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Catechu (Gambier): its microscopical characters*

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24 different commercial samples of catechu have been examined microscopically for the presence of vegetable fragments, starch and crystalline material. The components of the vegetable débris have been identified as being derived from the leaves and young shoots of *Uncaria gambier* Roxb. used in the preparation of the extract. The main microscopical characters of value in the identification of catechu are enumerated.

CATECHU is the dried aqueous extract prepared from the leaves and young shoots of *Uncaria gambier*, Roxb. family Rubiaceæ, a climbing shrub which is indigenous to Malaya, Borneo, Sumatra and Java. An excellent account of its preparation is given by Roebuck (1936) and descriptions of the various processes involved have also been summarised by Flückiger and Hanbury (1900), Burkill (1935), Howes (1953), Brumwell (1911) and others (Trease, 1960; Wallis, 1960). Although the methods used vary from one territory to another, the basic principles underlying the preparation are essentially the same; the process consists of boiling the young shoots and leaves of *Uncaria gambier* in water, straining, concentrating the resulting aqueous extract to a thick syrup, pouring it into a mould, allowing it to set, cutting the solidified extract into pieces of desirable shapes or forms and finally drying them in the sun.

In view of the method of preparation and the fact that frequently somewhat primitive filtering media have been employed for straining the extract (Brumwell, 1911; Howes, 1953), it is to be expected that the final product will contain varying amounts of vegetable material. Descriptions of the microscopical structures found in catechu are included in certain textbooks of pharmacognosy (Trease, 1960; Wallis, 1960) and some of the standard books on the microscopy of crude drugs (Barclay, 1900; Greenish, 1910; Parry, 1911; Schneider, 1921; Kay, 1938). Little attempt has been made, however, to relate these fragments to the plant, *Uncaria gambier* from which they presumably have been derived. It was decided, therefore, to carry out a full investigation of all the microscopical characters of commercial catechu and to compare their structure with that of the shoots of *Uncaria gambier* previously described (Leong & Jackson, 1964); in this way the origin of the fragments could be established.

Materials

Catechu occurs in commerce in more or less regular cubes, sometimes in blocks or angular masses or occasionally in the form of tablets or discs. It is usually light and friable. The colour of the external surface varies from light brown to almost black but the freshly broken surface

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is usually a light cinnamon brown colour, although it may be somewhat streaky. It has no odour and a bitter, astringent taste.

24 different commercial samples have been used in this investigation, including one (number 18, Table 1) obtained direct from one of the few remaining gambier factories in Malaya. 15 of these samples (Nos. 4, 6, 8–10, 13–17, 20–24, Table 1) had the characteristic form of most commercial catechu, that is, they occurred as more or less regular cubes. Of these, 8 (Nos. 15–17, 20–24, Table 1) were in the form of relatively large

TABLE 1. MICROSCOPICAL CHARACTERS OF COMMERCIAL CATECHU

Sample No.	Detached covering trichomes	Leaf and perianth fragments	Pollen grains	Starch	Extraneous crystalline material	Other identified microscopical structures
1	+++	+++	++	absent	absent	fragments of cork
2	+	+	absent	absent	absent	absent
3	+	+	absent	absent	absent	absent
4	+++	++++	++++	absent	absent	absent
5	+	+	+	present (rice)	absent	rice bran and rice husk
6	+	+	++	absent	hexagonal plates and silica	absent
7	+++	+++	++	absent	absent	absent
8	+	+	absent	absent	hexagonal plates	absent
9	+++	+++	++++	absent	absent	fragments of cork
10	++++	+++	++++	absent	absent	absent
11	+	+	++	absent	silica crystals	absent
12	+	+	++	absent	silica crystals	absent
13	+	+	++	absent	hexagonal plates and silica	absent
14	+++	+	++	present (rice)	absent	rice bran but no husk
15	+++	+++	++++	absent	common salt	fragments of cork
16	++++	+++	++++	absent	absent	fragments of cork
17	++++	+++	++++	absent	common salt	fragments of cork
18	++++	+++	+++	present (rice)	absent	rice bran and rice husk
19	+++	++	++	absent	absent	absent
20	++++	+++	++	absent	absent	absent
21	++++	+++	++++	absent	absent	absent
22	++++	+++	+++	absent	absent	fragments of cork
23	++++	+++	++	present (sago)	absent	absent
24	++++	+++	+++	absent	absent	fragments of cork

