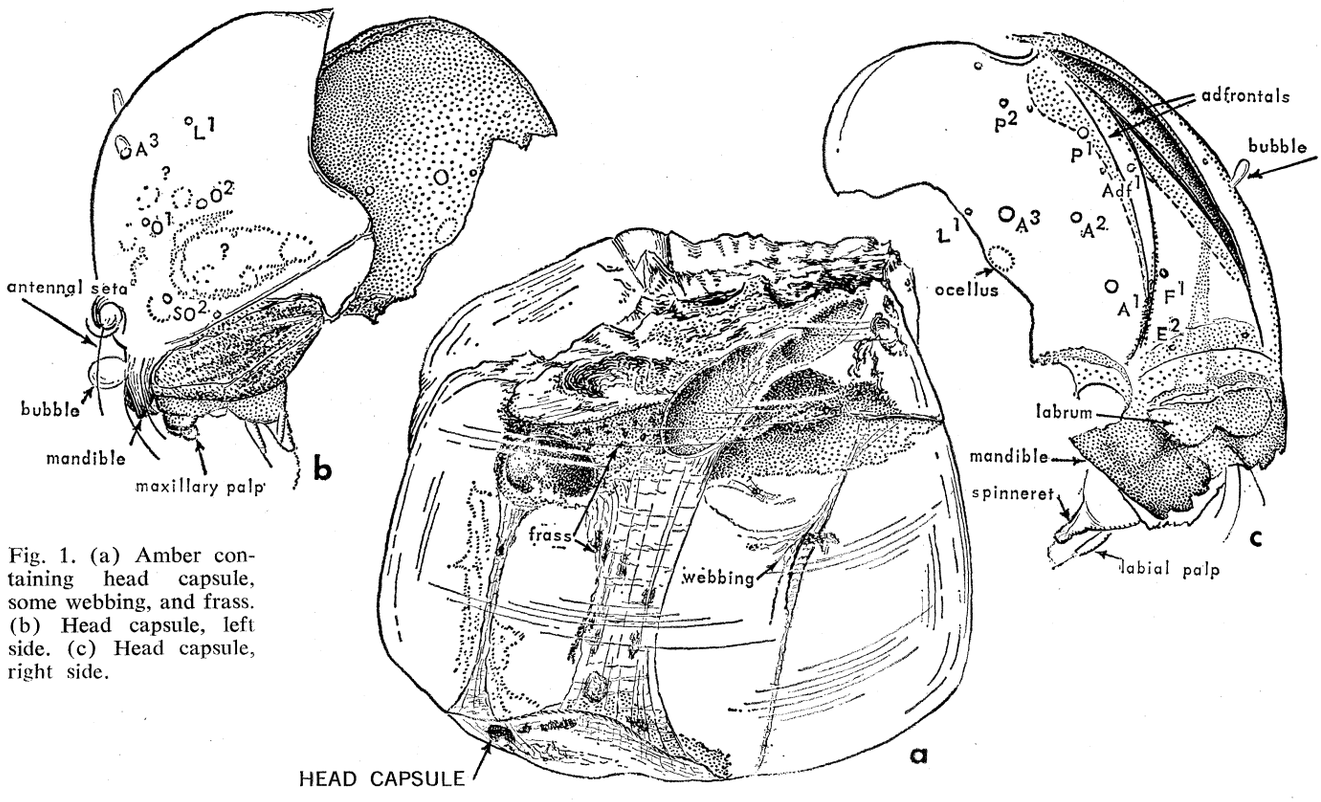

Fig. 1. (a) Amber containing head capsule, some webbing, and frass. (b) Head capsule, left side. (c) Head capsule, right side.

entire; two small pieces that lie nearby may be part of it. It appears to be split along the left adfrontal suture and the sides crushed together so that the left adfrontal is actually overlapped by the remainder of that side. Pressure has formed bubbles at some setal openings as illustrated. The positions of setae E^2 and the right seta F^1 on the frons are distinct, as are those of A^1, A^2, A^3, L^1, P^1, and P^2 on the right side. A cleared area anteroventral to A^3 on the right side could be ocellus I or II, probably the latter. On the left side, ocelli cannot be definitely distinguished, and the identity of the setal bases observed are doubtful. Seta L^1 appears to be farther from its corresponding seta A^3 on the left side than on the right side. If these setae are correctly identified, the difference in distances can be explained by light refraction. Other structures observed are the spinneret, which appears to be short, somewhat tapered toward the apex, with an apical silk pore; a labial palp; a maxillary palp; the labrum, with anterior margin emarginated and sides of notch forming an angle of slightly less than 90°; mandibles, which, in one view, show teeth more pointed than illustrated; and, lastly, what appear to be antennal and mandibular setae.

