# RESEARCH PAPERS

## THE ISOLATION AND IDENTIFICATION OF BEETLE FRAGMENTS FROM POWDERED VEGETABLE DRUGS

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### INTRODUCTION

VEGETABLE drugs are required by the British Pharmacopœia 1948 to be free from insects and other animal matter and it follows from this statement that such matter is not to be tolerated under the term "foreign organic matter." Most vegetable drugs whether whole or powdered are very prone to attack by insect pests especially when carelessly stored. The presence of whole insects is readily detectable by examining the material directly or by sieving. If infested material has been subsequently powdered, any eggs present are usually killed and unless reinfestation occurs no whole insects will be found. Detection must then be based on whatever insect fragments are present and since most drug pests are very small creatures, the weight of the more common beetles being of the order of 1 mg. and that of the identifiable fragments much less, even high infestations may be overlooked by direct microscopical examination. Some method of concentrating the insect fragments must therefore be adopted. The identification of fragments as being of insect origin is not however sufficient evidence that the material was infested. Certain drugs, particularly herbs such as hyoscyamus, not infrequently contain small insects which were associated with the growing plant and were not removed during the preparation for the market. Large insects such as the cockroach may also occasionally find their way into drugs and unless their identity was realised, the powdered material might be reported as highly infested. Thus a study of the diagnostic microscopical characters of drug pests appears desirable. In the present paper, methods for the isolation of insect fragments are considered, and the diagnostic microscopical characters of some of the more common beetle pests of drugs described.

### THE ISOLATION OF INSECT FRAGMENTS

For purposes of identification, the soft parts of insects may be ignored and only the exoskeleton need be considered. This consists of chitin impregnated with sclerotin and other substances, and is resistant to many chemical reagents, including boiling dilute mineral acids. Boiling caustic alkalies tend to remove the colouring and hardening materials without producing other visible changes. The exoskeleton is also water repellant but is readily wetted by petroleum and similar non-polar liquids.

Thus two methods of isolating insect fragments from powdered drugs are available; floating off with a non-polar liquid from an aqueous suspension, or solution of the vegetable material. Flotation methods are used extensively for the examination of foodstuffs and are particularly

suitable for starchy materials containing little cellulosic matter. In the author's experience, they have not afforded the complete separation of insect fragments when applied to powdered drugs. A method involving solution of the cellulosic material of infected drugs was devised by Greenish[1], but does not appear to have been generally adopted. It consists of boiling the defatted powder in 5 per cent. hydrochloric acid, macerating the washed residue for 18 to 48 hours in a mixture containing about 12·5 per cent. of sulphuric acid and 20 per cent. of chromic acid and separating the insect fragments by centrifugation. This method is not entirely satisfactory because solution of the vegetable material is not always complete and because of the time involved. The use of acetolytic methods appeared to the author to be more suitable and it was decided to investigate their practicability. Preliminary experiments with the usual laboratory methods of acetylation were made on absorbent cotton and on tow (lignocellulose). These materials dissolved most readily and completely in acetic anhydride containing 10 per cent. of concentrated sulphuric acid. With vegetable drugs however, solution was not always complete and an amorphous sludge sometimes remained. This was thought to be due to the non-cellulosic constituents since the crude fibre from these drugs was found to dissolve completely. After various trials the following method was evolved.

*Method.*—Boil about 5 g. of the powdered drug in a flask with 100 ml. of 10 per cent. w/w nitric acid in water for about 1 minute with frequent shaking. Filter through a No. 3 sintered glass filter, using suction, and wash the residue with hot water. Return the residue to the flask and boil for about 1 minute with 100 ml. of 2·5 per cent. sodium hydroxide solution, filter through the original filter and wash the residue with hot water. Remove the excess of water by suction and the last traces by passing a few ml. of glacial acetic acid through the filter. Transfer the residue as completely as possible to a flask of about 50 ml. capacity, and wash the remaining fragments into it with 10 ml. of acetic anhydride. Add a mixture of 10 ml. of acetic anhydride with 2 ml. of concentrated sulphuric acid, mix well and heat on a boiling water-bath until the crude fibre has dissolved. Solution is usually complete after about 10 to 15 minutes during which the liquid becomes dark reddish brown. Separate the residue by centrifugation, pour off the supernatant liquid, replace with glacial acetic acid and recentrifuge. After pouring off the glacial acetic acid, the residue of insect fragments may be mounted in any desired mountant for microscopical examination. Alternatively, the acetic anhydride can be hydrolysed before centrifugation by gradually adding the contents of the flask to about 10 ml. of water. Where infestation is heavy and the finest fragments are not required, the contents of the flask may be passed through the sintered glass filter and the residue washed with glacial acetic acid followed by water. It can then be removed by adding a few drops of water or mounting fluid to the filter and gently brushing with a small stiff brush. Passage of air the reverse way through the filter by connecting the side-arm of the receiving flask to a water tap facilitates the removal. If the drug contains much oil or fat it is pre-