Plus signs indicate the relative abundance of the particles.

irregular cubes exceeding 2 cm side and externally dark brown to almost black in colour; they were purchased from wholesale drug houses between 1953 and 1962, and seem to be typical of present-day material. The other cubes were all pre-1939 samples; they were reddish-brown in colour, about 2 cm side and were very regular in shape, having apparently been prepared more carefully than the preceding type; 3 of these samples (Nos. 6, 8, 13, Table 1) had a characteristic trade-mark symbol impressed on one of the sides of each cube.

The remaining 9 samples varied considerably in size and shape from flat circular plates to slender sticks, and from balls to irregular masses. All but two of these were Museum specimens manufactured before 1885 and it is unlikely that these forms of catechu are available on the present-day market; the other two samples (Nos. 18, 19) occurred in irregular masses and small angular pieces and were probably broken from large blocks, an alternative form in which catechu is occasionally manufactured at the present time.

Experimental methods

For the microscopical examination of all the post-1945 samples of catechu, in which the abundance of vegetable débris made its detection relatively easy, a small amount of the crushed material was mounted directly in solution of chloral hydrate (B.P.) and heated to clear. Most of the pre-1939 samples, however, contained only very small amounts of vegetable material and this was concentrated by boiling some of the powdered material in water and allowing to stand; mounts of the deposit were then made in solution of chloral hydrate as above.

Iodine (0·02N) was used to detect the presence of starch in the samples, and mounts were also made in solution of lactophenol (B.P.) to detect catechins and other crystalline material.

Microscopical characters

VEGETABLE FRAGMENTS

The following are the more important fragments found in the vegetable débris from the catechu samples; they were identified as being derived from the shoots of *Uncaria gambier* used in the preparation of the extract.

Covering trichomes. These are abundant and occur singly or in groups of up to 50 or more. They all are unicellular, but vary considerably in size, shape and degree of lignification and on the basis of these characters 4 types could be recognised:

(i) Large, stout, about 253–540 μ long and 21 to 43 μ wide; walls fairly thick and lignified; lumen nearly always filled with yellowish brown contents and often containing 1 or 2 thin, transverse, lignified septa; pitted at the base and only slightly enlarged; limb somewhat sinuous, narrowed near the base and gradually enlarging towards the centre, then remaining the same width to near the apex where it rapidly tapers to a point (Fig. 1, i).

