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EFFECT OF SUBSTITUENT AND RING CHANGES IN NATURALLY OCCURRING NAPHTHOQUINONES ON THE FEEDING RESPONSE OF LARVAE OF THE MEXICAN BEAN BEETLE, *Epilachna varivestis*

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Abstract—Behavioral evaluation of the antifeedant effect of 10 naturally occurring 1,4-naphthoquinones on larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant, was undertaken concurrently with that of a series of synthetic analogs and model compounds in order to assess structure–activity relationships. Plumbagin, 1,4-naphthoquinone, juglone, menadione, and naphthazarin, which were found to be active at 0.3% concentrations, were also bioassayed at 0.1, 0.05, and 0.01% at which concentration 1,4-naphthoquinone still retained some activity. The model studies suggest that two structural features might be operative independently against *E. varivestis*: one consisting of a properly substituted naphthoquinone moiety and the other requiring a benzo- or naphthohydroquinone. Within the naphthoquinone group, the relative activity is determined by a substituent effect which is the outcome of a complex interplay of electronic, steric, electrochemical, and positional requirements. Among the model compounds, 2-chloro-3-amino-1,4-naphthoquinone and α-naphthylamine displayed appreciable activity even at 0.01%. The results should enable selection of plant sources for naphthoquinones possessing larval inhibition properties.

Key Words—Antifeedant, Mexican bean beetle, *Epilachna varivestis*, secondary metabolites, naphthoquinones, hydroquinones.

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INTRODUCTION

Previous studies of larval growth inhibition of the spiny bollworm, *Earias insulana* (Boisd.), by a series of secondary plant compounds including the naphthoquinone lawsone (Weissenberg et al., 1986), led us to consider the potential activity of other naturally occurring naphthoquinones. Quite a large number of such compounds have been isolated from natural sources comprising bacteria, fungi, animals, and higher plants, and many of them were found to possess biocidal activity (Thomson, 1971). The present report deals with the effect exerted by 10 naphthoquinones (**1a–1j**) (Figure 1) widely distributed in nature on the feeding response of larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), a severe pest of snap and lima beans in North and Central America (Kogan, 1977). The insecticidal activity of these naphthoquinones has had sporadic investigation, and the early work done *inter*

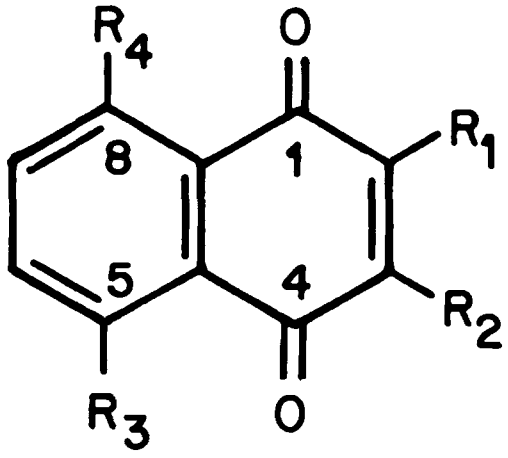

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FIG. 1. General structure of 1,4-naphthoquinones (**1a–1m**): **a** (1,4-naphthoquinone), **b** (juglone), **c** (naphthazarin), **d** (lawsone), **e** (menadione), **f** (plumbagin), **g** (shikonin), **h** (phthiocol), **i** (vitamin K_1), **j** (lapachol), **k** (2,3-dichloronaphthoquinone), **l** (2-chloro-3-aminonaphthoquinone), **m** (2-potassium sulfonate-naphthoquinone). **1a.** $R_1 = R_2 = R_3 = R_4 = H$. **1b.** $R_1 = R_2 = R_4 = H$; R_3, OH. **1c.** $R_1 = R_2 = H$; $R_3 = R_4 = OH$. **1d.** R_1, OH; $R_2 = R_3 = R_4 = H$. **1e.** R_1, CH_3; $R_2 = R_3 = R_4 = H$. **1f.** R_1, CH_3; R_3, OH; $R_2 = R_4 = H$. **1g.** R_1, $CHOHCH_2CH{=}C(CH_3)_2$; R_2, H; $R_3 = R_4 = OH$. **1h.** R_1, CH_3; R_2, OH; $R_3 = R_4 = H$. **1i.** R_1, CH_3; R_2, $CH_2CH{=}C(CH_3)-CH_2CH_2CH_2CH(CH_3)_3-CH_3$; $R_3 = R_4 = H$. **1j.** R_1, OH; R_2, $CH_2CH{=}C(CH_3)_2$; $R_3 = R_4 = OH$. **1k.** $R_1 = R_2 = Cl$; $R_3 = R_4 = H$. **1l.** R_1, Cl; R_2, NH_2; $R_3 = R_4 = H$. **1m.** R_1, SO_3K; $R_2 = R_3 = R_4 = H$.

