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Studies on Phenylphenol Derivatives with Biological Activity. Part V. Miticidal Activity and Effect on Oxidative Phosphorylation. Hong-Ming CHENG[1*], Morifusa ETO, Eiji TANIGUCHI, Shozo KUWATSUKA[2*], Yasuyoshi OSHIMA[3*] and Masaru KADO[**] (Department of Agricultural Chemistry, Kyushu University, Fukuoka and **Kumiai Chemical Co., Shimizu, Japan) Received September 26, 1969. *Botyu-Kagaku*, 34, 176, 1969.

23. フェニルフェノール誘導体の化学構造と生物活性に関する研究（第5報）殺ダニ性と酸化的リン酸化に対する影響とについて

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1969. 9. 26 受理

34種のフェニルフェノール誘導体を合成し，ニセナミハダニ *Tetranychus telarius* (Linnaeus) に対する殺ダニ性を検討した．その中で，4-クロル-2-フェニルフェノールおよび6-ニトロ-4-クロル-2-フェニルフェノールは強い殺成虫力を有し，4-フェニルフェニルアリルエーテルは殺卵力にすぐれていた．これら誘導体の殺ダニ性は置換基の種類と置換位置によって影響されるようである．

一方，マウス肝臓ミトコンドリヤを用い，その呼吸作用とATPase活性に対するフェニルフェノール類の影響を検討した．4-ニトロ-6-クロル-2-フェニルフェノールは ATPase の活性を高め，酸化的リン酸化のアンカップラーと考えられた．

Introduction

Previous studies in our laboratories have demonstrated that several derivatives of phenylphenols have not only herbicidal but also fungicidal and bactericidal activities, indicating that they have a broad spectrum of biological activities[1~4]. This paper deals with the relationship between structure and miticidal activity of phenylphenol derivatives. As a result, three of these tested chemicals were proved to have relatively high toxicity to the adults or eggs of *Tetranychus telarius* (Linnaeus).

In view of the mode of toxic action of these derivatives, it is of interest to study further the effects on mitochondrial oxidative phosphorylation and ATPase activity. It is well known that the toxic action of dinitrophenols is attributed to their abilities to uncouple the oxidative phosphorylation and to elicit ATPase activity in mitochondria[5]. The present investigation also reports that 4-nitro-6-chloro-2-phenylphenol and 4, 6-dinitro-2-phenylphenol act on mitochondria in the similar manner with unsubstituted dinitrophenol, indicating that *o*-aryl nitro- and chlorophenols are the uncouplers of oxidative phosphorylation.

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Experimental

Miticidal activity tests

For adult mites: Each of test chemicals was provided as a 10 percent wettable powder. The determination of miticidal activity was carried out according to a dipping method. Thirty to eighty adults of *Tetranychus telarius* were parasitized on the leaves of kidney beans (French bean) which were cultured in a green house and then were treated by dipping the leaves into a 0.1 percent test solution for a few seconds. Two days after the treatment, the mortality of the mites was determined. Only compounds whose mortality values were above ninety percent were further subjected to determine LC_{50} values.

For eggs: Ovicidal activity tests were also carried

out according to the dipping method. Several female adults of *Tetranychus telarius* were parasitized on the leaves of kidney beans which grew in a green house. Fifty to 130 eggs were laid on the surface of the leaves. The unhatched eggs were counted ten days after dipping and the mortality of eggs was determined. In the case that the mortality of eggs was higher than ninety percent, the test sample was further subjected to determine LC_{50}.