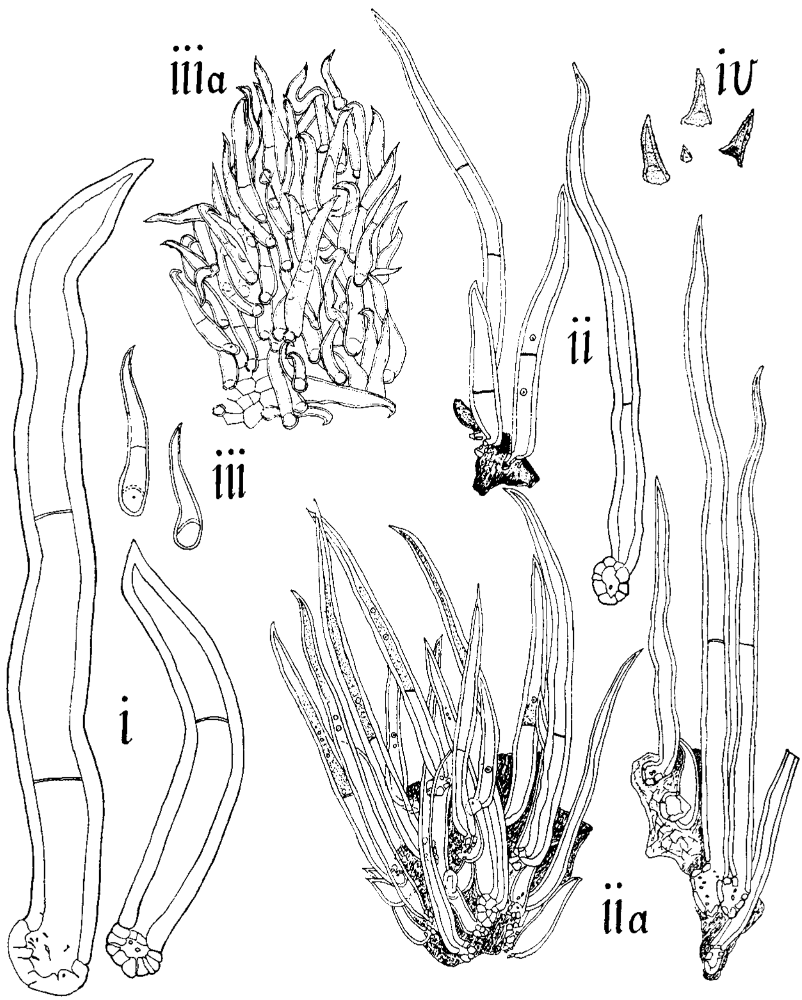

FIG. 1. Vegetable débris from pale catechu. Different types of covering trichomes: (*i*) from the inner epidermis of the petals; (*ii*) from the outer surface of the petals and the basal region of the calyx; (*iii*) from the outer epidermis of the lobes of the sepals; (*iv*) from the epidermis of the leaf midrib, stem or hook. (*iia*) and (*iiia*) show groups of trichomes of types (*ii*) and (*iii*) respectively massed together giving a flame-like appearance. All $\times 200$.

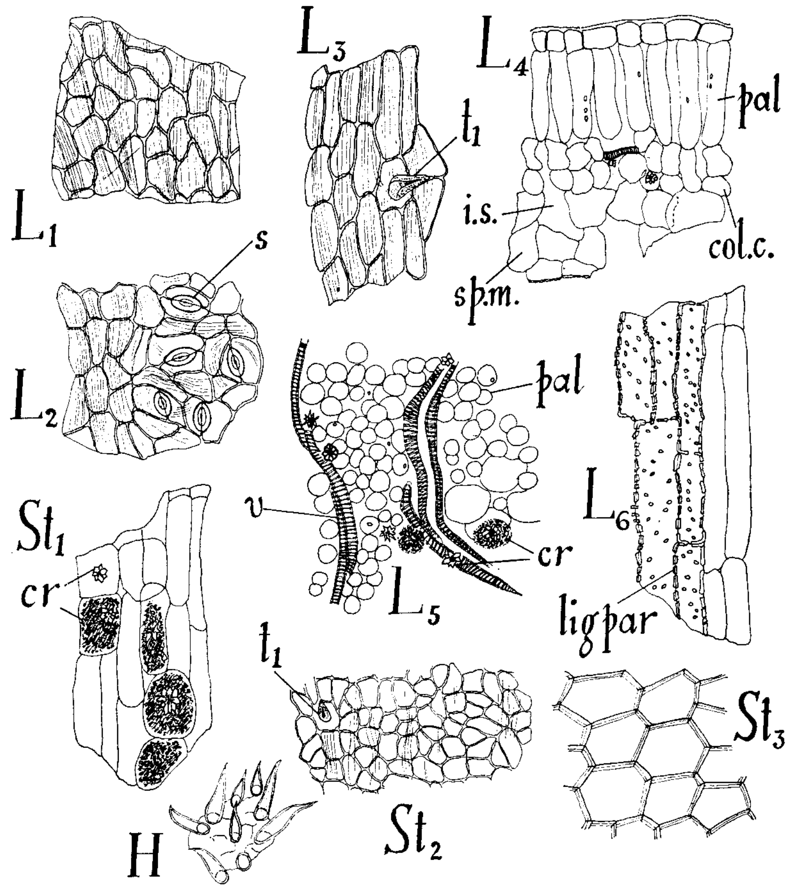

FIG. 2. Vegetable débris from pale catechu. Fragments from the leaves, stems and hooks. L_1 to L_6, fragments from the leaves; L_1, upper epidermis; L_2, lower epidermis; L_3, lower epidermis of the midrib; L_4, transverse section of the lamina; L_5, mesophyll seen in surface view; L_6, portion of the midrib medulla in longitudinal section; St_1 to St_3, fragments from the stems; St_1, portion of the phloem in longitudinal section; St_2, epidermis in surface view; St_3, cork; *H*, fragment of the epidermis of the hooks in surface view; *col.c.*, collecting cells; *cr*, crystals of calcium oxalate; *i.s.*, intercellular space; *lig.par*, lignified parenchyma; *pal*, palisade; *s*, stoma; *sp.m.*, spongy mesophyll; t_1, short covering trichomes; *v*, vessels. All ×200.

