Ketosteroids As Arrestance s to *Oryzaephilus surinamensis* (L.) from Wheat Flour Infested by the Same Weevil

Shunei Nakajima, Ako Okamura, Kenji Sugawara, Taro Takeda, Junkiichi Iwasa and Naomichi Baba

(Department of Bioreources Chemistry)

From hexane extract of wheat flour infested by the sawtoothed grain beetle (*Oryzaephilus surinamensis* (L.); Coleoptera; Silvandae), three ketosteroids, cholestane-3-one (3), ergostane-3-one (4) and stigmasteran-3-one (5), were obtained in a mixture and identified as arrestance to this weevil.

Key words: *Oryzaephilus surinamensis* (L.), infested wheat flour, arrestance, cholestane-3-one, ergostane-3-one and stigmasteran-3-one.

Introduction

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) is an economically important stored-product pest of worldwide distribution and is known to infest wheat graine which have been broken or milled by other stored-product insects. To understand the ecological aspects, we have studied the behavior of the insect in the infestation on the basis of pheromone chemistry. It was disclosed that in wheat flour infested by *O. surinamensis* some substances existed which were not contained in the fresh flour but had arrestance activity to the same pest.

From the hexane extract, two arrestance was isolated by us and identified as 13-oxo-(Z)-9-octadecenoic acid (1) and 15-oxo-(Z)-11-octadecenoic acid (2). (Fig. 1) During the course of isolation of the two compounds above, we found that the hexane extract still contained at least one other active substance.

In this report, purification and structural elucidation of such substances in the hexane extract of infested wheat flour are described.

![Diagram of Structures of Arrestance Isolated from Infested Wheat Flour](image)

Fig. 1 Structures of arrestance isolated from infested wheat flour.

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Materials and Methods

General

$^1$H NMR spectrum was recorded at 500 MHz on VXR 500 NMR (Varian, EIM) was measured on D 300 (GEOL), FT-IR on 710 GC (Nicolet) and UV on UV-3000 (Shimadzu) spectrometer while GC was determined on G 3000 Gas Chromatography (Hitachi) attached to Hitachi D-2200 Chromato-Integrator with $N_2$ as the carrier gas on an OV 1 column (0.25mm×5m). Column chromatography was done on silica gel 60 (Nacalai tesque Inc., 230-400 mesh). TLC was on Kieselgel 60 F$_{254}$ (Merck, Art. 5554, 9.2mm) and reverse phase was done using TLC RP-18, Merck, Art. 15685, 0.25mm) and Sep-Pak$^3$ (C18, Waters) cartridges.

Compounds on TLC were detected by four spray systems: Vanillin-sulfuric acid, 2,4-dinitrophenylhydrazine, anisaldehyde and antimony trichloride.

Experimental insects

The colonies of O. surfinaensis were maintained on wheat flour containing 5% (w/w) brewer’s yeast at 26-28°C in the dark. Test beetles were starved from 3 to 7 days prior to being used in bioassay.

Two-choice bioassay

A Petri dish (9 cm dia.) with two filter paper disks (5 cm dia.) was used as a test arena for arctic activity, and one of the two disks was treated with sample solution (sample) or solvent (control). Here, the assaying activity is defined as the number of insects arranged on a paper disk that is immersed with the sample solution to be tested. Percent (%) response was calculated by a formula $100(T-B)/N$, when $T$ and $B$ were the number of beetles on the treated and blank disks, respectively, after 10min at 26-28°C in the dark, and $N$ was total number of beetles released into the dish.

Preparation of Ketosteroids

The ketosteroids (3), (4) and (5) were prepar-
Results and Discussion

A hundred and ten grams of wheat flour infested for a few months by *O. surinamensis* at all life stages was passed through a sieve for removal of the insects and extracted with 500ml of hexane. After evaporation of the solvent, the residue (0.47 kg) was chromatographed repeatedly on silica gel eluted with hexane/ethyl acetate (0:1 and 95:5). Further purification was done on silanized precoated prep. TLC developed with MeOH followed by silver nitrate-impregnated prep. TLC developed with hexane/ethyl acetate (95:5). Two milligrams of the active substance were obtained as a single spot on TLC (hexane/ethyl acetate, 9:1). The arrestance activity is shown in Figure 3.

