apparent that the concentration of free sulfadimethoxine in plasma is increased by salicylic acid. Consequently, it is presumed that the increase of transfer of sulfadimethoxine from plasma to tissues, in cooperation with the inhibition of intestinal absorption of sulfadimethoxine by salicylic acid described above, results in the decrease in the blood levels of unchanged and total sulfadimethoxine by salicylic acid in rabbits. Further works on these problem are now under way and the details will be reported in near future.

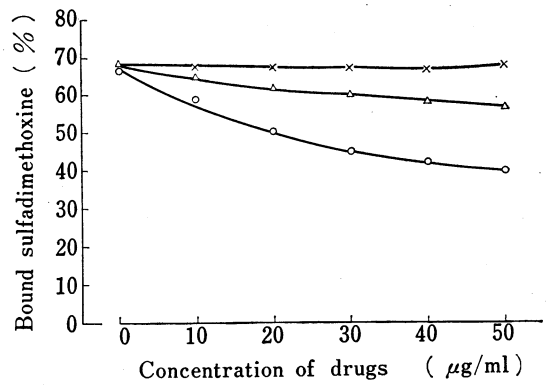

Fig. 4. Inhibitory Effect of Non-steroidal Anti-inflammatory Drugs on the Binding of Sulfadimethoxine and Bovine Serum Albumin

—○—: salicylic acid
—△—: indomethacin
—×—: benzydamine
initial concentration of sulfadimethoxine: 30 μg/ml
albumin concentration: 1.0 (w/v)%

Furthermore, the attainment of the maximum blood levels of unchanged and total sulfadimethoxine was delayed by simultaneous administration of indomethacin or benzydamine as shown in Fig. 1 and 2. Recently, Kato, *et al.*[9] reported that indomethacin or benzydamine evidently decreased the gastric emptying rate of phenol red in rats. As seen in Tables I and II, it is apparent that the absorption rate of sulfadimethoxine is much greater in the small intestine than the stomach, and that the primary site for the absorption of the drug is the small intestine. Accordingly, it may be considered that the simultaneous administration of indomethacin or benzydamine alters the absorption of sulfadimethoxine by delaying the gastric emptying and hence increasing the time for sulfadimethoxine to reach its primary absorption site.

9) R. Kato, A. Takanaka, K. Onoda, and Y. Omcri, *Nippon Yakurigaku Zasshi*, **67**, 134 (1971).

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Microbial Transformation of 2,4-D and Its Analogues

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2,4-Dichlorophenoxyacetic acid(2,4-D) and the analogous phenoxyacetic acids have been known to possess not only the growth regulating action on plants but also the antimicrobial activities against bacteria and moulds. It was during 1951 and 1958 that Naito, Tani, Kishi and Kojima examined antifungal activities of 2,4-D and its analogues, *i.e.* 2-chloro-, 2-methyl-, 2,4-dichloro-, 2-methyl-4-chloro-, and 2,4,5-trichloro-phenoxyacetic acids, against

1) Location: *a) 2-Chome, Ebara, Shinagawa-ku, Tokyo; b) Miki-machi, Kida-gun, Kagawa-ken.*

various moulds. They found the fact that the antifungal activities of these derivatives increased as cultivation proceeded, and, in explanation for this phenomenon, they postulated the production, in the culture broth, of growth-inhibiting principles by the moulds themselves from 2,4-D analogues.[2] And they actually isolated those active principles mostly in the state of crude yellow oils.[3]

The present authors cultivated three kinds of moulds, *Gloeosporium olivarum*, *Gloeosporium kaki* and *Schizophyllum commune*, on the media containing either 2,4-D or 2-methyl-4-chlorophenoxyacetic acid(M.C.P.) or 2,4,5-trichlorophenoxyacetic acid(2,4,5-T), and obtained the active principles as pure colorless crystals, in every case, and determined the structures thereof. It was also observed that the difference in the kind of moulds did not give different active principles.

