Synthesis of chiloglottones – semiochemicals from sexually deceptive orchids and their pollinators†

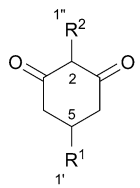
Jacqueline Poldy,[a,b] Rod Peakall[b] and Russell Allan Barrow*[a]

Received 2nd July 2009, Accepted 31st July 2009
First published as an Advance Article on the web 17th August 2009
DOI: 10.1039/b912233h

A five-step synthesis of monoalkyl- and 2,5-dialkyl-1,3-cyclohexanediones (**1**) is described *via* a sequence involving sequential Birch reductions and alkylations from the readily accessible and inexpensive starting material, 3,5-dimethoxybenzoic acid. Two approaches were considered in which alkylation at C-2 occurs either prior or subsequent to the proposed reduction. The successful route, in which Birch reduction of a 3-alkyl resorcinol derivative (**3**) precedes alkylation was applied in the synthesis of chiloglottone 1 (**1dc**), in 58% overall yield. Chiloglottone 1 is a member of a new class of natural products, representing a known sex pheromone of the thynnine wasp *Neozeleboria cryptoides* and pollinator attractant in the Australian sexually deceptive orchid genus *Chiloglottis*. The synthetic homologues were assessed for their biological activity *via* electroantennographic detection.

Introduction

Australian *Chiloglottis* orchids are known to release compounds representing an entirely new class of natural product[1] that mimic the female sex pheromones of their thynnine wasp pollinators. These chemical signals elicit sexual behavior in the male wasps, which can lead to copulatory attempts resulting in pollination of the orchid flower. In one example, chiloglottone 1 (**1dc**) has been confirmed from both the female insect *Neozeleboria cryptoides* and the flower of *C. trapeziformis* as the single unique chemical responsible for attraction of the male wasps.[2] In related orchid taxa, the prevalence of chiloglottones is demonstrated by the recent disclosure of biologically active analogues, chiloglottone 2 (**1fc**) and chiloglottone 3 (**1be**),[1] which differ in their substitution while conserving the 2,5-dialkyl-1,3-cyclohexanedione skeleton (Fig. 1; Table 1). It is predicted that these allomones are identical to the female released sex pheromone of the respective pollinators, but this has been difficult to confirm for all but one case,[2] as female



1

chiloglottone 1
R^1 = Pr, R^2 = Et (**1dc**)

chiloglottone 2
R^1 = Pent, R^2 = Et (**1fc**)

chiloglottone 3
R^1 = Me, R^2 = Bu (**1be**)

Fig. 1 Molecular structures of chiloglottones 1–3.

Table 1 Labelling for dialkyl-1,3-cyclohexanediones and related alkylated compounds

	R^1	H	Me	Et	Pr[a]	Bu	Pent
R^2		**a**	**b**	**c**	**d**	**e**	**f**
H	**a**	aa	ba	ca	da	ea	fa
Me	**b**	ab	bb	cb	db	eb	fb
Et	**c**	ac	bc	cc	dc	ec	fc[a]
Pr	**d**	ad	bd	cd	dd	ed	fd
Bu	**e**	ae	be[a]	ce	de	ee	fe
Pent	**f**	af	bf	cf	df	ef	ff
Hex	**g**	ag	bg	cg	dg	eg	fg

[a] Dialkyl-1,3-cyclohexanedione natural products, and analogues with R^1 = Pr, were prepared as representative targets using the synthetic route.

