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DEFENSIVE SUBSTANCES OF OPILIONIDS[1]

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Abstract—Two quinones (2,3-dimethyl-1,4-benzoquinone and 2,3,5-trimethyl-1,4-benzoquinone) and three phenols (2,3-dimethylphenol, 2-methyl-5-ethylphenol, and 2,3,4-trimethylphenol) were isolated from the defensive secretions of opilionids (Laniatores) from the Panama Canal Zone. The trimethylphenol was not previously reported as a natural product.

Key Words—Quinones, phenols, defensive secretions, Arachnida, Opiliones.

INTRODUCTION

Work on the defensive secretions of opilionids has been recently reviewed (Eisner et al., 1978). While it is clear that all three suborders of the order have defensive glands, chemical work has so far been done only on species of Palpatores and Laniatores. The Palpatores secrete short-chain acyclic compounds (references in Eisner et al., 1978) and naphthoquinones (Wiemer et al., 1978), while the Laniatores produce a variety of alkylated benzoquinones and phenols (references in Eisner et al., 1978). Given this chemical dichotomy at the subordinal level, as well as the considerable chemical diversity already apparent at the generic and specific levels, it seems likely that an in-depth study of opilionid secretions will eventually have phyletic and taxonomic implications. As part of our ongoing study of opilionid chemistry (Eisner et al., 1971, 1977, 1978; Jones et al., 1976, 1977; Meinwald et al., 1971; Wiemer et al., 1978), we here report on the secretory composition of four

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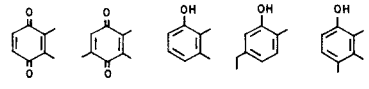
neotropical laniatores, two of the family Cosmetidae, and two of the Gonyleptidae.

METHODS AND MATERIALS

Sources of animals (Barro Colorado Island, Panama Canal Zone), procedures for "milking" them of secretion, and gas chromatographic (GC), infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopic (MS) techniques, were as previously described in connection with studies of other Laniatores (Eisner et al., 1977). Species identifications are to be considered tentative, and we are therefore depositing voucher specimens under special label (Robert E. Silberglied collector, T. Eisner exp. no. 903) in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

One specimen of *Nesopachylus* and all of *Zygopachylus* failed to emit secretion in response to the sort of stimulation (pinching of appendages; gentle squeezing of body with forceps) that ordinarily elicits glandular



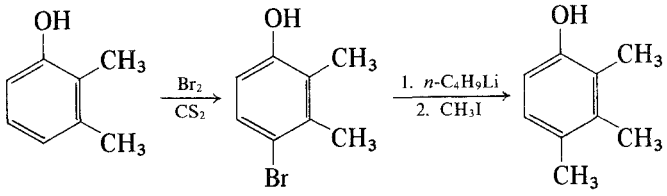
		I	II	III	IV	V
Cosmetidae						
Cynorta nannacornuta	M_{15}	17	10			
	M_4	18	9			
Eucynortula albipunctata	M_3			6	107	
Gonyleptidae						
Nesopachylus monoceros	M_1	35	11			
	D_1	230	100			
Zygopachylus albimarginis	D_{15}	13				5

FIG. 1. Benzoquinones (I, II) and phenols (III–V) in defensive secretions of species shown. The numbers give μg/individual. The letters with numerical subscript designate the nature of the chemical sample (M = secretion obtained by "milking" live specimens; D = secretion obtained by extracting glands dissected from freshly killed individuals; subscript = number of individuals whose secretion was pooled for the sample). The bracketed samples of *C. nannacornuta* are essentially comparable samples taken at two different times: the four individuals milked for the M_4 sample were survivors of the group of 15 milked to depletion 6 weeks earlier for the M_{15} sample; note that there was virtully no change in μg/individual over that period of time.

discharges in opilionids. These animals were killed by freezing, upon which their glands were promptly excised and extracted with methylene chloride.

Quantitative estimates of secretory components were made by comparing GC peak areas (Spectra-Physics Autolab Integrator model 23000-010) of a known portion of gland extract with peak area calibration curves obtained using standards of the compounds in question. In only one case (*Nesopachylus monoceros*) were these estimates based on secretion samples from single individuals. Pooled samples of secretion from several individuals were used with the other three species, and the estimates given for quantity of secretory component per individual are therefore calculated averages.

Chemical Identification and Synthesis. The chemical findings are summarized in Figure 1. Those benzoquinones and phenols previously known from other Laniatores (I–IV) were identified by direct GC-MS comparison with authentic samples. Compound V was recognized to be a trimethylphenol by its mass spectrum, but since the mass spectra of trimethylphenols are virtually indistinguishable (Sellier et al., 1974), it was essential to carry out direct comparisons with all six possible structural isomers. For this purpose, five of the trimethyl phenols were obtained from commercial sources (2,3,6-, 2,3,5-, and 2,4,6-trimethylphenol from Aldrich Chemical Co., Milwaukee, Wisconsin; 3,4,5-, and 2,4,5-trimethylphenol from Accurate Chemical & Scientific Co., Hicksville, New York). The sixth, 2,3,4-trimethylphenol, which proved identical to the natural product on the basis of GC-MS and thin-layer chromatographic analysis, was conveniently synthesized by the following two-step methylation sequence:



