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DEFENSIVE CHEMISTRY OF THE FLOUR BEETLE *Tribolium brevicornis* (LeC.): Presence of Known and Potential Prostaglandin Synthetase Inhibitors

RALPH W. HOWARD[1] and DELBERT D. MUELLER[2]

[1]*U.S. Department of Agriculture, Agricultural Research Service*
U.S. Grain Marketing Research Laboratory
1515 College Avenue, Manhattan, Kansas 66502
Department of Entomology, Kansas State University
Manhattan, Kansas 66506

[2]*Department of Biochemistry, Kansas State University*
Manhattan, Kansas 66506

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Abstract—The defensive secretion of *Tribolium brevicornis* contains 12 organic components, including quinones, hydroquinones, hydrocarbons, aromatic ketones, and aromatic esters. The two ketones, 2′-hydroxy-4′-methoxyacetophenone and 2′-hydroxy-4′-methoxypropiophenone, and the two esters, methyl 2,5-dihydroxy-6-methylbenzoate and methyl 2,5-dihydroxy-6-ethylbenzoate, represent ca. 0.25% of the biomass of the beetles. Mass spectral and NMR analyses were used to elucidate the structures of all components. The ketones are potent prostaglandin synthetase inhibitors (PSI), and the esters are suspected to be PSI.

Key Words—Biosynthesis, Coleoptera, Tenebrionidae, *Tribolium brevicornis*, prostaglandin, allomones, synthetase inhibitor, defensive secretion, 2′-hydroxy-4′-methoxyacetophenone, 2′-hydroxy-4′-methoxypropiophenone, methyl 2,5-dihydroxy-6-methylbenzoate, methyl 2,5-dihydroxy-6-ethylbenzoate.

INTRODUCTION

The defensive secretions of tenebrionid beetles often contain a diversity of natural products. The best known of these products are the ubiquitious quinones and olefins (Tschinkel, 1975a), but in addition compounds such as isocoumarins (Lloyd et al., 1978), terpenes (Gnanasunderam et al., 1981), aliphatic carbonyl

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compounds (Tschinkel, 1975b; Gnanasunderam et al., 1982), salicylate esters (Gnanasunderam et al., 1984), and β-hydroxy aromatic ketones (Suzuki et al., 1975; Howard et al., 1986) have also been found. The physiological significance of most of the nonquinone and hydrocarbon components is unknown. We recently reported, however, that the red flour beetle, *Tribolium castaneum* (Herbst), contains microgram per beetle levels of 2′-hydroxy-4′-methoxyacetophenone (**1**) and 2′-hydroxy-4′-methoxypropiophenone (**2**) (Howard et al., 1986) and that these aromatic ketones are potent inhibitors of both insect and mammalian prostaglandin synthetases. We have subsequently found that similar aromatic prostaglandin synthetase inhibitors (PSI) are present in a diversity of other insect exocrine secretions (Jurenka et al., 1986).

As part of our continuing efforts to identify insect-derived PSI, we have reexamined (Markarian et al., 1978; Wirtz et al., 1978) the defensive secretion of the North American flour beetle *Tribolium brevicornis* LeC. We have found that this insect contains substantial quantities of ketones **1** and **2** as well as two previously undescribed β-hydroxy aromatic esters. We report here the structure determination of these compounds and discuss their possible physiological significance.

METHODS AND MATERIALS

Insects. Cultures of *T. brevicornis* were obtained from Dr. R. Strong, Department of Entomology, University of California, Riverside, California, and from Dr. W. Burkholder, USDA-ARS, Department of Entomology, Madison, Wisconsin. They have gone through approximately five generations in our laboratory and have been reared on whole wheat flour mixed with 3% brewer's yeast in constant darkness at 30 ± 1°C, except for brief periods of examination in room light.

Isolation Methods. Beetles from each source population were chilled in an ice bath for ca. 10 min, causing many of the beetles to release their yellowish defensive secretion, which then solidified onto the cuticle. The solidified secretions were transferred with an insect pin to a 1-ml Reactivial,[3] diluted with diethyl ether, and examined by capillary gas chromatography–mass spectrometry (GC-MS). Other beetles were killed by freezing at −30°C, the entire insect extracted with diethyl ether, and then the extract was examined by GC-MS. Both methods with either population of beetles gave the same mixture of low-molecular-weight components in the same relative proportions. Therefore, all subsequent analyses utilized the whole-body extraction procedure.

Preparative Methods. Approximately 900 adult *T. brevicornis* (10.23 g)

[3] Mention of a proprietary product in this paper does not imply its approval by USDA to the exclusion of other products that may also be suitable.

were killed by freezing and then extracted 3× with 25-ml portions of diethyl ether. The combined extracts were concentrated in vacuo to yield 170 mg of a dark reddish yellow oil. This oil was chromatographed through a 2 × 20-cm column of BioSil A with 60-ml fractions of hexane, 1:9, 2:8, 4:6, 6:4, 8:2 ether–hexane, and 100% ether. Fractions were collected (7 ml) and monitored by GC-MS. Fractions containing aromatic components (10% ether and 20% ether) were concentrated in vacuo and further purified by thin-layer chromatography. Unactivated Kieselgel 60 F254 plates were predeveloped in $CHCl_3$, then streaked with CH_2Cl_2 solutions of fractions from the preparative column chromatography and redeveloped in $CHCl_3$, air dried, and examined under long-wavelength UV light. The β-hydroxy ketones, quinones, and esters all appeared as separate light-blue fluorescent bands. These bands were scraped from the plates and thoroughly extracted with CH_2Cl_2. The resulting CH_2Cl_2 solutions were concentrated in vacuo to yield the pure defensive secretion components. Individual components were stored in CH_2Cl_2 until used.

