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Antifeedants in Cyperaceae: coumaran and quinones from *Cyperus* spp.

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Abstract

Three antifeedants were isolated from the basal stem of *Cyperus nipponicus* and *C. distans*. One was identified by spectral analysis as the coumaran, remirol, and the others as the furoquinones, cyperaquinone and scabequinone. The significance of the above compounds in the chemical defense of weeds is discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Cyperus nipponicus*; Cyperaceae; Antifeedant; Coumarans; Quinones; Remirol; Cyperaquinone; *Spodoptera litura*

Introduction

The genus *Cyperus* includes common weeds found in upland and paddy fields in temperate to tropical regions. In Asian countries, *C. rotundus* and *C. scariosus* are used as traditional folk medicines. Cyperaceae are not affected by pests in upland and paddy fields. In studies with Cyperaceae, hexane extracts or essential oils show allelopathic properties and several natural products, viz. coumarins (Dini, Ramundo, Saturnino, Scimone, & Stagno d'Alcontres, 1992; Dini, Ramundo, Saturnino, Scimone, & Stagno d'Alcontres, 1993), quinones (Bureau, Fournet, & Bruneton, 1985) and sesquiterpenes, have been identified. The sesquiterpenic ketone, α-cyperone, a constituent of purple nutsedge (*C. rotundus*), shows insecticidal activity against diamondback moth (DBM) larvae (Dadang, Ohsawa, Kato, & Yamamoto, 1996). We suggest that Cyperaceae are seldom damaged by phytophagous insects, because they contain insect antifeedants. Our investigation has revealed insect antifeedant activity in hexane extracts from Cyperaceae against the tobacco cutworm (*Spodoptera litura*). In this paper, the identification and structural determination of these antifeedants is reported.

Results and discussion

Crude hexane extracts of most Cyperaceae from Japan exhibit antifeedant activities against the tobacco cutworm (Table 1). Plants of the genus *Cyperus* have been investigated extensively and are reported to contain coumarins and novel quinones (Dini et al., 1993). These quinones have a bi-furan moiety (Allan, Correll, & Wells, 1969). We separated and identified these compounds as insect antifeedants (Fig. 1). We also investigated the hexane extracts because they showed the same bioactivity as the ether extracts. The methanol extracts lacked antifeedant activity and the hexane extracts generally were not partially converted into artefacts through solvolysis.

Crude hexane extracts of Cyperaceae from Thailand are similarly bioactive (Table 2). Species with antifeedant activity were *C. nipponicus*, *C. distans*, *C. flavidus*, *C. serotinus*, *C. pilosus*, *C. orthostachyus* and *C. brevifolius* var. *leiolepis*.

The hexane extract from the basal stem of *C. nipponicus* afforded large amounts of the carmine pigment, cyperaquinone (**1**), and its precursor remirol (**2**) (Allan, Correll, & Wells, 1969), whereas the hexane extract from the subterranean parts of *C. distans* afforded considerable amounts of the yellow pigment, scabequinone (**3**) to about 7×10^{-3}% of its fresh weight. Analysis of the spectral data for the above compounds allowed us

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Table 1
List of hexane-extracted Cyperaceae species from Japan and AFI against tobacco cut-worm for the first screening at 1 mg/leaf-disc. Results are presented as means ± S.E. (where appropriate), $N=3$. AFI = antifeedant index

Species	Plant part	AFI ± S.E.
C. amuricus	whole plant	34.6 ± 5.9
C. brevifolis var. *leiopis*	leaves and stem	29.4 ± 16.8
	root and basal stem	16.7 ± 8.3
C. compressus	root and basal stem	40.9 ± 5.2
C. cyperoides	whole plant	35.3 ± 9.1
C. difformis	whole plant	27.9 ± 4.9
C. flavidus	leaves and stem	18.6 ± 3.5
	root and basal stem	23.3 ± 2.6
C. haspan	leaves and stem	32.6 ± 7.9
	root and basal stem	28.8 ± 10.6
C. iria	whole	22.7 ± 4.4
C. mircroiria	leaves and stem	34.4 ± 3.8
	root and basal stem	39.8 ± 12.7
C. monoplyllus	whole plant	24.9 ± 8.3
C. nipponicus	leaves and stems	26.1 ± 4.7
	root and basal stem	23.2 ± 2.6
C. odoratus	whole plant	22.1 ± 3.8
C. orthostachyus	leaves and stem	15.8 ± 7.0
	root and basal stem	19.0 ± 8.3
C. pilosus	leaves and stem	22.7 ± 5.4
	root and base stems	30.4 ± 11.4
C. rotundus	tuber (folk medicine)	24.3 ± 3.6
C. sanguinolentus	leaves and stem	41.7 ± 0.9
	root and basal stem	35.1 ± 9.2
C. serotinus	leaves and stem	29.7 ± 5.9
	root and basal stem	23.5 ± 7.4