The trichomes of this type are probably derived from the *inner surface* of the *petals.*

(ii) Usually shorter and more slender than type (i), about 234 to 522 μ long and 10 to 15 μ wide at the midpart of the limb; walls fairly thick and lignified; lumen frequently filled with granular substances or a yellowish brown fluid and often containing 1 to 3 (usually 2) lignified septa; bases sometimes slightly enlarged, densely pitted and rather irregular in outline; limb often bent at a right angle to the base and somewhat sinuous, slightly constricted near the base, then enlarging rapidly and remaining approximately the same width to near the apex, where it gradually tapers to a point (Fig. 1, ii).

These trichomes are derived from the *outer surface* of the *petals* and the *calyx tube.*

(iii) These are smaller than either of the preceding types, measuring approximately 151 to 270 μ long and 10 to 15 μ wide at the base; walls thin and only slightly lignified; lumen usually empty and may be divided by a single, slightly lignified septum; base rounded with few pits; limb somewhat sinuous and gradually tapering towards the pointed apex (Fig. 1, iii).

These trichomes are derived from the *outer surface* of the *calyx lobes.*

(iv) Small, conical, with a warty cuticle; about 25 to 43 μ long and 15 to 36 μ at the base, walls variable in thickness but unlignified; base usually very much enlarged with no pits; lumen with no septa, wide at the base but sometimes becoming occluded near the apex (Fig. 1, iv).

These trichomes are probably derived from the *epidermis* of the *leaves, hook* or *stem.*

Leaf fragments. These are most readily recognised when occurring as portions of the epidermis seen in surface view (Fig. 2, L_1 and L_2) when the diagnostic characters are: the polygonal epidermal cells with a finely striated cuticle, the cells of the upper surface with straight anticlinal walls and those of the lower surface with somewhat curved or slightly sinuous anticlinal walls; paracytic stomata present on the lower epidermis only and covering trichomes usually absent from both surfaces.

Occasionally the leafy fragments are seen in transverse section (Fig. 2, L_4), when they may be recognised by the following characters: the cells of the upper epidermis with a fairly thick, striated cuticle; the row of collecting cells beneath the single layer of palisade cells, and the occasional idioblasts in the spongy mesophyll each containing a single cluster crystal of calcium oxalate. The lower epidermis is generally absent from these fragments.

Fragments of epidermis from the veins of the leaf are also occasionally found; these consist of subrectangular or polygonal, thin-walled cells with a striated cuticle; some of the cells bear short conical trichomes of type (iv) (Fig. 2, L_3).

Other leafy fragments which occasionally occur are portions of the mesophyll with vascular strands and crystals of calcium oxalate (Fig. 2, L_5)

and, more rarely, small groups of lignified parenchyma probably derived from the medulla of the midrib (Fig. 2, L_6).

Stem fragments. (i) Groups of parenchyma from the phloem, some containing sandy crystals and cluster crystals of calcium oxalate (Fig. 2, St_1).

(ii) Portions of the epidermis with striated cuticle and occasional trichomes of type (iv). These are rather similar to the fragments of the epidermis of the midrib of the leaf, but can be distinguished from them by the presence of stomata, and sometimes by the shape and size of the epidermal cells (Fig. 2, St_2).

(iii) Fragments of cork, reddish-brown in colour, composed of 2 or 3 layers of thin-walled cells, seen in surface view (Fig. 2, St_3).

Hook fragments. Very occasionally, fragments of epidermis are found bearing short, conical trichomes in greater numbers than occur on the stem or leaf midrib; also some of these trichomes may have a smooth cuticle. Such fragments are probably small portions of the epidermis of the hook near the apex (Fig. 2, H).

Stipule fragments. These also occur only very rarely in the débris; they are recognised by the appearance and distribution of the trichomes and stomata on the epidermis (Fig. 3, Stp).

