Insect Antifeedant Activity of Furochromones: Structure-Activity Relationships

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INSECT ANTIFEEDANT ACTIVITY OF FUROCHROMONES: STRUCTURE-ACTIVITY RELATIONSHIPS

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ABSTRACT.—Thirty chromone derivatives, both synthetic and natural, were assayed for feeding deterrent activity against *Spodoptera litura* larvae to establish structure-activity relationships. Among the compounds tested, furochromones with alkoxy substituents at C-4 or C-9 exhibited maximum feeding deterrent activity. Loss of activity was noticed with the degradation or saturation of either of the heterocyclic rings. Substitution of C-7 methyl of the γ-pyrone ring and dealkylation of C-4 or C-9 methoxyl also caused considerable reduction in feeding deterrent activity.

Recent researches in multi-disciplinary areas of chemical and biological sciences provide ample evidences for the defensive role of secondary plant metabolites (1). Production of feeding deterrents is one of the novel defense mechanisms that makes plants unpalatable to insect predators. The antifeedants are species-specific and exhibit wide variations in structure (2). In an earlier communication, we have reported the antifeedant activities of constituents of the plant *Atalantia racemosa* (Rutaceae) and structure-activity relationships of coumarins (γ-benzopyrones) (3). Our recent studies on *Pimpinella monoica* Dalz. (Umbelliferae) have resulted in the isolation of γ-benzopyrones, khellin [**14**] and visnagin [**19**], as active feeding deterrent principles (4). This prompted us to undertake a comprehensive study on structure-activity relationships among γ-benzopyrones. The feeding deterrent activity of thirty chromones (benzopyrones) and related compounds was evaluated against *Spodoptera litura* larvae, and the results are discussed in the present communication.

EXPERIMENTAL

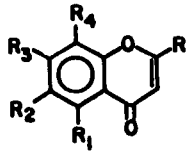
ANTIFEEDANT BIOASSAY.—*S. litura* larvae were raised on castor, *Ricinus communis*, leaves at 27° and 65–75% relative humidity. Freshly molted 4th instar larvae weighing 55–60 mg were selected from the stock culture, starved for 4 h, and used individually to assay antifeedant activity. Appropriate quantities of test compounds dissolved in Me_2CO were added to cellulose powder. The solvent was evaporated, and this cellulose powder was incorporated in the test diet (5). For feeding the control group of insects, the diet was prepared using cellulose powder treated with Me_2CO. The test compounds were evaluated for feeding inhibitory activity at a dose level of 100 ppm to establish structure-activity relationships. EC_{50} values were determined for compounds which caused more than 40% feeding inhibition at the above dose. Four concentrations (1000, 500, 100, and 50 ppm) were used for determining EC_{50} values. For each test concentration, 40 larvae were used. Food was provided ad libitum, and the larvae were allowed to feed for 48 h. The fecal pellets were collected, dried at 80° for 24 h, and weighed. Percent feeding deterrent activity was computed from $(C - T)/C \times 100$, where C is the average weight of fecal pellets produced by the control group of larvae and T is the average weight of fecal pellets produced by the larvae feeding on diet containing the test compound. The data were subjected to probit analyses for the computation of EC_{50} values (6).

TEST COMPOUNDS.—Chromones **1** and **2** and chromanones **5–7** were available in our laboratory (7). Furanochromones **14**, **19**, **23**, **25**, and **30** were isolated from *P. monoica* (4).

Khellinol [**15**] (8), 4,9-dimethoxy-7-(4′-methoxyphenyl)-furobenzopyran-5-one [**27**], and 4,9-dimethoxy-7-(3′,4′-dimethoxyphenyl)furobenzopyran-5-one [**28**] were prepared by the earlier described procedures (9). 4,9-Dimethoxy-7-(2′,4′-dimethoxyphenyl)furobenzopyran-5-one [**29**] was prepared by the directed condensation between compound **8** and 2′,4′-dimethoxybenzoyl chloride using lithium hexamethyldisilazide as base (10).

