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<td>MOTOYAMA, Naoki; SAITO, Tetsuo</td>
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Kyoto University
The mode of action of saligenin cyclic phosphorus esters should be different from that of ordinary organo phosphorus insecticides.

Summary

About 50 ring-substituted derivatives of saligenin cyclic phosphorus esters were prepared and examined for insecticidal activity. No compound which was superior in the activity than unsubstituted salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulfide) was found. It appears that the electronic character of substituent at para-position to phenolic ester linkage is not correlate with the insecticidal activity.

Acknowledgement

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Substrate Specificity of Cholinesterases in Mites. Naoki Motoyama and Tetsuo Saito (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Nagoya) Received May 8, 1968, Bolyu-Kagaku 33, 77, 1968

11. ハダニのコリンエスターゼの基質特異性 本山直樹・斎藤哲夫（名古屋大学農学部害虫学教室，名古屋市）43.5.8 受理

ナミハダニ，カンザワハダニ，ミカンハダニおよびイエバエのコリンエステラーゼの数種コリンエステル類に対する特異性を，Hestrin の比色法を用いて比較した。

イエバエでは，アセチルコリンおよびプロピオニルコリンに対して鈍性型の活性度－pS曲線が示された。またブチルコリンに対しては，過剰基質による阻害がおこらなかった。一方ハダニでは供試した3種類とともに，プロピオニルコリンに対してのみ鈍性型の活性度－pS曲線を示し，ブチルコリンおよびアセチルコリンに対しては過剰基質による阻害がみとめられなかった。従って少なくともアセチルコリンに対する反応に関して，ハダニと昆虫の間にコリンエステラーゼの性質の差異が想像される。

Introduction

It is generally accepted that two types of cholinesterase, true cholinesterase and pseudo cholinesterase, exist in vertebrates[1]. In order to distinguish the two types of cholinesterase various methods have been examined. Augustinsson[2] demonstrated that the typical bell-shaped activity-pS curve of true cholinesterase was found in an electric eel, and the typical S-shaped activity-pS curve of pseudo cholinesterase in serum of blood. Cholinesterases in most insect species have been considered to be analogous to true cholinesterase in vertebrates, owing to the bell-shaped activity-pS curve for acetylcholine (ACh) 31[13]. Aphids31[13] were the exception which showed
S-shaped activity-pS curve for ACh. Kanehisa\(^{10}\) supposed that the slender rice bug adult, the rice stink bug, and the rice green catapilar adult might contain both types of cholinesterase, since the activity was once inhibited by an excess of acetylcholine and it was recovered again with further increase of the substrate concentration thus showing both-shaped activity-pS curves.

The presence of the cholinergic system in the two-spotted spider mite was proved by Mehrotra\(^7\). Relationship between the cholinesterase activity and ACh concentrations in the two-spotted spider mite was first investigated by Voss\(^8\), who found that no inhibition of the activity occurred at the high concentration of substrate. Dauterman and Mehrotra\(^9\), studying on N-alkyl group specificity of cholinesterases of the two-spotted spider mite, reported the similar results. Voss and Matsumura\(^{10}\) also examined some basic properties of cholinesterases in organophosphorus-resistant and -susceptible strains of the mite. Recently, Sakai\(^{11}\) mentioned the same phenomena in the Kanzawa spider mite with acetylthiocholine as the substrate.

In the present paper, substrate specificity of cholinesterases in three species of mites was compared with that in the housefly in order to confirm the difference in cholinesterase properties between mites and insects.

**Materials and Methods**

Three species of mites tested were the two-spotted spider mite, *Tetranychus urticae*, the Kanzawa spider mite, *Tetranychus kanzawai*, both reared on kidney bean plants, and the citrus red mite, *Panonychus citri*, reared on young citrus trees. Strain and rearing method of the housefly, *Musca domestica*, was the same as described by Kanehisa\(^9\).

Acetylcholine chloride (ACh), propionyl choline-\(p\)-toluensulfonate (PCh) and butyrylcholine iodide (BCh) were used as substrates at the final concentrations of \(3 \times 10^{-6}\)M, \(10^{-5}\)M, \(3 \times 10^{-5}\)M and \(10^{-4}\)M. Homogenates of mites (50mg/ml) and the housefly (10 flies/3ml) were prepared in ice-cold 0.067M phosphate buffer, pH 7.3, using a Potter-Elvejhem glass homogenizer. The homogenates were centrifuged for 10min. at 3,000 r.p.m. Mixtures of 0.025ml of the supernatants and 0.025ml of the substrates were incubated for 2hr, at 37°C. At the end of the reaction period the enzyme-substrate solutions were diluted appropriately with the buffer for the following colorimetric assay since concentrations of the substrates tested were too high for the assay method except \(3 \times 10^{-4}\)M.

The remaining substrates were determined by the method of Hestrin\(^{12}\) using a photometer Shimazu “spectronic 20” with a microcuvette of 0.1ml capacity. Non-enzymatic hydrolysis of substrates and non-specific colour caused by the homogenates were corrected.