Assays of ATPase and oxidative phosphorylation activities

Materials: 2,4-Dinitrophenol (DNP) and 4-nitro-6-chloro-2-phenylphenol were recrystallized from benzene. 4,6-Dinitro-2-phenylphenol was recrystallized from ethyl alcohol. These compounds were pure on the basis of thin layer chromatography. Bovine serum albumin, disodium salts of ADP and ATP, cytochrome c were purchased from Sigma Chemical Co.. Solutions of all reagents were adjusted to pH 7.4 before addition to the incubation mixture. ADP, ATP and hexokinase solutions were prepared freshly before use. *Methods*: The mitochondrial suspension was prepared from adult male mouse (ddN) liver in 0.25M sucrose by the method of Schneider[6]. Using a teflon homoginizer the freshly isolated mouse liver was homogenized in cold 0.25M sucrose and then subjected to centrifuge at 1,000×g for 5 minutes at 0~3°C. The precipitates were discarded and the upper part of suspension was recentrifuged at 10,000×g for 10 minutes at 0~3°C. The supernatant was decanted and the mitochondria were resuspended in a volume of cold isotonic sucrose solution equal to a 4-fold weight of the liver. The protein content of mitochondria suspension was determined by a colorimetric method described by Lowry[7] using bovine serum albumin as a standard. The protein content of the final suspension of mitochondria used in this experiment was 2.5 to 3.25 mg/*ml*.

ATPase activity was measured in 10×150 mm test-tubes by the method of Weinbach[8]. The chilled tubes containing the reaction components except mitochondria were previously immersed in a thermostated water-bath before the addition of mitochondrial suspension to start the reaction. Inorganic phosphate was determined according to the Sumner's method[9] on aliquots of the reaction mixture after deproteinization with trichloroacetic acid. The assay of oxidative phosphorylation activity was carried out by the method of Hunter[10]. Oxygen consumption was determined by the Warburg method with air as an oxygen source. The reading was started after 5 minutes equilibrium in a water-bath at 25°C.

Results

Any phenylphenol derivatives used in this investigation did not show insecticidal activity against housefly at the dosage of 10 μg/fly. Many of them, however, have more or less toxicity against mites. Tables 1 to 6 show the relative miticidal activity of various structurally

Table 1. Miticidal activity of chloro-substituted phenylphenols to *Tetranychus telarius*.

Code no. KPP	Compound*	Mortality (%) Adults	Eggs	Remark
172	2-Phenylphenol, 6-chloro	9.8	0.0	—
175	4-chloro	92.4**	20.0	Phytotoxic
173	4,6-dichloro	26.8	0.0	Phytotoxic
178	4,6,(x)-trichloro	28.5	0.0	Phytotoxic
176	4-Phenylphenol, 2-chloro	63.3	40.0	Phytotoxic
177	2,6-dichloro	38.5	0.0	Phytotoxic

* 0.1% Solutions were used.
** The LD_{50} of KPP 175 is 0.04% for adults.

modified phenylphenols to the adults and eggs of *Tetranychus telarius*. Among these compounds, *p*-phenylphenyl allyl ether (KPP 114), 4-chloro-2-phenylphenol (KPP 175) and 4-chloro-6-nitro-2-phenylphenol (KPP 202) are the most effective compounds. The former is more effective in ovicidal activity, whereas the other two derivatives appear to be more effective to the adult mites than the eggs.

Miticidal activity of chloro-phenylphenols

The relation of structure to the miticidal activity of chloro-substituted phenylphenols is shown in Table 1. 4-Chloro-2-phenylphenol (KPP 175) is the most effective compound in this group against the adults and its LC_{50} is 0.04%, though it is of low ovicidal activity. However, its position isomer, 6-chloro-2-phenylphenol (KPP 172), shows very weak activity to the adults and is non-toxic to the eggs.

Dichloro- and trichloro-derivatives of 2-phenylphenol show weak activity to the adults and no ovicidal activity at all. Mono- and di-chlorinated 4-phenylphenols have moderate miticidal activity. 2-Chloro-4-phenylphenol (KPP 176) is not only highly effective against the adult of mites, but also moderately effective to the eggs. 2,6-Dichloro-4-phenylphenol (KPP 177) shows moderate activity to the adult mites but is inactive to the eggs. The introduction of a chlorine atom to 6-position results generally in the decrease of miticidal activity.

