References and Notes

1. Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.
2. S. Jensen, *Ambio* **1**, 123 (1972).
3. Dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[c,d]pentalene [see D. Shapley, *Science* **172**, 358 (1971)]. Mirex proved to be extremely toxic to crustaceans; toxic effects were observed at concentrations of 10^{-5} part per million in water [see C. G. Brookhout, A. J. Wilson, Jr., T. W. Duke, J. I. Lowe, *Water Air Soil Pollut.* **1**, 165 (1972); J. L. Ludke, M. T. Finley, C. Lusk, *Bull. Environ. Contam. Toxicol.* **6**, 89 (1971)]. Quite recently, mirex was also detected in seals from the Netherlands [see M. C. Ten Noever de Brauw and C. van Ingen, *Sci. Total Environ.* **2**, 196 (1973)].
4. The following states have used or are using mirex: Florida, North Carolina, South Carolina, Alabama, Georgia, Mississippi, Louisiana, Texas and Arkansas (*3*).
5. L. M. Reynolds, *Residue Rev.* **34**, 27 (1971).
6. A. S. Y. Chau and W. J. Wilkinson, personal communication; *Pesticide Analytical Manual* (Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C., 1971), vols. 1 and 2; L. D. Sawyer, *J. Assoc. Offic. Anal. Chem.* **56**, 1015 (1973).
7. The gas chromatographic determinations were made on a Tracor MT220 instrument with a flame ionization detector (for PCB analyses with electron capture detector), with stainless steel columns fitted for on-column injection under (i) isothermal conditions at 200°C and (ii) temperature-programmed from 110° to 260°C at 20°C per minute with each of the two columns. Column 1 was 1.8 m long and was packed with a mixed phase, 11 percent of a 1 : 1 mixture of OV 17 and QF-1, 80 to 100 mesh, Gas-Chrom Q; column 2 was 1.8 m long and was packed with 3 percent OV 1 on Chromosorb W, HP, 80 to 100 mesh.
8. E. J. Bonelli, *Anal. Chem.* **44**, 603 (1972); *Am. Lab. (Greens Farm, Conn.)* **3**, 27 (1971). The applied instrument was a Finnigan 1015 gas chromatograph–mass spectrometer with a Digital Corporation model PDP8E computer.
9. Additional settings were: ion source temperature, 120°C; electron energy, 70 ev.
10. "Polychlorinated biphenyls in the Great Lakes environment" (unpublished report of the Canada Centre for Inland Waters, Burlington, Ontario, February 1972).
11. R. M. Smith and R. West, *J. Org. Chem.* **35**, 2681 (1970); V. Mark and E. D. Weil, *ibid.* **36**, 676 (1971).
12. O. Johnson, *Chem. Week* **110**, 33 (1972); *ibid.* **111**, 17 (1972).
13. S. Uk, C. M. Himel, T. F. Dirks, *Bull. Environ. Contam. Toxicol.* **7**, 207 (1972).
14. For relative elution patterns of a variety of insecticides from Florisil, silica, and alumina columns, see L. M. Law and D. F. Goerlitz, *J. Assoc. Offic. Anal. Chem.* **53**, 1276 (1970).
15. D. Mackay and A. W. Wolkoff, *Environ. Sci. Technol.* **7**, 611 (1973).

5 March 1974; revised 10 April 1974

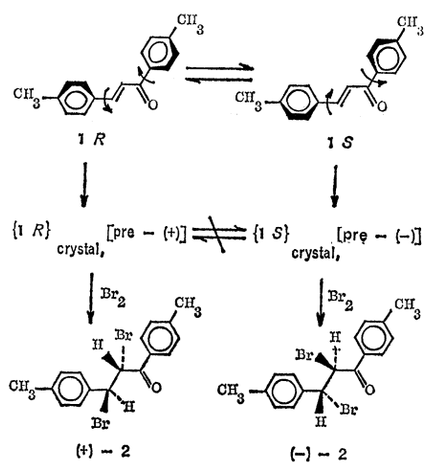
Mechanism for the Autocatalytic Formation of Optically Active Compounds under Abiotic Conditions

Abstract. *The bromination of chiral crystalline samples of 4,4′-dimethylchalcone was reinvestigated. In the presence of the optically active reaction product, (+)- or (−)-chalcone dibromide, crystallization from solutions of the achiral chalcone is specifically directed toward one-handedness. A feedback mechanism can thus be envisaged where optically active compounds are formed, generate additional material of the same chirality, and communicate this chirality to other regions, simply by cycles of solidification, reaction, and liquefaction.*

