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Isoflavanones from the allelopathic aqueous root exudate of *Desmodium uncinatum*

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Dedicated to the memory of Professor Jeffrey B. Harborne.

Abstract

Three isoflavanones, 5,7,2′,4′-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone (**1**), 4″,5″-dihydro-5,2′,4′-trihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone (**2**) and 4″,5″-dihydro-2′-methoxy-5,4′-dihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone (**3**) and a previously known isoflavone 5,7,4′-trihydroxyisoflavone [genistein (**4**)] were isolated and characterised spectroscopically from the root exudate of the legume *Desmodium uncinatum* (Jacq.) DC. We propose the names uncinanone A, B, and C for compounds **1**, **2** and **3**, respectively. Isolated fractions containing uncinanone B (**2**) induced germination of seeds from the parasitic weed *Striga hermonthica* (Del.) Benth. and fractions containing uncinanone C (**3**) moderately inhibited radical growth, the first example of a newly identified potential allelopathic mechanism to prevent *S. hermonthica* parasitism.

Keywords: *Striga hermonthica*; Scrophulariaceae; *Desmodium uncinatum*; Leguminosae; Allelopathy; Seed germination stimulant; Radical growth inhibitor; Uncinanone A, B and C

1. Introduction

The angiosperm genus *Striga* (Scrophulariaceae) comprises root parasites of many graminaceous cereal and leguminous crops, and causes considerable yield losses in Africa and some parts of Asia. *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L) Kuntz are serious pests on cereals while *Striga gesnerioides* (Willd.) Vatke parasitises legumes (Parker and Riches, 1993).

Striga species produce a large number of seeds with prolonged viability. Before a seed germinates, there must be an after-ripening process of pre-treatment or conditioning in a warm environment for 1–2 weeks and exposure to an external germination stimulant exuded by the roots of potential host or certain non-host plants (Parker and Riches, 1993). Germination stimulants such as strigol (Cook et al., 1966; Siame et al., 1993), sorgolactone (Hauck et al., 1992), alectrol (Muller et al., 1992; Yokota et al., 1998), orobanchol (Yokota et al., 1998) and dihydrosorgoleone (Chang et al., 1986) from host and non-host plants have been isolated and characterised. Some germacranolide, eudesmanolide and pseudoguaianolide sesquiterpene lactones have also been shown to induce germination of striga seeds (Fischer et al., 1989, 1990; Ruggutt and Ruggutt, 1997). Some reduction in striga damage on cereal crops in sub-Saharan Africa has been achieved by intercropping with legumes used as human food. The discovery that intercropping of cereals with the cattle forage legume *Desmodium uncinatum* significantly reduces *S. hermonthica* infestation (Khan et al., 2000) is now accepted and has become part of farming practice in areas of Kenya. Khan et al. (2002) demonstrated, in elegant greenhouse experiments, that the protection mechanism involves an allelopathic root exudation from *D. uncinatum* that is generated independently of the presence of *S. hermonthica*. Germination stimulation of *S. hermonthica* was observed and it was therefore hypothesised that in

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addition to germination stimulants present in *D. uncinatum* root exudate, there were additional factors affecting the growth and development of *S. hermonthica*. This latter biological activity subsequently prevented normal attachment to host plants. In this paper, we report the isolation of biologically active fractions from the root exudate of *D. uncinatum* and the structure elucidation of their major components by mass spectrometry and NMR spectroscopy.

2. Results and discussion

The previous demonstration that *D. uncinatum* root exudate prevents *S. hermonthica* parasitism of maize, without the presence of soil or microorganisms, justified use of this material in our investigation. The advantage of using root exudate is that there is far less bulk of initial material. However, very small quantities of biologically active material would need to be characterised.

Isolation of active root exudate material was investigated by trapping the exudate components from a hydroponic system with C-8, C-18 silica or activated charcoal adsorbent (Fig. 1). Trays of *D. uncinatum* seedlings were grown on distilled water for a period of 3 days, followed by nutrient B5 media (Ma et al., 1998) for a duration of 3 days, and then back to distilled water. This cycle continued for a period of 4 weeks. The trapping of root exudate material was carried out when the *D. uncinatum* plants were growing in the distilled water. Trapped material was desorbed with methanol and the total extract assayed for *S. hermonthica* germination stimulant activity. Adsorption on C-18 silica was found to be the most effective trapping material (Table 1) and 120 mg bulk crude root exudate was collected from a total of 60 000 *D. uncinatum* seedlings and 480 C-18 cartridge isolates. HPLC separation of the C-18 silica trap extract was performed on a reversed phase C-18 column, and eluting from 50% acetonitrile in water to 100% acetonitrile gave good separation with fractions 1–10 collected as shown on the UV detector trace (Fig. 2). Fraction 6 and 7 had higher germination activity while radical growth inhibition was highest from the very polar fractions 1–3 (Fig. 3). Of the three very polar fractions, fraction 3 was the most potent in inhibiting the growth of the radical (Table 2). However, HPLC analysis showed that fractions 1–3 comprised extremely complex mixtures of compounds and the isolation and identification of these bioactive compounds is currently being pursued. The UV detector trace of the HPLC analysis (Fig. 2) showed fractions 4, 6 and 8 contained more abundant material. As the quantity of material was so limited and these fractions did possess biological activity, albeit not as active as some other fractions, they were the initial target for structure elucidation. Bioassay-guided HPLC separation of the major components of fractions 4 (1.5 mg), 6 (0.5 mg) and 8 (3.0 mg, from a subsequent bulk root exudate) produced purified uncinanone A (**1**) (0.9 mg), uncinanone B (**2**) (0.3 mg) and C (**3**) (2.2 mg) respectively. Although genistein (**4**) is present in the root exudate of *D. uncinatum*, it was isolated in our studies by the chromatographic separation of a dichloromethane extract of macerated *D. uncinatum* root material. Commercially available genistein (**4**) and HPLC-purified uncinanone A (**1**) was not active at doses of 1–50 ppm either as a germination stimulant or radical growth inhibitor, despite the crude fractions in which they are found in possessing biological activity. Genistein **1** is inhibitory to radical growth at concentrations over 100 ppm, but this is probabaly too

