

NAPHTHOQUINONE INHIBITORS OF *PERIPLANETA AMERICANA* AND
SCOLYTUS MULTISTRIATUS FEEDING: ULTRAVIOLET DIFFERENCE
SPECTRA OF REACTIONS OF JUGLONE, MENADIONE, AND
1,4-NAPHTHOQUINONE WITH AMINO ACIDS AND THE INDICATED
MECHANISM OF FEEDING INHIBITION*

STEPHEN M. FERKOVICH AND DALE M. NORRIS

Department of Entomology, University of Wisconsin, Madison, Wisc. (U.S.A.)

(Received August 24th, 1970)

(Accepted April 22nd, 1971)

SUMMARY

The relative reactivity of 3 naphthoquinones, which are feeding inhibitors for *Periplaneta americana* and *Scolytus multistriatus*, with each of 7 amino acids was measured by ultraviolet difference spectroscopy. Juglone (5-hydroxy-1,4-naphthoquinone), menadione (2-methyl-1,4-naphthoquinone) or 1,4-naphthoquinone produced difference spectra immediately upon mixing with cysteine, but not with valine, serine, glutamic acid, arginine, tryptophan or proline in phosphate buffer (pH 7.0). The K_s values for the reactions indicated that the affinities of 1,4-naphthoquinone ($K_s = 4.4 \cdot 10^{-4} M$) and juglone ($K_s = 8.3 \cdot 10^{-4} M$) for cysteine were comparable, but were both approx. 10 times greater than that for menadione ($K_s = 3.2 \cdot 10^{-3} M$). The extinction coefficient of the complex formed by cysteine with juglone ($A = 3.448 \cdot 10^{-1} M$) was approx. 2 times greater than that of 1,4-naphthoquinone ($A = 1.290 \cdot 10^{-1} M$) or menadione ($A = 1.176 \cdot 10^{-1} M$). The importance of these results to explaining the mechanism of chemoreception in *P. americana* and *S. multistriatus* is discussed.

INTRODUCTION

In our continuing investigations of the energy-transduction mechanism involved in the neural stimulation or inhibition of gustation or olfaction by *Scolytus multi-*

* Approved for publication by the Director of the Research Division, College of Agricultural and Life Sciences. Studies were supported in part by grants No. GB-6580 and GB-8756 from the National Science Foundation; and by funds from the Elm Research Institute and the Wisconsin Department of Natural Resources.

striatus, NORRIS^{1,2} demonstrated the natural role of certain quinols and quinones, as chemical messengers, in the exchange of energy. Quinols (e.g. hydroquinone) were reported to stimulate; whereas, quinones (e.g. *p*-benzoquinone and 1,4-naphthoquinones) inhibited gustation in the insect. More recently, various 1,4-naphthoquinones were shown to inhibit feeding by *Periplaneta americana*³, and the order of the relative inhibition of the 1,4-naphthoquinones was the same as was found with the very different insect, *S. multistriatus*^{1,2}. This striking agreement between changes in molecular structure of the inhibitory ligand and relative inhibition of feeding in the 2 insects strongly suggested a similar mode of action. The *in vitro* binding³ of 3 naphthoquinones (juglone, 1,4-naphthoquinone and menadione) to antennal nerve cell membranes of *P. americana* subsequently corresponded to their comparative inhibition of feeding. Although NORRIS^{1,2} stated that the relative combined redox potential and intramolecular hydrogen bonding capabilities of the studied naphthoquinones were well correlated with their relative inhibition of feeding, changes in biological activity of such chemicals upon the introduction of various groups are difficult⁴ to interpret.

In the absence of adequate quantities of purified receptor protein from the insects, the relative capacities of 3 feeding-inhibitory quinones, juglone, menadione or 1,4-naphthoquinone, to react with each of several amino acids in a model system at physiological pH were studied. Such information should provide further insight into the relationship between chemical structures of 1,4-naphthoquinones and their relative binding and reactivity during inhibition of insect feeding. Our findings are presented in this paper.

