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 Universty, Nagoya) Received May 8, 1968, *Botyu-Kagaku* 33, 77, 1968

11. ハダニのコリンエステラーゼの基質特異性 本山直樹・斉藤哲夫（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鐘状型の活性度—pS曲線が示された．またブチリ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種類とも，プロピオニルコリンに対してのみ鐘状型の活性度—pS 曲線を示し，ブチリ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) [3)4)5)]. Aphids[5)6)] were the exception which showed

S-shaped activity-pS curve for ACh. Kanehisa[5] 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 occured at the high concentration of substrate. Dauterman and Mehrotra[9], studying on *N*-alkyl group specificity of cholinesterase 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[5].

Acetylcholine chloride (ACh), propionyl choline-*p*-toluensulfonate (PCh) and butyrylcholine iodide (BCh) were used as substrates at the final concentrations of 3×10^{-3}M, 10^{-2}M, 3×10^{-2}M and 10^{-1}M. Homogenates of mites (50 mg/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 3×10^{-3}M.

The remaining substrates were determined by the method of Hestrin[12] using a photometer Shimazu "spectronic 20" with a microcuvette of 0.1 ml 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.[8,9] The bell-shaped activity-pS curve was observed when PCh was used as the substrate. From the fact

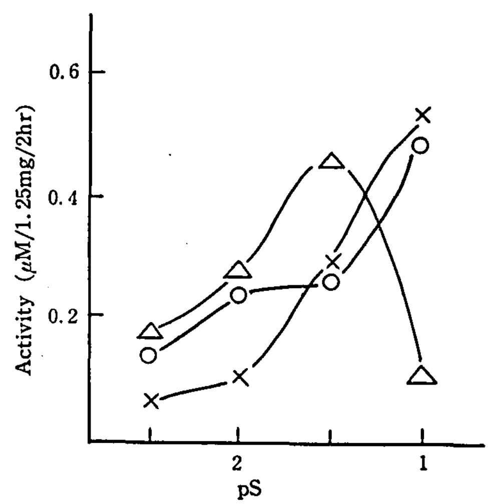

Fig. 1 Hydrolysis of acetylcholine (○), propionylcholine (△) and butyrylcholine (×) by cholinesterase of the two-spotted spider mite.
pS = −log substrate concentration

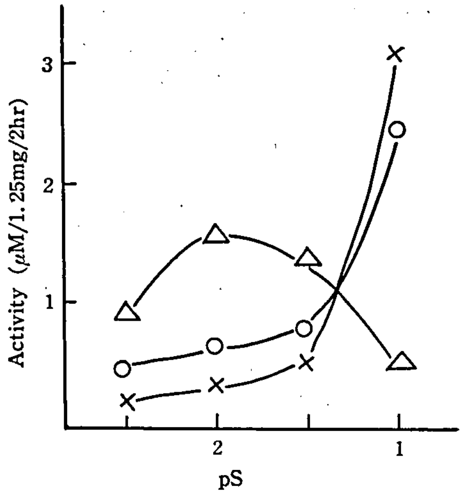

Fig. 2 Hydrolysis of acetylcholine (○), propionylcholine (△) and butyrylcholine (×) by cholinesterase of the Kanzawa spider mite.
pS=−log substrate concentration

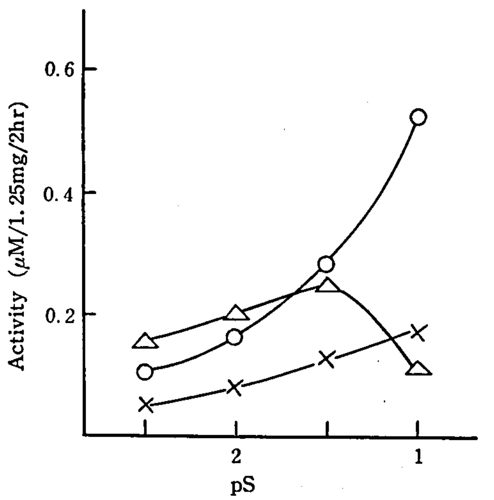

Fig. 3 Hydrolysis of acetylcholine (○), propionylcholine (△) and butyrylcholine (×) by cholinesterase of the citrus red mite.
pS=−log substrate concentration

