Punisterone [(20*R*,24*S*)-25-Deoxy-11α,20,24-trihydroxyecdysone]: A New Phytoecdysteroid from *Blandfordia punicea*

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A new phytoecdysteroid, punisterone [(20*R*,24*S*)-25-deoxy-11α,20,24-trihydroxyecdysone] (**1**), together with six known ones, ecdysone, 20-hydroxyecdysone, 5β,20-dihydroxyecdysone, ponasterone C, pterosterone, and turkesterone, have been isolated from the seeds of *Blandfordia punicea*.

Insect steroid hormone analogues (phytoecdysteroids) occur in significant concentrations in a wide range of ferns, gymnosperms, and angiosperms. These compounds are believed to contribute to the deterrence of invertebrate predation. Approximately 150 different phytoecdysteroids have been chemically identified to date.[1] The identification of new phytoecdysteroid structures is important for the determination of structure−activity relationships for the binding of ecdysteroids to the insect steroid hormone (ecdysteroid) receptor.[2] The genus *Blandfordia* (Blandfordiaceae) has not previously been reported to contain phytoecdysteroids. As part of our ongoing search for ecdysteroids from new plant sources,[3,4] we have examined two members of this genus (Table 1): *Blandfordia punicea* Sweet ("Tasmanian Christmas Bells") and *Blandfordia grandiflora* R. Br. ("Christmas Bells"), which are rhizomatous herbs endemic to eastern Australia.[5,6] Initial analysis of MeOH extracts of seeds with ecdysteroid-specific radioimmunoassays (RIA) and bioassays for insect ecdysteroid receptor agonists and antagonists[3] (Table 1) revealed high levels of ecdysteroids in both species of *Blandfordia*, which were detected with all three antisera and in the agonist bioassay. Analytical RP- and NP-HPLC separation of extracts with UV, RIA, and bioassay monitoring indicated the presence of several ecdysteroids. The extract from *B. grandiflora* seeds contained predominantly the known phytoecdysteroids 20-hydroxyecdysone (20*E*, **3**), 5β,20-hydroxyecdysone (polypodine B, 5,20*E*, **4**), and ecdysone (*E*, **2**). Because the extract of *B. punicea* (Figure 1) appeared to contain significant amounts of additional phytoecdysteroids, we isolated and identified the major ecdysteroids present in seeds of this species.
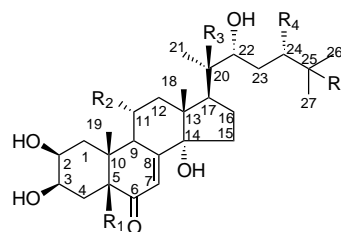
Seven phytoecdysteroids were isolated and characterized as (20*R*,24*S*)-25-deoxy-11α,20,24-trihydroxyecdy-

Table 1. Analysis of *Blandfordia* Seed Extracts for Ecdysteroid Agonists and Antagonists

	radioimmunoassay (μg ecdysone equivalents/g seed)			bioassay[a]	
species	black	white	DBL-1	agonist	antagonist
B. grandiflora	798.8	1299	2387	+++	−
B. punicea	403.1	764.5	1478	+++	−

[a] Bioassay results: active as neat extract (+), 10-fold dilution (++), 100-fold dilution (+++), and not active (−).

sone (named punisterone) (**1**), *E* (**2**),[1,7] 20*E* (**3**),[1,7] 5,20*E* (**4**),[1,8] ponasterone C (PoC, **5**),[1,9] pterosterone (**6**),[1,10] and turkesterone (**7**).[1,11] All except **1** were identified by direct comparison of their spectroscopic and HPLC characteristics with those of authentic samples. These ecdysteroids (**1**−**7**) can be envisaged as forming a biogenic sequence differing by the occurrence or absence of hydroxyl groups at C-5, C-11, C-20, C-24, and C-25.