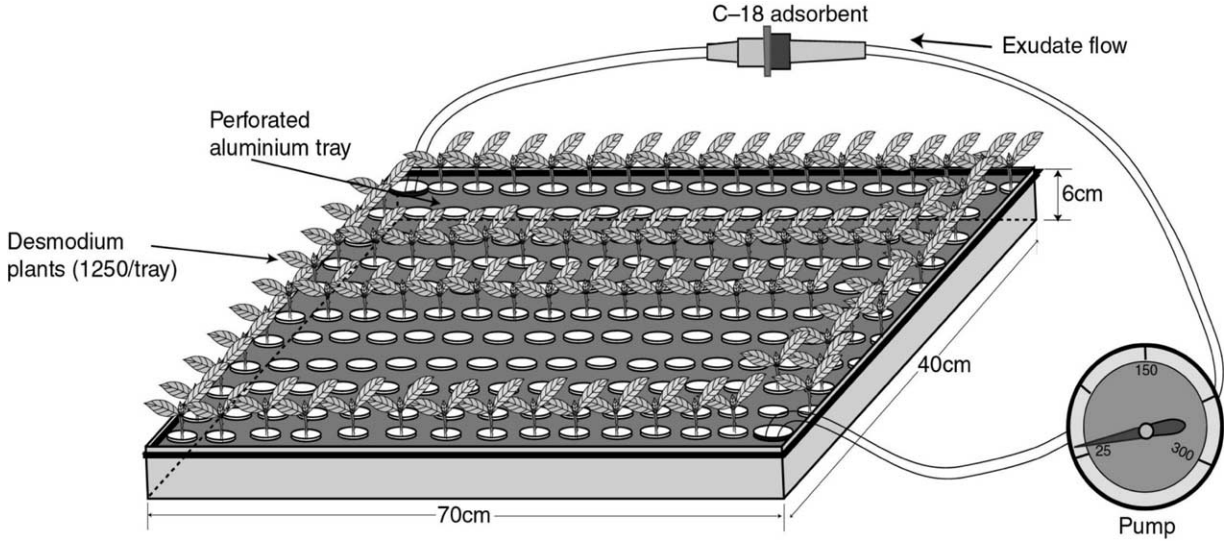

Fig. 1. Hydroponic cultivation of *D. uncinatum* showing the perforated aluminium sheet carrying desmodium plants on a perspex tray filled with water, and a pump and tubing with an adsorbent cartridge through which the exudate circulates.

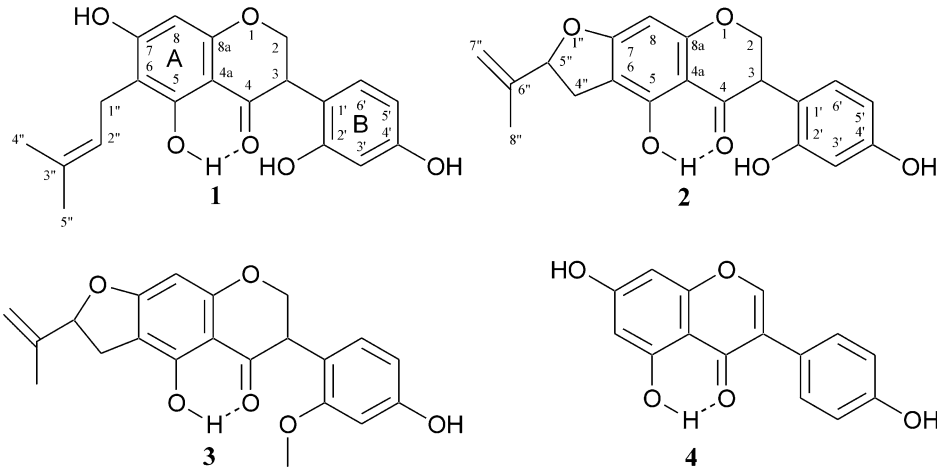

Table 1
Germination response of *S. hermonthica* to extracts (1 h extraction) from cyclic hydroponic system

Treatment	% Germination ± S.E. ($n = 10 \times 30$–40)		
	×1 dilution	×10 dilution	×100 dilution
C-18 silica	51.8 ± 5.93bc	76.8 ± 0.96a	11.2 ± 3.08f
C-8 silica	66.0 ± 7.56ab	33.7 ± 5.47cd	1.9 ± 0.99f
Activated charcoal	24.8 ± 3.92de	0.9 ± 0.55f	0.4 ± 0.37f
GR-24 1 ppm	75.4 ± 3.54a		

Means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test.

high to have ecological relevance. Fraction 6, which contained uncinanone B (**2**), however, possessed *S. hermonthica* germination activity at a lower concentration. When bioassayed at 50 and 10 ppm, the germination stimulant activity was not statistically different from that of the potent synthetic stimulant GR-24 (Thuring et al., 1997) at 1 ppm (Fig. 4). In contrast, HPLC-purified uncinanone C (**3**), prepared from the purification of fraction 8, inhibited growth of the radical (Fig. 5). The root exudate of *D. uncinatum* has therefore been shown to possess germination stimulation and radical growth inhibition activities and this combination may

