

THE TAKEI LABORATORY

Head : Prof. Dr. Sankichi Takei

The Takei Laboratory is under the direction of Prof. S. Takei who succeeded Prof. B. Suzuki in April, 1937, and has two rooms as the result of the extension of the institute building in September, 1937. The chemistry of derris root was studied by Miyajima *et al.* and in December, 1937 studies on pyrethrum flowers were taken up by Wakazono *et al.* A laboratory for the research of bioassay in the summer of 1943 and three experimental rooms were installed in 1948. After the War, Ohno, Takano, Nagasawa, Hamada, Inouye, Oiwa, *et al.* have been engaged in fundamental and applied researches for insecticides such as DDT, BHC, and synthetic pyrethrins together with previous researches.

1. Studies in the Quantitative Determination of the Active Principles of Derris Roots. II~X

- (1) **Deterioration of Rotenone and Degueline in Liquids and Effects by Some Additive Substances.**

By S. Takei and Y. Tada.

Bull. Inst. Chem. Res., Kyoto Univ., 11, 173 (1940).

- (2) **Comparison of Some Analytical Method with a New Method.**

By S. Takei and S. Miyajima.

Cyo-Kontyū, 3, No. 1, 1 (1941).

- (3) **Deterioration of Derris Powder and Some of its Preparations.**

By S. Takei and S. Miyajima.

Bull. Inst. Chem. Res., Kyoto Univ., 12, 101 (1941).

- (4) **Deterioration of Water Extracted Emulsions and Check of it.**

By S. Takei and S. Miyajima.

Ibid., 13, 49 (1944).

- (5) **Influence of Some Metallic Ions upon Water Extracted Emulsions.**

By S. Miyajima and S. Takei.

Ibid., 14, 51 (1947).

- (6) **Deterioration of Water Extracted Emulsion of Fresh Derris Roots.**

By S. Miyajima and S. Takei.

Ibid., 14, 55 (1947).

- (7) **Influence of the Sunlight on Water Extracted Emulsions.**

By S. Miyajima and S. Takei.

Ibid., 14, 59 (1947).

- (8) **Effects of Some Antiseptics on Water Extracted Emulsions.**
 By S. Miyajima and S. Takei.
Ibid., 14, 63 (1947).
- (9) **Deterioration of Water Extracted Emulsions by the Sunlight.**
 By S. Miyajima and S. Takei.
Ibid., 14, 66 (1947).

II. Studies on the Quantitative Determination of Pyrethrins. VIII~XIII

- (1) **Volumetric Method of Pyrethrin Determination by Use of Pure Mono- and Dicarboxylic Acids.**
 By S. Takei and K. Wakazono.
 J. Agr. Chem. Soc. Jap., 16, 399 (1940).
- (2) **Deterioration of Pyrethrins by the Sunlight and Temperature.**
 By S. Takei, K. Wakazono and K. Hiraoka.
Ibid., 17, 419 (1941).
- (3) **Studies on the Pyrethrin Content and Effectiveness of Mosquito-Spirals.**
 By S. Takei, K. Wakazono and K. Hiraoka.
Ibid., 17, 1107 (1941).
- (4) **Pyrethrin Contents in the Smokes of Mosquito-Spirals.**
 By S. Takei, K. Wakazono and K. Hiraoka.
Ibid., 18, 229 (1942).
- (5) **A New Volumetric Method by Means of Benzene Extraction.**
 By K. Wakazono, K. Hiraoka and S. Takei.
Ibid., 187, 66 (1942).
- (6) **Deterioration of Benzene Extracts.**
 By K. Wakazono and S. Takei.
Ibid., 20, 115 (1945);
 Bull. Inst. Chem. Res., Kyoto Univ., 14, 70 (1948).

III. On the Mosquito Incense Made from Pyrethrum and Mixed with Benzophenone, etc

- (1) **Pyrethrum Mixed with Benzophenone.**
 By T. Takano, M. Ueda, I. Murasawa and M. Ohno.
 Botyu-Kagaku, 7~9, 11 (1947).
- (2) **Pyrethrum Mixed with Benzophenone.**
 By T. Takano, M. Ueda, I. Murasawa and M. Ohno.
Ibid., 10, 12 (1948).