The active principle, obtained as colorless needles, from the neutral fraction of the culture grown on the media provided with M.C.P. melted at 60°. The infrared (IR) spectrum showed absorptions at 3300 cm^{-1} and 1050 cm^{-1} of hydroxyl, at 1250 cm^{-1} and 1080 cm^{-1} of phenolic ether, at 1600 cm^{-1}, 1500 cm^{-1}, 860 cm^{-1} and 798 cm^{-1} of 1,2,4-trisubstituted-phenyl, and at 2930 cm^{-1}, 2860 cm^{-1}, 1458 cm^{-1} and 1388 cm^{-1} of alkyl groups. The molecular weight was shown to be 187 by mass spectrum, and the elemental analysis agreed with the molecular formula $C_9H_{11}O_2Cl$. From these results, this active principle was postulated to be 2-(2-methyl-4-chlorophenoxy)ethanol, a reduction product of M.C.P.. This compound, whose melting point is described as 51—53° in the literature,[4] was newly synthesized by us by reduction of M.C.P. with $LiAlH_4$. And the synthesized one, which melted at 60°, was identified with the above cultivation product by mixed fusion and comparison of IR spectra.

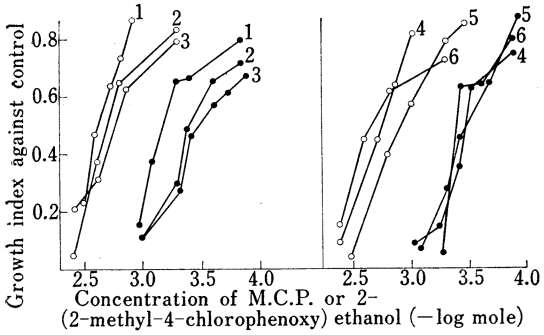

Fig. 1. Antifungal Activity of 2-(2-Methyl-4-chlorophenoxy)ethanol in Comparison of M.C.P.

Moulds: 1. *Schizophyllum commune*; 2. *Cephalothecium roseum*; 3. *Macrosporium porri*; 4. *Gloeosporium foliicorum*; 5. *Gloeosporium kaki*; 6. *Corticium gramineum*
Each mould was grown in Petri dish at 25° for 3 days on the peptone-salts agar media supplied with each compound. Growth index was calculated by dividing diameter of colonies by that of control.
—○—: 2-(2-methyl-4-chlorophenoxy)ethanol
—●—: M.C.P.

In the similar manner, by investigating into the IR spectrum, the mass spectrum and the elemental analysis, the active principle, which melted at 58° and 66°, of the cultures grown on the media containing either 2,4-D or 2,4,5-T were postulated to be, and identified with the authentic samples of 2-(2,4-dichlorophenoxy) ethanol and 2-(2,4,5-trichlorophenoxy) ethanol respectively.

The antifungal activity of 2-(2-methyl-4-chlorophenoxy)ethanol, now isolated as active principle from the culture filtrate, was compared with that of M.C.P., as shown in Fig. 1. It is clear from this figure that the former compound has much stronger antifungal activity than the latter.

From the above observation, it is certain that the antifungal activities of phenoxyacetic acid derivatives are operative after being reduced into phenoxyethanols. And it is a very interesting fact that this conclusion in the case of moulds is quite contrary to the wide-spread opinion[5] on the mechanism of the herbicidic activity of 2-(2,4-dichlo

2) N. Naito and T. Tani, *Ann. Phytopath. Soc. Japan*, **19**, 129 (1955).
3) N. Naito and T. Tani, *Jap. Journ. Bot.*, **15**, 152 (1956); *idem*, *Ann. Phytopath. Soc. Japan*, **21**, 74 (1956); N. Naito and Y. Kojima, *Tech. Bull. Fac. Agr. Kagawa Univ.*, **9**, 18 (1957); N. Naito, *Jap. Journ. Bot.*, **16**, 153 (1958).
4) D.B. McCaskey, U.S. Patent 2678336 [*C.A.*, **49**, 7596 (1955)].
5) L.J. Audus, *Nature*, **170**, 886 (1952).

rophenoxy)ethanol on higher plants, which insists that the phenoxyethanol is operative after being oxidized into 2,4-D.