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 occured at the high substrate concentrations of Ach as well as

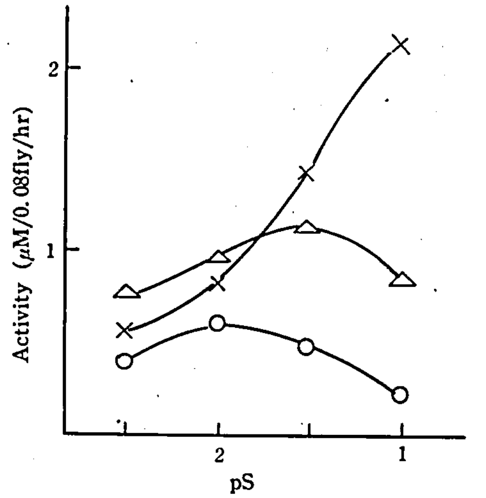

Fig. 4 Hydrolysis of acetylcholine (○), propionylcholine (△) and butyrylcholine (×) by cholinesterase of the housefly.
pS=−log substrate concentration

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[4)5)], 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 *Cryphalus fulvus* Niijima Living Beneath the Bark of Pine Tree. Studies on the Control of Forest Insects. III. Sumio NAGASAWA, Shoji ASANO, and Shizue FUSHIMI (Ihara Agricultural Chemicals Institute, Shimizu).
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12. マツの樹皮下に穿入したキイロコ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害する，いわゆる穿孔害虫の駆除を目的に調製された，殺虫剤の有効度を見積るための，ひとつの方法として，薬剤を処理して一定期間後に脱出した成虫の数をかぞえるだけの記録を，プロビット法によって解析し，その中央有効値をもとめる試みをおこなった．キイロコキクイムシの食害をうけた，クロマツの樹枝部を実験材料に，薬剤には BHC 乳剤をもちいて，まず，この虫の樹枝上における羽化数が，負の二項分布にしたがうことをたしかめた．その平均値と供試本数とで，各濃度段階においてえられた総羽化成虫数を割ることによって生存率をもとめ，普通のプロビット解析法の適用を可能ならしめた．なおこのときの重み係数は標本が負の二項分布にしたがっているときに適用される Anscombe の式によって算定した．

林木の樹皮下に喰い入って加害する，いわゆる穿孔害虫の薬剤による駆除試験は，通常所定量の薬剤を被害木に撒布し，一定期間経過した後に，その樹皮を剥離して，その中の生死の虫数を記録することによってなされている．しかしこの方法によって，満足するにたる殺虫率をえるための個体をあつめるには，とくに個体の分散が大きい，大型のカミキリムシなどにおいては，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，最もたしからしい薬物の有効度を見積るひとつの試案として，筆者ら[6]はさきに，クロマツを加害していたマツノマダラカミキリ *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．しかしこれとても，樹皮の剥離に多大の労力を要する欠点があり，また観察者による生死判定の誤差が大きく，ときに妥当な有効度の評価ができない場合がないでもない．筆者らは，そうした労力と誤差を排除して，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，薬剤を撒布して一定期間後に，供試木から脱出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をこころみた．そうしたことを意図した理由は，撒布した薬剤の濃度と脱出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脱出成虫数にもとづいて，各濃度段階における致死率を推定し，これからプロビットを用いる最尤法によって，濃度-致死率曲線の方程式が算定できるからである．この最尤推定法は Wadley[7] によって最初にのべられ，Finney[4] によってその直後詳細に体系づけられたもので，最近長沢ら[5] によって，昆虫の化学不妊剤の実験結果の解析にそのまま応用された．ところで Wadley[7] 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が，もしこれが負の二項分布にしたがっている場合は，かけるべき重みの計算に，Anscombe[1] 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，最尤推定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様式を，あらかじめ詳細に調査検討しておくことがのぞましく，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，計算に入らなければならない．ところでもし脱出する成虫の数が，負の二項分布にしたがう場合は，その分散の指標である母数 k の計算がまためんどうで，Bliss and Fisher[3] の最尤法によった場合などは，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