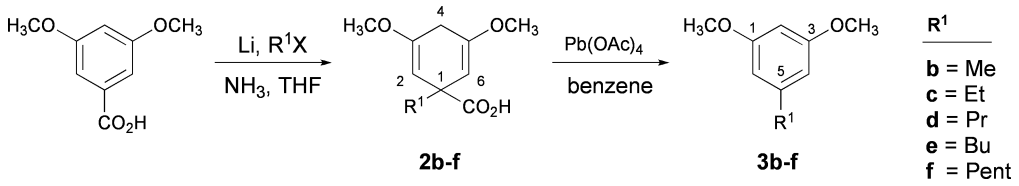
thynnines spend much of their lives underground emerging only to mate.[3,4]

As likely products of fatty acid biosynthetic pathways, it is anticipated that chiloglottones and related compounds represent a widespread class of new natural products that will be encountered across diverse groups of living organisms. To date, the known chiloglottones 1–3 exhibit a substitution pattern characterized by even numbers of carbons at the 2-position and odd numbers of carbons at the 5-position, consistent with biosynthesis from putative fatty acid precursors.[1] Dialkylresorcinols, with an apparent similarity to the chiloglottones, have been reported from *Pseudomonas sp.* displaying the same pattern of C-2 even, C-5 odd substitution,[5] resulting from head-to-head condensation of two fatty-acid derived subunits.[6] The biosynthesis of chiloglottones in *Chiloglottis* and related orchids is currently under investigation in our laboratories.

Recently we reported a synthetic route delivering a selection of mono and disubstituted chiloglottone 1 derivatives employing a cadmium mediated desymmetrisation as the pivotal step in a comparatively lengthy nine step synthesis.[7] As part of our ongoing interest in chiloglottone analogues, we report here an approach that substantially improves upon the efficiency of synthesis allowing rapid access (5 steps) to variably substituted

[a]*Research School of Chemistry, The Australian National University, Canberra, ACT, 0200, Australia. E-mail: rab@anu.edu.au; Fax: +61 2 6125 0760; Tel: +61 2 6125 3419*
[b]*Research School of Biology, The Australian National University, Canberra, ACT, 0200, Australia*

† Electronic supplementary information (ESI) available: Experimental procedures and analytical data for all compounds **2d–f**, **3a–f**, **4a–f** and **1da–dg**, along with copies of ^{1}H and ^{13}C NMR spectra for natural products and new compounds **1da–dg**, **1fc**, **1be**, **4c–e**. See DOI: 10.1039/b912233h



Scheme 1 Synthesis of alkyl resorcinol derivatives **3** from 1,3-dimethoxybenzoic acid.

2,5-dialkyl-1,3-cyclohexanediones, exemplified by the preparation of chiloglottones 1–3 and homologues **1da–dg** (Table 1). This approach will allow the timely preparation of these unstable molecules, facilitating identification and field studies that will elucidate the role of this evolving class of semiochemicals in orchid pollination.

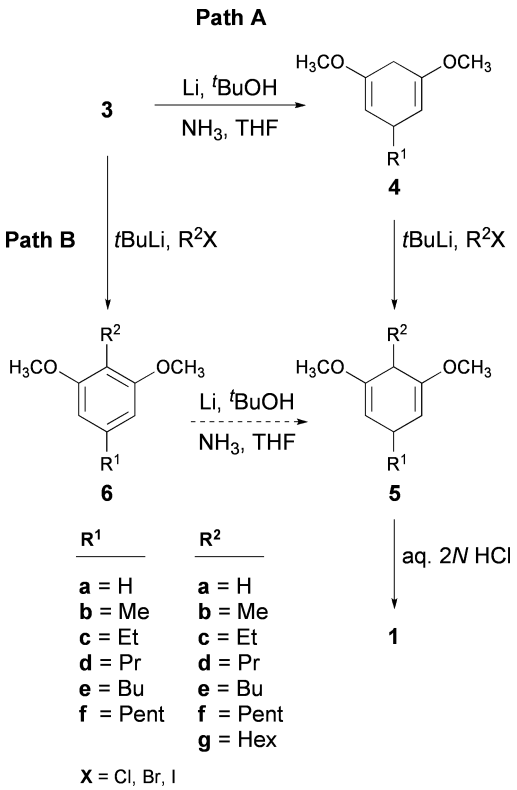
Results and discussion

Treating 3,5-dimethoxybenzoic acid with linear alkyl halides under Birch reductive-alkylation conditions readily afforded the diene acids **2b–f** in very good yields (77–99%) (Scheme 1).[8,9] As indicated by ^{1}H and ^{13}C NMR data, these compounds were of high purity and could generally be used without further purification. Where purification was necessary or desired the crude solids could be readily recrystallised from CH_2Cl_2, delivering clear colorless crystals.

