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EVALUATION OF DIMBOA ANALOGS AS ANTIFEEDANTS AND ANTIBIOTICS TOWARDS THE APHID *Sitobion avenae* IN ARTIFICIAL DIETS

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Abstract—A total of 25 compounds including benzoxazinones, benzoxazolinones, and *N*-glyoxylamide derivatives were tested as antifeedants and antibiotics towards the aphid *Sitobion avenae* in diet bioassays. The antifeedant and mortality indexes increased with the presence of electron-donating groups in the 7 position of the benzoxazinone moiety, the replacement of the oxygen atom by sulfur in the heterocyclic ring, the presence of a hemiacetal instead of an acetal at C-2 of the benzoxazine moiety (and hence the possibility of ring opening), and the presence of a hydroxyl group at C-4 of the benzoxazine moiety (hydroxamic acid) instead of a hydrogen atom (lactam). The results support earlier hypotheses on the chemical bases for the mode of action of these compounds.

Key Words—Aphids, *Sitobion avenae*, feeding deterrents, antibiotics, DIMBOA.

INTRODUCTION

2-β-*O*-D-Glucopyranosides containing a 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one moiety (**I**, Figure 1) are found mainly in the family Poaceae (Niemeyer, 1988) and confer the plant resistance against bacteria, fungi, and insects, including aphids (Niemeyer and Pérez, 1995). The naturally present

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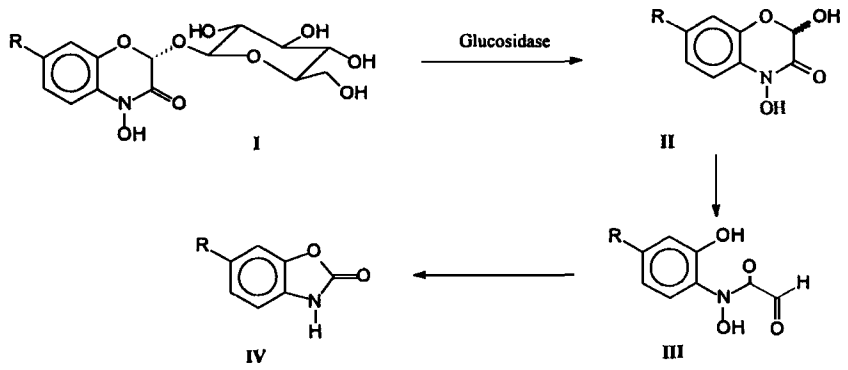


FIG. 1. **I:** 2-β-D-Glucopyranoside of a (2*R*)-2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one; **II:** a 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, the aglucone, liberated upon tissue damage by the action of a β-glucosidase. DIMBOA (R = OCH_3), TRIBOA (R = OH), DIBOA (R = H). **III:** the *N*-(2-hydroxyphenyl)-glyoxylo-hydroxamic acid produced in solution by the opening of the hemiacetal moiety; **IV:** the benzoxazolin-2(3*H*)-one produced as decomposition product of **II.**

glucosides are hydrolyzed by *endo*-β-glucosidases liberated following plant damage (Hofman and Hofmanova, 1969), producing the more toxic aglucones (**II**, Figure 1). In solution, these aglucones decompose through a mechanism involving the opening of the hemiacetal moiety to produce a *N*-(2-hydroxyphenyl)glyoxylohydroxamic acid (**III**, Figure 1), which further yields a benzoxazolin-2(3*H*)-one (**IV**, Figure 1) (Smissman et al., 1972; Bravo and Niemeyer, 1985). Recently the synthetic approaches, both to acetal glucosides and aglucones, have been reviewed (Sicker et al., 1997).

The main hydroxamic acid aglucone in wheat and maize extracts is 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), while in rye it is 2-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA). Trace amounts of 2,4,7-trihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (TRIBOA) are found in maize (Woodward et al., 1979).

