Insect Antifeedants from *Atalantia racemosa*

Devanand L. Luthria, Vijayaraghavan Ramakrishnan, Girja S. Verma, Bharathi R. Prabhu, and Asoke Banerji*

Seven coumarin derivatives have been isolated from the *n*-hexane and methanol extracts of aerial parts of *Atalantia racemosa* Wight and Arn. (Rutaceae) and tested for antifeedant activity against *Spodoptera litura* F. larvae. Among these, xanthotoxin is the most active and luvangetin is moderately active, while xanthyletin and racemosin are active only at higher concentrations. Rutarin, rutaretin, and umbelliferone failed to cause feeding inhibition even at a concentration of 1000 ppm. Antifeedant activities of 17 other structurally related coumarins have also been studied, to establish structure–activity relations.

Problems connected with excessive use of synthetic insecticides have stimulated the search for alternate methods for protection of crops from insect damage. In recent years greater emphasis has been laid on integrated pest management programs using behavior-modifying chemicals (Renwick and Radke, 1985). Antifeedants cause cessation of feeding by modifying gustatory behavior of insects (Warthen, 1979). Reports of isolation of insect antifeedant compounds from natural sources (Nakanishi, 1981; Van Beek and De Groot, 1986) have prompted us to screen Indian plants for insect feeding inhibitory substances. This report deals with the antifeedant activity of coumarin derivatives isolated from the plant *Atalantia racemosa* Wight and Arn. (N.O. Rutaceae). Feeding deterrency of several related coumarins has also been studied in order to establish structure–activity relations.

MATERIALS AND METHODS

Instruments and Conditions. Melting points were determined on Fisher-Johns melting point apparatus and are uncorrected. UV spectra were recorded in MeOH on a Shimadzu spectrophotometer (Model 240S). IR spectra were recorded on Perkin-Elmer spectrophotometer (Model 783). Mass spectral (EIMS, direct insertion) studies were carried out on a VG Micromass 7070F spectrometer at 70 eV. ^{1}H NMR spectra were recorded on Varian EM-360 (60-MHz) spectrometer. A laboratory ultrasonic cleaner (40 kHz) was used for ultrasonic irradiation.

Isolation and Identification of Feeding Inhibitors from *A. racemosa*. Stems and leaves (1.4 kg) of the plant *A. racemosa*, collected from Mahabaleshwar (Maharashtra, India), were air-dried, powdered, and extracted successively (Soxhlet) with *n*-hexane and methanol. The solvents were removed under reduced pressure, and the crude extracts were fractionated by column chromatography over silica gel and eluted with mixtures of solvents with increasing polarities (*n*-hexane, $CHCl_3$, EtOAc, MeOH). The procedure for isolation and purification of the chemical constituents is presented in Figure 1. The structures of the isolated compounds and their derivatives are shown in Figure 2.

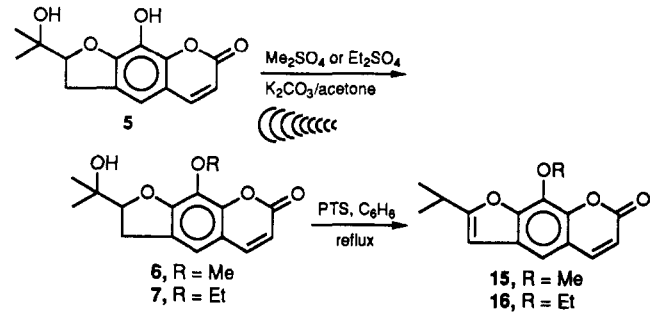
From the *n*-hexane extract four compounds were isolated and identified as xanthyletin (1) (Murray, 1978), racemosin (3) (Joshi et al., 1978), luvangetin (2) (Murray, 1978), and xanthotoxin (11) (Murray, 1978). Seven compounds, four (1–3, 11) of which were present in *n*-hexane extract, were also isolated from methanol extract. An additional three compounds were identified as umbelliferone (17) (Murray, 1978), rutaretin (5) (Schneider, 1967), and rutarin (4) (Murray, 1978). Identities of all isolated compounds were confirmed by chemical and physical (IR, UV, ^{1}H NMR, MS, melting point) methods and are in agreement with those reported in the literature.