	R₁	R₂	R₃	R₄	R₅
1	H	OH	OH	OH	H
2	H	H	H	H	OH
3	H	H	OH	H	OH
4	OH	H	OH	H	OH
5	OH	H	OH	OH	H
6	H	H	OH	OH	H
7	H	OH	OH	H	OH

Positive responses to ecdysteroid bioassay/RIA[12,13] and UV absorption (241.4 nm), characteristic for ecdysteroids, suggested **1** to be a phytoecdysteroid. CIMS revealed a molecular mass of 496 daltons (owing to the ready loss of a H_2O molecule; M − H_2O^+ instead of M⁺

a

Retention Time

b

Fraction Number

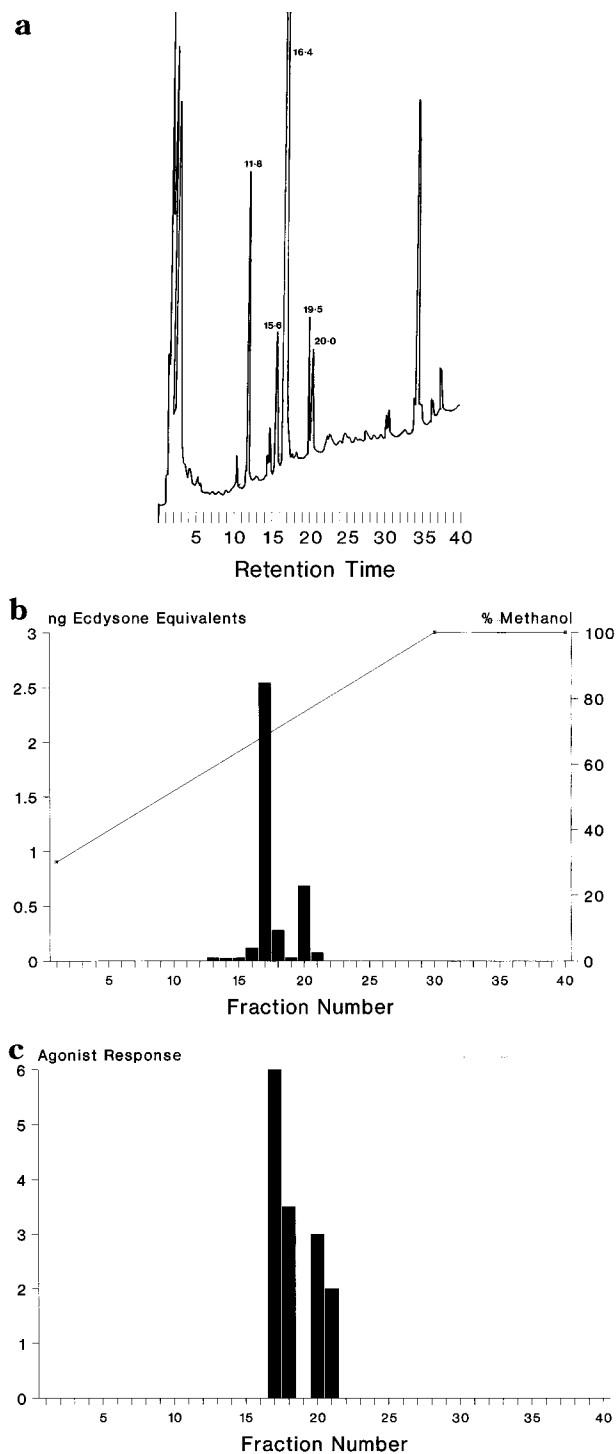**c**

Fraction Number

Figure 1. RP-HPLC separation of a MeOH extract of *B. punicea* seeds, monitored by (**a**) UV detection (242 nm), (**b**) RIA and, (**c**) agonist bioassay. Column D was eluted at 1 mL/min with a linear gradient from 30 to 100% MeOH over 30 min, followed by elution with MeOH for 10 min. The amount of extract injected was equivalent to 1.3 mg seed. Fractions (1 mL) were collected and aliquots (2 μL) were subjected to RIA (DBL-1 antiserum) and bioassay. The identities of the phytoecdysteroids in the UV-absorbing peaks are as follows: 11.8 min = turkesterone; 15.6 min = punisterone; 16.4 min = 20*E* and 5,20*E*; 19.5 min = *E*; 20.0 min = pterosterone and ponasterone C.

