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Defensive secretions in millipede species of the order Julida (Diplopoda)

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Abstract. The defensive compounds in the secretions of four species of millipede of the order Julida, *Cylindroiulus caeruleocinctus* (Wood, 1864), *C. latestriatus* (Curtis, 1845), *Choneiulus palmatus* (Nemec, 1895) and *Ommatoiulus sabulosus* (Linnaeus, 1758), were characterized using GC/MS analyses. The secretions contain mixtures of nine compounds of benzoquinones. A characteristic mixture of benzoquinones in the defensive secretions of the order Julida consists of 2-methyl-1,4-benzoquinone, 2-methoxy-3-methyl-1,4-benzoquinone, 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-methyl-1,4-benzoquinone, together with trace amounts of 1,4-benzoquinone and hydroquinone.

Key words. Defensive secretion, benzoquinones, GC/MS analyses, Millipedes, Diplopoda, Julida.

INTRODUCTION

The defensive secretions of millipedes differ in their content, depending on the taxonomic group. Repugnatorial glands are present in millipedes, except those in the orders Siphoniulida, Sphaerotheriida and Chordeumatida. The production of cyanogenic compounds is a characteristic of the order Polydesmida (Taira et al. 2003, Makarov et al. 2010, Kuwahara et al. 2011). Alkaloids are produced by the orders Glomerida and Polyzoniida (Mori & Takagi 2000, Shear et al. 2011). Compounds such as p-cresol and phenol are secreted by the orders Callipodida (Ćurčić et al. 2009, Shear et al. 2010) and Julida (Vujisic et al. 2011, Bodner & Raspotnig 2012, Sekulić et al. 2014). Benzoquinones are secreted by the orders Spirobolida, Spirostreptida (Eisner et al. 1978, Deml & Huth 2000, Kuwahara et al. 2002, Arab et al. 2003, Wu et al. 2007) and Julida (Huth 2000, Vujisic et al. 2011, Bodner & Raspotnig 2012).

The objective of this study was the identification of chemicals secreted by the following species of millipedes of the order Julida: *Cylindroiulus caeruleocinctus* (Wood, 1864), *C. latestriatus* (Curtis, 1845), *Choneiulus palmatus* (Nemec, 1895) and *Ommatoiulus sabulosus* (Linnaeus, 1758). Despite the fact that the chemical secretions of the two common species, *O. sabulosus* and *C. caeruleocinctus,* have been previously analyzed by Huth (2000), we included both these species in the study because of their tendency to achieve outbreaks levels in urban areas.

MATERIALS AND METHODS

The millipedes *Cylindroiulus caeruleocinctus* and *Ommatoiulus sabulosus* were collected at Kraków and Lublin, Poland, in fallow areas, gardens and from walls of residential buildings and in the case of *Cylindroiulus latestriatus* and *Choneiulus palmatus* in greenhouses in the Botanical Garden of the University Maria Curie-Skłodowska at Lublin, Poland.

Chemical Extraction

For the collection of defensive secretions, ten specimens of each species of millipede were put into a glass vial (1.5 ml), to which 200 µl of dichloromethane (DCM) was then added after which the vial was sealed and then shaken for two minutes on a shaker (Vortex type). The extract was collected by decantation followed by a gas chromatography-mass spectrometry (GC-MS) analysis.

Chemical analyses and Identification

GC/MS: Gas chromatography GC 450 (Varian, USA) with mass spectrometry detector MS 320 (Varian, USA) equipped with a CP-810 autosampler and a 30 m × 0.25 mm VF-5 ms column (Varian, USA), film thickness 0.25 µm, carrier gas He 0.5 ml/min., injector and detector temperature were used, respectively, at 250 °C and 270 °C; split ratio 1:40; inject volume 2 µl. A temperature gradient was applied (40 °C for 3 minutes, then incremented by 6 °C /min to 270 °C, kept at 270 °C for 0.67 minute, then incremented by 20 °C /min to 290 °C): ionization energy 70 eV; mass range: 45–400 Da; scan time 0.80 s.

The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra in the NIST library, and with data available in the literature on the compounds previously identified in the defensive secretions of millipedes (Arab et al. 2003, Deml & Huth 2000, Wu et al. 2007, Vujisić et al. 2011).

RESULTS

A total of nine benzoquinone derivatives were identified (Table 1) in the defensive secretions of the four species of the order Julida, viz. *C. caeruleocinctus*, *C. latestriatus, Ch. palmatus* and *O. sabulosus.* The chemical structures of these compounds are closely related. The two major constituents of the secretion are: 2-methyl-1,4-benzoquinone and 2-methoxy-3-methyl-1,4-benzoquinone. In the secretion produced by *O. sabulosus* the major constituent (34.28%) is 2-methyl-1,4-benzoquinone. The relative abundance of the major compound, 2-methoxy-3--methyl-1,4-benzoquinone, was significantly higher in *C. caeruleocinctus, C. palmatus,* and *O. sabulosus* than in *C. latestriatus* (44.47, 39.33, 53.79 vs. 0.31%). Furthermore, the relative content of 2-methyl-3,4-methylenedioxyphenol in the defensive exudates of *C. latestriatus* was significantly higher than in that of *C. caeruleocinctus*, *C. palmatus*, and *O. sabulosus* (50.16 *vs*. 9.69, 6.25, 1.52%). The other benzoquinones that are minor components in the defensive secretion of Julida are: 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-methyl-1,4-benzoquinone. Interestingly, we identified higher quantities of some minor compounds e.g. 2,3-dimethoxy-5-methyl-1,4-benzoquinone (comprising a total of 13.72% of chromatogram peak-area in the case of *C. caeruleocinctus*). Trace amounts of 1,4-benzoquinone and hydroquinone were also recorded.