(3) **Pyrethrum Mixed with BHC.**

By T. Takano, I. Murasawa and M. Ohno.

Ibid., 11, 15 (1949).**IV. Studies on Synthetic Pyrethrins**(1) **Synthesis of Chrysanthemum Monocarboxylic Acid.**

By Y. Inouye, Y. Katsuda, A. Nishimura, K. Kitagawa and M. Ohno.

Botyu-Kagaku, 16, 111 (1951).

The authors have synthesized chrysanthemum monocarboxylic acid, the acidic component of synthetic pyrethrins, by the addition of ethyl diazoacetate to 2,5-dimethyl-2,4-hexadiene which was obtained quantitatively by the catalytic isomerisation of 2,5-dimethyl-1,5-hexadiene over activated alumina at 200-230°, or Al_2O_3 -Cr catalyst at 250-270°. 2,5-Dimethyl-1,5-hexadiene was synthesized pure from methallyl chloride with magnesium in dry ether medium. The yield of 2,5-dimethyl-1,5-hexadiene was much raised by prolonged digestion of the reaction mixture on steambath with vigorous stirring. The procedures and yields at every stage were much improved by the authors. Another attempt to obtain 2,5-dimethyl-2,4-hexadiene directly by Wurtz condensation of isocrotyl chloride or bromide with molecular sodium (or activated magnesium) was doomed to failure because of the reversion of the halides to isobutene and low yield of the 2,5-dimethyl-2,4-hexadiene.

(2) **Synthesis of Cinerolone-Homologues.**

By Y. Katsuda, Y. Inouye, A. Nishimura, K. Kitagawa and M. Ohno.

Botyu-Kagaku, 16, 116 (1951).

The authors have synthesized a few kinds of cinerolone-homologues, and found that their chrysanthemates have an excellent insecticidal activities against the common house-fly, *Musca domestica vicina* Maq.

Every synthetic stage has been much simplified and the yields have been much improved. For instance, we have obtained better results in carbethoxylation of ketones by using the sodium methoxide under reduced pressure as the condensing agent than sodium hydride. The isolation of the α -substituted ethyl aceto-acetate in pure condition should be avoided to raise the yield. Substituted acetones and esters of 3-oxo-alkenoic acids were, however, purified by distillation. It was found advantageous not to distill hydroxydiketones before cyclization. These compounds are very subject to oxidation, especially in the presence of alkali, and should be protected during cyclization by excluding contact with air as much as possible.

(3) **Synthetic Pyrethroids and Toxicity.**

By Y. Inouye, Y. Katsuda, K. Kitagawa, A. Nishimura and M. Ohno.

Botyu-Kagaku, 16, in press.

The authors have synthesized the lower alkyl- and alkenyl-pyrethrins from the corresponding pyrethrolones and synthetic chrysanthemoyl chloride in the presence of dry pyridine (or quinoline, dimethylaniline) in dry benzene. Fifty kinds of

pyrethroids were synthesized from aromatic, terpenic, aliphatic, dialkylaminoethyl alcohols, monoalkyl-ethylene glycols and synthetic chrysanthemoyl chloride in an analogous manner.

The toxicity of these compounds was tested against the common house fly, mosquito pupa in kerosene space spray, emulsion and mosquito coil fume. Relative effectiveness was checked statistically by the Bliss' probit transformation method.

V. Studies on the Correlation between the Chemical Constitution and Insecticidal Activity of Halogenated Aromatic Compounds

Recently many synthetic insecticides such as DDT and BHC have been discovered and put to practical use. In order to find, therefore, some fundamental relations between the chemical constitution and insecticidal activity which may indicate the direction of pursuing more powerful insecticides, a series of research was undertaken. In the research many halogenated compounds related to DDT, BHC and benzophenone, were synthesized, and their effectiveness on several species of insects was tested, and then the relations between the chemical constitution and insecticidal activity were studied.

(1) Studies on DDT and its Related Compounds. I.