The effect of DIMBOA on aphids has been studied in detail. An antibiotic effect has been described. Thus, inverse relationships were found between the DIMBOA level in maize and infestation by *Rhopalosiphum maidis* Fitch (Long et al., 1977); a similar situation was also reported for wheat and *Metopolophium dirhodum* Walk. (Argandoña et al., 1980), *Schizaphis graminum* Rond. (Argandoña et al., 1981), and *Sitobion avenae* Fabr. (Bohidar et al., 1986). When added to artificial diets, DIMBOA decreased survival, weight gain, and reproduction of several cereal aphid species (Argandoña et al., 1980; Niemeyer et al., 1989; Givovich and Niemeyer, 1995). On the other hand, an antifeeding

effect has also been described. Thus, negative relationships were found between the DIMBOA content of wheat plants and the number of aphids feeding on them, and their honeydew production (Niemeyer et al., 1989); in choice tests, aphids preferred to settle and took longer time to attain a sustained phloem ingestion on wheat plants with lower DIMBOA content (Nicol et al., 1992; Givovich and Niemeyer, 1991); when added to artificial diets, DIMBOA inhibited aphid feeding (Argandoña et al., 1983).

The present work evaluates the effect of structurally related 2*H*-1,4-benzoxazin-3(4*H*)-ones, their decomposition products, and some open chain analogs on the aphid *S. avenae*, in choice and antibiosis tests employing artificial diets.

METHODS AND MATERIALS

Aphids. To start the cultures individuals of *S. avenae* were collected randomly in grass fields near the Laboratorio de Química Ecológica in Santiago, Chile, and the multiclonal cultures were maintained on oat (*Avena sativa* L. cv. Nehuén) for at least five generations in a greenhouse at 18–22°C, 18L : 6D photoperiod, and 50–70% relative humidity. Individuals used in the bioassays were chosen randomly from the culture, subject to the conditions that they had recently molted to adults and were in an active food site-searching mood.

Compounds. Melting points were determined in a Boetius micro hot-stage apparatus and are corrected. The NMR spectra were recorded on a Varian Gemini 200 spectrometer at 199.975 MHz for ^{1}H and at 50.289 MHz for ^{13}C in either $CDCl_3$ or DMSO-d_6 as solvent and hexamethyldisiloxane as internal standard. The IR spectra were obtained on an ATT Mattson spectrometer in potassium bromide. Mass spectra were recorded on a Finnigan MAT 212 spectrometer (70 eV EI ionization, source temperature 200°C). 2,4-Dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**1**, DIMBOA), was isolated from *Zea mays* L. cv. T55s according to the protocol published by Hartenstein et al. (1992); and benzoxazolin-2(3*H*)-one (**18**) was of commercial grade (Aldrich). The following compounds were synthesized as reported: 2-methoxy-4-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**2**) (Sicker et al., 1994); 2,4-dihydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**3**) (Sicker et al., 1994); 2-methoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**4**) (Zahn, 1923); 2,4,7-trihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**5**) (Kluge et al., 1995); 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**6**) (Sicker and Hartenstein, 1993); 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**7**) (Sicker et al., 1989); 2*H*-1,4-benzothiazin-3(4*H*)-one (**8**) (Sicker et al., 1994); 2-methoxy-4,7-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**9**) (Kluge et al., 1995); 2,7-dimethoxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**10**) (Atkinson et al., 1991); 2-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**11**)

(Sicker and Hartenstein, 1993); 4-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**12**) (Sicker et al., 1994); 2*H*-1,4-benzoxazin-3(4*H*)-one (**13**) (Honkanen and Virtanen, 1960); 2-methoxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**14**) (Atkinson et al., 1991); 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**15**) (Hartenstein and Sicker, 1994); 2-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**16**) (Atkinson et al., 1991); 6-methoxy-benzoxazolin-3(3*H*)-one (**17**) (Sicker, 1989); and 6-hydroxy-benzoxazolin-2(3*H*)-one (**19**) (Wieland et al., 1999). Other compounds were synthesized as described below.