to assign structures **1**–**3**. The absolute configuration of **3** at C-6 was elucidated to be *R* as compared with the rotation of the lactone, obtained by exhaustive ozonolysis of (+) isopropyl succinic acid (Allan, Dunlop, Kendall, Wells, & MacLeod, 1973). Similarily, we investigated the absolute configuration of **2**, which was also as *R*.

The TLC bioassay of antifeedant activity for *C. nipponicus* extract demonstrated a silica gel residual area with an R_f value around 0.3–0.4 on the TLC plate in

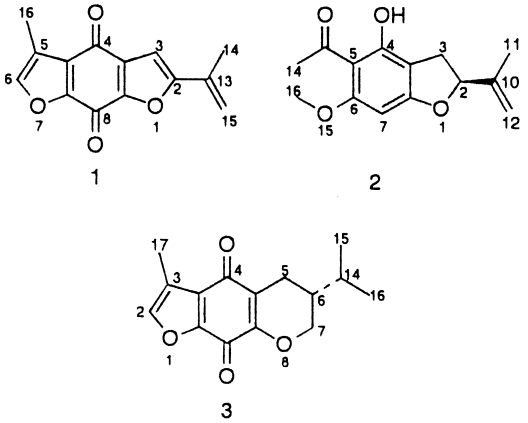

Fig. 1. Structure of antifeedants in Cyperaceae.

Table 2
List of hexane-extracted Cyperaceae species from Thailand and AFI against tobacco cut-worm for the first screening at 1 mg/leaf-disc. Results are presented as means ± S.E. (where appropriate), $N=3$. AFI = antifeedant index

Species	Plant part	AFI ± S.E.
C. cyperinus	whole plant	53.3 ± 8.1
C. diffusus	whole plant	34.7 ± 1.7
C. distans	leaves and stem	26.0 ± 8.9
	root	24.4 ± 10.8
C. iria	whole plant	31.6 ± 9.3
C. javanicus	whole plant	28.9 ± 1.7
C. nutans	whole plant	50.9 ± 4.1
C. pulcherrimus	whole plant	42.0 ± 0.8
C. stoloniferus	whole plant	25.2 ± 5.3
C. trialatus	whole plant	47.2 ± 6.6

system 1 (hexane–ethylacetate 10:1) (Escoubas, Fukushi, Lajide, & Mizutani, 1992). This region was scraped off and extracted with ethyl acetate, then again subjected to TLC analysis in system 1, thereby revealing the presence of **1** and **2**. The crude hexane extracts from *C. nipponicus* were evaporated to dryness and subjected to silica gel column chromatography. The insect antifeedant activity of the leaf-disc bioassay fraction was eluted as either a red or yellow pigment from column in system 1.

Three compounds in Cyperaceae showed various levels of antifeedant activity (Table 3), with the strongest activity being exhibited by scabequinone in *C. distans*.

Interestingly, these compounds have low phytotoxicity and medium levels of fish toxicity, but dihydrocyperaquinone, a cyperaquinone derivative, is acutely toxic to fish (Allan et al., 1969), more so than rotenone (Macleod, Worth, & Wells, 1978). Furthermore, chemical structure **2** resembles the C, D and E-ring moiety of rotenone: A/D ring-substituted derivatives of rotenone maintain electron transfer inhibitor activity, but B/C altered derivatives do not. This finding suggests that the activity of rotenone is related to the B/C ring configuration (Ahmad-Junan, Amos, Cockerill, Levett, & Whiting, 1994). Additionally, since tubaic acid poorly inhibits the electron transfer

Table 3
Activity of antifeedants from Cyperaceae and rotenone

Compounds	Insect antifeeding activity (*S. litura*)		Origin
	ED_{50} (mol/cm^2)	pED_{50}	
Cyperaquinone (1)	1.7×10^{-7}	6.77	*C. nipponicus*
Remirol (2)	1.3×10^{-7}	6.89	*C. nipponicus*
Scabequinone (3)	2.6×10^{-9}	8.59	*C. distans*
Rotenone	1.5×10^{-7}	6.82	*Derris elliptica*

system, the activity of **2** may not be related directly to the chemical structure of the rotenoids.

