A Procedure for Purification of Myrmica Venom: The Isolation of the Convulsive Component.

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During the last twenty years, many attractants, repellents, odor-trail substances, and sting lubricants were found in sting-bearing (Myrmicinae) and venom-spraying ants (Formicinae). These volatile odorous gland secretions are mainly terpenoids[+].

Formic acid is the predominant secrete of the venom gland of the Formicinae. As yet, little is known of the chemistry of non-volatile odorless venom components in ants. Because of the prevalence of the genus Myrmica in the south of Germany and the painful sting of the insects, the following studies were initiated: 1) to find a method for venom collection; 2) to isolate the venom components; 3) to study the pharmacological properties of the venom.

Isolation. The best method for collection of Myrmica venom, from a quantitative standpoint, was to excite the ants by means of electric shock operation. For this purpose it was prepared an electric shock apparatus, in which the ants were excited, and the secrete was extracted in water. In this apparatus it was possible to excite 250 Myrmica rubida LATR. or 700 Myrmica ruginodis NYL. in one procedure.

A second method for collection, while the least desireable from a purity standpoint, consisted of extracting the whole gaster of the ant in water. The supernant fluids were lyophilized and chromatographed by DEAE-sephadex A 50.

Further purification. The previously described electric shock technique for isolation of odorless venom components of Myrmica ruginodis has been extended by chromatography on a Sephadex G 50 column.

[+] For a review see: G.W.K. Cavill and P.L. Robertson 1965, Science 149, 1337-1345.

45 700 Myrmica ruginodis yield 152 mg proteinaceous and 1141 mg non-proteinaceous venom. The proteinaceous one consists of the minor component hyaluronidase (fraction 1), an enzyme with venom spreading properties and the so-called ant venom fraction 2, the major component, moving at pH 2,0 towards the cathode (Fig. 1).

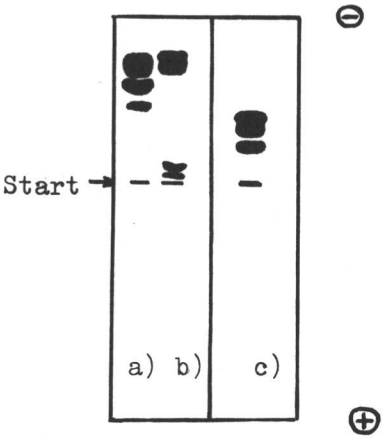

Fig. 1. Electrophoretic pattern of Myrmica venom: a) Bee venom melittin[+] (reference peptide, raw material), b) fraction 1 and 2 after 40 min. of electrophoresis at a potential gradient of 250 V at 1° C in urea-formic acid buffer, pH 2,0; c) Myrmica venom fraction 1 and 2 after 20 min. of electrophoresis at a potential gradient of 300 V in diethylbarbiturate buffer, pH 8,6, followed by staining with 0,2 % Ponceau S.

Both venom fractions are only roughly separable by gel filtration on Sephadex G 50. The average molecular weight of 14 900, determined on a Sephadex G 50 column, is calculated for the ant venom fraction 2.

[+] J. Jentsch 1968, Umschau in Wissenschaft u. Technik 68, 816-817.
J. Jentsch 1969, Z. Naturforsch. 24b, 33-35.
J. Jentsch 1969, Z. Naturforsch. 24b, 415-418.
J. Jentsch 1969, Z. Naturforsch. 24b, 597-598.