N-*(2′,4′-Dimethoxyphenyl)-2,2-diethoxyacetamide (**20**).* A mixture of 2,4-dimethoxyaniline (15.3 g; 0.1 mol), and 2,2-diethoxyethanoic acid (14.8 g; 0.1 mol) was refluxed in toluene for 4 hr in a Dean Stark apparatus. The solution was cooled, washed with water (2 × 250 ml), dried ($MgSO_4$), and evaporated under vacuum to give a colorless oil that crystallized in the freezer overnight to produce 22 g (77%) of **20** as colorless crystals (mp 36–37°C). IR: 1531, 1263 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.21 (t, 6H, 2 × CH_3, J = 7.2 Hz), 3.65 (q, 4H, 2 × CH_2), 3.76 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.95 (s, 1H, CH), 6.53 (dd, 1H, $H_{5'}$, $^4J_{5',3'}$ = 2.7 Hz, $^3J_{5',6'}$ = 8.8 Hz), 6.67 (d, 1H, $H_{3'}$, $^4J_{5',3'}$ = 2.7 Hz), 7.98 (d, 1H, $H_{6'}$, $^3J_{5',6'}$ = 8.8 Hz), 8.85 (s, 1H, NH); ^{13}C NMR (DMSO) δ: 15.3 (CH_3), 55.6 (OCH_3), 56.3 (OCH_3), 62.4 (OCH_2), 98.6 (CH), 99.1 (C-3′), 104.4 (C-5′), 119.9 (C-6′), 121.1 (C-1′), 150.3 (C-2′), 156.9 (C-4′), 165.3 (CO); MS: *m/z* 283 (M^+, 19), 238 (2), 209 (6), 152 (18), 103 (100).

N-*(2′,4′-Dimethoxyphenyl)glyoxylamide (**21**).* To a cooled (−25°C) solution of glyoxylic acid monohydrate (2.76 g; 0.03 mol) in THF (20 ml) was added a solution of dicyclohexylcarbodiimide (8.25 g; 0.04 mol) in THF (30 ml). When a white precipitate began to form, a cooled solution of 2,4-dimethoxyaniline (2.29 g; 0.015 mol) dissolved in THF (10 ml) was added portionwise. After 1 hr, the solid formed was filtered off and the organic fraction evaporated under vacuum. The product obtained was chromatographed (silicagel 60; toluene–ethyl acetate 2 : 1 v/v) to give 0.74 g (23.4%) of **21** as white solid (mp 212–214°C). IR: 1504, 1547 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 3.82 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.4 (dd, 1H, $H_{5'}$, $^3J_{5',6'}$ = 8.8 Hz, $^4J_{5',3'}$ = 2.7 Hz), 6.51 (d, 1H, $H_{3'}$, $^4J_{5',3'}$ = 2.7 Hz), 8.07 (d, 1H, $H_{6'}$, $^3J_{5',6'}$ = 8.8 Hz), 9.01 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 56.0 (OCH_3), 56.3 (OCH_3, 99.2 (C-3′), 104.4 (C-5′), 119.6 (C-1′), 121.3 (C-6′), 150.6 (C-2′), 157.5 (C-4′), 158.1 (CO), 189.1 (CHO); MS: *m/z* 209 (M^+, 100), 180 (37), 165 (24), 152 (89), 138 (61).

N-*(2′,4′-Dimethoxyphenyl)-2,2-dichloroacetamide (**22**).* A solution of 2,4-dimethoxyaniline (1.53 g; 0.01 mol) and 4-dimethylaminopyridine (1.50 g; 0.012 mol) in chloroform (50 ml) was cooled to 0°C by means of an ice-water bath. The solution was stirred and treated with 2,2-dichloroacetyl chloride (1.47 g; 0.01 mol), which was added in portions over 30 min, keeping the temperature between 2 and 5°C. The course of the reaction was monitored by TLC (toluene–ethyl acetate 1 : 2 v/v). When the reaction was complete, the solution was washed with

diluted HCl (2 × 60 ml). The organic layer was dried ($MgSO_4$) and evaporated under vacuum to give a white solid, which was recrystallized from cyclohexane to give 2.26 g (86%) of **22** as colorless crystals (mp 110–111°C). IR: 804, 836, 1036, 1124, 1554 cm^{-1}, ^1H NMR ($CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.04 (s, 1H, CH), 6.46–6.52 (m, 2H, $H_{3'}$ + $H_{5'}$), 8.17 (d, 1H, $H_{6'}$, $^3J_{5',6'}$ = 9 Hz), 8.69 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 56.0 (OCH_3), 56.4 (OCH_3), 67.5 ($CHCl_2$), 99.2 (C_3), 104.3 (C_5), 120.2 (C_6), 121.1 (C_1), 150.3 (C_2), 157.98 (C_4), 161.6 (CO); MS: *m/z* 263 (M^+, 73), 180 (34), 152 (100), 138 (14), 124 (24).