ferable to remove most of it by maceration for a short time with one or two changes of light petroleum or similar solvent before preparing the crude fibre.

## Identification of the Fragments

The beetles most frequently infesting stored vegetable drugs[2] are *Stegobium paniceum* L. (the drug-room beetle) and *Ptinus tectus** Boie. (the brown spider beetle). Less common but of frequent occurrence are *Niptus hololeucus* Fald. (the golden spider-beetle) and *Calandra granaria* L. (*the grain weevil*). *Lyctus brunneus* Steph., one of the powder post beetles, although primarily a pest of timber has been reported on vegetable drugs e.g. liquorice[3]. In these laboratories it has been found on Butea seeds and was recently introduced on Shensi rhubarb on which it appears to thrive. The following account is confined to these five species with the addition of a reference to the common cockroach *Blatta orientalis* L.

*Materials.*—A sample of Jamaica ginger was examined for freedom from infestation and then coarsely chopped. For each species 10 beetles were mixed with about 30 g. of the material and powdered in a steel laboratory end-runner mill until fine enough to pass through a No. 60 sieve. About 5 g. quantities were treated by the method described above and the residue mounted in cedar-wood oil for microscopical examination. Further mixtures with other drugs were subsequently prepared and the residue after removal of the vegetable material also examined. It consisted of fragments from the added beetles together with the acid-insoluble ash if any. With some drugs, especially leaves or herbs, the residue often contained pollen, some foreign to the particular drug, also various fungal spores and hyphæ.

The following descriptions were made from these fragments after reference to the whole insects cleared by boiling in 2·5 per cent. sodium hydroxide solution. With a few exceptions indicated by the absence of a surrounding line, the sketches were also made from the fragments. Drawing was done with the aid of a camera lucida at an original magnification of $\times 500$, or for mandibles and antennal joints $\times 200$.

*Microscopical Characters. General.*—The fragments consist of the cuticle of the beetle, the soft parts having been dissolved. Fragments from the body are irregular in outline, yellowish-brown and translucent. The larger joints of the appendages are also broken but the smaller ones such as those of the antennæ and tarsi are often intact. In life, the beetles are clothed with hairs all but a few of which become detached during the powdering process, but leave scars to mark their position and frequency. The detached hairs are usually too finely broken to be recognisable although occasional ones are found intact. The hairs of the species described are of two general types, bristle-like articulated setæ, and smaller and relatively more numerous clothing hairs. On the fragments, the positions of the former are marked by the setal scars which consist of a circular puncture enclosed within a concentric ring whereas the clothing

* Wallis mentions *P. brunneus* Dft. but *P. tectus* appears the more common of the two species.

hairs leave minute usually simple scars referred to subsequently as micro-punctures. The fragments proving of most value for identifying the beetles were derived from the elytra, the prothorax and the head, those from other segments being less characteristic. Elytral fragments are recognised by the presence of coarse punctures arranged in rows—the strial punctures, which usually have a densely coloured rim. They are surrounded by an oval or irregular area slightly darker than the general colour and apparently due to a local thickening or ingrowth of the cuticle. These areas may be absent from some fragments if the cuticle has laminated. In the majority of cases, the rows of strial-punctures are parallel except on fragments from the ends of the elytra. In certain species, coarse punctures resembling the strial punctures occur elsewhere than on the elytra. These can be recognised either by their irregular arrangement or if they are in rows, by their closeness to the rim of the segment which is usually also present.

The outer surface of the fragments may be smooth or raised into tubercles or ridges. The latter often enclose polygonal areas which probably correspond with the original cells of the epidermis. Mandibles are rarely found unbroken but the thick biting edge is often intact in which case the number and arrangement of the teeth is a useful character. The shape and size of the terminal joints of the antennæ is also of value, while their number together with that of the mandibles gives an indication of the number of beetles originally present.