Oxidative decarboxylation of compounds **2b–f** using lead tetraacetate in benzene, with minor modifications on a reported procedure,[8] returned the 5-alkylresorcinol derivatives **3b–f** in excellent yields (91–99%). These mobile oils could be purified by Kugelrohr distillation or chromatographed on silica (CH_2Cl_2 as eluent) as required, but generally gave products suitable for subsequent reaction.

At this point we considered the aromatic substrates **3** might represent a potential divergence in the synthesis. Thus we envisaged **Path A** (Scheme 2) would proceed with Birch reduction of **3** to give the 3-alkyl-1,5-dimethoxycyclohexadienes **4**, which would be alkylated to the 3,6-dialkyl-1,5-dimethoxycyclohexadienes **5** and then hydrolyzed under mild acid conditions to the target cyclohexanediones **1**. **Path B** sees an inversion of reduction and alkylation steps, with an initial directed *ortho* metalation to give the 2,5-dialkyl-1,3-dimethoxybenzenes **6**, with the anticipated Birch reduction on this aromatic material converging at the 1,5-dimethoxycyclohexadienes **5**.

In our investigations of **Path B**, directed *ortho* metalations of **3** gave the 2,5-dialkyl-1,3-dimethoxybenzenes **6** under mild conditions (**R^1** = Pr, **R^2** = Me, Et, Pr). However, these 1,2,3,5-tetrasubstituted aromatics failed to undergo Birch reduction under a variety of conditions, returning unaltered starting material. The ^{1}H NMR spectra showed no trace of the anticipated product **5** when either *t*-butyl alcohol or ethanol were employed as the source of protons in conjunction with lithium or sodium as the electron source. This finding is corroborated by an absence in the literature of *any* accounts of substrates of this nature undergoing Birch reductions. Indeed, we are aware of only one account of a resorcinol derivative with substitution at both C-2 and C-5 participating in such a reaction. Here the C-2 group is electron withdrawing,[10] in contrast to the mildly donating nature of our alkyl substituents.



Scheme 2 Two envisaged pathways to 2,5-dialkyl-1,3-cyclohexanediones **1** from 5-alkyldimethoxy benzenes **3**.

Progress *via* **Path A** was ultimately successful with Birch reduction of **3b–f**, using a large excess of lithium and *t*-butyl alcohol, readily affording the alkyl dienes **4b–f** (61–100%). The chemically inequivalent ring methylene protons of these alkyl dienes (H-6a and H-6b) appear in the ^{1}H NMR spectra as second order multiplets centred on $\delta_H \approx 2.8$ ppm. An unusual observation is the extensive degree of coupling of the methine (H-3) resonance at $\delta_H \approx 3.0$ ppm, displaying an AL_3X_2MN spin system. The ^{1}H NMR spectrum of **4b** presents a representative multiplet at $\delta_H = 3.04$ ppm (Fig. 2). A series of selective irradiations of the coupled resonances revealed that the thirteen clean constituent peaks comprise couplings of $^{3}J = 6.9$ Hz (quartet) to the adjacent methyl group (H-1′), $^{3}J = 3.3$ Hz (triplet) to the olefinic protons (H-2,4), and entirely unanticipated couplings of $^{5}J = 6.9$ Hz (doublet of doublets, pseudotriplet) to the ring methylenes (H-6a and H-6b). Similar behaviour was observed for the alkyl dienes **4c–f**.