Although cyperaquinones have only weak anti-microbial activities (Allan et al., 1969), they were found to have moderate antifeedant activity against polyphagous insects. Actually, various compounds have been reported as antifeedants and these constituents play a defensive role in plants (Norris, 1981). Closely related chromenes and coumarans have been reported as plant defense compounds (Isman & Proksch, 1985; Isman, Proksch, & Yan, 1987). We found that similar compounds act in the defense of Cyperaceae against phytophagous insects. They may prove useful as leads for new agrochemicals with reduced toxicity to mammals since they have minimal environmental persistence.

Experimental

General

^{1}H and ^{13}C NMR: $CDCl_3$ using TMS as int. standard. IR spectra were measured as KBr pellets. TLC was performed on silica gel F_{254} (Merck) using: hexane–ethyl acetate (10:1). Spots were visualized by their fluorescence at 254 nm or by spraying with 50% sulfuric acid.

Plant material

Aerial and basal stems of *Cyperus* genus were collected at the flowering stage from June to August 1996 at Nara and Kyoto Prefecture, Japan and Chiang Mai, Thailand.

Extraction and isolation

Plant materials were extracted separately from the aerial and basal stem parts with hexane, ether and MeOH at 4°C for 3 days.

Insect rearing

Tobacco cutworms (*Spodoptera litura* Lepidoptera Noctuidae) were reared on an artificial diet (Insecta LF, Nihon Nosan Kogyo Co.) in a controlled environment at 26.5°C and 60% humidity.

Leaf-disc bioassay

The experimental setting was based on that described (Escoubas, Lajide, & Mizutani, 1993). Leaf-discs, 2 cm in diameter, were prepared with a cork borer from fresh sweet potato (*Ipomoea batatas*) leaves that had been cultivated in the Kinki University farm (Nara Pref.).

Two discs were treated with an amount of the plant extract in an acetone solution and two other discs with acetone were used as a control. The 4 discs were set in alternating positions in the same petri dish. After complete removal of solvent, 15 larvae (3rd instar) were released into the dish. The dishes were then kept in an insect rearing room at 26.5°C under illumination for 2–5 h. Partially consumed leaf-discs were taped onto copy paper for monotone data conversion. The monotone data was photocopied and confirmed to contain no errors, then converted to digital data files with a digital scanner. Digital data analysis was performed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disc from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GEI). For each experiment, the data file of an intact disc was measured and compared to that of the treated disc. To measure the activity of the extracts, we adopted the antifeedant index described (Escoubas et al., 1992): AFI = % of treated discs consumed/(% of treated discs consumed + % of control discs consumed) × 100. A value of less than 30 indicates antifeedant activity, when the amount consumed for treated and control disc is set at an AFI value of 50.

TLC bioassay

TLC silica gel plates with a fluorescent indicator (Merck Silica Gel 60 F_{254} 0.25 mm thick) were cut into 4 × 10 cm pieces. The samples containing the hexane solution were applied as a band and the plate was developed in the appropriate solvent system for separation. Distilled water was added to the artificial diet to make a paste which was then applied onto a TLC plate in this layer. The TLC plate was placed in a plastic cup with 50 larvae (4th instar) for 1 day at 26.5°C. The location of the areas uneaten by the larvae were compared with the R_f values of the compounds detected on a reference TLC plate developed under the same conditions. Re-analysis was with UV light (254 nm) and 50% sulfuric acid heated to 150°C. Compounds with antifeedant properties contained silica gel residue (Escoubas et al., 1992).

Although these substances have been isolated previously, no bioactivity against insects has been reported.