By M. Hamada, T. Sasakawa and M. Ohno.
Botyu-Kagaku, 10, 9 (1948).

Eleven compounds were synthesized and tested on *Stephanitis nashi* E., *Armadillidium vulgare* L., *Calandra oryzae* L., and *Anthrenus verbaci* L. From the results obtained, the following conclusion has been obtained: As the number of Cl atoms of trichloromethyl radical of DDT are reduced one by one, the effectiveness decreases gradually. When Cl atoms of phenyl nucleus of DDT are substituted for Br, its effectiveness become selective, but if substituted for I or H, it is lost. When H atom combined to the central C atom is substituted for Cl or removed by dehydrochlorination, the effectiveness disappears.

(2) Studies on BHC and its Related Compounds. I.

By M. Hamada, T. Sasakawa and M. Ohno.
Botyu-Kagaku, 10, 17 (1948).

Ten compounds (isomers of $C_6H_6Cl_6$; crude mixtures of $C_6H_5Cl_7$ and of $C_6H_4Cl_8$; benzophenone hexachloride) were synthesized or separated, and tested on above-mentioned four species of insects. In these compounds only γ -isomer of BHC is far more effective than the other compounds, and it is about five times more toxic than DDT. Furthermore, γ -BHC was effective as a fumigant or repellent.

(3) Studies on diphenylmethan Series.

By M. Hamada, T. Sasakawa and M. Ohno.
Botyu-Kagaku, 10, 25 (1948).

Ten compounds (chlorine derivatives of benzophenone, diphenylmethane, and

diphenylethane) were synthesized and tested on above-mentioned four species of insects. But no definite conclusion was obtained from these experiments.

(4) Studies on DDT Related Compounds. II.

By **M. Hamada** and **M. Ohno**.

Botyu-Kagaku, **13**, 19 (1949).

Ten compounds related to DDT were synthesized and tested on the adult housefly (knockdown and kill). From the results obtained, the insecticidal activity depends so much upon the Cl atoms of para position of phenyl nucleus. Knock-down power of both p, p'-dimethoxy and -diethoxy analogues is stronger than that of p, p'-DDT. Bis-(p-chlorophenyl)-acetic acid, and its ester are almost ineffective.

(5) Studies on BHC Related compounds. II.

By **M. Hamada**, **T. Oiwa** and **M. Ohno**.

Botyu-Kagaku, **15**, 89 (1950).

Chlorinated products of γ -BHC and two isomers of $C_6H_5Cl_7$ and two isomers of $C_6H_4Cl_8$ were tested on the adult housefly. These compounds were much less effective than γ -BHC. So in technical preparation it is desirable to remove γ -BHC from the reaction system as soon as possible to avoid further chlorination.

Futhermore, halogenated derivatives of diphenylcyclopropane and diphenylpyrazoline were synthesized (Bull. Inst. Chem. Res., Kyoto Univ., **24**, 81 (1951)). The results of insecticidal tests will be reported later.

VI. Studies on the Determination of DDT and BHC

(1) Determination of p, p'-DDT by Dehydrochlorination.

By **T. Takano** and **M. Hamada**.

Botyu-Kagaku, **14**, 26 (1949).

A method is described for the determination of p, p'-DDT (most effective component of DDT) by the dehydrochlorination reaction of technical DDT. A sample (0.1 g) was dehydrochlorinated in 20 cc N/10 methanolic KOH solution at 40° for 40 mins. Then percentage of dehydrochlorination (y) was calculated from the following equation, $y = 35.46 (5.00 - n)$; where n is cc of thiocyanate solution consumed in Volhard titration. The relations between the concentration of p, p'-DDT (x) and the percentage of dehydrochlorination (y) in the above-mention reaction condition is as follows: $y = 0.733x + 26.7$.

So the p, p'-DDT content in technical DDT is calculable from the above-mentioned two equations. In case of DDT powder and emulsifiable, this procedure is applicable after ether extraction.

(2) Determination of p, p'-DDT in DDT Spray.

By **M. Hamada**, **T. Takano** and **M. Ohno**.

