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Insect antifeedant furanocoumarins from *Tetradium daniellii*

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Abstract

The dried fruits of *Tetradium daniellii* yielded a new linear furanocoumarin, 5-(6-hydroxy-3,7-dimethylocta-2,7-dienyloxy)psoralen, together with six other structurally related furanocoumarins. A similar chemical profile was recorded by HPLC analysis of a fragment of *T. daniellii* fruit obtained from an historic herbarium voucher specimen collected in September 1917 during an expedition to Yunnan province, China. Four of the compounds identified caused a potent feeding deterrent effect towards larvae of *Spodoptera littoralis* and *Heliothis virescens*.

Keywords: *Tetradium daniellii*; Rutaceae; Furanocoumarins; Geranyloxypsoralen; Xanthotoxin; Bergapten; Isopimpinellin; Antifeedant; *Heliothis virescens*; *Spodoptera littoralis*

1. Introduction

Tetradium daniellii (Benn.) T.G. Hartley (syns. *Evodia hupehensis* Dode., *E. daniellii* (Benn.) Hemsl.) (Hartley, 1981) is a small ornamental tree in the Rutaceae. The genus contains only nine species and has been allied to *Phellodendron* and *Zanthoxylum* on the basis of its morphological characteristics (Hartley, 1981) and ability to synthesise 1-benzyltetrahydroquinoline alkaloids (Ng et al., 1987). In addition to a wide variety of alkaloids (Wu et al., 1995) the genus is also known to produce several other classes of compounds including limonoids (Sugimoto et al., 1988), 8-prenylated flavanones (Grimshaw and Lamar-Zarawska, 1975) and coumarins (Wu et al., 1995). Several furanocoumarins have been isolated previously from the fruits of *T. daniellii* (as *E. hupehensis*) including 5-methoxypsoralen (bergapten), 8-methoxypsoralen (xanthotoxin) and 5,8-dimethoxypsoralen (isopimpinellin) together with the prenylated derivative isoimperatorin (Gellért et al., 1972; Reisch et al., 1985). In our search for compounds with anti-insect activity we have investigated further the chemistry of *T. daniellii* fruits and report on the isolation and antifeedant activity of a range of linear furanocoumarins.

2. Results and discussion

2.1. Structure determination

An acetone extract of the dried fruits of *T. daniellii* was analyzed by HPLC coupled to a photodiode-array detector and seven major components (**1**–**7**) were found (Fig. 1). Milligram quantities of **1**–**7** for structure elucidation and insect bioassays were obtained by repetitive isolation using analytical HPLC. UV–vis spectra of the seven compounds were similar to each other and to those of linear furanocoumarins, which show distinctive UV maxima at 300–310 nm and at 250 nm (Rashid et al., 1992).

^{1}H and ^{13}C NMR spectra were obtained for compounds **1**–**7** in CD_3OD. Their structures were determined independently of existing literature data using standard 1D and 2D experiments. Compounds **1**–**6** were identified as the linear furanocoumarins 8-methoxypsoralen (**1**), 5-methoxypsoralen (**2**), 5,8-dimethoxypsoralen (**3**), 8-geranyloxypsoralen (**4**), 5-geranyloxypsoralen (**5**) and 5-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy)psoralen (**6**). ^{1}H

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and ^{13}C NMR resonance assignments for these compounds are listed in the Experimental Section as complete data sets in CD_3OD have not been published previously.

A molecular formula of $C_{21}H_{22}O_5$ was determined for **7** by high resolution MS and confirmed that compound **7** had the same relative molecular mass as **6**. Comparison of the 1D ^1H and ^{13}C NMR spectra (Table 1) of **7** with those of **2**, **5** and **6** indicated that it was a 5-substituted psoralen with a side-chain similar to that of **6**. The site of substitution was confirmed from a long-range correlation in the HMBC spectrum of **7** from H-1″ at δ 5.04 (2H, *d*, $J = 6.9$ Hz) to C-5 (δ 150.7). The structure of the side-chain was determined using COSY, HSQC and HMBC data as summarised in Table 1. The most important points of difference with **6** were a $-CH_2CH_2CH(OH)-$ fragment from C-4″ to C-6″ (instead of a $-CH_2CH{=}CH-$ fragment) and a terminal methylene group at C-7″ (instead of a tertiary hydroxyl group). Compound **7** was therefore identified as 5-(6-hydroxy-3,7-dimethylocta-2,7-dienyloxy)psoralen, a new linear furanocoumarin.

