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

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From hexane extract of wheat flour infested by the sawtoothed grain beetle [*Oryzaephilus surinamensis* (L.) ; Coleoptera ; Silvanidae] , three ketosteroids, cholestan-3-one (**3**), ergostan-3-one (**4**) and stigmastan-3-one (**5**), were obtained in a mixture and identified as arrestants to this weevil.

Key words : *Oryzaephilus surinamensis* (L.), infested wheat flour, arrestants, cholestan-3-one, ergostan-3-one and stigmastan-3-one.

Introduction

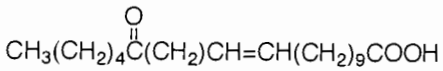
The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) is an economically important

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 arrestant activity to the same pest[1].

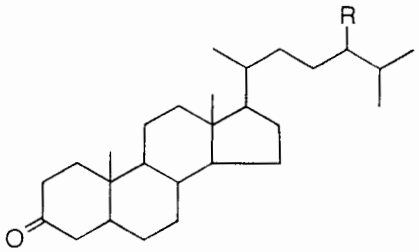
From the hexane extract, two arrestants was isolated by us and identified as 13-oxo-(*Z*)-9-octadecenoic acid (**1**) and 15-oxo-(*Z*)-11-icosenoic acid (**2**)[2]. (Fig. 1) During the course of isolation of the two compounds above, we found that the hexane extract still contained at least one other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

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

(1)



(2)



(3) R = H (cholestan-3-one)
(4) R = CH_3 (ergostan-3-one)
(5) R = C_2H_5 (stigmastan-3-one)

Fig. 1 Structures of arrestants 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), EIMS was measured on D 300 (JEOL), FT-IR on 710 GC (Nicolet) and UV on UV-3000 (Shimadzu) spectrometer while GC was determined on G 3000 Gas Chromatograph (Hitachi) attached to Hitachi D-2100 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, 0.2mm) and reverse phase was done using TLC (RP-18, Merck, Art. 15685, 0.25mm) and Sep-Pak® (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. surinamensis* were maintained on wheat flour containing 5% (w/w) brewer's yeast at 26-28°C in the dark. Test beetles were starved from 5 to 7 days prior to being used in bioassay.

Two-choice bioassay

A Petri dish (4cm dia.) with two filter paper disks (5mm dia.) was used as a test arena for arrestive activity, and one of the two disks was treated with sample solution (sample) or solvent (control). Here, the arrestant activity is defined as the number of insects arrested on a paper disk that is impregnated 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 the total number of beetles released into the dish.

Preparation of Ketosteroids

The ketosteroids (**3**),(**4**) and (**5**) were prepared from the corresponding sterols. (Fig. 2) For example, preparation of cholestan-3-one (**3**) was carried out in two steps as follows: a hundred milligrams of cholesterol (0.26mmol) was dissolved in 3ml of acetic acid, and platinum oxide (59mg) was added. The mixture was stirred under hydrogen atmosphere at room temp. for 24hr. After filtration, acetic acid was separated as the toluene azeotrop and solvent was evaporated under reduced pressure. Purification of the residue with silica gel column chromatography gave 70mg of cholestanol (0.18mmol) which was oxidized in acetone by constant stirring with chromium trioxide for 30min at 0°C. Usual workup and purification with silica gel column chromatography afforded 60mg of cholestan-3-one (**3**) in 60% total yields.

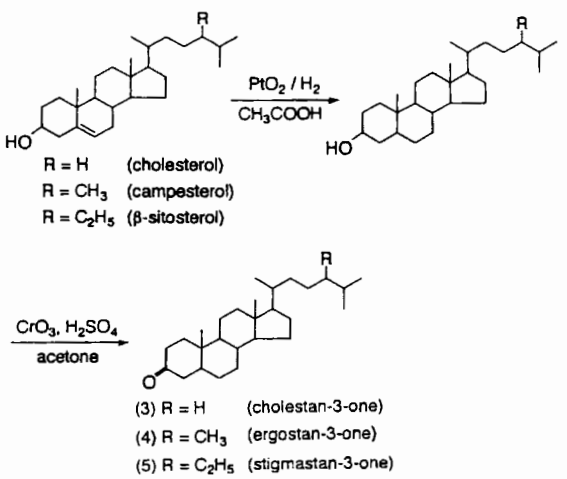

Fig. 2 Preparation of the three ketosteroids from phytosterols.

Ergostan-3-one (**4** , 64% yield) and stigmastan-3-one (**5** , 26% yield) were also prepared as above from the commercially available sterols, campesterol and β-sitosterol, respectively.

