28. ショウジョウバエにおける DDT の代謝. Ⅰ. 新代謝物の同定 塚本増久 (大阪大学 理学部 生物学教室) 34. 7. 31 受理.

一般に昆虫では，DDE を除いては今迄化学的に確認された DDT の代謝物は全く知られていない. キイロショウジョウバエにおいては，DDT はエーテル可溶性でしかも Schechter-Haller 法で赤色を呈する物質に代謝されることがわかった. しかし，この物質は DDE ではなく，また DDE からさらに代謝されたものでもないので，種々の方法によりその同定が行われた. ペーパークロマトグラフ法や カラムクロマトグラフ法 における DDT 代謝物の行動は，しらべられた既知の DDT 誘導体のうち Kelthane のそれと全く一致した. さらに カラムクロマトグラフ法で分離された代謝物の紫外部における吸光曲線や Fujiwara 法による呈色反応の結果からも，ショウジョウバエにおいては DDT はそのエタノール型の誘導体 Kelthane に代謝されることが確認された. このような代謝経路は昆虫類や哺乳類などで今迄全く知られていないので，その代謝機構は生化学的にも極めて興味深いものと思われる.

When DDT-resistant strains of *Drosophila melanogaster* were reared on DDT-containing media, no DDE was found in the ether-extract of larvae, pupae, and adults, using a non-aqueous paper chromatographic system, whereas another metabolite with an *Rf*-value lower than that for DDT was detected. When *Drosophila* was reared on DDE-containing media, no metabolite was produced, indicating that DDE is not a precursor of this unknown metabolite. Of the several derivatives of DDT tested, only Kelthane had the same chromatographic behavior as this metabolite, using both an aqueous and a non-aqueous paper chromatographic systems. The elution character for the unknown metabolite coincided with that for Kelthane in column chromatography. The ultraviolet absorption curve for the metabolite was also identical with that for Kelthane. The chloroform formation test by the Fujiwara reaction was positive for the metabolite and Kelthane, whereas DDT, DDE, TDE, FW-152, etc. were negative. From these evidences it is concluded that in *Drosophila* DDT is metabolized to its hydrol type derivative, Kelthane, and not to DDE.

In general, the metabolism of insecticides to non-toxic substances by insects is considered as one of the major mechanisms of resistance to insecticides. Particular attention has been paid to the detoxification of DDT for the past several years, because of the spectacular debut of DDT followed by the troublesome failure of this compound in pest control due to the development of insect resistance to this synthetic insecticide.

The first report on the metabolism of DDT in insects was the study of the large milkweed bug, *Oncopeltus fasciatus*, by Ferguson & Kearns[6]. In this insect injected DDT was rapidly converted into unknown metabolites which did not give the Schechter-Haller color reaction. In the housefly, *Musca domestica*, it was clearly demonstrated that the DDT-resistant strains could detoxify DDT to DDE[19,30] and there was a good correlation between the level of resistance and the ability of flies to dehydrochlorinate DDT both *in vivo*[14,20,21] and *in vitro*[31,32], i. e., the more resistant the strain, the greater the DDE formation. This dehydrochlorination of DDT was also demonstrated with the radioactive bromine analogue of DDT, DBrDT, in the housefly[39]. The activity of DDT-dehydrochlorinase was inherited along with the inheritance of DDT-resistance[13].

The additional metabolism of DDT to unknown metabolite (s), which did not respond to the Schechter-Haller test was indicated by the poor recovery of the total administered dose of DDT, and this non-Schechter-Haller portion was named metabolite X by Tahori & Hoskins[33]. More recently a water-soluble metabolite has been found in the excreta of the housefly after treatment with radioactive DDT[10,22,34]. This metabolite also does not give a color reaction by the Schechter-Haller procedure and hence is not DDA. The portion unaccounted for by the Schechter-Haller method was initially considered to be due to incomplete extraction or incomplete nitration. Although there is no direct evidence, it is now believed that metabolite X or non-Schechter-Haller metabolite may be considered as the water-soluble metabolite.

The problem is more complicated in the case of cockroaches, i.e., the metabolism of DDT to DDE apparently takes place, but several kinds of unknown metabolites are also detected by paper chromatography. When the American cockroach, Periplaneta americana, was treated topically with C[14]-labelled DDT, 80% of the radioactivity in the feces was associated with the more polar compounds, IV and V[21]. Hoskins & Witt[10] also demonstrated that the American cockroach could convert C[14]-labelled DDT to three metabolites moving more rapidly than DDT in an aqueous paper chromatographic system, and that the Rf-value for one of these metabolites coincided with that for 4,4-dichlorobenzophenone (DBP), but it was not yet identified chemically with DBP. According to Lindquist & Dahm[15], the Madeira roach, Leucophaea maderae, also excreted DDT, DDE and three unknown metabolites, A, B, and C, in the feces when roaches were treated with radioactive DDT. The latter two metabolites appeared to be more polar than DDT.

All the insect species tested and naturally resistant to DDT could degrade DDT to DDE and some of the species degrade the material further to an unidentified metabolite[25].

It has been reported by Perry & Buckner[25] that the DDT-resistant strain of the human body louse,

Pediculus humanus humanus, can metabolize DDT to a non-toxic water-soluble compound which behaves as if it were p-chlorobenzoic acid in the modified Schechter-Haller procedure.

The results in mammals, on the other hand, are slightly different from those in insects. The principal metabolite of DDT in mammals is DDA[4,16,38]. The presence of DDE[18] and of an unknown metabolite[11], probably complexed form of DDA, has also been demonstrated.