*STEGOBIUM PENICEUM* L.

*Elytra:* strial punctures 10 to 50μ apart in parallel rows, slit-like about 20 to 30μ long, with on each side one or rarely two rounded tubercles, each tubercle associated with a micro-puncture; the whole surrounded by an oval area about 30 to 50μ long: intervals between the rows 100 to 150μ broad, with setal scars about 10 to 15μ diameter, mainly in a single median row and separated by about 20 to 70μ: over the entire surface, numerous scattered micro-punctures separated by up to about 20μ. (Fig.1.C.)

*Prothorax:* tuberculate; tubercles prominent, rounded conical, furrowed and irregularly dentate at the base, about 10 to 20μ diameter, contiguous or separated by up to about 50μ, each closely associated with a setal scar; intervals micropunctate like the elytra. (Fig. 1.E.)

*Head:* dorsal surface similar to the prothorax but with less prominent tubercles; ventral surface ridged, ridges curved to semicircular, distinct or confluent forming crenate rows, each ridge overhanging a shallow depression containing a hair or its scar. (Fig. 1.A.)

*Antenna:* terminal joint ellipsoidal about 250μ by 70μ, constricted at the base, hairy. (Fig. 1.F.)

*Mandible:* biting edge about 150μ long, tridentate, the teeth diminishing in size from apex to base (Fig. 1.B); in the larva, the subterminal teeth prominent and connected by a cutting edge. (Fig. 1.G.)

*Hairs:* setæ; cylindrical, about 100 to 150μ long, acuminate, moderately thick walled, straight or slightly curved; clothing hairs similar but smaller, about 30 to 50μ long. (Fig. 1.D.)

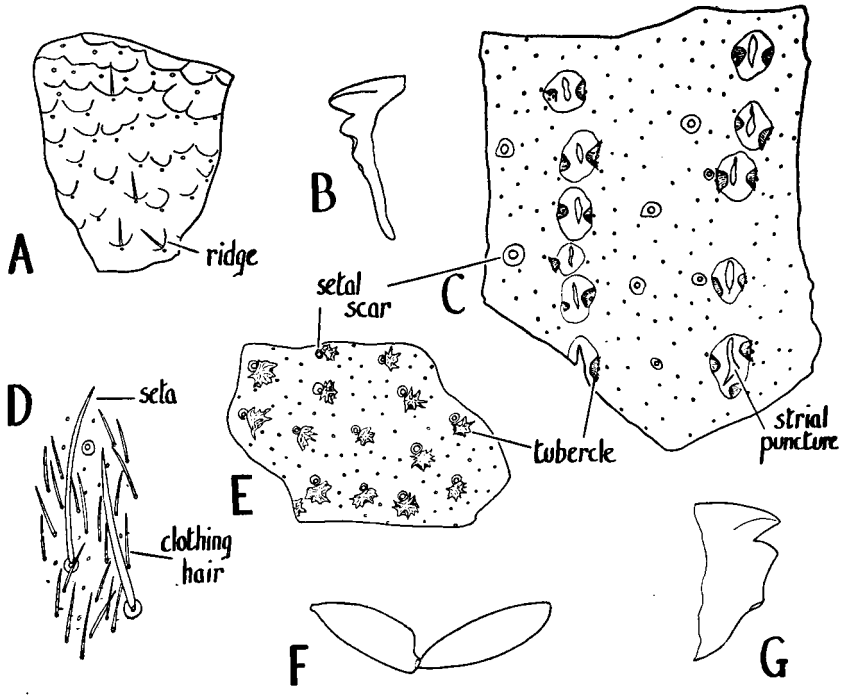


Fig. 1. *Stegobium paniceum* L.

A. Head, fragment from ventral surface showing ridges and an occasional hair, × 200. B. Biting edge of a mandible. G. The same from a larva, ×80. C. Elytron, fragment from the middle region showing two rows of strial punctures, × 200. D. Hairs from elytron of a whole insect, × 200. E. Prothorax, fragment showing tubercules with associated setal scars, × 200. F. Antenna, terminal and subterminal joints, hairs not shown, × 80.

*PTINUS TECTUS* BOIE.

*Elytra:* strial punctures up to about 30μ apart in parallel rows, oblong to elliptical about 25 to 50μ long with a thickened rim and surrounded by an oval or rounded area about 60 by 55μ; intervals between the rows 80 to 100μ broad, with a single median row of setal scars about 5 to 7μ diameter and separated by 40 to 60μ; over the entire surface, numerous scattered micropunctures separated by up to about 20μ. (Fig. 2.A.)