N-*(2',4'-Dimethoxyphenyl)-2-ethoxyglyoxylamide (23)*. A solution of 2,4-dimethoxyaniline (1.53 g; 0.01 mol) and 4-(dimethylamino)-pyridine (1.50 g; 0.012 mol) in chloroform (50 ml) was cooled to 0°C and under stirring was treated dropwise with ethyl oxalyl chloride (1.36 g; 0.01 mol) keeping the temperature between 2 and 5°C. After 4 hr, the solution was washed with diluted HCl (2 × 60 ml) and the organic layer dried ($MgSO_4$) and evaporated under vacuum to give a white solid, which was recrystallized from cyclohexane to give 1.83 g (72%) of **23** as colorless crystals (mp 145–147°C). IR: 830, 1266, 1542, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.42 (t, 3H, CH_3, $^3J_{1'',2''}$ = 7.2 Hz), 3.79 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.41 (q, 2H, CH_2, $^3J_{1'',2''}$ = 7.2 Hz), 6.49 (m, 2H, $H_{3'}$ + $H_{5'}$), 8.31 (d, 1H, $H_{6'}$ $^3J_{5',6'}$ = 9.6 Hz), 9.31 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 14.5 (CH_3), 56.0 (OCH_3), 56.3 (OCH_3), 63.8 (OCH_2), 99.2 (C-3), 104.3 (C-5), 120.3 (C-1), 121.2 (C-6), 150.3 (C-2), 153.8 (CO), 157.9 (C-4), 161.5 (CO); MS: *m/z* 253 (M^+, 78), 179 (100), 164 (21), 152 (66), 122 (61).

N-*(2',4'-Dimethoxyphenyl)-2-hydroxyacetamide (24)*. A mixture of 2,4-dimethoxyaniline (3.06 g; 0.02 mol), glycolic acid (1.52 g; 0.02 mol), and toluene (90 ml) was refluxed for 5 hr in a Dean Stark apparatus. The solution was cooled, and the white solid formed was filtered and recrystallized from cyclohexane to give 3.8 g (90%) of **24** as colorless crystals (mp 132–134°C). IR: 818, 1033, 1128, 1561, 3185 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.76 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.05 (s, 1H, OH), 6.52 (dd, 1H, $H_{5'}$, $^3J_{5',6'}$ = 8.8 Hz, $^4J_{3',5'}$ = 2.7 Hz), 6.67 (d, 1H, $H_{3'}$, $^4J_{3',5'}$ = 2.7 Hz), 8.10 (d, 1H, $H_{6'}$, $^3J_{5',6'}$ = 8.8 Hz), 8.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 55.6 (OCH_3), 56.2 (OCH_3), 61.9 (CH_2OH), 99.0 (C-3'), 104.4 (C-5'), 120.3 (C-6'), 120.5 (C-1'), 149.7 (C-2'), 156.4 (C-4'), 164.9 (CO); MS: *m/z* 211 (M^+, 56), 153 (64), 138 (46), 124 (16), 51 (100).

N-*(2',4'-Dimethoxyphenyl)-acetamide (25)*. A solution of 2,4-dihydroxyaniline (1.53 g; 0.01 mol) and 4-dimethylaminopyridine (1.50 g; 0.012 mol) in chloroform (50 ml) was cooled to 0°C by means of an ice-water bath. The solution was stirred and treated with acetic anhydride (1.22 g; 0.012 mol), which was added in portions over 30 min, keeping the temperature between 2 and 10°C. The course of the reaction was monitored by TLC (toluene–ethyl acetate 1 : 2 v/v. When the reaction was complete, the solution was washed with diluted HCl

(2 × 60 ml). The organic layer was dried ($MgSO_4$) and evaporated under vacuum to give a colorless solid, which was recrystallized from cyclohexane to give 0.8 g (41%) of **25** as colorless crystals (mp 115–116°C). IR: 800, 939, 1207, 1024, 1539 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.16 (s, 3H, $COCH_3$), 3.78 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.45 (m, 2H, $H_{3'} + H_{5'}$), 7.53 (s, 1H, NH), 8.22 (d, 1H, $H_{6'}$, $^3J_{5',6'} = 9.5$ Hz); ^{13}C NMR ($CDCl_3$) δ: 25.1 (CH_3), 55.9 (OCH_3), 56.1 (OCH_3), 99.0 (C-3), 104.2 (C-5), 121.3 (C-6), 121.8 (C-1), 149.7 (C-2), 156.9 (C-4), 168.4 (CO); MS: m/z 195 (M^+, 36), 153 (22), 138 (32), 122 (11), 42 (100).