Crystals of 4,4′-dimethylchalcone, **1**, space group $P2_12_12_1$, belong to the class of chiral (enantiomorphic, dissymmetric, optically active) crystals. In solution or in the melt, rotation about single bonds causes rapid interconversion between the right- and left-handed conformations, *R* and *S*. In the crystal, however, the conformations are locked in and cannot interconvert; further, as a result of the chiral crystal structure, all of the molecules in any single crystal have the same chiral conformation (*1*). Penzien and Schmidt found that the addition of bromine to monocrystals of **1** afforded optically active dibromide, **2**, some crystals yielding (+)-**2** and some yielding (−)-**2** in excess (*2*).

Penzien and Schmidt also noted inoculation effects. As a result of the more rapid growth of the initially formed precursors of (+) or precursors of (−) nuclei of **1** and their subsequent nucleation effects, polycrystalline samples of **1** also afforded **2** with varying degrees of optical activity. The spontaneous generation of optical activity by crystallization, of which this is a striking example, has been reported in other, widely different chemical systems (*3*, *4*).

When solutions of **1** in ethyl acetate were slowly evaporated, the resulting polycrystalline samples yielded, on treatment with bromine vapor, optically active dibromide, **2** (*5*). Both dextrorotary (+) and levorotary (−) material were formed (Fig. 1a) and, although the data show bias in favor of (+) material, this is most likely a result of the relatively small number of samples (*6*).



When solutions containing **1** and 3.97 mole percent of optically active (+)-dibromide [(+)-**2**] were evaporated, polycrystalline samples of **1** (containing some **2**) again resulted. But bromination of these samples as above now gave only levorotary [(−)-**2**] material (Fig. 1b) (*7*). The dibromide initially added was 62 percent optically pure, so that the solution contained only 0.025 mole of excess pure (+)-enantiomer per mole of **1** [or 0.042 g of pure (+)-enantiomer per gram of **1**] prior to crystallization.

The same experiment was repeated with (−)-**2** to induce crystallization of **1**. Twelve solutions of **1** and (−)-**2** (3.97 mole percent) in ethyl acetate were evaporated, and the resulting solids were powdered and brominated (*8*). All afforded (+)-**2** (Fig. 1c). In this experiment the (−)-**2** used to induce chirality was only 17 percent optically pure so that only 0.0067 mole of excess pure enantiomer was present per mole of dimethylchalcone, **1**.

In order to show that the presence of racemic (±)-**2** cannot direct the chirality of the crystallization of **1**, we evaporated and brominated solutions of the chalcone containing 5.2 mole percent of (±)-**2**. As we expected, both (+)- and (−)-dibromide were produced during the gas-solid reaction (Fig. 1d).

Another example illustrating that the crystallization of compounds which appear in chiral crystal structures can be profoundly affected by the presence of chiral materials is provided by Pincock *et al.*, who have found that the crystallization of a racemic melt of 1,1′-binaphthyl is directed toward the formation of (−)-samples in the presence of *l*-mandelic acid, while *d*-mandelic acid induces the crystallization of (+)-material (*6*). The resolution of racemates by chiral solvents is a related phenomenon (*9*). Our results indicate that the influence of even small quantities of optically active material in directing the chirality of crystals may be general. Further, the possibility of a feedback mechanism leading to the autocatalytic formation of optical activity is raised.

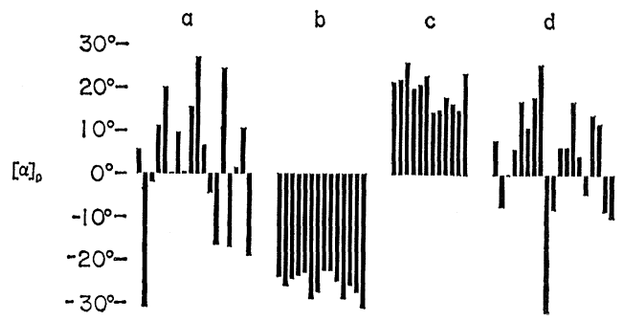


Fig. 1. Specific rotation of the dibromide, **2**, obtained by the bromination of 4,4′-dimethylchalcone, **1**, after evaporation of an ethyl acetate solution containing (a) no additive; (b) 3.97 mole percent (+)-**2**; (c) 3.97 mole percent (−)-**2**; and (d) 5.2 mole percent racemic (±)-**2**.