In previous papers[17,35,36] it was reported that DDT-resistance in Drosophila melanogaster was mainly due to a dominant gene located on the second chromosome. The present series of experiments was initiated to clarify the relationship between the gene action of the dominant resistant factor and the metabolism of DDT. Preliminary tests on the metabolite of DDT in resistant strains of Drosophila showed a reddish color by the Schechter-Haller test, and this suggested the presence of a metabolite or metabolites of DDT. Thus the identification of the metabolite was attempted to determine whether DDE is produced by Drosophila.

Materials

The insects used in these experiments were the various insecticide-resistant strains of Drosophila melanogaster : Hikone-R, WMB, WMD, WMH, etc. Descriptions of the strains will be given in the second part of this report.

The insecticides and their related compounds used were as follows:

DDT : 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane. Furnished by the Asahi Glass Co.; mp. 107-108°C. Paper chromatographically pure.

DDE : 1,1-bis(p-chlorophenyl)-2,2-dichloroethylene. Furnished by the Institute for Chemical Research, Kyoto University; mp. 88-89°C. Paper chromatographically pure.

TDE : 1,1-bis (p-chlorophenyl)-2,2-dichloroethane. Furnished by Rohm & Haas Co., and recrystallized by the author; mp. 111-112°C. Paper chromatographically pure.

Kelthane : 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol. Furnished by Rohm & Haas Co.; mp. 75-76°C. Some less polar impurity was

found by paper chromatography.

FW-152 : 1,1-bis (*p*-chlorophenyl)-2,2-dichloro-ethanol. Furnished by Rohm & Haas Co.; mp. 108-109°C. Some impurity was detected by paper chromatography.

Bis (*p*-chlorophenyl) methane : Furnished by the Asahi Glass Co. and recrystallized by the author; mp. 54-55°C.

DBP : 4,4'-dichlorobenzophenone. Prepared from *p,p'*-DDT by alkali dehydrochlorination and CrO₃ oxidation. Another sample was furnished by J. R. Geigy S. A.; mp. 145-147°C.

D₤T : 1,1-bis (phenyl)-2,2,2-trichloroethane. Furnished by J. R. Geigy S. A.; mp. 67-68° C.

DOHDT : 1,1-bis(*p*-hydroxyphenyl)-2,2,2-trichloroethane. Furnished by J. R. Geigy S. A.; mp. 192-194°C.

DDA : bis(*p*-chlorophenyl) acetic acid. Furnished by J. R. Geigy S. A.; mp. 168-170°C.

PCBA : *p*-chlorobenzoic acid. Furnished by J. R. Geigy S. A.; mp. 238-240°C.

Experimental Methods and Results

PREPARATION OF EXTRACTED SAMPLES

Adults of *Drosophila melanogaster* were reared at 25°C in milk jars (180 ml) containing larval medium consisting of 1.5% agar, 5% brewer's yeast powder, and 5% sucrose in water (w/v). Sometimes the usual Koji medium enriched with yeast powder was used. The chemicals to be tested were added to the larval medium immediately after boiling the medium. The medium was then thoroughly mixed in a Waring blendor, and was poured into the rearing jar. Usually 100-2000 μg/ml of DDT were mixed into the medium. Eggs were laid by adult flies onto the surface of cooled medium, and the hatched larvae were able to grow, to pupate and finally to emerge. After sufficient ovipositions(usually 2 days) the adults were removed from the jar to prevent over-population of larvae, and after 5-8 days 3rd-instar larvae and/or pupae were carefully collected by washing the inside surface of the jar with water and a spoon to avoid physical damage of the insect body. Adult flies were also collected after emergence. The body surface of larval and pupal materials was rinsed repeatedly and successively with water, ethanol

and ethyl ether to remove external contaminations. Newly emerged adults were rinsed directly with ether.

The sample of insects was ground thoroughly with sufficient amounts of anhydrous sodium sulfate with mortar and pestle, and was extracted with several successive portions of ether. One gram samples were usually extracted with a total of 50-60 ml of ether, and finally the solvent was evaporated on a water bath. The extracts were then subjected to chromatography or color reaction, directly or after further purification.

The DDT-metabolite was extractable from larvae, pupae and adults with common organic solvents such as ethyl ether, ethanol, acetone, benzene, carbon tetrachloride, n-hexane, cyclohexane, etc. In this series of experiments only the ether-extractable fraction was investigated because the water-soluble fraction contained too many kinds of interfering silver-reactants in paper chromatography and was rather difficult to perform the Schechter-Haller procedure.

PAPER CHROMATOGRAPHY OF DDT AND RELATED COMPOUNDS

The procedures followed were essentially based on those described by Mitchell[15].

Non-aqueous solvent system. —— Immobile : 2-phenoxy ethanol, 8%, in ether(v/v) ; Mobile: 2,2,4-trimethyl pentane.

Aqueous solvent system. ——Immobile : refined soybean oil, 1%, in ethyl ether (v/v) ; Mobile: aqueous methanol.

Chromogenic agent. —— 1.7g AgNO₃ was dissolved in water, then 10 ml 2-phenoxy ethanol and 50 ml ethanol were successively added, and finally the solution was diluted with water to 200 ml.