MATERIALS AND METHODS

Juglone (5-hydroxy-1,4-naphthoquinone), menadione (2-methyl-1,4-naphthoquinone) and 1,4-naphthoquinone from Aldrich Chemical Company, Milwaukee, Wisc., were recrystallized from redistilled benzene. Valine, serine, glutamic acid, arginine, tryptophan, cysteine (hydrochloride) and proline from Nutritional Biochemical Corporation, Cleveland, Ohio, were of reagent grade and used without further purification. Both the naphthoquinones and amino acids were used as freshly prepared solutions in 0.1 M KH_2PO_4 - Na_2HPO_4 buffer (pH 7.0). The distilled water utilized was deionized by a mixed-bed column deionizer (Deem-a-Flow, model DF-400).

Difference spectra were recorded with a Cary model-15 spectrophotometer using a 0.0–1.0 slide wire. Absorption cells with a path length of 1 cm were used throughout, and the reference and sample compartments were thermostated at 25°. Base lines were first recorded with naphthoquinone in both sample and reference compartments. An amino acid was then added to the naphthoquinone solution in the sample compartment, and the differential spectrum was immediately obtained. The concentration of naphthoquinone was held constant ($6 \cdot 10^{-5}$ M) and was smaller than the concentration of amino acid ($3 \cdot 10^{-4}$ M to $9.6 \cdot 10^{-3}$ M).

RESULTS

Of the 7 amino acids tested, a difference spectrum in the ultraviolet and visible regions was only produced when cysteine was added to the naphthoquinone. Difference spectra of mixtures of cysteine with 1,4-naphthoquinone are shown in Fig. 1.

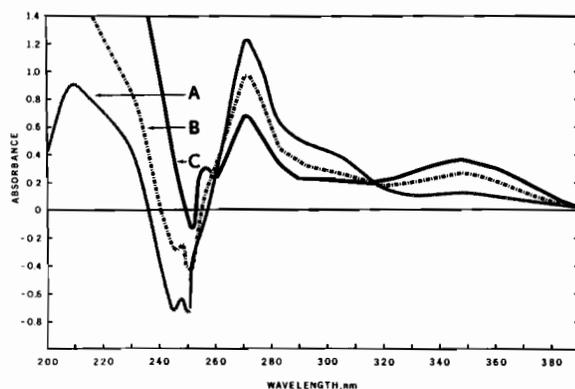


Fig. 1. Difference spectra of (A) $6 \cdot 10^{-4}$ M cysteine, (B) $2.4 \cdot 10^{-3}$ M cysteine or (C) $9.6 \cdot 10^{-3}$ M cysteine in $6 \cdot 10^{-5}$ M 1,4-naphthoquinone vs. $6 \cdot 10^{-5}$ M 1,4-naphthoquinone alone in 0.1 M $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH 7.0) in the reference cell.

Positive peaks at approx. 210, 270 and 347 nm; and 2 negative peaks between 240 and 260 nm appeared with the addition of cysteine. As the cysteine concentration was increased ($6 \cdot 10^{-4}$ M to $9.6 \cdot 10^{-3}$ M), the positive maxima at about 210 and 347 nm increased; the negative peaks between 240 and 260 nm increased toward, or passed, the zero absorbance line; and the positive peak at 270 nm decreased.