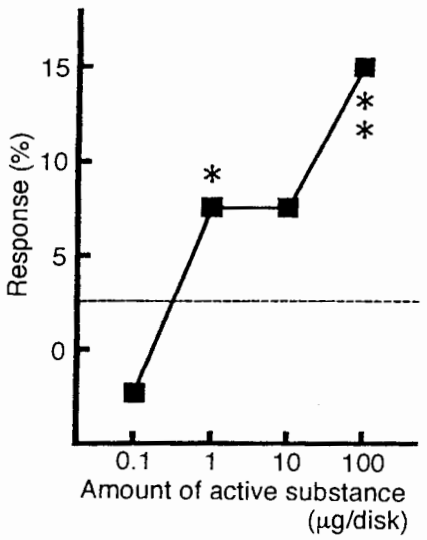

Fig. 3 Response by mixed-sex *O. surinamensis* in two-choice bioassay. Significant response (paired-sample t test) to the active components indicated by : *$p<0.1$; **$p<0.05$

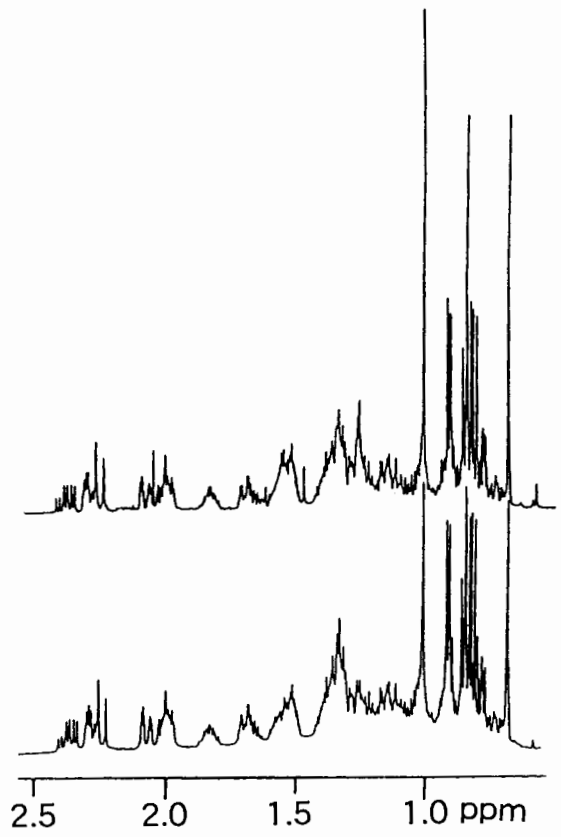

Fig. 4 ^{1}H NMR spectra of the mixture obtained from infested flour (upper) and that of the prepared mixture (lower).

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 g) was chromatographed repeatedly on silica gel eluted with hexane / ethyl acetate (9 : 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 arrestant 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-dinitrophenylhydrazine 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 δ2.5 were observed, suggesting the absence of double bonds and oxygenated methylene and/or methine 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 400 and a small one at m/z 386 which were separated from the highest mass ion at m/z 414 by CH_2 and $(CH_2)_2$, respectively. It is to be expect-

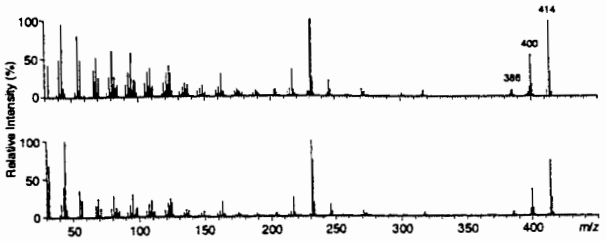

Fig. 5 EIMS spectra of the mixture obtained from infested flour (upper) and that of the prepared mixture (lower).

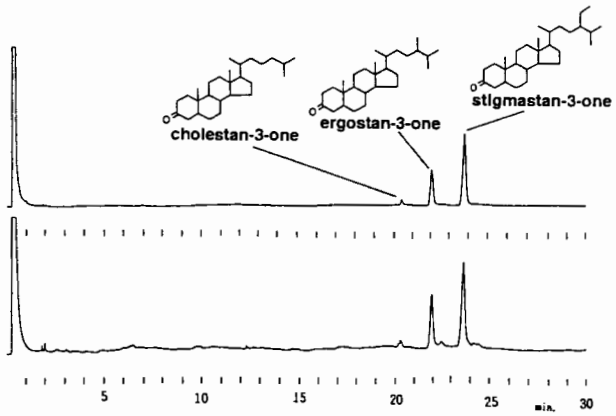

Fig. 6 GC charts of the mixture obtained from infested flour (upper) and that of the prepared mixture (lower).(OV 1, 0.25 mm×5 m, 100-270°C, 10°C/min)

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. 6). Although further purification of the mixture was attempted, it was impossible to separate out to 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[3]. 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 ketosteroids, 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. 2)

These three synthesized ketosteroids were mixed at the same ratio calculated from the natural mixture and such an artificial 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 ^{1}H NMR spectrum, EIMS spectrum and also GC retention time. Since the blend of three synthetic ketosteroids also showed arrestant activity equivalent to the natural one, the proposed structures of these compounds were confirmed.

Some steroids with pheromonal activity[4,5] have been identified in vertebrates and ecdysteroids as insect hormones represent a widespred family of steroid found in many invertebrate as well as in many plants[6,7]. However, steroids are uncommon in insect pheromones, except for a trail pheromone of the tent caterpillar, *Malacosoma americanum*[8,9], identified as 5β-cholestan-3, 24-dione[10] and recently, two aggregation pheromones of the German cockroach, *Blattella germanica*, identified as characteristic chlorinated steroid glucosides, blattellastanoside-A and B[11]. It is, therefore, interesting that *O. surinamensis* may produce a mixture of ketosteroids which are utilized by themselves for intraspecific communication.

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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テロイド

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世界的に著名な貯穀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ヘキサン抽出物中には，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．本研究では種々の機器分析，および市販化合物からの誘導などにより，未知の活性物質が cholestan-3-one，ergostan-3-one，stigmastan-3-one の混合物であると同定した．