Comparative Effects of Aristolochic Acids, Phenanthrene, and 1,3-Benzodioxole Derivatives on the Behavior and Survival of *Spodoptera litura* Larvae

Labunmi Lajide,* Pierre Escoubas, and Junya Mizutani

Research and Development Corporation of Japan, Eniwa RBP, Megumino Kita 3-1-1, Eniwa-Shi, Hokkaido 061-13, Japan

In a leaf disk choice bioassay against *Spodoptera litura* larvae, aristolochic acids **2** and **4** showed the strongest antifeedant activity when compared to phenanthrene, 1,3-benzodioxole, and reduced aristolochic acid derivatives. Of the phenanthrene analogs, only phenanthridine had a significant feeding-deterrent activity at a 1% concentration. The results of the diet incorporation bioassay of these compounds at a concentration of 1000 ppm showed that growth inhibition and survival of *S. litura* larvae varied according to the structure and that modifications of the aristolochic acid structure significantly reduced the anti-insect activity of the derivatives. Only 3,4-(methylenedioxy)-10-nitrophenanthrene (**5**) showed severe growth inhibition, while 5-nitro-1,3-benzodioxole (**12**) from the 1,3-benzodioxole series had the strongest toxicity. Phenanthridine (**14**) and phenanthrene (**16**) were very toxic, while 1,10-phenanthroline (**15**) showed strong growth inhibition. The observed results suggest that the differences might be due to different modes of action and that neither the phenanthrene analogs nor the 1,3-benzodioxole is as active as the natural aristolochic acids.

INTRODUCTION

Homocyclic or heterocyclic ring systems constitute part of the molecular structure of many bioactive plant secondary metabolites. This activity often is expressed in some biological system external to the plant such as insect feeding inhibition, insect growth regulation, toxicity, antimicrobial activity, and plant growth inhibition (Whittaker and Feeny, 1971; Chapman, 1974; McLaren, 1986; Cardellina, 1988; Jermy, 1990). The study of those structures has therefore a great potential for the discovery of novel agrochemical agents or new chemical leads. The precise role of all structural features of the compounds is often not well-defined with respect to their contribution to the overall activity. The understanding of structure–activity relationships for a given compound may lead to the development of simpler, less toxic analogs that may be commercially viable.

In the course of our investigation of plant–insect interaction, with the purpose of identifying potentially useful botanical pesticides, we have screened a number of tropical plants from Nigeria. The medicinal plant *Aristolochia albida* demonstrated strong antifeedant activity against the larvae of the tobacco cutworm, *Spodoptera litura* (Lajide *et al.*, 1992), and yielded aristolochic acid (**2**) as the most active component. Since this compound displayed a very strong feeding-deterrent activity, we decided to further investigate its structure–activity relationship through the study of structurally related compounds.

Phenanthrene and its derivatives were included in this study since aristolochic acid has the same basic skeletal structure. The various inhibitory activities of naturally occurring phenanthrene-based compounds have been previously reported. These include inhibition of protein synthesis by phenanthrene alkaloids (Donaldson *et al.*, 1968), anti-inflammatory effects of phenanthrene compounds from *Catasetum barbatum* (Shimizu *et al.*, 1988a), platelet aggregation inhibitors from *Salvia miltiorrhiza* (Onitsuka *et al.*, 1983), and activity of liriodenine from *Pachygona ovata*, a plant reputed to have insecticidal or insect-repellent properties (El-Kawi *et al.*, 1984). Phenanthraquinone is also known as an inhibitor of various enzymes (Chung *et al.*, 1987; Shimizu *et al.*, 1988b; Bironaite *et al.*, 1991). Structure–activity relationship studies of prostaglandin synthesis inhibition by phenanthrene analogs have also been conducted (Levine and Hong, 1977). The phenanthridines have been shown to exhibit a variety of physiological activities, including antitrypanosomal, antibacterial, antiviral, RNA polymerase and DNA polymerase I inhibition (Aktipis and Panayotatos, 1977), and inhibition of plasmodial nucleic acid anabolism (Lantz and Van Dyke, 1972). Structure–activity relationship studies have also been done for phenanthridine alkaloids such as lycorine, and their physiological activity has been attributed to their ability to inhibit ascorbic acid biosynthesis (Evidente *et al.*, 1986).