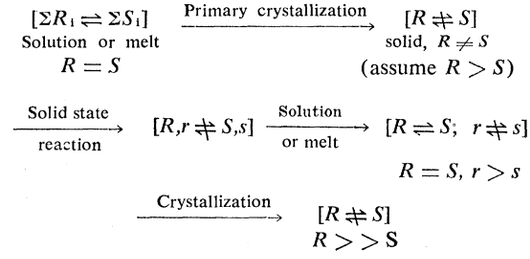


Fig. 2. Scheme illustrating one cycle of crystallization, solid state reaction, and liquefaction leading to the spontaneous generation of optically active product, *r*, in the presence of which the crystallization step of the second cycle is stereospecifically directed toward the formation of excess *R* crystals.

In the experiments we describe, the chiral reaction product **2** induces crystallization of **1**, which finally affords **2** of the opposite chirality. However, this situation occurs by chance; it was a priori equally possible for (+)-**2** to have induced crystallization of **1**, which would have led to more (+)-**2**. A mechanism is thereby suggested for absolute asymmetric synthesis which has several attractive features. The pathways are illustrated in Fig. 2.

When a solution or melt containing equal numbers of enantiomers, *R* and *S*, or right- and left-handed conformations, ΣR_i and ΣS_i, crystallizes, the number of *R* crystals and *S* crystals may not be equal (as is typically observed with 4,4′-dimethylchalcone). If a solid state reaction now takes place (the reaction is $R \rightarrow r$ and $S \rightarrow s$), to produce a chiral product, the product will be optically active, $r \neq s$. Although the first crystallization can produce *R* or *S* crystals with equal probability, subsequent crystallization is now predetermined to produce only *R* crystals (assuming *r*, produced in excess, induces crystallization of *R*). A small enantiomeric excess formed in the first crystallization can be enormously amplified by partial reaction followed by crystallization and further reaction; the results in Fig. 1c were obtained with about seven molecules of excess optically pure enantiomer for every 1000 molecules to be crystallized.

Two additional aspects of this scheme should be noted. First, once a chiral product is produced it can, by transfer to other regions, induce crystallization which will lead to additional material of the same chirality. Second, if *r* is being produced in excess in one region and *s* predominates in a different region, once these two regions come into contact with one another, that region with the larger quantity of pure enantiomer will "neutralize" the second region, forming a quantity of (*r,s*) racemic material, and now both regions will be under the influence of the same excess enantiomer and both will favor the future formation of this enantiomer alone.

The requirements for the scheme shown in Fig. 2 are a molecular species (*R,S*) which (i) is racemized in the liquid state, (ii) crystallizes in a chiral crystal structure, (iii) can undergo a solid state reaction to yield a chiral product, (iv) is not racemized under the conditions which racemize *R,S*; and (v) where the dissymmetric product formed induces the crystallization of the reactant in that chirality which gives rise to more product of the same handedness (*r* induces the crystallization of *R*).

One immediate conclusion of this work is the following: although the presence of optical activity in meteorites and other nonterrestrial material has been carefully sought, with the assumption that it would imply the involvement of living material, optically active compounds may clearly be produced under abiotic conditions.

Finally, although dissymmetric crystallization processes have often been considered as sources for the origin of optical activity in living systems, (*10*) they have generally been dismissed because the required conditions appeared too demanding (*4*): (i) crystallization is necessary; (ii) although crystallization in any given area may show predominance of one type of handedness, the overall result from crystallization in many areas will inevitably be racemic; and (iii) crystallization of substances in enantiomorphic crystal structures is very rare. Our data indicates that the second argument is not valid. The crystallization requirement is certainly not a demanding one: perhaps the most common geochemical phenomenon is melting and solidification, solution and crystallization. Typical of the possibilities would be cool evenings (or winters) when moisture condenses, thus dissolving the sample, followed by hot days (or summers) when the water evaporates leaving behind crystals that can now undergo thermal or photochemical solid state reactions. Last, crystallization in chiral space groups, especially of molecules that lack chiral centers and are thus most relevant for consideration, is a common phenomenon among inorganic and organic substances; perhaps 15 percent of the crystals investigated thus far are dissymmetric (*11*).