Miticidal activity of nitrated phenylphenols

Table 2 indicates the effect to miticidal activity of structural modification of nitrated phenylphenols. Like the case of chloro derivatives, ortho substituted 2-phenylphenol, *i.e.* 6-nitro-2-phenylphenol, has very weak activity but its *p*-isomer, 4-nitro-2-phenylphenol, is much more effective to both the adults and eggs. 4,6-Dinitro derivative of 2-phenylphenol shows very weak adulticidal activity, while has higher toxicity to the eggs than mononitro derivatives have. The adulticidal activity of a mononitro 4-phenylphenol is greater than a dinitro derivative but both two derivatives of 4-phenylphenol show no ovicidal activity at all. On the contrary, nitrated 2-phenylphenol show more or less ovicidal activity. In general, there is no distinguished effective compound in this series.

Table 2. Miticidal activity of nitro-substituted phenylphenols to *Tetranychus telarius*.

Code no. KPP	Compound	Mortality (%) Adults	Eggs	Remark
154	5-Phenylphenol, 6-nitro	5.7	10.0	Phytotoxic
155	4-nitro	33.3	20.0	Phytotoxic
157	4,6-dinitro	8.6	40.0	Phytotoxic
156	4-Phenylphenol, 2-nitro	35.9	0.0	Phytotoxic
158	2,6-dinitro	7.7	0.0	Phytotoxic

Table 3. Miticidal activity of chloro-nitro-substituted phenylphenols to *Tetranychus telarius*.

Code no. KPP	Compound	Mortality (%) Adults	Eggs	Remark
201	4-Nitro-6-chloro-2-phenylphenol	50.0	30.0	Phytotoxic
202	4-Chloro-6-nitro-2-phenylphenol	100.0*	50.0	—
203	2-Nitro-6-chloro-4-phenylphenol	11.1	0.0	—

* The LC_{50} of KPP 202 is 0.01% for adults.

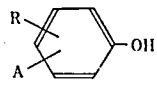
Miticidal activity of chloro-nitro-substituted phenylphenols

Table 3 indicates the effect on miticidal activity by the introduction of nitro group to monochlorophenylphenols. It is observed that the nitration of monochloro-2-phenylphenols enhances toxicity to both the eggs and adults of *Tetranychus telarius*. 4-Nitro-6-chloro-2-phenylphenol (KPP 201) is more effective than the corresponding unnitrated compound, 6-chloro-2-phenylphenol (KPP 172), both in adult toxicity and in ovicidal toxicity. It also shows greater activity than a corresponding unchlorinated compound, 4-nitro-2-phenylphenol (KPP 154). The chlorination of 6-nitro-2-phenylphenol extremely increased the miticidal activity. 4-Chloro-6-nitro-2-phenylphenol (KPP 202) is the most effective derivative among all of the tested compounds. Its LC_{50} value for the adults is 0.01%. This compound shows also moderate ovicidal action. Since this compound is less phytotoxic, so it seems to be a prospective miticide. The nitration of 2-chloro-4-phenylphenol induced only a negative effect on the activity. The nitrated product (KPP 203) is almost completely inactive to both the adults and eggs.

Miticidal activity of allyl-, acetyl- and amino-phenylphenols

The miticidal activity of allyl-, acetyl- and amino-phenylphenol derivatives are shown in Table 4. No compound in this group shows any ovicidal effect to *Tetranychus telarius*. However, all the compounds except 4-amino-2-phenylphenol (KPP 190) show more or less adulticidal activity. It appears to be of interest to note that there is a common feature that the derivatives of 4-phenylphenol are more effective than the derivatives of 2-phenylphenol except allyl derivatives. 2-Acetyl-4-phenylphenol (KPP 118) and 6-allyl-2-phenylphenol (KPP 180) show moderate adulticidal activity and all others show weak activity.

Table 4. Miticidal activity of allyl, acetyl and amino-phenylphenols to *Tetranychus telarius*.