The distinct adfrontal sclerites are diagnostic of Lepidoptera. These sclerites are never found on larvae of other orders (*5*). The labrum, the opposable mandibles, the spinneret, and labial palp are also typically lepidopteran in shape. The apparently long adfrontals extending to the back of the head, the position of the setae, in fact, the overall appearance of the head capsule are characteristic of many Microlepidoptera. Some indication of family relationship might have been obtained from the number, comparative size, and arrangement of the ocelli if the ocelli could have been observed. But even an attempt to fit the small adjacent pieces, one of which seems to show at least one ocellus, into the missing areas of the capsule was not successful. If there are actually fewer than the usual six ocelli present, a possible tineid connection could be suggested. Unfortunately, the family to which the head capsule belongs cannot be determined with certainty.

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21 October 1969

Elm Bark Derived Feeding Stimulants for the Smaller European Elm Bark Beetle

Abstract. *The principal feeding stimulants for the beetle* Scolytus multistriatus *Marsham from the twig bark of* Ulmus americana *L. have been identified as (+)-catechin-5-β-*D*-xylopyranoside and lupeyl cerotate.*

The selection of a host by phytophagous insects results, in many cases, from the presence of specific chemicals in that host (*1*). The occurrence in elm of chemotactic factors, as well as thigmotactic stimuli, that induce feeding in *Scolytus multistriatus* Marsham has been indicated (*2*, *3*). We report here

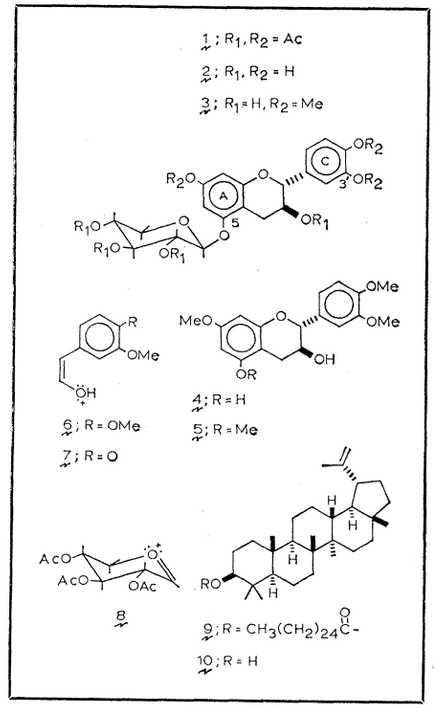
the isolation and identification of two compounds from the extract of the bark of *Ulmus americana* L., which account for most of the feeding stimulation activity in that extract in the smaller European elm bark beetle (*S. multistriatus*).

The bioassay results of the fractions from the elm extract are summarized in Table 1. Twigs (2- to 4-year growth) were collected locally, and stripped of the bark; the bark (6 kg) was pulverized (through 80 mesh) and exhaustively extracted at room temperature with 95 percent ethanol. The solvent was evaporated at reduced pressure at 40°C, and the residue (922 g) (A) was assayed for feeding stimulant activity. Initially, the bioassay was performed with the use of agar pegs (*4*). Later, we used elderberry pith disks (12-mm diameter) in a modification of the method of Norris and Baker (*5*). Two disks, one treated with a quantity of the test fraction and a solvent-treated control were fixed about 5 cm apart to the bottom of the test arena with 8-mm thumbtacks. The raised edge of the tack provided the necessary thigmotactic stimulus. One newly emerged beetle was placed in each dish, and 100 beetles (50 males and 50 females) were used in each test (done in replicate). The test containers with the beetles were stored in the dark for 48 hours at controlled temperature and humidity (29°C, 85 percent relative humidity), and the disks with a feeding hole or holes were regarded as showing a positive response.