Preparation of Derivatives. *Xanthotoxol (12).* Two methods were used for the preparation of compound 12:

Method A. It was prepared in 50% yield by the method described by Schonberg and Aziz (1953) using aniline hydrochloride.

Chemical Ecology Section, Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

Scheme I. Preparation of 2-Isopropylxanthotoxol Derivatives



Method B. To a cold solution of xanthotoxin (11) (88 mg) in methylene chloride (3 mL) at −30 °C was added BBr_3 (0.2 mL). After being stirred at room temperature for 16 h, the reaction mixture was poured into ice. Xanthotoxol (12), which separated out, was filtered. Evaporation of the methylene chloride layer gave additional quantity of xanthotoxol (12) that was mixed with the earlier lot and crystallized from acetone: yield 66 mg (80%); mp 252–253 °C (lit. mp 251–252 °C; Livingstone, 1977).

Xanthotoxol Acetate (13). Acetylation of xanthotoxol (12) with Ac_2O/pyridine (16 h, 30 °C) followed by the usual workup furnished compound 13: mp 176–179 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3, $COCH_3$), 6.38 (d, 1, J = 10 Hz, H-6), 7.80 (d, 1, J = 10 Hz, H-5), 7.56 (s, 1, H-4), 6.83 and 7.70 (furan ring protons).

Xanthotoxol Ethyl Ether (14). A mixture of compound 12 (60 mg), K_2CO_3 (500 mg), and diethyl sulfate (0.2 mL) in acetone (10 mL) was irradiated with ultrasound at room temperature until the reaction was complete (monitored by TLC, 3 h). The reaction mixture was worked up in the usual way. Compound 14 was purified by preparative TLC followed by crystallization (acetone/*n*-hexane): yield 48 mg (71%); mp 104–106 °C (lit. mp 104–106 °C; Banerjee et al., 1982).

Rutaretin 9-Methyl and 9-Ethyl Ethers (6 and 7). These compounds were prepared by sonochemical O-alkylation of rutaretin (5) with dimethyl sulfate/diethyl sulfate as described above (Scheme I). Rutaretin 9-methyl ether (6): yield 72%; mp 132 °C (lit. mp 132–133 °C; Schneider et al., 1967). Rutaretin 9-ethyl ether (7): yield 75%; crystallized from EtOAc/*n*-hexane as colorless needles; mp 102–104 °C; IR, ν_{max} (KBr pellet) 3470, 2980, 1710, 1620, 1585, 1485, 1430, 1290, 1150, 1090, 1085, 1015 cm^{-1}; UV, λ_{max} (methanol) 252 nm (ε 4557), 262 (ε 4842), 334 (ε 16145); ^{1}H NMR ($CDCl_3$) δ 1.24 and 1.37 (2 s, 6, $C(CH_3)_2$), 1.40 (t, 3, OCH_2CH_3), 1.97 (s, 1, O*H*), 3.23 (d, 2, J = 9 Hz, C-3 CH_2), 4.30 (q, 2, J = 7 Hz, OCH_2CH_3), 4.76 (t, 1, J = 7 Hz, C-2 C*H*), 6.18 (d, 1, J = 10 Hz, H-6), 6.94 (s, 1, H-4), 7.57 (d, 1, J = 10 Hz, H-5); MS (70 eV) m/e 290 (M^+), 275, 273, 257, 232, 230, 219, 204, 203, 59. Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.18; H, 6.25. Found: C, 66.32; H, 6.31.

