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Pheromones and analogs from *Neozeleboria* wasps and the orchids that seduce them: a versatile synthesis of 2,5-dialkylated 1,3-cyclohexanediones

Jacqueline Poldy [a,b], Rod Peakall [b], Russell A. Barrow [a,*]

[a] *Department of Chemistry, The Australian National University, Canberra, ACT 0200, Australia*
[b] *School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia*

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Abstract

Chiloglottone, a wasp pheromone and attractant of sexually deceptive *Chiloglottis* orchids, and several structural analogs were synthesized. The synthetic approach is facile, high yielding and versatile, enabling rapid divergence to generate dialkylated analogs of chiloglottone. The key transformation was an organocadmium-mediated desymmetrization of glutaric anhydride derivatives. This library of synthetic 2,5-dialkylated 1,3-cyclohexanediones may assist in future identification of natural products in further species.

Keywords: Cyclohexanedione; Pheromone; Organocadmium reagents; Sexual deception; *Chiloglottis*; *Neozeleboria*

The orchids (family Orchidaceae) exhibit a profusion of non-rewarding species that utilize deceptive strategies to exploit their insect pollinators. Of these pollination syndromes, sexual deception is exclusive to the Orchidaceae, where it has evolved independently in European, African, Central and South American, and Australian genera.[1] The pollination syndrome is characterized by exquisite examples of floral mimicry used to attract male insects to the flower where copulation attempts ('pseudocopulation') can lead to pollination.[2] Of primary importance in eliciting mating behavior in pollinators are floral odor bouquets that mimic the female sex pheromone.[3] Endorsed with other sensory cues, many insects use olfactory stimuli as their principal means of discrimination in sex attraction and recognition.[4] In the Australian orchid genus *Chiloglottis* all species employ sexual deceit,[5] suggesting that odor might be important for floral isolation and maintaining species integrity,[6] on account of the necessary specificity of the pollinators' pheromone. Gas chromatographic analysis in conjunction with electroantennographic detection (GC EAD) has detected attractive compounds from floral extracts of *Chiloglottis* species. In *C. trapeziformis*, pollinated by the thynnine wasp *Neozeleboria cryptoides*, the structure of the active constituent from orchid labella and female wasp extracts was subsequently determined via mass spectral analysis from both sources, and confirmed through synthetic replication of the natural product 2-ethyl-5-propyl-1,3-cyclohexanedione, 'chiloglottone' (**5a**).[7] Likewise, the same compound has been identified as a sexual attractant to *N. monticola*, the pollinator of *C. valida*, with important ecological and evolutionary implications.[8]

The desire to investigate speciation and reproductive isolation within this orchid genus and the pheromone systems of the wasp pollinators is substantially facilitated by available synthetic compounds, including known attractive orchid metabolites and their analogs. Furthermore, as biological material is available in very low abundance (< 0.1 μg/labellum), insufficient for extensive data acquisition,[7] synthetic material is necessary for structural clarification. We proposed to develop a versatile route that would

* Corresponding author. Tel.: +61 2 6125 3419; fax: +61 2 6125 0760.
E-mail address: rab@anu.edu.au (R. A. Barrow).

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deliver the natural product **5a** as well as mono and 2,5-dialkylated cyclohexanedione analogs **5b–f**, some of which we anticipate may be represented in the natural environment. The selection of target compounds represents an assortment of structural isomers and analogs, but is by no means an exhaustive array (Fig. 1).

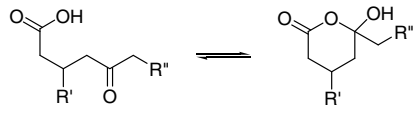
An earlier synthesis of **5a** has been reported via a conjugate addition and Dieckmann-type condensation of two asymmetric starting fragments, with subsequent decarboxylation yielding the desired cyclohexanedione.[7] However, the sequence would require *de novo* preparation of the advanced precursors to generate a diversity of compounds with alternative 2,5-dialkyl groups. Here, we describe a general approach to 2,5-dialkylated cyclohexanediones and report the first full characterization of chiloglottone (**5a**).

