$TCID_{50}$ per 0.1 ml. The infectivity titer was reduced from $10^{3.5}$ to $10^{0.05}$ $TCID_{50}$ per 0.1 ml after the cell-cultured agent was heated at 56°C for 30 minutes.

For electron microscopy, specimens obtained by ultracentrifugation of supernatants of BHK-21 cell cultures infected with sixth cell-culture passage of the Porton agent associated with African Green monkey were inactivated with osmium tetroxide, mixed with sodium phosphotungstate stain, and sprayed onto carbon-coated grids. These preparations contained many bizarre cylindrical particles (Fig. 1) with a uniform diameter of 90 to 100 nm. Their length varied from 130 to more than 2600 nm. Particles had rounded ends, but many had large blebs of membranous material attached to one end. Many bent into looped or "fish hook" configurations around their terminal bleb. Rarely, branched particles were observed (Fig. 2). Cross-striations and an inner cylindrical structure were observed in particles penetrated by the stain; the cross-striation interval was approximately 5 nm, and the core diameter was 45 nm. Electron microscopy (thin sections) of infected cell cultures revealed extracellular particles which, when sectioned transversely, consisted of concentric electron-opaque rings consonant with the surface and core appearing in negative-contrast preparations. Sectioned particles appeared to be covered with an additional irregular surface layer.

The same kind of particles was observed in ultrathin sections of liver from guinea pigs infected with Porton material that had been passaged twice through guinea pigs or with Flak isolate passaged four times through guinea pigs. In areas of periportal necrosis, particles accumulated in large numbers within the lumina of debris-filled sinusoids (Fig. 3). Infrequently, budding of particles was observed from the cytoplasmic membrane of hepatocytes (Fig. 4). This budding, which is observed in thin sections, may mean that the blebs attached to free particles in negative-contrast preparations were a likely consequence of avulsion of attached membrane at the time of particle release. These particles were not observed in uninoculated cell cultures or normal guinea pig tissues examined in parallel with infected specimens.

Because we often found the particles in passage materials from Porton specimens as well as in passage materials from human specimens received directly from Frankfurt and observed the spatial and temporal relation between these particles and cytopathological changes both in guinea pig liver and in cell cultures, we conclude that these particles are probably the etiological agent of the fatal human disease. This conclusion is supported by our serological results. Moreover, the characteristics of ether and heat lability, resistance to the metabolic inhibitor 5-bromodeoxyuridine, and the cross-striated cylindrical structure suggestive of helical symmetry, indicate that the agent is viral. Similarities to viruses of the Stomatoviridae or rhabdovirus (*6*, *7*) family are evident. Cross-striation interval, core structure, suggestion of surface projections, and mode of maturation are similar to vesicular stomatitis virus, the prototype virus of the family. However, the particle diameter measured here (90 to 100 nm as compared to 65 to 75 nm for vesicular stomatitis virus) and, primarily, the remarkable length and extreme variation in length among particles serves to emphasize the uniqueness of this agent.

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20 February 1968

Dihydromatricaria Acid: Acetylenic Acid Secreted by Soldier Beetle

Abstract. *The aposematic cantharid beetle* Chauliognathus lecontei *produces a defensive secretion, from glands in its thorax and abdomen, containing 8-cis-dihydromatricaria acid. Similar acetylenic compounds are known only from certain fungi and flowering plants.*

Chauliognathus lecontei is a large red and black beetle (Fig. 1A) of the family Cantharidae (soldier beetles). A native of the southwestern United States and northwestern Mexico (*1*), it commonly occurs in dense and conspicuous aggregations on various flowering herbs and shrubs, sometimes in association with mimics (*2*). Like other members of its genus, it possesses a series of exocrine defensive glands in pairs on the prothorax and on the first to eighth abdominal segments where their openings are visible as small pores near the lateral margins of the segments. Each gland, a sac-like invagination of the integument, consists of a cuticular lining, a secretory epithelium, and a surrounding layer of compressor muscles. When the beetles are handled, pinched with forceps, or otherwise disturbed, they discharge their glands, and droplets of a white secretion emerge from the openings (Fig. 1, B and C) (*3*). We now report isolation and identification of an acetylenic acid that is a major component of the secretion.

Beetles (1500) were "milked" by gentle squeezing with forceps and absorption of the secretion with pieces of filter paper. Extraction of the papers with methylene chloride yielded a yellow viscous oil showing strong infrared absorption at 3.43, 3.48 (shoulder), 5.72, 5.79 (shoulder), and 8.80 μ, in addition to a region of weaker absorption between 2.85 and 4 μ, indicative of a carboxylic acid. Thin-layer chromatography also suggested the presence of an acidic function; the material was therefore separated into acidic and neutral fractions with aqueous sodium bicarbonate.