The chromatographic paper employed was Toyo Filter Paper, No. 51. The extracted sample and standard compounds were dissolved in a small quantity of ether, and were spotted onto the paper. After spraying an immobile phase, the paper was developed with a mobile solvent by ascending method. The spraying of the immobile solution and the temperature appeared to be the most critical factors, and the speed of ascending or the *Rf*-value varied according

to these conditions. The time required for a 15 cm run was usually 2-3 hours at 20±2°C.. When the solvent front reached the 15 or 20 cm line, the paper was removed from the chamber, dried and sprayed with the chromogenic agent. Chromatograms were partially dried in the air, and then exposed to strong ultraviolet light. If the paper was still moist with chromogenic agent, the spot would be dull for detection.

When the ether-extracts of several resistant strains reared on DDT-containing media were tested by ascending chromatography, only a single silver reacting spot was significant as DDT-metabolite. This unknown metabolite ascended more slowly than DDT or DDE in the non-aqueous solvent system. If the body surface of insects to be tested was well rinsed with ether, only traces of DDT were detected in the internal extract. Neither DDE nor DDA was found on the chromatograms so far tested.

Furthermore, when *Drosophila* was reared on 2000μg/ml DDE-containing media, no metabolite was found on the chromatogram.

In order to compare the migration characteristics of the unknown metabolite with those of known compounds, several standard chemicals were tested with both the aqueous and non-aqueous systems. A definite Rf-value for these compounds could not be established because of varying temperature, but the order of ascending in non-aqueous system was always as follows : DDE>bis(p-chlorophenyl)methane≧DφT>DDT >DBP≒TDE>Kelthane>FW-152>p-chlorobenzoic acid≒DDA≒DOHDT. When the temperature stays below 20°C, the separation of these compounds is good. At the summer laboratory temperature, however, the former three compounds moved together with the solvent front, and hence they did not separate from each other. In the aqueous system, the order of ascending was inverted from that in the non-aqueous system.

Figures 1 and 2 present typical chromatograms of the *Drosophila* metabolite and some known compounds for comparison in non-aqueous and aqueous solvent systems, respectively.

Decrease of the number of chlorine atom at

the ethane position or addition of a polar group such as the hydroxyl radical give a lower Rf-value than that of the parental DDT in the non-aqueous solvent system. Of the several
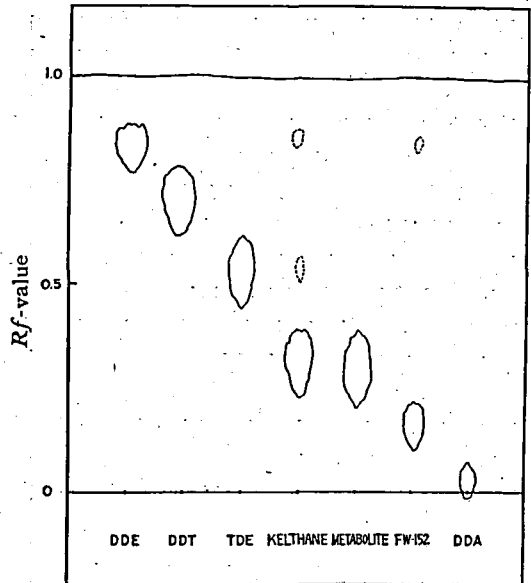

Fig. 1. A typical chromatogram tested with the non-aqueous system at 22°C. Dotted spots show some impurities contained in the standard.
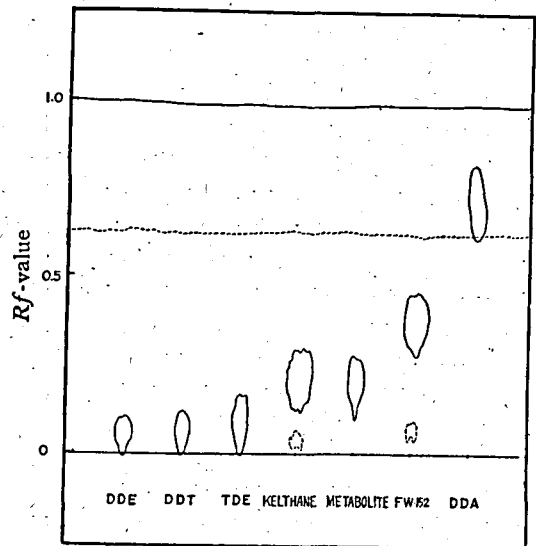

Fig. 2. Atypical chromatogram tested with the aqueous system at 23-24°C. Dotted spots show some impurities. A dark curtain exists between the dotted line and the solvent front.

derivatives of DDT tested, the location of the unknown metabolite exactly coincided with only that of Kelthane. DDA, *p*-chlorobenzoic acid and DOHDT were too polar.

The ether-extract contained some substances which interfered in the ascension of the metabolite and caused "tailing" especially in the aqueous solvent system. Column chromatography effectively removed some of such interfering substances.

COLUMN CHROMATOGRAPHY

1. Modified Davidow column The ether-extract of insect samples contains large amounts of fatty substances which interfere with analyses by paper chromatography, colorimetry and spectrophotometry. Fats can be removed by a celite column[5] in which a mixture of concentrated sulfuric acid and fuming sulfuric acid is employed as the stationary phase. This column, however, does not easily elute large quantities of DBP and DDA or other mammalian metabolite of DDT[4,18]. Jensen *et al.*[11] used an improved Davidow column in which the mixture of acids was replaced by concentrated sulfuric acid alone. The *Drosophila* metabolite appeared to be more polar than DDT, and thus the modified column was employed in this series of experiments. The *Drosophila* metabolite of DDT was not destroyed by concentrated sulfuric acid or by the mixture of acids.