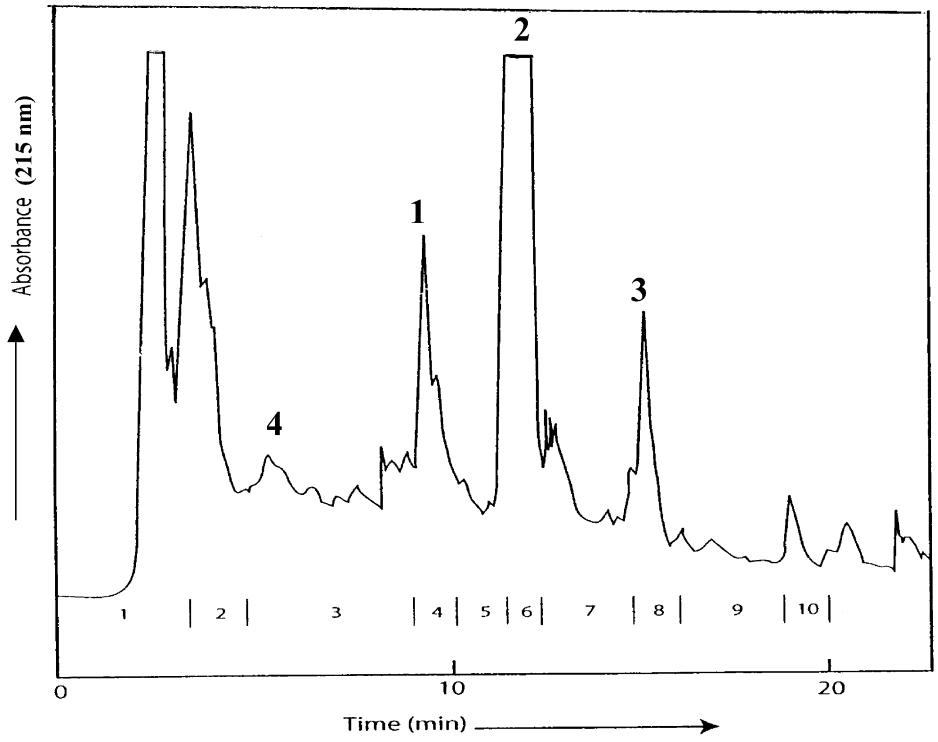

Fig. 2. HPLC profile of the crude extract of the aqueous root exudate of *D. uncinatum* with peaks corresponding to compounds **1–4** and fractions 1–10 marked.

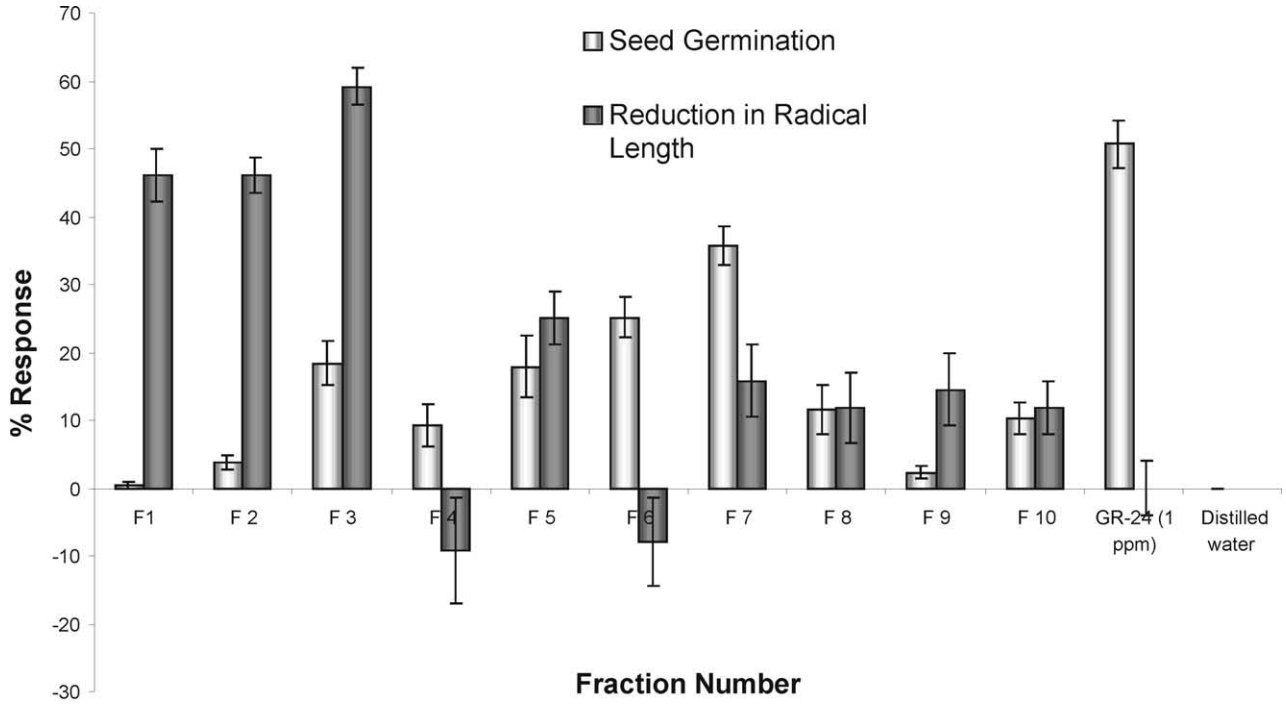

Fig. 3. Germination ($n = 5 \times 30$–40) and radical growth inhibition ($n = 30$) of *S. hermonthica* to fractions from the hydroponic extract of *D. uncinatum* (×1 dilution, see Experimental).

Table 2
Radical growth inhibition of germinated *S. hermonthica* seeds to fractions 1, 2 and 3 from *D. uncinatum* hydroponic root extract

Treatment	Conc. (ppm)	Radical length (mm) ($n = 30$)	% Radical growth inhibition
Fraction 3	100	0.51 ± 0.03f	58.9
Fraction 2	100	0.62 ± 0.03ef	50.0
Fraction 1	100	0.92 ± 0.05bcd	25.8
Fraction 3	10	0.82 ± 0.05de	33.9
Fraction 2	10	0.88 ± 0.07cd	29.0
Fraction 1	10	1.16 ± 0.05ab	6.5
Fraction 3	1	1.10 ± 0.08ab	11.3
Fraction 2	1	1.27 ± 0.05a	−2.4
Fraction 1	1	1.20 ± 0.07a	3.2
GR-24	1	1.24 ± 0.06a	0.0

Means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test.

comprise part of the mechanism of protection against *S. hermonthica* parasitism.