*Cyperaquinone (**1**)*

Red crystal. M.p. 182–183°C. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 242 $[M]^+$ (100), 213 (15.6), 197 (33.7),

185 (23.5), 167 (21.4), 157 (18.6), 149 (46.1), 57 (74.3). UV λ_{max}^{MeOH} nm: 473, 347, 259. IR ν_{max}^{KBr} cm^{-1}: 3112, 2916, 2848, 1706, 1664, 1498, 1386, 1334, 1294, 1029, 995, 937, 732. ^1H NMR (270 MHz $CDCl_3$): δ 2.12 (3H, s, H-14), 2.32 (3H, d, $J=1.0$ Hz, H-16), 5.30 (1H, s, H-15), 5.88 (1H, s, H-15), 6.73 (1H, s, H-3), 7.48 (1H, d, $J=1.0$ Hz, H-6). ^{13}C NMR (270 MHz $CDCl_3$): δ 8.6, 19.1, 104.2, 116.4, 122.2, 127.5, 132.2, 132,4, 145.6, 149.8, 153.3, 161.2, 163.8, 178.5.

*Remirol (5-acetyl-4-hydroxy-2-isopropenyl-6-methoxy-2,3-dihydrobenzofuran, **2**)*

Orange plate. M.p. 76–77°C. EIMS (probe) 70 eV, *m/z* (rel. int): 248 $[M]^+$ (72.4), 233 $[M-CH_3]^+$(100), 215 (22.0), 205 (8.6), 191 (12.7), 177 (8.7), 105 (2.7). UV λ_{max}^{MeOH} nm: 293, 235 (sh.), 212. IR ν_{max}^{KBr} cm^{-1}: 2927, 2854, 1733, 1635, 1610, 1465, 1433, 1290, 1238, 1203, 1141, 1110, 790, 763. ^1H NMR (270 MHz $CDCl_3$): δ 1.75 (3H, s, H-11), 2.52 (3H, s, H-14), 2.85 (1H, dd, $J=15.0$, 7.5 Hz, H-3), 3.21 (1H, dd, $J=15.0$, 9.5 Hz, H-3), 3.85 (3H, s, H-16), 4.85 (1H, s, H-12), 5.01 (1H, s, H-12), 5.23 (1H, dd, $J=9.5$, 7.5 Hz, H-2), 6.00 (1H, s, H-7), 14.12 (1H, s, OH-4). ^{13}C NMR (270 MHz $CDCl_3$): δ 16.9, 30.8, 33.0, 55.7, 85.4, 88.1, 104.8, 106.1, 112.4, 143.4, 161.8, 164.3, 166.8, 203.2.

*Scabeqinone (**3**)*

Yellow needle. M.p. 108–110°C. EIMS (probe) 70 eV, *m/z* (rel. int): 260 $[M]^+$ (91.8), 245 $[M-CH_3]^+$ (39.8), 217 (100), 204 (16.9), 189 (36.4), 163 (27.7), 109 (16.4), 82 (18.3), 70 (32.9), 56 (22.1). UV λ_{max}^{MeOH} nm: 430, 322, 268, 220. IR ν_{max}^{KBr} cm^{-1}: 3128, 2962, 2875, 1683, 1647, 1610, 1577, 1523, 1467, 1355, 1232, 1120, 1043, 1001, 916, 744. ^1H NMR (270 MHz $CDCl_3$): δ 1.07 (6H, d, $J=6.5$ Hz, H-15 and H-16), 1.55-1.75 (2H, m, H-6 and H-14), 2.11 (1H, dd, $J=18.0$, 10.0 Hz, H-5), 2.28 (3H, d, $J=1.0$ Hz, H-17), 2.68 (1H, ddd, $J=18.0$, 5.0, 2.5 Hz, H-5), 3.79 (1H, t, $J=10.0$ Hz, H-7), 4.48 (1H, dt, $J=10.0$, 2.5 Hz, H-7), 7.46 (1H, d, $J=1.0$ Hz, H-2). ^{13}C NMR (270 MHz $CDCl_3$): δ 8.6, 19.6, 20.2, 22.0, 29.2, 36.9, 70.8, 118.4, 121.4, 127.5, 145.9, 149.7, 153.8, 169.7, 183.2.

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