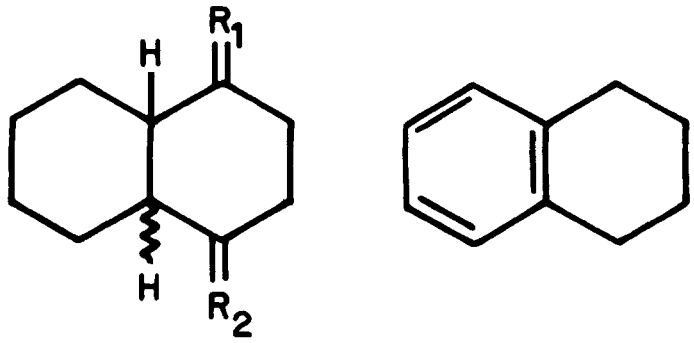

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FIG. 2. General structure of decahydronaphthalene (decalin) derivatives (decalin, **2a**; *trans*-decalin-1,4-dione, **2b**) and molecular structure of tetrahydronaphthalene (tetralin; **3**). **2a.** $R_1 = R_2 = H_2$ (5α- and 5β-H). **2b.** $R_1 = R_2 = O$ (5α-H).

alia on some of the compounds that were active in our study has been summarized (Norris, 1986; Hassanali and Lwande, 1989). The compounds include 1,4-naphthoquinone itself (**1a**), which was demonstrated to occur in plants (Müller and Leistner, 1976), and whose annular frame, devoid of any other substitution pattern, provides a convenient reference structure for comparison to the variously substituted quinonoid and/or benzenoid rings appearing in other naphthoquinones, including the related synthetic derivatives **1k–1m,** and in some analogs and model compounds, **2–8** (Figures 2–4), tested concurrently. We wish to present herein, along with our results, certain considerations on structure–activity relationships concerned with the annular effect on the activity, as well as an extensive appraisal of the substituent effect.

METHODS AND MATERIALS

Test Compounds

Twenty-six pure substances including naphthoquinones and model compounds were bioassayed (Tables 1 and 2). The following were of commercial origin: 2,3-dichloro-1,4-naphthoquinone, juglone, lawsone, menadione, naphthazarin, *p*-naphthohydroquinone, 1,4-naphthoquinone, vitamin K_1 (Fluka AG, Buchs, Switzerland), 1,4-benzoquinone, decalin, hydroquinone, lapachol, quinhydrone (Aldrich Chemical Co., Milwaukee, WI, USA), α-naphthol, α-naphthylamine (B.D.H. Ltd., Poole, UK), 1,3-dihydroxynaphthalene, 2-potassium sulfonate-1,4-naphthoquinone (Eastman Kodak Co., Rochester, NY,

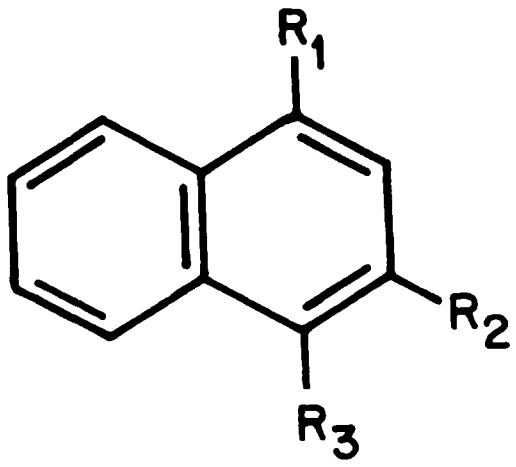

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FIG. 3. General structure of naphthalene derivatives (**4a–4g**): **a** (naphthalene), **b** (α-naphthylamine), **c** (α-naphthol), **d** (1,3-dihydroxynaphthalene), **e** (1,4-dihydroxynaphthalene), **f** (1,4-diacetoxynaphthalene), **g** (carbaryl). **4a.** $R_1 = R_2 = R_3 = H$. **4b.** R_1, NH_2; $R_2 = R_3 = H$. **4c.** R_1, OH; $R_2 = R_3 = H$. **4d.** $R_1 = R_2 = OH$; R_3, H. **4e.** $R_1 = R_3 = OH$; R_2, H. **4f.** $R_1 = R_3 = OAc$; R_2, H. **4g.** R_1, $OCONHCH_3$; $R_2 = R_3 = H$.