Uncinanone A (**1**) gave $[M]^+$ and $[M+H]^+$ ions at 356 and 357 in the EIMS and ESIMS spectra respectively. Features observed by NMR spectroscopy were that of a hydrogen bonded phenol at 12.02 ppm, a spin-coupled system containing protons at 4.63, 4.76 and 3.93 ppm, and a prenyl group (methyl singlets at 1.69 and 1.75 ppm with a 2H doublet at 3.26 ppm and a 1H triplet at 5.17 ppm). In addition, upfield aromatic signals indicated two oxygenated aromatic groups. Three of these aromatic protons (δ 7.23, *d*, $J = 8.2$ Hz, 6.42, *d*, $J = 2.4$ Hz and 6.37, *dd*, $J = 2.4$, 8.2 Hz) showed characteristic coupling of the 1,2,4-trisubstituted aromatic ring. Fragment ions at m/z 221 $[A_1+H]^+$ and 136 $[B_1]^+$ (Fig. 6) arising from a *retro*-Diels–Alder reaction showed that the A-ring has the isoprenyl group and the B-ring has two hydroxyl groups (Shirataki et al., 1999; Monache et al., 1996). HMBC correlations between the two protons of C-1″ and the carbons C-5, C-6 and C-7 placed the isoprenyl unit on C-6 (Table 3), while H-3 correlated into the B-ring. A NOE between H-2″ and the singlet at 1.69 ppm defined this methyl group H-5″ as *cis* to the double bond proton, and was confirmed by a NOE between H-1″ and the H-4″ methyl singlet at 1.75 ppm.

Uncinanone B (**2**) gave $[M]^+$ and $[M+H]^+$ ions at 354 and 355 in the EIMS and CIMS spectra respectively. The ^{1}H NMR spectrum showed a signal at 11.80 ppm characteristic of a hydrogen-bonded phenol. However, this signal appeared as two peaks with a very small difference in shift of 5 ppb. Further examination of the other proton resonances revealed the existence of two compounds with almost identical NMR spectra. A set of NMR resonances that appeared to be derived from an isoprene group gave a second spin-system (2.91, 3.26 and 5.32 ppm on ^{13}C resonances at 30.3 and 88.8 ppm respectively) and suggested the presence of a dihydrobenzofuran ring. HMBC correlation of the chelated hydroxyl group and two protons on C-4″ with the carbon at δ 106.5 showed that C-4″ should be joined to C-6

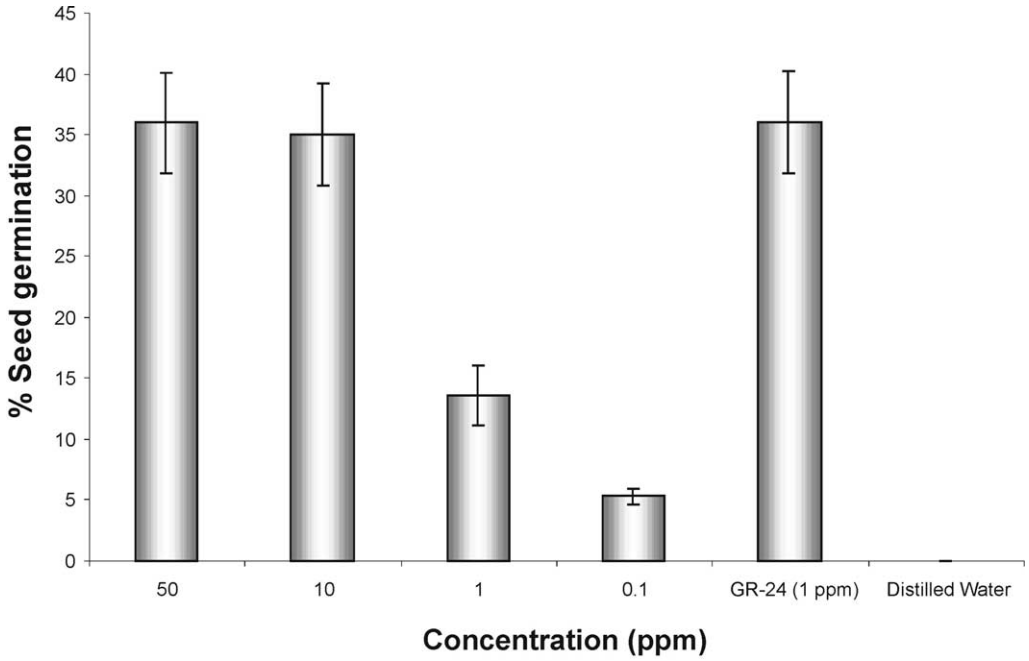

Fig. 4. Germination response of *S. hermonthica* to fraction 6 of the hydroponic extract of *D. uncinatum* and GR-24 (1 ppm) ($n = 10 \times 30$–40).

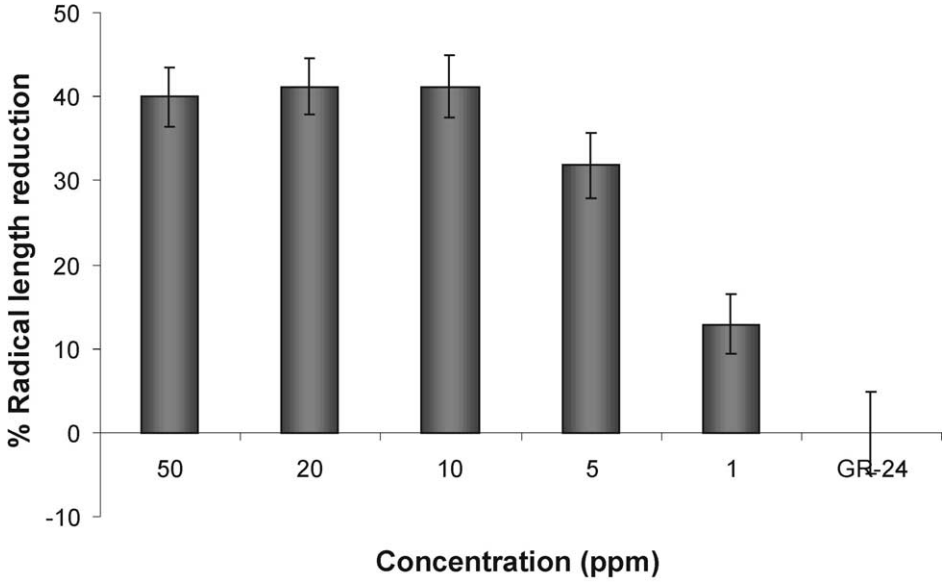

Fig. 5. Radical growth inhibition ($n = 30$) of germinated *S. hermonthica* seeds exposed to purified uncinanone C (**3**).