Compounds **4** represent a point of diversification in that a selection of alkyl groups can be incorporated. The six homologues, **4a–f**, can be rapidly diverged to give an expansive array of mono and dialkyl dienes **5aa–fg** (Table 1). NMR data show a distribution of stereoisomers with regard to the relationship between the

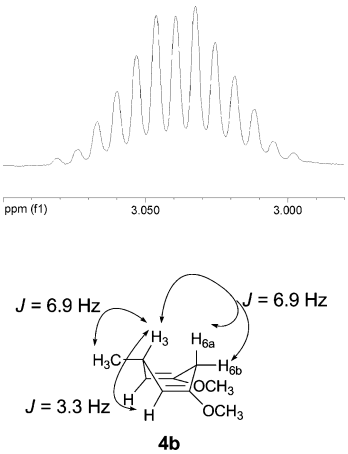


Fig. 2 The highly coupled resonance from H-3 of **4b** (500 MHz, $CDCl_3$) with *J* values distinguished through selective irradiation of coupled resonances.

3- and 6-alkyl substituents. We chose not to attempt to isolate these, recognising that hydrolysis of the vinyl ethers would remove the stereochemical differences due to epimerization about C-2. Finally, acid hydrolysis yielded compounds of **1** as white solids, with spectroscopic data consistent with those of 2,5-dialkylated-1,3-cyclohexanediones.[7] Acquisition of ^1H and ^{13}C NMR spectra in CD_3OD simplifies spectral interpretation by confining the compounds to their vinylogous acids, rather than the tautomeric distribution present in less polar solvents such as $CDCl_3$.

To demonstrate the effectiveness of the route, we aimed to use the synthesis in the preparation of biologically active compounds, including natural products chiloglottones 1, 2 and 3 (**1dc**, **1fc** and **1be**, respectively). Synthesis of chiloglottone 1 commenced from commercially available 3,5-dimethoxybenzoic acid. Birch reductive alkylation with bromopropane delivered 3,5-dimethoxy-1-propyl-2,5-cyclohexadienecarboxylic acid (**2d**) in almost quantitative yield (99%). Spectroscopic and physical data agreed with previously reported values.[9] Decarboxylation returned the aromatic compound **3d** as a clear mobile oil, in excellent yield (99%). 1,5-Dimethoxy-3-propyl-1,4-cyclohexadiene **4d** was furnished in 88% yield when **3d** was treated with lithium in refluxing liquid ammonia (–33 °C), using *t*-butyl alcohol as the source of protons. Directed metalation using *t*-butyllithium at –78 °C and iodoethane as alkylating agent gave 6-ethyl-1,5-dimethoxy-3-propyl-1,4-cyclohexadiene (**5dc**) as a stereoisomeric mixture, which was treated with aqueous 2*N* HCl in acetone. The resulting white solid (**1dc**) (82% over two steps) was characterized on the basis of its ^1H and ^{13}C APT NMR, HR EIMS and melting point. Chiloglottones 2 and 3 were similarly prepared†.

We prepared a selection of chiloglottone 1 homologues, with substitution at C-2 ranging from the unalkylated homologue through to the hexyl-substituted compound (**1da–dg**). These variably substituted 2-alkyl-5-propyl-1,3-cyclohexanediones were assessed for their biological activity *via* electroantennographic detection (EAD). Compounds that are electrophysiologically perceived by an insect antenna are indicated by a response in the electoantennograph. Male wasps belonging to the *Neozeleboria monticola* complex (pollinators of *Chiloglottis valida*) were captured by baiting with chiloglottone 1 in Kosciuszko National Park during December of 2008. The excised antennae were exposed to synthetic materials through GC-EAD, using the known attractant **1dc** as a positive control. In addition to chiloglottone 1, two compounds (**1db** and **1dd**) yielded an antennal response (Fig. 3) in seven of eight GC-EAD trials, while the C-2 unalkylated material (**1da**) and the higher homologues (**1de–dg**) showed no activity across a total of 15 experiments.

