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ISOLATION AND IDENTIFICATION OF THE MAJOR COMPONENT OF SETAL EXUDATE FROM *CORYTHUCHA CILIATA*

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ABSTRACT.—Clear microdroplets are associated with secretory, abdominal setae on nymphs of the sycamore lace bug *Corythucha ciliata*. The major component of this exudate material has been identified as 3,6-dihydroxy-2-[1-oxo-10(*E*)-tetradecenyl]cyclohex-2-en-1-one [**1**]. Preliminary observations of these insects suggest a defensive function for these exudate droplets.

Developing lace bug nymphs form large aggregations on the undersides of leaves on host plants and, unlike many other gregarious feeding insects, are relatively free of predators and parasites. Various types of processes extend as outgrowths of the body wall of nymphs. Many species have additional setae which are secretory and considered important to defense. In the case of *Corythucha ciliata* Say (Hemiptera; Tingidae), potential predators exhibit aversion behavior after tactile engagement with microdroplets of setal exudate.

As part of a comparative study of setal exudate material from lace bugs, we have previously reported the presence of 2-alkyl-5-hydroxychromones and the corresponding chromanones and diketones from the azalea lace bug *Stephanitis pyrioides* (1), and dihydroxyphenyl-1,3-diketones and the corresponding chromones and chromones possessing an additional phenolic oxygen from the rhododendron lace bug *Stephanitis rhododendri* (2). The presence of these unusual compounds in *Stephanitis* prompted us to explore the genus *Corythucha*. Generally restricted to the Western Hemisphere, many of the 68 species of *Corythucha* are strikingly similar to each other, and each species is limited to one or a few host plants (3).

RESULTS AND DISCUSSION

Initial samples of setal exudate from the sycamore lace bug (*C. ciliata*) were obtained by wicking of the microdroplets onto small, triangular pieces of filter paper that were then extracted with CH_2Cl_2. Compared to previous tingids examined by this wicking procedure (1,2), a much smaller amount of material was available from *C. ciliata*. Analysis of this material by gc indicated the presence of six components, the most abundant of which constituted 53% of the total. A threshold effect was observed during gc analysis, which suggested the components were extremely polar. The combination of ei, ci, and deuterium exchange gc/ms established a molecular weight of 336 and the presence of two exchangeable hydrogens for the major component **1**. Five less abundant components had molecular weights of 338+336 (chromatographically unresolved; 4% of total exudate material), 362 (9%), 346 (6%), and 362 (28%); each possessed two exchangeable hydrogens except for the compound with molecular weight of 346, which had one. The mass spectra of the five less abundant components resembled **1** in that some ions were in common but differed considerably in intensity. None of the ei mass spectra closely resembled those of compounds we have isolated from other tingids (1,2), nor did the mass spectra of the six unknowns exhibit a high degree of correla-

tion with any entry in the data system mass spectral library. Hrms analysis provided an empirical formula of $C_{20}H_{32}O_4$ for the molecular ion. In addition to supporting empirical formula assignments listed in the experimental section, hrms also indicated a curious loss of C_2H_4O to yield a weak (3%) ion at *m/z* 292.

Preliminary gc/ms of a much larger sample obtained by whole body rinsing of ca. 10 g of insects and cast molt skins with CH_2Cl_2 revealed, not surprisingly, numerous components in addition to the six observed in the initial wicked sample. Many of the spurious compounds were hydrocarbons and probably of cuticular origin as well as derived from leaf material co-aspirated with cast skins. A sample of only stellate hairs, the predominant leaf structure that was co-aspirated, was collected, extracted, and by gc/ms was found not to contain any of the six compounds present in the wicked sample.

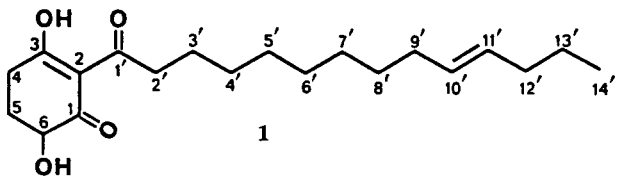
Only the major component **1** was obtained in sufficient amount for final trapping by gc for subsequent spectroscopic analysis.