of the A-ring. A peak at 6.04 ppm was assigned to H-8 while the coupling pattern observed at 7.37, 6.43 and 6.49 ppm assigned these to H-6′, H-5′ and H-3′ respectively. The two sets of peaks were present in a ratio close to 50:50 and it is possible that in the proposed structure, which contains two stereogenic centres at C-3 and C-5″, one or both of the centres is racemic. Further studies on the stereochemistry at these two centres are being pursued. The physical distance between these two centres in such a planar molecule could explain the high spectral similarities between diastereoisomers. In the EIMS spectrum, the appearance of fragments at *m*/*z* 136 and *m*/*z* 219 again resulting from *retro*-Diels–Alder reaction, is in agreement with placement of two hydroxyl groups in B-ring. The structure of uncinanone B (**2**) was confirmed through HMQC and HMBC experiments and was characterised as 4″, 5″-dihydro-5, 2′, 4′-trihydroxy-5″-isopropenylfurano-(2″,3″,7,6)-isoflavanone.

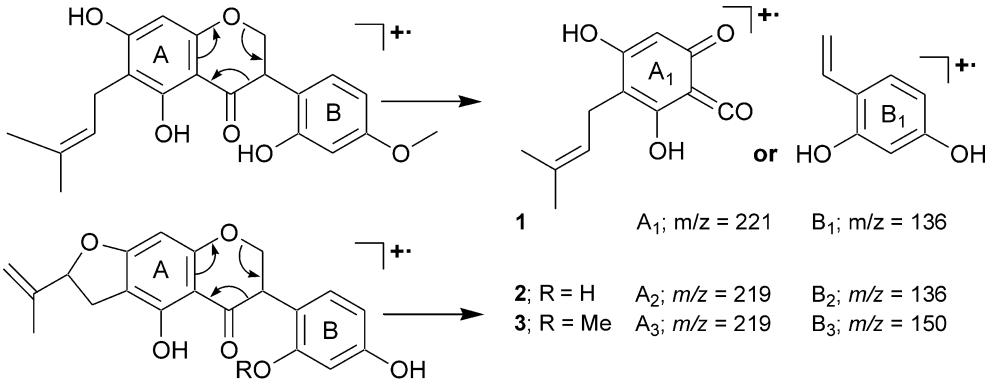

Fig. 6. *retro*-Diels–Alder EIMS reaction of **1–3**.

The ^{1}H NMR spectral data and the MS fragmentation pattern of uncinanone C (**3**), with $[M]^+$ at 368, were similar to that of **2** but revealed the existence of one methoxyl group in place of one hydroxyl group. The EIMS peaks resulting from *retro*-Diels–Alder reaction were at *m*/*z* 150 and 219, and showed that the methoxyl group was therefore attached to the B-ring. The methoxyl protons and H-3 all correlated with C-2′ in the HMBC spectrum and NOE difference spectra showed a strong NOE between the methoxyl protons and H-3′, but not H-5′, assigning the methoxyl to C-2′. Another NOE was observed between H-3 and H-6′. The *exo*-methylene proton signals could also be stereochemically assigned by a NOE between the signals at 4.96 and the methyl signals of both diastereoisomers at 1.78 and 1.80 and also between the other *exo*-methylene signals at 5.10 and the H-5″ protons of both diastereoisomers (Fig. 7).

Compound **4** exhibited spectral data identical to that of genistein, a known isoflavone (Hanawa et al., 1991).

Compounds **1–3** are currently the targets of synthetic studies to verify chemical structure and to examine the stereochemistry of the natural compounds. In addition, purified synthetic material will allow the biological activity of these compounds to be further examined.

3. Experimental

3.1. General experimental procedure

UV spectra were recorded on a 168 diode array detector module attached to a Beckman System Gold 126 HPLC system. Mass spectra were recorded on a Fisons VG platform II spectrometer at electron energy of 70 eV. HREIMS (70 eV) were recorded with a VG Autospec mass spectrometer. Nuclear magnetic resonance spectra were recorded using Joel JNM-400 (400 MHz) and Bruker Avance 500 MHz NMR spectrometers.

3.2. Plant material

Seeds of *S. hermonthica* were harvested in 1997 and *D. uncinatum* seeds purchased from the Kenya Seed Company, Nairobi, Kenya.

3.3. Germination of plant seeds

A perspex tray (70 cm×40 cm×6 cm high) was covered with a thin sheet of cotton and made wet with distilled water. Nine filter papers, Nigel 27 cm diameter, were placed on the wet cotton. *D. uncinatum* seeds (20 g) were soaked in 1% NaOCl solution for 10 min, washed thoroughly with tap water, rinsed three times with distilled water and placed on the wet filter paper in the tray. The tray was wrapped in black polythene sheet and kept at room temperature for 6 days.

3.4. Preparation of germinated seeds for exudate production

Six-day-old plant seedlings (1250) were transferred to an aluminium sheet (41 cm×71 cm, perforated with 3 mm diameter holes, 1 cm apart) placed on a perspex tray (40 cm×70 cm×6 cm) containing 12.5 l of distilled water. The roots of the seeds emerged from the bottom side of the tray into the water (Fig. 1).

3.5. Isolation of root exudates

Trays of *D. uncinatum* seedlings were alternated between distilled water for a period of 3 days, and nutrient B5 media (Ma et al., 1998) for a duration of 3 days. Root exudate was collected from the distilled water phase (Fig. 1) using a peristaltic pump with a flow rate of 4 ml/min, passing the water through a cartridge containing 0.5 g C-18 silica (25–40 μM, Merck) which was replaced every 12–16 h and which constituted a run. After 1 month, 60 runs had been completed and new plants were cultivated to prepare a total of 480 runs.