USA), 2-chloro-3-amino-1,4-naphthoquinone (Frinton Laboratories, S. Vineland, NJ, USA), 2,3-epoxy-2,3-dihydro-1,4-naphthoquinone (Janssen, Beerse, Belgium), naphthalene (Frutarom, Haifa, Israel), plumbagin (Carl Roth GmbH & Co., Karlsruhe, Germany), and tetralin (Riedel-De Haën AG, Seelze-Hannover, Germany). Three compounds were gifts: 5α-decalin-1,4-dione (Dr. Clive Newton, Stanford University, CA, USA), phthiocol (Prof. Kazuhiro Maruyama, Kyoto University, Japan) and shikonin (Dr. Hiroshi Fukui, Kyoto University,

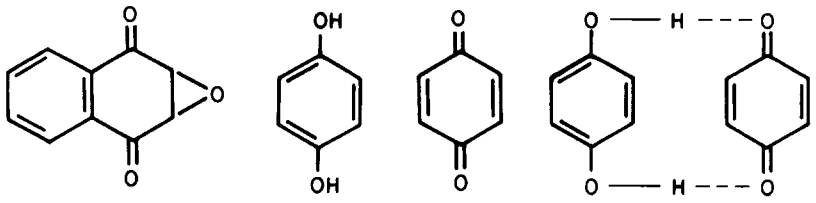

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FIG. 4. Molecular structures of 2,3-epoxy-2,3-dihydro-1,4-naphthoquinone (**5**), hydroquinone (**6**), 1,4-benzoquinone (**7**), and quinhydrone (**8**).

Japan). *p*-Naphthohydroquinone diacetate was prepared according to a known procedure (Fieser, 1948). The test compounds were dissolved in methyl alcohol to give solutions of appropriate concentration for bioassays.

Chemical Modifications of 1,4-Naphthoquinones and Subsequent Application of the Products Formed in Situ

Reduction with Sodium Borohydride. Solutions of naphthoquinones **1a, 1b,** and **1d–1f** (30 mg each) in methyl alcohol (10 ml) were treated separately with $NaBH_4$ (5 mg; Fluka). The color of solutions changed instantly. After 5 min at room temperature, the solutions of the corresponding naphthohydroquinones formed *in situ* (TLC evidence) were applied separately on leaves, as described for the bioassay. Controls with solutions of $NaBH_4$ (5 mg) in methyl alcohol (10 ml) were run in parallel.

Epoxidation with Sodium Hypochlorite. Solutions of naphthoquinones **1a, 1b,** and **1d–1f** (30 mg each) in methyl alcohol (10 ml) were treated separately with NaOCl (0.5 ml of an ~ 1 N solution in 0.1 N NaOH; B.D.H.). Color change occurred instantly. After 5 min at room temperature, the solutions of the corresponding epoxides formed *in situ* (TLC evidence) were applied separately on leaves, as described for the bioassay. Controls with solutions of NaOCl (0.5 ml) in methyl alcohol (10 ml) were run in parallel.

Insect Rearing Technique

Larvae of *E. varivestis* were reared on bean leaves (*Phaseolus vulgaris* L. var. 'Brittle Wax') at 25 $\pm$ 1°C, as described previously (Ascher et al., 1987).

Antifeedant Bioassay

The experiments were conducted with 20- to 30-mg *E. varivestis* larvae on bean leaves, in a previously described test device (Ascher et al., 1987). Solutions of the test compounds in methyl alcohol at various concentrations were pipetted on leaves and then spread over the entire leaf area with a small paint brush. Controls with methyl alcohol-treated leaves and with untreated leaves were run in parallel. The larvae (20 per treatment) were allowed to feed on the treated and control leaves for 48 hr at 21–23°C and then reweighed. Following the 48-hr feeding period the injured, moribund, or dead insects were noted and discarded, and the survivors were weighed. The test compounds were first bioassayed at a primary screening concentration of 0.3% (w/v) and the most active compounds were subsequently tested at 0.1, 0.05, and 0.01%. Percentage larval starvation was calculated from the ratio of the mean weight change of larvae to 100% starvation assessed by considering the mean weight loss of starved larvae (Ascher and Nissim, 1965).