Compounds **1**–**5** are widespread in Rutaceae, whereas compound **6** is scarce and was first isolated from *Notopterygium incisum* Ting ex Ho-t. Chang (Kozawa et al., 1983). Gellért et al., (1972) found **1**, **2** and **3** in the pericarps of *T. daniellii* (as *E. hupehensis*) together with the prenylated derivative isoimperatorin and Reisch et al., (1985) identified the same compounds together with xanthotoxol (8-hydroxypsoralen), the linear pyranocoumarin xanthyletin and other coumarins including fraxidin and marmesin from the same species. In the present study only three of the coumarins (**1**, **2** and **3**) previously identified in *T. daniellii* were detected. The HPLC profile of the extract used to isolate **1**–**7** was compared with that of an extract prepared using a fragment of fruit material from a plant that had been growing wild in its natural habitat. This fruit material was obtained from a herbarium specimen (Forrest 14772) collected by George Forrest in Yunnan, China (September 1917) which was referred to specifically by Hartley in his revision of the genus (Hartley, 1981). The extract of this specimen contained compounds **1**–**7**, although the relative amounts of the compounds varied (Fig. 2). The profiles of furanocoumarins in our two samples do not match the data published previously by both Gellért et al. (1972) and Reisch et al. (1985). It is possible that Gellért et al., (1972) and Reisch et al. (1985) may have investigated material that was not *T. daniellii* or that cultivated genotypes differ from wild genotypes. Neither Gellért et al. (1972) nor Reisch et al (1985) refer to herbarium vouchers for the material they analysed. The present work illustrates that analysis of plant fragments from herbarium specimens can be a useful method for the validation of living plant material (Fig. 2) and could in principle be extended to a comparison of wild and cultivated plants.

Table 1
^1H and ^{13}C NMR chemical shift assignments, coupling constants and long-range heteronuclear connectivities for **7**

	$\delta^{13}C$	δ ^1H	HMBC	COSY
2	163.3			
3	112.8	6.28 *d*, (10.0)	C-2, C-4a	H-4
4	141.2	8.27 *d*, (10.0)	C-2, C-5, C-8a	H-3
4a	109.2			
5	150.7			
6	116.0			
7	159.8			
8	94.6	7.21 *br s*	C-4a, C-6, C-7, C-8a	
8a	153.9			
2′	105.9	7.79 *d*, (2.4)	C-7	H-3′
3′	147.0	7.16 *dd*, (2.4, 0.9)	C-2′	H-2′
1″	70.6	5.04 *d*, (7.1) 2H	C-2″, C-3″, C-5	H-2″
2″	120.7	5.58 *tq*, (7.1, 1.3)		H-1″, 9″-CH_3 (^4J)
3″	143.9			
4″	36.4	2.07 *m* 2H		5″-CH_2
5″	29.9	1.64 (*m*)		H-4″, H-6″
	1.49 (m)			H-4″, H-6″
6″	89.3	4.15 *t*, (6.8)	C-4″, C-5″, C-8″,	5″-CH_2
7″	145.7			
8″ (*E*)	114.2	4.92 *m*		10″-CH_3 (^4J)
(*Z*)		4.87 *m*[a]		10″-CH_3 (^4J)
9″-CH_3	16.4	1.66 *br s*	C-2″, C-3″, C-4″	H-2″
10″-CH_3	17.0	1.69 *m*	C-6″, C-7″, C-8″	H-8″

[a] Indicates NOE correlation to 10″-CH_3.