Table 3
^{1}H NMR spectral data ($CDCl_3$) of **1–3** and the associated HMBC correlations to carbon resonances

	Uncinanone A (**1**)		Uncinanone B (**2**)[a]		Uncinanone C (**3**)[a]	
	δ_H, *J* (Hz)	HMBC correlations	δ_H, *J* (Hz)	HMBC correlations	δ_H [No., mult., *J* (Hz)]	HMBC correlations
H-2*A*	4.76, 1H, *dd*, *J* = 4.3, 12.6	160.9 196.9, 115.1	4.88, 2H, m, contains *J* = 11.8	164.4, 196.7	4.54, 1H, *t*, *J* = 10.9 4.54, 1H, *t*, *J* = 11.0	164.5, 197.9
H-2*B*	4.63, 1H, *dd*, *J* = 4.6, 12.6		4.75, 1H, *d*, *J* = 11.9 4.74, 1H, *d*, *J* = 11.9	115.5, 164.4, 196.7	4.44, 1H, *dd*, *J* = 5.4, 10.8 4.44, 1H, *dd*, *J* = 5.4, 10.8	47.0, 115.2, 164.5, 197.9
H-3	3.93, 1H, *t*, *J* = 4.6	115.1, 128.2, 156.3, 196.9	3.94, 2H, *br s*	128.0	4.31, 1H, *dd*, *J* = 5.7, 11.1 4.29, 1H, *dd*, *J* = 5.5, 11.2	70.0, 115.2, 131.3, 159.2, 197.9
H-8	5.94, 1H, *s*	102.0, 107.2, 160.9, 164.8	6.04, 1H, *s* 6.04, 1H, *s*		6.02, 2H, *s*	104.7, 164.5, 169.3
H-3′	6.42, 1H, *d*, *J* = 2.4	108.4, 115.1, 156.7	6.49, 2H, *s*		6.48, 2H, *br s*	108.0, 115.2, 157.9
H-5′	6.37, 1H, *dd*, *J* = 2.4, 8.2		6.43, 2H, *d*, *J* = 8.3		6.42, 2H, *br d*, *J* = 8.0	100.0, 115.2
H-6′	7.23, 1H, *d*, *J* = 8.2	156.3, 156.7	7.37, 1H, *d*, *J* = 8.0 7.36, 1H, *d*, *J* = 8.0		6.95, 2H, *br d*, *J* = 8.1	47.0, 157.9
H-1″	3.26, 2H, *d*, *J* = 7.0	107.2, 121.1, 136.0, 162.0, 164.8				
H-2″	5.17, 1H, br t, *J* = 7.0					
H-4″*A*	1.75, 3H, *s*	25.8, 121.1, 136.0	2.91, 1H, m 2.89, 1H, m	89.1, 106.5, 143.2, 159.5, 170.2	2.95, 2H, *dd*, *J* = 7.5, 15.2	88.7, 105.7, 143.5, 159.7, 169.3
H-4″*B*			3.26, 1H, *dd*, *J* = 9.7, 15.2 3.25, 1H, *dd*, *J* = 9.7, 15.2	106.5, 143.2, 159.5, 170.2	3.30, 2H, *dd*, *J* = 9.8, 15.2	88.7, 105.7, 143.5, 159.7, 169.3
H-5″	1.69, 3H, *s*	17.9, 121.1, 136.0	5.32, 1H, *t*, *J* = 9.0 5.30, 1H, *t*, *J* = 9.1		5.32, 2H, br t, *J* = 8.6	17.4, 113.2
H-7″			5.09, 1H, *s* 5.08, 1H, *s*	17.1, 89.1, 143.2	5.10, 2H, *br s*	17.4, 88.7, 143.5
			4.96, 1H, *s* 4.95, 1H, *s*	17.1, 89.1	4.96, 2H, *br s*	17.4, 88.7
H-8″			1.77, 3H, *s* 1.75, 3H, *s*	89.1, 113.5, 143.2	1.80, 3H, *s* 1.78, 3H, *s*	88.7, 113.2, 143.5
OH	5.00, 6.31, 7.77		7.99, 1H, *br s* 7.93, 1H, *br s*			
5-OH	12.02, 1H, *s*	102.0, 107.2, 162.0	11.80, 1H, *s* 11.80, 1H, *s*	101.6, 106.5, 159.5	12.45, 2H, *s*	104.7, 105.7, 159.7
4′-Ome					3.80, 6H, *s*	159.2

[a] Proton resonances are shown for both compounds of the diastereoisomeric mixture.

Material was desorbed from the cartridges with methanol to give a crude extract (120 mg) which was dissolved in methanol (2.5 ml) and subjected to semi-preparative HPLC. Analytical HPLC was performed on a Beckman System Gold 126 equipped with a 168 diode array detector module using a Beckman ultrasphere C-18 column (250×4.6 mm), and semi-preparative HPLC was performed on a Varian 5000 LC with Beckman C-18 (250×10 mm, 5 μm) and ultrasphere C-18 columns (250×10 mm, 5 μM). (Solvents, flow rate described at the appropriate stages below). Using a reversed phase C-18 column (eluting from 50% acetonitrile in water to 100% acetonitrile in 19 min and then 100% acetonitrile at 2.5 ml/min and monitored at 215 nm) fractions 1 (1.2 mg), 2 (17.8 mg), 3 (3.0 mg), 4 (1.5 mg), 6 (0.5 mg), and less than 0.1 mg for each of the fractions 5 and 7–10 were isolated. Biologically active fractions that caused *S. hermonthica* seed germination [fractions 4 (1.5 mg)

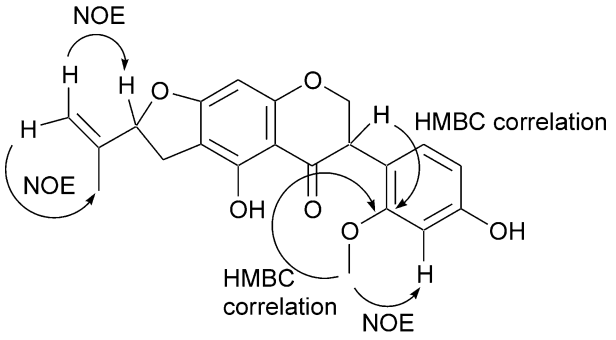

Fig. 7. Gradient HMBC correlations defining the methoxyl position and NOEs defining *exo*-methylene proton stereochemistry for uncinanone C (**3**).