Perianth fragments. These are commonly found in most samples of catechu and, like the leaf fragments, are most readily recognised when occurring as portions of the epidermis seen in surface view; they are distinguished from the leaf fragments by the presence of numerous epidermal trichomes, also the petal fragments are reddish-brown in colour.

(i) The outer epidermis of the *corolla lobes* is more readily recognised than the inner; it consists of polygonal cells with straight or slightly sinuous anticlinal walls which are very thin and not always clearly visible, being frequently obliterated by a layer of covering trichomes of type (ii) which lie with their apices pointing in the same direction, thus giving the fragments a "flame-like" appearance; frequently some of the trichomes are broken off leaving a characteristic cicatrix which is somewhat irregular in outline, finely pitted, lignified and usually surrounded by 4 to 6 epidermal cells; stomata infrequent (Fig. 3, P_1).

(ii) Fragments of *corolla tubes* occur less frequently than those from the corolla lobes; the outer epidermis is composed of very thin-walled, elongated cells, the length of which is about three to four times greater than the width; numerous covering trichomes are present, lying parallel to the epidermal cells with their apices pointing in the same direction; they are of type (ii) but measure only up to 234 μ in length and usually contain a single lignified septum situated in the lower half of the limb; the slightly sinuous anticlinal walls of the underlying mesophyll cells may also be visible as faint, disjointed lines giving a somewhat "beaded" appearance (Fig. 3, P_2).

(iii) The *calyx lobes* are the only recognisable fragments from the sepals; the outer epidermis consists of thin-walled, polygonal or sub-rectangular cells with very slightly sinuous anticlinal walls; paracytic