The inclusion of 1,3-benzodioxole derivatives in this study implies the use of simpler model compounds bearing functional groups similar to the ones present in **2**. Moreover, 1,3-benzodioxole derivatives have been implicated in many biological activities such as toxicity (Fujitani *et al.*, 1992), insecticide synergism (Vaidyanathaswamy *et al.*, 1977), cytochrome P-450 inhibition (Dahl and Brezinski, 1985), and chemosterilant properties against the housefly, *Musca domestica* (Jurd *et al.*, 1979).

Often, more than one structural factor is involved in a given biological activity of a molecule. In this paper, an attempt is made to further understand the structure–activity relationships of aristolochic acid, phenanthrene, and 1,3-benzodioxole derivatives using behavioral and physiological responses of larvae of the tobacco cutworm, *S. litura*, in feeding experiments.

EXPERIMENTAL PROCEDURES

Instruments. All melting points were determined with a Yanaco micromelting point apparatus and are uncorrected. ^{1}H NMR (270 MHz) and ^{13}C NMR (67.88 MHz) spectra were obtained on a JEOL EX 270 (270 MHz) spectrometer. EIMS and high-resolution MS were recorded on a JEOL HMX DX 300.

* Author to whom correspondence should be addressed.

0021-8561/93/1441-2426$04.00/0

Chemicals. Unless otherwise indicated, chemicals were purchased from either Aldrich Chemical Co., Milwaukee, WI [aristolochic acids 1 and 2, phenanthridine (14), phenanthrene-9-carboxaldehyde (17), phenanthrol (18)] or Wako Pure Chemical Industries, Osaka, Japan [3,4-(methylenedioxy)-6-nitro-benzaldehyde (10), 1,10-phenanthroline (15), phenanthrene (16), and 9,10-phenanthradione (19)].

Insects. *S. litura* larvae were reared on an artificial diet (Insecta LF, Nihon Nosan Kogyo Co.) in a controlled-environment chamber (27 °C and 70% relative humidity).

Leaf Disk Bioassay. Feeding-deterrent activity was evaluated in a choice bioassay using disks from sweet potato leaves (*Ipomoea batatas*) as previously described (Escoubas *et al.*, 1992). Compounds were tested at an initial concentration of 10 000 ppm (100 $\mu g/cm^2$) and at further dilutions (5000, 1000, 500, and 100 ppm) for compounds 2–4. An antifeedant index was calculated as $[T/(C-T)] \times 100$, where T is the percentage of the surface of treated disks consumed, and C = % of the surface of control disks consumed. An index value of less than 20 was considered to indicate significant activity.

Growth Inhibition Bioassay. The samples diluted at an appropriate concentration were incorporated into the dry diet using cellulose powder (1 g) as an inert carrier. After complete evaporation of the solvent under partial vacuum, the cellulose powder was mixed with the dry diet (9 g) and water added to obtain the final diet (10 g). Usually, 10 mg of the compound was incorporated into 10 g of the diet to obtain a final concentration of 1000 ppm (w/w). In control experiments, solvent only was applied.

Four batches of five neonate *S. litura* larvae were fed a portion of the diet, in 55 mm diameter plastic Petri dishes lined with wet filter paper. After 1 week, their individual weights as well as the mortality were recorded. Percentage of mortality was calculated as percent dead T/percent dead C × 100 and percentage of growth inhibition as mean weight of T/mean weight of C × 100, where T indicates treatment and C indicates control (N = 20).

Synthesis of Aristolochic Acid Derivatives. The syntheses of compounds 3, 6, 8, and 9 have been described previously (Lajide *et al.*, 1992).

3,4-(Methylenedioxy)-10-nitrophenanthrene (5). Decarboxylation of 8-demethoxyaristolochic acid (1, 30 mg) by using copper(I) oxide and 2,2′-bipyridyl in a manner similar to the synthesis of 6 gave 5 (21 mg, 87%), which recyrstallized from chloroform as yellow needles: mp 166–168 °C; EIMS Found 267.0522 $[M]^+$, $C_{15}H_9NO_4$, Calcd. 267.0532 (100), 221 (55), 209 (20), 163 (65), 149 (74), 104 (31), 83 (27), 57 (70), 55 (82), 43 (34); ^1H NMR ($CDCl_3$) δ 9.12 (1H, d, J = 8 Hz, C1-H), 8.23 (1H, s, C9-H), 8.02 (1H, d, J = 8 Hz, C5-H), 7.94 (1H, d, J = 8 Hz, C2-H), 7.77 (1H, m, C6-H), 7.67 (1H, m, C7-H), 6.33 (2H, s, $-OCH_2O$).