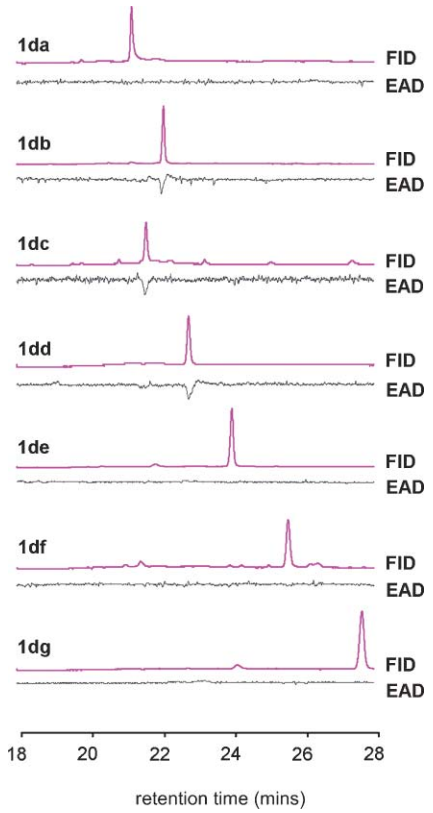


Fig. 3 Gas chromatographic analysis of synthetic cyclohexanediones **1da–dg** with simultaneous flame ionization detection (FID) and electroantennographic detection (EAD) using antennae from male *N. monticola* wasps captured when attracted to chiloglottone 1.

An absence of response does not preclude the possibility of a compound being active in other thynnine species. Equally, EAD activity does not in itself confirm that a compound will exhibit a behavioural function in the field.[11] While the substitution pattern of chiloglottones 1–3 characterized by even numbers of carbons at the C-2 position and odd numbers of carbons at the C-5 position suggests a probable biosynthesis from fatty acid precursors,[1] our 2,5-dialkyl-1,3-cyclohexanedione array includes alternative possibilities (Table 1), some of which display an EAD response but are less likely to represent behaviourally active natural products. We are currently completing this array (Table 1) to fill out the range of 2- and 5-alkyl homologues and explore their activity in related taxa.

Coupled with the recent, systematic investigation of mass spectral data from 2,5-dialkyl-1,3-cyclohexandiones,[1] this synthetic route will advance the discovery of new natural products in this class.

Conclusions

We have developed a new and general method for the synthesis of 2,5-dialkylated-1,3-cyclohexanediones **1** from commercially

available and inexpensive starting materials, *via* a sequence of Birch reduction and alkylation steps. Substitution at C-5 of compound **1** is imparted through an initial *in situ* Birch reductive alkylation of 3,5-dimethoxybenzoic acid (Scheme 1), whereas the C-2 substituent is delivered through a two-step Birch reduction/metalation process (Scheme 2, **Path A**). This methodology allows an efficient approach to construct an extensive array of analogues of chiloglottone 1 (**1dc**), which may be simultaneously female released sex pheromones of thynnine wasp and allomones in sexually deceptive *Chiloglottis* orchids to attract their male pollinators.

Experimental

General experimental methods

^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz respectively, unless indicated otherwise. When acquired in deuterated chloroform, ^{1}H NMR spectra are referenced to the resonance from residual $CHCl_3$ at 7.26 ppm, and ^{13}C NMR spectra to the central peak in the signal from $CDCl_3$, at 77.0 ppm. In d_4-methanol ^{1}H NMR spectra are referenced to the central peak of the resonance from residual CHD_2OD at 3.30 ppm, and ^{13}C NMR spectra to the central peak in the signal from CD_3OD, at 49.0 ppm. Peak assignments were established using APT, HMQC, HMBC and COSY experiments where assignment was otherwise ambiguous.