TABLE 1. MEAN WEIGHT CHANGE AND STARVATION OF *Epilachna*

1,4-Naphthoquinone						
		0.3			0.1	
		Weight change (mg)[a]		Starvation (%)[a]	Weight change (mg)	
Structure	Name	Control	Treatment		Control	Treatment
1a	1,4-Naphthoquinone	8.2 ± 0.8	−8.3 ± 1.0	102.4 ± 6.0[b]	13.5 ± 1.6	−4.2 ± 0.8
1b	Juglone	10.7 ± 0.8	−7.4 ± 0.7	97.3 ± 4.5	15.2 ± 0.9	10.9 ± 1.5
1c	Naphthazarin	8.6 ± 1.0	−5.4 ± 0.8	84.8 ± 6.1	8.6 ± 1.0	3.5 ± 1.0
1d	Lawsone	11.4 ± 0.7	−2.3 ± 0.4	71.0 ± 5.6	10.6 ± 1.4	0.5 ± 1.4
1e	Menadione	8.9 ± 1.0	−5.6 ± 0.3	85.9 ± 3.6	13.6 ± 0.8	8.2 ± 0.8
1f	Plumbagin	9.3 ± 0.7	−9.1 ± 0.9	106.9 ± 6.5	10.9 ± 0.9	−2.5 ± 0.8
1g	Shikonin	12.6 ± 1.9	1.4 ± 1.0	54.6 ± 1.0		
1h	Phthiocol	9.7 ± 1.1	−3.8 ± 1.1	76.7 ± 4.3		
1i	Vitamin K_1	12.2 ± 1.3	8.7 ± 1.1	17.4 ± 4.9		
1j	Lapachol	8.6 ± 0.6	−2.8 ± 0.7	69.0 ± 6.2	12.1 ± 0.9	8.4 ± 2.2
1k	2,3-Dichloronaphthoquinone	9.7 ± 0.7	−7.6 ± 0.7	98.3 ± 3.8	9.7 ± 0.7	−4.2 ± 0.7
1l	2-Chloro-3-amino-naphthoquinone	10.0 ± 0.7	−3.4 ± 0.8	74.9 ± 2.6	10.0 ± 0.7	−0.7 ± 0.8
1m	2-Potassium sulfonate-naphthoquinone	7.8 ± 0.8	4.5 ± 0.7	21.0 ± 4.2		

[a] Value ± standard error.
[b] Values of percentage starvation higher than 100% are presumably due to stress-induced weight loss, in addition to that caused by starvation.

RESULTS AND DISCUSSION

The results of the antifeedant bioassays of naturally occurring naphthoquinones against *E. varivestis* are presented in Table 1. Plumbagin, 1,4-naphthoquinone, juglone, menadione, and naphthazarin were found to be highly active antifeedants at 0.3% concentration. Lesser activity was shown by phthiocol, lawsone, lapachol, and shikonin, and practically none by vitamin K_1. The most active compounds were further bioassayed at lower concentrations. At 0.1%, 1,4-naphthoquinone and plumbagin still showed marked activity, followed by lawsone. At 0.05%, lawsone displayed *ca.* 50% activity, whereas 1,4-naphthoquinone and plumbagin retained about 30% activity. At 0.01%, only 1,4-naphthoquinone still possessed *ca.* 30% activity.

The quinonoid structure is associated with a wide range of biological activity, and variously substituted naphthoquinones—both natural and synthetic—

varivestis LARVAE TREATED WITH 1,4-NAPHTHOQUINONES

Concentration (%, w/v)						
	0.05			0.01		
Starvation (%)	Weight change (mg)		Starvation (%)	Weight change (mg)		Starvation (%)
	Control	Treatment		Control	Treatment	
82.7 ± 8.6	13.5 ± 1.6	7.6 ± 2.6	27.6 ± 9.2	13.5 ± 1.6	6.8 ± 1.4	31.3 ± 7.1
18.6 + 9.3	15.2 ± 0.9	14.2 ± 1.4	4.3 ± 5.1	15.2 ± 0.9	10.8 ± 1.5	19.0 ± 5.6
30.9 ± 6.9						
54.6 ± 8.8	10.6 ± 1.4	1.8 ± 0.7	47.5 ± 5.7	10.6 ± 1.4	7.3 ± 1.8	17.8 ± 10.1
25.1 ± 3.8						
71.3 ± 4.3	10.9 ± 0.9	5.2 ± 1.4	30.3 ± 2.0	10.9 ± 0.9	9.5 ± 1.5	7.4 ± 7.7
18.5 ± 4.5						
78.9 ± 3.8	9.7 ± 0.7	6.7 ± 1.3	17.0 ± 6.6	9.7 ± 0.7	8.6 ± 1.1	6.2 ± 6.2
59.8 ± 4.7	7.3 ± 0.5	−4.4 ± 0.3	77.0 ± 1.9	7.3 ± 0.7	−3.1 ± 0.7	68.4 ± 4.4

were shown to display insecticidal, fungicidal, herbicidal, molluscicidal, termiticidal, miticidal, aphicidal, antiviral, antibacterial, antibiotic, coccidiostatic, antitrypanosomal, antineoplastic, anticercarial, antimalarial, tuberculostatic, and tumor-inhibitor effects (Marston et al., 1984; Clark, 1985; Jacobsen and Pedersen, 1986; Spencer et al., 1986). Naphthoquinones are involved in cellular respiration and photosynthesis, participating competitively with the cell components in the cell electron transport (Holmes et al., 1964; Ambrogi et al., 1970). Many of them are believed to act on oxidative phosphorylation (Jacobsen and Pedersen, 1986). Since it was inferred that the presence of certain substituents in the quinone ring, or a vacant quinonoid position, would be essential for activity (Sankaram et al., 1975), a model study was undertaken to investigate the relevance of electronic, steric, electrochemical, and location effects of various substituents in both the quinone and the phenyl nucleus, as well as that of the quinonoid and benzenoid moieties, to antifeedant activity for the larvae of *E. varivestis*; the conclusions arrived at are discussed below.