8-Methoxy-3,4-(methylenedioxy)phenanthrene-9-carboxaldehyde (7). This compound, in which the nitro group has been removed, was obtained as one of the products in the lithium aluminum hydride ($LiAlH_4$) reduction of methyl aristolochiate (3) reported in the literature (Lajide *et al.*, 1992). The faster moving band using a short silica gel column (30% EtOAc/hexane) gave 7 (5 mg, 13%) and recrystallized from chloroform as pale yellow needles: mp 170–171 °C; EIMS Found 280.0725 $[M]^+$, $C_{17}H_{12}O_4$, Calcd. 280.0728 (100), 265 (26), 250 (55), 237 (14), 221 (9), 193 (9), 179 (11), 163 (28), 150 (16), 82 (11), 44 (16); ^1H NMR ($CDCl_3$) δ 10.07 (1H, s, CHO), 8.92 (1H, d, J = 9.5 Hz, C10-H), 8.69 (1H, d, J = 8 Hz, C5-H), 8.26 (1H, d, J = 9.5 Hz, C9-H), 7.75 (1H, s, C2-H), 7.59 (1H, t, J = 8 Hz, C6-H), 7.07 (1H, d, J = 8 Hz, C7-H), 6.37 (2H, s, $-OCH_2O-$), 4.05 (3H, s, OCH_3).

6-Nitro-1,3-benzodioxole-5-carboxylic Acid (11). To a solution of the aldehyde 10 (5.36 g) in 1,2-dimethoxyethane (100 mL) and water (25 mL) was added a solution of sulfamic acid (3.5 g), acting as chlorine scavenger, and sodium chlorite (80%, 2 g) in water (30 mL). The reaction mixture was stirred at room temperature for 30 min and then diluted with water and extracted with ether. The ether solution was washed with brine and dried (Na_2SO_4). Evaporation of the solvent and crystallization of the residue from acetone gave the acid 11 (5 g, 86%) as yellow needles: mp 169–170 °C [Lit. mp 166–167 °C (Mitscher *et al.*, 1978)]; EIMS Found 211.0347 $[M]^+$, $C_8H_5NO_6$, Calcd 211.0348 (100); ^1H NMR ($CDCl_3$) δ 11.01 (1H, bs, COOH), 7.45 (1H, s, C5-H), 7.23 (1H, s, C2-H), 6.31 (2H, s, OCH_2O).

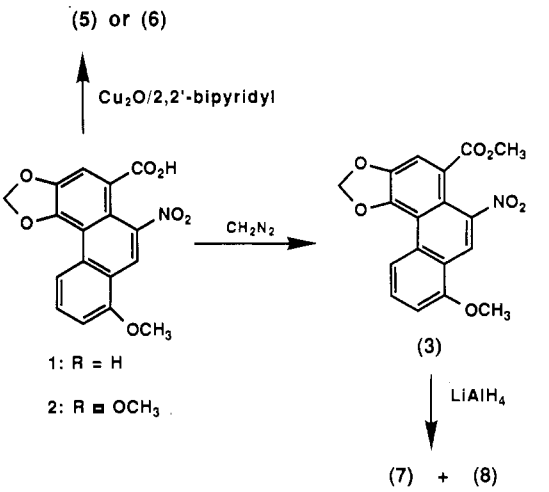


Figure 1. Synthetic scheme of aristolochic acid derivatives 3 and 5–8.

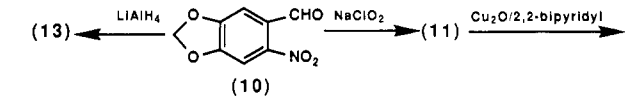


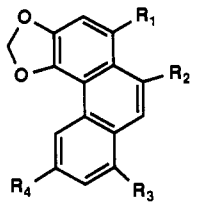
Figure 2. Synthetic scheme of 1,3-benzodioxole derivatives 10–13.

5-Nitro-1,3-benzodioxole (12). A solution of the acid 11 (1.5 g), copper(I) oxide (200 mg), and 2,2′-bipyridyl (500 mg) in anhydrous dimethylformamide was gently refluxed in an atmosphere of nitrogen for 3 h. The cooled reaction mixture was poured into cold dilute hydrochloric acid (1 M) and the precipitate filtered. The precipitate and the filtrate were extracted with ether, and the combined ethereal solution was washed with saturated sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The residue obtained on evaporation of the solvent gave the nitro compound 12 (1 g, 84%), which recrystallized from chloroform as yellow needles: mp 146–148 °C; EIMS Found 167.0398 $[M]^+$, $C_7H_5NO_4$, Calcd 167.0400 (100); ^1H NMR ($CDCl_3$) δ 7.88 (1H, dd, J = 2.3, 9 Hz, C6-H), 7.66 (1H, d, J = 2.3 Hz C2-H), 6.86 (1H, d, J = 9 Hz, C5-H).