Quantitative Analysis of Individual Insects. Individual live beetles were placed in microvials constructed from Pasteur pipets and 50 μl of CH_3CN containing 0.5 μg vanillin/μl (as an internal standard) was then added. After 20 min, 1-μl aliquots of the CH_3CN solution were analyzed by capillary GC using a flame ionization detector. A comparison of peak areas of the internal standard to those of the quinones, ketones, and esters was used to calculate the abundance of these chemicals in individual beetles. Some of the beetles were subsequently crushed in the microvial and reanalyzed to assess how much of the defensive secretions was not recovered by the 20-min soaking.

Derivatization. A 10-μl portion of the ether solution from our preliminary isolation was treated with 100 μl Trisil® (Pierce Chemical Company, Rockford, Illinois), held at room temperature for 30 min, and then examined by GC-MS.

Instrumentation. GC-MS analyses were conducted on a Hewlett Packard model 5790A capillary GC (Hewlett Packard, Inc., Palo Alto, California) equipped with a 30-m × 0.2-mm DB-1 capillary column (J & W Scientific, Inc., Rancho Cordova, California), interfaced to a Hewlett Packard model 5970 mass selective detector operated at 70 eV, and a Hewlett Packard model 5730A gas chromatograph containing a 12.5-m × 0.2-mm HP cross-linked methyl silicone capillary column with a flame ionization detector. Nitrogen was the carrier gas, and injections utilized the splitless mode. Signals from the GC were stored and analyzed using a Hewlett Packard model 3380 Integrator. [^{1}H]NMR spectra were acquired on a Bruker WM-400 spectrometer (USA Bruker Instruments, Inc., Billerica, Massachusetts) using a 5 mm C/H probe, 32 K data points over a 6024-Hz spectral width, a 2.0-sec delay and, unless otherwise indicated, the spectra were processed with a 0.1-Hz line broadening. The samples were dissolved in "100%" $CDCl_3$ (Aldrich Chemical Co., Milwaukee, Wisconsin), and the chemical shifts were referenced to the protiochloroform peak taken as 7.26 ppm relative to tetramethylsilane (0 ppm). Specific reso-

nance homonuclear decouplings were done with 10L to 20L (low) settings using a 5-sec total delay time. All of the specific compound resonances reported integrated to the correct number of protons within ±10% when $C(O)CH_3$ or OCH_3 were taken as 3.0, except for the methyl protons of the 6-methyl compound **3**, which indicated approximately four hydrogens due to an overlapping signal from an unidentified impurity.

Sources of Chemicals. Reference compounds were either purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, or were synthesized by R. Howard using standard synthetic procedures. Dr. Ronald Bentley, University of Pittsburgh, Pittsburgh, Pennsylvania, generously provided a series of aromatic esters as model compounds for our mass spectral studies.

RESULTS

Individual beetles contain substantial quantities of quinones, ketones, and esters in their defensive secretions (Table 1). A comparison of the amount extracted by simply soaking the intact beetle to that obtained by crushing the insect in the solvent indicates that soaking removes only about 20% of the actual amount present (Table 1). Since individual beetles weigh ca. 10 mg, their quinones and aromatic defensive secretion components together comprise ca. 2% of their biomass, with the ketones and esters representing ca. 0.25% of the insects biomass.

The acetophenone **1** and propiophenone **2**, which we isolated from *T. brevicornis*, were identical in all respects with the compounds previously isolated from *T. castaneum* (Howard et al., 1986). Our previous structure proofs relied on mass spectral and infrared spectral data coupled with total synthesis. In this paper we report 400-MHz [^{1}H]NMR data which further confirm the two reported structures (Figure 1A and 1B; Table 2). Compound **1** showed two methyl peaks, three aromatic protons, and a singlet far downfield at 12.716 ppm. The

TABLE 1. QUANTITIES OF QUINONES, AROMATIC KETONES, AND AROMATIC ESTERS EXTRACTED FROM WHOLE AND CRUSHED *Tribolium brevicornis* LeC.

Compound		Amount (μg per beetle, $\bar{X}$ ± SEM)	
		Whole (N = 18)	Crushed (N = 5)
2-Methylbenzoquinone		13.1 ± 3.1	51.9 ± 9.0
2-Ethylbenzoquinone		32.1 ± 7.2	122.9 ± 24.7
2′-Hydroxy-4′-methoxyacetophenone	**1**	0.4 ± 0.1	1.3 ± 0.2
2′-Hydroxy-4′-methoxypropiophenone	**2**	3.7 ± 0.8	20.6 ± 5.3
Methyl 2,5-dihydroxy-6-methylbenzoate	**3**	0.4 ± 0.1	3.8 ± 0.6
Methyl 2,5-dihydroxy-6-ethylbenzoate	**4**	0.4 ± 0.1	2.8 ± 0.4

most upfield of the aromatic resonances (Figure 1A) at 6.360 ppm was a doublet with a 2.4-Hz coupling constant, whereas the signal just downfield from it (6.390 ppm) was a doublet of doublets with 8.8- and 2.5-Hz coupling constants. The most downfield of the aromatic peaks (7.575 ppm) showed only the 8.8-Hz coupling. The observed coupling pattern strongly suggests ortho coupling between the 7.575 and 6.390 ppm resonances, combined with meta coupling between the 6.390 and 6.360 ppm protons, as expected for the trisubstituted aromatic ring of **1.** Table 2 compares the observed chemical shifts to those previously reported for **1** (Suzuki et al., 1975).