TABLE 2. MEAN WEIGHT CHANGE AND STARVATION OF *Epilachna varivestis* LARVAE TREATED WITH MODEL COMPOUNDS AND ANALOGS OF NAPHTHOQUINONE DERIVATIVES

Compound		Concentration	Weight change (mg)[a]		Starvation
Structure	Name	(%, w/v)	Control	Treatment	(%)[a]
2a	5α-Decalin-1,4 dione	0.3	5.5 ± 0.4	3.8 ± 0.9	12.7 ± 0.6
2b	Decalin	0.3	20.1 ± 1.3	17.7 ± 1.9	8.6 ± 6.0
3	Tetralin	0.3	9.7 ± 1.9	9.5 ± 1.0	1.1 ± 1.8
4a	Naphthalene	0.3	5.5 ± 0.5	5.0 ± 0.9	3.7 ± 3.7
4b	α-Naphthylamine	0.3	10.0 ± 1.0	−3.4 ± 0.5	62.4 ± 2.8
4c	α-Naphthol	0.3	5.5 ± 0.5	−1.3 ± 0.5	50.7 ± 4.0
4d	1,3-Dihydroxynaphthalene	0.3	6.9 ± 0.7	3.9 ± 0.9	20.3 ± 6.3
4e	1,4-Dihydroxynaphthalene	0.3	6.7 ± 0.4	−8.0 ± 0.6	100.0 ± 4.0
4e	1,4-Dihydroxynaphthalene[b]	0.3	6.7 ± 0.4	−8.3 ± 0.5	100.5 ± 5.5
4f	1,4-Diacetoxynaphthalene	0.3	6.9 ± 0.7	−5.8 ± 0.8	85.8 ± 8.0
4f	1,4-Diacetoxynaphthalene	0.46[c]	5.5 ± 1.1	−7.9 ± 1.0	100.0 ± 4.6
5	2,3-Epoxy-2,3-dihydro-naphthoquinone	0.3	9.9 ± 0.7	−3.5 ± 0.6	75.0 ± 5.3
6	Hydroquinone	0.3	9.6 ± 0.7	−8.0 ± 1.3	100.0 ± 4.3
7	1,4-Benzoquinone	0.3	10.4 ± 0.6	6.2 ± 0.8	22.9 ± 0.8
8	Quinhydrone	0.3	10.4 ± 0.6	2.2 ± 0.8	44.8 ± 0.8

[a] Value ± standard error.
[b] In the presence of vitamin C (0.5%).
[c] Concentration equivalent to 0.3% of the corresponding free diol.

Effect of Substituent Location

The results of our work suggest that substitution at position 2 of the quinone ring of naphthoquinone **1a** (methyl, hydroxyl, and sulfonate groups) decreases activity markedly (**1e, 1d,** and **1m**). However, introduction of the hydroxyl substituent at position 5 in the phenyl ring of **1a** reduces activity only slightly (**1b**), the latter thus becoming much more active than the isomeric **1d.** Substitution at both quinonoid positions 2 and 3 with chlorine tends to diminish activity very slightly (**1k**), but an appreciable decrease occurs when one of the chlorine substituents is replaced in the latter by an amino group (**1*l***). When a hydroxyl group is already present in position 5 of naphthoquinone, as in the case of juglone (**1b**), introduction of a methyl substituent at C-2 enhanced activity (**1f**). Further addition of a *peri* hydroxyl group to **1b** to yield naphthazarin (**1c**) seems to decrease the antifeedant effect, and substitution at C-2 in the last system with a hydroxymethylpentenyl substituent significantly reduces the activity (**1g**).

Examination of the results obtained with the other group of naphthoquinones, which are already substituted at C-2 in the quinone ring, shows that

introduction of the hydroxyl group at C-5 in the phenyl ring of 2-methylnaphthoquinone (**1e**) enhances the activity markedly (**1f**), whereas further substitution at the vicinal C-3 position of the quinonoid system with hydroxyl or phytyl substituents leads to a considerable reduction in effectiveness (**1h** and **1i,** respectively). In the case of 2-hydroxynaphthoquinone (**1d**), further substitution at C-3 with a methyl group slightly increases activity (**1h**), while a 3-methylbutenyl group slightly lowers it (**1j**).