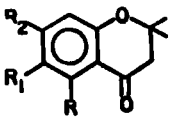
Visnaginol [**20**] (8) and khellindiol [**16**] (11) were prepared by BBr_3-demethylation of **19** and **14**. Khellinol ethyl ether [**17**] and khellindiol diethyl ether [**18**] were prepared by sono-mediated *O*-alkylation of **15** and **16**, respectively (3). Visnaginol ethyl ether [**21**] and khellol methyl ether [**24**] were prepared from **20** and **23** following the same procedure (3).

CHROMONES



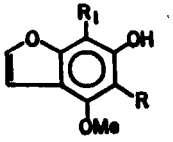
1 R=Me, R_2=R_3=OMe, R_1=R_4=H
2 R=COOH, R_2=R_3=OMe, R_1=R_4=H
3 R=Me, R_1=OMe, R_2=−CHO, R_3=OH, R_4=H
4 R=Me, R_1=R_4=OMe, R_2=−CHO, R_3=OH

CHROMANONES



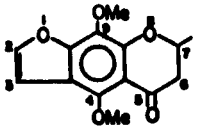
5 R=R_1=H, R_2=OMe
6 R=R_2=OH, R_1=H
7 R_1=OH, R=R_2=H

BENZOFURANS



8 R=Ac, R_1=OMe
9 R=−COOH, R_1=H
10 R=−COOH, R_1=OMe

HYDROGENATED PRODUCTS



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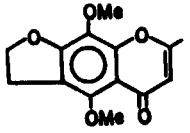

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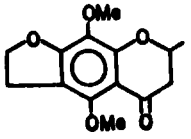
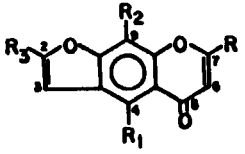


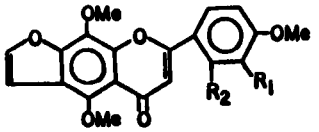
13

FURANOCHROMONES



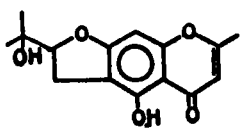
14 R=Me, R_1=R_2=OMe, R_3=H
15 R=Me, R_1=OH, R_2=OMe, R_3=H
16 R=Me, R_1=R_2=OH, R_3=H
17 R=Me, R_1=OEt, R_2=OMe, R_3=H
18 R=Me, R_1=R_2=OEt, R_3=H
19 R=Me, R_1=OMe, R_2=R_3=H
20 R=Me, R_1=OH, R_2=R_3=H
21 R=Me, R_1=OEt, R_2=R_3=H
22 R=Me, R_1=OMe, R_2=H, R_3=Ac
23 R=−CH_2OH, R_1=OMe, R_2=R_3=H
24 R=−CH_2OMe, R_1=OMe, R_2=R_3=H
25 R=−CH_2OH, R_1=R_2=OMe, R_3=H
26 R=−Me, R_1=R_2=OAc, R_3=H

FUROFLAVONES



27 R_1=R_2=H
28 R_1=OMe, R_2=H
29 R_1=H, R_2=OMe

α-HYDROXYISOPROPYL DIHYDROFURANOCHROMONE



30

2-Acetylvisnagin [**22**] was prepared by ultrasonic irradiation of a mixture of 18-Crown-6, K_2CO_3 and chloroacetone (10). The reaction mixture after usual workup gave **22** in 55% yield: mp 204–206°; uv λ max nm (log ε) (MeOH) 329 (4.00), 277 (4.42), 239 (4.24); ^{1}H nmr ($CDCl_3$) δ 2.36 (3H, s, Me), 2.63 (3H, s, Ac), 4.26 (3H, s, OMe), 6.06 (1H, s, H-6), 7.26 (1H, s, H-9 overlapping with $CDCl_3$), 7.76 (1H, s, H-3), ms *m/z* $[M]^+$ 272, 243, 228, 201, 187, 173.

