Studies on the Absolute Stereochemistry in Metabolism and Activity Development of Insecticides

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INTRODUCTION

Since the living organisms are essentially constituted of optically active materials like proteins and nucleic acids, biologically active substances such as pesticides and medicines, when developing activity in the organisms, are principally dependent upon the chirality of their molecules. Many of biologically active substances are optically active, and most of them exhibit different activities between their enantiomers.

Here is a miserable example which we have never forgotten being caused by a medicine, thalidomide (1), which had been practically used as the racemic mixture and resulted in causing serious malformation on human embryos. In 1979, Blaschke et al. succeeded in the optical resolution of the racemic mixture of thalidomide, and clarified that each enantiomer exhibited different biological activity. It was very surprising that the (S)-(−)-1, which has the same absolute stereochemistry as natural glutamic acid, induced teratogenic activity, whereas its antipodal (R)-(+)1 showed no toxicity.

Most of the past synthetic pesticides do not have an asymmetry in their molecules, because the basic structures to exhibit pesticidal activity consist of functional groups such as phosphate, carbamate, amide, etc.

The author here describes the stereochemical studies concerning metabolism of achiral fenitrothion (2) and propaphos (3) and biological activity of chiral chlordene (4).

ENANTIOTOPIC DEMETHYLATION OF ACHIRAL FENITROTHION INTO CHIRAL DESMETHYLFENITROTHION

Fenitrothion (2) is a pesticide practically used for protection of agricultural crops from a wide
variety of insect damage. Desmethylfenitrothion (5) was identified as one of the major degradation products of fenitrothion, when the pesticide was metabolized by mammals, insects and soil organisms. Desmethylfenitrothion was nontoxic to animals, and the metabolic conversion to this compound was considered to be one of the detoxifying enzymatic reactions mediated with glutathione-S-transferase.

1. Absolute Stereochemistry of Desmethylfenitrothion

The absolute stereochemistry of desmethylfenitrothion (5) was determined first by derivatizing the diastereomers, 6 and 7, from racemic 5 and (S)-(−)-α-phenethylamine, and then by comparative analysis of 1H-NMR spectra with the consideration of their most stable conformation. The determination was supported by 1H-NMR analysis of the isopropylamido and 1,1-diphenylmethylamido derivatives (8 and 9).2)

2. Enantiotopic Demethylation of Fenitrothion by Mice and Houseflies

A pair of diastereomers, 6 and 7, were used as reference compounds for chromatographic determination of an enantiomeric ratio of metabolic 5. Metabolic experiments of 2 with mouse liver homogenate as well as mice were conducted. Details in the metabolic experiments were described in the publication.3) The results show that an enantiotopic demethylation reaction clearly occurs in both in vitro and in vivo metabolism of fenitrothion, producing a larger amount of (R)P-(+)-desmethylfenitrothion with a lesser amount of (S)P-(−)-enantiomer: the enantiomeric ratios are 68:32 and 65:35, respectively (Fig. 1).

As a separate experiment in the metabolism of 2 with both fenitrothion-susceptible and resistant strains of houseflies, we obtained that an enantiotopic demethylation did not occur in both the species, i.e., the susceptible and resistant houseflies equally demethylated two methyl ester groups of fenitrothion without any enantiotopic discrimination.3)

3. Direct Analysis of an Enantiomeric Composition of Desmethylfenitrothion by Chiral HPLC

A free form of racemic desmethylfenitrothion has not yet been analyzed of its enantiomeric composition. Metabolic product of 2 with rat liver enzyme was analyzed with the chiral HPLC (Fig. 2), where an enantiomeric ratio gave 58% of (R)r-(+)-desmethylfenitrothion and 42% of (S)r-(−)-enantiomer. This datum was less enantiotopic compared with that obtained in the above experiment with mice.4)

These studies that we conducted using rat, mice and houseflies suggest that there are dif-
ferences among species of living organisms in an extent of enantiotopic demethylation reaction of fenitrothion to desmethylfenitrothion.

ENANTIOSELECTIVITY IN SULFOXIDATION
OF ACHIRAL PROPAPHOS INTO
PROPAPHOS SULFOXIDE WITH
PLANT AND INSECT

Propaphos (3) is used to control the rice leaf hopper and the rice leaf beetle. The compound 3 is known to be metabolized into propaphos sulfoxide (10) in rice plants and insects, but the stereochemistry of the product (10) has not been investigated.

