

Application of attenuated total reflection infrared spectroscopy in the study of *Peruphasma schultei* defensive secretion

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Abstract. In this short communication, we present the first Fourier Transform Infrared Absorbance (FT-IR) study of peruphasmal; a defensive secretion from *Peruphasma schultei*. The spectral data collected are representative of the natural product structure proposed by Dossey et al. This study demonstrates the viability of FT-IR as another tool in the physical and biological chemist's repertoire for use in determining important structural characteristics from minute amounts of available sample.

Keywords: Peruphasmal, secretion, nuclear magnetic resonance, infra-red spectrum assignments, FT-IR, NMR

1. Introduction

Individual organisms often produce natural product compounds in extremely minute amounts. However, relatively large quantities of these products are typically needed for meaningful structural studies [1–3]. Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for structural studies [3,4], but has a large sample requirement [2,3]. Recently, Dossey et al. [2] used a special 1.0-mm diameter NMR probe, invented by Brey et al. [4], that significantly reduces the sampling requirement for NMR [2]. Dossey et al. [2] were able to use this technology to study the defensive secretions from individual walkingstick insects (Order Phasmatodea). Another physical technique that can be used to study the structures of natural products is Fourier Transform Infrared Spectroscopy (FT-IR) [5,6]. Recent advances in infrared technology allow the study of extremely small amounts of natural products. In this note, we report the infrared study of peruphasmal [2], an important constituent of the defensive secretion of *Peruphasma schultei*. *P. schultei* is a recently discovered species of phasmid, which habituates the Cordillera del Condor in Northern Peru [7]. It produces a defensive secretion that contains glucose and peruphasmal, an isomer of anismorphal [8,9] and dolichodial [9–11]. In aqueous solution, and in nature, these compounds are in equilibrium with their congruous diol [2] (see Fig. 1). To our knowledge, this is the first FT-IR study of peruphasmal. We were able to make spectral measurements using approximately only 140 µg of peruphasmal dissolved in 10 µl of deuterated chloroform (CDCl₃); a distinct advantage over other physical methods of structural analysis which require significantly greater sample size.

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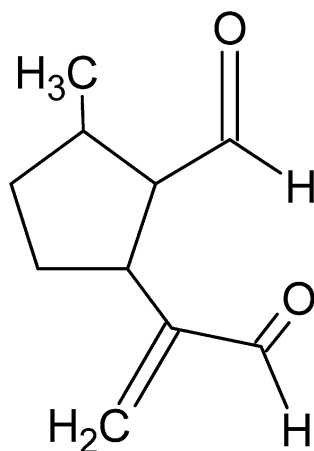


Fig. 1. Proposed non-stereospecific structure of peruphasmal suggested by Dossey et al. [2].

2. Materials and methods

The sample was prepared from a single spray of about 20 μl from a large adult female that was collected by agitating the insect while a glass vial was put over the defensive gland. The secretion was dissolved in 2 ml of D_2O followed by extraction with 500 μl of CDCl_3 (99.98% Cambridge Isotope Laboratories, Inc.). NMR, Gas Chromatography (GC) and Mass Spectrometry (MS) were performed on the sample to check its quality and purity (see Figs 2, 3a and 3b). All peaks observable in the NMR spectrum are consistent with peruphasmal and the proton impurity of CHCl_3 in the CDCl_3 solvent [2].

Sample preparation for GC-MS consisted of adding 3 μl of CDCl_3 extract to a vial containing 500 μl of methyl *tert*-butyl ether (MTBE). Analysis was performed using an Agilent 6890 N gas chromatograph and a 5975 B ion trap mass spectrometer in chemical ionization mode (isobutane) that acquired full-scan spectra over the ranges m/z 40–400 at 0.85 s per scan. Holox (Charlotte, NC) high-purity helium was used as a carrier gas (1.4 ml/min). Cool on-column injection at 40°C was used (1 μl); transfer-line and manifold temperatures were 240 and 220°C, respectively. The oven program was as follows: isothermal for 2 min, heating from 40 to 260°C at 10°C/min, and then isothermal for 10 min. GlasSeal connectors (Supleco) fused three silica columns in series: a primary deactivated column (8 cm long, 0.53 mm i.d.), an HP-1MS retention gap column (2 m long, 0.25 mm i.d., $df = 0.25 \mu\text{m}$), and a J&W DB-5 analytical column (30 m long, 0.25 mm i.d., $df = 0.25 \mu\text{m}$).