Choice Tests. To determine the effect of the compounds on the feeding activity of aphids, 10 aphids were confined in a horizontally placed Plexiglas cylinder (3.0 cm long, 2.5 cm ID). To one end of the cylinder a sachet made of two Parafilm M membranes (American Can Co.) and containing 200 μL of a solution consisting of sucrose (25% w/v) and a small amount of dimethylsulfoxide (DMSO, 0.05% w/v) was attached. This side was named "control" (C). To the other end of the cylinder, a similar sachet containing the compound to be tested (0.5 mg/ml), was attached [treatment (T)]. The aphids were allowed to choose between the diets in a dark room maintained at 18–22°C and 50–70% relative humidity. After 18 hr, the number of feeding aphids in the treatment side (T) and the number of feeding aphids on the control side (C) were recorded. Feeding was judged by the immobility of the aphids and their antennae pointing backwards. An antifeedant index was defined as $(C - T)/(C + T)$ (Powell et al., 1997) in order to estimate the repellent or stimulant effects of the compounds on feeding activity. The antifeedant index may vary between 1 and −1, where 1 means maximum antifeedant activity; 0 no activity, and −1 maximum phagostimulant activity. The experiment was replicated 25 times for each compound tested.

Mortality Test. Ten aphids were confined in a vertically placed Plexiglas cylinder (3.0 cm high, 2.5 cm ID). One end of the cylinder was closed with a plastic net, the other with a sachet made of two Parafilm M membranes, containing 200 μl of a sucrose solution (25% w/v), a small amount of DMSO (0.05% w/v), and the compound to be tested at a concentration of 2 mM. Control cylinders were prepared in the same way but contained only 200 μl of sucrose solution (25% w/v) and DMSO (0.05% w/v). The cylinders containing the aphids were maintained at 18–22°C, 18L : 6D photoperiod and 50–70% relative humidity. After 89 hr, the number of dead aphids in the treatment and in the paired control cylinders were recorded. When aphid reproduction occurred, the progeny were not taken into account. A mortality index was defined as the mean of the number of dead aphids in the treatment minus that of the paired control. Twenty-five replicates were performed for each compound tested.

Statistical Analysis. Comparisons of activities within sets of chemically related compounds (the sets are defined in the Results section) were performed

with a nonparametric one-way ANOVA (Kruskal-Wallis test) followed by the Bonferroni correction. When significant differences between activities were found, a posteriori multiple comparisons were performed (Siegel and Castellan, 1988).

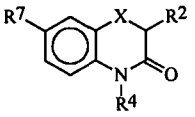
RESULTS

Bioassays. In the choice bioassay, the mean number of dead aphids at the end of the experiments was 0.078 ± 0.00006 (N = 625), and the mean number of aphids neither dead nor feeding on the diets at the end of the experiment was 2.14 ± 0.00025 (N = 625). In the antibiosis bioassay, the mean number of dead aphids in the control cylinder at the end of the experiments was 1.25 ± 0.0022 (N = 625), and the mean number of aphids neither dead nor feeding in the diet at the end of the experiment was 2.21 ± 0.0022 (N = 625) in the control cylinders and 2.16 ± 0.00045 (N = 625) in the treatment cylinders.

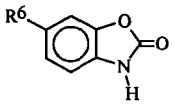
Activities. The antifeedant and the mortality indexes are tabulated as average values followed by their standard errors in Table 1. Five sets of compounds were defined that addressed different chemical questions through pairwise comparisons: (1) nature of the substituent at C-7 (pairs **7–5**, **7–1**, **14–9**, and **14–10**); (2) substitution of oxygen by sulfur at position 1 of the heterocyclic ring (pairs **7–3**, **13–8**, **14–2**, **15–12**, and **16–4**); (3) hydroxyl or methoxyl group at C-2, i.e., hemiacetal or acetal (pairs **9–5**, **10–1**, **14–7**, and **16–6**); (4) hydroxyl or hydrogen on nitrogen, i.e., hydroxamic acid or lactam (pairs **4–2**, **11–1**, **16–14**, **6–7**, **8–12**, and **13–15**); and (5) benzoxazinone- or benzoxazolinone-type compounds (pairs **1–17**, **5–19**, and **7–18**).