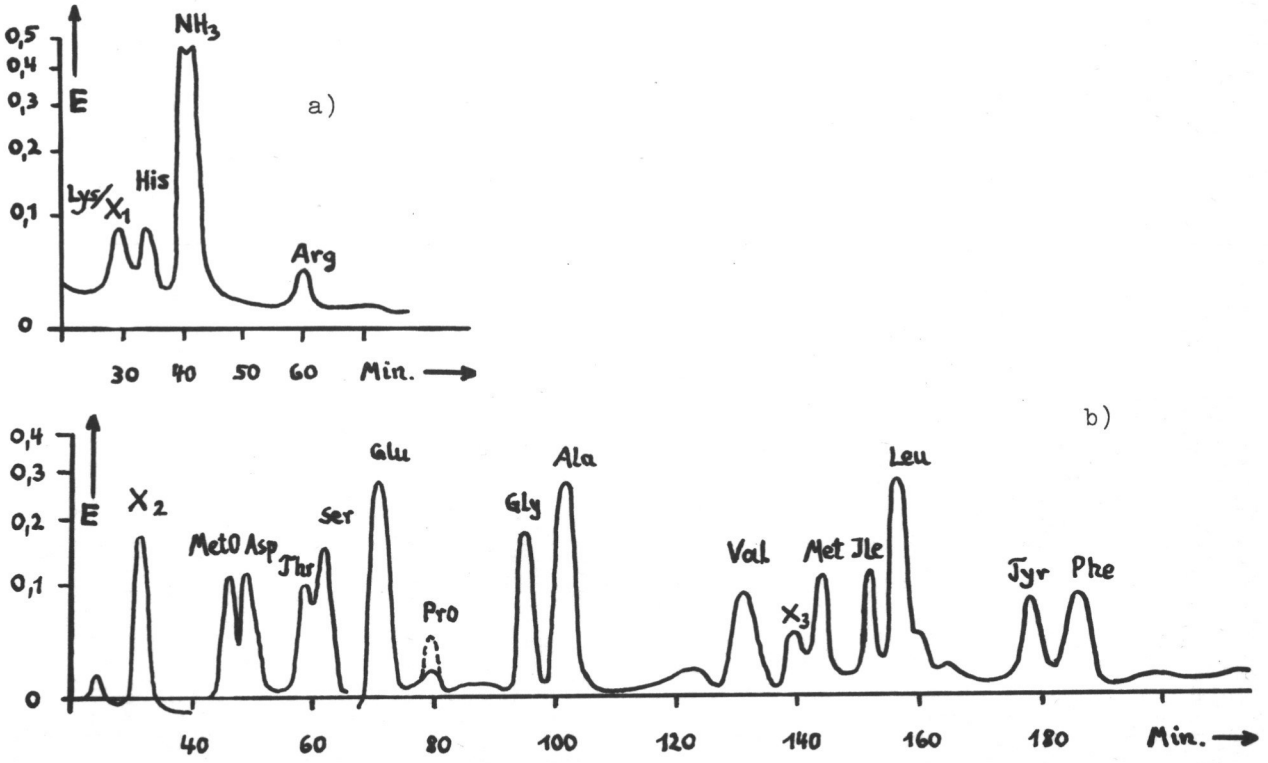

Fig. 2. Amino acid composition of fraction 3: a) Basic components, b) neutral and acidic components.

Separation of the whole proteinaceous venom by means of electrophoresis in urea-formic acid buffer delivers two additional venom components, also stainable with Ponceau S (Fig. 1). Neither phospholipases nor any hemolyzing proteins were found in Myrmica ruginodis, which are known in the toxins of many animals.

The Myrmica venom may not correspond to the peptide melittin, known to be the toxic main component in bee venom. It seems to be more toxic in insects than in vertebrates, as resulted from later experiments with the american cockroach. The venom seems to be directed mainly against insects.

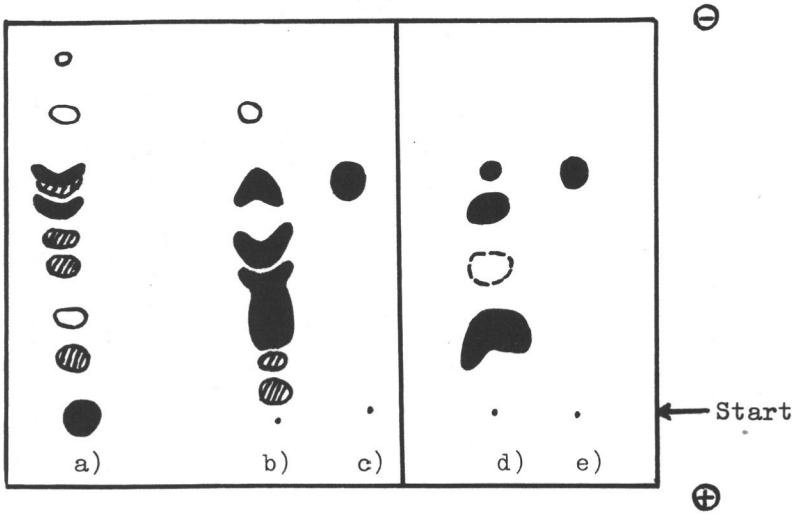

Fig. 3. Thin layer chromatogram and electropherogram of Myrmica venom: a) Venom from 23 dissected venom reservoirs, b) fraction 3, c) histamine (reference substance); below 0,08 mm silica gel; eluent: chloroform-ammonia; d) fraction 3, e) histamine; electrolyt: pyridine (100 ml)/water (900 ml)/acetic acid, pH 6,5, after 10 min. of electrophoresis at a potential gradient of 1400 V, followed by staining with 0,3 % ninhydrin.

The proteinaceous venom fractions 1 and 2 appear to be relatively stable. They were not destroyed on lyophilization and not lost their lethal effect for many months in the cold.

Fig. 4. Venom apparatus of Myrmica ruginodis from STITZ[+].

When the non-proteinaceous portion of the venom (fraction 3) is subjected to amino acid analysis according to the method of SPACKMAN, STEIN, and MOORE[++] 16 naturally occuring amino acids were found in the eluats followed by the ninhydrin reaction (Fig. 2). Cystine, cysteine and tryptophane are absent. In addition, fraction 3 contains the so-called substances X_1, X_2, X_3, and histamine (biogenic amine). The compounds X_2 and X_3 are perhaps peptides.