A one-dimensional (1D) ^1H NMR spectrum was acquired on 10 μl of the CDCl_3 extract at 27°C using a Bruker Avance II 600 MHz spectrometer (Bruker 600 UltrashieldTM magnet) with a 1-mm triple resonance high temperature superconducting probe developed by Brey et al. [4]. NMR protocol involved a simple 1D pulse program with a 2 second presaturation/relaxation delay, 8 scans, 2 dummy scans, 8k real data points over a 12 ppm spectral width, and a 45 degree tip angle. CHCl_3 was assigned to 7.24 ppm and its integral was used to estimate an approximate concentration of peruphasmal in the final sample used for FT-IR. The data were analyzed using Bruker Topspin 1.0 and the post-script files made with XWinPlot.

FT-IR spectra were recorded on a Bruker Vector 33 spectrometer equipped with a KBr beam splitter and deuterated triglycine sulfate (DTGS) room temperature detector. The spectra were recorded in the

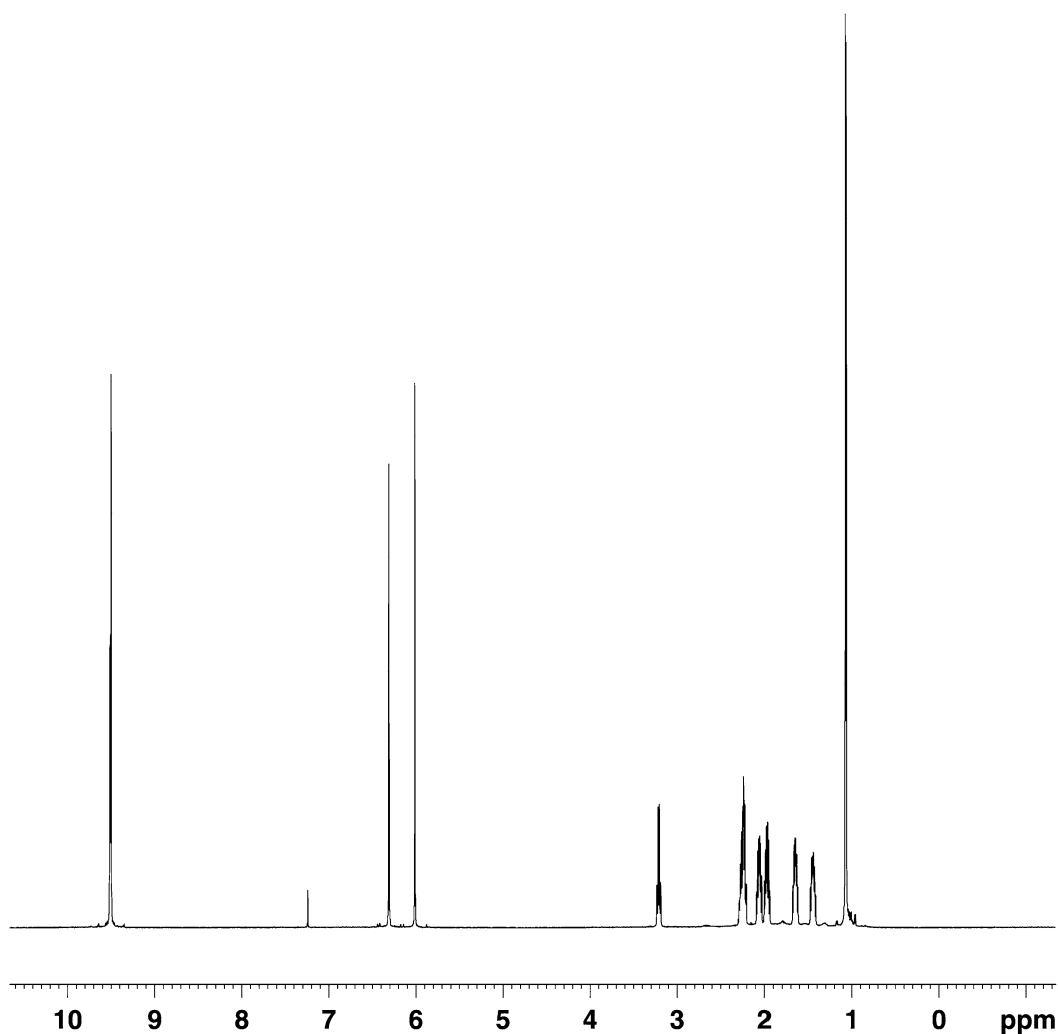


Fig. 2. NMR spectrum of peruphasmal sample to demonstrate appropriate purity. All peaks observable in the spectrum are consistent with resonances from peruphasmal and CHCl_3 .