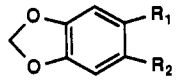
5-(Hydroxymethyl)-6-nitro-1,3-benzodioxole (13). A suspension of $LiAlH_4$ (130 mg) in anhydrous ether was added to a solution of the aldehyde 10 (2.6 g) in anhydrous ether (75 mL) and stirred at 0 °C for 5 min. The reaction mixture was diluted with ethyl acetate and poured into a 2% HCl solution. The organic layer was washed with water and brine and dried (Na_2SO_4). The crude alcohol obtained on evaporation of the solvent was purified on a short silica gel column with 20% ethyl acetate/hexane as eluent to give the pure benzyl alcohol 13 (2.3 g, 88%), which crystallized from chloroform as pale yellow needles: mp 115–117 °C; EIMS Found 197.0521 $[M]^+$, $C_8H_7NO_5$, Calcd 197.0522 (100); ^1H NMR ($CDCl_3$) δ 7.70 (1H, s, C5-H), 7.15 (1H, s, C2-H), 6.16 (2H, s, OCH_2O), 4.89 (2H, s, CH_2OH).

RESULTS AND DISCUSSION

The syntheses of aristolochic acid derivatives 5–9 and the 1,3-benzodioxole derivatives 10–13 proceeded in fairly good yield as described under the Experimental Procedures (Figures 1 and 2). The structures of all the compounds are shown in Figure 3. The antifeedant index values of the compounds tested, including commercially available compounds (14–19), are given in Table I. Aristolochic acids 2 and 4 demonstrated the strongest antifeedant activity, with compound 2 showing activity even at 100 ppm, while 4 loses activity very quickly at lower concentrations (Table II). Among the remaining compounds only phenanthridine (14) and phenanthrene-9-carboxaldehyde (17) showed any activity, with antifeedant index values of 12.01 and 23.98, respectively. This activity was, however, very weak, considering the fact that they were tested at a fairly high concentration (10 000 ppm). Our experience



A



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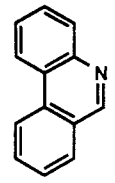

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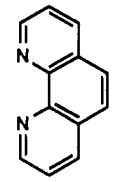

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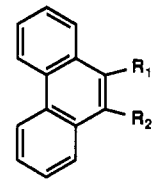

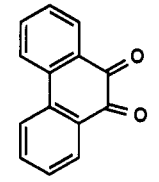

F

A:	R_1	R_2	R_3	R_4
(2)	CO_2H	NO_2	OCH_3	H
(3)	CO_2Me	NO_2	OCH_3	H
(4)	CO_2H	NO_2	OCH_3	OH
(5)	H	NO_2	H	H
(6)	H	NO_2	OCH_3	H
(7)	CHO	H	OCH_3	H
(8)	CH_2OH	NO_2	OCH_3	H
(9)	CHO	NO_2	OCH_3	H

B:	R_1	R_2
(10)	CHO	NO_2
(11)	CO_2H	NO_2
(12)	H	NO_2
(13)	CH_2OH	NO_2

C: Phenanthridine (14)

D: 1,10-Phenanthroline (15)

E:	R_1	R_2
(16)	H	H
(17)	H	CHO
(18)	H	OH

F: 9,10-Phenanthradione (19)

Figure 3. Structures of compounds tested 2–19.

Table I. Antifeedant Index (at 10 000 ppm for 100 μg/cm²), Percentage of Growth Inhibition, and Percentage of Mortality (at 1000 ppm w/w) of *S. litura* Larvae for Compounds 2–19

compd	antifeedant index ± SD	% mortality	% growth inhibition
control	66.75 ± 13.97	0.00	0.00
2	0.00 ± 0.00	5.00	97.64
3[a]	–	0.00	0.72
4	0.00 ± 0.00	65.00	99.35
5	57.60 ± 9.42	5.00	81.71
6	45.61 ± 7.79	10.00	4.51
7	35.15 ± 5.63	5.00	30.51
8	36.20 ± 9.51	5.00	44.14
9	33.68 ± 10.93	0.00	7.59
10	60.24 ± 3.76	10.00	3.52
11	49.38 ± 10.80	25.00	39.88
12	31.30 ± 7.99	60.00	92.70
13	44.75 ± 8.19	10.00	38.75
14	12.01 ± 6.52	100.00	100.00
15	42.79 ± 2.41	55.00	91.60
16	31.25 ± 10.60	80.00	91.33
17	23.98 ± 10.99	20.00	49.52
18	31.87 ± 3.76	5.00	24.72
19	54.41 ± 7.10	0.00	23.64

[a] Tested only at 1000 ppm for antifeedant activity.