was observed), which accounted for $C_{27}H_{44}O_8$. The absence of an $[M]^+$ ion due to dehydration is a general characteristic for 11α-hydroxyecdysteroids.[1] In its ^{1}H-NMR spectrum (Table 2), the signals for the protons of the steroidal part indicated an ecdysteroid having 2β-,

3β-, or 11α-hydroxy groups and a 5β-A/B-ring junction[14] and were comparable to those exhibited by ajugasterone C[15,16] and paxillosterone.[1] For a better comparison, the ^{1}H-NMR spectrum of a standard sample of ajugasterone C was also obtained in D_2O and is presented together with the data for **1** (Table 2). In the C_8 side chain of **1**, like those of ajugasterone C and paxillosterone, the absence of a C-25 hydroxy group was confirmed from the methyl doublets (δ 0.89 and δ 0.96, J = 6.8 Hz) owing to the couplings between H-25 and the methyls (Me-26 and Me-27). An additional −OH group on the side chain (when compared to ajugasterone C) was evident from a deshielded 1H multiplet (δ 3.71) observed in the −CHOH region of the ^{1}H-NMR spectrum and from the MS fragment ion m/z 362 [M + H − H_2O − 117], which was due to the loss of a C_6 unit from the side chain. The splitting patterns of H-22 (δ 3.61, br d, J = 10 Hz) and H-23a (δ 1.47, ddd, J = 13, 10, 7 Hz) signals and the signal for H-25 (δ 1.78), which was more deshielded than that of ajugasterone C (δ 1.55) (Table 2), indicated that this extra hydroxy group should be affixed to C-24, rather than to C-23. The assignment of a C-24 OH group was confirmed from the ^{1}H−^{1}H correlations (TOCSY first relay)−26/27-Me (δ 0.89, δ 0.96) → H-25 (δ 1.78) → H-24 (δ 3.71), and from ^{1}H−^{13}C correlations (pulsed-field gradient HMBC)−^2J from 26/27-Me (δ 1.24) to C-25 (δ 31.2) and ^3J to C-24 (δ 75.5) (Table 3). The ^{1}H-NMR and a ^{1}H−^{1}H TOCSY-NMR spectra of **1** were also obtained in pyridine-d_5 in order to have direct comparison for the chemical shifts and coupling patterns for the protons in the side chain of **1** with those for pterosterone and 24-*epi*-pterosterone (Table 4), and, on the basis of this comparison, the chiralities at C-20, C-22, and C-24 were determined, respectively, as *R*, *R*, and *S*. Owing to the paucity of sample, a good ^{13}C-NMR spectrum could not be obtained, but the proton-detected ^{1}H-^{13}C PFG-HMBC and HMQC experiments revealed all the major ^{1}H−^{13}C correlations and thus enabled unequivocal assignments of all the carbons (precision of the ^{13}C chemical shifts = ±0.1 ppm). Thus, the structure of punisterone was assigned unambiguously as **1**.

The genus *Blandfordia*, previously classified in the family Liliaceae,[5] has been placed recently into a new family, Blandfordiaceae, which comprises only one genus.[6] Phytoecdysteroids have been detected in certain species within the Liliaceae (*sensu* Hooker and Jackson[5]): *Agapanthus* spp. (Dinan *et al.*, unpublished), *Allium sativum*,[17] *Asparagus* spp. (Dinan *et al.*, unpublished), *Paris* spp.,[18,19] and *Trillium* spp.[17,20,21] The accumulation of ecdysteroids, specially **3** and **4**, in high concentrations might have some chemotaxonomic significance in the Blandfordiaceae, as was observed for the Chenopodiaceae.[22] Although the ecological function of these ecdysteroids still remains as an open question, it has been suggested, on the basis of circumstantial evidence, that they might constitute a qualitative defense for plants and confer some protection against nonadapted phytophagous insects.[23]

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-2101PC spectrophotometer. The MS were obtained with a Riber 10-10B aparatus (Nermag S.A.) using a chemical desorption mode with NH_3 as a reagent gas.[24] ^{1}H, TOCSY, PFG-