Botyu-Kagaku, **16**, 45 (1951).

In case of DDT spray, total DDT was extracted by nitromethane in 6-8 hours at 140-145° (bath temperature), using a successive extracting apparatus. Then,

nitromethane was removed and the residue was filled up to a certain definite amount by methanol, and was pipetted out a certain amount equivalent to 0.1g of technical DDT. Then, p, p'-DDT content was determined by the method mentioned above.

(3) Determination of γ -BHC in BHC Spray.

By M. Hamada and T. Oiwa.

Unpub.

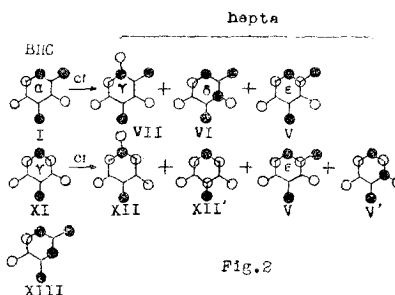
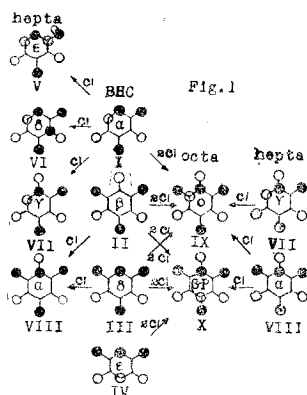
In case of BHC spray it was difficult to determine the γ -BHC content directly by the polarographic method. So the partition Chromatographic method (supporting medium; silicic acid, mobile solvent: petroleum ether saturated with nitromethane, immobile solvent: nitromethane) was used to separate off petroleum, and the separated crystalline parts were collected, and then γ -BHC was determined by the ordinary polarographic method.

VII. Studies on the Molecular Structures of BHC and its Related Compounds

The authors^{1,2,3,4)} have been investigating organic chemically the structures of isomers of BHC (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) and its related compounds such as 1, 1, 2, 3, 4, 5, 6-heptachlorocyclohexane (hepta) and 1, 1, 2, 3, 4, 4, 5, 6- and 1, 1, 2, 2, 3, 4, 5, 6-octachlorocyclohexane (octa). As a result they discovered ϵ -hepta (mp. 55~55.5°), and determined the structures of α -BHC (mp. 157~158°), γ -BHC (mp. 112~113°), δ -BHC (mp. 138~139°), ϵ -BHC (mp. 218.5~219°), α -hepta (mp. 153~154°), γ -hepta (mp. 85~86°), δ -hepta (mp. 139~140°), ϵ -hepta, β -p-octa (mp. 265°), and o-octa (mp. 149~150°).

Studies in physical chemistry have recently proved that the actual molecule of cyclohexane, almost without exception, have a chair-form. As a result, difference of isomers of BHC, hepta and octa which are chlorine substitution products of the cyclohexane may be considered to consist in the difference of situations of chlorine and hydrogen atoms in the chair-form cyclohexane ring. Accordingly, thirteen, twenty, and twenty nine geometric isomers can be expected theoretically in BHC, hepta, and octa respectively, but actual number becomes much less on account of the repulsions among the atoms. The number of isolated so far is five, five, and four, respectively.

The authors chlorinated the isomers of BHC and hepta, and the products were separated by partition chromatography with help of polarographic and partly of infrared analyses. The results of the reactions are shown in Fig. 1 and 2. In these figures the cyclohexane ring is represented by a hexagonal ring, chlorine atom in an equatorial bond by a circle attached to a short line, chlorine atom in a polar bond by a circle on the hexagonal ring, and black and white circles represent chlorine atoms above and under the central plane.