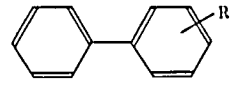


Code no. KPP	Compound R	A	Mortality (%) Adults	Eggs
180	6-$CH_2CH=CH_2$	2-C_6H_5	44.5	0.0
181	2-$CH_2CH=CH_2$	4-C_6H_5	18.0	0.0
190	4-NH_2	2-C_6H_5	0.0	0.0
191	2-NH_2	4-C_6H_5	14.3	0.0
192	4-$CH_2N(CH_3)_2$	2-C_6H_5	9.3	0.0
193	2-$CH_2N(CH_3)_2$	4-C_6H_5	29.6	0.0
117	4-$COCH_3$	2-C_6H_5	6.6	0.0
118	2-$COCH_3$	4-C_6H_5	48.3	0.0

Miticidal activity of ether and oxyacetic acid derivatives of phenylphenols

As shown in Table 5, the miticidal activities of oxyacetic acid derivatives of phenylphenols are weak to both the adults and eggs. Their sodium salts are almost completely inactive. The loss of activity may be due to the change of permeability. In contrast, allyl ethers of phenylphenols are found to be highly toxic to the adults and eggs. It is very interesting to note that each allyl ether of 2-and 4-phenylphenols has strikingly different selectivity in ovicidal and adulticidal activities. The former (KPP 113) is much more toxic to the adults of *Tetranychus telarius* than to the eggs. On the contrary, the latter (KPP 114) is much more toxic to the eggs than to the adults.

Table 5. Miticidal activity of ether and oxyacetic acid derivatives of phenylphenols to *Tetranychus telarius*.



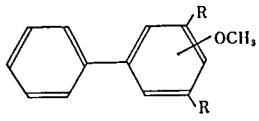
Code no. KPP	Compound R	Mortality (%) Adults	Eggs
140	2-OCH_2COOH	10.4	6.0
141	4-OCH_2COOH	24.6	6.0
142	2-OCH_2COONa	0.0	0.0
143	4-OCH_2COONa	8.6	0.0
113	2-$OCH_2CH=CH_2$	72.0	8.0
114	4-$OCH_2CH=CH_2$	6.0	90.0*

* The LC_{50} of KPP 114 is 0.05% for eggs.

The miticidal activity of sulfonic acid derivatives of methoxydiphenyls

As shown in Table 6, all the sulfonic acid derivatives of methoxydiphenyls have weak toxicity to the adult mite and no ovicidal activity at all.

Table 6. Miticidal activity of sulfonic acid derivatives of methoxydiphenyl to *Tetranychus telarius*.



Code no. KPP	Compound OCH_3	3,5-diR	Mortality (%) Adults	Eggs
123	2-OCH_3	SO_2Cl	10.8	0.0
124	4-OCH_3	SO_2Cl	4.1	0.0
125	2-OCH_3	SO_2NH_2	3.1	0.0
127	2-OCH_3	SO_2NHCH_3	10.0	0.0
128	4-OCH_3	SO_2NHCH_3	27.0	0.0
130	4-OCH_3	$SO_2NHCH_2CH_2OH$	11.0	0.0

Effects on oxidative phosphorylation

Oxygen uptake by mitochondria in the absence of ADP was inhibited (65%) by 4-nitro-6-chloro-2-phenylphenol at the concentration of 5×10^{-5}M, while it was not by DNP at the same concentration. When the former was used in lower concentration (5×10^{-6}M), the oxygen consumption was maintained on a high level as shown in Fig. 1. The uptake of inorganic phosphate was, however, completely inhibited at this concentration. 4,6-Dinitro-2-phenylphenol inhibited the oxygen uptake even at 5×10^{-6}M. Apparently, phenyl-substituted nitrophenols have an ability to inhibit the oxygen uptake of mitochondria in higher concentrations and also seem to have an ability to uncouple the oxidative phosphorylation even in lower concentrations.

4,6-Dinitro- and 4-nitro-6-chloro-2-phenylphenols showed a DNP-like action on the ATPase activity of mitochondria. As shown in Table 7, the freshly isolated mitochondria have little ATPase activity, but by the addition of these toxic substances, a large amount of inorganic phosphates was released from ATP, indicating they have an action to evoke the ATPase of mitochondria.