DISCUSSION

The data reveal that the defensive chemicals produced by the millipede orders Spirobolida, Spirostreptida and Julida, first described as "quinone millipedes" by Eisner et al. (1978), are chemically similar. The most common compounds in the defensive secretions of the four julid millipede species studied are mainly 2-methyl-1,4-benzoquinone and 2-methoxy-3-methyl-1,4--benzoquinone, which is in agreement with previous data on Julida (Huth 2000, Vujisić et al. 2011, 2014, Bodner & Raspotnig 2012, Sekulić et al. 2014), Spirostreptida (Williams & Singh 1997, Deml & Huth 2000) and Spirobolida (Kuwahara et al. 2002, Arab et al. 2003, Wu et al. 2007). The major compound in the defensive secretion of *C. caeruleocinctus*, *C. palmatus* and *O. sabulosus* is 2-methoxy-3-methyl-1,4-benzoquinone. The compound 2-methyl-3,4-methylenedioxyphenol is recorded in the defensive secretion of Spirobolida (Wu et al. 2007) and julid millipede species (Vujisić et al. 2011, Sekulić et al. 2014) and confirmed by our analyses. The minor components 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-methyl-1,4-benzoquinone are recorded in the defensive secretion of *Acladocricus setigerus* (Silvestri, 1897) (Wu et al. 2007), the julid millipede species *Allajulus dicentrus* (Latzel, 1884) (Bodner & Raspotnig 2012) and *Unciger*

Table 1. Chemical compounds in the defensive secretions produced by four julid millipede species. Abbreviations: RI – retention index obtained from GC/MS data, nd – not detected

no	compound	RI	relative abundance (content in %)			
			Cylindroiulus caeruleocinctus CC	*Cylidroiulus latestriatus* CL	*Choneiulus palmatus* CP	*Ommatoiulus sabulosus* OS
1.	1,4-benzoquinone	9.146	nd	nd	0.08	0.65
2.	2-methyl-1,4- benzoquinone	11.870	12.16	29.66	28.99	34.28
3.	2-methoxy-3-methyl-1,4-benzoquinone	16.208	44.47	0.31	39.33	53.79
4.	hydroquinone	18.244	0.72	0.04	0.33	0.33
5.	2-methoxy-5-methyl-2,5-cyclohexadiene-1,4-dione	20.074	nd	nd	0.27	0.12
6.	2-methyl-3,4-(methylenedioxy)phenol	21.236	9.69	50.16	6.25	1.52
7.	2-methyl-1,4-benzenodiol	20.100	2.48	4.45	4.93	3.35
8.	2,3-dimethoxy-5-methyl-1,4-benzoquinone	21.760	13.72	2.51	3.49	0.43
9.	2,3-dimethoxy-1,4-benzoquinone	21.893	1.43	0.30	1.99	1.75

transsilvanicus (Verhoeff, 1899) (Sekulić et al. 2014), which is in accordance with the results of the present study.

We detected 1,4-benzoquinone and hydroquinone, which confirms results of previous studies on julid species (Huth 2000, Vujisić et. al. 2011, Bodner & Raspotnig 2012, Sekulić et al. 2014). We identified trace amounts of 1,4-benzoquinone in the defensive fluids of the stripped millipede *O. sabulosus,* which confirms previous data recorded for the stripped millipede (Huth 2000) and *Megaphyllum bosniense* (Verhoeff, 1897) (Vujisić et. al. 2011).

The benzoquinones in the defensive secretions of julid millipedes have antibacterial, antifungal and antihelminthic qualities (Williams & Singh 1997). Moreover, benzoquinones secreted by tropical species cause staining and burning of the skin and, in the case of eyes, causes lacrimation, keratitis and ulceration of the cornea (Haddad et al. 2000, Buden et al. 2004, De Capitani et al. 2011). Therefore, the benzoquinone secretions of *Cylindroiulus latestriatus, Choneiulus palmatus, C. caeruleocinctus* and *O. sabulosus,* which occur in urban areas, may be dangerous for humans.

REFERENCES

Arab A., Zacarin G. G., Fontanetti C. S., Camargo-Mathias M. I., Santos M. G. & Cabrera A. C. 2003: Composition of the defensive secretion of the Neotropical millipede Rhinocricus padbergi Verhoeff 1938 (Diplopoda: Spirobolida: Rhinocricidae). *Entomotropica* **18**: 79–82.