A confirmatory NMR analysis of the propiophenone **2** proved to be less straightforward. Its spectrum (Figure 1B) showed a singlet for the methoxy methyl resonance at 3.837 ppm, an upfield triplet and quartet indicative of an ethyl group, a complex set of peaks in the aromatic region, and a hydroxy proton signal at 12.845 ppm. Homonuclear decoupling of the 6.439 ppm resonance essentially collapsed the 7.664 ppm multiplet, indicating that the apparent complexity was due to second-order effects. Chemical shifts and coupling constants for the three aromatic protons were therefore obtained by simulation techniques using the Bruker PANIC program. The observed and calculated aromatic region spectra are compared in Figure 1B and C, and the calculated shifts and coupling constants for all protons are given in Table 2. These results confirm the 1,2,4-trisubstitution pattern of **2.**

The structures of the two esters **3** and **4** were established using mass spectral and NMR analyses in conjunction with selected derivatizations. The mass spectrum of **3** (Figure 2) showed a prominent molecular ion at *m/z* 182 which

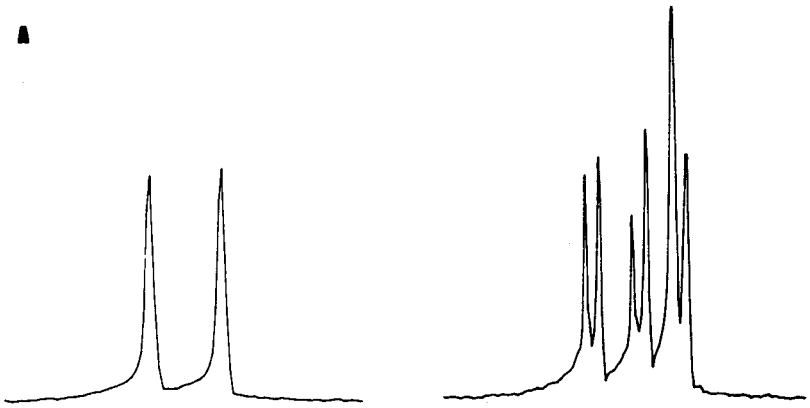

FIG. 1A. [^{1}H]NMR spectra at 400 MHz of the aromatic region of the ketones of *T. brevicornis* in $CDCl_3$. The spectra in A and B were processed with −0.25 Hz line broadening. (A) Compound **1,** 2′-hydroxy-4′-methoxyacetophenone.

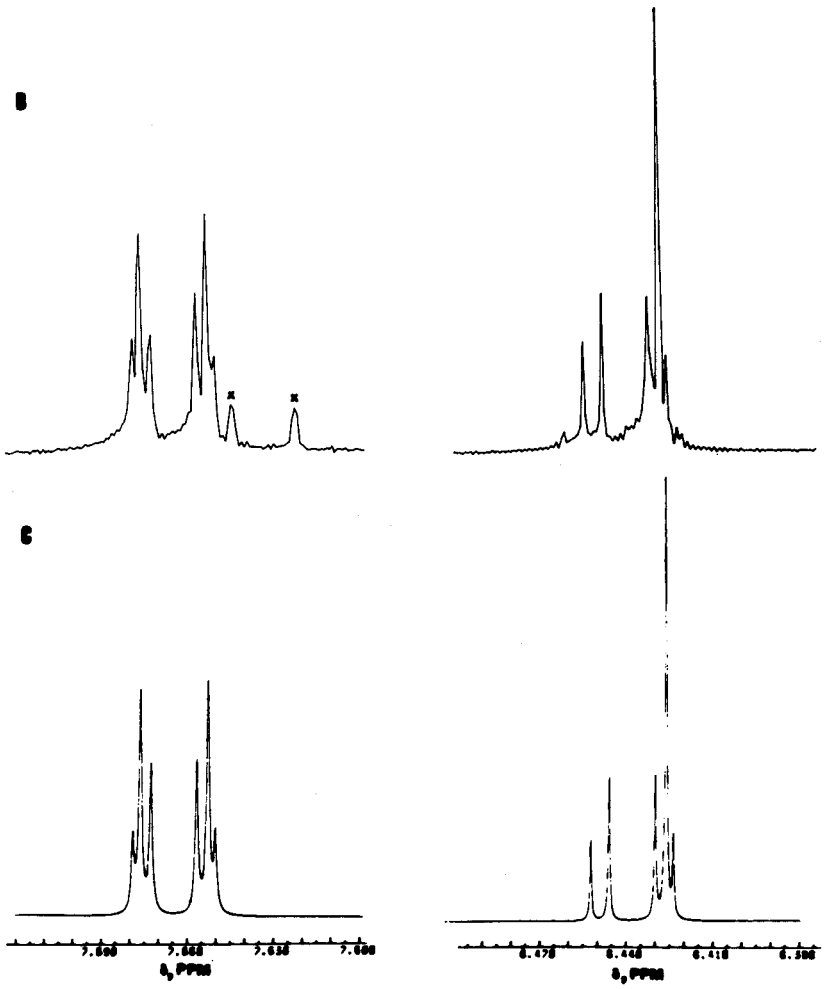

FIG. 1B, C. [^{1}H]NMR spectra at 400 MHz of the aromatic region of the ketones of *T. brevicornis* in $CDCl_3$. (B) compound **2**, 2′-hydroxy-4′-methoxypropiophenone; (C) simulated aromatic region spectrum of **2** using the Bruker PANIC program. In this spectrum the downfield set of peaks are displayed with a line width of 0.3 Hz and the upfield sets of peaks with a 0.5 Hz line width. The peaks marked × are from an unidentified contaminant.

