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Antifeedant activity of an anthraquinone aldehyde in *Galium aparine* L. against *Spodoptera litura* F.

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Abstract

The insect antifeedant anthraquinone aldehyde nordamnacanthal (1,3-dihydroxy-anthraquinone-2-al) was identified in *Galium aparine* L., and isolated from the root powder of akane (*Rubia akane*), a member of the Rubiaceae. Structure–activity relationship (SAR) studies using a series of anthraquinone analogues suggested that the aldehyde group on the anthraquinone was more important than the quinone moiety for antifeedant activity against the common cutworm (*Spodoptera litura*). High levels of nordamnacanthal were found in the seed leaf stage and in callus tissue induced from seedlings of *G. aparine*, but its concentration decreased with plant development. Since these compounds are natural pigments for dying textiles, we also evaluated the antifeedant activity against the carpet beetle (*Attagenus japonicus*), a textile pest was also evaluated. While nordamnacanthal had strong antifeedant activity against the common cutworm, it did not show any antifeedant activity against the carpet beetle. The most effective antifeedant against the carpet beetle was the major constituent in the extract of *R. trictorum*, lucidin-3-*O*-primeveroside, a food pigment.

Keywords: *Galium aparine* L; *Rubia akane*; Rubiaceae; Anthraquinones; Nordamnacanthal; *Spodoptera litura*; *Attagenus japonicus*

1. Introduction

Galium aparine L. (bedstraw), a member of the Rubiaceae, is an infamous annual weed in Japan. It is not harmed by phytophagous insects in the field and occasionally damages farm crops. On the other hand, some members of the Rubiaceae have historically been used as a source of natural dyes for textiles and anthraquinone dyes have recently been used commercially. Anthraquinones found in the Rubiaceae (Halim et al., 1992; El-Gamal et al., 1995) and iridoids in *Galium* spp. (Iavarone et al., 1983) showed weak cytotoxic activity (Halim et al., 1992; Kawasaki et al., 1992; El-Gamal et al., 1997). In this study, the structure–activity relationships of a series of anthraquinones as insect antifeedants against a herbivorous pest, the common cutworm, and a textile pest, the carpet beetle, are reported.

2. Results and discussion

In the screening of Japanese wild plants against the common cutworm *Spodoptera litura*, the hexane extract of *G. aparine* was found to have potent insect antifeedant activity, but the responsible compound (nordamnacanthal **1**, see below) was not stable and occurred only in small quantities in the mature plant, based on TLC analysis. A large amount of presumably the same insect antifeedant was present in the hexane extract of the *Rubia akane* root, hence this plant material was used for bulk collection and analysis of the responsible compound **1**. Compound **1** was purified by silica gel column chromatography, recrystallized from hexane, and its structure confirmed to be nordamnacanthal **1**, by comparison of NMR spectral data with literature values (Zhou et al., 1994). The MS fragmentation pattern and physicochemical properties of this compound were similar to those of the active compound in the hexane extract of *G. aparine* and its calli, thus identifying the insect antifeedant in *G. aparine* as the known compound **1** (Figs. 1 and 2).

Calli produced from seedlings of *G. aparine* were germinated in the dark at 10 °C, and these also accumulated **1**.

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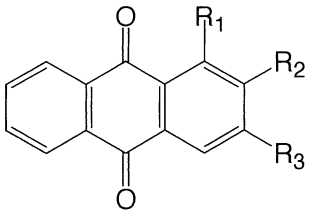

	R_1	R_2	R_3
1	OH	CHO	OH
2	OH	CH_2OH	O-Prim
3	OH	OH	H
4	OH	CH_2OH	OH
5	OH	CH_2OH	O-Glu
6	H	CH_2OH	H
7	H	COOH	H
8	H	CHO	H
9	H	CH_3	H

(See table 1 for chemical nomenclatures)

Fig. 1. Nordamnacanthal and alizarin anthraquinones tested for antifeedant activity.