1. Enantioselective Metabolism of Propaphos into Propaphos Sulfoxide with Rice Seedlings

Rice seeds treated with propaphos were cultured at 25°C for 2 weeks. From the rice seedlings, metabolic 10 was extracted with acetone and was purified from the solvent extract by column chromatography to afford a pure colorless oil. The positive optical rotation of the metabolite 10 ([α]D +27.2°) showed that asymmetric induction had taken place during oxidative bioconversion of achiral 3 into chiral 10.

In order to determine the enantiomeric composition, the isolated 10 was analyzed by chiral HPLC. Two peaks A and B appeared on the chromatogram (Fig. 3), and from their peak areas calculated, the enantiomeric composition of product 10 was concluded to have the ratio of 73:27.5.

The absolute stereochemistry of the metabolite 10 whose major component was the (+)-enantiomer was determined by circular dichroism (CD), compared with the reference compound of (R)S-(+)-methyl p-tolyl sulfoxide (11) to be (R)S configuration.
2. Enantioselective Sulfoxidation of Propaphos with German Cockroaches

Adult male German cockroaches were used for the metabolism of 3. The solvent extract of the insects were purified by preparative tlc to afford the metabolite 10, which was subjected to chiral HPLC under the same conditions as above. The chromatogram showed that the ratio of \((R)\) to \((S)\)-enantiomer was 56:44. This result showed a similar tendency to that obtained with rice seedlings, but the extent of enantioselectivity to produce an excess of \((R)\)-enantiomer was less than the case of rice. 7

**COMPARATIVE METABOLISM OF ENANTIOMERS OF CHLORDENE AND CHLORDENE EPOXIDE**

Synthetic cyclodiene insecticides, heptachlor (12) and chlordane (13), are chiral, but optical resolution of these cyclodiene compounds had not been succeeded before the author. 8, 9

1. Synthesis of Both Enantiomers of Chlordene and Chlordene Epoxide

Both enantiomers (optically pure) of chlordene epoxide (15) were synthesized via optical resolution of racemic 1-hydroxychlordene as shown in Fig. 4. 8 The absolute stereochemistry of optically active chlordene (4) and chlordene epoxide (15) was determined by CD of dechlorinated tricyclic ketone derivatives (16a and 16b) of \((-)\)- and \((+)\)-14, compared with the reference compounds (17a and 17b) 9 as shown in Fig. 4.

![Fig. 4 Synthetic scheme of the enantiomers of chlordene (4) and chlordene epoxide (15).](image)

Both optically pure enantiomers of 4 and 15 were thus tested against German cockroaches. \((+)\)-Chlordene and \((-)\)-chlordene epoxide were insecticidal, whereas both the antipodes were nontoxic. 8

2. Metabolic Experiments of the Enantiomers with German Cockroaches and Identification of the Metabolic Products

Adult male German cockroaches were topically applied with approximately two-third
dose of LD_{50} of toxic each of (+)-chlordene and (-)-chlordene epoxide. The metabolism with a pair of enantiomers of chlordene gave four kinds of metabolic products, and that of chlordene epoxide, three ones (Fig. 5). The most noticeable difference in metabolism between the enantiomers was observed on chlordene epoxide, whose production from toxic (+)-chlordene was about seven times larger than that of nontoxic (-)-enantiomer. The results show that (-)-chlordene epoxide itself is toxic, while (+)-chlordene is insecticidal after metabolic conversion into the corresponding (-) epoxide. The present study established firmly that the absolute stereochemistry played an important role in chiral pesticides to develop their biological activities.

CONCLUSION

The author has investigated the relationships between stereochemistry and biological activity in the course of metabolism of racemic and achiral insecticides. Based on these results, it is considered that stereochemical approaches in both the in vivo metabolism of pesticides and their binding with chiral insect receptor organs related with their insecticidal activity will be increasingly more important in future.

REFERENCES

1) G. Blaschke, H. P. Kraft & F. Koehler: Arzneim.-Forsch./Drug Res. 29 (II), 1640 (1979)