Table 2. ^{1}H-NMR Data of Ajugasterone C and **1** and ^{13}C-NMR data of **1**[a]

position	δ ^{1}H (ppm) **1**[f]	δ ^{1}H (ppm) ajugasterone C[f]	δ ^{13}C (ppm)[b] **1**
1$_{ax}$	1.39 (1H, t, J = 13)	1.39 (1H, t, J = 13)	36.9
1$_{eq}$	2.48 (1H, **dd**, J = 13, 3.5)	2.48 (1H, **dd**, J = 13, 3.5)	
2$_{ax}$	4.09 (1H, m, $w_{1/2}$ = 12)	4.09 (1H, m, $w_{1/2}$ = 12)	66.9
3$_{eq}$	4.09 (1H, m, $w_{1/2}$ = 12)	4.09 (1H, m, $w_{1/2}$ = 12)	66.9
4$_{ax}$	1.75[c]	1.75[c]	31.4
4$_{eq}$	1.75[c]	1.75[c]	
5	2.32[c]	2.30[c]	51.1
6			206.4
7	5.97 (1H, **br d**, J = 2)	5.98 (1H, **d**, J = 2)	121.5
8			164.9
9$_{ax}$	3.12 (1H, **br dd**, J = 9, 2)	3.12 (1H, **br dd**, J = 9, 2.1)	41.1
10			38.2
11$_{ax}$	4.22 (1H, m, $w_{1/2}$ = 24)	4.22 (1H, m, $w_{1/2}$ = 24)	68.1
12$_{ax}$	2.05[c]	2.05[c]	41.5
12$_{eq}$	2.27 (1H, **dd**, J = 13, 5.9)	2.27 (1H, **dd**, J = 13, 5.9)	
13			47.1
14			84.3
15a	2.06[c,d]	2.05[c,d]	30.2
15b	1.66[‡c]	1.65[c,e]	
16a	1.90[c,d]	1.86[c,d]	19.9
16b	1.80[c,e]	1.80[c,e]	
17	2.30[c]	2.35[c]	48.5
18	0.86 (3H, s)	0.86 (3H, s)	17.4
19	1.09 (3H, s)	1.09 (3H, s)	22.9
21	1.27 (3H, s)	1.24 (3H, s)	18.9
22	3.61 (1H, br d, J = 10)	3.43 (1H, br d, J = 10)	75.2
23a	1.47 (1H, **ddd**, J = 13, 10, 7)	1.25[c]	34.7
23b	1.80[c]	1.60[c]	
24a	3.71 (1H, m, $w_{1/2}$ = 12)	1.37[c]	75.5
24b		1.26[c]	
25	1.78[c]	1.55[c]	31.2
26	0.89 (3H, **d**, J = 6.8)	0.89 (3H, **d**, J = 6.8)	15.1
27	0.96 (3H, **d**, J = 6.8)	0.91 (3H, **d**, J = 6.8)	18.5

[a] Spectra obtained in D_2O and referenced to TSP-d_4. [b] Data obtained from PFG-HMQC and PFG-HMBC experiments. [c] δ obtained from cross peaks in 2D ^{1}H−^{1}H TOCSY. [d,e] Values could be reversed. [f] J (coupling constant) and $w_{1/2}$ (width at half-height) are in Hz, and ax = axial, eq = equatorial.

Table 3. ^{1}H-^{13}C PFG-HMQC Direct Correlation (^1J) and ^{1}H−^{13}C PFG-HMBC Long-Range Correlation (^2J and ^3J) in **1**[a]