Table II. Antifeedant Activity of Aristolochic Acids 2 and 4 and Methyl Ester 3 at 1000, 500, and 100 ppm

compd	1000 ppm	500 ppm	100 ppm
2	0.00 ± 0.00	0.00 ± 0.00	3.73 ± 1.86
3	30.86 ± 2.35	41.12 ± 2.58	51.04 ± 1.86
4	0.00 ± 0.00	21.42 ± 6.64	53.67 ± 17.37

in this field indicates that compounds which do not exhibit complete feeding inhibition at this concentration cannot be considered significantly active as antifeedants. In a previous study on the antifeedant activity of aristolochic acid derivatives against *S. litura* larvae (Lajide *et al.*, 1993), we demonstrated that the location of a –COOH group in close proximity to an $-NO_2$ group was essential for antifeedant activity and that modification of the –COOH group in **2** resulted in significantly lower antifeedant activity.

It is interesting to note that none of the phenanthrene-based structures (**16–19**) elicited a strong antifeedant response as shown by their high index values. This suggests that the basic phenanthrene skeletal structure by itself is not sufficient to induce a negative feeding response in the larvae. The 1,3-benzodioxole compounds (**10–13**) were also not active.

In the series of aristolochic acid derivatives (**5–9**), compounds **7–9** have relatively lower index values than **5** and **6**. This result is in accordance with observations made by previous authors, which have determined that compounds with antifeedant activity are the ones which possess the more oxidized or unsaturated structures. For example, *p*-hydroquinone is a feeding stimulant, while the more oxidized derivative, *p*-benzoquinone, is an antifeedant (Norris, 1986). This appears clearly in the aristolochic acid series, where the highest activity is associated with the presence of a carboxylic group (**2**) and where activity lessens upon reduction to the alcohol (**8**) or the aldehyde (**9**).

The results of the growth inhibition bioassay and mortality observed for the different compounds are also given in Table I. In the aristolochic acid series, aristolochic acids **2** and **4** again demonstrated the strongest growth inhibition. The derivative **5** also evoked a strong growth inhibition response. This was followed by **8** and **7** with fairly weak activity, while **3**, **6**, and **9** hardly affected the growth of the *S. litura* larvae.

It is also interesting to note that the activity of **4** is associated with a rather significant mortality (65%),

whereas none is observed for **2** and very low mortality is seen for all other compounds in the series. The observed growth inhibition may therefore be due to several different mechanisms: in the case of **2**, larvae probably starve rather than feed on the spiked diet. (Actually the toxicity of aristolochic acid **2** upon ingestion cannot be verified since the amount ingested is probably negligible.) However, the acid **4** does seem to act by toxicity. Although it is also a good antifeedant, a small amount ingested is sufficient to provoke a high percentage of mortality. This result demonstrates that a small modification of structure, such as the introduction of a hydroxy group, can drastically affect the balance existing between the two types of action.

Compound **5** shows yet another type of activity; although not antifeedant and not toxic, it induces a strong reduction of weight gain in the insects. Another physiological mechanism is therefore probably involved here. The structural difference between **5** and **6**, which does not show growth inhibition, is the presence of a methoxy group in the 8-position, thus demonstrating again how a subtle change in the structure can affect the biological activity.

Finally, the importance of the group in position 1 is stressed again upon examination of compounds **2** (–CO_2H), **6** (–H), **8** (–CH_2OH), and **9** (–CHO). None of them shows toxicity, but their effect on growth varies greatly with the degree of oxidation of the substituent in position 1.

Growth inhibition is a complex phenomenon, which can result from the interaction of the allelochemicals with different biological processes such as hormonal control, metabolic enzymes, or detoxification. It is therefore difficult to offer a satisfactory hypothesis at this point. It can be suggested, however, that the interaction of the compounds with a receptor protein or a binding site will be influenced by the ability of the compounds to form bonds with a nucleophilic site in the receptor. This was proposed as an explanation of the mode of action of benzoquinone antifeedants (Norris, 1986) and may be a relevant model for explaining the activity of phenanthrene-based compounds. In this model, the oxidation state of key functional groups was deemed to be critical for activity, in a similar way to our observations.