attenuated total reflection (ATR) mode with a single bounce MIRacle[®] ATR accessory from PIKE technologies, Inc., Madison, WI. The accessory was equipped with a split pea shaped ZnSe ATR crystal. To record spectra, about 10 μl of sample solution in CDCl_3 was poured on the ATR crystal and allowed to dry. This produced a uniform film of peruphasmal on the ATR crystal whose single beam spectrum was recorded and then rationed against the single beam spectrum of the bare crystal to obtain the absorbance spectrum. Spectra were recorded with a resolution of 4 cm^{-1} and 64 scans were averaged for each spectrum. The spectrometer's optics were sealed from the atmosphere but its compartment was not purged during measurements.

The spectra were run and processed with a Bruker OPUS program. To check the reproducibility of the spectral measurements, spectra were recorded on three different days and were found reproducible to within 5% or better throughout the spectral range.

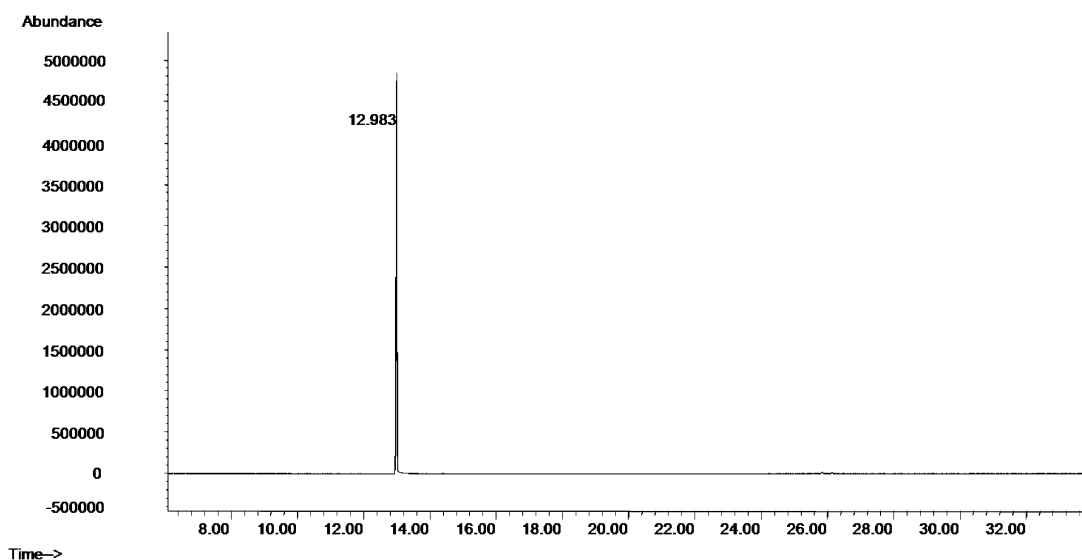


Fig. 3a. Total ion gas chromatograph of peruphasmal sample. This further demonstrates the sample's purity.