proton	δ ^{13}C ^1J	δ ^{13}C ^2J	δ ^{13}C ^3J
H$_2$-1	36.9 (C-1)	66.9 (C-2), 38.2 (C-10)	66.9 (C-3), 41.1 (C-9)
H-2	66.9 (C-2)	36.9 (C-1), 66.9 (C-3)	38.2 (C-10), 31.4 (C-4)
H-3	66.9 (C-3)	31.4 (C-4), 66.9 (C-2)	36.9 (C-1), 51.1 (C-5)
H$_2$-4	31.4 (C-4)	51.1 (C-5)	
H-5	51.1 (C-5)	66.9 (C-3)	
H-7	121.5 (C-7)	164.9 (C-8), 206.4 (C-6)	51.1 (C-5), 84.3 (C-14)
H-9	41.1 (C-9)	68.1 (C-11)	
H-11	68.1 (C-11)	41.1 (C-9)	164.9 (C-8), 47.1 (C-13)
H$_2$-12	41.5 (C-12)	68.1 (C-11), 47.1 (C-13)	41.1 (C-9), 84.3 (C-14), 17.4 (C-18)
H$_2$-15	30.2 (C-15)		
H$_2$-16	19.9 (C-16)		
H-17	48.5 (C-17)		84.3 (C-14)
H-22	75.2 (C-22)	77.6 (C-20)	75.5 (C-24), 48.5 (C-17), 18.9 (C-21)
H$_2$-23	34.7 (C-23)		
H-24	75.5 (C-24)	34.7 (C-23), 31.2 (C-25)	75.2 (C-22), 15.1 (C-26), 18.5 (C-27)
H-25	31.2 (C-25)		
Me-18	17.4 (C-18)	47.1 (C-13)	84.3 (C-14), 48.5 (C-17), 41.5 (C-12)
Me-19	22.9 (C-19)	38.2 (C-10)	51.1 (C-5), 41.1 (C-9), 36.9 (C-1)
Me-21	18.9 (C-21)	77.6 (C-20)	48.5 (C-17), 75.2 (C-22)
Me-26	15.1 (C-26)	31.2 (C-25)	75.5 (C-24), 18.5 (C-27)
Me-27	18.5 (C-27)	31.2 (C-25)	75.5 (C-24), 15.1 (C-26)

[a] Spectra obtained in D_2O.

HMQC, and PFG-HMBC NMR spectra were recorded on a Bruker AMX500 (Bruker AMX400 in the case of ^{1}H and ^{13}C NMR of the 20E and 5,20E compounds) instrument using standard Bruker microprograms. Sep-Pak Vac 35 mL (10 g) C_{18} cartridges (Waters) were used for initial fractionation of extracts. HPLC separation was performed with a Gilson model 811 HPLC coupled with Gilson 160 diode-array detector and using Gilson Unipoint computer program. Technoprep 10C8

preparative C_8 (column A), Apex II Diol 5 μm (Jones Chromatography) semipreparative (column B), Apex II Diol 5 μm (Jones Chromatography) analytical, Zorbax Silica semipreparative (column C), and Spherisorb ODS-2 5μm analytical (column D) columns were used. The chromatographic separations were monitored at 242 nm.

Plant Material. Seeds of *B. grandiflora* R. Br. and *B. punicea* Sweet were purchased, respectively, from

Table 4. ^{1}H-NMR Data (in Pyridine-d_5) for the Side Chain of **6**, 24-*epi*-Pterosterone, and **1**[a]

position	**6**[b]	**1**	24-*epi*-pterosterone[c]
		δ ^{1}H ppm	
21	1.59 (3H, s)	1.59 (3H, s)	1.66 (3H, s)
22	4.12 (1H, **br d**, $J = 10.6$)	4.13 (1H, **br d**, $J = 10.0$)	4.13 (1H, br s)
23a		1.80 m	1.87 m
23b		2.00 m	2.03 m
24a	3.94 (1H, dt, $J = 9.0, 4.0$)	3.94 (1H, m, $W_{1/2} = 22$)	4.50 (1H, br d, $J = 10.1$)
25		1.70 m	1.85 m
26	1.00 (3H, d, $J = 6.6$)	1.00 (3H, d, $J = 6.8$)	1.01 (3H, d, $J = 6.4$)
27	1.01 (3H, d, $J = 6.6$)	1.01 (3H, d, $J = 6.8$)	1.10 (3H, d, $J = 6.4$)

[a] J (coupling constant) and $W_{1/2}$ (width at half-height) are in Hz. [b] Data obtained from Lafont and Wilson (1992).[1] [c] Data obtained from Ohta *et al.* (1996).[25]

Chiltern Seeds, Cumbria, U.K. (cat. no. 204J), and B & T World Seeds, Whitnell House, Fiddington, Somerset, U.K. (cat. no. 38049).