Within the benzoxazinone series, the antifeedant index consistently showed a tendency to increase (an asterisk denotes a significant difference i.e., $P < 0.05$) upon substitution with electron-donating groups at C-7 (**7** < **5**, **7** < **1***, **14** < **9***, and **14** < **10**), replacement of the heterocyclic oxygen atom by sulfur (**7** < **3***, **13** < **8***, **14** < **2***, **15** < **12***, and **16** < **4***), and the presence of a hydroxyl group instead of a methoxy group as substituent at C-2 (**9** < **5***, **10** < **1***, **14** < **7***, and **16** < **6***). The replacement of hydrogen by hydroxyl on the heterocyclic nitrogen atom tended to increase the antifeedant index in some cases (**4** < **2***, **11** < **1***, and **16** < **14**) and to decrease it in others (**6** > **7**, **8** > **12***, and **13** > **15**). The mortality index showed a tendency to increase upon substitution with electron-donating groups at C-7 (**7** < **1**, **14** < **9***, and **14** < **10**; however, **7** > **5***), replacement of the heterocyclic oxygen atom by sulfur (**7** < **3**, **13** < **8***, **14** < **2***, **15** < **12**, and **16** < **4***), and the presence of a hydroxyl group instead of a methoxy group as substituent as C-2 (**10** < **1***, **14** < **7***, and **16** < **6***; however, **9** > **5**). The replacement of hydrogen by hydroxyl on the heterocyclic nitrogen atom tended to increase the antifeedant index in some cases (**4** < **2***, **6** < **7***, **11** < **1***, and **16** < **14***) and to decrease it in others (**8** > **12*** and **13** > **15**).

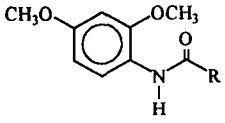
TABLE 1. ACTIVITY OF COMPOUNDS TESTED



Compound	X	R^2	R^4	R^7	Antifeedant index[a]	Mortality[a]
1	O	OH	OH	OCH_3	0.547 ± 0.027	5.52 ± 0.14
2	S	OCH_3	OH	H	0.462 ± 0.024	5.09 ± 0.11
3	S	OH	OH	H	0.418 ± 0.030	5.62 ± 0.09
4	S	OCH_3	H	H	0.375 ± 0.025	1.78 ± 0.20
5	O	OH	OH	OH	0.347 ± 0.020	1.04 ± 0.15
6	O	OH	H	H	0.294 ± 0.022	0.88 ± 0.12
7	O	OH	OH	H	0.271 ± 0.024	5.46 ± 0.17
8	S	H	H	H	0.232 ± 0.021	5.34 ± 0.15
9	O	OCH_3	OH	OH	0.204 ± 0.028	1.24 ± 0.16
10	O	OCH_3	OH	OCH_3	0.124 ± 0.032	0.52 ± 0.16
11	O	OH	H	OCH_3	0.114 ± 0.031	1.52 ± 0.16
12	S	H	OH	H	0.087 ± 0.028	0.28 ± 0.07
13	O	H	H	H	0.041 ± 0.024	0.72 ± 0.09
14	O	OCH_3	OH	H	0.018 ± 0.028	0.48 ± 0.10
15	O	H	OH	H	−0.035 ± 0.023	0.19 ± 0.14
16	O	OCH_3	H	H	−0.103 ± 0.030	0.03 ± 0.11



	R^6		
17	OCH_3	0.435 ± 0.018	0.25 ± 0.16
18	H	0.257 ± 0.023	0.48 ± 0.07
19	OH	0.136 ± 0.028	1.04 ± 0.10



	R		
20	$CH(OEt)_2$	0.497 ± 0.024	0.96 ± 0.13
21	CHO	0.451 ± 0.018	1.62 ± 0.22
22	$CHCl_2$	0.125 ± 0.027	0.63 ± 0.05
23	COOEt	−0.218 ± 0.024	0.57 ± 0.04
24	CH_2OH	−0.253 ± 0.026	0.62 ± 0.04
25	CH_3	−0.407 ± 0.028	0.34 ± 0.03

[a]Mean ± standard error.