Bands at 3470, 1667, and 966 cm^{-1} in the ir spectrum of **1** suggested, respectively, the presence of a hydroxyl in a single bridge intramolecular hydrogen bond configuration (4), an α,β-unsaturated ketone (5), and a *trans* olefinic bond.

Ozonolysis of compound **1** provided the derivative 3,6-dihydroxy-2-(1,10-dioxodecyl)cyclohex-2-en-1-one, which was demonstrated by eims and cims to have many ions in common with **1**, and to have a molecular weight of 296, thereby indicating a loss of a four-carbon fragment from **1**.

The uv spectrum of **1** with λ max at 270 and 232 nm closely resembled that of 2-acetylcyclohexane-1,3-dione [272 and 232 nm (6)] and suggested a tri-ketone structure. An examination of the literature for compounds with these characteristics led to Mudd's investigation of 2-acylcyclohexane-1,3-diones isolated from larvae of *Ephestia kuehniella* Zeller (7). Among the compounds reported by Mudd was 3,6-dihydroxy-2-[1-oxo-9(*Z*)-octadecenyl]cyclohex-2-en-1-one [**2**]. The ei mass spectrum of **2**, isolated from *E. kuehniella* and generously supplied by Dr. Mudd, was very similar to that of **1**, the major difference being the molecular ion at 392 for compound **2** vs. 336 for compound **1**. The ir spectra of **1** and **2** were nearly congruent except for the presence of a bond at 966 cm^{-1} in the spectrum of **1** that was absent in that of **2**, and minute differences in the regions between 1220-1245 cm^{-1} and 1150-1200 cm^{-1}.

^{1}H-nmr spectra (Table 1) indicated a single terminal methyl, an isolated double band, and a strongly hydrogen-bonded enolic H (δ 18.24 in $CDCl_3$). Chemical shifts of ring protons were in agreement with those published by Mudd (7), and the assignments were confirmed by decoupling experiments. Comparison of the ^{1}H spectrum of **1** obtained in C_6D_6 to the $CDCl_3$ spectrum was interesting; the linear chain methylene groups 3′–8′, and 13′ shifted from δ 1.54-1.27 to 1.38-1.22. This anisotropic shift revealed underlying signals that permitted assignments of ring protons on C-4 at δ 1.62 (2H, m) and those on C-5 at δ 1.76 (2H, m). Also, the resonance of the side chain 3′



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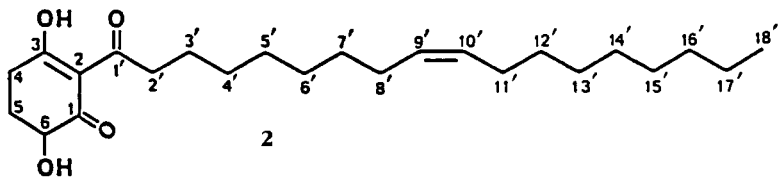

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protons was observed at δ 1.62 (2H, m); these assignments were also confirmed by ir-radiation experiments. Additional support of structure **1** was obtained by comparing the ^{13}C-nmr spectrum (Table 1) with other published ^{13}C-nmr data (7). The steady state pulse technique employed on the limited amount of sample available (ca. 1 mg) did not permit assignment of ring carbons 1 and 3. Slight differences between the observed ^{13}C chemical shifts and reported ^{13}C chemical shifts are due to the difference in solvents used. No attempt has yet been made to determine the configuration of the chiral center at C-6.