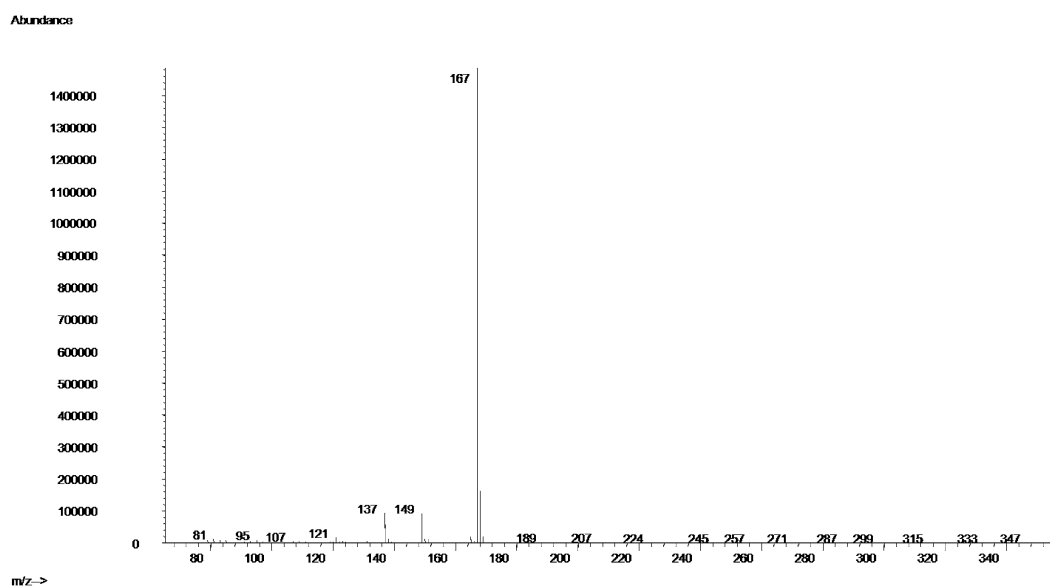


Fig. 3b. Mass spectrum (chemical ionization) of the major peak in the gas chromatograph (a). The molecular weight of peruphasmal is 166 daltons (peak shows $M + 1$ ion).

3. Results and discussion

The CH stretching ($3150\text{--}2600\text{ cm}^{-1}$) and fingerprint regions ($1800\text{--}900\text{ cm}^{-1}$) of the infrared spectrum of the dialdehyde isomer of Peruphasmal are shown in Figs 4 and 5. The spectral region between 2600 to 1800 cm^{-1} is not shown as it was devoid of any spectral peaks. The major peaks in these spectra arise from CH stretching, C=O stretching, CH bending, CH rocking and CH wagging modes. Table 1 lists the frequencies of major peaks and their assignments. These assignments are based upon previous