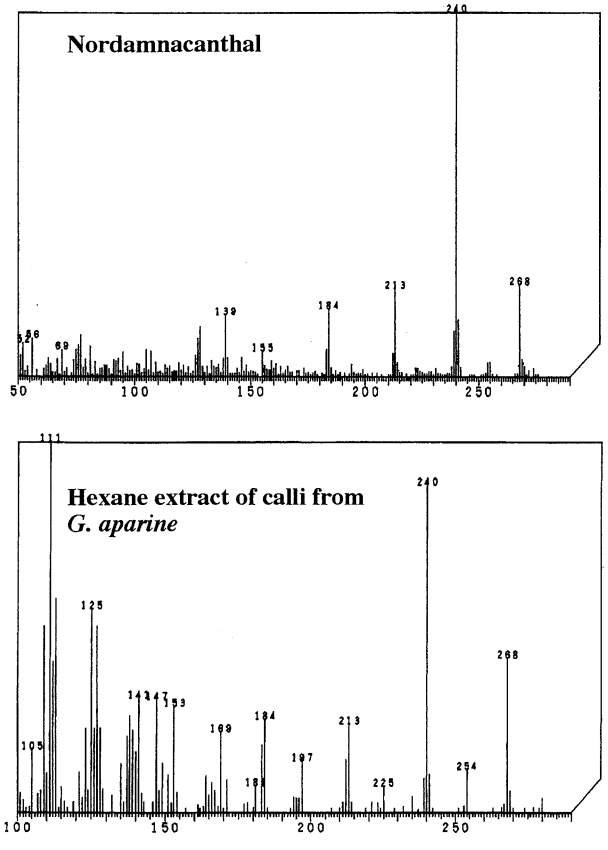

Fig. 2. Comparison with MS spectrum fragment pattern of nordamncanthal and its hexane extract of calli from *G. aparine*.

Indeed, both **1** and its decomposition products from hexane extracts of calli were readily detected by TLC. On the other hand, while the total amount of **1** in the plant remained essentially the same based on the HPLC analysis, plant growth caused an increase in fresh

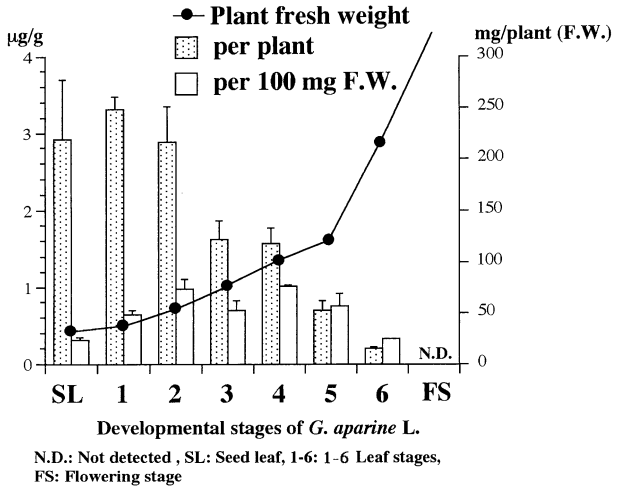

Fig. 3. Change in nordamnacanthal concentration in aerial parts of *G. aparine* with development.

Table 1
Antifeedant activities of test anthraquinones against *S. litura* and *A. japonicus*

Compound	*S. litura*		*A. japonicus*
	ED_{50} (μmol/cm^2)	(95% CI[a])	FIR[b] (%)
1 Nordamnacanthal	0.12	(0.111–0.1342)	Inactive
2 Lucidin-3-*O*-primerside	Inactive		88.9
3 Alizarin	Inactive		Inactive
4 Lucidin	Inactive		Inactive
5 Lucidin-3-*O*-glucoside	Inactive		Inactive
6 2-Hydroxymethylanthraquinone	Inactive		85.6
7 Anthraquinone-2-carboxylic acid	Inactive		Inactive
8 Anthraquinone-2-aldehyde	0.75	(0.656–0.825)	Inactive
9 2-Methylanthraquinone	3.02	(2.026–4.556)	58.3

[a] CI: confidence interval.