Bodner M. & Raspotnig G. 2012: Millipedes that smell like bugs: (E)-alkenals in the defensive secretion of the julid diplopod Allajulus dicentrus. *Journal of Chemical Ecology* **38**: 547–556.

Ćurčić B. M., Makarov S. E., Tešević V. V., Jadranin M. B. & Vujisić L. V. 2009: Identification of Secretory Compounds from the European Callipodidan Species Apfelbeckia insculpta. *Journal of Chemical Ecology* **35**: 893–895.

Buden D. W., Attygalle A. & Wu X. 2004: Distribution of the Chunk Islands Giant Millipede, Acladocricus setigerus (Spirobolida: Rhinocricidae), and identification of its defensive compounds. *Pacific Science* **58**: 625–636.

De Capitani E. M., Vieira R. J., Bucaretchi F., Fernandes L. C. R., Toledo A. S. & Camargo A. C. 2011: Human accidents involving Rhinocricus spp., a common millipede genus observed in urban areas of Brazil. *Clinical Toxicology* **49**: 187–190.

Deml R. & Huth A. 2000: Benzoquinones and hydroquinones in defensive secretions of tropical millipedes. *Naturwissenschaften* **87**: 80–82.

Eisner T., Alsop D., Hicks K. & Meinwald J. 1978: Defensive secretions of millipedes. Pp.: 41–72. In: Bettini S. (ed.): *Handbook of Experimental Pharmacology Arthropod Venoms*. *Vol. 48.* Berlin: Springer, 977 pp.

Haddad Jr. V. & Cardoso J. L. C. 2000: Accidents provoked by millipede with dermatological manifestations: report of two cases. *Anais Brasileiros de Dermatologia* **75**: 471–474.

Huth A. 2000: Defensive secretions of millipedes: more than just a product of melting point decrease? Pp.: 191–200. In: Wytwer J. & Golovatch S. I. (eds): *Progress in Studies on Myriapoda and Onychophora. Fragmenta Faunistica* **43**(Supplement): 1–395.

Kuwahara Y., Noguchi S., Mori N. & Higa Y. 2002: Identification of benzoquinones and hydroquinones as the secretory compounds from three species of Okinawan millipedes. *Japanese Journal of Environmental Entomology and Zoology* **13**: 117–124.

Kuwahara Y., Shimizu N. & Tanabe T. 2011: Release of hydrogen cyanide via a post-secretion Schotten-Baumann reaction in defensive fluids of Polydesmoid millipedes. *Journal of Chemical Ecology* **37**: 232–238.

Makarov S. E., Curcić B. P., Tesević V. V., Jadranin M. B., Vujisić Lj. V., Curcić S. B., Mandić B. M., Sekulić T. Lj, & Mitic B. M. 2010: Defensive secretions in three species of polydesmids (Diplopoda, Polydesmida, Polydesmidae). *Journal of Chemical Ecology* **36**: 978–982.

Mori K. & Takagi Y. 2000: Enantioselective synthesis of polyzonimine and nitropolyzonimine, spirocyclic compounds from the defensive glands of a millipede, Polyzonium rosalbum. *Tetrahedron Letters* **41**: 6623–6625.

Sekulić T. Lj., Vujisić Lj. V., Ćurčić B. P. M., Mandić B. M., Antić D. Ž., Trifunović S. S., Godevac D. M., Vajs E. V., Tomić V. T. & Makarov S. E. 2014: Quinones and non-quinones from the defensive secretion of Unciger transsilvanicus (Verhoeff, 1899) (Diplopoda, Julida, Julidae), from Serbia. *Archives of Biological Sciences* **66**: 385–390.

Shear W. A., McPherson I. S., Jones T. H., Loria S. F., & Zigler K. S. 2010: Chemical defense of a troglobiont millipede, Tetracion jonesi Hoffman (Diplopoda, Callipodida, Abacionidae). *International Journal of Myriapodology* **3**: 153–158.

Shear W. A, Jones T. H. & Wesener T. 2011: Glomerin and homoglomerin from the North American pill millipede Onomeris sinuata (Loomis, 1943) (Diplopoda, Pentazonia, Glomeridae). *International Journal of Myriapodology* **4**: 1–10.

Taira J., Nakamura K. & Higa Y. 2003: Identification of secretory compounds from the millipede, Oxidus gracilis C. L. Koch (Polydesmida: Paradoxosomatidae) and their variation in different habitats. *Applied Entomology and Zoology* **38**: 401–404.

Williams L. A. D. & Singh P. D. A. 1997: Biology and biological action of the defensive secretion from a Jamaican millipede. *Naturwissenschaften* **84**: 143–144.

Wu X., Buden D. W. & Attygale B. 2007: Hydroquinones from defensive secretion of a giant Pacific millipede, Acladocricus setigerus (Diplopoda: Spirobolida). *Chemoecology* **17**: 131–138.

Vujisić L. V., Makarov S. E., Ćurčić B. P. M., Ilić B. S., Tešević V. V., Godevac D. M., Vučković I. M., Ćurčić B. S. & Mitić B. M. 2011: Composition of the defensive secretion in three species of European millipedes. *Journal of Chemical Ecology* **37**: 1358–1364.