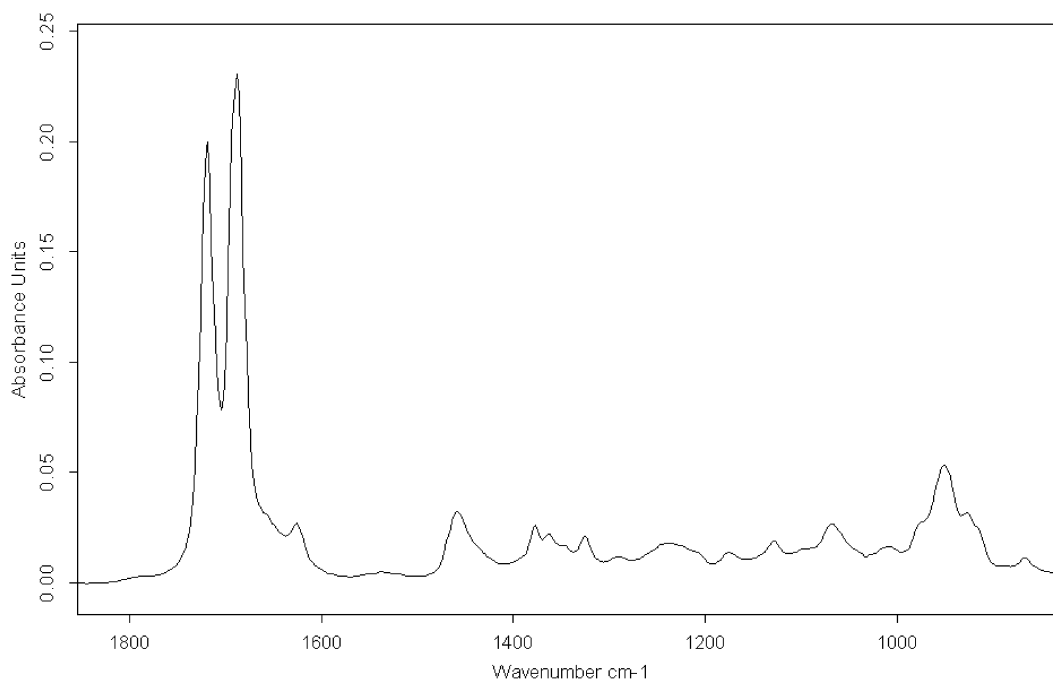


Fig. 4. Fingerprint region of FT-IR spectrum of the peruphasmal sample.

infrared work on molecules, which are approximate fragments of the structure of the dialdehyde isomer of peruphasmal. Most of the peaks above 1300 cm^{-1} can be attributed to various functional groups of the dialdehyde isomer of peruphasmal with a high degree of certainty, as these peaks are fairly well resolved. However, the assignments below 1300 cm^{-1} should be considered tentative, because of the spectral congestion that is caused by overlapping modes (bending, rocking, wagging) due to the very similar nature of the methyl, cyclic and alkenic CH oscillators.

The most notable features of the infrared spectrum of Fig. 4 are the two carbonyl peaks at 1718 and 1689 cm^{-1} that arise from C=O groups of methylcyclopentylcarbaldehyde and acroleinic parts of peruphasmal. It is interesting to note that the acroleinic C=O peak of peruphasmal absorbs at lower frequency (1689 cm^{-1}) compared with C=O peak (1698 cm^{-1}) in acrolein. This is most likely due to the electron donating properties of the methylcyclopentylcarbaldehyde group attached to the 2 carbon of acroleinic aldehyde, which may cause the downward shift in frequency.

Acrolein has been associated with vascular toxicity [12], axonal membrane disruption [13], bladder epithelium toxicity [14], inhibition of T-cell responses [15], disregulation of neutrophil apoptosis [16], hepatocyte cytotoxicity [17], lipid peroxidation [18] and neurotoxicity [19]. Studies by Eder and Deininger [20] and Takamoto et al. [21] along with structural similarities between peruphasmal and methyl, ethyl, and propyl substituted acroleins [20,22] suggest that peruphasmal may have similar chemical mechanisms that drive their biological activities. This literature and our FT-IR spectral data seem to support the hypothesis that the methylcyclopentylcarbaldehyde may act as an electron donating group leading to nucleophilic and electrophilic susceptibility as per the mechanism put forth in Fig. 6. However, this hypothesis has yet to be investigated.