With juglone (5-hydroxy-1,4-naphthoquinone) plus cysteine, the differential

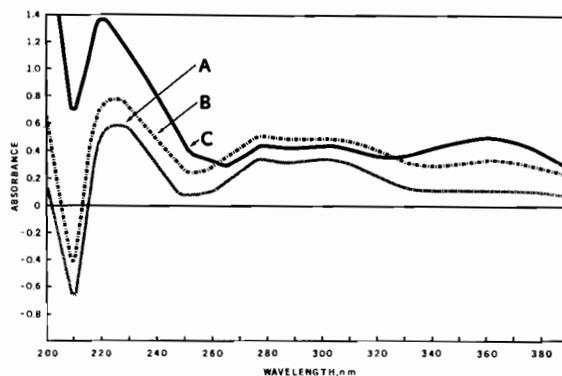


Fig. 2. Difference spectra of (A) $3 \cdot 10^{-4}$ M cysteine, (B) $6 \cdot 10^{-4}$ M cysteine or (C) $2.4 \cdot 10^{-3}$ M cysteine in $6 \cdot 10^{-5}$ M juglone vs. $6 \cdot 10^{-5}$ M juglone alone in 0.1 M $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH 7.0) in the reference cell.

spectra exhibited positive maxima at about 225, 280–300 and 360 nm; and a negative peak at 210 nm (Fig. 2). Increasing the concentration of cysteine ($3 \cdot 10^{-4} M$ to $2.4 \cdot 10^{-3} M$) produced a progressive decrease in the negativity of the band at 210 nm, and an increase in absorbance of the 225-nm band. However, the absorbance at 280 nm increased through the $6 \cdot 10^{-4} M$ cysteine concentration but then declined with $2.4 \cdot 10^{-3} M$ cysteine; simultaneously the peak at 360 nm in the visible region increased with concentration of cysteine.

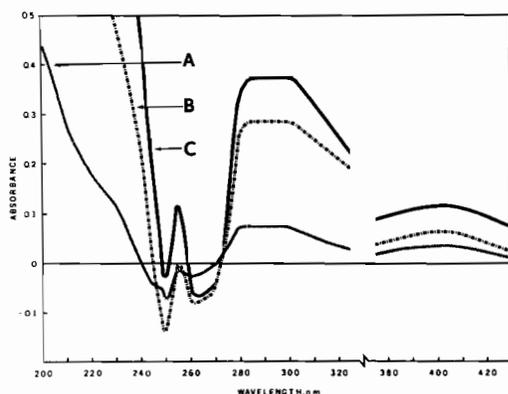


Fig. 3. Difference spectra of (A) $6 \cdot 10^{-4} M$ cysteine, (B) $2.4 \cdot 10^{-3} M$ cysteine or (C) $4.8 \cdot 10^{-3} M$ cysteine in $6 \cdot 10^{-5} M$ menadione vs. $6 \cdot 10^{-5} M$ menadione alone in $0.1 M$ KH_2PO_4 - Na_2HPO_4 (pH 7.0) in the reference cell.

Menadione plus cysteine ($6 \cdot 10^{-4} M$ to $4.8 \cdot 10^{-3} M$) produced spectra as shown in Fig. 3. The principle features of these spectra are negative peaks at about 250 and 260–261 nm and 2 positive maxima at about 280–300 and 405 nm. The peaks at about 250 and 260–261 nm increased in negativity as the cysteine concentration was raised from $6 \cdot 10^{-4} M$ to $2.4 \cdot 10^{-3} M$ and then declined at the $4.8 \cdot 10^{-3} M$ concentration. The bands at about 280–300 and 405 nm increased in absorbance as the concentration of cysteine was increased.

From the variation in absorbance at the maximum in the difference spectrum in the visible region (347 nm, 1,4-naphthoquinone; 360 nm, juglone; and 405 nm, menadione), plots of inverse absorbance at a given wavelength against the inverse concentration of cysteine gave straight lines for each naphthoquinone according to the LINEWEAVER-BURK method⁵ (Figs. 4 and 5). The reciprocal of the initial reaction velocity (V_0) was measured as the rate of change in absorbance and from the following LINEWEAVER-BURK relationship; values of $1/V_{max}$ and K_s/V_{max} were determined from the intercept and slope of the curve, respectively.