TABLE 1. Nmr Spectral Data for **1** and **3**

Atom[a]		^{1}H nmr			^{13}C nmr	
		Compound			Compound	
		1 (C_6D_6)	**1** ($CDCl_3$)	**2** ($CDCl_3$)[b]	**1** (C_6D_6)	**2** ($CDCl_3$)[b]
RING						
C1/C1	—	—	—	—	—	197.8
C2/C2	—	—	—	—	109.69	110.3
C3/C3	(–OH)	—	18.24 (s)	18.3	—	195.5
C4/C4	(–H2)	1.87 (m)	2.78 (m)	2.76 (m)	30.87	27.2
C5/C5	(–H2)	1.76 (m)	1.9-2.2 (m)	1.95	30.04	31.4
C6/C6	(–H)	3.53 (dd)	4.09 (dd)[c]	4.09 (dd)[c]	71.59	71.6
C6/C6	(–OH)	4.15 (br)	4.01 (s)	4.04 (br, s)	—	—
SIDE CHAIN						
C1′/C1′	—	—	—	—	208.6	206.0
C2′/C2′	(–H2)	2.99 (d-m)	3.03 (m)	3.03 (m)	40.51	40.2
C3′/C3′	(–H2)	1.61 (m)	1.27-1.54 (m)	1.26-1.30	24.91	24.6
C4′/C4′	(–H2)	1.26 (m)	1.27-1.54 (m)	1.26-1.30	29.49	29.3
C5′-7′/C5′-7′	(–H2)	1.26 (m)	1.27-1.54 (m)	1.26-1.30	29.75	29.3
C8′/	(–H2)	1.26 (m)	1.27-1.54 (m)	1.26-1.30	27.35	—
C9′/C8′	(–H2)	2.00 (m)	1.95 (m)	1.9-2.1 (m)	33.08	27.2
C10′/C9′	(–H)	5.45 (m)	5.36 (m)	5.35 (m)	130.5	130.0
C11′/C10′	(–H)	5.45 (m)	5.36 (m)	5.35 (m)	130.9	130.0
C12′/C11′	(–H2)	2.00 (m)	1.95 (m)	1.9-2.1 (m)	35.14	27.2
C13′/C17′	(–H2)	1.26 (m)	1.27-1.54 (m)	1.26-1.30	23.12	22.7
C14′/C18′	(–H3)	0.88 (t)[d]	0.86 (t)[d]	0.88 (m)	13.82	14.0

[a]Compound **1**/compound **2**.
[b]From Mudd (7).
[c]J=13 & 4 Hz.
[d]J=7 Hz.

From the above data we conclude that the major component from the setal exudate of *C. ciliata* is 3,6-dihydroxy-2-[1-oxo-10(*E*)-tetradecenyl]cyclohex-2-en-1-one [**1**]. This class of compounds is comparatively rare. We know of only two reports of these compounds being isolated from nature. The first is the discovery by Mudd of several compounds of this type from the mandibular gland of the lepidopteran larvae of *E. kuehniella* (7). The second occurrence is in the fruit of the Brazilian tree *Virola elongata* (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Low resolution ei, ci, and deuterium exchange mass spectra were obtained using a Finnigan Model 4510 quadrupole instrument coupled to an Incos data system. CH_4

and NH_3 were used as ci reagent gases, and deutero-ammonia was employed for deuterium exchange. Samples were admitted to the ionizing chamber via a 30 m×0.32 mm i.d. fused silica column with a 0.25 μm DB-1 film (J and W Scientific), which was temperature-programmed from 175° to 260° at 2°/min. Gas-liquid chromatographic trapping was performed on a Varian Model 3700 equipped with a thermal conductivity detector and a 15 m×0.528 mm i.d. Megabore column with a 1.5 μm film of DB-1 (J and W Scientific) and operated at 210°. High resolution mass spectral analyses were performed by Midwest Center for Mass Spectrometry (Lincoln, Nebraska). ^{1}H-nmr spectra were obtained from a Nicolet NT-300 (300 MHz) Fourier transform spectrometer using both $CDCl_3$ and C_6D_6 as solvents and TMS as an internal standard. ^{1}H chemical shifts are reported in δ from TMS and coupling constants are in Hz. ^{13}C-nmr spectrum was recorded in C_6D_6 at 75.4 MHz using steady state pulse techniques with a tip angle of 42°. Uv spectra, 190-400 nm, were obtained with a Perkin-Elmer Model 559 using MeOH as solvent. Ir spectra were measured in CCl_4 and CS_2 from 400 to 4000 cm^{-1} with a Perkin-Elmer Model 580 B equipped with an ir Data Station.

INSECT REARING AND COLLECTION.—Lace bugs were maintained on 1-2.5 m high container-grown cloned sycamore saplings (*Platanus occidentalis* L.) on greenhouse benches. Saplings were occasionally pruned and were watered daily to stimulate branching for maximum leaf production. Cast skins from developing nymphs were collected periodically from the undersides of leaves by aspiration into Pasteur pipettes for extraction. (Adult lace bugs were identified to species by J.W.N. Voucher specimens do not exist, as there is no question of species identity.)