Stereoelectronic Substituent Effect

The quinone ring of naphthoquinones is the structural feature assumed to be involved in much of their antifeedant activity (Norris, 1986), and electronic and steric effects exerted by substituents at C-2 and C-3 could conceivably influence their mechanism of action on the target pathogen by affecting the strong electron-withdrawing character of the quinone carbonyls. Yet, under the conditions of our bioassay, the unsubstituted naphthoquinone **1a** proved to be one of the most active antifeedant substrates against larvae of *E. varivestis* (Table 1), and the presence of either an electron-repelling (methyl) or an electron-attracting (hydroxyl) group at one of the β positions resulted in a decrease in activity (**1a** vs **1e** and **1d,** respectively). The 2-hydroxy group becomes strongly acidic as a result of the increased stability of the anion, due to interaction of the *p*- and *o*-quinone anions induced by the *ortho* quinoid character of the resultant system (Moore and Scheuer, 1966), which should affect the stability of the ring. Steric hindrance by larger substituents at C-2 might also interfere with the participation of the quinonoid system in any biocidal mechanism and, thus, decrease activity (**1g, 1i,** and **1m**).

Activity is retained when the position next to the hydroxyl substituent in the quinone ring is occupied by an electron-repelling alkyl group (**1h** and **1j**). All the same, further substitution of menadione (**1e**) at the vicinal position of the quinonoid system with either an electron-attracting (hydroxyl) or an electron-repelling (phytyl) group, leads to a decrease in activity (**1d** and/or **1i**). Disubstitution of naphthoquinone on the quinoidal ring with electronegative chlorine substituents (**1k**) retains the high activity, presumably due to an increased degree of conjugation, which would facilitate addition–elimination reactions (Clark, 1985). However, the activity level drops appreciably when one of the beneficial chlorine substituents is replaced by the weaker electronegative amino group (**1l**).

Turning now to substitution into the aromatic ring, it appears that addition of a strong electron-attracting hydroxyl group at the *peri* 5 position does not affect activity much (juglone; **1b**), whereas a second *peri*, strongly hydrogen-bonded hydroxyl group at C-8 seems to decrease it (naphthazarin; **1c**), probably owing to rapid tautomerism of the naphthazarin system, which results in simultaneous occurrence of benzenoid and quinonoid properties in both rings (Moore

and Scheuer, 1966). Conversely, although the strong electron-withdrawing character of the quinone carbonyls contributes to isolating the aromatic moiety from the quinoidal double bond and its substituents (Singh et al., 1968), addition of a *peri* 5-hydroxyl group to 2-methyl-naphthoquinone (**1e**) raises the activity appreciably (plumbagin; **1f**). On the other hand, substitution of juglone (**1b**) at one of the β positions of the quinoidal ring with an electron-repelling methyl group brings about a slight increase in activity (plumbagin; **1f**). When naphthazarin is substituted at one of the β positions with an alkyl group (shikonin; **1g**), the principal tautomer shows quinonoid character of the ring bearing the substituents, as substantiated by C-13 NMR evidence (Papageorghiu, 1980); yet the activity drops markedly, and this may well be due to steric hindrance by the bulkier alkyl group present at C-2 in shikonin (**1g**).

Electrochemical Effect of Substituent

The quinoidal moiety of naphthoquinones displays an electrochemical system prone to reversible reduction-oxidation reactions of the quinol–quinone type, characterized by a redox potential (E_0) or a related half-wave potential ($E_{1/2}$). Substituents in both rings of the naphthoquinone frame might conceivably modify the electron density around the quinoid carbonyl groups and thus cause a shift in either E_0 or $E_{1/2}$ values which could, arguably, be correlated with the deterrent effect. Accordingly, a substituent which reduces the E_0 of a deterrent naphthoquinone, would be expected to reduce the latter's deterrent effect (Norris, 1969). For substituents on the aromatic ring (and even in the *peri* position), the mutual interaction with the quinoid carbonyl groups is less pronounced than for the substituents on the quinoid ring (Fieser and Fieser, 1935), and they therefore have a lesser effect on the $E_{1/2}$ shift (Zuman, 1962). Indeed, substituents at C-2 in the quinoidal ring reduce E_0 more than they would at other positions (Fieser and Fieser, 1935). Among them, electron-attracting groups, which increase the electron density at the quinoid grouping as a result of their effect being transmitted through the conjugated system of the aromatic ring, shift $E_{1/2}$ toward negative values, whereas electron-repelling groups, due to their electron unsaturation, cause a reduction in the electron density at the quinoid ring which facilitates reduction and, consequently, cause $E_{1/2}$ to shift toward positive values (Vladimirtsev and Stromberg, 1957). In fact, examination of our results suggests that reduced E_0 might account for the decrease in deterrent effect with both electron-repelling (methyl) and electron-attracting (hydroxyl) substituents at C-2, the former being more of a deterrent than the latter, as would be expected. Likewise, the $E_{1/2}$ value of the most active naphthoquinones in our assays fits quite well the activity range between -160 and -530 mV suggested for competitive participation in electron transport with cell components in bac-