In our synthesis (Scheme 1), alkylation of diethyl malonate delivered an assortment of derivatives **1**,[9] substituted at what was to become the 5-position of the cyclohexanedione products. The corresponding diols[10] generated on $LiAlH_4$ reduction were mesylated in good to excellent yield prior to homologation through reaction with KCN in DMSO to return the dinitriles **2**.[10a,11] These transformations were conveniently monitored by IR spectroscopy, by means of the conspicuous CN absorption at ca. 2240 cm^{-1}. Hydrolysis of the pure nitriles to glutaric acid derivatives[12] was followed by intramolecular condensation to deliver the symmetric 4-alkyl substituted glutaric anhydride **3**[10a,13] in excellent yields, representing the key intermediates in the synthesis.

With the cyclic anhydrides in hand, the synthesis became divergent through a dialkylcadmium mediated desymmetrization, to deliver the appropriate 3-alkyl substituted δ-keto carboxylic acids. On treatment with diazomethane, the corresponding methyl esters[14] **4** were obtained in quantitative yield. ^{13}C NMR spectra showed resonances at δ 210 and δ 173 symptomatic of ketone and carboxyl carbon resonances. Subsequent addition to the resulting ketone is avoided due to the inherently inferior reactivity of alkylcadmium species with respect to their magnesium counterparts.[15,16]

Attempts to isolate crude keto acids in an aqueous $NaHCO_3$ extraction gave low returns, despite good to excellent crude yields (79–100%). Somewhat unexpectedly, the organic phase was found to retain the bulk of the material. We propose that the biphasic distribution is attributable to tautomerism between the keto acids and their isomeric hydroxy lactones (Scheme 2). This elucidation is consistent with accounts of similar equilibria, particularly in structures capable of forming thermodynamically favorable 6-membered heterocycles.[17,18]

Ultimately, when the δ-keto esters **4** were treated with freshly prepared KO^{t}Bu the desired mono and 2,5-dialkylated 1,3-cyclohexanediones **5** were obtained. It is interesting to note that these compounds exist exclusively as their enol tautomers in CD_3OD solution, as indicated from their NMR data.[19] Typical overall yields ranged from 26%



Scheme 2. Proposed tautomerism of δ-keto acids with hydroxy lactones.

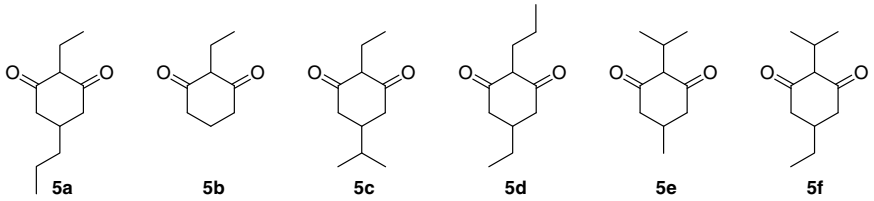
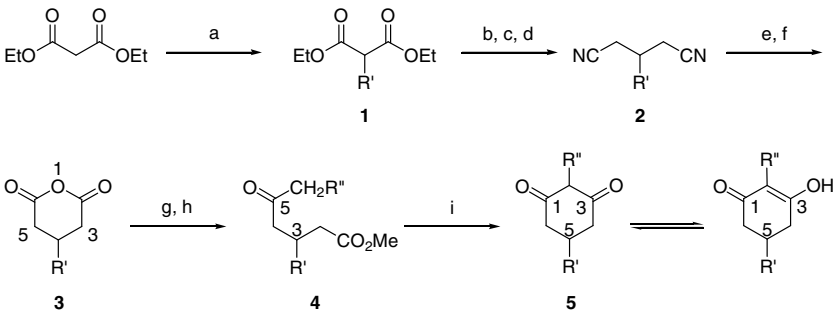


Fig. 1. A selection of mono and 2,5-dialkylated 1,3-cyclohexanediones demonstrating the versatility of the reported method.