The neutral fraction consisted of a semisolid polymeric material and a nonpolar component that, isolated by preparative thin-layer chromatography, showed strong infrared maxima at 5.75 and 8.70 μ. On standing, the latter compound was shortly converted to a polymeric species and was examined no further (*4*).

In contrast, the bicarbonate phase, after thorough washing with methylene chloride and acidification, afforded colorless, odorless platelets (6.4 mg); the melting point was 55° to 57°C after crystallization from pentane. Inspection of the infrared spectrum [bands at 2.86, 3.13 to 3.85 (weak), 3.44, 5.73 (shoul-

der), 5.84, and 8.82 μ] showed resemblance to the spectra of long-chain carboxylic acids. Mass-spectral and ultraviolet-absorption data rapidly restricted the structural possibilities; a base peak at m/e 162, combined with ultraviolet-absorption maxima at 228 mμ (log ϵ, 3.00), 239 mμ (log ϵ, 3.43), 251.5 mμ (log ϵ, 3.78), 265.5 mμ (log ϵ, 3.98), and 281.5 mμ (log ϵ, 3.88), leads to the molecular formula $C_{10}H_{10}O_2$ and a chromophore of type I (*5*).

$$-\overset{|}{C}=\overset{|}{C}-C\equiv C-C\equiv C-$$

I

The nuclear magnetic resonance spectrum at 60 Mhz showed the following characteristics: a 3-proton quartet (coupling constants $J \cong 7$ and 1.5 hz) at τ8.14 (terminal, allylic methyl), a 4-proton singlet at τ7.28, and a 2-proton complex olefinic region at τ3.95 to τ4.60. From these data, structure II, 8-decene-4,6-diynoic acid, was derived, the τ7.28 singlet being assigned to the 4 methylene protons, while the complex region at lower field was attributed to the olefinic protons. On the basis of melting-point values (II*a*, 60.5°C; II*b*, 144°C) (*5*), the *trans* isomer II*b* was discounted, and complete agreement of the spectral properties (including infrared, ultraviolet, nuclear magnetic resonance, and mass spectra) of the natural product with those of authentic 8-*cis*-decene-4,6-diynoic acid (8-*cis*-dihydromatricaria acid) II*a* fully substantiated this assignment.

Structure II*a* was confirmed by comparison of methyl esters derived from the *Chauliognathus* acid and from authentic 8-*cis*-dihydromatricaria acid. These esters, prepared by treatment with diazomethane, were purified by preparative gas-liquid chromatography on a column of 8-percent SE30 silicone gum at 140°C. The infrared (5.75, 7.17, 8.30, and 8.52 μ) and mass spectra of both substances were superimposable (high-resolution molecular weight: measured, 176.0838; calculated, 176.0837), and their retention times on gas-liquid chromatography were identical. Ultraviolet absorption at 228 mμ (log ϵ, 3.47), 239 mμ (log ϵ, 3.80), 251.5 mμ (log ϵ, 4.10), 266 mμ (log ϵ, 4.27), and 281.5 mμ (log ϵ, 4.15) completely excluded the *trans* isomer II*b* (*5*).

A separate study of the vulnerability of *Chauliognathus* to predators will be reported elsewhere. Suffice it to say that the beetles are rejected by jays (*Cyanocitta cristata* and *C. stelleri*), grasshopper mice (*Onychomys torridus*), carabid beetles (*Calosoma prominens*), and ants (*Pogonomyrmex occidentalis*). They are acceptable to toads (*Bufo cognatus*), praying mantids (*Stagmomantis* sp.), assassin bugs (*Apiomerus* sp.), centipedes (*Scolopendra polymorpha*), solpugids (*Eremobates* sp.), and tarantulas.

Neither dihydromatricaria acid nor similar acetylenic substances have hitherto been isolated from insects or other animals, but various acetylenic compounds are known from certain fungi and flowering plants. Diversity is greatest in the Compositae, where the known compounds include the methyl ester of II*a*, as well as methyl esters III, IV, and V (*6*).