fits a molecular formula of $C_9H_{10}O_4$. The base peak at *m/z* 150 (M-32) and the prominent ion at *m/z* 122 (M-32-28) strongly suggested that compound **3** was a substituted salicylate (Budzikiewicz et al., 1967). The two subsequent losses of mass 28 to give the ions at *m/z* 94 and *m/z* 66 indicated an aromatic molecule

TABLE 2. ^{1}H CHEMICAL SHIFTS AND COUPLING CONSTANTS OF 2′-HYDROXY-4′-METHOXYACETOPHENONE **1** AND 2′-HYDROXY-4′-METHOXYPROPIOPHENONE **2**

Compound	[^{1}H]NMR parameter[a]	H atom							
		3-H	5-H	6-H	C(O)CH$_3$	C(O)$\underline{CH_2}$CH$_3$	C(O)CH$_2$$\underline{CH_3}$	OCH$_3$	2-OH
1	δ	6.360(d)	6.390(dd)	7.575(d)	2.504(s)			3.787(s)	12.716(s)
	δ^b	6.33	6.40	7.58	2.51			3.80	12.72
	J		8.8	8.8					
		2.4	2.5						
2	δ^c	6.428(dd)	6.439(dd)	7.664(dd)		2.955(q)	1.230(t)	3.837(s)	12.845(s)
	J^c		8.79	8.79		7.33	7.34		
		2.50	2.50						
		0.51		0.51					

[a] δ is in ppm downfield from tetramethylsilane (0 ppm) as referenced to the $CHCl_3$ solvent peak taken as 7.26 ppm. J values are in Hz.
[b] Suzuki et al. (1975).
[c] The aromatic proton δ and J values were obtained from simulated spectra (see Results).

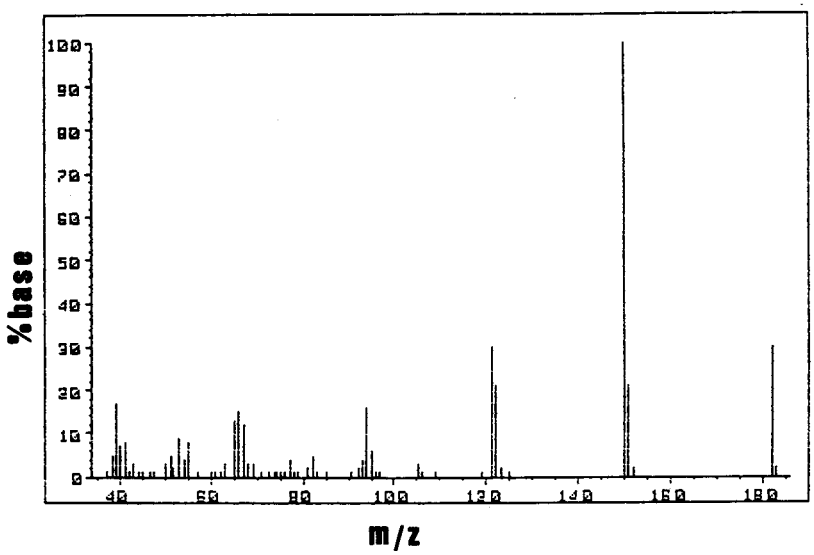

FIG. 2. EI mass spectrum of methyl 2,5-dihydroxy-6-methylbenzoate **3**.

with two ring hydroxyls, and by subtraction the remaining substituent on the ring had to be a methyl group. Derivatization of **3** with TMS yielded a product with a molecular ion (base peak) at *m/z* 268, corresponding to the addition of one methyl group and one trimethylsilyl residue to the starting ester. Other major fragment ions from this derivative occurred at *m/z* 253 (loss of methyl), *m/z* 237 (loss of $-OCH_3$), and *m/z* 73 (trimethylsilyl). These data thus confirmed our conclusion regarding the presence of two hydroxyl groups on the aromatic ring.

To this point the data argued for a trisubstituted methyl benzoate with one of the substituents being a hydroxy ortho to the carboxylate function. The placement of the methyl group could not be unequivocally determined from mass spectral data alone, although we suspected it to be at the other ortho position to the carboxylate functionality, from biosynthetic considerations and by analogy to the esters reported by Gnanasunderam et al. (1984). Similarly, the precise location of the second hydroxyl could not be determined solely from mass spectral evidence. It was possible, however, to exclude the second hydroxyl being at C-3 of the aromatic ring from the following considerations. Hydroquinones are known to undergo facile oxidation to the corresponding quinones in the hot injection ports of gas chromatographs, and indeed the production of such quinones was always observed in our analyses. Orthoquinones have very prominent M + 2 ions in their mass spectra which frequently are as intense as the M^+ ions (Zeller, 1974). The quinone derived from **3** has a prominent M^+