$$\frac{1}{V_0} = \frac{K_s}{V_{max}} \times \left(\frac{1}{S} + \frac{1}{V_{max}} \right)$$

The extinction coefficients were obtained from the $1/V_{max}$ values which may be equated with $1/A$, the reciprocal of the extinction coefficient of a complex or adduct

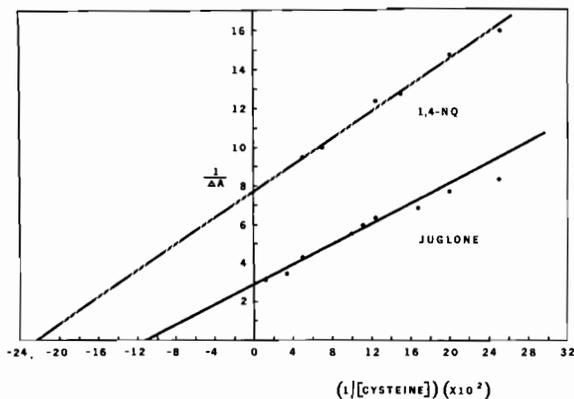


Fig. 4. LINEWEAVER-BURK plot for determination of the dissociation constant (K_s) and the extinction coefficient (A) of the complex between cysteine and naphthoquinone. $1/\Delta A$ represents the reciprocal of the magnitude of the difference spectrum measured as difference in absorption of the apparent cysteine-naphthoquinone complex at 347 nm for 1,4-naphthoquinone and 360 nm for juglone. The concentration of naphthoquinone was kept constant ($6 \cdot 10^{-5} M$) and was smaller than the concentration of cysteine ($3 \cdot 10^{-4}$ – $6 \cdot 10^{-3} M$).

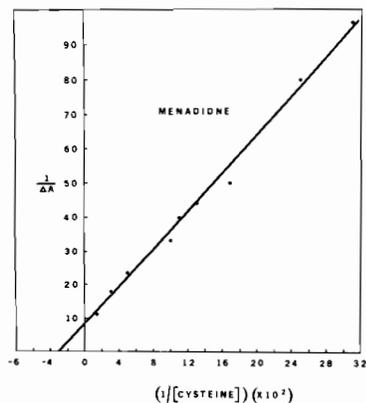


Fig. 5. LINEWEAVER-BURK plot for determination of the dissociation constant (K_s) and the extinction coefficient (A) of the complex between cysteine and menadione. $1/\Delta A$ represents the reciprocal of the magnitude of the difference spectrum measured as difference in absorption of the cysteine-menadione complex at 405 nm. The concentration of naphthoquinone was held constant ($6 \cdot 10^{-5} M$) and was smaller than the concentration of cysteine ($3 \cdot 10^{-4}$ – $6 \cdot 10^{-3} M$).

between two molecular species according to the BENESI-HILDEBRAND⁶ plot.

The A and K_s values are given in Table I.

DISCUSSION

The reaction of cysteine with each of the naphthoquinones produced a linear

TABLE I

DISSOCIATION CONSTANTS (K_s) AND EXTINCTION COEFFICIENTS (A) FOR THE BINDING OF $6 \cdot 10^{-5} M$ NAPHTHOQUINONE TO CYSTEINE

Naphthoquinone	Cysteine concn. (M)	A (M)	K_s (M)
2-Methyl-1,4-naphthoquinone	$3 \cdot 10^{-4}$ – $6 \cdot 10^{-3}$	$1.176 \cdot 10^{-1}$	$3.2 \cdot 10^{-3}$
5-Hydroxy-1,4-naphthoquinone	$3 \cdot 10^{-4}$ – $2.4 \cdot 10^{-3}$	$3.448 \cdot 10^{-1}$	$8.3 \cdot 10^{-4}$
1,4-Naphthoquinone	$4 \cdot 10^{-4}$ – $9.6 \cdot 10^{-3}$	$1.290 \cdot 10^{-1}$	$4.4 \cdot 10^{-4}$

