原　著

Effect of Rotenone and its Derivatives on the Glutamic Dehydrogenase in Insects. Junichi FUKAMI* and Chôjiro TOMIZAWA (National Institute of Agricultural Sciences, Nishigahara, Tokyo) Received Nov. 5, 1957. *Botyu-Kagaku* **23**, 1, 1958.

1. 昆虫のグルタミン酸脱水素酵素に及ぼすロテノーン及びその誘導体の影響

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前報（深見，1956）において，ロテノーンは昆虫体内で神経および筋肉の組織呼吸を抑制してこれらを麻痺せしめ，致死させることを証明した．かつまたその組織呼吸抑制の一部はグルタミン酸脱水素酵素の抑制であると結論した（深見，富沢，1956）．次はこの実験の追証と，化学構造と作用性の関係を検討するために，ロテノーン誘導体 dihydro-rotenone, rotenolone-I, rotenolone-II, dehydro-rotenone の in vitro での昆虫グルタミン酸脱水素酵素に対する作用を調べたところ，in vivo の毒力と密接な関連があることが判明した．

Many works have been done concerning the relationship between the chemical structure of organo-phosphorus insecticides and their inhibition of cholinesterase activity (Metcalf, 1955), while the data concerning the influence of insecticide on respiratory enzyme system were very few. It has been reported (Anderson et al., 1954; Brown and Brown, 1956; Chadwick, 1952; Perry and Sacktor, 1955) that DDT and its derivatives, some of which have no insecticidal toxicity, inhibit the cytochrome oxidase activity in insect body. In this case, since the parallelism between their insecticidal toxicity and the inhibition of cytochrome oxidase activity was not held, it is doubtful that the primary action of DDT against insects was the inhibition of cytochrome oxidase activity.

The authors concluded in the previous reports (Fukami, 1956; Fukami and Tomizawa, 1956) that rotenone inhibit the respiration of the muscle and the nerve in insect body, and the inhibition of the respiratory metabolism was partly due to the inhibition of the glutamic dehydrogenase activity in insect body. The present study was undertaken with the objects of determining whether rotenone and its derivatives inhibit the glutamic dehydrogenase activity in insect body, and of gaining information as to the chemical structure of rotenone and its derivatives and their physiological action.

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Materials and Methods

Test insect : Adults of the American cockroach, Periplaneta americana L., reared at 27-30°C on a dry diet made from baker's yeast, were used throughout the experiment.

Isolation of the mitochondrial fraction of insect muscle : The mitochondrial fraction of insect muscle was isolated by a slight-modification of the techniques already reported (Fukami and Tomizawa, 1956). The mitochondrial fraction was prepared from the muscle of each fifteen individual of male and female cockroaches, and the pH of the muscle homogenate was adjusted over 7.0 by adding 0.4 M tris (hydroxymethyl) aminomethane buffer (pH 9.0) during the isolation of the mitochondrial fraction.

Measurement of oxygen uptake by the mitochondrial fraction : 0.2 ml (or 0.3 ml) of 10% aqueous acetone only, or 10% aqueous acetone containing 10^{-5} M rotenone or its derivative was added to 1.8 ml (or 2.7 ml) of the mitochondrial fraction. After pre-incubation for 10 minutes at 30 °C,

* This is an account of investigations performed during the post-graduate course of Tokyo University.

oxygen uptake measurements were made in a conventional Warburg apparatus.

Rotenone and its derivatives tested in this experiment : Rotenone, rotenolone-I, rotenolone-II, dehydro-rotenone and dihydro-rotenone were used. The chemical structure of these compounds should be present later.

Results

At first, the inhibition of glutamic dehydrogenase activity was compared between rotenone and dihydro-rotenone. As shown in Table 1 and Figure 1, dihydro-rotenone inhibited the glutamic dehydrogenase activity as well as rotenone did, although the degree of the inhibition was less slightly.

Rotenolone-I also inhibited the glutamic dehydrogenase activity, but the inhibition by rotenolone-II or dehydro-rotenone was only slight. The results are shown in Table 2 and Figure 2.

Table 1. In vitro effects of rotenone and dihydro-rotenone on the O_2 uptake of mitochondrial fraction of the muscle of the American cockroach in the presence of 0.02 M of l-glutamate.
Incubated for 1 hr. at 30°, pH 7.2. Total volume, 2 ml. Final concentration of insecticides, 10^{-5}M.