Khellindiol diacetate [**26**] was prepared by acetylation of **16** with Ac_2O/pyridine (16 h, 30°) followed by usual workup, yielding **26** (10): mp 156–158°; ^{1}H nmr ($CDCl_3$) δ 2.33 (3H, s, Me), 2.5 (6H, s, 2 × OAc), 6.07 (1H, s, H-6), 6.90 (1H, d, $J = 2$ Hz, H-3), 7.70 (1H, d, $J = 2$ Hz, H-2).

6,7-Dihydrokhellin [**11**], 2,3-dihydrokhellin [**12**], and 2,3,6,7-tetrahydrokhellin [**13**] were prepared by the catalytic hydrogenation (PtO_2) of **14** and separation of the individual compounds by preparative tlc (10).

6-Formyl-5-methoxy-7-hydroxy-2-methylchromone [**3**] was prepared by $K_2Cr_2O_7$ oxidation of **19** (8). 6-Formyl-5,8-dimethoxy-7-hydroxy-2-methylchromone [**4**] was prepared by the RuO_4 ($NaIO_4$, $RuCl_3 \cdot H_2O$) oxidation of **14** (10). Compound **4**: mp 198–200° [lit. (12) mp 199–202°] uv λ max nm (log ε) 304 (3.70), 270 (4.43), 262 (4.44), 221 (4.81); ^{1}H nmr ($CDCl_3$) δ 2.40 (3H, s, Me), 4.00 (3H, s, OMe), 4.06 (3H, s, OMe), 6.06 (1H, s, H-3), 10.40 (1H, s, CHO), 12.26 (1H, s, OH).

6-Hydroxy-4-methoxybenzofuran-5-carboxylic acid [**9**] and 6-hydroxy-4,7-dimethoxybenzofuran-5-carboxylic acid [**10**] were prepared from **19** and **14** by oxidative alkaline degradation (H_2O_2, NaOH) (8).

Khellinone [**8**] was prepared by the method described by Spath and Gruber (13).

RESULTS AND DISCUSSION

Structures of various chromones and their analogues are shown. Feeding deterrency values for these compounds are presented in Table 1. Furanochromones khellin [**14**] and visnagin [**19**], which were the major feeding deterrents isolated from *Pimpinella monoica*, were selected as models for structural modifications. Alteration in all the parts of the molecule [furan (segment A), pyrone (segment B), and alkoxy (segment C)] were carried out (Figure 1). Feeding deterrent activity of these compounds was assessed to establish structure-activity relationships.

SEGMENT A: FURAN RING.—Among the compounds tested, **19** showed strong feeding deterrency (4). But its close analogue, 2-acetylvisnagin [**22**], did not show significant activity even at 1000 ppm. Therefore, it was concluded that substitution at the 2 position lowers the activity of **19**. Similar results were obtained in the case of the furanocoumarins (3). Saturation of the 2,3 double bond as in 2,3-dihydrokhellin [**12**] also diminishes the activity compared to that of **14**. This observation indicates that the presence of the 2,3 double bond is essential for imparting feeding deterrency. Absence or cleavage of the furan ring also results in a drastic reduction in activity, as revealed by the poor deterrency of chromones **1–4** and chromanones **5–7**. Visammiol [**30**], an α-hydroxyisopropyldihydrofuranochromone, and 2,3,6,7-tetrahydrokhellin [**13**] with both the heterocyclic rings reduced showed weak antifeedant activity.

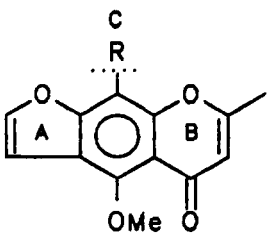
SEGMENT B: PYRONE RING.—Changes of the substituents at the pyrone ring (position 7) also caused a drastic reduction in activity. Thus, **25** and **23**, which contain a CH_2OH group at position 7 and are analogues of **19** and **14**, were significantly less active. The furanoflavones **27–29**, the aryl analogues of **14**, also did not show significant feeding deterrency.