Representative proceedure for preparation of 3,5-dimethoxy-1-alkyl-2,5-cyclohexadienecarboxylic acids 2

Reductive alkylations were performed with adaptations to published procedures.[8,9] To a solution of 3,5-dimethoxybenzoic acid (1 equiv.) in dry THF (2 mL/mmol) liquid NH_3 (approx. 5 mL/mmol) was condensed. Lithium (2.2 equiv.) was added in portions at −33 °C until a deep blue color persisted. The appropriate alkyl halide (1.2 equiv.) was added dropwise, causing an immediate reversion of the color change through orange to colorless. NH_3 was evaporated under a stream of N_2 overnight. The residue was partitioned between Et_2O and H_2O, the aqueous layer chilled to 0 °C and acidified to pH 3–4 with careful addition of 2*N* HCl. The aqueous layer was reextracted (EtOAc), the organic phase washed (H_2O), dried ($MgSO_4$) and concentrated *in vacuo*. The solid residue was recrystallized from CH_2Cl_2 to return the diene acid **2**.

3,5-dimethoxy-1-propylcyclohexa-2,5-dienecarboxylic acid **2d** was obtained in 99% yield as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.68 (2H, *s*, H-2,6), 3.60 (6H, *s*, 3,5-OCH_3), 2.75 (2H, *s*, H-4), 1.73–1.67 (2H, *m*, H-1″), 1.31–1.20 (2H, *m*, H-2′), 0.90 (3H, *t*, $^3J = 7.2$, H-3′); ^{13}C APT NMR (75 MHz, $CDCl_3$): δ 182.8 (C, *C*O_2H), 153.0 (C, C-3,5), 94.8 (CH, C-2,6), 54.4 (CH_3, 3,5-O*C*H_3), 49.9 (C, C-1), 43.4 (CH_2, C-1′), 31.1 (CH_2, C-4), 17.6 (CH_2, C-2′), 14.2 (CH_3, C-3′); *m*/*z* (Electrospray ionization) 227.1289 [(M+H)$^+$. $C_{12}H_{19}O_4$ requires 227.1283 ($\Delta = 2.8$ ppm)].

Representative procedure for synthesis of 1,3-dimethoxy-5-alkylbenzenes 3

Following a published account with minor modifications;[8] to a rapidly stirred solution of **2** (1 equiv.) in benzene (20 mL/mmol) was added $Pb(OAc)_4$ (1.3 equiv.). After 30–40 min, by which time the mixture had become colorless, H_2O (approx. equivolume to benzene) was added and the mixture filtered under vacuum through a plug of silica. The aqueous phase was extracted with Et_2O and the combined organic extracts washed (sat. aqueous $NaHCO_3$ solution), dried ($MgSO_4$) and the solvents removed *in vacuo* to give **3** as a pale mobile oil.

1,3-dimethoxy-5-propylbenzene **3d** was prepared in 99% yield as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.35 (2H, *d*, $^4J =$ 2.1, H-4,6), 6.30 (1H, *t*, $^4J = 2.1$, H-2), 3.78 (6H, *s*, 3,5-OCH_3), 2.53 (2H, *t*, $^3J = 7.5$, H-1′), 1.63 (2H, *tq*, $^3J = 7.5$, $^3J = 7.5$, H-2′), 1.23 (3H, *t*, $^3J = 7.5$, H-3′); ^{13}C NMR APT NMR (75 MHz, $CDCl_3$): δ 160.6 (C, C-1,3), 145.1 (C, C-5), 106.5 (CH, C-4,6), 97.5 (CH, C-2), 55.2 (CH_3, 3,5-O*C*H_3), 38.4 (CH_2, C-1′), 24.3 (CH_2, C-2′), 13.9 (CH_3, C-3′); *m*/*z* (EI) 180.1149 [$M^{+\bullet}$ $C_{11}H_{16}O_2$ requires 180.1150 ($\Delta = 0.7$ ppm)].

