Chemical defence of phalangodid harvestmen: *Bishopella laciniosa* (Crosby & Bishop) and *Texella bifurcata* (Briggs) produce 2-methyl-5-ethylphenol (Opiliones: Grassatores: Phalangodidae)

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Summary

This is the first report of the chemistry of the defensive secretions of harvestmen of the family Phalangodidae. Using gas chromatography/mass spectroscopy and infrared spectroscopy, we identified 2-methyl-5-ethylphenol as the sole methanol-extractable component from specimens of *Bishopella laciniosa* (Crosby & Bishop) from North Carolina, and from a single specimen of *Texella bifurcata* (Briggs) from Oregon. This compound has been recorded in Opiliones before as a component of the secretion of the cosmetid species *Cynorta astora* Goodnight & Goodnight and *Eucynortula albipunctata* (Pickard-Cambridge), the gonyleptid *Pachyloidellus goliath* Acosta, and the stygnommatid *Stygnomma spinifera* (Packard).

Introduction

Among arachnids, harvestmen (Order Opiliones) are well-known for the diversity of their chemical defences, recently reviewed by Gnaspini & Hara (2007). The majority of species that have been studied belong to the infraorder Grassatores, especially the endemic Neotropical families Cosmetidae and Gonyleptidae (see also Hara *et al*., 2005). The two families are thought to be closely related (Giribet & Kury, 2007) and both are members of the superfamily Gonyleptoidea. Numbers of different benzoquinones and phenols have been detected in cosmetids and gonyleptids (Gnaspini & Hara, 2007; Hara *et al*., 2005). The single species of Stygnommatidae (Samooidea) studied, *Stygnomma spiniferum* (Packard), secretes a mixture of three methylethylphenols. No species of the other two superfamilies of Grassatores, Zalmoxoidea and Phalangodoidea, have been studied up to now.

Methods and Results

Specimens of *Bishopella laciniosa* (Crosby & Bishop) were collected by WAS at the Western Carolina University Cullowhee Mountain Preserve, Cullowhee, Jackson Co., North Carolina, USA, directly into vials containing less than 1 ml USP methanol. A single specimen of *Texella bifurcata* (Briggs) was collected by Casey Richart in Curry Co., Oregon, USA, likewise directly into a vial with less than 1 ml USP methanol. To avoid contamination, the glass vials had caps lined with Teflon. The specimens, now preserved in 70% ethanol, have been placed as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia, USA. The methanol extract of the specimens was analysed using a Shimadzu QP-5000 GC/MS equipped with an RTX-5, 30 m × 0.25-mm i.d. column. A single volatile component was observed in the methanol extracts of both species that had a mass spectrum m/z=136 (M^+, 44), 121 (100), 107 (8), 103 (3), 91 (16), 77 (15), suggesting an ethyl, methylphenol. An authentic sample of 2-methyl-5-ethylphenol was prepared (Morgan & Pettit, 1934) and this had an identical mass spectrum and gas chromatographic retention time to those of the natural material. The virtually identical vapour phase infrared spectra (Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m × 0.25 mm RTX-5 amine column) of the natural and synthetic 2-methyl-5-ethylphenol confirmed the geometry of the natural material from the harvestmen (Fig. 1).