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References and Notes

1. The absolute configurations of the reactants and products are not known, and our assignments are only illustrative. The addition of chlorine or iodine monochloride vapor to single crystals of **1** also leads to optically active dihalides (B. S. Green and L. Heller, unpublished).
2. K. Penzien and G. M. J. Schmidt, *Angew. Chem. Int. Ed. Eng.* **8**, 608 (1969).
3. E. Havinga, *Chem. Weekbl.* **38**, 642 (1941); *Biochim. Biophys. Acta* **13**, 171 (1954); A. C. D. Newman and H. M. Powell, *J. Chem. Soc.* (1952), p. 3747; R. E. Pincock and K. R. Wilson, *J. Am. Chem. Soc.* **93**, 1291 (1971); A. Elgavi, B. S. Green, G. M. J. Schmidt, *ibid.* **95**, 2058 (1973). See also: H. Morawetz, *Science* **152**, 705 (1966); F. Wudl, D. A. Lightner, D. J. Cram, *J. Am. Chem. Soc.* **89**, 4099 (1967); D. Y. Curtin and A. R. Stein, *Can. J. Chem.* **47**, 3637 (1969), footnote 7; R. S. Miller, D. Y. Curtin, I. C. Paul, *J. Am. Chem. Soc.* **94**, 5117 (1972).
4. G. Wald, *Ann. N.Y. Acad. Sci.* **69**, 352 (1957), and references therein.
5. Solutions containing 200 mg of **1** in 6 ml of ethyl acetate were evaporated during a 1-week period. The resulting solids were powdered and exposed to bromine vapor in a desiccator; the reaction was monitored by nuclear magnetic resonance spectroscopy (increase in methine absorption relative to methyl resonance). When reactions were 50 to 80 percent complete the reaction mixtures were

dissolved in chloroform and passed through a short column of silica gel to remove colored impurities. The rotation of a weighed sample (80 mg) in chloroform (2 ml) was then measured (Perkin-Elmer model 141 polarimeter, 1-ml, 10-cm cuvettes) and the specific rotation $[\alpha]_D$ was calculated. For enantiomerically pure **2**, $[\alpha]_D = 167°$ (*2*).

6. R. E. Pincock, R. R. Perkins, A. S. Ma, K. R. Wilson, *Science* **174**, 1018 (1971).
7. Solutions containing 180 mg of **1** and 12 mg of **2** (having $[\alpha]_D = 103°$) in 6 ml of ethyl acetate were evaporated to dryness overnight. The samples were then powdered and exposed to bromine for 1 week, leading to about 55 percent reaction. The rotations of weighed samples (about 80 mg) were corrected for the rotation of the dibromide added initially. In a separate experiment, six methylene chloride solutions, each containing 90 mg of **1** and 8 mg of **2** ($[\alpha]_D = 137°$), were evaporated and then brominated (70 to 90 percent reaction). All gave levorotary material, mean $[\alpha]_D = 25.5°$.
8. The solutions contained 210 mg of **1** and 14 mg of **2** ($[\alpha]_D = -28.4°$) and were evaporated over a 1-day period. Bromination for 1 week led to 40 to 60 percent reaction. The rotations were corrected for the presence of (—)-**2** initially present. This experiment was repeated on ten samples, each containing 260 mg of **1** and 22 mg of **2** ($[\alpha]_D = -135°$) in 6 ml of ethyl acetate. All afforded, after treatment as above, dextrorotary dibromide, mean $[\alpha]_D = 26.2°$.
9. R. M. Secor, *Chem. Rev.* **63**, 297 (1963).
10. The scheme in Fig. 2 is an illustration of the stereospecific autocatalytic production of enantiomer excess as formulated by Calvin [M. Calvin, *Chemical Evolution* (Oxford Univ. Press, London, 1969), pp. 149–152]. See also: A. Dauvillier. The *Photochemical Origin of Life* (Academic Press, New York, 1965), pp. 107–115; F. F. Seelig, *J. Theor. Biol.* **34**, 197 (1972), and references therein; L. G. Harrison, *ibid.* **39**, 333 (1973).
11. See J. H. Stout and L. H. Jensen, *X-ray Structure Determination* (Macmillan, London, 1968), pp. 134–135.