2-Isopropylxanthotoxin (15). A mixture of compound 6 (60 mg) in benzene (5 mL) and *p*-toluenesulfonic acid (60 mg) was refluxed. The completion of the reaction was monitored by TLC (4 h). The reaction mixture was successively washed with aqueous $NaHCO_3$, water, and brine. The dried (Na_2SO_4) organic layer on removal of solvent gave crude compound 15, which was purified by preparative TLC ($CHCl_3$, double run) followed by crystalli-

0021-8561/89/1437-1435$01.50/0

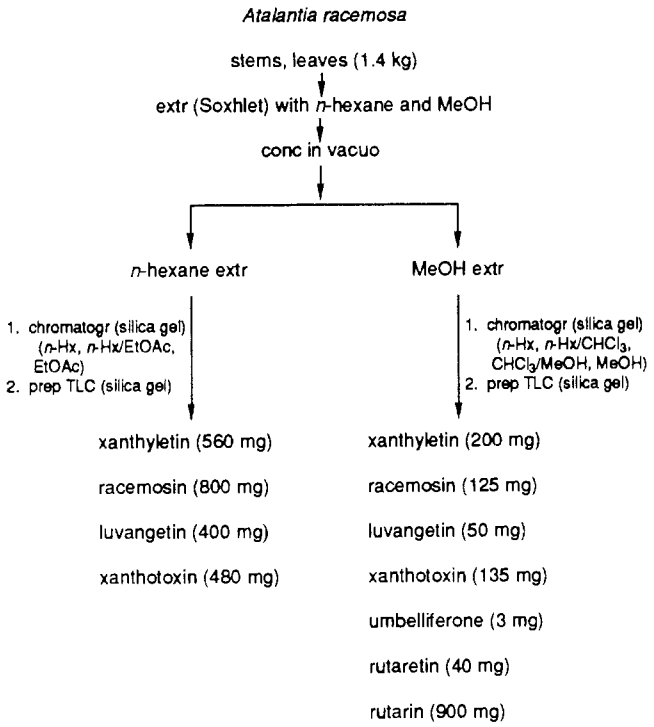


Figure 1. Isolation of feeding inhibitors from *A. racemosa*.

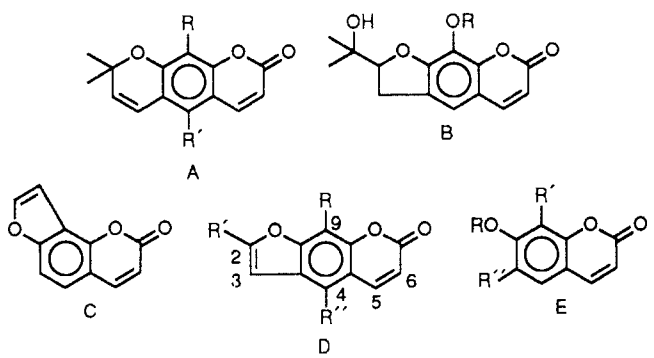


Figure 2. Structures of compounds tested for antifeedant activity against *S. litura*. A, pyranocoumarins: xanthyletin (1) R = R′ = H; luvangetin (2) R = OMe, R′ = H; racemosin (3) R = R′ = OMe. B, 2-(α-hydroxyisopropyl)dihydrofuranocoumarins: rutarin (4) R = glucosyl; rutaretin (5) R = H; rutaretin 9-methyl ether (6) R = Me; rutaretin 9-ethyl ether (7) R = Et. C, furanocoumarin: angelicin (8). D, furanocoumarins: psoralen (9) R = R′ = R″ = H; isopimpinellin (10) R = R″ = OMe, R′ = H; xanthotoxin (11) R = OMe, R′ = R″ = H; xanthotoxol (12) R = OH, R′ = R″ = H; xanthotoxol acetate (13) R = OAc, R′ = R″ = H; xanthotoxol ethyl ether (14) R = OEt, R′ = R″ = H; 2-isopropylxanthotoxin (15) R = OMe, R′ = *i*-Pr, R″ = H; 2-isopropylxanthotoxol ethyl ether (16) R = OEt, R′ = *i*-Pr, R″ = H. E, coumarins: umbelliferone (17) R = R′ = R″ = H; umbelliferone methyl ether (18) R = Me, R′ = R″ = H; umbelliferone ethyl ether (19) R = Et, R′ = R″ = H; umbelliferone 7-apioglucoside (20) R = apioglucosyl, R′ = R″ = H; daphnetin (21) R = R″ = H, R′ = OH; daphnetin 7-methyl ether (22) R = Me, R′ = OH, R″ = H; hydrangetin (23) R = R″ = H, R′ = OMe; scopoletin 7-apioglucoside (24) R = apioglucosyl, R′ = H, R″ = OMe.

zation (ether/*n*-pentane): yield 35 mg (75%); mp 90–91 °C (lit. mp 90–91 °C; Schneider et al., 1967).