[b] FIR: feeding inhibition rate against carpet beetle. Staining dose: **1**–**4** (1 mg/cm^2 cloth), **5**–**9** (3 mg/cm^2 cloth). Inactive: antifeedant activity was below 50% at 0.33 mg/cm^2 against *S. litura* and 3 mg/cm^2 against *A. japonicus*.

weight, and so the concentration of anthraquinone per plant accordingly decreased (Fig. 3). In a similar manner, it has been reported that in the Cyperaceae, the total amount of JH III (juvenile hormone III), which is part of the plant's chemical defense system, decreases with plant development (Bede et al., 1999).

We next compared other natural pigments in the Rubiaceae, including lucidin-3-*O*-primeveroside **2** and alizarin **3**, as well as authentic anthraquinone analogues (**4**–**9**) in a SAR study of anthraquinones. Among the natural compounds in Rubiaceae, **1** showed the strongest insect antifeedant activity (Table 1), with complete inhibition at 83 μg/cm^2. Since quinones and compounds with aldehyde groups have been reported to be insect antifeedants (Blaney et al., 1987; Morimoto et al., 1999), the importance of an aldehyde group at C-2 of the anthraquinone for antifeedant activity against the common cutworm was evaluated. Anthraquinones **2**–**7**,

which lack an aldehydic group at the C-2 position, did not show insect antifeedant activity, whereas anthraquinone-2-aldehyde **8** and 2-methylanthraquinone **9**, still had an antifeedant activity against common cutworms (Table 1). These results suggest that aldehydic and/or methyl groups at the C-2 position, are important for antifeedant activity against the common cutworms.

The extract of *R. tinctorum* protect textiles from the carpet beetle (Nakashima and Doi, 1997). However, our antifeedant assays for anthraquinones using carpet beetles showed that only **2** (1 mg/cm^2) significantly inhibited feeding, whereas **1** and **8** had no antifeedant activity against the carpet beetle (Table 1). While the carpet beetle was sensitive to **2**, this activity disappeared with its conversion to **5**. These results suggest that dyes from *R. akane* and *R. tinctorum* may protect against textile pests partially due to the presence of **2**. These compounds cannot, however, be used commercially since they need to be applied in very high doses. Nevertheless, the results may be useful for the development of other functional textile pigments.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR: $CDCl_3$ with TMS as an int. standard. IR spectra were measured using a KBr tablet. TLC was performed on silica gel F_{254} (Merck) using *n*-hexane–EtoAc (3:1). Spots were visualized by fluorescence at 254 and 365 nm or by spraying with 50% H_2SO_4. Nordamnacanthal has an orange fluorescence when irradiated at 365 nm. Authentic anthraquinones **3** and **6** were purchased from Tokyo Kasei Co., Ltd., **9** was purchased from Wako Chemical Co., Ltd., and **7** was purchased from Aldrich Chemical Co., Ltd. The food pigment from *R. tinctorum* was provided by San-Ei Gen F.F.I. Co., Ltd.

3.2. Plant material

Bedstraw, *G. aparine* L., was collected near Kinki University, Nara, Japan, from November to May 1998–2000.

3.3. Extraction and isolation

Fresh aerial portions of *G. aparine* (3 kg) were extracted with *n*-hexane for 3 days at 4 °C, and the resulting extract was concentrated under reduced pressure to give a yellowish green oil (4.19 g). The root powder of *R. akane* (2 kg), was also extracted with *n*-hexane at 4 °C for 3 days, with the extract concentrated under reduced pressure to give a brown oil (5.37 g). The extracts were separated by silica gel column chromatography, eluted with *n*-hexane–EtOAc (3:1), and purified by recrystallization from *n*-hexane.

3.4. Preparation of test compounds

Compound **2** purified in crystalline form, was obtained from the food pigment made from the extract of *R. tinctorum* dissolved in glycerol, and stored at 4 °C for 1 month. Compound **4** was prepared by hydrolysis of **5** using 6 M HCl aq. at 90 °C, whereas compound **5** was obtained by hydrolysis of **2** using 1.2 M HCl aq., compound **2** (140 mg, 0.25 mmol) dissolved in 1.2 M HCl aq. (30 ml) at 90 °C for 1.5 h. After neutralization using NaOH aq. we obtained the crystalline **5** by filtration (89.5 mg, yield 83.5%).