Bioassay. The biological activities (ecdysteroid agonist or antagonist) of extracts, Sep-Pak, and HPLC fractions were determined with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line.[12]

Radioimmunoassay (RIA). RIA was performed according to a procedure described previously[13] using ecdysteroid-specific antisera, DBL-1, Black and White, which were donated by Prof. J. Koolman, University of Marburg, FRG. The cross-reactivities of these antisera with a number of phytoecdysteroids are given elsewhere.[3]

Microextraction and Analytical HPLC Analysis. Seeds were ground with a pestle and mortar. Samples (<25 mg) were extracted three times with MeOH (1 mL) at 55 °C. The pooled extracts were mixed with 1.3 mL of H_2O and 2 mL of *n*-hexane. The aqueous MeOH phase was analyzed for ecdysteroid content by RIA, bioassay, and HPLC (Figure 1).

Large-Scale Extraction and Isolation. Powdered seeds of *B. punicea* (10 g) were extracted with MeOH (3 × 200 mL, 3 × 24 h) at 55 °C, with continuous stirring using a magnetic stirrer. The extracts were pooled and diluted to a 70% aqueous MeOH solution. After being defatted with *n*-hexane, the extract was concentrated (<45 °C). Sep-Pak fractionation of the concentrated extract (redissolved in 10% aqueous MeOH) applying MeOH–H_2O step-gradient elution, followed by bioassay/ RIA, revealed the presence of ecdysteroids in the fractions eluted with 25% and 60% aqueous MeOH. The RP-HPLC analysis of the fraction (25% aqueous MeOH) using column A (isocratic, 30% MeOH in H_2O, 5 mL/ min) resulted in the isolation of **7**, the purity of which was checked with a standard sample in column C (isocratic, cyclohexane–2-propanol–H_2O, 100:40:3, 4 mL/min). Similar treatment (isocratic, 55% MeOH in H_2O, 5 mL/min) of the other fraction (60% aqueous MeOH) yielded six fractions, numbered in increasing order of their retention times. NP-HPLC of fraction 4 using column B (isocratic, 4% MeOH in CH_2Cl_2, 2 mL/ min) afforded **1**, which was further purified using column C (isocratic, cyclohexane–2-propanol–H_2O, 100: 40:3, 4 mL/min). Similar treatment of fraction 5 yielded compounds **3** and **4**, fraction 6 yielded **2**, and a mixture of **5** and **6**, which were separated in column C (isocratic, cyclohexane–2-propanol–H_2O, 100:40:3, 4 mL/min).

Punisterone [(20*R***,24***S***)-25-deoxy-11α,20,24-trihydroxyecdysone] (1)** (0.4 mg): amorphous; UV λ max (log ϵ) (MeOH) 241.4 (4.08) nm; ^{1}H-NMR data, see Table

2; CIMS m/z 496 [M + H + NH_3 − H_2O]$^+$, 479 [M + H − H_2O]$^+$, 461 [479 − H_2O]$^+$, 443 [461 − H_2O]$^+$, 425 [443 − H_2O]$^+$, 407, 391, and 362 [M + H − 117]$^+$.

Ecdysone (2) (0.8 mg): amorphous; HPLC, UV, ^{1}H-NMR, and CIMS data in agreement with literature.[1,7]

20-Hydroxyecdysone (3) (20 mg): amorphous; HPLC, UV, ^{1}H-NMR, ^{13}C-NMR, and CIMS data in agreement with literature.[1,7]

5β,20-Dihydroxyecdysone (4) (14.0 mg): amorphous; HPLC, UV, ^{1}H-NMR, ^{13}C-NMR, and CIMS data in agreement with literature.[1,8]

Ponasterone C(5) (0.25 mg): amorphous; HPLC, UV, ^{1}H-NMR, and CIMS data in agreement with literature.[1,9]

Pterosterone (6) (0.20 mg): amorphous; HPLC, UV, ^{1}H-NMR, and CIMS data in agreement with literature.[1,10]

Turkesterone (7) (1.5 mg): amorphous; HPLC, UV, ^{1}H-NMR, and CIMS data in agreement with literature.[1,11]