*Prothorax:* central region of the pronotum coarsely punctate, punctures irregularly arranged, deep, circular to irregularly oblong about 40 to 80μ long, resembling the strial punctures of the elytra but sometimes lacking the surrounding area; intervals with scattered setal scars and micropunctate like the elytra, Fig. (2.D): remaining regions locally thickened, thickened areas isolated, more or less circular and surrounding a setal scar, or confluent, irregular and enclosing up to about 5 setal scars; surface over the areas micro-punctate, intervals impunctate. (Fig. 2.C.)

*Head:* coarsely punctate, punctures numerous scattered and separated by up to about 50μ, very shallow, circular to oval about 30 to 80μ diameter each associated with a setal scar; intervals micropunctate like

the elytra. (Fig. 2.B.) Similar fragments from the thoracic and abdominal segments also occur.

*Antenna:* terminal joint ellipsoidal, about 270 by 100μ and 30 to 40μ in diameter at the base, hairy. (Fig. 2.F.)

*Mandible:* biting edge about 120μ long with two teeth, apical tooth acute, basal tooth subacute. (Fig. 2.G.)

*Hairs:* setae; resembling those of *S. paniceum* but rather longer and

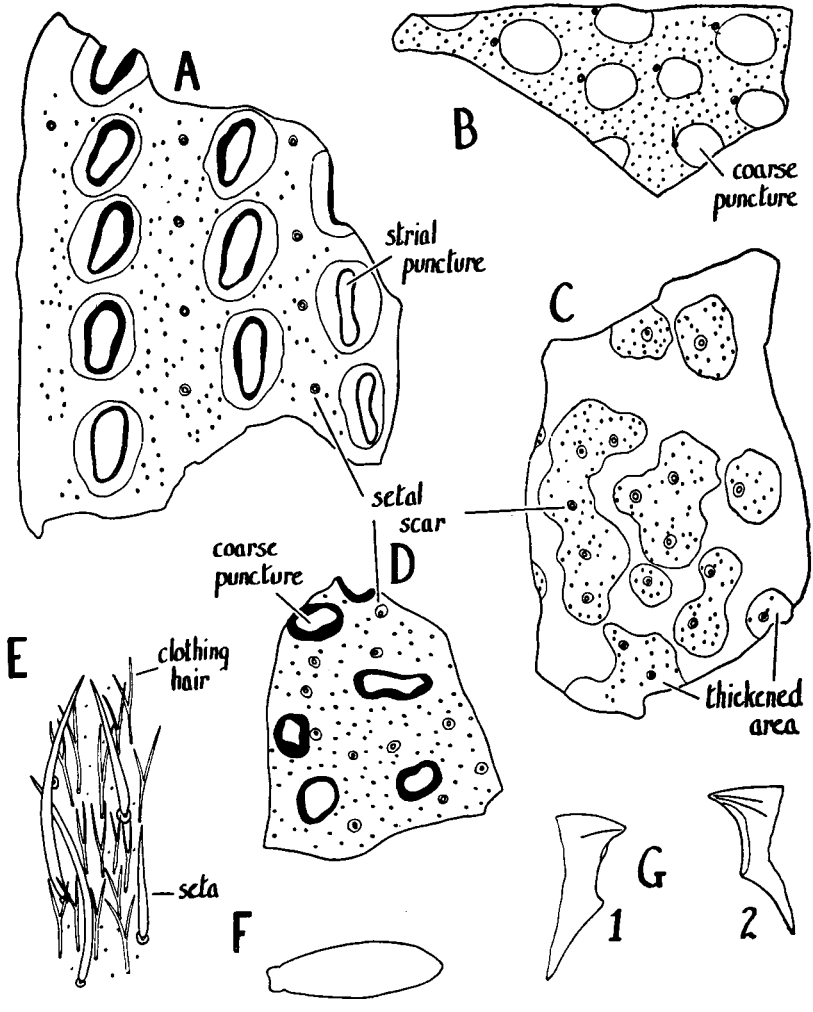


Fig. 2. *Ptinus tectus* Boie.
A. Elytron, fragment from middle region. B. Head, fragment showing coarse shallow punctures with associated setal scars. C. and D. Prothorax; C, fragment from near the head showing isolated locally thickened areas; D, from central region of the pronotum showing coarse deep punctures. E. Hairs from elytron of a whole insect. A to E, × 200. F. Terminal joint of antenna, hairs not shown, × 80. G. Biting edge of mandibles: 1. larval; 2. adult, × 80.

wider; clothing hairs Y-shaped, cylindrical with acuminate arms, about 40 to 60μ long. (Fig. 2.E.)