Experimental[6]

Cultivation——Three moulds, *i.e. Gloeosporium olivarum*, *Gloeosporium kaki* and *Schizophyllum commune*, were grown, in the same manner, in 50 Roux bottles each containing 200 ml of the media, which was made by dissolution of 10 g of sucrose, 2 g of peptone, 0.2 g of KH_2PO_4, 0.04 g of anhydrous $MgSO_4$, and either 0.1 g of 2,4-D or 0.04 g of M.C.P. or the same gram of 2,4,5-T in water. Administration of more respective phenoxyacetic acid caused too strong inhibition of growth of the moulds. Cultivation was continued for 3 weeks at the temperature of 28°.

Isolation of the Active Principle from the Culture on the Media Containing M.C.P.——The culture filtrate was acidified to pH 2.0 by adding HCl, and extracted with ether. The ethereal extract was washed twice with 4% aqueous NaOH then with water, and dried over anhydrous Na_2SO_4. The crude yellow crystals that remained after evaporation of ether were decolorized by use of carbon and recrystallized from hexane as colorless needles, mp 60°. The crystals obtained from each mould were identified with each other by mixed fusion and comparison of IR spectra. The yields were 1.89 g at *Gloeosporium olivarum*, 1.49 g at *Gloeosporium kaki*, 0.99 g at *Schizophyllum commune*. *Anal.* Calcd. for $C_9H_{11}O_2Cl$: C, 57.92; H, 5.94. Found: C, 58.24; H, 6.16. Mass Spectrum *m/e*: 187 (M^+). This substance was identified with the 2-(2-methyl-4-chlorophenoxy)ethanol, synthesized from M.C.P. in the next column, by mixed fusion and comparison of IR spectra.

Synthesis of 2-(2-Methyl-4-chlorophenoxy)ethanol——To a suspension of 1 g of $LiAlH_4$ in 100 ml of absolute ether was added 1.5 g of M.C.P. in small portions, and then the mixture was boiled for 10 min. After cool, the excess $LiAlH_4$ was cautiously decomposed by addition of water, and the reaction mixture was filtered. The ethereal washing of the mass on filter was combined with the ethereal layer of the filtrate, and the combined ether was washed with 4% aqueous NaOH then with water, and dried over anhydrous Na_2SO_4. After evaporation of ether, crude yellow crystals were obtained as much as 1.3 g, which were recrystallized from hexane as colorless needles of mp 60°. *Anal.* Calcd. for $C_9H_{11}O_2Cl$: C, 57.92; H, 5.94. Found: C, 58.23; H, 6.17.

Isolation of the Active Principle from the Culture on the Media Containing 2,4-D——After the similar procedure as in the case of M.C.P., colorless crystals of mp 58°, indifferent to the kind of moulds, were obtained. Yields were 1.14 g at *Gloeosporium olivarum*, 1.25 g at *Gloeosporium kaki*, and 1.32 g at *Schizophyllum commune*. This substance was identified with the authentic sample of the 2-(2,4-dichlorophenoxy)ethanol, which was synthesized by the procedure of Kjeldgaard,[7] by mixed fusion and IR spectra.

Isolation of the Active Principle from the Culture on the Media Containing 2,4,5-T——After the similar procedure as in the case of M.C.P., colorless needles of mp 66°, indifferent to the kind of moulds, were obtained. Yields were 0.82 g at *Gloeosporium olivarum*, 0.52 g at *Gloeosporium kaki*, 0.83 g at *Schizophyllum commune*. This substance was identified with the authentic sample of 2-(2,4,5-trichlorophenoxy)ethanol, which was synthesized by the procedure of Kjeldgaard,[7] by mixed fusion and comparison of IR spectra.

6) All melting points were not corrected. Hitachi model 215 and Hitachi RMS-4 apparatus were used for measurement of IR and mass spectra, respectively.

7) K. Kjeldgaard, *Farm. Tidende*, **66**, 33 (1956).