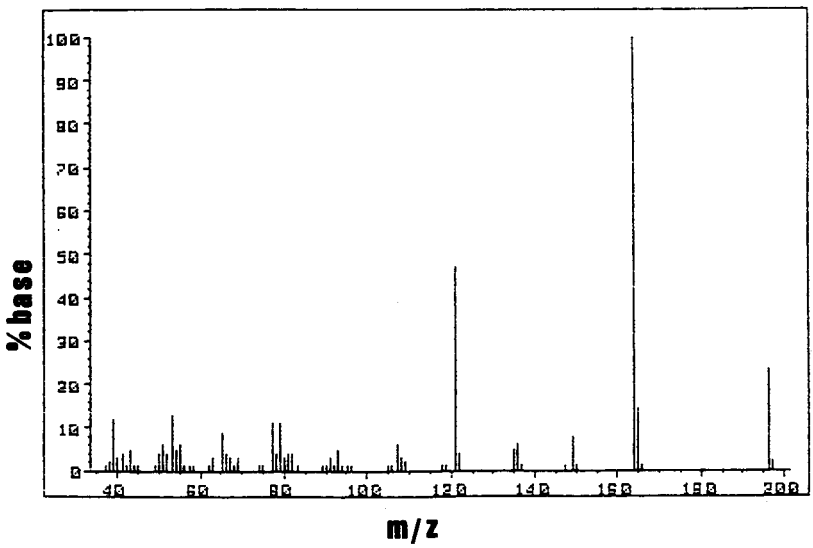

FIG. 3. EI mass spectrum of methyl 2,5-dihydroxy-6-ethylbenzoate **4**.

ion at *m/z* 180 and an M + 2^+ ion abundance of ca. 3% which clearly does not match known *o*-quinone behavior.

Inspection of the mass spectrum of compound **4** (Figure 3) suggested that it was probably an alkyl homolog of **3**. A prominent molecular ion at *m/z* 194 suggested a molecular formula of $C_{10}H_{12}O_4$, and the base peak again arose by a loss of methanol. The three subsequent successive losses of mass 28 producing ions at *m/z* 136, 108, and 80 again indicated a dihydroxy methyl benzoate with one of the hydroxyls ortho to the carboxylate group. The prominent ion at *m/z* 121 at first did not seem consistent with **4** being the ethyl homolog of methyl substituted **3**, and we considered the possibility that **4**, like **1** and **2**, contained a methoxy group. This possibility was tested by subjecting **4** to derivatization with the TMS reagent and examining the mass spectrum of the resulting derivative. That product had an M^+ at *m/z* 282 corresponding, as in **3**, to the addition of both CH_3 and trimethylsilyl to the starting ester, thus demanding that both ring oxygen functionalities be hydroxyls. As with ester **3**, the second hydroxyl was not at carbon 3 because of the low abundance of the M + 2 ion of the corresponding quinone. Therefore, other possible mechanisms that might lead to the ion at *m/z* 121 from the base peak at *m/z* 164 were considered. If the *m/z* 164 ion had a 6-ethyl substituent, it rationally could produce the *m/z* 121 ion by a loss of the methyl group from the ethyl side chain in conjunction with a ring expansion and 1,2-H shift to produce a keto tropylium ion, which in turn could lose carbon monoxide to produce a resonance stabilized

p-hydroxybenzene acylium ion of mass 121. Placement of an ethyl group elsewhere on the ring would not be expected to lead to a prominent *m/z* 121 ion nor would any dimethyl structure that we considered (Budzikiewicz et al., 1967). Our analysis to this point for compounds **3** and **4** strongly suggested that we were dealing with methyl 2,X-dihydroxy-6-alkylbenzoates.

From biosynthetic considerations it seemed likely that the second hydroxyl was at C-5. We accordingly synthesized methyl 2,5-dihydroxybenzoate **5** from the corresponding acid and used it as a standard for NMR comparisons. In Figure 4A, the [^{1}H]NMR spectrum of **5** is compared with that of ester **3** from *T. brevicornis* (Figure 4B). The natural product showed an additional 3H singlet resonance at 2.432 ppm due to the ring methyl. The chemical shift for the 6-methyl protons was in good agreement with the 2.48 ppm chemical shift reported for that group in methyl 2-hydroxy-6-methylbenzoate (Chan and Brownbridge, 1980). The 2.432 ppm singlet clearly is not a methoxy methyl since those chemical shifts are at 3.8 ppm or higher (Table 2). In addition, Chan and Brownbridge (1980) report the chemical shift of the C-4 methyl in methyl 2-hydroxy-4,6-dimethylbenzoate to be at 2.25 ppm.

In the aromatic region the alkylated benzoate **3** has only two resonances, 6.750 (d) and 6.933 ppm (d) compared to three for compound **5:** 6.872 (d), 7.010 (dd), and 7.273 ppm (d) (Figure 4A and B). That the missing proton at 7.273 ppm corresponded to the 6-position followed directly from the assignments of the ring hydrogens of **5.** Since the 7.010 ppm signal of **5** was a doublet of doublets with coupling constants of 8.9 and 3.1 Hz, that resonance corresponded to H-4, because 3.1 Hz would be unrealistically large for para coupling between H-3 and H-6 and therefore must have arisen from four-bond coupling between H-4 and H-6. The same coupling of 3.1 Hz was observed only at the 7.273 ppm resonance which identifies it as H-6.