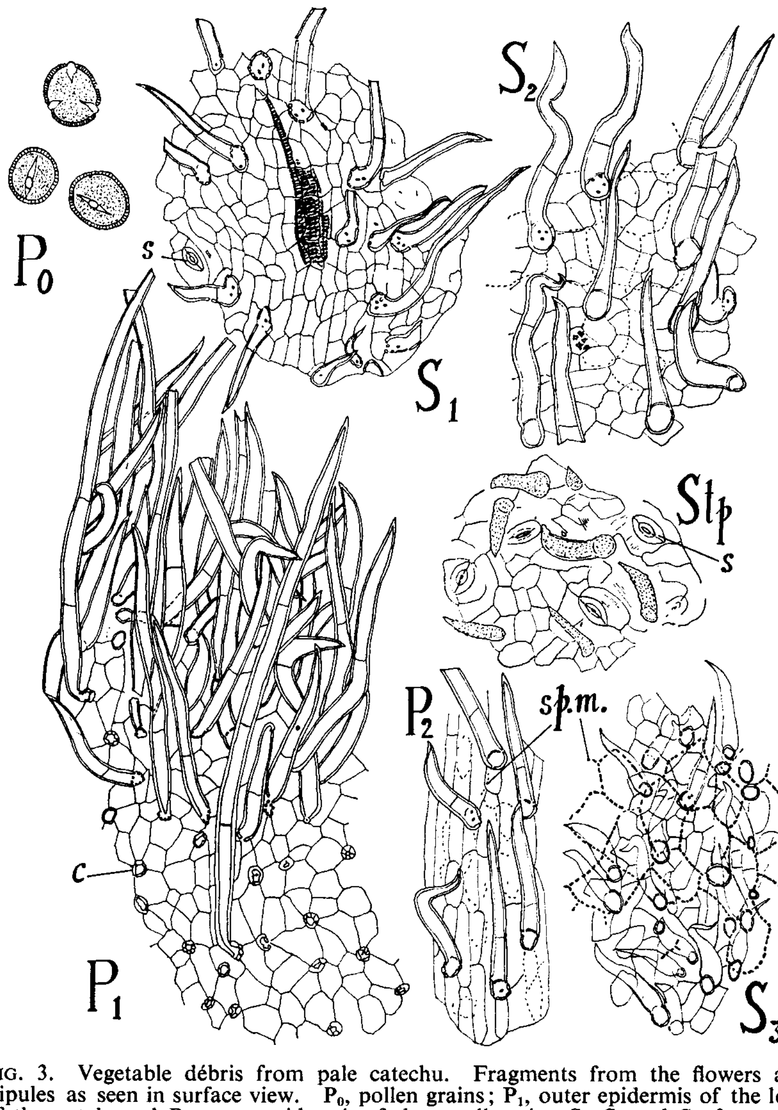

FIG. 3. Vegetable débris from pale catechu. Fragments from the flowers and stipules as seen in surface view. P_0, pollen grains; P_1, outer epidermis of the lobe of the petals and P_2, outer epidermis of the corolla tube; S_1, S_2 and S_3, fragments from the outer epidermis of the central region of the lobes of the sepals; S_1 and S_2 show the underlying vascular tissue and mesophyll respectively; S_3 shows the mesophyll lying uppermost and the epidermis underneath; *Stp*, lower epidermis of the stipule; *c*, cicatrix; *s*, stoma; *sp.m.*, spongy mesophyll. P_0, $\times 450$, others $\times 200$.

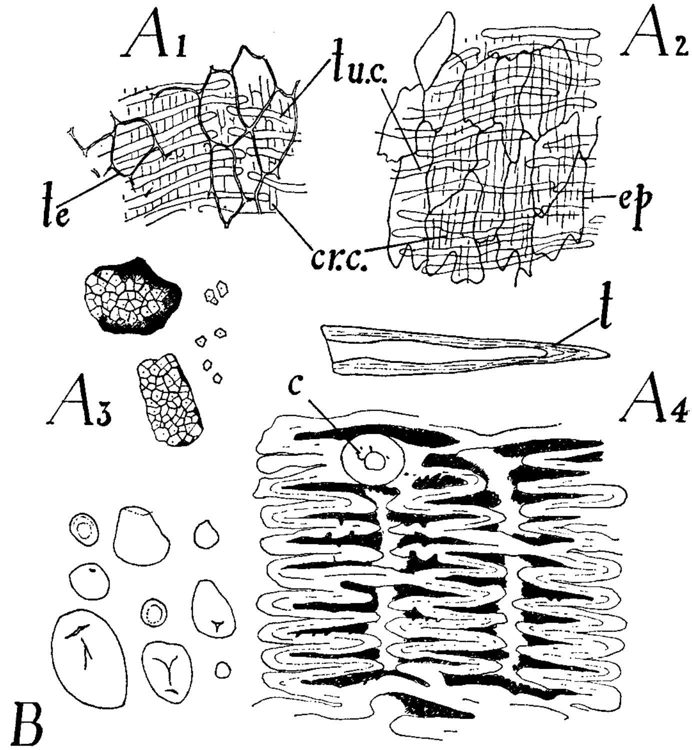

FIG. 4. Starch and other foreign material found in pale catechu. *A*, rice, *Oryza sativa* L.; A_1, surface view of the inner layers of the pericarp and adherent testa; A_2, surface view of the outer layers of the pericarp; A_3, starch granules; A_4, outer epidermis of the palea in surface view; *B*, starch granules from sago. All $\times 230$. *c*, cicatrix; *cr.c.*, cross cells; *ep*, epicarp; *t*, covering trichome from the palea; *te*, testa or seed coat; *tu.c.*, tubular cells.

stomata fairly numerous but absent over the veins; covering trichomes of type (iii) fairly abundant, also scattered rounded or ovoid cicatrices which are lignified and frequently pitted; the underlying spongy mesophyll cells with "beaded" anticlinal walls and occasional groups of slender annular and spiral vessels are frequently visible; the inner epidermis is thin-walled, with very few trichomes (Fig. 3, S_1, S_2, S_3).

Pollen grains. These were present in most of the samples and although an occasional extraneous pollen grain was seen, the majority were similar to those of *Uncaria gambier* previously described (Leong & Jackson, 1964) and were obviously derived from the flowers present in the material used for making the extract (Fig. 3, P_0).