and 6 (0.5 mg)] were further purified on an ultrasphere C-18 column (250×4.6 mm) using acetonitrile/water (1:1) at 1 ml/min and monitored at 240 nm. This yielded uncinanone A (**1**) (0.9 mg, 0.8% yield, $R_t = 8.5$ min) and B (**2**) (0.3 mg, 0.3% yield, $R_t = 11.5$ min) respectively. The above production, isolation and purification procedure was repeated to yield fraction 8 (3 mg) from a subsequent crude extract (880 mg) which was purified to give (**3**) (2.2 mg, 0.25% yield, $R_t = 15$ min).

In parallel, a dichloromethane extract of macerated *D. uncinatum* root extract (4.5 g) was applied to a silica gel column (28 cm×2.2 cm id) and eluted with hexane–acetone (10:0–0:10). The fraction that eluted with 40–50% acetone (0.5 g) was further chromatographed on silica gel using dichloromethane/ethyl acetate (10:0–7:3). The fraction collected with 10% ethyl acetate in dichloromethane (200 mg) was subjected to semi-preparative HPLC using a nucleosil C-18 column (250 mm×10 mm, 5 μm), methanol/water (6:4) at a rate of 4 ml/min, monitored at 240 nm, to give fractions containing uncinanone A (**1**) (1.6 mg) and genistein (**4**) (1.2 mg).

3.6. Bioassay

All bioassay data were subjected to Analysis of variance (ANOVA) using Tukey's Studentised Range (HSD) Test (SAS Statistical package, version 6.04).

3.6.1. Germination bioassay

The germination tests were performed in accordance with Thuring et al. (1997). Seeds of *S. hermonthica* (30–40/glass-fibre filter paper disc) were conditioned at 25 °C for 14 days. Fractions 1–10 were obtained from HPLC separation of 6 mg of crude root exudate. From these solutions, a 1 ml aliquot was reduced to 0.1 ml and made up to 0.5 ml with distilled water to make a test solution of ×1 dilution. Alternatively a known quantity of the test sample was dissolved in a minimum amount of methanol and diluted with distilled water to the desired concentration in parts per million. GR-24 (Thuring et al., 1997) was used as a positive control as a 1 ppm solution in distilled water and distilled water only was the negative control. The amount of methanol was never greater than 0.5% v/v in the final solution. Glass-fibre filter paper discs containing conditioned seeds were placed on filter paper (Whatman No. 1) to remove excess moisture. The discs were then returned to the petri dishes and 50 μl of test solution applied to each disc. The petri dish was sealed with parafilm and placed in an incubator at 35 °C. Germinated seeds were counted under a dissecting microscope 24 h later, and the percentage of germinated seeds was computed.

3.6.2. Radical growth inhibition assay of crude extracts

A graticule was calibrated at magnification of 20. This scale was used in all radical length measurements. Each glass fibre disc containing conditioned *S. hermonthica* seeds (30–40/disc) was exposed to 50 μl of the test solution and 1 ppm GR-24 or maize root exudate to induce germination. The discs were then placed in petri dishes sealed in parafilm and kept in an incubator at 35 °C for 24 h and at 25 °C for a further 24 h. Radical length was measured after 48 h using the graticule mounted on a dissecting microscope, and the percentage inhibition $(1-lt/lc)\times 100$, where lt = radical length of germinated

Table 4
^{13}C NMR spectral data ($CDCl_3$) of **1–3**

C-atom	Uncinanone A (**1**)	Uncinanone B[a] (**2**)	Uncinanone C[a] (**3**)
2	69.4	69.7	70.0
3	44.8	45.0	47.0
4	196.9	196.7	197.9
4a	102.0	101.6	104.7
5	162.0	159.5	159.7
6	107.2	106.5	105.7
7	164.8	170.2	169.3
8	95.5	91.0	90.7
8a	160.9	164.4	164.5
1′	115.1	115.5	115.2
2′	156.3	156.9	159.2
3′	105.0	105.7	100.0
4′	156.7	156.9	157.9
5′	108.4	108.8	108.0
6′	128.2	128.9	131.3
1″	21.1		
4″	17.9	30.3	30.7
2″	121.1		
5″	25.8	88.8	88.7
3″	136.0		
6″		143.2	143.5
7″		113.5	113.2
8″		17.1	17.4
4′-Ome			56.0

[a] ^{13}C data were obtained indirectly by inverse detection using 2D gradient HMQC and gradient HMBC spectra. With this technique, the two diastereomeric sets of ^{13}C data for **2** and **3** are not resolved.

seed exposed to treatment, and *lc* = radical length of control germinated seed) computed.

3.7. Structural characterisation

3.7.1. 5,7,2′,4′-Tetrahydroxy-6-(3-methylbut-2-enyl)-isoflavanone—uncinanone A (1)

UV (λ_{max}, MeOH/H_2O; 49:1), 290, 340 nm; IR (ν cm^{-1}) 3055, 2990, 1640, 1603, 895; ^1H and ^{13}C NMR (Tables 3 and 4); EIMS *m/z* (rel.int.): 356 $[M]^+$ (58), 338 $[M-H_2O]^+$(19), 323 $[M-H_2O-Me]^+$(5), 301 $[M-C_4H_7]^+$(10), 300 $[M-H-C_4H_7]^+$(11), 283 $[M-H_2O-C_4H_7]^+$ (22), 221 $[A_1+H]^+$ (57), 220 (14), 205 (7), 194 (37), 179 (31), 165 (100), 139 $[B_1+H]^+$ (69), 136 (25), 43 (57); ESIMS *m/z* (rel. int.): 357 $[M+H]^+$ (100), 735 $[2M+Na]^+$ (25); HREIMS *m/z* 356.1294 (calc. for $C_{20}H_{20}O_6$: 356.1259).