Scheme 1. Preparation of mono and 2,5-dialkylated 1,3-cyclohexanediones exhibiting diverse alkyl substituents. Reagents and conditions: (a) NaOEt, R′X (82–95%); (b) $LiAlH_4$ (95–98%); (c) py, MsCl (73–93%); (d) KCN (80–98%); (e) aq NaOH (75–98%); (f) TFAA (97–100%); (g) $Cd(CH_2R'')_2$ (78–99%); (h) CH_2N_2 (quant.); (i) KO^{t}Bu (73–98%).

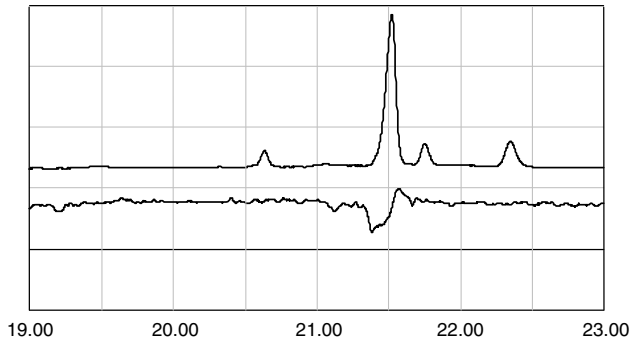


Fig. 2. GC FID (top) and GC EAD (bottom) of chiloglottone (**5a**).

to 48% over nine steps. Reaction mixtures were assessed by GC MS to determine the extent and efficiency of the cyclizations. Two compounds for which cyclization proved most challenging exhibited a 2-isopropyl substituent (**5e, 5f**). Despite more forcing conditions and longer reaction times low yields of the desired products were returned along with starting material.

The mono and 2,5-dialkylated 1,3-cyclohexanediones generated using this divergent methodology represent structural isomers (**5c, 5d, 5f**) and smaller homologs (**5b, 5e**) of the confirmed natural product **5a**. Chiloglottone (**5a**) produced the expected electroantennographic response (EAD) on freshly mounted *N. cryptoides* antennae (Fig. 2) and field studies confirmed its attractive nature toward male wasps. Interestingly, when **5b–f** were evaluated employing the EAD assay we also observed electroantennographic responses comparable to those achieved with the naturally occurring pheromone **5a**. Although this in vitro activity is not necessarily sufficient to confer a behavioral effect in field trials, it supports the notion that diverse 2,5-dialkylated 1,3-cyclohexanediones could represent sex attractants, which may yet be detected in related taxa.

In summary, we have demonstrated a facile and versatile method for synthesizing mono and 2,5-dialkylated 1,3-cyclohexanediones that proceeded in good to excellent yields. The protocol is applicable to a range of alkyl halides to impart various 2- and 5-alkyl substituents. Moreover, the glutaric acid derivatives represent stable and convenient intermediates that enable rapid divergence to dialkylated analogs. The availability of a synthetic library of analogs provides a practical tool with which to probe the behavioral activity of the pheromones, and anticipates potential natural products which may yet be encountered. Efforts are currently directed at adapting the synthesis to further broaden the existing library and encapsulate putative pheromones and will be reported as part of a full paper.

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Supplementary data

Experimental procedures and characterization data for compounds **1–5**; ^{1}H, ^{13}C NMR spectra and GC EI-MS of chiloglottone (**5a**) are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.037.