Examination of the [^{1}H]NMR spectrum of ester **4** indicated it to be very similar to that of compound **3.** Differences include the absence of the 3H singlet at 2.432 ppm and the presence of a triplet at 1.191 ppm and a quartet at 2.917 ppm with identical coupling constants ($J = 7.4$ Hz), thus confirming the presence of an ethyl group in this compound. The shift of 2.917 ppm for the methylene protons excludes the possibility of an ethoxy derivative since in that case the shift would have been 4.0 ppm or greater (Jackman and Sternhell, 1969). The chemical shifts and coupling constants for compounds **3, 4,** and **5,** as well as literature (Gnanasunderam et al., 1984) values for methyl 6-methyl- and methyl 6-ethylsalicylate (**6** and **7,** respectively) are listed in Table 3.

The chemical shift values for the hydroxyl protons reported in Table 3 varied somewhat from sample to sample, apparently depending on the states of dryness of the sample. This variability is a well-known phenomenon for phenols, as evidenced by the range of chemical shift values reported for salicylates in the Sadtler Standard NMR Spectra (1970) (5 to ca. 13 ppm in $CDCl_3$). For compounds **3** and **4,** two OH peaks were observed near 12.75 and 10.5 ppm.

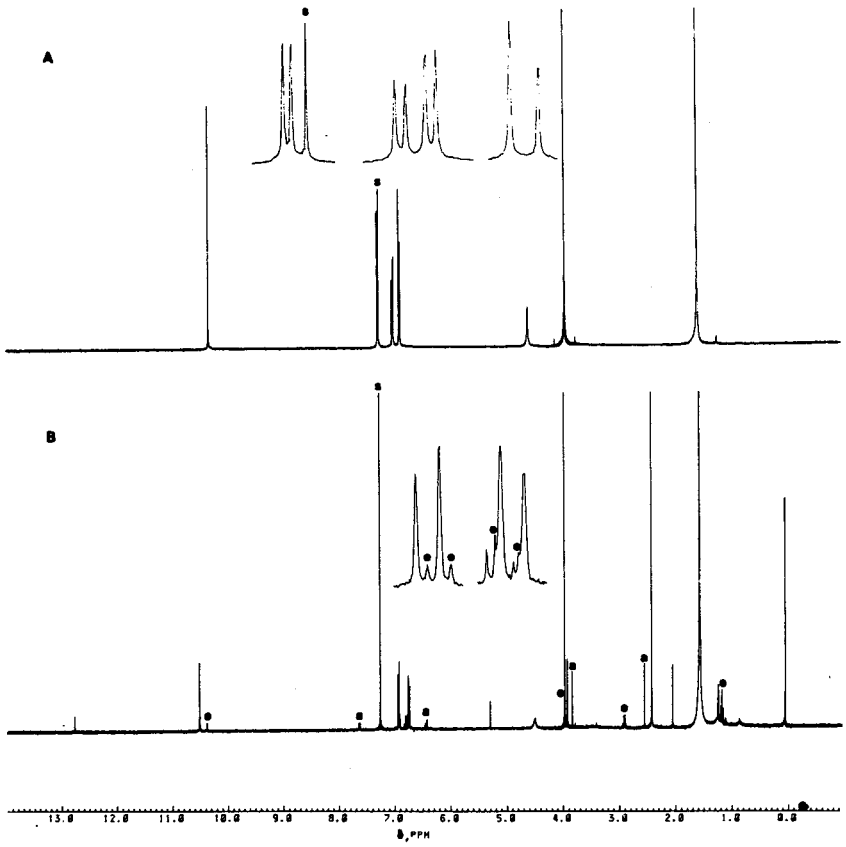

FIG. 4. [^{1}H]NMR spectra of the methyl benzoates at 400 MHz in $CDCl_3$. (A) Authentic methyl 2,5-dihydroxybenzoate. The expanded peaks are those between 6.8 and 7.3 ppm of the full spectrum with "s" denoting the protiochloroform peak. The large peak at ca. 1.5 ppm is an impurity from the isolation procedures, probably hexane. (B) Methyl 2,5-dihydroxy-6-methylbenzoate from *T. brevicornis*. The expanded peaks are those between 6.7 and 7.0 ppm of the full spectrum. The letter "e" above a peak denotes that it is from the corresponding 6-ethylbenzoate **4** and "a" indicates the acetophenone **1.** The other smaller peaks between 5.5 and 1.0 ppm, as well as the larger peaks at ca. 1.5 and 0 ppm, are contaminants from the extraction procedures.

Unfortunately, the spectrum of compound **5** (Figure 4A) gave only one signal in this region (10.4 ppm), but showed a broad feature in the water region at 5.5 ppm. The 2-O<u>H</u> chemical shifts in the β-hydroxy ketones **1** and **2** (Table 2) came at ca. 12.8 ppm, and the corresponding hydroxy signals for methyl 2-hydroxy-6-methyl benzoate **6** and methyl 2-hydroxy-4,6-dimethylbenzoate **8** are reported to occur at 11.03 and 11.00 ppm, respectively (Chan and Brownbridge,

TABLE 3. ^{1}H CHEMICAL SHIFTS AND COUPLING CONSTANTS OF METHYL 6-ALKYL-2,5-DIHYDROXYBENZOATES (**3, 4**), METHYL 2,5-DIHYDROXYBENZOATE (**5**), AND METHYL 6-ALKYL-2-HYDROXYBENZOATES (**6, 7**)