When the Fujiwara color reaction[7] is attempted, carbon tetrachloride cannot be used as a solvent for chromatography prior to this color reaction because of its interference with the analysis. In such cases, the modified Davidow column was further modified by replacing carbon tetrachloride with *n*-hexane.

2. Activated alumina column The column described by Sternburg & Kearns[20] was effective for the separation of DDT and its metabolite. Freshly activated alumina (Aluminium Oxide Standard, E. Merck, Germany) was packed into a chromatographic tube of about 1 cm inner diameter to a depth of 5-6 cm. The procedures used were almost the same as in the original description.

DDE and bis (*p*-chlorophenyl) methane were found in the petroleum ether eluate. As described above, none of the *Drosophila* extract obtained from flies emerged from DDT-containing media has been found to contain DDE, and hence the use of petroleum ether was omitted in subsequent experiments. DDT, DDE and TDE were found in the carbon tetrachloride eluate, and the benzene eluate contained DBP. Kelthane, FW-152, insect fats, and 4,4'-dichlorobenzhydrol, if present, will be contained in the last eluate of ether. After the ether-extracts were separated by this column into four fractions, further analyses were carried out by paper chromatography and/or the Schechter-Haller method to detect the fraction into which the known and unknown compounds were eluted.

The unknown metabolite of DDT was found to be eluted into the ether fraction. When a commercial activated alumina (Wako Junyaku, Ltd., 200-300 mesh) was used, the *Drosophila* metabolite, Kelthane and FW-152 were eluted into the benzene fraction, even after fresh reactivation.

3. Ion-exchange resin column After removal of some impurity in the ether-extract by the modified Davidow column, further separation or purification of the metabolite was attempted using ion-exchange resin columns (Seki & Tsukamoto, unpublished). This procedure was based on the non-ionic adsorption of aromatic substances on ion-exchange resins. Seki[27] has used partially esterified carboxylic acid type resin for the separation of oestrogens. The resins employed in these experiments were Duolite CS-101 and Amberlite CG-50 (200-300 mesh), and the resin was esterified by refluxing a mixture of *iso*-propanol and 2N HCl (3:1 by volume) for more than 30 hours. The solvent system employed was the *iso*-propanol-water mixture (3:2 or 5:4 by volume). The esterified resin was washed with this solvent and the suspension was poured into a chromatographic tube (0.8 cm inner diameter, 120cm in height). Care must be taken that no small air bubbles remain in the column. Bubbles can be prevented by pouring the solvent mixture into the tube

prior to pouring in the suspension of resin. After settling of the column of resin in a tube (column size : 0.8×88cm), the sample to be examined was dissolved in one ml of solvent mixture, and was placed onto the resin column just before the solvent disappeared into the column. When the sample solution had been just absorbed by the column a large quantity of the solvent was poured into the tube, and the tube was placed on automatic fraction collector. About 0.9 ml of eluate was collected in a small test tube as one fraction. Each fraction was diluted with solvent to give 3.0 ml and its ultraviolet absorption was measured at appropriate wave lengths using a spectrophotometer.

If the tube is filled with the solvent, the column can be used repeatedly and the elution pattern of the DDT-derivatives is quite reproducible at the same temperatures. When a mixture of *iso*-propanol-water (3 : 2) was used, the metabolite and Kelthane were eluted into fraction numbers 40-50 at 18°C, 38-46 at 22°C, and 35-43 at 24°C. In the case of a 5:4 solvent mixture system, the metabolite and Kelthane were eluted into fraction numbers 50-60, and DDT did into 80-90 at 180°C. Additions of methanol to the solvent system did not improve the separation of DDT from DDE.

A typical elution pattern of the standard compounds and that of the ether-extract after purification with the celite column are shown in figure 3. These elution curves indicate that the unknown metabolite is Kelthane or a closely similar substance.

ULTRAVIOLET ABSORPTION CHARACTERISTICS

Attempts to determine the absorption spectrum in the ultraviolet regions of the *Drosophila* metabolite by direct cyclohexane extraction with the aid of concentrated sulfuric acid[32] were unsuccessful because of interfering substances.

The modified Davidow column followed by the alumina column could remove most of the interfering fatty substances and less polar impurities. Traces of carbon tetrachloride or benzene, used as the solvent in these chromatographic procedures, produce interfering absorption in the ultraviolet regions. After
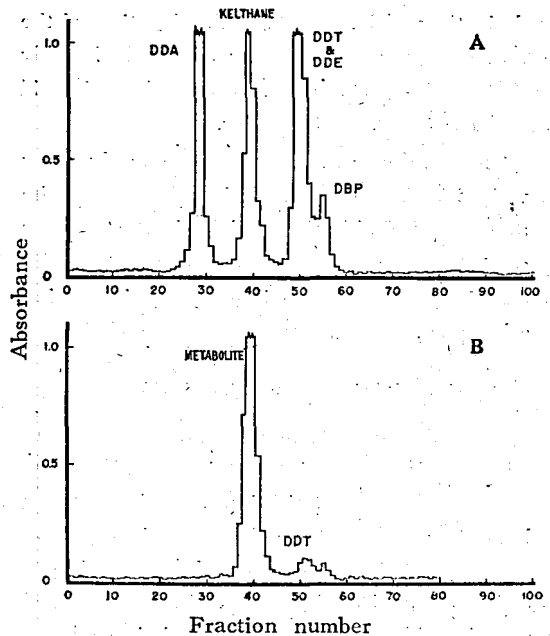

Fig. 3. Elution pattern of standard compounds and the metabolite of DDT, using Duolite CS-101 resin column and 3:2 solvent system at 24°C.

elution through the ion-exchange resin, no appreciable interference by carbon tetrachloride or benzene was demonstrated in fraction numbers 1-150. Figure 4 illustrates the ultraviolet absorption curves of known compounds and of the purified metabolite in aqueous *iso*-propanol.