Benzofurans **9** and **10**, obtained by the cleavage of the γ-pyrone rings of **14** and **19**, failed to deter feeding of the larvae. Saturation of the 6,7 double bond of the pyrones caused drastic reduction in activity. This is exemplified by the insignificant feeding deterrency of **11**. These observations suggest that the presence of an intact pyrone ring with methyl substitution at position 7 is essential for imparting high levels of feeding deterrent activity.

SEGMENT C: ALKOXY SEGMENT.—Alkoxy groups on the aromatic ring also play an important role in changing the bioactivity. Complete or partial dealkylation of **14** or

TABLE 1. Antifeedant Activity of Chromones and Their Analogues Tested Against *Spodoptera litura*.

Compound	% Feeding Inhibition at 100 ppm	EC_{50} in ppm
Chromones		
6,7-Dimethoxy-2-methylchromone [**1**]	10.4	—
6,7-Dimethoxychromone-2-carboxylic acid [**2**]	6.2	—
6-Formyl-5-methoxy-7-hydroxy-2-methylchromone [**3**]	3.8	—
6-Formyl-5,8-dimethoxy-7-hydroxy-2-methyl chromone [**4**]	5.4	—
Chromanones		
7-Methoxy-2,2-dimethyl-4-chromanone [**5**]	0	—
5,7-Dihydroxy-2,2-dimethyl-4-chromanone [**6**]	0	—
6-Hydroxy-2,2-dimethyl-4-chromanone [**7**]	0	—
Benzofurans		
Khellinone [**8**]	27.7	—
6-Hydroxy-4-methoxy-benzofuran-5-carboxylic acid [**9**]	15.2	—
6-Hydroxy-4,7-dimethoxy-benzofuran-5-carboxylic acid [**10**]	0	—
Reduced products		
6,7-Dihydrokhellin [**11**]	14.0	—
2,3-Dihydrokhellin [**12**]	26.2	—
2,3,6,7,-Tetrahydrokhellin [**13**]	13.1	—
Furanochromones		
Khellin [**14**]	44.9	107.9
Khellinol [**15**]	0	—
Khellindiol [**16**]	0	—
Khellinol ethyl ether [**17**]	65.1	49.8
Khellindiol diethyl ether [**18**]	74.8	28.1
Visnagin [**19**]	63.0	62.2
Visnaginol [**20**]	0	—
Visnaginol ethyl ether [**21**]	78.0	25.7
2-Acetyl visnagin [**22**]	8.6	—
Khellol [**23**]	12.12	—
Khellol methyl ether [**24**]	26.8	—
Ammiol [**25**]	22.7	—
Khellindiol diacetate [**26**]	0	—
Furanoflavones		
4,9-Dimethoxy-7-(4′-methoxyphenyl)-furobenzopyran-5-one [**27**]	12.0	—
4,9-Dimethoxy-7-(3′,4′-dimethoxyphenyl)-furobenzopyran-5-one [**28**]	21.1	—
4,9-Dimethoxy-7-(2′,4′-dimethoxyphenyl)-furobenzopyran-5-one [**29**]	7.3	—
Miscellaneous		
Visamminol [**30**]	14.1	—



14 R=OMe
19 R=H

FIGURE 1. Segments (A,B,C) of the furanochromone molecule subjected to structural alterations.

19 as in **15**, **16**, and **20** caused total loss of activity. On the other hand, ethyl ether **17** showed increased activity compared to the natural products.

Results obtained from the structure-activity correlation studies suggest that among furochromones the presence of an unsubstituted furan ring and an alkoxy substitution at positions C-4 and/or C-9 are essential for imparting feeding deterrent activity. Both the heterocyclic (furan and pyrone) rings are essential. Cleavage or saturation of either of the heterocyclic rings, as well as replacement of 7-Me by CH_2OH or aryl groups, diminishes the activity.

ACKNOWLEDGMENTS

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