The crude extract residue (A) was partitioned between chloroform and water to give two active fractions B (water soluble; 767 g) and C (chloroform soluble; 152 g). The chloroform-soluble residue (C) was partitioned further between petroleum ether (b.p. 60°–110°C) and 10 percent aqueous methanol; this partitioning yielded two active fractions D (aqueous methanol) and E (petroleum ether). Fraction D (71 g) was partitioned between 50 percent aqueous methanol and chloroform, and the inactive chloroform fraction (F, 60 g) and the active methanol fraction (G, 10 g) were obtained. Column chromatography of fraction D or fraction G on alumina, silicic acid, or nylon powder failed to resolve the mixture cleanly, or at all, and the appearance of activity occurred only on elution with very polar solvents. Acetylation of fraction G in pyridine and acetic acid gave an inactive acetate mixture (12 g) which on mild hydrolysis (0.02*N* methanolic KOH, 1 hour under reflux in a nitrogen atmosphere) yielded a mixture with restored activity. The infrared spectra of fraction G and of the hydrolysis product were essentially identical, and the lack of bands in the carbonyl region eliminated the possibility that any hydrolyzable carbonyl derivatives were present originally in fraction G. Chromatography of the acetate mixture (8 g) on silicic acid resulted in nine fractions (dry-weight peak analysis) of which only one had activity after hydrolysis. This fraction crystallized readily from ethanol to give the heptaacetate **1** (0.33 g), which was characterized as follows: $C_{34}H_{36}O_{17}$; m.p. 182°–183°C; specific rotation, −13.9° (0.57 percent in chloroform). On saponification and deionization on Dowex 50W (H), compound **1** yielded the amorphous feeding stimulant (+)-catechin-5-β-D-xyloside (**2**) (0.19 g) with the following characteristics: specific rotation, −25° (0.45 percent in methanol); an absorption maximum at 280 nm (log molar extinction 3.53). The structure was proved as follows.

Table 1. Feeding responses of *S. multistriatus* to the elm bark extract and fractions thereof. These are typical values obtained from 100 beetles (50 males, 50 females) each placed in a petri dish with two elderberry disks, one containing the test fraction, the other the control.

Fractions	Amount (mg/disk)	Percent feeding	
		Treated	Control
A	5.0	76	16
B	2.5	50	2
C	1.5	38	2
B + C	2.5 + 1.5	70	16
D	0.5	24	2
E	1.0	26	4
B + E	2.5 + 0.25	74	4
F	1.0	2	0
G	2.5	38	2
H	2.5	10	6
H (hydrolyzed)	2.5	34	2
1	0.25	5	1
2	0.2	23	13
9	0.01	31	11
2 + **9**	0.2 + 0.01	52	14



Acetylation of compound **2** yielded the heptaacetate quantitatively. Methylation of compound **2** with diazomethane gave the nonphenolic methyl ether **3** (m.p. 192°–194°C) which, on acid hydrolysis, yielded a water-insoluble phenolic aglycone **4** and a sugar. The aglycone **4** gave a positive Gibbs test (for phenols with no substituent in the *para* position) (*6*), and on methylation afforded (+)-catechin tetramethyl ether (**5**) identical (as judged by mixture melting point, infrared, ultraviolet, and nuclear magnetic resonance spectra, and specific rotation) to an authentic sample (*7*). The mass spectrum of the methyl ether **3** showed peaks consistent with the proposed structure (*8*). Of immediate interest were those at *m/e* (mass/charge) 180 (relative abundance 100 percent) and 165 (12 percent) corresponding to fragment ions **6** and **7**, respectively; these results eliminated the possibility that position 3′ in ring *C* might be the point of attachment for the sugar. The sugar must therefore be placed at position 5. On paper chromatographic analysis the carbohydrate from the hydrolysis showed the same R_F as authentic xylose in three solvent systems (*9*), and the peracetate (prepared with sodium acetate and acetic anhydride) was identical (as judged by mixture melting point, infrared and nuclear magnetic resonance spectra, and specific rotation) to β-D-xylose tetraacetate (*10*). The presence of a pyranose ring in the active glycoside **2** was supported by the presence of peaks at *m/e* 259 (16 percent), 199 (14 percent), 157 (38 percent), and 97 (49 percent) which dominate the mass spectrum of the heptaacetate **1**. Metastable ions were observed for the sequence as indicated. Additional characteristic peaks at *m/e* 43 (100 percent) and 139 (46 percent) support the predicted fragmentation of the ion **8** (*11*). Hydrolysis of the glycoside **2** by emulsin was taken as evidence for the presence of the β-glycosidic linkage.