Insecticide	mm^3 of O_2 uptake/hr.	Per cent inhibition
Control	147.9	
Rotenone	17.0	88
Dihydro-rotenone	25.6	83

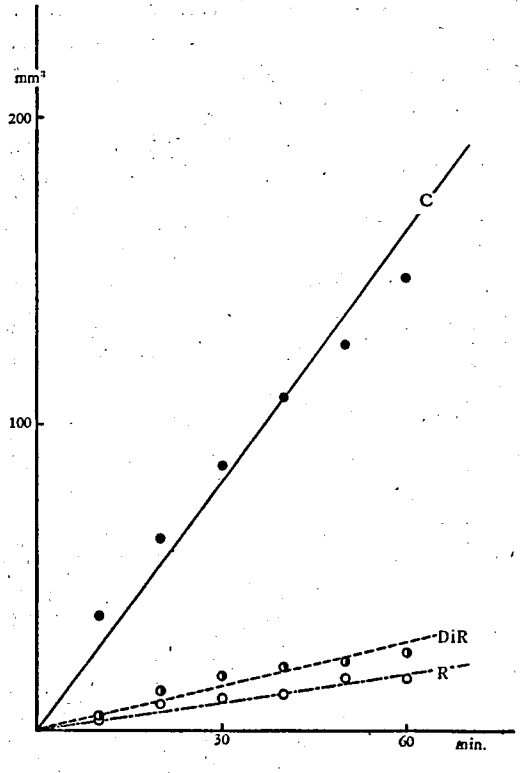


Fig. 1 In vitro effects of rotenone and dihydro-rotenone on the O_2 uptake of mitochondrial fraction of the muscle of the American cockroach in the presence of 0.02 M l-glutamate.
C : Control
DiR : 10^{-5} M Dihydro-rotenone
R : 10^{-5} M Rotenone

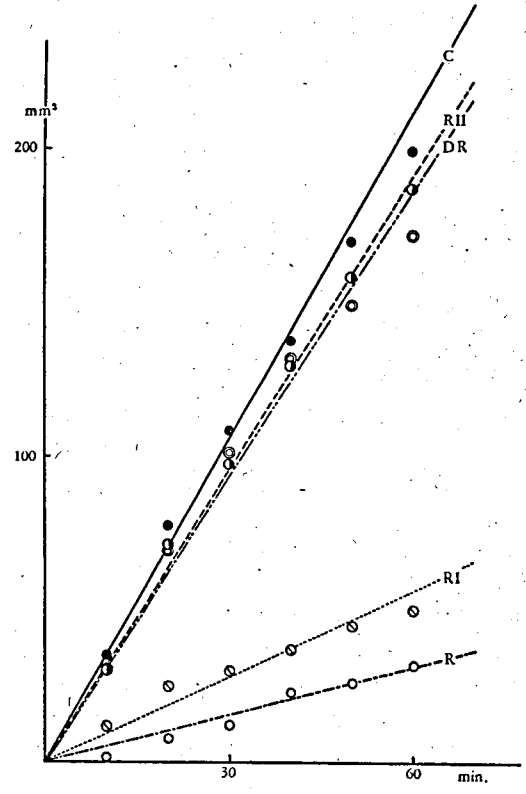


Fig. 2 In vitro effects of rotenone, rotenolone-I, rotenolone-II and dehydro-rotenone on the O_2 uptake of mitochondrial fraction of the muscle of the American cockroach in the presence of 0.02 M l-glutamate.
C : Control
RII : 10^{-5} M Rotenolone-II
DR : 10^{-5} M Dehydro-rotenone
RI : 10^{-5} M Rotenolone-I
R : 10^{-5} M Rotenone

Table 2. In vitro effects of rotenone, rotenolone-I, rotenolone-II and dehydro-rotenone on the O_2 uptake of mitochondrial fraction of the muscle of the American cockroach in the presence of l-glutamate.
Incubated for 1 hr. at 30°, pH 7.2. Total volume, 3 ml. Final concentration of insecticides, 10^{-5} M.

Insecticide	mm^3 of O_2 uptake/hr.	Per cent inhibition
Control	198.8	
Rotenone	31.5	84
Rotenolone-I	50.4	75
Rotenolone-II	186.5	6
Dehydro-rotenone	171.2	14

Discussion

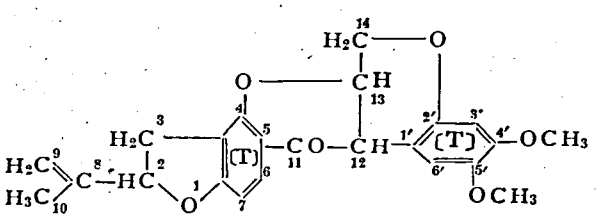
The relative toxicities of rotenone and its derivatives against insects and fishes which has been reported up to date are summarized in Table 3. Rotenone and dihydro-rotenone are toxic to the test organisms, but rotenolone-II and dehydro-rotenone are very low in their toxicity. Data concerning the toxicity of rotenolone-I has not been published. Examining the lethal dosages of rotenone, dihydro-rotenone and dehydro-rotenone against guinea pig and rabbit, Ambrose and Haag (1937) showed that the order of their toxicities was similar to those against insects and fishes such as in Table 3.