2-Isopropylxanthotoxol Ethyl Ether (16). It was prepared by dehydration of compound **7** by the same method as described above: yield 71%; mp 65–66 °C; IR, ν_{max} (KBr pellet) 2980, 1735, 1715, 1630, 1605, 1590, 1410, 1400, 1320, 1215, 1185, 1160, 1110, 1090 cm^{-1}; UV, λ_{max} (methanol) 252 nm (ε 31 717), 302 (ε 12 926), 348 (ε 3658); ^1H NMR (CCl_4) δ 1.33 and 1.45 (d, 6, J = 7 Hz, $CH(CH_3)_2$), 1.50 (t, 3, J = 7 Hz, OCH_2CH_3), 3.10 (m, 1, $CH(CH_3)_2$), 4.47 (q, 2, J = 7 Hz, OCH_2CH_3), 6.16 (d, 1, J = 10 Hz, H-6), 6.30 (br s, 1, C-3 C*H*), 7.06 (s, 1, H-4), 7.60 (d, 1, J = 10 Hz, H-5); MS, (70 eV) m/e 272 (M^+). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.50; H, 5.93. Found: C, 70.52; H, 5.91.

Table I. Feeding Inhibitory Activity of the Compounds Isolated from *A. racemosa* and the Related Coumarins against *S. litura*

compd	EC_{50}, ppm
Pyranocoumarins	
xanthyletin (1)	870
luvangetin (2)	220
racemosin (3)	778
Coumarins	
umbelliferone (17)	**[a]
umbelliferone methyl ether (18)	**
umbelliferone ethyl ether (19)	**
umbelliferone 7-apioglucoside (20)	**
daphnetin (21)	**
hydrangetin (22)	**
daphnetin 7-methyl ether (23)	**
scopoletin 7-apioglucoside (24)	**
2-(α-Hydroxyisopropyl)dihydrofuranocoumarins	
rutarin (4)	**
rutaretin (5)	**
rutaretin 9-methyl ether (6)	**
rutaretin 9-ethyl ether (7)	**
Furanocoumarins	
angelicin (8)	616
psoralen (9)	170
isopimpinellin (10)	5
xanthotoxin (11)	31
xanthotoxol (12)	**
xanthotoxol acetate (13)	**
xanthotoxol ethyl ether (14)	23
2-isopropylxanthotoxin (15)	**
2-isopropylxanthotoxol ethyl ether (16)	**

[a] No feeding inhibition at 1000 ppm.

Besides the above compounds, the coumarins angelicin (8), psoralen (9), umbelliferone methyl ether (18), umbelliferone ethyl ether (19) (available in our laboratory), daphnetin (21), daphnetin 7-methyl ether (22), and hydrangetin (23) (supplied by Prof. M. R. Parthasarathy, Delhi University, Delhi); two coumarin glycosides umbelliferone 7-apioglucoside (20) and scopoletin 7-apioglucoside (24) (supplied by Prof. P. S. Rao, Kakatiya University, Warangal); and isopimpinellin (10) isolated from *Pimpinella monoica* (Banerji et al., 1988) were also included in our investigation for studying the structure–activity relations among various coumarin derivatives.