EXTRANEOUS VEGETABLE FRAGMENTS

In addition to the fragments derived from *Uncaria gambier* described above, 3 of the samples (Nos. 5, 14, 18, Table 1) also contained *Rice bran*; this was identified by reference to Winton (1945) and Wallis (1957) and confirmed by comparison with the bran and husks removed from whole fruits of *Oriza sativa.* The fruit wall, portions of which constitute rice bran, is composed of a number of layers of thin-walled cells and several of these adhere together; the epidermis, or epicarp, is a single layer of elongated, somewhat wavy-walled cells and beneath this a layer of very thin-walled elongated transverse cells is faintly discernible, followed by a layer of tubular cells with intercellular spaces; these cells are orientated with their long axes at right angles to those of the cells of the other layers, thus giving a very characteristic appearance to the fragments (Fig. 4, A_2). Frequently the testa is also adherent to the layers of the fruit wall; this consists of a layer of rectangular cells with straight, slightly thickened anticlinal walls, and in the fragments in which this layer is lying uppermost the underlying tubular cells of the fruit wall are clearly seen with the very faint striations of the transverse cells lying underneath (Fig. 4, A_1).

In two of these samples, in addition to the rice bran described above, fragments of the *Rice husks*, or paleae, were also present; these were recognised by the structure of the outer epidermis which is very characteristic (Fig. 4, A4), being composed of a layer of cells with very deeply sinuous, thickened and partially lignified walls and occasional covering trichomes which are unicellular, elongated and sharply pointed at the apex and have thickened, partially lignified walls; these trichomes frequently become detached from the epidermis, leaving a characteristic cicatrix (Fig. 4, A_4, c), and occur scattered in the vegetable débris (Fig. 4, A_4, t).

Starch. The 3 samples (5, 14, 18) which contained rice bran also contained starch and, as is to be expected, this was typical rice starch; it consisted of small, polygonal granules, 3 to 8 μ in diameter, occurring singly or more frequently in large groups closely packed together to form angular masses, some granules with a faintly discernible central hilum (Fig. 4, A_3).

Starch was also found in one other sample (23); the granules were much larger, measuring 7 to 45 to 69 μ in diameter, some ovoid to subspherical but the majority muller-shaped indicating that they were components of original compound granules; hilum a split, usually 1/3 to 1/5 eccentric; striations not visible, but many granules with deep cracks or fissures (Fig. 4, B). Reference to the literature (Wallis, 1957) indicated that the starch was commercial sago, and this was confirmed by comparison with authentic material.

CRYSTALLINE MATERIAL

Of all the microscopical structures found in commercial catechu the most characteristic and diagnostic are the acicular crystals of *catechin* which constitute the main bulk of the solid extract. They occur in compact, yellowish-brown masses, and are birefringent; the size of the individual crystals varies in different samples and appears to be larger in those containing only a small amount of vegetable débris (Fig. 5, A).

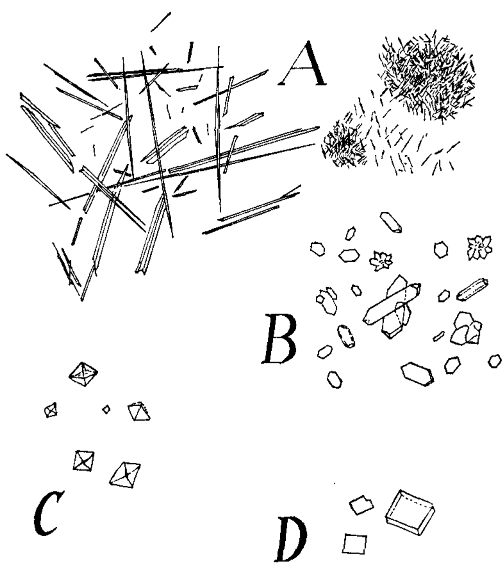

FIG. 5. Crystalline material from pale catechu. *A*, acicular crystals of catechin from two different samples, showing the variation in size; *B*, crystals of unknown composition in single hexagonal plates and in clusters; *C*, silica crystals; *D*, common salt crystals. All $\times 330$.

Varying amounts of other crystalline materials, in characteristic forms, were also found in a number of the samples; these included common salt crystals, silica crystals and some crystalline substance of unknown composition. *Common salt crystals* were found in two samples, and they were identified chemically and also microscopically by comparison with pure sodium chloride; they occurred in the form of very thin, relatively square plates (Fig. 5, D).

Silica crystals were occasionally seen in nearly all the samples but the amount present was usually very small; they occurred in the form of small, birefringent hexahedrons or double pyramids (Fig. 5, C).