4 February 1974

5-Hydroxyindoleacetic Acid in the Lumbar Fluid: A Specific Indicator of Spinal Cord Injury

Abstract. *In cats, 19 days after the lower thoracic cord was injured, the concentrations of 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid in the lumbosacral cord and that of 5-hydroxyindoleacetic acid in the lumbar fluid decreased. At the same time the concentrations of these substances in the cord above the lesion and that of 5-hydroxyindoleacetic acid in the cisternal fluid was not significantly altered. Since high concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid are present in the human lumbosacral cord, it appears that the concentration of 5-hydroxyindoleacetic acid in the lumbar fluid of animals and man reflects the biochemical changes of 5-hydroxytryptamine in the normal and injured spinal cord.*

The number of injuries of the spinal cord in man is increasing. Efforts are being made to advance the care and study of injuries to the spinal cord in humans (*1*).

In an effort to develop a method for studying the biochemical changes in the injured spinal cord in animals and potentially in man in vivo, we considered two relevant findings on animals. (i) After transection of the spinal cord, the degeneration of the descending serotonergic nerve fibers takes place in the cord below the lesion with simultaneous decrease of 5-hydroxytryptamine (5-HT, serotonin) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (*2*). (ii) The concentration of 5-HIAA in the spinal fluid of lumbosacral region (lumbar fluid) reflects the metabolism of 5-HT in the spinal cord (*3, 4*). Thus, after injury of the serotonergic fibers, a decrease of 5-HT and 5-HIAA in the spinal cord below the lesion followed by a similar decrease of 5-HIAA in the lumbar fluid might be expected. If so, this would open the possibility of studying the metabolism of 5-HT in the injured spinal cord by analysis of the lumbar fluid, which can be obtained by lumbar puncture in man in vivo. We now report that the concentration of 5-HIAA in the lumbar fluid reflects the changes of 5-HT metabolism in the injured spinal cord of cats.

Adult cats were anesthetized with thiopental sodium, and a partial laminectomy at T_{11} vertebrae was performed. The exposed spinal cord of each cat was squeezed epidurally with a fine dissecting forceps for 10 minutes. Control animals underwent laminectomy, but their spinal cords were left intact. The wound in each animal was sprinkled with xanthocillin and sewed up. The animals with injury of the cord developed clinical evidence of complete loss of function below the lesion (paraplegia, urine retention, and loss of sensitivity). After the operation the cats were treated daily with procaine penicillin (200,000 international units), and urine retention was relieved by manual expression. There was no evidence of blockage of communication between spinal fluid above and below the site of injury (*5*).

Nineteen days after the operation, the spinal cord (about 2 g) below the lesion (lumbosacral cord), part of thoracic cord (about 1 g) above the lesion, and samples of cisternal (about 1 ml) and lumbar (about 0.3 ml) fluid were taken for analysis in animals under thiopental anesthesia (*3, 4*); the samples were then frozen (— 20°C). Determinations of 5-HT (*6*), 5-HIAA (*6*), and norepinephrine (*7*) in the spinal cord and of 5-HIAA in the cerebrospinal fluid (*8*) were performed the next day. The concentration of 5-HT and 5-HIAA in the lumbosacral cord in humans was also measured.

Nineteen days after injury to the cat's spinal cord, there was a 75 percent decrease of 5-HT in the cord below the lesion (lumbosacral cord), while the concentration of 5-HT in the thoracic cord above the lesion was not changed. The concentration of 5-HIAA in the lumbosacral cord and in the lumbar fluid was lowered (by 80 and 68 percent, respectively; $P < .001$), while the concentration in the thoracic cord as well as in the cisternal fluid was not significantly altered ($P > .05$) (Fig. 1). Since the decrease of 5-HT and 5-HIAA in the injured lumbosacral cord was followed by a corresponding decrease of 5-HIAA in the lumbar fluid (Fig. 1), it appears that by measuring the concentration of 5-HIAA in the lumbar fluid we can obtain a better understanding of the metabolism of 5-HT in the injured spinal cord. Further, after blockage of the active transport of 5-HIAA from the spinal cord and spinal fluid by probenecid (*4, 9*), we found a greater increase of 5-HIAA in the lumbosacral cord and lumbar fluid in control as compared to injured animals. This indicates that the

Table 1. Concentration of 5-HT and 5-HIAA in the human lumbosacral cord.

Subject	Age	Sex	Cause of death	Time from death to autopsy (hours)	5-HT (ng/g)	5-HIAA (ng/g)
S.M.	78	Female	Auto accident	23	454	371
T.Z.	35	Female	Cerebral edema	17	557	491
P.L.	77	Male	Pulmonary embolism	10	454	673
D.Z.	27	Female	Auto accident	3	650	617
S.M.	84	Male	Pneumonia	9	563	799
				Mean ± S.E.M.	536 ± 37	590 ± 74