The purified metabolite has an absorption peak at 231 mμ. DDE and DBP have maximum absorptions at 247 mμ and 264 mμ, respectively, and hence they can be eliminated as possible metabolite of DDT. TDE, Kelthane, FW-152 and DDA have absorption curves rather similar to one another with maximum absorption around the 230 mμ region, i.e., 231.5 mμ, 231 mμ, 230 mμ and 230 mμ, respectively. The absorption curve of the purified metabolite was exactly identical to that of Kelthane. Kelthane was the only compound assumed to be a likely metabolite which was not eliminated on the basis of the chromatographic behavior.

The metabolite was hydrolyzable under mildly alkaline conditions, and the hydrolysis product had an absorption curve with a maximum around 265-267 mμ. This is exactly similar to
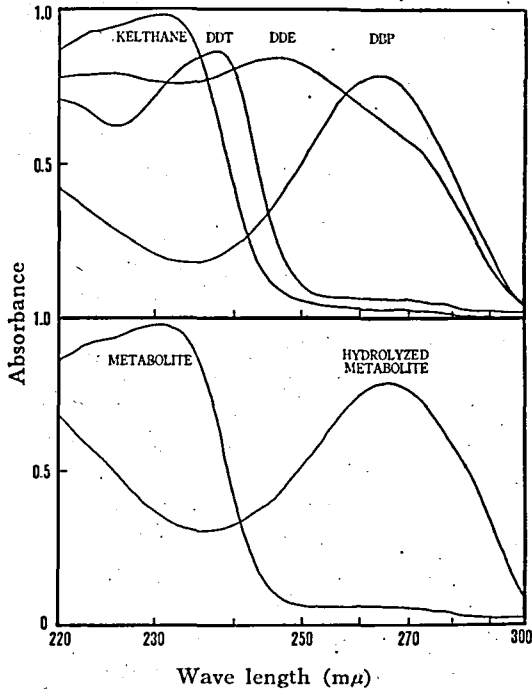
146

Fig. 4. Ultraviolet absorption curves of Kelthane, DDT, DDE, DBP and the *Drosophila* metabolite.

Fig. 5. Absorption spectra of the Schechter-Haller products of the metabolite, Kelthane, DDT and an extract of untreated flies.

the absorption curve of DBP which has been produced by the alkaline hydrolysis of Kelthane.

COLOR REACTION

1. The Schechter-Haller test Nitrated products of DDT and its related compounds give blue, purple, red or orange color reactions with sodium methylate[6]. This method was employed with slight modifications in the analysis of extracted samples of *Drosophila* at desired steps of the isolation or purification processes. Ninety minutes' severe nitration was employed on the boiling water. After color development with 2.0 ml benzene and 4.0 ml sodium methylate solution, absorption curves were recorded by a spectrophotometer. The mixture of benzene and sodium methylate solution (1:2 by volume) was used as a reference solution.

The *Drosophila* metabolite of DDT yielded red color products by this procedure. Figure 5 shows absorption curves of the nitrated metabolite, DDT, Kelthane, etc. Since most derivatives of DDT have the same or a closely similar
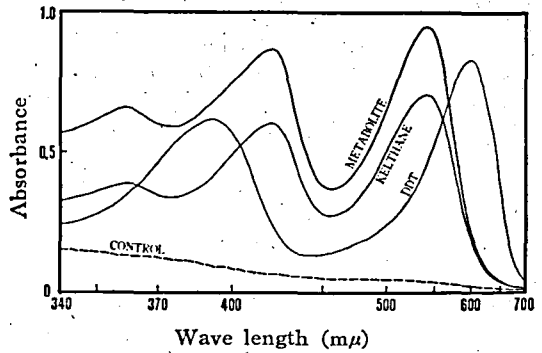
absorption curve as DDE when subjected to the Schechter-Haller test, it is not sufficient to conclude from this color reaction alone that a DDT-metabolite is DDE. When alumina column chromatography was employed prior to analyses, the petroleum ether eluate of the ether-extract did not form a red-colored complex. This indicates no DDE formation by this insect species. On the contrary, the Schechter-Haller positive metabolite behaved in a manner similar to Kelthane.

2. The Fujiwara test Small amounts of chloroform and carbon tetrachloride can be detected by the formation of red-colored complex with alkali and pyridine[7]. The principle of this Fujiwara reaction is also applicable to chloroform-producing pesticides such as Kelthane[2)] and Dipterex (O, O-dimethyl-2, 2, 2-tri-chloro-1-hydroxyethyl phosphonate)[1,8].

A test tube which contained the sample to be analysed was chilled by ice cold water. Exactly 8.0 ml of pyridine and 2.0 ml of 25% NaOH solution (w/w) were added into the tube. Then the chilled tube was transferred into the boiling water, and after 2 minutes of a vigorous shaking the color of the upper layer was recorded by a spectrophotometer. The fatty substances extractable with ether did not interfere with the formation of the red-colored dye but did cause a troublesome gelation and turbidity. Care must be taken to avoid any contamination with polychloride solvents such as chloroform or carbon tetrachloride. For this reason the

147

Davidow column cannot be applied prior to the Fujiwara reaction. Modification of the Davidow column was, therefore, accomplished by replacing carbon tetrachloride with n-hexane.
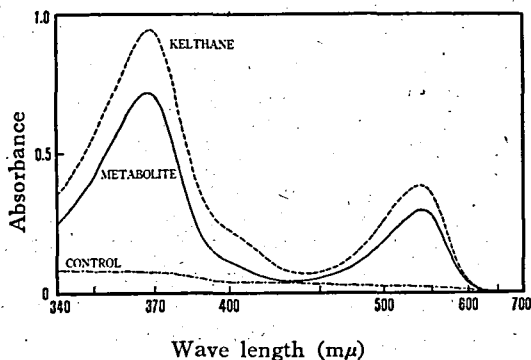

Fig. 6. Absorption characteristics of the Fujiwara reaction products of Kelthane, the *Drosophila* extracts from treated and untreated insects.