In three of the pre-1939 samples other crystals were found which were birefringent and in the form of thin, relatively regular hexagonal plates, usually lying singly with their flat faces uppermost but occasionally clustered together in small groups. They occurred only in relatively small amounts and were not identified (Fig. 5, B).

Discussion

Table 1 summarises the composition of the débris in the 24 samples examined. As the *hook* and *stipule* fragments occurred relatively infrequently they are not included under a separate heading but they were usually present in all samples which had an abundance of leaf and perianth segments. Portions of parenchyma and epidermis of the *stem* also occurred in most samples but cork fragments, indicating the use of older stems in the preparation of the extract, were only found in 7 of the samples and these are indicated in the last column of the Table.

Reference to the Table shows that *covering trichomes* occurred in varying amounts in all samples; they were particularly numerous in those manufactured since 1945 (i.e., Nos 15 to 24). Portions of the *leaves* and *perianth segments* were also found in all samples, but generally they were slightly less abundant than the covering trichomes. *Pollen grains* were not detected in all the samples but they were present in considerable quantities in those which contained abundant trichomes and other vegetable fragments. Of the four samples which contained *starch*, in only one was vegetable débris relatively scarce, suggesting that the reported practice (Brumwell, 1911; Howes, 1953) of the addition of starch to the extract is not associated with a particular manufacturing process. It is interesting to note that such addition of starch is still practised, as indicated by the abundance of rice starch in sample 18, recently obtained from Malaya. The two samples which contained *common salt crystals* also contained abundant vegetable débris, including fragments of cork; as salt is an unusual adulterant of catechu it is possible that these 2 samples, although they were obtained at different times, originally came from the same source. The other extraneous crystalline materials found—namely *silica* and the *unidentified hexagonal crystals*—were associated with only a small amount of vegetable débris, and were found only in samples manufactured before 1939.

Catechin crystals have not been included in Table 1 as they were found in very large quantities in all the samples examined.

Of the fragments of the leaves and shoots of *Uncaria gambier* present in commercial catechu, it is interesting to note that most of them are derived from superficial tissues, e.g. covering trichomes, epidermises of leaves and perianth segments and pollen grains, which would readily become detached from the plant when the marc is stirred during the extraction process. The almost complete absence of any internal structures from the core of the plant, e.g., vessels and fibres, indicates that the harvested leafy twigs are not subjected to comminution before the extraction process; this is in accordance with the normal manufacturing procedure as reported.

DIAGNOSTIC CHARACTERS

The diagnostic microscopical characters which are of most value in the identification of commercial and pharmaceutical catechu are as follows:

1. The abundant crystalline masses consisting of numerous *acicular crystals of catechin.*

2. A very large number of unicellular *covering trichomes*, either whole or fragmented, occurring singly or in groups still attached to pieces of epidermises, and showing different characters depending on the part of the plant from which they have been derived.

Isolated, whole covering trichomes are of four types, as described on page 410 (Fig. 1).

3. Fragments from the *lobes of the petals* showing the outer epidermis and, occasionally, the underlying spongy mesophyll, as described on page 414 (Fig. 3).

4. Fragments from the *central region of the lobe of the sepals* showing the outer epidermis and frequently also the underlying spongy tissues, the mesophyll being occasionally traversed by slender vascular strands; cells of the epidermis with slightly sinuous anticlinal walls; stomata numerous and paracytic; covering trichomes of type (iii) fairly numerous; cicatrices, scattered and usually rounded or ovoid in outline.

5. A large number of *pollen grains* of *Uncaria gambier*, subspherical in shape, about 11 to 18 μ in diameter, with three pores and three germinal furrows and a warty exine.

6. Fragments of *leaf* showing polygonal epidermal cells with striated cuticle. The details are described on page 413 (Fig. 2).

7. Fragments of *cork.*

All the above are derived from the source material, young twigs of *Uncaria gambier.*

If *rice bran* or *rice husks* are present in the extract, the additional diagnostic microscopical characters are:

(a) *Rice bran:* fragments of the pericarp in surface view, showing the epicarp, very frequently with underlying layers of *transverse* and *tubular* cells; described on page 417; see also Fig. 4.

(b) *Rice husk:* fragments of the outer epidermis of the paleæ (Fig. 4) described on page 417.

(c) The abundant angular masses of *starch granules* typical of rice.

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