For example, compound **5** has the greatest possibility of producing a reactive intermediate after initial microsomal oxidation of the methylenedioxy group first to a catechol and then to a quinone intermediate (**20**) (Jurd *et al.*, 1979) (Figure 4). The increase in the lipophilicity of **5** compared to the rest of the aristolochic acid derivatives might also be one of the factors enhancing the activity.

In the 1,3-benzodioxole group, **12** was the most toxic compound with 60% mortality, while **11** and **13** with additional hydrophilic substituents demonstrated moderate growth inhibition and low toxicity. The aldehyde **10** did not show any activity at all. The observed reduction of growth parallels toxicity, suggesting that poisoning might be responsible for this result, as mentioned in the previous paragraph. It can therefore be hypothesized that the cellular target of these compounds could be different from that of the aristolochic acid derivatives since the latter did not exhibit any toxicity except for **4**. Here again, the modification of one functional group induces a broad variation of activity. The activity of **12** might possibly be due to its transformation to a reactive quinone intermediate **21** (Figure 5). The reactivity of quinones, *p*-quinones, and quinone methides is well-known (Turner, 1964; Norris, 1986) and has been implicated in oxidative phosphorylation and nucleophilic reactions as mentioned above.

Phenanthridine (**14**) and 1,10-phenanthroline (**15**), which incorporate nitrogen into the phenanthrene structure, were particularly active. **14** showed some antifeedant activity and was also very toxic to the larvae. The

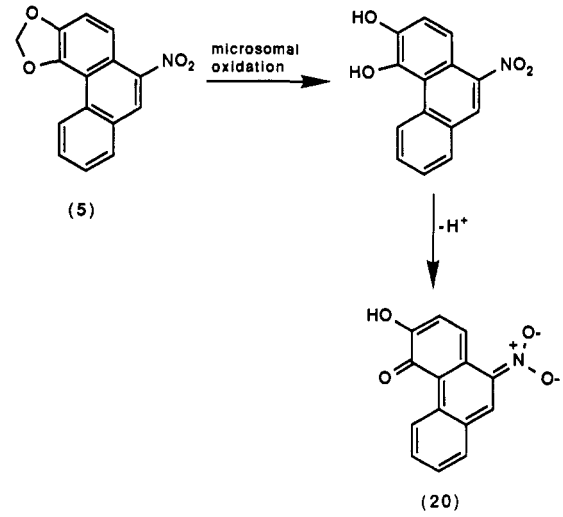

Figure 4. Formation of quinone intermediate **20** from **5**.

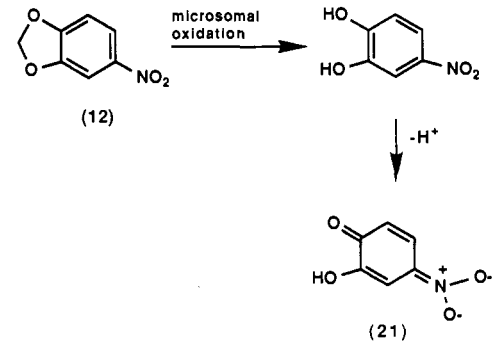

Figure 5. Formation of quinone intermediate **21** from **12**.

introduction of nitrogen into the phenanthrene ring structure when compared with oxygenated phenanthrenes **17–19** seems to provoke a significant increase in biological activity, probably due to an increase in its nucleophilicity as demonstrated by lycorine alkaloids (Evidente *et al.*, 1986).

The last series of compounds tested were phenanthrene analogs **16–19**). Phenanthrene **16** was, remarkably, the most potent of the four compounds. The aldehyde **17** reduced the growth of the larvae by *ca.* 50%, while the phenol **18** and the dione **19** demonstrated *ca.* 25% growth inhibition when compared to control. Compounds **17–19** possessed very low toxicity, but phenanthrene **14** was highly toxic to the larvae. Here again the degree of oxidation in the compounds **17–19** negatively affected its growth inhibition activity when compared to that of the aristolochic acid derivatives. The observed results possibly indicate a different mode of action. An investigation of the exact molecular target is, however, necessary for a complete interpretation of those results.

The activity of phenanthrene was rather unexpected, but it can be hypothetized that its lipophilicity might be a leading factor in the enhanced activity of **16**. Lipolysis is an important physiological process in living organisms, and it is necessary for the breakdown of water-insoluble long-chain triglycerides prior to cellular absorption of the breakdown products. It has been reported that hydrophobic molecules that can dilute the interfacial substrate concentration can inhibit interfacial activation in enzyme-catalyzed lipolysis in cells. This type of inhibition has been demonstrated by phenanthrene (Ferreira and Patton, 1990).