Benzoxazinones tended to be more active than the respective benzoxazolinones to which they decompose [**1** > **17***, **5** > **19***, and **7** > **18**). Within the open-chain analogs, higher antifeedant indexes were associated with the presence of electronegative substituents at the amidic carbonyl group, the trend being less clear in the mortality indexes.

DISCUSSION

Bioassay conditions represent, in general, a stress to the test individuals. Particularly in the antibiosis test, which used a minimal diet and lasted 89 hr, aphids may have been significantly stressed. However, the low mean mortality observed in the control experiments indicated that this stress did not compromise the survival of the test aphids. Moreover, the low number of aphids found neither dead nor feeding and the similarity between those found in the test and control experiments validated the bioassays for comparative purposes.

A chemical mechanism to account for the biological activities of hydroxamic acids containing the 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one moiety has been proposed in which the electrophilic groups in **II** and the derived compound **III** (Figure 1) react and modify nucleophilic targets in metabolically relevant molecules in the biological environment (Niemeyer and Pérez, 1987). As bases for this proposal, hydroxamic acids of structure **II** react with thiols via a redox reaction involving the hydroxamic nitrogen atom and addition reactions involving the aldehydic carbonyl group of intermediate **III** (Pérez and Niemeyer, 1985) and with amines via the aldehydic and the amidic carbonyl groups of intermediate **III** (Pérez and Niemeyer, 1985) and with amines via the aldehydic and the amidic carbonyl groups of intermediate **III** (Pérez and Niemeyer, 1989b). They also inhibit the enzymes papain (Pérez and Niemeyer, 1989a), α-chymotrypsin (Cuevas et al., 1990), and aphid cholinesterases (Cuevas and Niemeyer, 1993) by reaction with nucleophilic residues in them.

The redox reaction with thiols is blocked when hydroxamic acids are transformed into the corresponding amides, and the formation of **III** is more favorable in the case of amides as compared with hydroxamic acids (Atkinson et al., 1991) or is completely blocked when the hemiacetal is transformed into an acetal. Furthermore, the reactivity of the amidic carbonyl group in **III** may be enhanced by electron-withdrawing substituents on it. In accordance with this chemical description, substitution of hydroxyl by methoxyl at C-2 decreased activity, some lactams were less active than the corresponding hydroxamic acids, and the presence of an α,β-dicarbonyl moiety or electron-withdrawing groups at the amidic carbonyl group in the open-chain compounds produced comparatively higher antifeedant and mortality indexes.

The finding that substitution of oxygen by sulfur at position 1 of the het-

erocyclic ring and the incorporation of electron-donating groups at C-7 of the benzoxazinones increased the activity of compounds in both bioassays can not be explained on the basis of the mechanism presented above. Chemical studies with compounds such as **II**, which lacked the hydroxyl group at C-2, showed that the hydroxamic hydroxyl group was transformed by acylation into a better leaving group, which, upon departure, formed a highly electrophilic nitrenium ion-type intermediate (Hashimoto et al., 1979, 1991; Hashimoto and Shudo, 1996; Ishizaki et al., 1992). This electrophile has been shown to further react with biologically relevant molecules possessing nucleophilic sites. Formation of the electrophilic intermediate should be favored by electron-donating groups. In accordance with this chemical description, substitution of hydrogen by either hydroxyl or methoxyl in the aromatic ring and also substitution of oxygen by sulfur in the heterocyclic ring increased the antifeedant and mortality indexes.

Two comparisons can not be rationalized in the chemical terms described above: compound **5** showed significantly higher indexes than compound **12**, in spite of being a lactam with no possibility of ring opening, and compound **7** showed significantly higher mortality than compound **5**, in spite of lacking an electron-donating substituent in the aromatic ring. It is likely that other chemical interactions with biological substrates, such as hydrophilic–hydrophobic interactions, predominate in these cases.

The results presented indicate that the antifeedant and antibiotic activities of hydroxamic acid derivatives can not be rationalized in terms of a single mechanism accounting for the chemical reactivity of the compounds tested. This is not surprising since, most likely, taste and susceptibility to the toxic effects of xenobiotics in an aphid are complex phenomena in which more than one cascade of events leads to the same final effect. Consequently, different compounds may show the same final effect, albeit by affecting different pathways or different steps in a given pathway, presumably by different chemical mechanisms, depending on the structural features of the xenobiotic at hand.

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