Hartley (1981) transferred several species from *Euodia* [now known as *Evodia* (IPNI, 1999)] to *Tetradium* on the basis of morphological characters. More recently Ng et al. (1987) and Wu et al. (1995) suggested that the presence of benzophenanthridine alkaloids in the extracts of root bark from species of *Tetradium* (but not *Evodia*) is a character that supports both Hartley's revision of the genus and the proposal that *Tetradium* is closely allied to *Zanthoxylum* and *Phellodendron*. Similarly, furanocoumarins have been isolated from both *Zanthoxylum* (Saquib et al., 1990) and *Tetradium* (Gellért et al., 1972; Reisch et al., 1985 and this study) but not from *Euodia* species. If furanocoumarins are subsequently found as constituents of the fruit of *Phellodendron* then this group of phenolics would have a strong association with the "proto-Rutaceae" (*Tetradium, Zanthoxyllum* and *Phellodendron*) (Ng et al., 1987) and might be a valuable chemosystematic marker for this group. The present work indicates that furanocoumarins can be detected effectively as marker compounds even in historic specimens and emphasises that analysis of both living and herbarium material has an important role in chemosystematic studies of this kind.

2.2. Biological activity

Compounds **1**–**7** were tested against the larvae of two species of Lepidoptera, *Spodoptera littoralis* and *Heliothis virescens*. Both species were deterred from feeding on discs treated with four (**1**–**3**, **5**) of the seven furanocoumarins isolated from the acetone extract of *T. daniellii* (Table 2). Overall, *S. littoralis* showed a greater response to these compounds than *H. virescens*. The composition of functional groups at C-5 and C-8 modulated the activity of the furanocoumarins. For example, a comparison of the activity of **4** and **5** shows that activity decreased when the geranyloxy moiety was attached to the psoralen at C-8 (**4**) rather than at C-5 (**5**). In fact, the antifeedant activity of **4** was lost when tested against *H. virescens*. Compound **5** showed potent activity against both species but the activity decreased when C-7″ and C-6″ were substituted by an hydroxyl group, as in **6** and **7**, respectively. Previous research has shown that compounds **1**–**3** are antifeedant to *S. littoralis* (Calcagno et al., 2002).

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were acquired on a Bruker 400 MHz instrument in CD_3OD at 30 °C. All chemical shift values (δ) are given in ppm with TMS used as a primary reference. Positive ion first-order MS were recorded using LC-MS (Thermo Finnigan LCQ) with an electrospray ionization (ESI) source. High-resolution electrospray ionisation mass spectra (HR-ESI-MS) were acquired using a Micromass LCT mass spectrometer calibrated with a PEG calibration solution (50:50 acetonitrile:water). HPLC was carried out using a Waters system consisting of a 600E pump, 717 autosampler and 996-photodiode-array detector.

3.2. Plant material

Fruits of *Tetradium daniellii* (Benn.) T.G. Hartley were collected from plants growing at the Royal Botanic Gardens, Kew (Acc. no. 1977-6618). A small fragment of fruit of *T. daniellii* from a herbarium specimen (Forrest 14772) collected in Yunnan, China in September 1917 was also used for extraction and HPLC analysis.

3.3. Extraction and isolation

Fruits of *T. daniellii* (5 g fresh Acc. 1977-6618 and 100 mg Forrest 14772) were ground to a fine powder and extracted with Me_2CO at room temperature for 24 h. After filtration, the residual plant material was washed with Me_2CO. The combined extracts were filtered and the solvent removed under reduced pressure. The residue was re-dissolved in MeOH and analysed by HPLC