response upon plotting $1/S$ versus $1/V_0$ ($1/A$). The K_s values for 1,4-naphthoquinone ($K_s = 4.4 \cdot 10^{-4} M$) and juglone ($K_s = 8.3 \cdot 10^{-4} M$) were comparable, but 1,4-naphthoquinone exhibited a slightly greater affinity for cysteine. Menadione showed the least affinity for cysteine ($K_s = 3.2 \cdot 10^{-3} M$); its K_s value was approx. 10 times greater than that of either 1,4-naphthoquinone or juglone. The relative affinity of the naphthoquinones for cysteine may be explained by the number and accessibility of acceptor sites in the naphthoquinone molecule. 1,4-Naphthoquinones may react with thiol groups by formation of mono- and di-complexes⁴. Our data revealed the formation of such energy-transfer complexes. All 3 naphthoquinones exhibited a positive band between 265 and 300 nm. As the concentration of cysteine was increased, the band at about 270 nm for 1,4-naphthoquinone decreased in absorbance as the band at 347 nm in the visible region increased. As the concentration of cysteine was increased, the di-complex of 1,4-naphthoquinone with cysteine apparently increased. MORRISON *et al.*⁷ found that the di-complexed form of *p*-benzoquinone predominated when the amino acid tested was held in excess. The 270-nm band for 1,4-naphthoquinone and the 280–300-nm band for juglone apparently represent the mono-complexed forms; and the peaks appearing in the visible region for these quinones, the di-complexed naphthoquinone. This is supported by the reaction of cysteine with menadione. Menadione apparently reacts with sulfhydryls only, or predominately, at the 3 position⁸. Like juglone, the differential spectrum for menadione plus cysteine showed a peak at 280–300 nm which agreed with the mono-complex. In contrast to juglone, as the cysteine concentration increased, the absorbance of this peak with menadione always increased markedly, while the peak in the visible region increased relatively little.

Because none of the maxima of the reduced naphthoquinones alone coincided with the following bands, the positive ones at 270 and 347 nm for 1,4-naphthoquinone, the ones at 280–300 and 360 nm for juglone, and the bands at 280–300 and 405 nm for menadione were attributable to naphthoquinone–cysteine complexes and were not produced by ketoreduction of the naphthoquinones.

The K_s value, of an inhibitory naphthoquinone, correlated positively with its redox potential⁴ at pH 7.0 (1,4-naphthoquinone, + 0.07; juglone, + 0.033; and menadione, + 0.02). However, the affinity of the 3 naphthoquinones for natural binding sites on antennal nerve cell membrane fractions of *Periplaneta americana*, as determined from previous studies³ *in vitro*, was of the following order: juglone > 1,4-naphthoquinone > menadione. Moreover, the relative feeding inhibition of the

3 naphthoquinones to the same insect and also to *Scolytus multistriatus* followed a similar pattern¹⁻³.

Thus, K_s values and redox potentials alone do not fully explain the relative feeding inhibition caused by these naphthoquinones; relative intramolecular hydrogen bonding capabilities of the naphthoquinones apparently are also important components of the total inhibitory action of these chemicals. A hydroxyl group in the 5 position has the greatest chance for intramolecular hydrogen bonding, and 5-hydroxy-1,4-naphthoquinone was the best feeding inhibitor for *Scolytus multistriatus* and *Periplaneta americana*^{2,3}.

The relative concentrations of the complexes formed by cysteine with the naphthoquinones giving the absorption bands in the visible region were obtained from the molar extinction coefficients A . The difference between the extinction coefficients of the complex formed with menadione ($A = 1.176 \cdot 10^{-1} M$) and of that formed with 1,4-naphthoquinone ($A = 1.290 \cdot 10^{-1} M$) was small. However, the A value for juglone ($A = 3.448 \cdot 10^{-1} M$) was approx. 2 times greater than that of 1,4-naphthoquinone or menadione.