Absorption at 1717 cm⁻¹ on FT-IR spectrum of the active substance as well as coloration on TLC with 2, 4-dinitrophenyldihydrazine and antimony trichloride suggested the presence of steroids having a carbonyl group.

On the ^1^H NMR spectrum (Fig. 4), no proton signals in the field lower than 4.25 were observed, suggesting the absence of double bonds and oxygenated methylene and/or methane groups. The spectrum also showed that the substance had a long alkyl side chain.

The EIMS spectrum (Fig. 5) showed prominent peak at m/z 414, accompanied by a peak at m/z 460 and a small one at m/z 386 which were separated from the highest mass ion at m/z 414 by CH₂ and (CH₃)₂, respectively. It is to be expect-
ed that such mass differences are encountered when these species of ions are produced by a mixture of homologous components in the active substance. Additionally, three major peaks were detected on the capillary GC chromatogram (Fig. 8). Although further purification of the mixture was attempted, it was impossible to separate out each component by conventional methods.

On the other hand, all insects need a dietary source of sterols for normal growth and reproduction because they lack the biosynthetic pathway of steroid skeleton from mevalonic acid and are unable to synthesize sterols. Since the wheat flour used in this experiment is rich in phytosterols, it is considered that O. surinamensis uses these phytosterols or their fatty acid esters for dietary requirements and probably modifies a part of them to oxidative products.

Based on such speculation and all of the above spectral data, the active substance seems to be a mixture of three saturated keto steroids, i.e., cholestan-3-one (3), ergostan-3-one (4) and stigmastan-3-one (5), which might be converted by this insect from corresponding phytosterols in fresh wheat flour.

Thus, for the confirmation of the structures of the individual components, three commercially available sterols, cholesterol, campesterol and β-sitosterol, which reflect the possible origin of the active substance, were chosen for starting materials and chemically modified by hydrogenation and Jones oxidation to the desired ketosteroids. (Fig. 3)

These three synthesized ketosteroids were mixed at the same ratio calculated from the natural mixture and such an artifi-cial mixture was submitted to GC and spectral analysis. The result was that the artificial mixture was identical to the natural product with respect to the pattern of the 1H NMR spectrum, EI MS spectrum and also GC retention time. Since the blend of three synthetic ketosteroids also showed an assay activity equivalent to the natural one, the proposed structures of these compounds were confirmed.

Some steroids with pheromonal activity have been identified in vertebrates and inverte-roids as insect hormones represent a widespread family of steroid found in many invertebrates as well as in many plants. However, steroids are uncommon in insect pheromones, except for a trail pheromone of the tent caterpillar, Malacosoma americanum, identified as 5α-cholestan-3, 24-dione and recently, two aggregation pheromones of the German cockroach, Blattella germanica, identified as characteristic chlorinated steroid glucosides, blattellastanoside-A and B. It is, therefore, interesting that O. surinamensis may produce a mixture of ketosteroids which are utilized by themselves for intra-specific communication.

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Arrestants in infested wheat root

ノコギリヒラタムシ食害小麦に含まれる
定着活性ケトストロイド

中島 修平・呑村 愛子・菅原 敬二・竹田 太郎
岩佐 順吉・馬場 直道  

(生命農業開発学科)

世界的に著名な害虫であるノコギリヒラタムシによって食害された小麦のハキサン抽出物には、未
食害の小麦には含まれない、数種のノコギリヒラタムシ定着活性物質が存在し、このうち2種の活性物質が
既に構造解明された。本研究では数種の基盤成分、および市場化物から誘導などにより、未食の活性物
質がcholestan-3-one, ergostan-3-one, stigmastan-3-oneの混合物であると同定した。