**Results and Discussions**

The effects of substrate concentration on the hydrolysis of ACh, PCh and BCh by cholinesterases of the two-spotted spider mite, the Kanzawa spider mite and the citrus red mite, are respectively illustrated in Figs. 1, 2 and 3. No inhibition of the cholinesterase activity at the high concentrations of ACh and BCh occurred in all the species of mites tested. The present result for ACh agrees with that obtained manometrically in the two-spotted spider mite.\(^{13}\) The bell-shaped activity-pS curve was observed when PCh was used as the substrate. From the fact

![Fig. 1 Hydrolysis of acetylcholine (○), propionylcholine (△) and butyrylcholine (×) by cholinesterase of the two-spotted spider mite. pS = −log substrate concentration](https://example.com/fig1.png)
that the similar shapes of the activity-pS curves were also detected in the Kanzawa spider mite and the citrus red mite, it seems reasonable to suppose that the phenomena are considered as a general pattern of cholinesterases in mites.

Activity-pS curves for ACh, PCh and BCh with the housefly cholinesterase are given in Fig. 4. In contrast to those for mites, a distinct inhibition of the cholinesterase activity occurred at the high substrate concentrations of ACh as well as PCh. For BCh, however, no inhibition of the cholinesterase activity was observed even an excess of the substrate. Since it has been recognized that cholinesterases in the most species of insects studied except aphids show the similar pattern as found in the housefly, it may be said that there are some differences in nature of cholinesterase for the hydrolysis of ACh between the most species of mites and insects. However, since the enzyme preparations used in the present experiments were crude, it is still difficult to conclude that the difference found in the activity-pS curves is responsible truly for the difference in cholinesterase properties between mites and insects. In order to confirm this point, further experiments are necessary using more purified enzymes.

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Effectiveness of BHC Emulsifiable Concentrate on Adults of Cryptalus fulvus Niijima Living Beneath the Bark of Pine Tree. Studies on the Control of Forest Insects. III. Sumio NAGA
Received June 24, 1968. Botyu-Kagaku, 33, 80, 1968 (with English Summary, 85)

12. マンの樹皮下に穿入したキロロキクイムシに対する BHC 乳剤の有効度。林業害虫の
防除に関する研究。第 3 報、長沢純夫・浅野昌司：伏見静枝（イハラ農薬研究所）

林木の樹皮下に穿入して食する、いわゆる穿孔害虫の駆除に目的を有する殺虫剤の有効度
を測定するため、とくに乳剤として、乳剤を処方して一定期間経過した後に、その樹皮を剥
離して、その中の木の細菌を記録することによって
さされている。しかしこの方法によって、調査に
なる穿孔害虫をえた木の個体をあつめるには、とくに
個体の分散が大きい、大型のカミキリなどにおい
ては、きわめて困難である。こうした不都合を、ある程
度少なくして、最もたちらしい実験の有効度を観察
るひとつの試案として、筆者らは、マツマツを
出典していたマンノマグサカミキリ Monochamus
alternatus Hope の駆除に用いた、ホリサイド乳剤の
有効度の評価に、少数例を用いる個体別記録の解析法
を応用した。しかしこれら試案、乳剤の散布に多大の
労力を要する欠点があり、また観察者の生業判定
の誤差が大きく、ときに妥当な有効度の評価がで
ない場合が少なくない。筆者ら、その二つと誤差を
排除して、より能率的に正確な薬剤の効果を判定
することを目的に、乳剤を散布して一定期間後に、供試
木から観察する成虫の数だけをかぞえる方法によって、
有効度の評価をおこなうことがここにあたる。そうした
ことを意図した理由は、散布した薬剤の濃度と観察し
た成虫の数との関係がえられれば、無処理対照区にお
ける観察成虫数にもとづいて、各濃度レベルにおける致
死率を推定し、これからプロットを用いる最低効率
によって、濃度一時産率の方程式が算定できるからで
ある。この最低効率法は Wadley7 の方法で、最初のものと
べられ、Finney6 によってその直線解に体験して
られたもので、最近長沢ら9によって、昆虫の化学不
処剤の実験結果の解析にそのまま応用された。ところ
で Wadley7 の方法は、一供試単位あたりの虫の数が
ポアソン分布にしたがっているときに適用できるで
、もしこれが供試の二番分散布にしたがっている場合は、か
けるべき重みの計算に、Anscombe10 の modification
が必要である。それ故、林木の穿孔害虫の駆除薬剤の
試験結果を、最小二乗法によって整理する場合は、ま
ずそれらの供試木上における分布図を、あらかじめ
詳細に調査検討しておくことがおそらく、上記のい
ずれの分布にしたがっているかを決定した上で、計算
に入らないべきなら、とところもみたる散布の成
虫の数が、供試の二番分散布にしたがい、その分布の
指標である母数の計算がたのめんでどう、Bliss
and Fisher10 の最小二乗法によって場合などは、とくに
わずらわしく、そのために多大の時間を要する。しか