Representative procedure for synthesis of 1,5-dimethoxy-3-alkyl-1,4-cyclohexadienes 4

To a solution of **3** (1 equiv.) in dry THF (approx. 4.5 mL/mmol) and *t*BuOH (approx. 4.5 mL/mmol), NH_3 (approx. 10–15 mL/mmol) was condensed. Lithium (17 equiv.) was added in portions at −33 °C and the solution allowed to warm slowly to room temperature. NH_3 was evaporated under a stream of N_2 and the residue partitioned between Et_2O and sat. aqueous NH_4Cl solution. The aqueous was reextracted (Et_2O), the combined organics dried ($MgSO_4$) and concentrated under vacuum to return the diene **4**.

1,5-dimethoxy-3-propylcyclohexa-1,4-diene **4d** was prepared in 88% yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.60–4.59 (2H, *m*, H-2,4), 3.56 (6H, *s*, 1,5-OCH_3), 3.03–2.93 (1H, *m*, H-3), 2.77–2.75 (2H, *m* [apparent *d*], $J = 6.9$, H-6), 1.37–1.34 (4H, *m*, H-1′ and H-2′), 0.91 (3H, *t*, $^3J = 7.0$, H-3′); ^{13}C APT NMR (75 MHz, $CDCl_3$): δ 151.6 (C, C-1,5), 96.0 (CH, C-2,4), 54.1 (CH_3, 1,5-O*C*H_3), 40.6 (CH_2, C-1′), 35.4 (CH_2, C-3), 31.3 (CH_2, C-6), 19.4 (CH_2, C-2′), 14.3 (CH_3, C-3′); *m*/*z* (Electrospray ionization) 183.1383 [(M+H)$^+$. $C_{11}H_{19}O_2$ requires 183.1385 ($\Delta = 1.1$ ppm)].

Representative procedure for synthesis of 1,5-dimethoxy-3,6-dialkyl-1,4-cyclohexadienes 5

Alkylations were achieved in a similar manner to previously reported methods.[12] A solution of **4** (1 equiv.) in dry THF (10 mL/mmol) was cooled to −78 °C. *t*BuLi (1.1 equiv., 1.25 M in pentane) was added dropwise *via* syringe. The solution was stirred for 30 min at −78 °C before dropwise addition of the required alkyl halide (1.6 equiv.). After 10–15 min at −78 °C the suspension was slowly warmed to r.t. and quenched with H_2O. The aqueous residue was extracted with Et_2O, the combined organic phases dried ($MgSO_4$) and the solvents removed *in vacuo*, returning **5**, which was immediately hydrolyzed to **1**, without separation of diastereomers.

Representative procedure for synthesis of 2,5-dialkyl-1,3-cyclohexanediones 1

With modifications on a reported method,[13] crude **5** (1 equiv.) was dissolved in acetone (5 mL/mmol) and aq. 2*N* HCl (3 equiv.) added. The resulting solution was stirred overnight at r.t. The

acetone was evaporated under reduced pressure and the residue diluted (H_2O), basified (aq. 1*N* NaOH) and washed with Et_2O. The aqueous layer was reacidified (pH 1–3 aq. 2*N* HCl) and extracted with EtOAc. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to return **1** as a white solid.

2-ethyl-5-propyl-1,3-cyclohexanedione **1dc** (chiloglottone 1) was synthesized in 82% over two steps. m.p. 124–126 °C; IR (neat): 2957, 2928, 2872, br. 2640, 1557, 1383, 1263, 1244, 1105 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 2.46 (2H, *dd*, $^2J = 16.5$, $^3J = 4.3$, H-4eq, 6eq), 2.25 (2H, *q*, $^3J = 7.5$, H-1″), 2.14 (2H, *dd*, $^2J = 16.5$, $^3J = 11.3$, H-4ax, 6ax), 2.07–2.01 (1H, *m*, H-5), 1.39–1.34 (4H, *m*, H-1′ and H-2′), 0.94 (3H, *t*, $^3J = 6.5$, H-3′), 0.90 (3H, *t*, $^3J = 7.5$, H-2″); ^{13}C APT NMR (125 MHz, CD_3OD): δ 176.5 (br, C, C-1,3), 118.5 (C, C-2), 39.3 (CH_2, C-4,6), 38.8 (CH_2, C-1′), 34.4 (CH, C-5), 20.7 (CH_2, C-2′), 16.0 (CH_2, C-1″), 14.4 (CH_3, C-3′), 13.6 (CH_3, C-2″); *m/z* (EI) 182.1307 [$M^{+\bullet}$ $C_{11}H_{18}O_2$ requires 182.1307 ($\Delta = 0.1$ ppm)], 182 (39%), 167 (5), 154 (6), 139 (17), 125 (42), 111 (30), 97 (100), 84 (35), 69 (39), 55 (78).