Compound	[^{1}H]NMR parameter[a]	H atom									
		3-H	4-H	5-H	6-H	CO_2CH_3	6-CH_3	6-$\underline{CH_2}CH_3$	6-$CH_2\underline{CH_3}$	2-OH	5-OH
3	δ	6.750(d)	6.933(d)			3.970(s)	2.432(s)			12.754(s)	10.501(s)
	J	8.8	8.9								
	δ(5-3)	0.122	0.077			−0.049					−0.113
	δ(6-3)	−0.02	0.34			0.01	<0.1			−1.47	
4	δ	6.758(d)	6.923(d)			3.975(s)		2.917(q)	1.191(t)	12.755(s)	10.369(s)
	J	8.8	8.8					7.4	7.4		
	δ(5-3)	0.114	0.087			−0.054					0.019
	δ(7-4)	0.00	0.35			−0.255[b]		0.02	0.01	−1.48	
5[c]	δ	6.872(d)	7.010(dd)		7.273(d)	3.921(s)				ca 5.5(s, br)	10.38(s)
	J	8.9	8.9								
			3.1		3.1						
6[d]	δ	6.73	7.27(dd)	6.85(d)		3.98(s)	2.5(s)			11.28(s)	
	J	7.4	7.4	8.2							
			8.2								
7[d]	δ	6.76(d)	(7.27)[e]	6.86(d)		3.72(s)		2.94(q)	1.2(t)	11.3(s)	
	J	7.3		8.0				7.4	7.5		

[a] The chemical shifts, δ, are in ppm downfield from tetramethylsilane (0 ppm) as referenced to the solvent $CHCl_3$ peak taken as 7.26 ppm. The coupling constants, *J*, are in Hz.
[b] This difference is probably too large because the CO_2CH_3 shift of 3.72 ppm reported for 7 seems to be in error.
[c] Authentic methyl 2,5-dihydroxybenzoate (see Methods and Materials).
[d] Gnanasunderam et al. (1984), who used δ = 7.27 ppm for the $CHCl_3$ reference. Assignments of 3-H and 5-H for **6** and **7** are based on the current results for **3** and **4.**
[e] Peak obscured by $CHCl_3$.

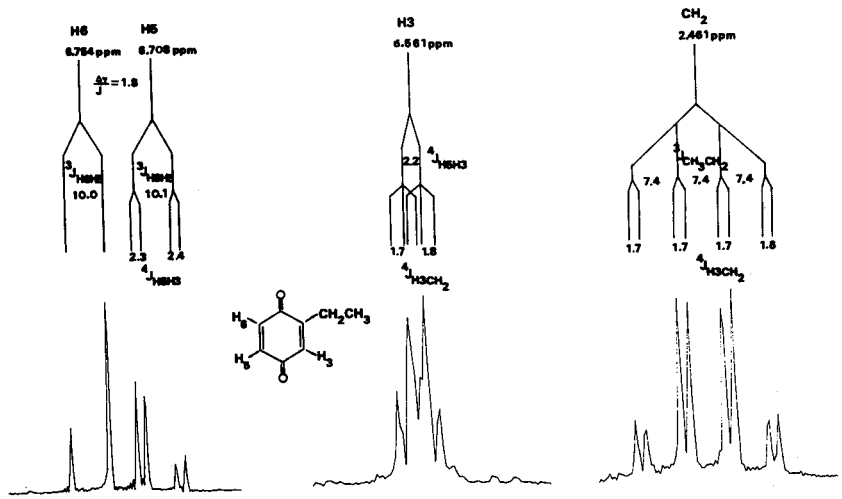

FIG. 5. [^{1}H]NMR multiplets of 2-ethylbenzoquinone from *T. brevicornis* at 400 MHz in $CDCl_3$. The triplet at 1.136 ppm for CH_2CH_3 did not show any long-range coupling and is not shown in this figure. The spectrum was processed with a −0.25 Hz line broadening. The coupling constants values shown are those obtained directly from the spectrum at each multiplet.

1980). Although we cannot unequivocally assign chemical shifts to the 2-O<u>H</u> and 5-O<u>H</u> resonances of **3** and **4,** we are tentatively assigning the most downfield resonance to the 2-O<u>H</u> hydrogen since it is involved in internal hydrogen bonding in $CDCl_3$ and matches most closely to the observed chemical shift for the β-hydroxy ketones of **1** and **2.** Thus considering all of the data, the structures of the *T. brevicornis* esters can be assigned as methyl 2,5-dihydroxy-6-methylbenzoate for **3** and methyl 2,5-dihydroxy-6-ethylbenzoate for **4.**

Although 2-methyl- and 2-ethylbenzoquinone are well-known defensive secretion products, we obtained a NMR spectrum of the ethyl derivative (Figure 5) in preparation for biosynthetic studies. Observation at 400 MHz of the long-range coupling between H-3 and the methylene protons of the 3-ethyl group rendered the proton assignments straightforward (Table 4). We note that even at 400 mHz, the aromatic protons still show slight second order effects.