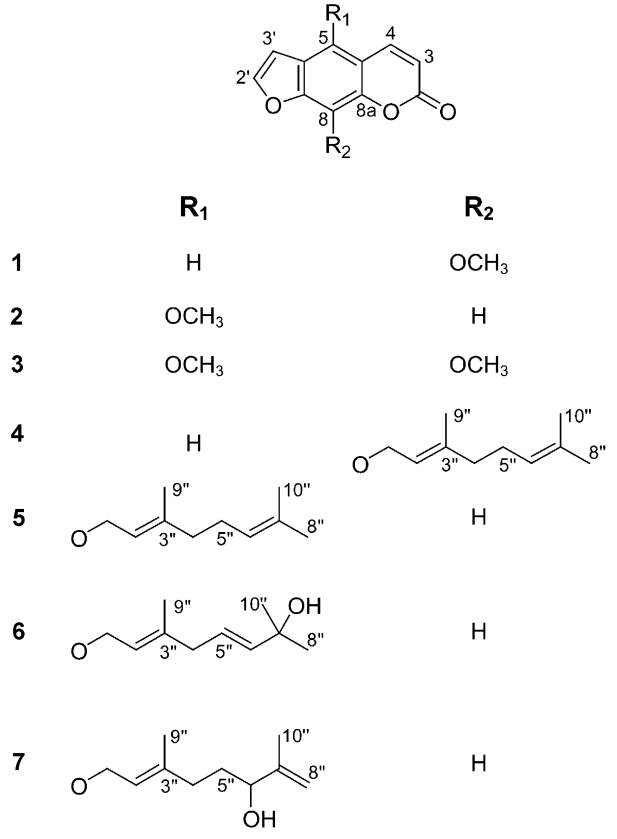

Fig. 1. Structures of furanocoumarins **1**–**7** isolated from fruits of *Tetradium daniellii*.

(Merck LiChrospher, 250 × 4.0 mm i.d., 5 μm particle size) at 1 ml min^{-1} flow rate, using a linear solvent gradient of MeCN:H_2O 40:60 to MeCN:H_2O 100:0 over 20 min. Compounds **1**–**7**, the major UV absorbing components of the Me_2CO extract, were detected at 310 nm and eluted at 12.9 (**1**), 15.3 (**2**), 14.4 (**3**), 24.3 (**4**), 26.0 (**5**), 20.9 (**6**) and 21.2 (**7**) min. **1**, **2**, **3**, **4**, and **5** were isolated by repeated collection from an analytical column to give final yields of **1** (7.4 mg), **2** (3.8 mg), **3** (4.5 mg), **4** (2.3mg) and **5** (3.2 mg). Compounds **6** (0.3 mg) and **7** (2.3 mg) required preliminary isolation as a single fraction using the method described above followed by their