Bioassay. Tobacco caterpillar, *Spodoptera litura F.*, larvae were raised on *Ricinus communis* L. leaves at 27 °C and 65–70% RH. Freshly moulted fourth-instar larvae weighing 60–65 mg were selected from the stock culture, starved for 4 h, and used individually for the assay of antifeedant activity. Crude extracts (1000 ppm) and synthetic/isolated compounds (1–1000 ppm) were incorporated in cellulose powder and used for the preparation of test diet (Verma et al., 1986). For each test concentration, 40 larvae were used. Control diet contained appropriate solvent-treated cellulose powder. Food was provided ad libitum. The fecal pellets were collected after 48 h, dried at 80 °C, and weighed. Percent feeding inhibition was computed from $(C - T)/C \times 100$, where C = the average weight of fecal pellets in control group and T = the average weight of fecal pellets in treated group. The data of percent inhibition of feeding were subjected to Probit analysis (Busvine, 1957) for computation of EC_{50} values.

RESULTS AND DISCUSSION

Antifeedant Activity of Coumarins Isolated from *A. racemosa*. The antifeedant activities of various coumarins are expressed as EC_{50} values in Table I. Of the compounds isolated from *A. racemosa*, furanocoumarin xanthotoxin (11) showed the highest antifeedant activity. This compound was also found to be the antifeedant principle of *Orixa japonica* (Yajma et al., 1977). However, variation in the reported EC_{50} value with that of the

present study could be due to differences in the method of assays employed. 2-(α-Hydroxyisopropyl)dihydrofuranocoumarins rutaretin (5) and its glucoside rutarin (4) were not active. Umbelliferone (17), a biosynthetic precursor of coumarins isolated from *A. racemosa* also lacked feeding inhibitory activity. Among pyranocoumarins, luvangetin (2) showed moderate activity while xanthyletin (1) and racemosin (3) were active only at higher concentrations. Xanthyletin (1) is reported to be an antifeedant principle of the plant *Boenninghausenia albiflora* Reichard (Hosozawa et al., 1974). However, the feeding inhibitory activity of luvangetin (2) and racemosin (3) has not been reported in the literature.

Structure–Activity Relationship. The varying degrees of antifeedant activity exhibited by the coumarins isolated from *A. racemosa* prompted us to evaluate the activity of other structurally related coumarins to get insight into structure–activity relationships. Since xanthotoxin (11) showed the highest antifeedant activity, several xanthotoxin derivatives and other structurally related compounds were tested to identify structural features contributing to antifeedant activity. Among xanthotoxin derivatives, xanthotoxol ethyl ether (14) showed highest feeding inhibition. Demethylated products of xanthotoxin, xanthotoxol (12) and its acetate (13), totally lacked feeding inhibitory activity. Methyl and ethyl ethers of rutaretin (6 and 7), which are 2-(α-hydroxyisopropyl)dihydrofurano analogues of xanthotoxin, were found to be inactive. 2-Isopropylxanthotoxin (15) and its ethyl analogue 16 also failed to exhibit any feeding deterrency. Psoralen (9), which like xanthotoxin (11) has a linearly fused furan ring but lacks a methoxyl group, is moderately active but its isomer angelicin (8), with an angularly fused furan ring, shows severalfold reduction in biological activity. Compared to xanthotoxin (11), isopimpinellin (10), which has an additional methoxyl group at the 4-position, showed a 6-fold increase in bioactivity. It is interesting to observe that luvangetin (2), racemosin (3), and xanthyletin (1), which are corresponding pyrano analogues of xanthotoxin (11), isopimpinellin (10), and psoralen (9), respectively, have much less feeding deterrency. Substituted coumarins (17–24) without furano and pyrano moieties did not show any significant activity.

Several conclusions may be drawn from the studies presented above. A linearly fused furan ring along with alkoxy groups at positions 9 and 4 play a critical role in imparting antifeedant activity to xanthotoxin (11), its ethyl analogue (14), and isopimpinellin (10). 2-Isopropylxanthotoxin (15) and its ethyl analogue (16) also did not exhibit significant feeding deterrency, suggesting that a substituent in the furan ring also causes a loss in antifeedant activity.

ACKNOWLEDGMENT

Financial assistance from the Department of Science and Technology, Government of India, is gratefully acknowledged. D.L.L. is grateful to the Department of Atomic Energy, Government of India, for the award of a Jr. Research Fellowship.