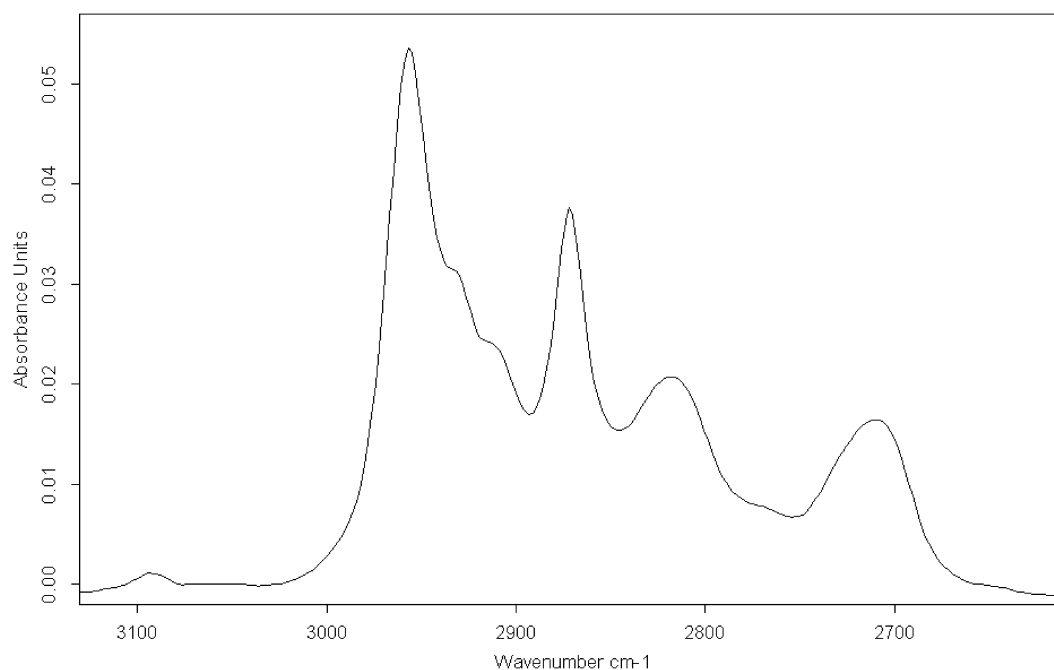


Fig. 5. C–H stretching region of FT-IR spectrum of the peruphasmal sample.

Table 1

Assignment of the major peaks of peruphasmal FT-IR spectrum

FT-IR frequency (cm ⁻¹)	Assignment
3090	=C–H stretching
2956	Antisymmetric cyclo C–H stretching
2931	Combination
2909	Symmetric C–H stretching
2871	Antisymmetric cyclo C–H stretching
2816	Symmetric C–H stretching
2709	Aldehyde C–H stretching
1718	Methylcyclopentylcarbaldehyde C=O
1689	Acroleinic C=O
1627	C=C stretching
1459	CH ₂ deformation
1380	CH ₃ deformation
1325	CH ₂ rocking
1231	CH ₂ rocking
1178	CH ₂ wagging
1128, 1069, 1008, 973	CH bending modes
951	CH ₃ rock

4. Conclusions

FT-IR spectroscopy in the attenuated total reflection mode is a viable tool for studying the structure of small amounts of venom from phasmid samples. The NMR technique of Dossey et al. [2] using the probe invented by Brey et al. [4], in combination with FT-IR technique has lead to a better understanding

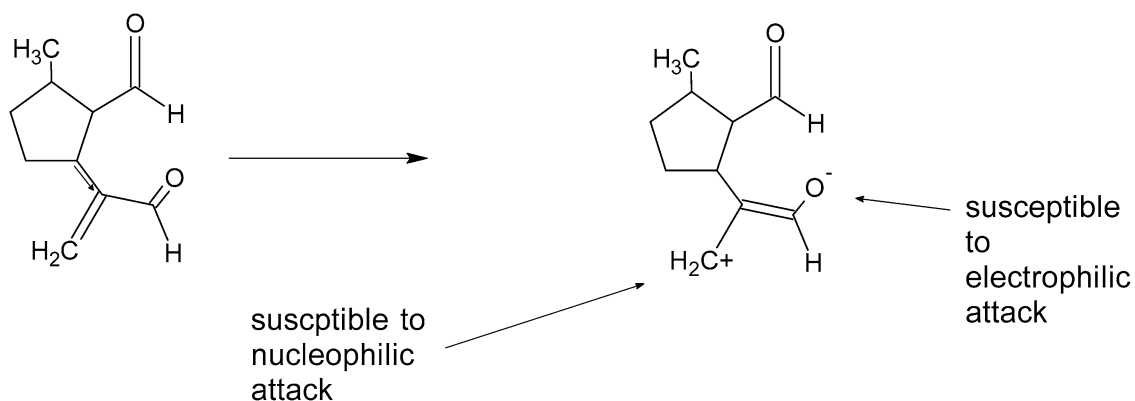


Fig. 6. Hypothetical nucleophilic and electrophilic susceptibility to acroleinic portion of peruphasmal.

of the chemical structure and nature of peruphasmal. Future use of this method as well as the structural information gathered in this study will inevitably lead to a more thorough comprehension of other natural products that exist in relatively small quantity.

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