Electroantennographic studies

Gas chromatography with electroantennographic detection (GC-EAD) of the synthetic materials **1da–1dg** were performed according to previously published procedures.[2] Solutions of compounds **1da–1dg** were prepared at approximately 0.1 mg/mL. 4–5 μL of each sample were injected splitless (subsequently opened after 1 min, split ratio 1:1) into a gas chromatograph (HP 5890), equipped with a BP21 column (30 m × 0.25 mm) and a retention gap represented by a 5 m deactivated wide-bore fused silica column mounted between injector and analytical column with helium serving as carrier gas. The temperature profile commenced at 40 °C (1 min), then was ramped at 10 °C min^{-1} to 230 °C, and held for 10 min. Injector, EAD outlet and flame ionisation detector (FID) temperatures were at 250 °C. At the end of the column, the outlet was split 1:1 with one outlet directed over a male wasp's antenna prepared according to Mant *et al.*[11] The other outlet was directed to the FID. EAD signals and FID responses were recorded simultaneously.

Acknowledgements

This work was supported by an Australian Research Council grant to RP (DP0451374) and The Australian National University (ANU). JP acknowledges the ANU for an Australian Postgraduate Award.

Notes and references

1 S. Franke, F. Ibarra, C. M. Schulz, R. Twele, J. Poldy, R. A. Barrow, R. Peakall, F. P. Schiestl and W. Francke, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 8877–8882.
2 F. P. Schiestl, R. Peakall, J. G. Mant, F. Ibarra, C. Schulz, S. Franke and W. Francke, *Science*, 2003, **302**, 437–438.
3 R. Peakall, *Funct. Ecol.*, 1990, **4**, 159–167.
4 F. P. Schiestl and R. Peakall, *Funct. Ecol.*, 2005, **19**, 674–680.
5 A. Pohanka, J. Levenfors and A. Broberg, *J. Nat. Prod.*, 2006, **69**, 654–657.
6 B. Nowak-Thompson, P. E. Hammer, D. S. Hill, J. Stafford, N. Torkewitz, T. D. Gaffney, S. T. Lam, I. Molnár and J. Ligon, *J. Bacteriol.*, 2003, **185**, 860–869.
7 J. Poldy, R. Peakall and R. A. Barrow, *Tetrahedron Lett.*, 2008, **49**, 2446–2449.
8 M. J. Gunter and L. N. Mander, *Aust. J. Chem.*, 1981, **34**, 675–678.
9 A. J. Leipa, J. S. Wilkie and K. N. Winzenberg, *Aust. J. Chem.*, 1989, **42**, 1217–1225.
10 D. H. R. Barton, L. Bould, D. L. J. Clive, P. D. Magnus and T. Hase, *J. Chem. Soc. C*, 1971, **12**, 2204–2215.
11 J. G. Mant, F. P. Schiestl, R. Peakall and P. H. Weston, *Evolution*, 2002, **56**, 888–898.
12 F. J. Sardina, A. D. Johnston, A. Mourino and W. H. Okamura, *J. Org. Chem.*, 1982, **47**, 1576–1578.
13 E. Piers and J. R. Grierson, *J. Org. Chem.*, 1977, **42**, 3755–3757.