EXTRACTION AND ISOLATION.—Each collection of cast skins was placed into a fritted glass filter funnel and rinsed repeatedly with CH_2Cl_2. The color of the rinses progressed from an initial dark brown to a pale yellow. Rinses from all collections (ca. 10 g cast skins) were combined and evaporated to provide 0.53 g of a nearly black residue, which was dissolved in warm MeOH, cooled, and solids removed by filtration. The filtrate (0.32 g) was chromatographed on Unisil Si gel (25 g) using 75 ml C_6H_6 followed by 50 ml volumes of Et_2O in C_6H_6 increasing in 1% steps. Beginning with the 3% Et_2O portion, 4-ml fractions were collected, and by gc analysis, **1** was detected in fractions 13 through 35. These fractions were combined and evaporated to yield 84 mg of a greenish residue. The residue was chromatographed on 20% $AgNO_3$/Unisil (10 g) using 25 ml hexane followed by 25-ml volumes of Et_2O in hexane, beginning at 2% and increasing in 1% steps. Fractions covering the 15-18% range yielded 28 mg of a pale yellow residue which contained **1**. The material was rechromatographed on a 20% $AgNO_3$/Unisil column using 300 ml 10% Et_2O in hexane followed by 100 ml 12% Et_2O in hexane with 3.5-ml fractions being collected. Fractions 60 through 115, which contained **1**, were combined and chromatographed on rphplc (C_{18}) using 2.0 ml/min of H_2O-MeCN-iPrOH (20:60:20) monitored at 210 nm to yield two major components: 6.1 min (3.4 mg, **1**) and 7.0 min. Final purification of **1** was achieved via trapping from a megabore capillary gc column.

OZONOLYSIS OF **1** TO 3,6-DIHYDROXY-2-(1,10-DIOXODECYL)CYCLOHEX-2-EN-1-ONE.—A mixture of 10 μg (0.03 μmol) **1** in 10 μl CH_2Cl_2 (−70°) and 12 μl of CH_2Cl_2 (−70°) saturated with O_3 was allowed to stand for 0.5 h at −70°, after which 20 μg (0.3 μmol) Me_2S was added and the vial allowed to come to room temperature.

3,6-DIHYDROXY-2-[1-OXO-10(*E*)-TETRADECENYL]CYCLOHEX-2-EN-1-ONE [**1**].—Uv λ max MeOH 270, 232 nm; ir ν max (CS_2) 3470, 2955, 2925, 2853, 1667, 1389, 1121, 966, 895 cm^{-1}; eims *m/z* (% rel. int.) 336 $[M]^+$ (72), 318 $[M-H_2O]^+$ (2), 292 $[M-C_2H_4O]^+$ (3), 183 $[C_9H_{11}O_4]^+$ (43), 168 $[C_8H_8O_4]^+$ (41), 155 $[C_7H_7O_4]^+$ (13), 137 $[C_7H_5O_3]^+$ (17), 126 $[C_6H_6O_3]^+$ (23), 55 (100%); CH_4 cims 337 $[M+H]^+$ (100), 365 $[M+C_2H_5]^+$ (16), 377 $[M+C_3H_5]^+$ (5); NH_3 cims 354 $[M+NH_4]^+$ (100); ND_3 cims 360 $[M+ND_4]^+$ (100); *Anal.* calcd for $C_{20}H_{32}O_4$: 336.2301. Found (ms): 336.2299; ^{1}H nmr (300 MHz), see Table 1; ^{13}C nmr (75.4 MHz), see Table 1.

3,6-DIHYDROXY-2-(1,10-DIOXODECYL)CYCLOHEX-2-EN-1-ONE.—Eims *m/z* (% rel. int.) 296 (3), 278 (2), 268 (57), 253 (51), 183 (100), 170 (52), 168 (31), 153 (32), 140 (48), 126 (40), 55 (95); NH_3 cims 314 $[M+NH_4]^+$ (100).

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