*NIPTUS HOLOLEUCUS* FALD.

*Elytra:* strial punctures about 100μ apart in parallel rows, circular about 5 to 10μ diameter with a deeply pigmented rim about 20μ diameter, each surrounded by a rounded to oblong concentrically striated area about 120 by 80μ having an indented margin and sometimes exhibiting a number of fissures radiating from the puncture; co-linear with the strial punctures, a row of setal scars, one associated with each puncture and separated from it by 20 to 40μ*; intervals between the rows about 200μ broad, with setal scars about 5 to 10μ diameter mainly in a single median row and separated by about 50 to 100μ; over the entire surface numerous scattered micropunctures separated by up to about 20μ. (Fig. 3.A.)

*Prothorax:* over the greater part, scattered setal scars and micro-punctures similar to those of the elytra; near the margin one or sometimes two rows of coarse punctures separated by about 30 to 70μ, punctures deep, circular to oblong about 20 to 40μ long with a thickened rim and sometimes an enclosing circular to oval area about 40 to 80μ long. (Fig. 3.C.)

*Head:* over the greater part, scattered setal scars and micro-punctures; near the margin tuberculate, tubercles contiguous or separated by up to about 20μ, conical, each subtending a short hair; extreme margin reticulately ridged, the ridges enclosing elongated polygonal areas about 15 to 60μ long and 15μ wide. (Fig. 3.F.)

*Other regions; meso- and metosternites, some joints of the appendages:* sparsely micropunctate and reticulately ridged, the enclosed areas more or less regular penta- or hexagonal, about 10 to 15μ long and 5 to 10μ wide. (Fig. 3.G.)

*Antennæ:* terminal joint ellipsoidal to subcylindrical, about 400 by 100μ and 50μ in diameter at the base, hairy. (Fig. 3.B.)

*Mandibles:* biting edge about 200μ long with two teeth and resembling that of *P. tectus.* (Fig. 3.E.)

*Hairs:* setæ, cylindrical to narrow fusiform, about 100 to 150μ long, acute or bifurcate at the tip, relatively thick walled; clothing hairs, flattened scale-like about 40 to 60μ long by 10 to 20μ wide, cleft into three or occasionally four long fine points. (Fig. 3.D.)

*CALANDRA GRANARIA* L.

*Elytra:* surface corrugate with intervals of 40 to 100μ: in the furrows, one or rarely two rows of circular punctures about 5 to 10μ diameter each situated at the bottom of a circular or oval saucer-shaped depression about 15 to 30μ long and separated by about 40 to 70μ, each depression also containing a circular puncture about 3 to 5μ diameter or an occasional hair: intervals, reticulately ridged the ridges enclosing regular or somewhat elongated hexagonal areas about 10 to 30μ long and 10μ wide, and also exhibiting an occasional punctured depression like those of the furrows. (Fig. 4.B.)

* Hinton[4] states that the setæ arise from the strial punctures.