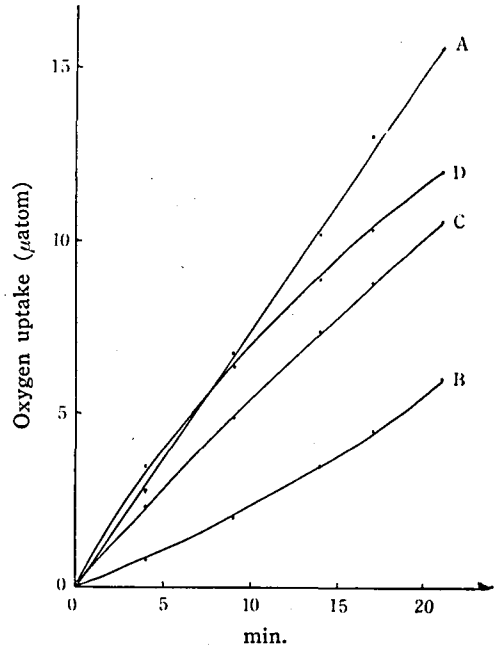


Fig. 1. Effect of nitrophenols on oxygen uptake by mouse liver mitochondria.

Each flask contained 1.5×10^{-2}M potassium phosphate, pH 7.4, 6.6×10^{-3}M magnesium chloride, 1×10^{-2}M succinate, 2.6×10^{-5}M cytochrome c and 1 m*l* of mitochondrial suspension containing 3.25 mg protein. Each side arm contained 0.25 m*l* of hexokinase solution (1% in 0.25M glucose). Final volume was made up to 3.0 m*l* with 0.25M sucrose.

A=with 8×10^{-4}M ADP; B=without ADP;
C=with 5×10^{-6}M 4-nitro-6-chloro-2-phenylphenol;
D=with 5×10^{-5}M 2,4-dinitrophenol.

Discussion

It is well known that several *o*-alkyl dinitrophenols are used not only as fungicides and herbicides but also as useful insecticides and miticides. The detailed studies of these compounds by several investigators have scattered throughout the literatures[11~17]. The present investigation shows *o*-aryldinitrophenol has less miticidal activity in comparison with *o*-alkyldinitrophenol, though the former is as active to kill weeds as the latter is[1].

Tables 1 to 6 indicate the following general

Table 7. Elicitation of ATPase activity by nitrophenols from mouse liver mitochondria*.

Phenols added	Concentration (M)	Phosphate liberated (μM)
Control		0.5
2,4-Dinitrophenol	1×10^{-4}	9.3
4-Nitro-6-chloro-2-phenylphenol	1×10^{-4}	8.1
4,6-Dinitro-2-phenylphenol	1×10^{-4}	7.5

* Each test tube contained 0.03 M tris buffer, pH 7.4, 0.003M $MgCl_2$, 0.072M KCl, 0.001M EDTA, 0.01M ATP, 0.3 m*l* of mitochondrial suspension containing 0.9mg of protein. Final volume up to 1.5 m*l* with 0.25M sucrose. Incubated at 25°C for 15 minutes.

order of the effectiveness of phenolic ring substituents against adult mites: for *o*-phenylphenol series, *p*-Cl > *p*-NO_2 > *p*-$CH_2N(CH_3)_2$ > *p*-$COCH_3$ > *p*-NH_2; for *p*-phenylphenol series, *o*-Cl > *o*-$COCH_3$ > *o*-NO_2 > *o*-$CH_2N(CH_3)_2$ > *o*-$CH_2CH{=}CH_2$ > *o*-NH_2; as disubstituents of *o*-phenylphenol, 6-NO_2, 4-Cl > 6-Cl, 4-NO_2 > 4,6-diCl > 4,6-diNO_2. However, there is rather little correlation between chemical structure and activity.

The miticidal activity of phenylphenol derivatives against *Tetranychus telarius* may be considered to be associated more or less with substituted group in the phenolic ring and the position of substituents which may serve to orient the molecule at the proper physiological site and to impart the proper degree of chemical reactivity. For example, 4-chloro-2-phenylphenol(KPP 175) was more effective than an isomeric 6-chloro-2-phenylphenol (KPP 172) in miticidal activity, and the chemical reactivity of both compounds has a somewhat difference (the pKa of 4-chloro-isomer is 10.67 and 6-chloro-isomer is 9.76). Thus, it would be suggested that the difference in the activity between two compounds might be attributed to not only the chemical reactivity but also the effect of chlorine atom on the approach of the molecule to the physiological site. 4-Chloro-6-nitro-2-phenylphenol (KPP 202, pKa 6.38) and 4-nitro-6-chloro-2-phenylphenol (KPP 201, pKa 5.64) also showed different miticidal activity each other. This may be explained by the same consideration as in the case of mono-chloro-substituted 2-phenylphenol. It seems hard to rationalize that each allyl ether of *o*-phenylphenol and *p*-phenylphenol has strikingly different selectivity in ovicidal and adulticidal action. Some factors such as permeability or steric effect should be considered.