The ether-extract of *Drosophila* emerged from DDT-containing media or the purified metabolite gave a red color by the Fujiwara reaction, and its absorption curve was identical with that of Kelthane (Figure 6). Distilled water was used as a reference solution in spectrophotometry. DDT, DDE, TDE and FW-152 did not give such a reddish color reaction by this procedure.

Discussion and Conclusion

Hoskins & Witt[10] have recently classified the DDT-metabolism in various insect species into three types: 1) absorbed DDT remains largely unchanged, 2) much of it is metabolized to DDE, and 3) the chief metabolite(s) does not respond to the Schechter-Haller test.

The question remains whether all the metabolite which has been reported as DDE by the Schechter-Haller test alone is true DDE, because several DDT-derivatives other than DDE also yield reddish colors by this test method. Another type of the DDT-metabolism has been described by Perry & Buckner[23] as a result of studies with the human body louse. In this insect, the DDT-metabolite gives a red color by the modified Schechter-Haller procedure, but the metabolite is water-soluble and is not easily extractable with ordinary organic solvents.

The metabolism of DDT by *Drosophila* also does not fall among these four types, and hence is a new or fifth type of DDT-metabolism in insects.

When the ether-extractable fraction of DDT-treated *Drosophila* was developed by the non-aqueous paper chromatographic system, only one silver-reacting spot with a lower Rf-value than DDE, DDT or TDE was detected on the paper. This suggests that the non-DDE metabolite is more polar than DDT. As is well known, DDE is considered as a primary metabolite in many insects, and there is no identified metabolite of DDT other than DDE. It seemed rather reasonable to suppose that DDE was produced at first, and was then rapidly converted into the more polar substance. This assumption was, however, rejected as a result of the experiment in which DDE was fed to larvae of resistant strains of *Drosophila*. From these results of experiments it seems much more likely that the unknown metabolite is not produced via DDE but comes directly from DDT through a relatively simple biochemical step reaction.

DDT can be converted to TDE under the condition for polarography. In the non-aqueous system of paper chromatography, the Rf-value for TDE was lower than that for DDT but higher than that for the metabolite. FW-152, the hydrol type derivative of TDE, was also eliminated by its lower Rf-value than that for the metabolite. Only Kelthane, the hydrol type derivative of DDT, had exactly the same ascending behavior as the unknown metabolite. Kelthane is one of the effective acaricides, but is almost non-toxic to *Drosophila*. For example, Canton-S, one of the most susceptible strains to DDT, can emerge from a dose of 300 μg/ml of Kelthane, whereas it could not emerge from more than 50 μg/ml dose of DDT.

On the basis of these chromatographic properties alone, it is not permissible to conclude that the toxic DDT is metabolized to Kelthane by *Drosophila*. Consequently this attractive hypothesis was examined by the various techniques at present available. The results obtained from column chromatography and ultraviolet

spectrophotometry·supported the·idea of transformation of DDT to Kelthane.

More convincing is direct detection of a tertiary hydroxyl group. By means of the usually available techniques, the micro-detection of a tertiary alcohol is more difficult than detection of primary or secondary alcohols. Fortunately, the chloroform test by the Fujiwara reaction is applicable to Kelthane, because this acaricide is broken down by alkali into DBP and chloroform. FW-152, the dichloroethanol derivative, gave only a yellowish color in this reaction compared to the red color given by Kelthane, the trichloroethanol derivative of DDT. The fact that the *Drosophila* metabolite gave a positive Fujiwara reaction proved that the metabolite possessed a trichloroethanol moiety in its molecule. Ultraviolet absorption spectra also supported the presence of a DBP-like compound after alkali treatment of the purified metabolite. From these evidences it can be concluded that in *Drosophila melanogaster*, DDT is metabolized to its hydrol type derivative, Kelthane, and not to DDE.

The actual biochemical mechanism of the formation of Kelthane from DDT is still unknown. Chemically DDT can be converted to 1,1-bis(p-chlorophenyl)-1,2,2,2-tetrachloroethane and then to Kelthane. This intermediate is also practically non-toxic to insects. It seems, however, unlikely that such a chlorination of DDT occurs *in vivo*. Oxidation and hydroxylation of toxic substances are common mechanism for detoxication in animals, whereas oxidation in the α-position of alkyl hydrocarbons is less common. The question hence remains whether the hydrogen atom at the tertiary ethane carbon is replaced with a hydroxyl group or is directly oxidized to a hydrol. In any case such a reaction may be catalyzed by an enzyme system *in vivo*, for example, by a dehydrogenase or oxidase. DDT-dehydrochlorinase from the housefly is activated by glutathione *in vitro* but not by BAL, cysteine, methionine, etc.[31,32] The *Drosophila* homogenates, was not activated by glutathione, and no metabolite of DDT was produced *in vitro* under the condition suitable

for the action of the housefly DDT-dehydrochlorinase (Tsukamoto & Okubo, unpublished). It is, therefore, assumed that the DDT-detoxifying enzyme system, if one is present, of *Drosophila* differs far from that of the housefly. Kearns (personal communication) also did not find any DDT-dehydrochlorinase system in an American DDT-resistant strain of *Drosophila melanogaster*.