teriostasis (Holmes et al., 1964; Ambrogi et al., 1970). Insofar as antimicrobial (Ambrogi et al., 1970) and antifeeding (Norris, 1986) activity imply reaction between a membrane receptor protein and the electrophilic 1,4-naphthoquinones by a combination of redox interaction and Michael-type (1,4-addition) reaction, $E_{1/2}$ can provide only an initial indication, other factors involved being stereoelectronic features of the compounds, their hydrogen bonding ability, and their increased degree of conjugation. Inasmuch as behavioral effects of plant secondary metabolites elicit different responses from different pathogens, most compounds found to be active in our study also exhibited antifeeding activity—albeit not necessarily in the same order of relative feeding inhibition—in early studies done with other insects such as *Scolytus multistriatus* (European elm bark beetle) (Norris, 1969), *Periplaneta americana* (American cockroach) (Norris et al., 1970), *Culex pipiens quinquefasciatus* (mosquito) (Desmarchelier and Fukuto, 1974), *Acalymma vittata* (F.) (striped cucumber beetle) (Reed et al., 1981), *Aedes aegypti* (mosquito) (Hassanali and Lwande, 1989), and *Actias luna* (luna moth) (Thiboldeaux et al., 1994).

Summing up, the substituent influence on the activity of naphthoquinones is seemingly the outcome of a complex interplay of electronic, steric, electrochemical, and positional effects operating in the reaction between the insect lipoprotein receptors and naphthoquinones, which is involved in energy transduction and in the insect perception of the latter compounds as antifeedants (Norris, 1986, 1988). Other factors include electrophysiological, biophysical, and biochemical aspects (Norris, 1988). Different potential functions have been variously suggested for plumbagin (**1f**), such as inhibition of chitin synthetase (Kubo et al., 1983), of ecdysone 20-monooxygenase (Mitchell and Smith, 1988), and of ecdysteroid production (Joshi and Sehnal, 1989), as well as interference with the neuroendocrine system of the insect and its integration of molting processes (Gujar and Mehrotra, 1988), induction of sterilization and of immunodepression (Saxena and Tikku, 1988), an antilipid peroxidative effect (Sankar et al., 1987), and deactivation of polyphenol oxidase due to formation of six-membered chelate complexes (Sankaram et al., 1975). The antifungal action of lawsone (**1d**) was attributed to inhibition of nitrate reductase (Tripathi et al., 1980). Antimicrobial naphthoquinones were found to inhibit the succinate oxidase system and respiration by acting in the region of cytochrome *c*/cytochrome *b* in the respiratory enzyme chain, to inhibit and/or uncouple oxidative phosphorylation (Howland, 1963), to inhibit electron transfer by acting as vitamin K or ubiquinone antagonists (Holmes et al., 1964), and to inhibit mitochondrial electron transport (Fieldgate and Woodcock, 1973). The synthetic 2,3-dichloro-1,4-naphthoquinone (**1k**) is employed commercially as an agricultural fungicide under the name dichlone, and its activity is presumably linked with the ability to react with vital amines and thiols present in biological systems (Sankaram et

al., 1975) and to form hydrogen bonds (Ambrogi et al., 1970). Furthermore, fungicide activity was shown for juglone (Hedin et al., 1980) and for naphthoquinones found in bark-inhabiting beetle larval defensive secretions and suggested to act under bark as effective fungicides (Dettner, 1993).

Effect of Rings

Bicyclic Frame. Attempts were made to assess the relevance, if any, of the bicyclic backbone to activity. Thus, the saturated bicyclic derivatives decalin (**2a**) and *trans*-decalin-1,4-dione (**2b**) (the latter having the arrangement of the carbonyl groups found in 1,4-naphthoquinones) did not display activity (Table 2). Likewise, aromatization of one ring (tetralin; **3**) or both (naphthalene; **4a**) did not induce activity.