Compound **5** (5.3 mg, 12.3 nmol) dissolved in 6 M HCl aq. (15 ml) and hydrolysed at 90 °C for 4 h. We obtained the crystalline **4** by filtration (3.0 mg, yield 90.6%).

Compound **8** was prepared by oxidation of **4** with PCC. Compound **6** (630 mg, 2.64 mmol) was dissolved in CH_2Cl_2 (12.5 ml), and PCC (1.97 g, 9.2 mmol) was added to the solution along with 3A molecular sieves (2 g). After 1 h, the products were extracted with ethyl acetate, concentrated by evaporation, passed through silica gel, and recrystallized from hexane to give pure crystalline **8** (60 mg, 9.6%).

White crystal. mp 188–191 °C EIMS (probe) 70 eV, *m*/*z* (rel. int.): 236 (M^+, 100), 207 (24.2), 151 (34.7). HR-EIMS (probe) *m*/*z*: 236.11799 ($C_{15}H_8O_3$, requires 236.04732). ^{1}H NMR (270 MHz $CDCl_3$): δ 10.25 (1H, *s*, CHO), 8.81 (1H, *s*, Ar-H), 8.49 (1H, *d*, $J=7.9$ Hz), 8.39–8.33 (2H *m*., Ar-H), 8.30 (1H, *s*, Ar-H), 7.90–7.83 (2H, *m*, Ar-H). ^{13}C NMR (270 MHz $CDCl_3$): δ 190.9, 182.3, 182.3, 139.9, 134.7, 133.2, 129.7, 128.3, 127.6.

3.5. Insect rearing

Common cutworms (*Spodoptera litura* Lepidoptera, Noctuidae) purchased from Sumika Techno Service Co. Ltd. (Takarazuka, Japan), were reared on an artificial diet (Insecta LF, Nihon Nosan Kogyo Co. Ltd.) in a controlled room environment at 26.5 °C and 60% humidity. Carpet beetles (*Attagenus japonicus,* Dermestidae) were a gift from Dr. T. Nakashima (Kinki University, Japan) and reared on dried bonito shavings in a controlled room environment at 28 °C.

3.6. Antifeedant bioassay

The experimental conditions have been described previously (Morimoto et al., 1999). Leaf-disks, 2 cm in diameter, were prepared from fresh sweet potato (*Ipomoea batatas*) leaves using a cork borer. Two disks were treated with a specified amount of the plant extract or test compounds in an acetone solution and two other

disks that were treated only with acetone were used as a control. Acetone-insoluble test compounds were applied using an arabic gum paste. The four disks were placed in alternating positions in the same petri dish. After complete removal of the solvent, 15 larvae (third instar) were released into the dish. To evaluate antifeedant activity against textile pests, the substrate was changed from sweet potato leaves to swatches of wool fabrics (1 cm × 1 cm). The incubation period was 6 h in the dark for the common cutworm. The slower-feeding carpet beetle was allowed to feed for 2 weeks.

AFI (antifeedant index) = % of treated disks consumed/(% of treated disks consumed + % of control disks consumed) × 100. A value of less than 30 was considered to reflect antifeedant activity. The ED_{50} values of the test compounds were calculated by the probit method after changing the AFI value to a feeding inhibition rate.

3.7. Quantitative analysis of nordamnacanthal in G. aparine L. by HPLC

Quantitative analysis was performed by HPLC with an internal standard using a Shimadzu VP-10 HPLC system. The aerial part of the plant was collected from an observation field at Kinki University Nara Campus. Plants were weighed and extracted with hexane (20-fold volume of fresh weight) at room temperature without previous washing with water. Hexane extracts were evaporated to dryness and MeOH containing 10 ppm 2-methylanthraquinone was added as an internal standard. The samples were measured by HPLC (column: GL science ODS-3, 0.25 mm ϕ, 25 cm, mobile phase: acetonitrile 65% in water, 2 ml/min, UV detector: 256 nm).

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