In DDT-resistant strains of *Aëdes* mosquitoes, attempts to demonstrate a DDT-dehydrochlorinase activity *in vitro* were unsuccessful under the condition suitable for the housefly homogenates, whereas DDE was produced *in vivo*[2,3].

Reports on DDE formation in insects other than the housefly should be reinvestigated unless the produced Schechter-Haller positive metabolite has been well identified as DDE by adequate procedures. The evidence of no DDE formation *in vitro* suggests a possibility that even the metabolic product *in vivo* might not be true DDE in these insects. From *Sarcophaga crassipalpis* and the American cockroach, an unknown metabolite of DDT that was eluted through the alumina column in a manner similar to 4,4'-dichlorobenzhydrol was isolated[29]. This unknown metabolite had an absorption peak at 540 mμ when subjected to the Schechter-Haller method, but the metabolite was found to be stable to treatment with alcoholic alkali[27]. Since the *Drosophila* metabolite of DDT is unstable to alkali, the roach metabolite seems to be not identical with the *Drosophila* metabolite. Hoskins & Witt[10] suspected from the paper chromatographic behavior that one of three polar metabolites of DDT was DBP. In an aqueous solvent system Kelthane has a higher Rf-value than DBP, and is easily degraded to DBP under alkaline condition. It will, therefore, not be surprising if Kelthane is shown to be produced as a metabolite of DDT not only in *Drosophila* but also in other insect species. Sometimes, the presence of trace of DBP was suggested in *Drosophila* sample by paper or colum chromatography. But it is not yet conclusive at present whether DBP is true metabolite or is due to

artificial degradation during the course of preparation of samples.

In the present paper only the qualitative data for identification of the metabolite have been reported. Quantitative and comparative studies on DDT-metabolism from the genetical viewpoint will be given in succeeding papers.

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Résumé

Malgré les nombreuses recherches sur le métabolisme du DDT dans les insectes, il n'y a que le DDE qui a été bien identifié chimiquement comme le métabolit du DDT.

Les résultats des études chromatographiques et spectrophotométriques sur un nouveau métabolit du DDT ont conduit à la conclusion suivante: chez *Drosophila melanogaster* le DDT est converti au le Kelthane ou 1,1-bis(*p*-chlorophényl)-2,2,2-trichloroéthanol, mais non au DDE.

Reference Cited

1) Arthur, B. W. and J. E. Casida : J. Agr. Food Chem. 5, 186 (1957).

2) Brown, A. W. A. : Bull. Wld. Hlth. Org. 14, 807 (1956).

3) Brown, A. W. A. and A. S. Perry : Nature 178, 368 (1956).

4) Burns, E. C., P. A. Dahm and D. A. Lindquist : J. Pharmac. Exp. Therap. 121, 55 (1957).

5) Davidow, B. J. : J. Assoc. Offic. Agr. Chem. 33, 130 (1950).

6) Ferguson, W. C. and C. W. Kearns : J. Econ. Ent. 42, 810 (1949).

7) Fujiwara, K. : Sitz. Nat. Ges. Rostock 6, 33 (1916).

8) Giang, P. A., W. F. Barthel and S. A. Hall : J. Agr. Food Chem. 2, 1281 (1954).

9) Gunther, F. A. and R. C. Blinn : J. Agr. Food Chem. 5, 517 (1957).

10) Hoskins, W. M. and J. M. Witt : Proc. Xth Intern. Congr. Ent. 2, 151 (1958).

11) Jensen, J. A. et al. : J. Agr. Food Chem. 5, 919 (1957).

12) Lindquist, D. A. and P. A. Dahm : J. Econ. Ent. 49, 579 (1956).

13) Lovell, J. B. and C. W. Kearns : Bull. Ent. Soc. Amer. 2, 17 (1956).

14) March, R. B. and R. L. Metcalf : Proc. Calif. Mosq. Cont. Assoc. Conf. 18, 17 (1950).

15) Mitchell, L. C. : J. Assoc. Offic. Agr. Chem. 40, 294 (1957).

16) Neal, P. A. et al. : Public Hlth. Rept. 61, 403 (1946).

17) Ogaki, M. and M. Tsukamoto : Botyu-Kagaku 18, 100 (1953).

18) Pearce, G. W., A. M. Mattson and W. J. Hayes, Jr. : Science 116, 254 (1952).

19) Perry, A. S. and W. M. Hoskins : Science 111, 600 (1950).

20) Perry, A. S. and W. M. Hoskins : J. Econ. Ent. 44, 850 (1951).

21) Perry, A. S., R. W. Fay and A. J. Buckner : J. Econ. Ent. 46, 972 (1953).

22) Perry, A. S., J. A. Jensen and G. W. Pearce : J. Agr. Food Chem. 3, 1008 (1955).

23) Perry, A. S. and A. J. Buckner : Amer. J. Trop. Med. Hyg. 7, 620 (1958).

24) Robbins, W. E. and P. A. Dahm : J. Agr. Food Chem. 3, 500 (1955).

25) Rosenthal, I., G. J. Frisone and F. A. Gunther : J. Agr. Food Chem. 5, 514 (1957).

26) Schechter, M. S. et al. : J. Ind. Eng.