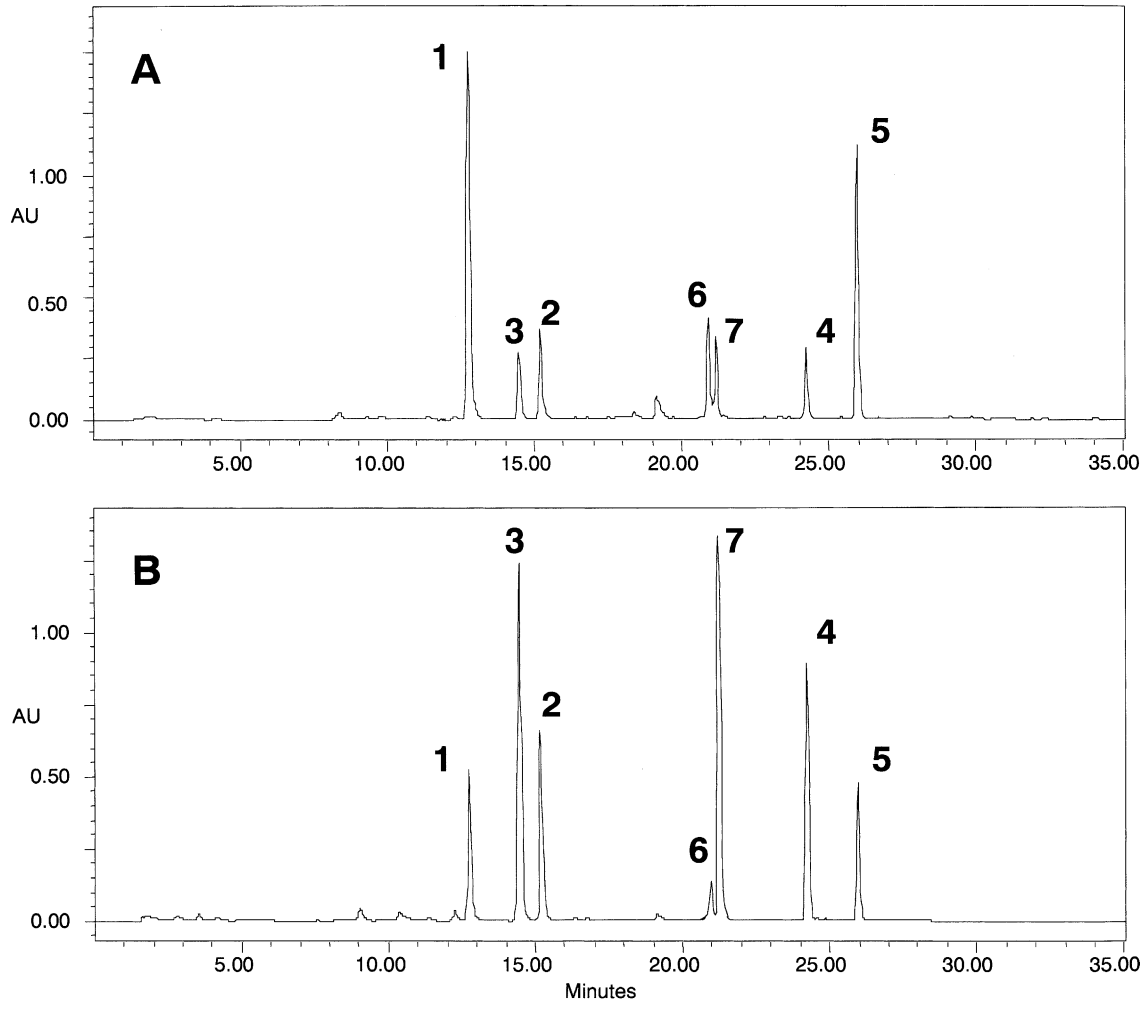

Fig. 2. HPLC profile of furanocoumarins from fruits of *Tetradium daniellii*: (a) living material, (b) herbarium specimen.

Table 2
Effect of furanocoumarins on feeding of larvae of *Spodoptera littoralis* and *Heliothis virescens* ($n = 5$–10 per concentration)

Compound[a]	Feeding index[b]		FI_{50}[c]	
	S. littoralis (mean ± S.E.M.)	*H. virescens* (mean ± S.E.M.)	*S. littoralis* conc. (M)	*H. virescens* conc. (M)
1	100 ± 0.0*	100 ± 0.0*	7.4×10^{-4}	6.6×10^{-4}
2	100 ± 0.0*	100 ± 0.0*	7.1×10^{-5}	5.2×10^{-4}
3	100 ± 0.0*	100 ± 0.0*	6.4×10^{-4}	6.6×10^{-4}
4	61 ± 12.7*	10 ± 9.3	8.0×10^{-4}	ndr
5	92 ± 5.6*	100 ± 0.0*	4.4×10^{-4}	6.1×10^{-4}
6	4 ± 22.0	20 ± 10.2	$>10^{-3}$	ndr
7	25 ± 19.1	13 ± 10.3	$>10^{-3}$	ndr

[a] See Fig. 1 for structures of compounds.
[b] Feeding index ((C–T)/(C + T))% mean ± standard error of the mean at 1×10^{-3} M.
[c] FI_{50} conc. (M) required to elicit a Feeding Index of 50%; ndr = no linear dose-dependent response.
* $P < 0.01$ Wilcoxon matched-pairs test.

final separation using a convex solvent gradient of MeCN:H_2O 50:50 to MeCN:H_2O 100:00 over 20 min.

3.4. 8-Methoxypsoralen (xanthotoxin) (**1**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.01 (1H, *d*, $J=9.6$ Hz, H-4), 7.87 (1H, *d*, $J=2.2$ Hz, H-2′), 7.54 (1H, *s*, H-5), 6.95 (1H, *d*, $J=2.2$ Hz, H-3′), 6.37 (1H, *d*, $J=9.6$ Hz, H-3), 4.24 (3H, *s*, 8-OCH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 162.8 (C-2), 149.2 (C-7), 148.6 (C-2′), 146.7 (C-4), 144.3 (C-8a), 133.9 (C-8), 128.1 (C-6), 118.0 (C-4a), 115.1 (C-3), 114.9 (C-5), 108.0 (C-3′), 61.8 (8-OCH_3); ESIMS *m*/*z*: 217 $[M+H]^+$.