Bromination of phenols in CS_2 had been shown to proceed preferentially in the position para to oxygen (Gilman and Blatt, 1944). Making use of this observation, 2,3-dimethylphenol (1 g, 8.2 mmol, Aldrich Chemical Co., 97%) was dissolved in CS_2 (10 ml) and cooled to 0° C. Bromine (1.4 g, 9.0 mmol) in CS_2 (3 ml) was added dropwise over 30 min to the stirred solution. After an additional hour at 0° C, the reaction mixture was warmed to room temperature and the solvent was removed in vacuo. The remaining brown solid was distilled (bulb-to-bulb, 90° C, 0.3 mm Hg) to give 1.5 g of a mixture of 4-bromo-2,3-dimethylphenol and 6-bromo-2,3-dimethylphenol (9:1, estimated on the basis of GC peak areas, 8 ft. OV-1 on Chromosorb Q). Recrystallization from chloroform gave 4-bromo-2,3-dimethylphenol (1.2 g, 73%) as

white needles: mp 94–95°C (92°C; Heicken, 1939); IR ($CHCl_3$) 3570(m), 1580(m), 1465(s), 1280(vs), 1165(m), 1065(s), 790(m, in CS_2); NMR ($CDCl_3$)δ 7.20 (1H, d, $J = 8$ Hz), 6.47 (1H, d, $J = 8$ Hz), 4.80 (1H, broad singlet, exchanges with D_2O), 2.33 (3H, s), 2.18 (3H, s); MS *m/e* (rel. intensity) 202 (84), 200 (89), 187 (7), 185 (8), 121 (100), 103 (14), 91 (45), 77 (39).

4-Bromo-2,3-dimethylphenol (200 mg, 1.0 mmol) was dissolved in freshly distilled, dry tetrahydrofuran (3 ml) and cooled to −100°C (dry ice–ether) under an argon atmosphere. *n*-Butyllithium (2.3 eq) in hexane was added very slowly, and the mixture was stirred for an hour. After warming to 0°C, the mixture was quenched with methyl iodide (156 mg, 1.1 mmol) and stirred for an additional 20 min. The solvent was removed in vacuo, and the remaining solid was dissolved in CH_2Cl_2 (10 ml). This solution was extracted twice with 5% NaOH (10 ml), and the combined base extracts were acidified to pH 1 (conc. HCl). Extraction of the acidic phase with CH_2Cl_2 followed by solvent removal and preparative gas chromatography (3% OV-1 on Chromosorb Q) gave 2,3,4-trimethylphenol (45 mg, 32%, yield unoptimized): mp 69–70°C (69°C; Lejeune et al., 1957); IR ($CHCl_3$) 3580(s), 1610(m), 1475(s), 1280(s), 1060(s), 790 (vw, in CS_2), 760 (s, in CS_2); NMR ($CDCl_3$) 6.84 (1H, d, $J = 8$ Hz), 6.52 (1H, d, $J = 8$ Hz), 4.47 (1H, s, exchanges with D_2O), 2.20 (3H, s), 2.17 (6H, s); MS *m/e* (rel. intensity) 136 (70), 121 (100), 91 (16), 77 (7).

DISCUSSION

It is clear from the results (Figure 1), that two species produce only quinones (I and II), one species produces only phenols (III and IV), and a fourth, *Zygopachylus albimarginis*, produces a quinone (I) and a phenol (V). Since exclusive production of quinones or phenols had previously been demonstrated in Laniatores (see Eisner et al., 1978, for a list of the Laniatores known to produce compounds I–IV), only *Zygopachylus* stands out as unusual. Moreover, the particular phenol produced by *Zygopachylus*, which we had previously detected but were unable to characterize because only a single individual of this species had been available to us (Eisner et al., 1977, 1978), is a natural product hitherto unreported from either animals or plants. Whether the unusual 2,3,4-substitution pattern of this phenol conveys a special functional property upon the molecule remains to be seen.

Although it is clearly premature to tell whether a knowledge of the glandular chemistry of the Laniatores will eventually corroborate or throw into questions the existing taxonomy of the group, it is apparent already that secretory composition does not vary in accord with preestablished generic lines. For example, while *Cynorta nannacornuta*, as here shown, secretes two quinones, produced also by *Nesopachylus monoceros*, its congener, *Cynorta*

astora, produces phenols (Eisner et al., 1977), as does *Eucynortula albipunctata*.

Refinement of modern chemical techniques is such that minute amounts of material often suffice for characterization of chemical components. This is particularly true for the quinonoid and phenolic products produced by Laniatores. Only relatively few specimens of *Zygopachylus* sufficed for identification of the new phenol V, despite the fact that the average phenol content per individual in this small species was only 5 μg. With bigger species, such as *Nesopachylus monoceros*, which may store upward of 300 μg of secretion, even single individuals can contain sufficient material for analysis. It appears, therefore, that with the Laniatores, chemical studies could provide an unorthodox and potentially fruitful basis for investigation of the evolutionary relationships within the group. Studies of the Palpatores have proven more laborious because of the special nature of the secretory products involved. But the results with this group would be of no lesser phyletic value, and the greater efforts invested would be offset by the potentially greater chemical interest of these findings.

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