Chem., Anal. Ed. **17**, 704 (1945).

27) Seki, T. : Nature **181**, 768 (1958).

28) Sternburg, J. and C. W. Kearns : J. Econ. Ent. **45**, 497 (1952a).

29) Sternburg, J. and C. W. Kearns : J. Econ. Ent. **45**, 505 (1952b).

30) Sternburg, J., C. W. Kearns and W. N. Bruce : J. Econ. Ent. **43**, 214 (1950).

31) Sternburg, J., C. W. Kearns and H. Moorefield : J. Agr. Food Chem. **2**, 1125 (1954).

32) Sternburg, J., E. B. Vinson and C. W. Kearns : J. Econ. Ent. **46**, 513 (1953).

33) Tahori, A. S. and W. M. Hoskins : J. Econ.

Ent. **46**, 302 (1953).

34) Terriere, L. C. and R. D. Schonbrod : J. Econ. Ent. **48**, 736 (1955).

35) Tsukamoto, M. and M. Ogaki : Botyu-Kagaku **18**, 39 (1953).

36) Tsukamoto, M. and M. Ogaki : Botyu-Kagaku **19**, 25 (1954).

37) Vinson, E. B. and C. W. Kearns : J. Econ. Ent. **45**, 484 (1952).

38) White, W. C. and T. R. Sweeney : Public Hlth. Rept. **60**, 66 (1945).

39) Winteringham, F. P. W., P. M. Loveday and A. Harrison : Nature **167**, 106 (1951).

Darstellung von n-Hexin-1-olen und n-Hexen-1-olen. Akikazu Hatanaka, Masayuki Hamada und Minoru Ohno (Institut für Chemische Forschung, Universität Kyoto) Eingegangen am 30. Juli 1959. *Botyu-kagaku* **24**, 151, 1959, (mit deutscher Zusammenfassung 155).
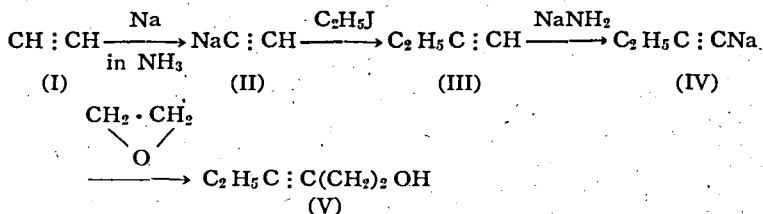
29. n-Hexin-1-ol および n-Hexen-1-ol 類の合成 * 畑中顕和・浜田昌之・大野 稔 (京都大学 化学研究所 大野研究室) 34. 7. 30 受理

未知物質をふくむ *n*-Hexin-1-ol の位置異性体4種と *n*-Hexen-1-ol の位置および幾何異性体7 種全部を純粋に合成した。まず *n*-Hexin-1-ol 類すなわち 2-,3-,4- および 5-Hexin-1-ol は、 すべて Acetylen を出発物質として液安中での反応を経て好収量で合成した。ついで *n*-Hexen- 1-ol 類のうち 2-,3-,4-*trans*- および 5-Hexen-1-ol は、相当する Hexinol 類を液安中で Na 還元により、又 2-,3- および 4-*cis*-Hexen-1-ol は Palladium-Bariumsulfat を触媒として半水 添してえた。これら Alkohol 類はすべて 3,5-Dinitrobenzoat を経て精製した。最後にこれら *n*-Hexin-1-ol および *n*-Hexen-1-ol 類の赤外吸収スペクトルについて検討した。

青葉アルコールすなわち 3-*cis*-Hexen-1-ol は生葉特有のにおいの主成分であり、遊離或いはエステルの形で広く植物青葉中に含まれている。このものは童葉アルデヒド[2]、胡瓜アルコール[3] および Jasmon[1] のような興味ある香気成分の構成分であり、古くから植物生理学的、香料化学的見地から、更には二重結合の幾何異性の検討を目的として研究されてきた。最近になって *n*-Hexen-1-ol 類の昆虫誘引性について 2, 3 の研究が発表された[4,5,6]。このように *n*-Hexen-1-ol 類は興味ある物質であるが、その二重結合の幾何および位置異性にもとずく7つの異性体のうち、未知のも

のもあり、又既に合成されているものゝうちにも幾何異性体として不純なものも多いので、著者らはこの点に留意しながら7つの異性体をそれぞれ相当する4つの *n*-Hexin-1-ol の部分水添によって全部純粋に合成した。

まず 3-Hexin-1-ol(V) は1938年以来 M. Stoll[7]、 S. Takei[8] および L. Crombie[9] らによって複雑な経路を経て合成されていたが、1950年 F. Sondheimer[10] が Acetylen を出発物質とするつぎのような方法に改めたが、著者らはこの方法を更に改良してより高い収率でこのものを合成した。

$$CH \vdots CH \xrightarrow[\text{in } NH_3]{Na} NaC \vdots CH \xrightarrow{C_2H_5J} C_2H_5C \vdots CH \xrightarrow{NaNH_2} C_2H_5C \vdots CNa$$

$$\text{(I)} \qquad\qquad \text{(II)} \qquad\qquad \text{(III)} \qquad\qquad \text{(IV)}$$

$$\overset{\displaystyle CH_2 \cdot CH_2}{\underset{\displaystyle O}{\diagdown \diagup}}$$

$$\xrightarrow{\hspace{2cm}} C_2H_5C \vdots C(CH_2)_2\, OH$$

$$\text{(V)}$$

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