3.5. 5-Methoxypsoralen (bergapten) (**2**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.27 (1H, *dd*, $J=10.0$, 0.5 Hz, H-4), 7.77 (1H, *d*, $J=2.4$ Hz, H-2′), 7.25 (1H, *dd*, $J=2.4$, 1.0 Hz, H-3′), 7.16 (1H, *br s*, H-8), 6.27 (1H, *d*, $J=10.0$ Hz, H-3), 4.31 (3H, *s*, 5-OCH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 163.3 (C-2), 160.1 (C-7), 154.0 (C-8a), 151.4 (C-5), 146.7 (C-2′), 141.3 (C-4), 114.3 (C-6), 113.0 (C-3), 107.6 (C-4a), 106.4 (C-3′), 94.3 (C-8), 61.0 (5-OCH_3); ESIMS *m*/*z*: 217 $[M+H]^+$.

3.6. 5,8-Dimethoxypsoralen (isopimpinellin) (**3**)

White solid; UV λ_{max} MeOH nm: 254, 306 nm; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.24 (1H, *d*, $J=9.8$ Hz, H-4), 7.82 (1H, *d*, $J=2.4$ Hz, H-2′), 7.21 (1H, *d*, $J=2.4$ Hz, H-3′), 6.29 (1H, *d*, $J=9.8$ Hz, H-3), 4.20 (3H, *s*, 5-OCH_3), 4.11 (3H, *s*, 8-OCH_3); ^{13}C NMR (CD_3OD, 100MHz) δ 162.7 (C-2), 151.7 (C-7), 147.1 (C-2′), 146.2 (C-5), 144.9 (C-8a), 141.4 (C-4), 129.3 (C-8), 116.5 (C-6), 113.2 (C-3), 108.8 (C-4a), 106.4 (C-3′), 62.1 (8-OCH_3), 61.5 (5-OCH_3); ESIMS *m*/*z*: 247 $[M+H]^+$.

3.7. 8-Geranyloxypsoralen (**4**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.03 (1H, *dd*, $J=9.6$, 0.2 Hz, H-4), 7.88 (1H, *d*, $J=2.3$ Hz, H-2′), 7.57 (1H, *s*, H-5), 6.95 (1H, *d*, $J=2.3$ Hz, H-3′), 6.36 (1H, *dd*, $J=9.6$, 0.2 Hz, H-3), 5.52 (1H, *tq*, $J=7.3$, 1.3 Hz, H-2″), 5.00 (1H, *d*, $J=7.3$ Hz, H-1″), 4.94 (1H, *m*, H-6″), 1.96 (1H, *m*, H-4″), 1.95 (1H, *m*, H-5″), 1.62 (3H, *br s*, 9″-CH_3), 1.59 (3H, *br s*, 8″-CH_3), 1.53 (3H, *br s*, 10″-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 162.9 (C-2), 150.3 (C-7), 148.5 (C-2′), 146.8 (C-4), 145.4 (C-8a), 144.8 (C-3″), 132.6 (C-7″), 132.3 (C-8), 127.8 (C-6), 124.8 (C-6″), 120.7 (C-2″), 118.0 (C-4a), 115.3 (C-5), 115.0 (C-3), 108.0 (C-3′), 70.7 (C-1″), 40.6 (C-4″), 27.5 (C-5″), 25.8 (C-8″), 17.7 (C-10″), 16.5 (C-9″); ESIMS *m*/*z*: 337 $[M+H]^+$.