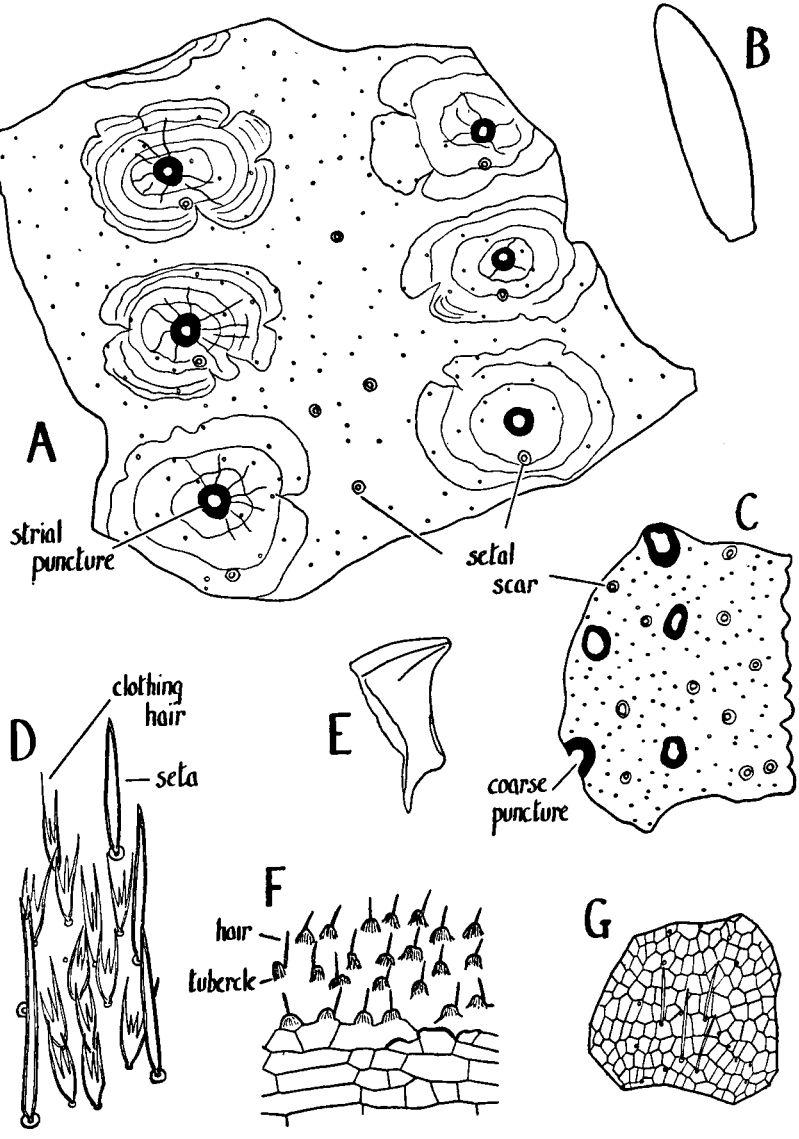


Fig. 3. *Niptus hololeucus* Fald.

A. Elytron, fragment from middle region showing strial punctures with surrounding areas and associated setal scars, x 200. B. Terminal joint of antenna, hairs not shown, × 80. C. Prothorax, fragment including the margin, showing coarse punctures, ×200. D. Hairs from elytron of a whole insect, × 200. E. Biting edge of a mandible, × 80. F. Head, fragment from near the margin showing tubercles with associated hairs and reticulate ridging, × 200. G. Fragment from mesosternite showing reticulate ridging and occasional hairs, × 200.

*Prothorax, head and other regions:* sculpture like that of the elytra, punctured depressions often larger, up to 60μ diameter, scattered irregularly or arranged in more or less regular rows, and separated by about

15 to 160μ, reticulations more regularly hexagonal than on the elytra, about 5 to 10μ long. (Fig. 4.A.)

*Antennæ:* terminal joint obovate, up to about 300 by 150μ, diameter at the base about 50μ, apical fourth with numerous very small hairs, remainder almost glabrous. (Fig. 4.D.)

*Mandibles:* biting edge curved, about 100μ long with 3 or 4 closely arranged serrate teeth. (Fig. 4.C.)

*Hairs:* arising from the punctured depressions, about 25 to 50μ long, somewhat flattened, multifid, with 5 to 10 linear truncate segments often unequal in length; the base sometimes elevated on a bun-shaped tubercle.

*LYCTUS BRUNNEUS* STEPH.

*Elytra:* strial punctures 10 to 25μ apart in parallel rows, narrow elliptical to slit-like about 5 to 15μ long, with a thickened rim and enclosed in an oval or irregularly oblong area about 15 to 30μ long: in-

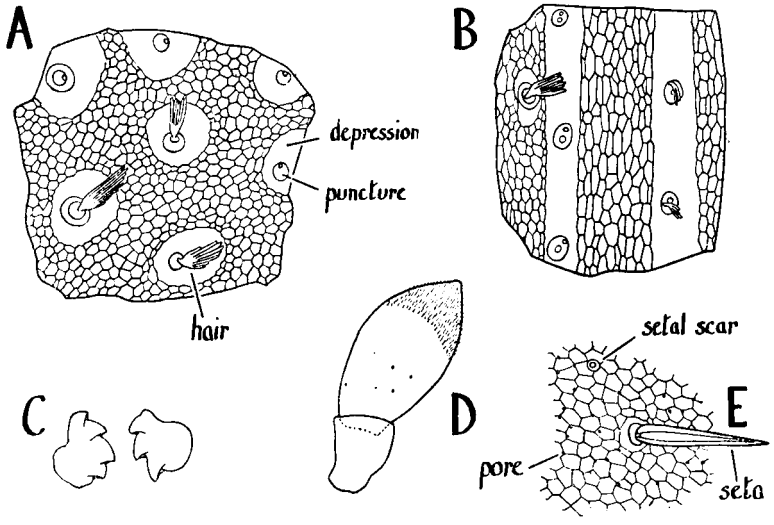


Fig. 4. *Calandra granaria* L.