It is reasonable to assume that the mechanism of the toxic action of chloro-and nitro-phenylphenols may be similar to that of some pesticidal chloro- and nitrophenols, which have been recognized as the uncouplers of oxidative phosphorylation. Many investigators[8,18~21] have, however, reported that the toxic action of the nitro-and chloro-phenols is not only attributed to their ability to uncouple the oxidative phosphorylation but also due to the specific effect on the metabolism of amino acids in poisoned animals and insects.

The results presented in this report on the effects of 4-nitro-6-chloro-2-phenylphenol and 4,6-dinitro-2-phenylphenol upon oxidative phosphorylation in mitochondria indicate that they are, similarly to DNP, the uncouplers of oxidative phosphorylation to elicit the ATPase activity in mitochondria. From these experimental facts, it is assumed that the toxic action of these compounds are, at least partially, due to their uncoupling effect on oxidative phosphorylation.

Summary

The purpose of the present researches is to examine whether phenylphenol derivatives would have miticidal activity. Three of 34 compounds tested were highly effective against *Tetranychus telarius*. 4-Chloro-2-phenylphenol and 6-nitro-4-chloro-2-phenylphenol were more effective in adulticidal activity, but less in ovicidal activity. On the contrary, 4-phenylphenyl allyl ether was shown to have the strongest toxicity to eggs. The miticidal activity of phenylphenol derivatives appeared to be influenced by the kind of and the position of substituents.

Using mouse liver mitochondria, the effects of 4-nitro-6-chloro-2-phenylphenol on the oxidative phosphorylation and ATPase activity were investigated. This compound in low concentration (5×10^{-6}M) inhibited the uptake of inorganic

phosphate during oxidation of substrates and also had a strong ability to induce the liberation of inorganic phosphate from ATP by the freshly prepared mouse liver mitochondria.

Acknowledgment: This work was carried out partially with a support from Sanko Chem. Co., to which thanks are due.

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抄　　録

合成フェロモン類によるキクイムシの集合行動

Aggregation Behavior of *Dendroctonus brevicomis* in Response to Synthetic pheromone. J. P. Vité and G. B. Pitman. J. Insect physiol, 15, 1617～1622 (1969).

6種の 6, 8-dioxabicyclo〔3. 2. 1〕octane基本骨格を持ったフェロモン類縁体の誘引効果を野外試験した．供試キクイムシの1種 western pine beetle (*Dendroctonus brevicomis* Lec.) に誘引集合性を持った化合物は，この虫のフェロモンとして知られている2化合物 Frontalin (1, 5-dimethyl-6, 8-dioxabicyclo〔3. 2. 1〕-octane) と Brevicomin (*exo*-7-ethyl-5-methyl-6, 8-dioxabicyclo〔3. 2. 1〕octane) のみであった．Frontalin, Brevicomin および oleoresin の混合または単用した供試薬剤1～2mg を，小型三角フラスコ中のガラス毛細管に入れ，トラップの誘引源とする簡単な方法で効果的な野外試験が行なえた．上記3種混合物は天然の誘引物質と同程度の活性を示し，誘引された虫の性比は1対1に近く，天然の被害材に観察される場合と同様であった．雌の食入している木材片を誘引源とした場合と，Brevicomin および oleoresin の場合の集合虫数は同程度で性比は雄が多い．Frontalin および oleoresin の場合には雌が雄の 2.6 倍多く集まる．雌には Brevicomin が多く含まれている．以上の結果より Frontalin と oleoresin が主に雌の集合食入を促進し，雌の食入後 Brevicomin を分泌して雄を誘引するものと考えられる．しかし詳細な集合の機作については解明されていない．

（桑原保正）