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19. *Characterization data for selected compounds*: Compound **3** (R′ = Pr): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (2H, dd, $^{2}J = 17.1$, $^{3}J = 4.5$, H-3a, 5a), 2.41 (2H, dd, $^{2}J = 17.1$, $^{3}J = 10.3$, H-3b, 5b), 2.16 (1H, m, H-4), 1.39–1.37 (4H, m, CH_2-1′, CH_2-2′), 0.92 (3H, m, CH_3-3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5 (C, C-2, 6), 36.6 (CH_2, C-1′), 36.0 (CH_2, C-3, 5), 28.4 (CH, C-4), 19.5 (CH_2, C-2′), 13.7 (CH_3, C-3′); EI-MS *m*/*z* (%): 157 (52, $[M+H]^+$), 97 (40), 84 (79), 70 (67), 69 (66), 56 (94), 55 (81), 43 (99), 42 (100). *3-Propyl-5-oxooctanoic acid*: IR ν_{max} 3050, 2960, 2934, 2875, 1700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.1 (1H, br, s, 1-CO_2H) 2.46–2.24 (7H, m, CH_2-2, CH_2-4, CH_2-6, H-3), 1.57 (2H, tq, $^{3}J = 7.4$, $^{3}J = 7.4$, CH_2-7), 1.39–1.25 (4H, m, CH_2-1′, CH_2-2′), 0.88 (6H, m, CH_3-8, CH_3-3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.6 (C, C-5), 178.8 (C, C-1), 46.6, 45.1 (CH_2, C-4, C-6), 38.2 (CH_2, C-2), 36.3 (CH_2, C-1′), 30.4 (CH, C-3), 19.8 (CH_2, C-2′), 17.1 (CH_2, C-7), 14.0, 13.7 (CH_3, C-8, C-3′); GC EI-MS *m*/*z* (%): 200 (<1, $[M]^{+\cdot}$), 182 (2), 157 (26), 129 (14), 111 (19), 97 (20), 87 (37), 83 (37), 71 (85), 69 (43), 55 (49), 43 (100); HREI-MS: found 200.1413 (calculated for $C_{11}H_{20}O_3$ 200.1412). Compound **4** (R′ = Pr, R″ = Et): IR ν_{max} 2959, 2934, 2874, 1738, 1713 cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 3.60 (3H, s, CO_2CH_3) 2.45–2.20 (7H, m, CH_2-2, CH_2-4, CH_2-6, H-3), 1.54 (2H, tq, $^3J = 7.4$, $^3J = 7.4$, CH_2-7), 1.39–1.22 (4H, m, CH_2-1′, CH_2-2′), 1.86 (6H, m, CH_3-8, CH_3-3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.2 (C, C-5), 173.2 (C, C-1), 51.3 (CO_2CH_3), 46.8 (CH_2, C-4), 45.0 (CH_2, C-6), 38.2, 36.4 (CH_2, C-2, C-1′), 30.6 (CH, C-3), 19.8 (CH_2, C-2′), 17.1 (CH_2, C-7), 14.0, 13.6 (CH_3, C-8, C-3′); EI-MS m/z (%): 214 (6, $[M]^{+\cdot}$), 183 (22), 171 (79), 143 (25), 129 (93), 128 (33), 111 (25), 97 (77), 83 (47), 71 (78), 69 (64), 55 (63), 43 (100); HREI-MS: found 214.1573 (calculated for $C_{12}H_{22}O_3$ 214.1569). Compound **5a** (R′ = Pr, R″ = Et, chiloglottone): ^{1}H NMR (500 MHz, CD_3OD) δ 2.46 (2H, dd, $^2J = 16.5$, $^3J = 4.3$, H-4_{eq}, 6_{eq}), 2.25 (2H, q, $^3J = 7.5$, CH_2-1″), 2.14 (2H, dd, $^2J = 16.5$, $^3J = 11.3$, H-4_{ax}, 6_{ax}), 2.04 (1H, m, H-5), 1.38 (4H, m, CH_2-1′, CH_2-2′), 0.94 (3H, t, $^3J = 6.5$, CH_3-3′), 0.90 (3H, t, $^3J = 7.5$, CH_3-2″); ^{13}C-APT NMR (125 MHz, CD_3OD) δ 176.5 (C, C-1, 3), 118.1 (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, 13.6 (CH_3, C-3′, C-2″); EI-MS m/z (%): 182 (60, $[M]^{+\cdot}$), 167 (12), 154 (6), 139 (23), 125 (27), 112 (66), 97 (100), 84 (64), 69 (95), 55 (89), 43 (48), 41 (60); HREI-MS: found 182.1307 (calculated for $C_{11}H_{18}O_2$ 182.1307).