Adult *C. lecontei* commonly aggregate on composites and possibly they may obtain their acetylenic compound from the diet; but they aggregate on other plants also. Our beetles were drawn from three separate aggregates: two from composites (*Psilostrophe sparsiflora* and *Baccharis glutinosa*); the other from a species of Asclepiadaceae (*Asclepias subverticillata*), a family not known to produce acetylenic compounds. Samples of secretion from the three groups of beetles were initially examined separately, and chromatographic and spectrophotometric evidence showed them to be chemically indistinguishable. Of course, under natural conditions, beetles may move between aggregations, and it is possible that those on *Asclepias* obtained their acetylenic compound during previous sojourn on composites.

$$CH_3-CH=CH-C\equiv C-C\equiv C-CH_2-CH_2-COOH$$

II (*a*, *cis*; *b*, *trans*)

$$CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-COOCH_3$$

III

$$CH_3-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3$$

IV

$$CH_3-C\equiv C-C\equiv C-C\equiv C-CH=CH-COOCH_3$$

V

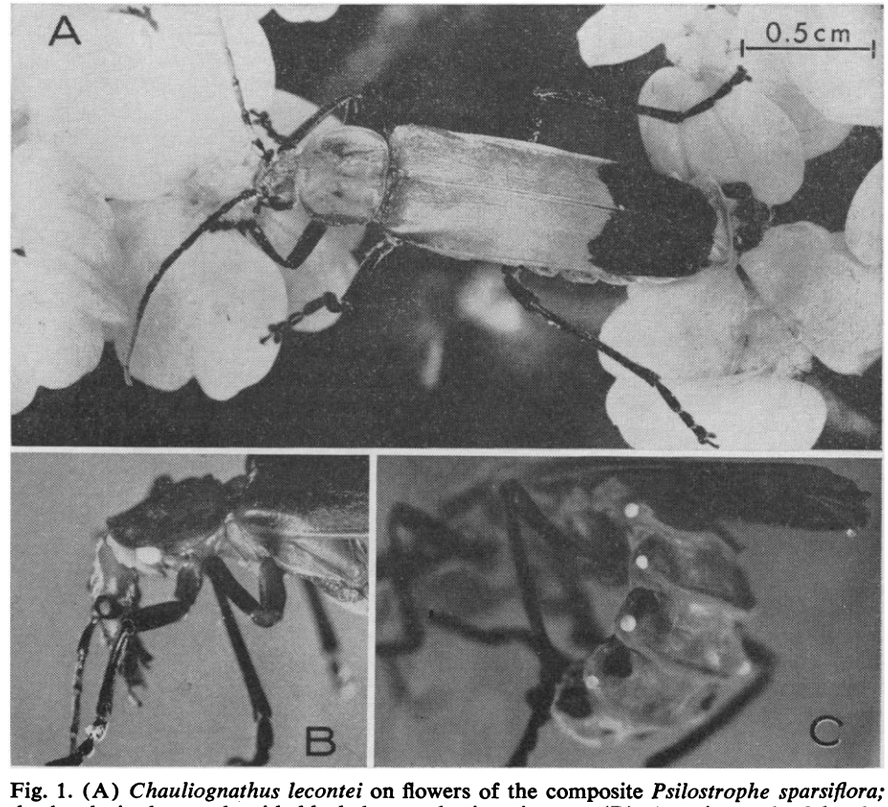

Fig. 1. (A) *Chauliognathus lecontei* on flowers of the composite *Psilostrophe sparsiflora;* the beetle is deep red, with black legs and wing tips. (B) Anterior end of beetle, showing white droplet of secretion discharged from left gland of prothorax. (C) Rear view of beetle, with abdomen curved downward, discharging secretion from four of its left abdominal glands.

The larvae of *Chauliognathus* are carnivorous (*7*) and have glands that are apparently homologous to those of the adult (*8*). Nothing is known about the chemistry of their secretion, which is white and sticky like that of the adult (*9*).

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10. Aided by NIH grant AI-02908. We thank W. J. Dress and P. A. Hyypio for identifying the composites, Rosalind Alsop for collecting the secretion, and S. Schrader of Cornell University's Mass Spectrometer Facility (NIH grant FR 00355). Also we thank N. A. Sörensen for 8-*cis*-dihydromatricaria acid and for providing ultraviolet-absorption data on the previously unknown *yne-ene-yne* chromophore, which is quite different in character. Report No. 22 of our series Defense Mechanisms of Arthropods.

30 January 1968

Cross-link in Fibrin Polymerized by Factor XIII: ε-(γ-Glutamyl)lysine

Abstract. *ε-(γ-Glutamyl)lysine has been isolated from enzymatic hydrolyzates of cross-linked human fibrin. This compound was not detected in "non-cross-linked" fibrin prepared with ethylenediaminetetraacetic acid, which inhibits factor XIII; intermediate amounts were observed when the fibrin was prepared with glycine ethyl ester, which inhibits factor XIII competitively. These and ancillary experiments furnish conclusive evidence that ε-(γ-glutamyl)lysine cross-links form in human fibrin during polymerization catalyzed by factor XIII.*

The cross-linking of fibrin is the terminal step in blood clotting. This reaction is catalyzed by an enzyme; the inactive precursor of this enzyme is now designated factor XIII, though it has been known as serum factor, Laki-Lorand factor (LLF), fibrinase, fibrin-stabilizing factor (FSF), and plasma transglutaminase (*1*, *2*).