The molecular structure of β -BHC has already been determined by X-ray analysis. Consequently, the structures of α -hepta and o- and β -p-octa are determined from the reactions as VIII, IX, and X, respectively. Among the theoretical isomers of BHC, I and III are the only structures which can produce o-octa besides II. Experimentally, besides β -BHC, α - and δ -BHC could produce o-octa. Therefore, either I or III must be α -BHC, and the rest δ -BHC. Of these two structures, III can produce α -hepta, and I cannot. Experimentally, α -hepta was produced from δ -BHC. The conclusion is, therefore, that III is the molecular configuration of δ -BHC, and I of α -BHC. Among the theoretical isomers of BHC, the structures which can produce β -p-octa are II, III, and IV, but two of these are β -BHC and δ -BHC. Experimentally, from ϵ -BHC, β -p-octa was also produced. As a result IV must be the structure of ϵ -BHC. Among the theoretical isomers of hepta, the structures which can produce o-octa of IX are VII and VIII, but VIII is the structure of α -hepta. As o-octa was also produced from γ -hepta, experimentally, VII must be the structure of γ -hepta.

The possible isomers of hepta, which can be derived from α -BHC of I are VII, VI, and V. Since VII is γ -hepta, one of the two forms left is of δ -hepta, and the other of ϵ -hepta. Now, taking into account the fact that the forms VI and V can be also derived from the forms XI and XIII of 16 possible isomers of BHC, respectively, and that the experimental result that ϵ -hepta is also produced by chlorination of γ -BHC, it must be considered that one of the two, XI or XIII, is the molecular configuration of γ -BHC. As has been pointed out by Y. Morino *et al.*, the calculated values of dipole moments of the two forms are 3.19-2.93 D (XI) and 1.88 D (XIII), and the experimental value for γ -BHC is 2.80 D. Consequently, XI should be the molecular configuration of γ -BHC. The isomers of hepta, which can be derived from XI, are XII, XII', V, and V', but among them only V can be obtained by the chlorination of both α - and γ -BHC. It is, therefore, concluded that V is ϵ -hepta and VI is δ -hepta.

- 1) T. Oiwa, R. Yamada, M. Hamada, M. Inouye and M. Ohno: *Botyu-Kagaku*, **14**, 43 (1949).
- 2) T. Oiwa, R. Yamada, M. Hamada, M. Inouye and M. Ohno: *Ibid.*, **15**, 32 (1950).
- 3) T. Oiwa, R. Yamada and M. Ohno: *Ibid.*, **15**, 86 (1950).
- 4) T. Oiwa, R. Yamada and M. Ohno: *Ibid.*, **16**, 11 (1951).

VIII. Studies on the Biological Assay of Insecticides

In 1947, when communication with abroad was resumed after years of seclusion as a result of World War II, literature and samples of various new insecticides began to flow into this country from U.S.A. and others. It appears that the studies of insecticides in this country has undergone a complete transformation since then. Until this year studies on the biological assay of insecticides in our laboratory as well as in other laboratories of our country was so inactive that only a few papers had been published. Though the literatures of Dr. Bliss' probit transformation method for analysing the sigmoid response curves was introduced in this country ten years ago, few authors have applied this excellent analytical method to their data of insecticide assay, but most reports contains nothing but tabulated experimental data without statistical treatments. In 1947, for the first time, W. Ohsawa and S. Nagasawa analytically explained the principles of transforming the cumulative dosage-mortality curve to the linear regression line, of which the mathematical theory was omitted altogether in Bliss' paper, and by means of a certain simple algebraic combination of the two parameters of the regression line, the median, M , and the standard deviation, σ , defined several measures for indicating the effectiveness, i. e. the k -th order effective lethal dose (for common uses, adopted the 3rd order), the absolute degree of effectiveness, the effective equivalent, the deviation of effectiveness and the effective equivalent concentration. They further discussed the conception of grading the effectiveness using an effective equivalent and the deviation of effectiveness. Since then, based on this formulation, we have been carrying out many investigations on biological assay of various insecticides. On the other hand, we have been studying the mechanism of the lethal effect of so-called "inert" pulverized materials to insects.

As a result of these biological researches, we have reached the following conclusion: the current method of inspecting insecticides in this country which is based only on the chemical analyses or physical tests of effective component is not adequate, and the practical value of insecticides should be appraised by the results of biological assay. Preparation of an insecticide must be, therefore, criticized from both chemical and biological points of view. In this connection we urged the establishment of the official control test insecticide in each toxicant or formulation.