3.8. 5-Geranyloxypsoralen (bergamottin) (**5**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.24 (1H, *d*, $J=9.8$ Hz, H-4), 7.78 (1H, *d*, $J=2.4$ Hz, H-2′), 7.18 (1H, *br s*, H-8), 7.15 (1H, *dd*, $J=2.4$. 1.0 Hz, H-3′), 6.27 (1H, *d*, $J=9.8$ Hz, H-3), 5.54 (1H, *tq*, $J=7.1$, 1.3 Hz, H-2″), 5.04 (1H, *s*, H-6″), 5.03 (1H, *d*, 7.1 Hz, H-1″), 2.08 (2H, *m*, H-5″), 2.07 (2H, *m*, H-4″), 1.67 (3H, *br s*, 9″-CH_3), 1.63 (3H, *br s*, 8″-CH_3), 1.57 (3H, *br s*, 10″-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 163.3 (C-2), 159.8 (C-7), 153.9 (C-8a), 150.5 (C-5), 146.9 (C-2′), 144.4 (C-3″), 141.5 (C-4), 132.8 (C-7″), 124.8 (C-6″), 120.5 (C-2″), 116.1 (C-6), 113.1 (C-3), 109.0 (C-4a), 106.3 (C-3′), 94.9 (C-8), 70.9 (C-1″), 40.6 (C-4″), 27.3 (C-5″), 25.8 (8″-CH_3), 17.8 (10″-CH_3), 16.7 (9″-CH_3); ESIMS *m*/*z*: 337 $[M+H]^+$.

3.9. 5-(7-Hydroxy-3,7-dimethylocta-2,5-dienyloxy)psoralen (notoptol) (**6**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR (CD_3OD, 400 MHz), δ 8.26 (1H, *dd*, $J=9.8$, 0.5 Hz, H-4), 7.79 (1H, *d*, $J=2.4$ Hz, H-2′), 7.21 (1H, *m*, H-8), 7.15 (1H, *dd*, $J=2.4$, 0.9 Hz, H-3′), 6.29 (1H, *d*, $J=9.8$ Hz, H-3), 5.59 (1H, tq, $J=7.1$, 1.3 Hz, H-2″), 5.58 (1H, *m*, H-6″), 5.56 (1H, *m*, H-5″), 5.03 (1H, *d*, 7.1 Hz, H-1″), 2.76 (2H, *br d*, $J=6.2$ Hz, H-4″), 1.65 (3H, *br s*, 9″-CH_3), 1.26 (2 × 3H, 2 × *s*, 8″-CH_3 and 10″-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 163.3 (C-2), 159.8 (C-7), 153.9 (C-8a), 150.4 (C-5), 147.0 (C-2′), 143.2 (C-3″), 141.6 (C-4), 138.1 (C-6″), 132.3 (C-7″), 128.2 (C-5″), 121.2 (C-2″), 116.4 (C-6), 113.2 (C-3), 109.2 (C-4a), 106.2 (C-3′), 95.0 (C-8), 82.4 (C-6″), 71.0 (C-1″), 43.3 (C-4″), 25.0 (8″-CH_3 and 10″-CH_3), 16.8 (9″-CH_3); ESIMS *m*/*z*: 355 $[M+H]^+$.

3.10. 5-(6-Hydroxy-3,7-dimethylocta-2,7-dienyloxy) psoralen (**7**)

White solid; UV λ_{max} MeOH nm: 254, 306; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 355 $[M+H]^+$, HR-EIMS *m*/*z*: 355.1553 $[M+H]^+$ (calc. for $C_{21}H_{23}O_5$, 355.1545).

3.11. Biological assays

Compounds **1**–**7** were dissolved in acetone and applied to glass-fibre discs in a binary choice test as described previously (Simmonds et al., 1990) and tested against final stadium larvae of *Spodoptera littoralis* Boisd. and *Heliothis virescens* F., 36–48 h into the stadium, which had been deprived of food for 2–3 h prior to the bioassay. The compounds were tested at a range of conc. between 5×10^{-5} and 1×10^{-3} M. The Feeding Index ((C–T)/(C + T)) × 100 was calculated using the amount of control (C) and treatment (T) discs eaten

during the 18 h bioassay. The Wilcoxon matched-pairs test was used to evaluate the significance of the amount of the C and T discs eaten at 1×10^{-3} M. The conc. required to elicit a Feeding Index of 50% (FI_{50}) has been calculated by regression. Both the Feeding Index at 1×10^{-3} M and the FI_{50} values are presented so comparisons can be made with other published data on antifeedants.

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