Earlier reports indicated that cross-linking occurred through the joining of fibrin monomer molecules by a transamidation (or transpeptidation) reaction involving glutaminyl (or asparaginyl residues of the "acceptor" polypeptide chain and free amino groups on the "donor" chain. This concept was based on (i) reduction in the number of amino-terminal glycine residues in cross-linked fibrin, (ii) inhibition of cross-linking by certain glycine derivatives, and (iii) liberation of ammonia during cross-linking (*2*).

There is evidence that the lysine of the "donor" chain rather than glycine inhibits the cross-linking (*3*). Fuller and Doolittle (*4*) and Lorand *et al.* (*5*) postulated that the cross-link is ε-(γ-glutamyl)lysine. Since active factor XIII has transamidase activity toward glutaminyl residues (*6*) and various tissue transamidases catalyze a cross-linking of fibrin (*7*), the postulated ε-(γ-glutamyl)lysine bond seemed reasonable and highly tenable; however, neither group proposing this cross-link was able to demonstrate it directly.

We now report that the ε-(γ-glutamyl)lysine bond (Fig. 1) is in fact the cross-link in fibrin polymerized by factor XIII. ε-(γ-Glutamyl)lysine was isolated from total enzymic hydrolyzates of polymerized fibrin, and the amount of this dipeptide was compared with that in hydrolyzates of fibrin monomer. This experiment was feasible because ε-(γ-glutamyl)lysine is not hydrolyzed by common peptidases and proteases (*8*).

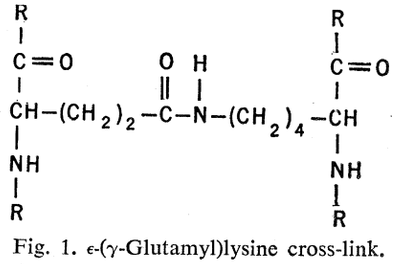

Fig. 1. ε-(γ-Glutamyl)lysine cross-link.

The human fibrinogen used [Blombäck (*9*) fraction I-2, precipitated three times in succession in the presence of ε-aminocaproic acid to remove plasminogen (*10*)] was more than 92 percent clottable; the amount of factor XIII present as a contaminant was enough to yield a totally acid-insoluble clot (*11*). Samples containing 22 mg of fibrinogen were dissolved in 0.1*M* tris-(hydroxymethyl)aminomethane chloride (tris) buffer, *p*H 7.5, and were placed in small dialysis sacs. Solutions prepared in the same buffer (and adjusted to *p*H 7.5 if necessary) were added to obtain the desired final concentrations in a total volume of 2 ml. All samples were adjusted to 0.015*M* with respect to cysteine and all except those containing ethylenediaminetetraacetic acid (EDTA) were made up to contain 0.008*M* $CaCl_2$; EDTA (0.008*M*) was used in certain samples to prevent cross-linking by removing Ca^{++} ions required for the activity of factor XIII; glycine ethyl ester (GEE), a competitive inhibitor of factor XIII (*2*), was used in others at a concentration of 0.1*M*. (The EDTA and GEE concentrations were chosen to give fibrin clots soluble in 1 percent monochloroacetic acid, 2 percent acetic acid, and 8*M* urea, reagents which are used to detect cross-linking.)

The samples were clotted with human thrombin (*12*), incubated at 37°C for 90 minutes, and dialyzed at 5°C, first against 0.1*M* NH_4HCO_3, then against 0.1*M* NH_4HCO_3 containing 0.01*M* $CaCl_2$. After dialysis, each sac was cut into small pieces; the mixture of tubing and clot fragments was incubated with 1 mg of trypsin at 37°C for 24 hours, and the samples were freeze-dried and dissolved in 2.00 ml water. Half of each sample was then digested successively with pronase (1.33 mg/ml), prolidase (460 unit/ml), and leucine aminopeptidase (110 unit/ml), essentially by the procedure of Pisano *et al.* (*13*). After the last incubation, tritiated (*14*) synthetic ε-(γ-glutamyl)lysine was added to each sample, and the protein was precipitated with an equal volume of 24 percent trichloroacetic acid. After centrifugation, the supernatant was diluted with five volumes of water and applied to a column (1 by 10 cm) of Bio-Rad AG 50-X4 (200 to 400 mesh; H^+ form). The column was washed with 30 ml of water, then eluted with 1*M* pyridine until the radioactivity was recovered (about 45 ml). The radioactive eluate was freeze-dried, dissolved in