the two independent molecules differ markedly from the conformations found for double-helical RNA (5). A comparison of the torsion angles of the two independent molecules of UpA with those of double-helical RNA is given in Table 1. The largest differences among the three structures occur in the torsion angles involving O(5')—P and P-O(3') phosphodiester bonds. Molecule 1 (Fig. 1a) is in the extended or open conformation with the phosphodiester bonds exhibiting the (-) gauche-trans conformation (1, 6, 7). Molecule 2 (Fig. 1b) is in the closed (inverted) conformation with the (+) gauche-(+) gauche conformation about the phosphodiester bonds.

Table 1. A comparison of the backbone conformations in UpA and double-helical RNA. The torsion angles are about the backbone bonds; A is the adenine part and U the uridine part. The torsion angles for RNA (1) are for eleven-fold viral RNA (5). Molecule is in the open conformation and molecule 2 is in the closed (inverted) conformation.

Bond	Torsion angles		
	Mole- cule 1	Mole- cule 2	RNA
AC(3')(4')	94°	83°	83°
AC(4') - C(5')	52	57	74
AC(5') - O(5')	- 167	- 159	165
O(5')—P	- 88	84	- 78
P-0(3')	164	84	- 87
UO(3') - C(3')	- 138	- 159	- 134
UC(3') - C(4')	81	87	83
UC(4') - C(5')	54	49	74

SCIENCE, VOL. 174