DISCUSSION

The defensive secretion of *T. brevicornis* was previously reported by Markarian et al. (1978) and Wirtz et al. (1978) to contain 2-methylbenzoquinone, 2-ethylbenzoquinone, the corresponding hydroquinones, and 1-pentadecene. These are indeed the major components of this beetle's defensive secretion, but

TABLE 4. ^{1}H CHEMICAL SHIFTS AND COUPLING CONSTANTS OF 2-ETHYLBENZOQUINONE

[^{1}H]NMR parameter[a]	H atom				
	3-H	5-H	6-H	$\underline{CH_2}CH_3$	$CH_2\underline{CH_3}$
δ	6.561(dt)	6.708(dd)	6.754(d)	2.46(qd)	1.136(t)
J		10.1	10.0	7.4	7.4
	2.2, 1.8	2.4		1.7	

[a] δ is in ppm downfield from tetramethylsilane (0 ppm) as referenced to $CHCl_3$ of the solvent (δ = 7.26 ppm).

we have also found substantial quantities of at least 10 other components, including six additional olefins (Howard, 1987), the two ketones, and the two esters which are the subject of this paper. Complex blends such as these are typical of insect defensive secretions. However, the functions of all components are not always so clear. Components such as the quinones and hydroquinones are presumably cytotoxicants or irritants, whereas the olefins most likely are serving as "biosolvents" to aid in the cuticular penetration of the toxicants (Blum, 1981) and the prostaglandin synthetase inhibitors.

Prostaglandins are local cell hormones which have been isolated from nearly every type of mammalian tissue (Bergstrom et al., 1968), from several nonmammalian vertebrates and marine invertebrates (Bundy, 1984), and from insects (Brady, 1983; Lange, 1984; Murtaugh and Denlinger, 1982; Wakayama et al., 1986). In vertebrates, prostaglandins have been shown to modulate adenylate cyclase activity and facilitate ion transport, nerve transmission, platelet aggregation, and gastrointestinal function (Samuelsson et al., 1978, 1980). In invertebrates, prostaglandins have been shown to mediate ion regulation in mollusks (Graves and Dietz, 1979; Freas and Grollman, 1980), to produce fevers in crayfish (Casterlin and Reynolds, 1978) and scorpions (Cabanac and Guelte, 1980), to regulate oviposition behavior in two species of crickets (Destephano et al., 1982; Loher et al., 1981) and a silkmoth (Yamaja Setty and Ramaiah 1980), and to be required for the normal emergence and flight capabilities of a mosquito (Dadd and Kleinjan, 1984). Future studies will undoubtedly show that prostaglandins play as important a role in invertebrate physiology as they do in mammals.

Given that prostaglandins do play such a vital role in the normal physiology of animals, it is not surprising that insects have evolved chemicals that would inhibit the biosynthesis of prostaglandins in their competitors, parasites, or predators. At least three orders of insects (Coleoptera, Hemiptera, and Hymenoptera) have been reported to possess exocrine secretions that contain aromatic compounds analogous to those that we have found in *T. brevicornis*

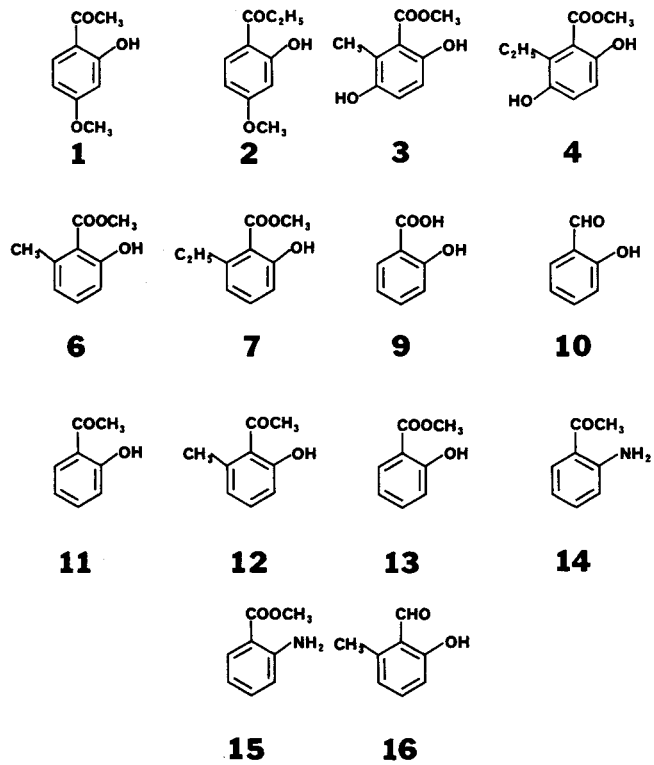

FIG. 6. Insect exocrine chemicals with known or suspected prostaglandin synthetase activity.

(Blum, 1981) (Figure 6). The common feature of all these compounds is the presence of the β-hydroxy or β-amino carbonyl moiety. Compounds **1, 2, 10, 13, 14,** and **15** (Figure 6) have been shown to be excellent prostaglandin synthetase inhibitors in both mammalian and insect systems (Howard et al., 1986; Jurenka et al., 1986). Although salicylic acid (**9,** Figure 6) has been reported to be only minimally active as a PSI, it has been shown to be converted in vivo to 2,5-dihydroxybenzoic acid, which is a very active PSI compound (Robinson and Vane, 1974). Such evolutionary "backfiring" of an organism's detoxification mechanism is of course well known to insecticide toxicologists (Matsumura, 1975). We consider it highly likely that the compounds in Figure 6, including the *T. brevicornis* esters **3** and **4,** which have not yet been tested for their PSI activity, will also prove to be excellent prostaglandin synthetase inhibitors, as will other compounds of a similar structure from other insects. Studies are now underway in our laboratories to identify such chemicals, to elucidate their ecological roles, and to further clarify the physiological roles of prostaglandins in insects.

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