Certain quinones may complex with and/or oxidize thiols, and our results indicated that the sulfhydryl group of cysteine, and also of the chemoreceptor site in our test insects³ reacted with inhibitory naphthoquinones at physiological pH by energy-transfer complexing. However, the possible involvement of simple oxidation of sulfhydryl groups cannot be excluded. Because several investigations have shown that sulfhydryl groups near or in cell membranes are important in the regulation of cell permeability⁹⁻¹², it is reasonable to conclude that an important energy-transduction mechanism of action by which feeding-inhibitory naphthoquinones function at the molecular level is *via* complex formation with and/or oxidation of the SH groups in receptor macromolecules in the neuron. NORRIS¹ showed that hydroquinone stimulated and benzoquinone inhibited feeding by *S. multistriatus*. Thus, a characteristic shift in a critical balance between SH groups and S-S bonds in receptor macromolecules in the neural cell membrane must be involved in the stimulation or inhibition of feeding in the test insect by quinols or quinones, respectively. Such shifts can cause changes in nerve membrane protomer conformation and allow the altered flow of inorganic ions which is certainly involved in the generation of the electrical potential which may fire the neuron.

REFERENCES

- 1 D. M. NORRIS, Quinol stimulation and quinone deterrence of gustation by *Scolytus multistriatus*, *Ann. Entomol. Soc. Am.*, 63 (1970) 476-478.
- 2 D. M. NORRIS, Energy transduction mechanism in olfaction and gustation, *Nature*, 222 (1969) 1263-1264.
- 3 D. M. NORRIS, S. M. FERKOVICH, J. E. BAKER, J. M. ROZENTAL AND T. K. BORG, Energy transduction in quinone inhibition of insect feeding, *J. Insect Physiol.*, 17 (1971) 85-97.
- 4 J. L. WEBB, Quinones, in *Enzyme and Metabolic Inhibitors*, Vol. III, Academic Press, New York, 1966, pp. 421-594.
- 5 H. LINEWEAVER AND D. BURK, The determination of enzyme dissociation constants, *J. Am. Chem. Soc.*, 56 (1934) 658-666.

- 6 H. A. BENESI AND J. H. HILDEBRAND, A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons, *J. Am. Chem. Soc.*, 71 (1949) 2703-2707.
- 7 M. MORRISON, W. STEELE AND D. J. DONNER, The reaction of benzoquinone with amines and proteins, *Arch. Biochem. Biophys.*, 134 (1969) 515-523.
- 8 L. F. FIESER AND R. B. TURNER, The addition of sulfhydryl derivatives to 2-methyl-1,4-naphthoquinone, *J. Am. Chem. Soc.*, 69 (1947) 2335-2341.
- 9 A. FARAH, N. D. YAMODIS AND N. PESSAH, The relation of changes in sodium transport to protein-bound disulfide and sulfhydryl groups in the toad bladder epithelium, *J. Pharmacol. Exptl. Therap.*, 170 (1969) 132-144.
- 10 K. M. SCOTT, V. A. KNIGHT, C. T. SETTLEMIRE AND G. P. BRIERLEY, Differential effects of mercurial reagents on membrane thiols and on the permeability of the heart mitochondrion, *Biochemistry*, 9 (1970) 714-724.
- 11 A. KARLIN AND E. BARTELS, Effects of blocking sulfhydryl groups and of reducing disulfide bonds on the acetylcholine-activated permeability system of the electroplax, *Biochim. Biophys. Acta*, 126 (1966) 525-535.
- 12 F. BERNHEIM, The effect of sulfhydryl compounds and mercurials on the swelling rate of cells of *Pseudomonas aeruginosa* in sodium and potassium buffers, *Proc. Soc. Exptl. Biol. Med.*, 133 (1970) 841-844.

Chem.-Biol. Interactions, 4 (1971/72) 23-30