

recorded for 72 hours. Basic emergency medical care was available and volunteers were monitored under direct supervision of medical staff.

Preparation of Extract from Abdominal Glands and Chemical Analysis

Workers ($n = 7$) were thawed in a refrigerator and then their abdominal gland structure, attached to the sting apparatus (Figures 2B and 2C), was pinched out in distilled water with the aid of a dissecting microscope (Stemi SR®, Zeiss, Germany). Dissected venom glands were placed in 0.5 mL microtubes and disrupted by ultrasonic waves (UP 400S®, Hielscher, Germany) for three minutes in a cycle mode of 0.5 second, 50 kHz in either 250 μ L hexane (chromatography grade, Merck, Germany) or 50 μ L of a 9:1 solution of acetonitrile and trifluoroacetic acid. The resulting mixtures were shaken for one minute and membranes were removed by centrifugation for five minutes at 3000 r/m. The hexane-based extract was concentrated by nitrogen flow and examined by GC-MS on a Varian Star 3800® instrument linked to a Varian Saturn 2000® Mass Selective Detector, set to monitor m/z 40-350 at 70 eV with a scanning speed of 1 scan/sec. The samples were analyzed using a fused silica capillary column (50 m \times 0.25 mm ID, 0.12 μ m FT) coated with CP-Sil8cb (5% phenyl, 95% polydimethylsiloxane). The oven temperature was programmed to rise from 50°C to 300°C at 15°C/minute. The carrier gas was helium at 1.2 mL/min. The injector port temperature was held at 200°C. The peak area of each component in the chromatogram was determined and then the percentage of each substance in the gland calculated by the Saturn® GC/MS Workstation computer package Saturn view® version 5.2.1, 1989-1998 (Varian Associates, Inc., USA).

High-performance liquid chromatography (HPLC) was performed using a Waters 6000A® pump and Waters R401® (USA) differential refractometer detector. Ten microliters of the acetonitrile/trifluoroacetic acid extract was injected into a C₁₈ reserved phase column (5 μ m, particle size; 220 \times 2.1-mm column; Vydac) and separation was monitored at a flow rate of 20 μ L/minute (28).

Chemical Identification

Identification was primarily accomplished by using spectra from the NIST library (NIST Mass Spectrometry, 2007). Precise identification was based on comparison of GC retention time and mass spectra with either authentic or the laboratory-