The range of compounds tested during this study was not meant to be comprehensive but to indicate trends in activity among a number of compounds related to aris-

tolochic acid, phenanthrene analogs, and 1,3-benzodioxole possessing similar functional groups as **2**. In comparing antifeedant activity, growth inhibition, and toxicity against *S. litura* larvae, a very clear distinction must be made between behavioral and physiological effects of the tested compounds. The observed effects are the result of a complex mode of action, and similar observations (*e.g.*, no weight gain) may result from the interaction of the compounds with very different cellular receptors. The elucidation of these as well as hypotheses on modes of action was not considered in the present study, which was concerned with a strict and limited comparison of effects under identical conditions. Our goal was to identify the portions of functional groups in the molecule necessary for activity and possibly to provide insights on possible lead structures modeled on naturally occurring bioactive compounds for future development of plant protection chemicals.

In conclusion, there seems to be no single structural factor emerging predominantly among the compounds tested for antifeedant, growth inhibition, and toxicity against *S. litura* larvae, when compared to aristolochic acid **2**. Antifeedant activity seems to be strongly reduced upon modification of the natural aristolochic acid structure, in particular the carboxylic acid group. Compounds that possess the ability to form reactive quinone intermediates, such as **5** and **12**, as well as phenanthrene, are quite toxic, and other derivatives show growth inhibition without toxicity, suggesting different mechanisms of action and different molecular targets.

It might be expected that more than one structural factor operates in a plant constituent with very high activity, containing several different substituents in the molecule as seen in aristolochic acid. Appropriately substituted phenanthrene-based skeletal structures certainly offer a unique model for possible lead structures for insect control.

ACKNOWLEDGMENT

We thank Y. Matsuzawa for technical assistance and K. Watanabe and E. Fukushi for EIMS/HRMS. This project was funded and supported by the Research Development Corp. of Japan (JRDC) under the ERATO program.

LITERATURE CITED

Aktipis, S.; Panayotatos, N. The inhibitory effect of Phenanthridines on the synthesis of Ribonucleic Acid catalyzed by Deoxy-ribonucleic acid-Dependent Ribonucleic acid Polymerase. *Mol. Pharmacol.* **1977**, *13*, 706–718 (and references cited therein).

Bironaite, D. A.; Cenas, N. K.; Kulys, J. J. The Inhibition of Glutathione Reductase by Quinones. *Z. Naturforsch.* **1991**, *46C*, 966–968.

Cardellina, J. H. Natural Products In the Search for New Agrochemicals. In *Biologically Active Natural Products: Potential Use in Agriculture;* Cutler, H. G., Ed.; ACS Symposium Series 380; American Chemical Society: Washington, DC, 1988; pp 305–315.

Chapman, R. F. The Chemical Inhibition of Feeding by Phytophagous Insects: A review. *Bull. Entomol. Res.* **1974**, *64*, 339–363.

Chung, H.; Harvey, R. G.; Armstrong, R. N.; Jarabak, J. Polycyclic aromatic Hydrocarbon quinones and glutathione thioethers as substrates and Inhibitors of the Human placental NADP-linked 15-Hydroxyprostaglandin Dehydrogenase. *J. Biol. Chem.* **1987**, *267*, 12448–12451.

Dahl, A. R.; Brezinski, D. A. Inhibition of rabbit nasal and Hepatic Cytochrome P-450-Dependent Hexamethylphosphoramide N-demethylase by methylenedioxyphenyl compounds. *Biochem. Pharmacol.* **1985**, *34*, 631–636.

Donaldson, G. R.; Atkinson, M. R.; Murray, A. W. Inhibition of Protein synthesis in Ehrlich ascites-tumor cells by Phenanthrene alkaloids: tylophorine, tylocrebrine and cryptopleurine. *Biochem. Biophys. Res. Commun.* **1968**, *31*, 104–109.

El-Kawi, M. A.; Slatkin, D. J.; Schiff, P. L., Jr.; Dasgupta, S.; Chattopadhyay, S. K.; Ray, A. B. Additional alkaloids of *Pachygone ovata*. *J. Nat. Prod.* **1984**, *47*, 459–464.

Escoubas, P.; Lajide, L.; Mizutani, J. An improved leaf-disk antifeedant bioassay and its application for the screening of Hokkaido plants. *Entomol. Exp. Appl.* **1993**, *66*, 99–107.