In this division the following papers have been published so far.

- 1) The effectiveness of insecticides and methods of indication. W. Ohsawa and S. Nagasawa: Botyu-Kagaku, **7**, **8**, **9**, 1-10 (1947).
- 2) Lethal effect of the diatomaceous earth against the azuki bean weevil, *Callosobruchus chinensis* L., especially on the problem of the relations of this lethal effect to the moisture. S. Nagasawa: *Ibid.*, **7**, **8**, **9**, 38-44 (1947).
- 3) The statistico-physiological analysis of the lethal action of water, kerosenes, and pyrethrin against the worker of *Cremastogaster brunnea matsumurai* Forel. W. Ohsawa and S. Nagasawa: *Ibid.*, **10**, 42-59 (1948).
- 4) Comparison of the toxicity of γ -BHC, 1068 and p, p' -DDT to the pupa of the common house mosquito (*Culex pipiens* var. *pallens* Coquillett). (A preliminary report). S. Nagasawa: *Ibid.*, **11**, 20-23 (1949).

- 5) Testing the larvicidal effect of household insecticidal emulsions to the larva to of the common housefly. W. Ohsawa and S. Nagasawa: *Ibid.*, **12**, 9-12 (1949).
- 6) On the lethal effects of household pyrethrum emulsion to the pupa of the common house mosquito (*Culex pipiens var. pallens* Coquillett). S. Nagasawa: *Ibid.*, **12**, 12-18 (1949).
- 7) On the lethal effects of the household pyrethrum emulsion to the larva of the common house mosquito (*Culex pipiens var. pallens* Coquillett). S. Nagasawa: *Ibid.*, **13**, 37-41 (1949).
- 8) On the knock down effects of the mosquitocide incense made of pyrethrum mixed with benzophenone against the adult of common housefly (*Musca domestica* L.). S. Nagasawa and C. Uruha: *Ibid.*, **14**, 31-41 (1949).
- 9) On the toxicity of DDT powder to the adult of common housefly (*Musca domestica* L.), with special reference to the comparison of the toxicity estimated biologically with the *p, p'*-DDT content determined by the dehydrochlorination method. S. Nagasawa and T. Takano: *Ibid.*, **15**, 46-53 (1950).
- 10) On the lethal effect of the powder of silicon carbide to the adult of azuki bean weevil (*Callosobruchus chinensis* L.), with special reference to the relations between the lethal effect and the particle size. (A preliminary report). S. Nagasawa: *Ibid.*, **15**, 79-85 (1950).
- 11) Comparison of the toxicity of Toxaphen, Thanite and *p, p'*-DDT against the pupa of common house mosquito (*Culex pipiens var. pallens* Coquillett). S. Nagasawa and Y. Inoue: *Ōyo-Kontyu*, **6**, 41-45 (1950).
- 12) On the lethal effect of the powder of Yamagata Bentonite to the adult of azuki bean weevil (*Callosobruchus chinensis* L.), with special reference to the relations between the lethal effects and the particle size. S. Nagasawa and C. Uruha: *Botyu-Kagaku*, **15**, 178-180 (1950).
- 13) The statistico-physiological analysis of the vital resistabilities to water, kerosene, and pyrethrins of the workers of *Cremastogaster brunnea matsumurai* Forel. W. Ohsawa and S. Nagasawa: *Trans. 8th Int. Congr. Ent.*, Stockholm. 1-12 (1948).
- 14) On the inspection of mosquitocide incense. S. Nagasawa, S. Sumita, and S. Hirai: *Botyu-Kagaku*, **15**, 203-217 (1950).
- 15) On the knock down effect of the BHC powder to the adult of the common housefly (*Musca domestica* L.). S. Nagasawa: *Bull. Chem. Inst. Univ. of Kyoto*, **24**, 32-41 (1951).
- 16) On the lethal effect of the powder of Volclay Bentonite and Panther Creek Bentonite to the adult of the azuki bean weevil (*Callosobruchus chinensis* L.). S. Nagasawa and M. Yoshinobu: *Botyu-Kagaku*, **16**, 35-40 (1951).