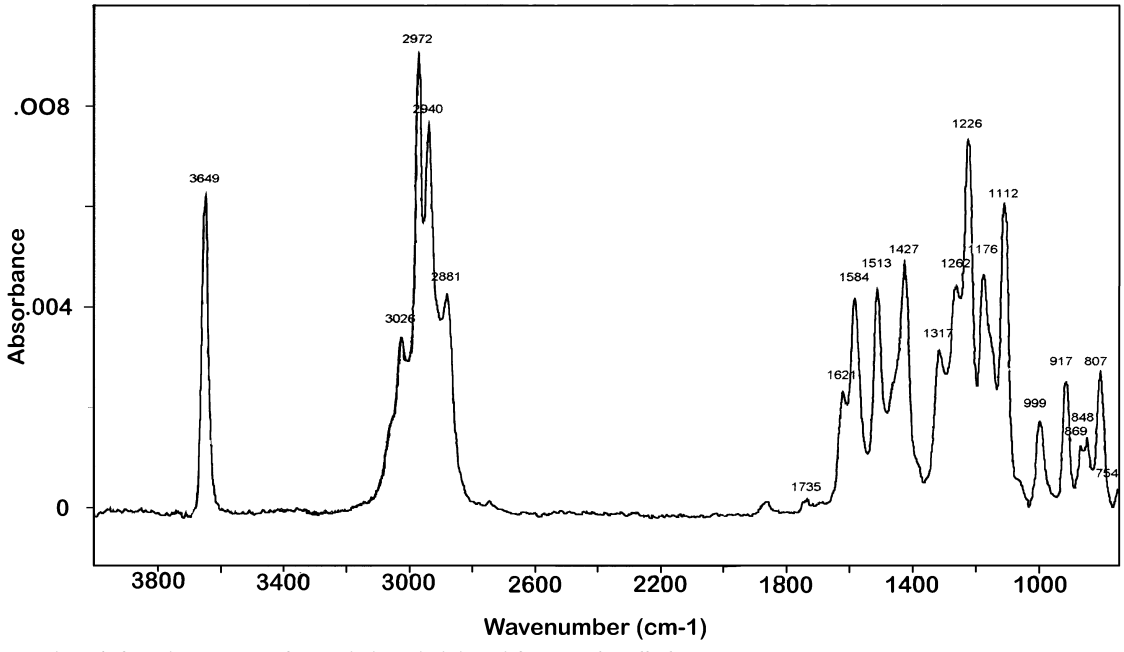

Fig. 1: Vapour phase infrared spectrum of 2-methyl-5-ethylphenol from *Bishopella laciniosa*.

Species	Secretion components	Reference
Cynorta astora	2,3-dimethylphenol **2-methyl-5-ethylphenol**	Eisner *et al.* (1977)
Eucynortula albipunctata	2,3-dimethylphenol **2-methyl-5-ethylphenol**	Roach *et al.* (1980)
Pachyloidellus goliath	2,3-dimethylphenol 2-methyl-5-ethylphenol **2-methyl-5-ethylphenol** (and three alkylated benzoquinones)	Acosta *et al.* (1993)
Stygnomma spinifera	2,3-dimethylphenol 2,3-dimethyl-5-ethylphenol **2-methyl-5-ethylphenol**	Duffield *et al.* (1981)

Table 1: Defensive secretion chemistry of grassatorean Opiliones utilising 2-methyl-5-ethylphenol.

Discussion

Juberthie (1976) described the anatomy and defensive behaviours of a European phalangodid, *Scotolemon lespesi* Simon, but did not chemically analyse the secretion. The internal repugnatorial glands of *S. lepesi* are large, extending posteriorly from the location of the ozopores nearly to the posterior border of the carapace. As in a number of other laniatorids, the secretion is conducted posteriorly by a well-defined cuticular groove that runs from the pore to the last free abdominal tergite. Although we did not make observations of the behaviour of *B. laciniosa*, a very similar groove is found in that species, as in *Stygnomma spinifera* and several gonyleptids in which secretion has been observed.

2-methyl-5-ethylphenol was found to be the sole methanol-extractable volatile from the two phalangodid species we examined. The same molecule has been detected before in four species of harvestmen, namely *Cynorta astora* Goodnight & Goodnight, from Panama (Eisner *et al.*, 1977), *Eucynortula albipunctata* (Pickard-Cambridge), from Costa Rica (Roach *et al.*, 1980), *Pachyloidellus goliath* Acosta, from Argentina (Acosta *et al.*, 1993), and *Stygnomma spinifera* (Packard), from Florida, USA (Duffield *et al.*, 1981). The first two named are in the family Cosmetidae, *P. goliath* is in the subfamily Pachylinae of the family Gonyleptidae, and *S. spinifera* is a member of the family Stygnommatidae. The Gonyleptidae and Cosmetidae are grouped in the superfamily Gonyleptoidea, and Stygnommatidae is a family of the Samooidea (Giribet & Kury, 2007).

In the two cosmetids, 2-methyl-5-ethylphenol was accompanied by 2,3-dimethylphenol, and in the pachyline by 2,3-dimethylphenol, 2,3-dimethyl-5-ethylphenol and three benzoquinones. In *S. spinifera*, the compounds 2,3-dimethylphenol and 2,3-dimethyl-5-ethylphenol were also detected (Table 1).

Thus the two phalangodids we studied were unique among the studied species producing 2-methyl-5-ethylphenol in that this compound was the sole component of their secretion. We collected our material by whole-body extraction in methanol, while the four other species (as far as we could ascertain from the literature) were studied from secretions collected from live animals, induced to secrete by leg-pinching or by pressing on the body. It is common for the secretion from the repugnatorial glands themselves to be mixed with an oral regurgitant (Gnaspini & Hara, 2007), so it is possible that the presence of multiple but similar compounds in the four non-phalangodid species is due to the interaction of a single component produced in the gland with enzymes or other factors in the regurgitant. To test this hypothesis, it will be necessary to compare secretion collected by whole-body extraction (or gland dissection) with secretion emitted by live animals.

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