Evidente, A.; Arrigoni, O.; Luso, R.; Calabrese, G.; Randazzo, G. Further experiments on structure-activity relationship among lycorine alkaloids. *Phytochemistry* **1986**, *25*, 2739–2743.

Ferreira, G. C.; Patton, J. S. Inhibition of Lipolysis by hydrocarbons and fatty alcohols. *J. Lipid Res.* **1990**, *31*, 889–897.

Fujitani, T.; Ando, H.; Fujitani, K.; Ikeda, T.; Kojima, A.; Kubo, Y.; Ogata, A.; Oishi, S.; Takahashi, H. Subacute toxicity of piperonyl butoxide in F344 rats. *Toxicology* **1992**, *72*, 291–298.

Jermy, T. Prospects of antifeedant approach to Pest Control. *J. Chem. Ecol.* **1990**, *16*, 3151–3160.

Jurd, L.; Fye, R. L.; Morgan, J., Jr. New Types of Insect Chemosterilants. Benzylphenols and Benzyl-1,3-benzodioxole Derivatives as Additives to Housefly Diet. *J. Agric. Food Chem.* **1979**, *27*, 1007–1016.

Lajide, L.; Escoubas, P.; Mizutani, J. Antifeedant Activity of *Aristolochia albida* Root Metabolites against the Tobacco Cutworm, *Spodoptera litura*. *J. Agric. Food Chem.* **1993**, *41*, 669–673.

Lantz, C. H.; Van Dyke, K. *Plasmodium berghei*: Inhibited incorporation of AMP-8-^{3}H into nucleic acids of erythrocyte-free malaria parasites by acridines, phenanthridines and 8-aminoquinoline. *Exp. Parasitol.* **1972**, *31*, 255–261.

Levine, L.; Hong, S. L. Analogues of Anthracene, phenanthrene and benzoflavone inhibit prostaglandin biosynthesis by cells in culture. *Prostaglandin* **1977**, *14*, 1–9

McLaren, J. J. Biologically active substances from higher plants. Status and future potential. *Pestic. Sci.* **1986**, *17*, 559–578.

Mitscher, L. A.; Grace, H. E.; Clark, G. W., III; Suzuki, T. Quinolone antimicrobial agents: Versatile new synthesis of 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids. *J. Med. Chem.* **1978**, *21*, 485–489.

Norris, D. M. Antifeeding compounds. In *Chemistry of Plant Protection. Sterol biosynthesis, Inhibitors and anti-feeding compounds*; Bowers, W. S., Ebing, W., Fukuto, T. R., Martins, D., Weigler, R, Yamamoto, I., Eds.; Springer-Verlag: Secaucus, NJ, 1986; pp 97–143.

Onitsuka, M.; Fujio, M.; Shinma, N.; Maruyama, H. B. New platelet aggregation inhibitors from "Tan-Shen"; Radix of *Salvia miltiorrhiza*, BUNGE. *Chem. Pharm. Bull.* **1983**, *31*, 1670–1675.

Shimizu, M.; Shogawa, H.; Hayashi, T.; Arisawa, M.; Suzuki, S.; Yoshizaki, M.; Morita, N.; Ferro, E.; Basualdo, I.; Berganza, L. H. Anti-inflammatory constituents of topically applied drugs III. Constituents and anti-inflammatory effect of Paraguayan crude drug "Tamanda cuna" (*Catasetum barbatum*, LINLE). *Chem. Pharm. Bull.* **1988a**, *36*, 4447–4452.

Shimizu, S.; Hattori, S.; Hata, H.; Yamada, H. A novel fungal enzyme NADPH-dependent carbonyl reductase showing high specificity to conjugated polyketones. *Eur. J. Biochem.* **1988b**, *174*, 37–44.

Turner, A. B. Quinone methides. *Q. Rev. Chem. Soc.* **1964**, *18*, 347–360.

Vaidyanathaswamy, R.; Parmar, B. S.; Attri, B. S.; Singh, R. P.; Mukerjee, S. K. Alpha alkyl and beta alkyl substituted cinnamates as pyrethrum synergists. *J. Agric Food Chem.* **1977**, *25*, 1401–1404.

Whittaker, R. H.; Feeny, P. P. Allelochemics: Chemical interactions between species. *Science* **1971**, *171*, 757–770.

Received for review February 9, 1993. Revised manuscript received June 8, 1993. Accepted August 17, 1993.®

® Abstract published in *Advance ACS Abstracts,* October 1, 1993.