3.7.2. 4″,5″-Dihydro-5,2′,4′-trihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone—uncinanone B (2)

UV (λ_{max} MeCN/H_2O; 3:2) 220, 240, 290 nm; ^1H and ^{13}C NMR (Tables 3 and 4); EIMS *m/z* (rel. int.) 354 $[M]^+$ (20), 336 $[M-H_2O]^+$ (3), 321 $[M-H_2O-Me]^+$ {4), 219 $[A_2+H]^+$ (99), 203 (19), 177 (24), 151 $[C_7H_3O_4]^+$ (5), 136 $[B_2+H]^+$ (40), 123 $[C_7H_7O_2]^+$ (17), 121 (24), 44 (100); HREIMS *m/z* 354.1169 (calc. for $C_{20}H_{18}O_6$: 354.1103).

3.7.3. 4″,5″-Dihydro-2′-methoxy-5,4′-dihydroxy-5″-isopropenylfurano-(2″,3″,7,6)-isoflavanone—uncinanone C (3)

^1H and ^{13}C NMR (Tables 3 and 4); EIMS *m/z* (rel. int.) 368 $[M]^+$ (39), 219 $[A_3+H]^+$ (100), 203 (5), 150 $[B_3+H]^+$ (10), 135 (12), 107 (12). HREIMS *m/z* 368.1309 (calc. for $C_{21}H_{20}O_6$: 368.1259).

3.7.4. 5,7,4′-Trihydroxyisoflavone—genistein (4)

UV (λ_{max} MeCN/H_2O; 9:10) 259, 330 nm; ^1H NMR (500 MHz, acetone-d_6) δ 6.31 (1H, *d*, *J* = 2.4 Hz, H-6), 6.45 (1H, *d*, *J* = 2.4 Hz, H-8), 6.92 (2H, *d*, *J* = 8.5 Hz, H-3′/5′), 7.47 (2H, d, *J* = 8.5Hz, H-2′/6′), 8.20 (1H, s, H-2), 8.62 (1H, brs, OH-4′), 9.87 (1H, brs, OH-7), 13.06 (1H, s, OH-5); EIMS *m/z* (rel. int.): 270 $[M]^+$ (31), 269 $[M-H]^+$ (7), 153 (43), 152 (25), 124 (46), 118 (56), 89 (69), 69 (85), 45 (100); HREIMS *m/z* 270.0506 (calc. for $C_{15}H_{10}O_5$: 270.0582).

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References

Chang, M., Netzly, D.H., Butler, L.G., Lynn, D.G., 1986. Chemical regulation of distance: characterization of the first natural host germination stimulant for *Striga asiatica*. J. Am. Chem. Soc. 108, 7858–7860.

Cook, C.E., Whichard, L.P., Turner, B., Wall, M.E., Egley, G.H., 1966. Germination of witchweed (*Striga lutea* Lour): isolation and properties of a potent stimulant. Science 154, 1189–1190.

Fischer, N.H., Weindenhamer, J.D., Bradow, J.M., 1989. Dihydroparthenolide and other sesquiterpene lactones stimulates witchweed germination. Phytochemistry 28, 2315–2317.

Fischer, N.H., Weindenhamer, J.D., Riopel, J.L., Quijano, L., Menelaou, M.A., 1990. Stimulation of witchweed germination by sesquiterpene lactones: a structure activity study. Phytochemistry 29, 2479–2483.

Hauck, C., Muller, S., Schildnecht, H., 1992. A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. J. Plant Physiol. 139, 474–478.

Hanawa, F., Tahara, S., Mizutani, J., 1991. Isoflavonoids produced by *Iris pseudocorus* leaves treated with cupric chloride. Phytochemistry 30, 157–163.

Khan, Z.R., Pickett, J.A., van den Berg, J., Wadhams, L.J., Woodcock, C.M., 2000. Pest. Manag. Sci. 56, 957–962.

Khan, Z.R., Hassanali, A., Overholt, W., Khamis, T.M., Hooper, A.M., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 2002. Control of the witchweed *Striga hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic. J. Chem. Ecol. 28, 1871–1885.

Ma, Y., Babiker, A.G.T., Sugimoto, Y., Inanaga, S., 1998. Effect of medium composition on production of *Striga hermonthica* (Del.) Benth germination stimulants by *Menispermum dauricum* (DC.) root cultures. J. Agric. Food Chem. 46, 1587–1592.

Monache, G.D., Botta, B., Vinciguerra, V., De Mello, J.F., Chiappeta, A.A., 1996. Antimicrobial isoflavanones from *Desmodium canum*. Phytochemisty 41, 537–544.

Muller, S., Hauck, C., Schildknecht, H., 1992. Germination stimulant produced by *Vigna unguiculata* Walp cv Saunders Upright. J. Plant Growth Regul. 11, 77–84.

Parker, C., Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford, UK.

Ruggutt, J.K., Ruggutt, K.J., 1997. Stimulation of *Striga hermonthica* seed germination by 11β,13-dihydroparthenolide. J. Agric. Food Chem. 45, 4845–4849.

Siame, B.A., Weerasuyira, Y., Wood, K., Ejeta, G., Butler, L.G., 1993. Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. J. Agric. Food Chem. 41, 1486–1491.

Shirataki, Y., Matsuoka, S., Komatsu, M., Ohyama, M., Tanaka, T., Iinuma, M., 1999. Four isoflavanones from the roots of *Sophora tetraptera*. Phytochemistry 50, 695–701.

Thuring, J.W.J.F., Nefkens, G.H.L., Zwanenburg, B., 1997. Asymmetric synthesis of all stereoisomers of the strigol analogue GR-24. Dependence of absolute configuration on stimulatory activity of *Striga hermonthica* and *Orobanche crenata* seed germination. J. Agric. Food Chem. 45, 2278–2283.

Yokota, T., Sakai, H., Okuno, K., Yoneyama, K., Takeuchi, Y., 1998. Alectrol and orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. Phytochemistry 49, 1967–1973.