Registry No. 1, 553-19-5; 2, 483-92-1; 3, 68421-13-6; 4, 20320-81-4; 5, 13895-92-6; 6, 121498-42-8; 7, 121498-43-9; 8, 523-50-2; 9, 66-97-7; 10, 482-27-9; 11, 298-81-7; 12, 2009-24-7; 13, 10386-19-3; 14, 83934-65-0; 15, 121498-44-0; 16, 121498-45-1; 17, 93-35-6; 18, 531-59-9; 19, 31005-02-4; 21, 486-35-1; 22, 19492-03-6; 23, 485-90-5; 24, 121569-48-0.

LITERATURE CITED

Banerjee, S. K.; Gupta, B. D.; Singh, K. J. A simple one-step synthesis of phenyl ethers from phenyl acetate. *J. Chem. Soc., Chem. Commun.* **1982**, 815–816.

Banerji, A.; Luthria, D. L.; Ramakrishnan, V. Bio-active compounds from *Pimpinella monoica*. *Abstracts of Papers*, VIIIth Annual Conference of Indian Council of Chemists, S.V. University, Tirupati, India, 1988; 00–71.

Busvine, J. R. Toxicological Statistics. In *A critical review of the techniques for testing insecticide*; Commonwealth Institute of Entomology: London, 1957; pp 174–178.

Hosozawa, S.; Kato, N.; Munakata, K.; Chen, Y. L. Antifeedant active substance for insect in plant. *Agric. Biol. Chem.* **1974**, *38*, 1045–1048.

Joshi, B. S.; Gawad, D. H.; Ravindranath, K. R. Chemical constituents of *Atalantia racemosa* Wt. and Arn. Structure and synthesis of racemosin, a novel pyranocoumarin. *Proc. Indian Acad. Sci.* **1978**, *87A*, 173–179.

Livingstone, R. Benzo[b]pyrans (5, 6-benzopyrans, chromenes) and their derivatives. In *Rodd's chemistry of carbon compounds*; Coffey, S., Ed.; Elsevier Publishing: Amsterdam, 1977; Vol. IVE, p 128.

Murray, R. D. H. Naturally occurring plant coumarins. *Prog. Chem. Org. Nat. Prod.* **1978**, *35*, 199–429.

Nakanishi, K. Insect antifeedants from plants. In *Insect biology in future*; Locke, M., Smith, D. S., Eds.; Academic: New York, 1981; pp 603–611.

Renwick, J. A. A.; Radke, C. D. Constituents of host and non-host plants deterring oviposition by the cabbage butterfly, *Pieris rape*. *Entomol. Exp. Appl.* **1985**, *39*, 21–26.

Schneider, G.; Muller, H.; Pfaender, P. Rutaretin, ein neues furocoumarin aus *Ruta graveolens* L. *Arch. Pharm.* **1967**, *300*, 73–81.

Schonberg, A.; Aziz, G. Furochromones and coumarins VI. Demethylation of xanthotoxin, khellin and khellol with aniline hydrochloride and magnesium iodide. *J. Am. Chem. Soc.* **1953**, *75*, 3265–3266.

Van Beek, T. A.; De Groot, A. Terpeniod antifeedants Part I An over review of terpenoid antifeedants of natural origin. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 513–527.

Verma, G. S.; Ramakrishnan, V.; Mulchandani, N. B.; Chadha, M. S. Insect feeding deterrents from the medicinal plant *Tylophora asthmatica*. *Entomol. Exp. Appl.* **1986**, *40*, 99–101.

Warthen, J. D., Jr. *Azadirachta indica*, a source of insect feeding inhibitors and growth regulators. USDA Review Manual; ARM-NE4; U.S. GPO: Washington, DC, 1979; pp 1–21.

Yajma, T.; Kato, N.; Munakata, K. Phloroglucinol type furocoumarins, a group of potent naturally occurring insect antifeedants. *Agric. Biol. Chem.* **1977**, *41*, 1263–1268.

Received for review October 31, 1988. Accepted March 17, 1989.