Quinoidal Ring. Another set of experiments was devised in order to evaluate the importance of the quinone ring for activity. The quinoidal moiety is, of course, characterized by the 1,4-carbonyl groups conjugated to the 2,3-double bond. Any modification of these substituents would conceivably alter the quinoidal character of the compounds and, in the process, demonstrate its relevance to activity. The most convenient modifications are reduction of the carbonyl groups and epoxidation of the conjugated double bond. We chose sodium borohydride as the reducing agent and sodium hypochlorite as the epoxidizing agent. The progress of reactions was followed by TLC and color change, and the products were first bioassayed *in situ*, in parallel with the appropriate controls containing the same reagents and solvents, in order to ascertain quickly whether the activity would be affected. It was found that the quinone system was readily reduced to the related hydroquinone using a slight excess of sodium borohydride, enough to prevent both air-oxidation of the product back to the original quinone, and its possible degradative hydrogenolysis (Moore et al., 1966). Likewise, the reaction of naphthoquinones with sodium hypochlorite led to the formation of the corresponding epoxides after only a few minutes (Marmor, 1963). Consequently, both reactions were carried out for periods of 5 min, in order to minimize possible formation of by-products, and submitted immediately to bioassays. Five model naphthoquinones, variously substituted at both rings, were selected for these experiments (**1a, 1b, 1d–1f**). The results showed that naphthoquinones unsubstituted at the quinoidal ring (**1a** and **1b**) experienced a significant drop in activity following both reactions (down to 50–60% starvation), while the 2-substituted naphthoquinones (**1d–1f**) under these conditions displayed practically the same activity as before, for reasons which were not immediately apparent (*vide infra*). At any rate, all five compounds still showed a notable level of activity after the reactions (even **1a** and **1b**), which might indicate that the reaction products would act by yet another mechanism, obviously different from that of the original naphthoquinones.

Since the main products of the reduction and/or epoxidation of naphthoquinone **1a** are *p*-naphthohydroquinone (1,4-dihydroxynaphthalene; **4e**) and 2,3-epoxy-2,3-dihydronaphtoquinone (**5**), respectively, the assays were also conducted with pure samples of these compounds, and the results indicated appreciable activity for both of them, especially the former (Table 2). High activity was also recorded when **4e** was bioassayed in the presence of vitamin C as antioxidant to prevent oxidation back to **1a,** thus demonstrating that the naphthoquinone derivative is not involved in this case. Clearly, besides the activity induced by the properly substituted naphthoquinone system, there is another mechanism of activity at stake here, related to the hydroquinone moiety.

In order to gain more insight into this intriguing assumption, we prepared the 1,4-diacetate of naphthohydroquinone (**4f**) where, of course, both hydroxyl groups are derivatized and thus unable to undergo reoxidation to the quinone system. We bioassayed the diacetate at the same concentration as the other compounds and also at a concentration equivalent to that of the corresponding free diol, and in both cases high activity was observed. Even if not involved in back-oxidation to 1,4-naphthoquinone, the *para* arrangement of the two hydroxyl groups seems to be important for activity, since the isomeric 1,3-dihydroxynaphthalene (**4d**) proved to be largely inactive. Contrariwise, the isomeric 1,5-dihydroxynaphthalene, along with **4e,** were found in another bioassay to display antifungal activity comparable to that of juglone (Hedin et al., 1980). α-Naphthol (**4c**), which has a 1-hydroxyl group only, showed some weak activity. Additional substitution in the hydroquinone ring at the C-2 position (**1d–1f**) would apparently not interfere with activity (*vide supra*), whereas discrepancy between the results of the crude reaction products of **1a** and the related pure compounds (Table 2, **4e** and **5**) might be ascribed to the presence of degradation products in the former.

More evidence on the relevance of the hydroquinone moiety to activity was obtained by bioassaying the single-ring hydroquinone (**6**) which exhibited high activity, comparable to that of naphthohydroquinone (**4e**). Conversely, the related 1,4-benzoquinone (**7**) was found in our experiments to be virtually inactive, whereas quinhydrone (**8**), which is the equimolecular adduct of **6** and **7,** displayed some activity intermediate between that of the components alone (Table 2). Incidentally, α-naphthylamine (**4b**), which has a 1-amino group, displayed moderate activity even at a 0.01% concentration (results not shown), perhaps through a carbaryl (**4g**)-like mechanism. This result recalls similar activity exerted by the synthetic 2-chloro-3-amino-1,4-naphthoquinone **1l** (Table 1) and suggests a possible effect of the amino group.

Our results show that two structural features might independently be operative against *E. varivestis:* one consisting of a properly substituted naphthoquinone moiety and the other requiring a benzo- or naphthohydroquinone. Within the naphthoquinone group, the relative activity is determined by a substituent

effect emerging from a subtle balance of steric, electronic, electrochemical, and positional requirements. The results should enable selection of plant sources for naphthoquinones possessing larval inhibition properties, notwithstanding that the ecological effect of plant–pathogen interaction would be settled by the global biochemical features of plant tissues rather than by individual secondary metabolites (Roddick et al., 1988). Likewise, the high activity showed by hydroquinones warrants further study to assess their potential for practical application. The activity displayed by epoxynaphthoquinone (**5**) is puzzling, especially compared with the inactive decalin-dione (**2a**), and deserves more investigation.

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