The chemical structures of rotenone and its derivatives are the following: Dihydro-rotenone is characterized by the saturation of the double bond in isopropenyl side chain with hydrogen. Rotenolone-I, rotenolone-II and dehydro-rotenone are regarded as the oxidation product of rotenone. Martin (1937) suggested that the hydrogen atoms of carbon 12 and 13 in the central ring are important in determining the toxicity of rotenoide. The reason for this suggestion are (1) saturation of the isopropenyl side chain of rotenone to produce dihydro-rotenone has little effect on toxicity, (2) oxidation to dehydro-rotenone which introduces a double bond in the central ring between carbon 12 and 13, destroys the toxicity, and (3) the methyl ether of enolized rotenone

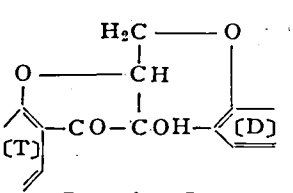
Table 3. Relative toxicities of rotenone and its derivatives.

Compound	Silk* worm	Army** worm	House** fly	Gold*** fish	Common**** loach
Rotenone	100	100	100	100	100
Dihydro-rotenone	33	—	70	100	—
Rotenolone-I	—	—	—	—	—
Rotenolone-II	—	—	—	—	1
Dehydro-rotenone	0.15	0	—	—	—

* Shepard and Campbell (1932)
** Haller, Goodhue and Jones (1942)
*** Gersdorff (1935)
**** Takei, Koide and Miyazima (1930)



Rotenone



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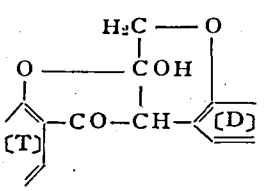

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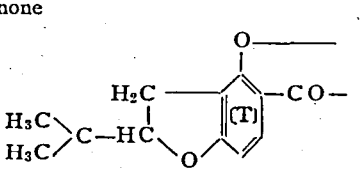
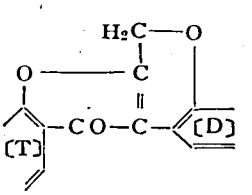
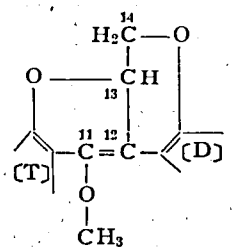


Dihydro-rotenone



Dehydro-rotenone

having the following structure, is much less effective. Considering the relationship between the chemical structure and the physiological action, Läuger et al. (1944) deduced that the lactone ring of rotenone is important on the insecticidal toxicity.



From these and the present data, a parallelism was found between the in vivo toxicity of rotenone and its derivatives against insects and the degree of the inhibition of the glutamic dehydrogenase activity by each of them. Rotenolone-I inhibited the glutamic dehydrogenase activity, but rotenolone-II did not inhibit as shown in Table 2 and Figure 2. Although the data concerning the insecticidal toxicity of rotenolone-I is unable to be available, the difference of the degree of the inhibition of the glutamic dehydrogenase activity between rotenolone-I and rotenolone-II is presenting an interesting problem concerning the chemical structure of rotenone derivatives and their physiological action.

Being different with the case of the inhibition of cytochrome oxidase activity by DDT or its derivative, a rather close correlation exists between the degree of the inhibition of the glutamic dehydrogenase activity by rotenone or its derivative and their toxicity against insects. This fact seems to present an evidence that the inhibition of the respiratory metabolism of insects by rotenone is partly due to the inhibition of the glutamic dehydrogenase.

Summary

Inhibition of the glutamic dehydrogenase activity in the muscle of the American cockroach was compared among rotenone, dihydro-rotenone, rotenolone-I, rotenolone-II and dehydro-rotenone.

1. Dihydro-rotenone inhibited the glutamic dehydrogenase activity as well as rotenone did, although the degree of the inhibition was less slightly. Rotenolone-I also inhibited the glutamic dehydrogenase activity, but the inhibition by rotenolone-II or dehydro-rotenone was only slight.

2. It was found from the above result that a rather close correlation exist between the in vivo toxicity of rotenone and its derivatives against insects and the degree of the inhibition of the glutamic dehydrogenase activity by each of them.

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