A. Prothorax, fragment showing hairs arising from the saucer-shaped depressions, with reticulately ridged intervals, × 200. B. Elytron, fragment from middle region showing two furrows, × 200. C. Mandibles, × 80. D. Antenna, terminal and subterminal joints, × 80. E. *Blatta orientalis* L. fragment showing reticulate ridging, × 200.

tervals between the rows about 40 to 60μ broad with a single median row of setal scars about 5μ diameter and separated by about 20 to 40μ: surface smooth and impunctate. (Fig. 5.B.)

*Prothorax, head and other regions:* coarsely punctate, punctures numerous, contiguous or separated by up to about 20μ, very shallow, circular oval or irregular about 20 to 30μ diameter, each enclosing an eccentric setal scar: intervals smooth and impunctate. (Fig. 5.C.)

*Antennæ:* terminal joint rounded-conical about 160 by 100μ and 50μ diameter at the base, surface sparsely finely hairy, subterminal joint sometimes attached, obconical of similar dimensions. (Fig. 5.D.)

*Mandibles:* biting edge about 50μ long with two prominent closely set teeth. (Fig. 5.A.)

*Hairs:* setæ, about 80 to 100μ long, slender and thin walled but otherwise similar to those of *S. paniceum;* clothing hairs lacking from the body. (Fig. 5.E.)

### *Blatta orientalis* L.

*Elytra* lacking. All sclerites reticulately ridged, the ridges enclosing more or less regular hexagonal areas about 10 to 15μ long, some having a minute pore in one angle; setal scars, scattered at intervals of about 70 to 100μ, each about 5 to 15μ diameter and sometimes surrounded by a circular unridged area.

*Setæ,* about 80 to 100μ long, conical acuminate and thick-walled. Clothing hairs lacking. (Fig. 4.E.)

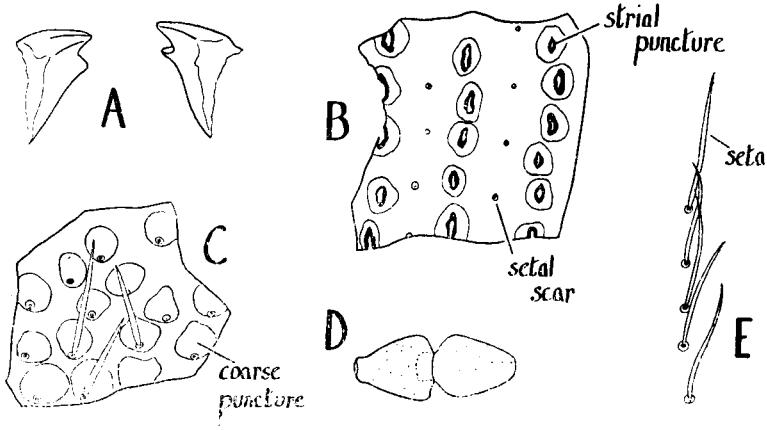


Fig. 5. *Lyctus brunneus* Steph.
A. Biting edge of mandibles, × 80. B. Elytron, fragment from middle region, × 200. C. Prothorax, fragment showing coarse shallow punctures with enclosed setæ or setal scars, × 200. D. Antenna, terminal and subterminal joints, × 80. E. Setæ from elytron of whole insect, × 200.

## Summary

1. A rapid and effective method of isolating insect fragments from powdered infested drugs is described.

2. The method depends on solution of the crude fibre of the drug in acetic anhydride containing 10 per cent. v/v of concentrated sulphuric acid.

3. The diagnostic microscopical characters of five of the more common beetle pests of drugs are described and illustrated.

In conclusion I wish to express my thanks to Mr. W. D. Hincks, M.P.S., F.R.E.S., of the Manchester Museum, for his assistance in confirming the identity of the species described.

## References

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