References and Notes

(1) Lafont, R. D.; Wilson, I. D. *The Ecdysone Handbook*; Chromatographic Society: Nottingham, U.K., 1992.
(2) Dinan, L. In *Ecdysone*, Koolman, J., Ed.; Thieme Verlag: Stuttgart, 1989; pp 345−354.
(3) Dinan, L. *Eur. J. Entomol.* **1995**, *92*, 271−283.
(4) Sarker, S. D.; Girault, J.-P.; Lafont, R.; Dinan, L. N. *Biochem. Syst. Ecol.*, in press.
(5) Hooker, D.; Jackson, B. D. *Index Kewensis*, Clarendon Press: Oxford, U.K., 1893; Vol. 1, p 311.
(6) Brummitt, R. K. *Vascular Plant Families and Genera*, Royal Botanic Gardens: Kew, U.K., 1992; p 702.
(7) Girault, J.-P.; Lafont, R. *J. Insect Physiol.* **1988**, *34*, 701−706.
(8) Nishimoto, N.; Shiobora, Y.; Fujino, M.; Inoue, S. S.; Takemoto, T.; De Oliveira, F.; Akisue, G.; Akisue, M. K.; Hashimoto, G.; Tanaka, O.; Kasai, R.; Matsuura, H. *Phytochemistry* **1987**, *26*, 2505−2507.
(9) Nakanishi, K.; Koreeda, M.; Chang, M. L.; Hsu, H. Y. *Tetrahedron Lett.* **1968**, 1105−1110.
(10) Takemoto, T.; Hikino, Y.; Arai, T.; Kawahara, M.; Konno, C.; Arihara, S.; Hikino, H. *Chem. Pharm. Bull.* **1967**, *15*, 1816.
(11) Usmanov, B. Z.; Gorovitz, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1975**, 466−470.
(12) Clément, C. Y.; Bradbrook, D. A.; Lafont, R.; Dinan, L. *Insect Biochem. Molec. Biol.* **1993**, *23*, 187.
(13) Dinan, L. *Phytochem. Anal.* **1992**, *3*, 132−138.
(14) Girault, J.-P.; Bathori, M.; Varga, E.; Szendrei, K.; Lafont, R. *J. Nat. Prod.* **1990**, *53*, 279−293.
(15) Imai, S.; Murata, E.; Fujioka, S.; Koreeda, M.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1969**, 546−547.

(16) Kubo, I.; Klocke, J. A.; Ganjian, I.; Ichikawa, N.; Matsumoto, T. *J. Chromatogr.* **1983**, *257*, 157−161.

(17) Matsuoka, T.; Imai, S.; Sakai, M.; Kamada, M. *Annu. Rept. Takeda Res. Lab.* **1969**, *28*, 221−271.

(18) Novoselskaya, N. L.; Gorovits, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1981**, 402−403.

(19) Singh, S. B.; Thakur, R. S. *Tetrahedron* **1982**, *38*, 2189−2194.

(20) Imai, S.; Toyosato, T.; Sakai, M.; Sato, Y.; Fujioka, S.; Murata, E.; Goto, M. *Chem. Pharm. Bull.* **1969**, *17*, 335−339.

(21) Imai, S.; Toyosato, T.; Sakai, M.; Sato, Y.; Fujioka, S.; Murata, E.; Goto, M. *Chem. Pharm. Bull.* **1969**, *17*, 340−342.

(22) Dinan, L. *Eur. J. Entomol.* **1995**, *92*, 295−300.

(23) Bergamasco, R.; Horn, D. H. S. In *Endocrinology of Insects*; Downer, R. G. H., Laufer, H., Eds.; A. R. Liss: New York, 1983; Vol. 1, pp 627−654.

(24) Lafont, R.; Beydon, P.; Mauchamp, B.; Sommé-Martin, G.; Andrianjafintrimo, M.; Krien, P. In *Regulation of Insect Development and Behaviour*; Sehnal, F., Zabza, A., Menn, J. J., Cymborowski, B., Eds.; Technical University of Wrocław: Wrocław, Poland; 1981; pp 125−144.

(25) Ohta, S.; Guo, J.-R.; Hiraga, Y.; Suga, T. *Phytochemistry* **1996**, *41*, 745−747.

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