The active petroleum ether fraction (E) was chromatographed on two silicic acid columns in sequence and the most

active fraction crystallized from acetone to give lupeyl cerotate (**9**), with m.p. 84°–85°C; specific rotation −16.1°(0.56 percent in chloroform); infrared bands at 1720 cm^{-1} (ester) and 1640 cm^{-1} (olefin). Its structure was established on the basis of the products obtained after hydrolysis (1*N* KOH, 90 percent ethanol, 5 hours). Lupeol was separated from the potassium salt of the fatty acid by ether extraction, then passed through a short basic alumina column (benzene to chloroform), and crystallized from ethanol, m.p. 216°–217°C. Comparison (as judged by mixture melting point, infrared and nuclear magnetic resonance spectra, and specific rotation) of the isolated lupeol (**10**) with an authentic sample showed them to be identical; their acetates were also identical (*12*). The potassium salt of the fatty acid was suspended in dilute HCl and warmed until globules formed; the mixture was cooled and filtered; the fatty acid was crystallized from benzene and identified as cerotic (hexacosanoic) acid, m.p. 88°–90°C, with infrared bands at 3500 to 2500 cm^{-1} (carboxylic acid OH) and 1705 cm^{-1} (carboxyl). The mass spectrum showed a molecular ion at *m/e* 396.3923 (71 percent) (calculated mass for $C_{26}H_{52}O_2$ is 396.3967 and prominent peaks at *m/e* 339 (4 percent), 297 (9 percent), 241 (7 percent), 185 (13 percent), 129 (36 percent), 73 (63 percent), and 43 (100 percent), a pattern characteristic for an unbranched long-chain fatty acid (*13, 14*).

The aqueous extract residue (B) was examined for its active constituents by a number of procedures including extraction, precipitation, and chromatography; but in the end the separation was accomplished by chromatographing the total acetates (as with fraction G) on silicic acid. Acetylation of 10 g of residue B gave 15 g of acetates which were separated into nine fractions of which the most active yielded 0.64 g of the heptaacetate **1**. The glycoside **2** is present in a larger amount in fraction B, and that isolated from G apparently represents a quantity carried over as a result of a slightly favorable partitioning into chloroform.

The combination of the two new isolated substances, (+)-catechin xyloside (**2**) and lupeyl cerotate (**9**), elicits a feeding response approaching 60 to 70 percent of that obtained from the residue of the initial extract. The difference probably represents the effect of other, unidentified constituents acting additively or synergistically, but these were not readily indicated in our study.

Since *S. multistriatus* is the principal vector in the spread of the Dutch elm disease fungus, *Ceratocystis ulmi* (Buisman) C. Moreau, knowledge related to the chemical factors influencing the feeding behavior of the insect might be of use in measures designed to control this disease.

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30 October 1969

5S RNA Synthesized by Escherichia coli in Presence of Chloramphenicol: Different 5′-Terminal Sequences

Abstract. Escherichia coli *cells, grown in the presence of chloramphenicol, synthesize a low molecular weight RNA (CM-5S RNA) not bound to ribosomes which is similar to ribosomal 5S RNA. Oligonucleotide patterns derived from ribonuclease digests of 5S RNA and of CM-5S RNA are indistinguishable except that the 5′-terminal oligonucleotides differ. Whereas the nucleotide sequence of the 5′-terminus of normal 5S RNA is pUpG-, there are three alternate sequences of the 5′-terminus of CM-5S RNA: pUpUpG-, pUpUpUpG-, and pApUpUpUpG-.*

In the presence of chloramphenicol, *Escherichia coli* cells synthesize an RNA (CM-5*S* RNA) (*1*) of low molecular weight. The CM-5*S* RNA, in contrast to normal ribosomal 5*S* RNA, is not bound to ribosomes (or to "chloramphenicol particles") but is found free in the 100,000*g* supernatant fraction of the centrifuged cell homogenates (*2*). Although CM-5*S* RNA is eluted from Sephadex G-100 columns in the same position as normal 5*S* RNA, it is eluted from methylated albumin kieselguhr (MAK) columns somewhat later than normal 5*S* RNA (*2*). We now report a difference in primary structure between CM-5*S* RNA and normal ribosomal 5*S* RNA.

Normal *Escherichia coli* EA2 cells and those treated with chloramphenicol (100 μg/ml) were grown in a low phosphate medium containing ^{32}P; the RNA was prepared by extraction of the whole cells with phenol at 4°C, in the presence of whole cell, nonradioactive, carrier RNA (1 mg/ml). The RNA was precipitated by alcohol, and then fractionated by Sephadex G-100 gel filtration. The fractions corresponding to 5*S* RNA were collected, precipitated in alcohol, and purified by MAK column chromatography. The purified 5*S* RNA and CM-5*S* RNA were digested by pancreatic ribonuclease and ribonuclease T1 and analyzed by two-dimensional oligonucleotide fractionation (*3*).

The two-dimensional fractionation