The chromatogram of fraction 3 is roughly the same as a pattern of the extract from 23 dissected venom reservoirs, besides the highmolecular compounds, which were separated by gel filtration. But it could be, that a part of the amino acids was excreted from the crop region of the ants (Fig. 3).

[+] H. Stitz 1939, Hymenoptera: Formicidae. In F. Dahl, "Die Tierwelt Deutschlands", Vol. 37, Jena 1939.

[++] D.H. Spackman, W.H. Stein and S. Moore 1958, Analyt. Chem. 30, 1190-1206.

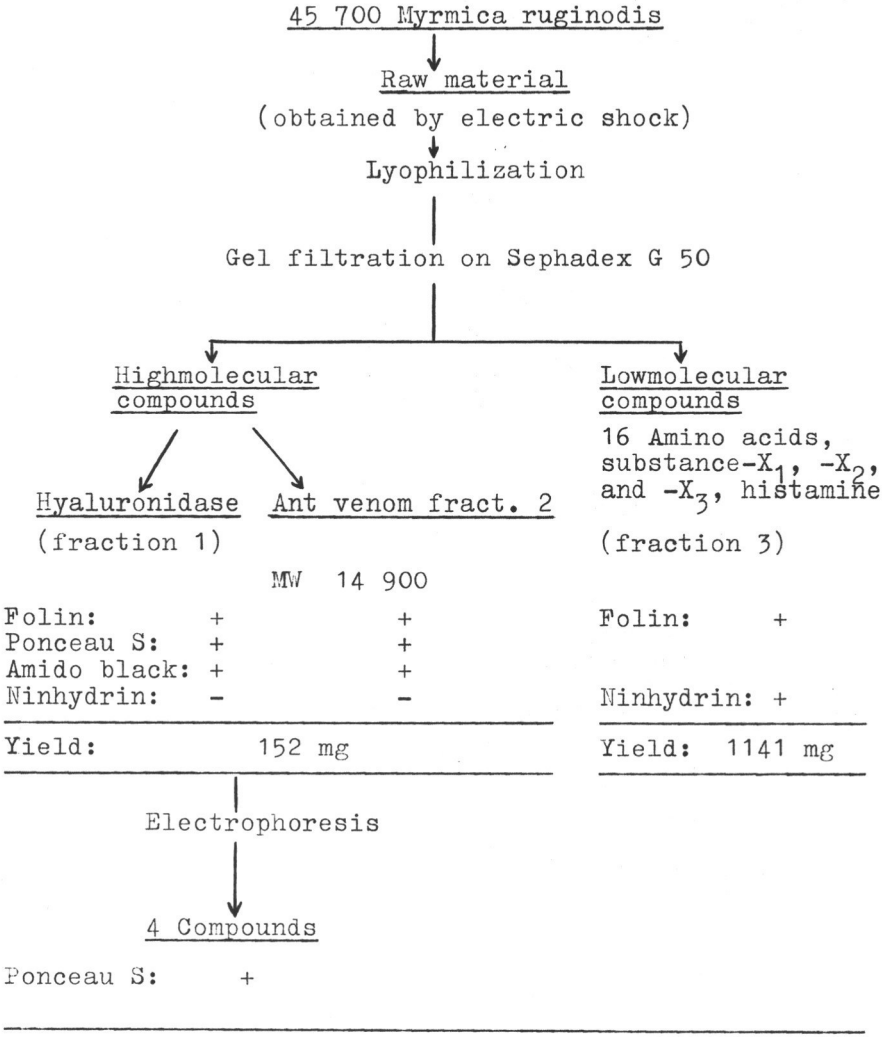

Fig. 5. Scheme for purification of Myrmica venom.

The occurrence of amino acids in animal toxins was demonstrated in 1964[+].

When dissecting the venom reservoir of Myrmica ruginodis (see Fig. 4), a clear colorless aqueous solution was obtained. Thus, the water-insoluble volatile alarm secretions, attractants, and repellents cannot originate from this region of the system of exocrine glands. - Fig. 5 reviews the purification steps of Myrmica venom.

Pharmacological properties. A long lasting sting of Myrmica in the arm of a human test subject results in an extreme sting pain, a large flare and a small edematous area at the site of injection. Sweating, feverishness and piloerection of the area and axillary pain are observed (Pogonomyrmex barbatus similar tested gave the same results[++]). - A sublethal intraveneous dosage of the whole venom (fraction 1/2 and 3) in female white mice results in extreme lethargy and piloerection. After injection of lethal dosages of either the whole Myrmica venom or fraction 1/2 the following effects are observed: extreme sensibility when touching the mouse, occasionally paralysis of the behind extremities, followed by clonic convulsion, respiratory distress and death. - The reaction of the american cockroach against the venom mixture was also tested.

The venom is toxic to female mice and when given intraveneously the LD_{50} is in the range of 50 - 60 mg per kilogram of body weight.

[+] H. Michl and H. Bachmayer 1964, Monatsh. Chem. 95, 480-484.

[++] M.W. Williams and C.S. Williams 1964, Proceed. Soc. Exp. Biol. Med. 116, 161-163.