The discovery of 2,5-dialkylcyclohexan-1,3-diones as a new class of natural products

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Orchids employing sexual deceit attract males of their pollinator species through specific volatile signals that mimic female-released sex pheromones. One of these signals proved to be 2-ethyl-5-propylcyclohexan-1,3-dione (chiloglottone1), a new natural product that was shown to be most important in the relations between orchids of the genus *Chiloglottis*, native to Australia, and corresponding pollinator species. Systematic investigations on the mass spectrometric fragmentation pattern of 2,5-dialkylcyclohexan-1,3-diones identified key ions providing information about the structures of the substituents at positions 2 and 5. Results enabled us to identify 2-ethyl-5-pentylcyclohexan-1,3-dione (chiloglottone2) and 2-butyl-5-methylcyclohexan-1,3-dione (chiloglottone3) as new natural products that play a decisive role in the pollination syndrome of some *Chiloglottis* species. During field bioassays, pure synthetic samples of chiloglottone1–3 or mixtures thereof proved to be attractive to the corresponding orchid pollinators. Because of their likely biogenesis from ubiquitous fatty acid precursors, 2,5-dialkylcyclohexan-1,3-diones may represent a hitherto overlooked, widespread class of natural products.

2-butyl-5-methylcyclohexan-1,3-dione | 2-ethyl-5-pentylcyclohexan-1,3-dione | *Chiloglottis* | semiochemical | mass spectrometry

The family Orchidaceae is well known for its large number of species and its diverse and often specialized pollination systems (1, 2). Pollination by deception in which flowers attract pollinators without providing them with any reward (pollen, nectar) for the service of pollination is particularly prevalent in orchids (3). One unusual form of deceptive pollination is sexual deception. This pollination strategy is particularly well developed in the European terrestrial orchid genus *Ophrys* and in multiple terrestrial orchid genera in Australia (4–6). In Australian orchids, males of specific hymenopteran pollinators, predominantly thynnine wasps (Hymenoptera: Tiphiidae) are attracted to the orchid flowers through specific volatiles that mimic the sex pheromones released by their females. At close range, visual and tactile mimicry of the females may also play a role. The males are deceived into releasing mating behavior on the orchid labellum with the transfer of pollen occurring either during precopulatory behavior (7) or attempted mating with the flower, so called "pseudocopulation" (8, 9). Female thynnine wasps are parasitoids that spend much of their lifetime as adults underground, searching for hosts on which to lay eggs. When ready to mate, the wingless female emerges from the ground and "calls" for mates by emitting a pheromone while perched in a prominent position on vegetation or within the leaf litter. The winged males typically respond in seconds with the first to arrive picking up the female and carrying her in copula where mating and feeding occur (7, 10).

Although chemical mimicry has long been proposed as the basis of the extreme pollinator specificity in sexually deceptive orchids (4, 8), we have only recently begun to identify the semiochemicals accounting for this phenomenon. This breakthrough has been achieved by combining gas chromatography with electroantennographic detection (GC/EAD) to identify candidate compounds, followed by coupled gas chromatography/mass spectrometry (GC/MS) to determine their chemical structures. Confirmation of biological activity with synthetic compounds during field bioassays was the final essential step (11–13).

A milestone in the understanding of thynnine wasp pheromones and the attractive principles of orchids that mimic them has been the identification of 2-ethyl-5-propylcyclohexan-1,3-dione, chiloglottone1, formerly called chiloglottone, as the decisive compound involved in the chemical mimicry used by *Chiloglottis trapeziformis* (13). This compound, showing a unique molecular structure, was found to be released by flowers of the orchid and at the same time proved to be the female-produced sex pheromone of its pollinator, the thynnine wasp *Neozeleboria cryptoides*. Chiloglottone1 has also been shown to be the sexual attractant used by the orchid *Chiloglottis valida* to attract its thynnine wasp pollinator *Neozeleboria monticola* (10). Hybridization between these two orchids is known but is largely avoided because their core ranges are nonoverlapping (14).

Chiloglottone1 was the first 2,5-dialkylcyclohexan-1,3-dione found in nature. Its substitution pattern and the cyclohexan-1,3-dione substructure suggest a biosynthesis involving activated 3-oxohexanoic acid and 2-hexenoic acid: a Claisen-type condensation between the acid moiety of the hexenoic acid and position 4 of the 3-ketoacid precursor and a Michael-type addition of the unsaturated precursor to position 2 of the 3-ketoacid would produce 2-ethyl-5-propylcyclohexan-1,3-dion-4-carboxylate, a precursor bearing an additional carboxylic moiety. This cyclic 3-ketoacid would yield chiloglottone1 upon decarboxylation (Fig. 1: R^2 = Et, R^1 = *n*Prop.).

Because 3-ketoacids and 2,3- unsaturated acids are general intermediates in fatty acid formation in nature, we postulated that activated fatty acid derivatives other than C6 may be involved in the biosynthesis of analogs of chiloglottone1. If so, this compound could be the first representative of a new class of possibly widespread natural products, namely cyclohexan-1,3-diones characterized by alkyl-substituents with even numbers of carbon atoms at position 2 and uneven numbers at position 5. The most simple compound of this type would then be 5-methylcyclohexan-1,3-dione (Fig. 1: R^2 = H, R^1 = Me), involving acetoacetate and crotonate.

Similar to other behavior-mediating volatiles of insects and plants, chiloglottone1 proved to be present both in the orchids and the pollinators in very small amounts, only, and it was impossible to isolate enough material for NMR investigations. As a consequence, structure elucidation was based only on GC/MS and on GC/FTIR and comparison of corresponding data with those of synthetic reference compounds (13). There is not much information available on EI mass spectra of alkylated

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The authors declare no conflict of interest.

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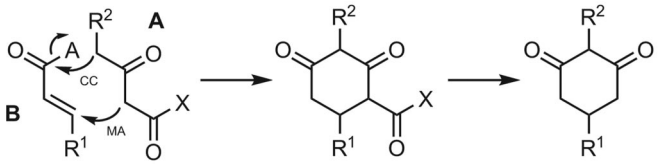


Fig. 1. Presumed biosyntheses of 2,5-dialkylcyclohexan-1,3-diones from a 3-ketoacylprecursor A and a 2,3-unsaturated acylprecursor B; X indicates an activator. The order of the two steps forming the nucleus, Claisen condensation (CC) or Michael-type addition (MA), is not defined.

cyclohexan-1,3-diones although formation of some key fragments has been described. Basic investigations have been carried out by Vandewalle et al. (15, 16), whereas Goto et al. (17) and Maquestiau and Lejeune (18) focused on spectra of dimedone derivatives. Therefore, it appeared highly desirable to better understand the mass spectrometric fragmentation pattern of chiloglottone1 and its analogs to identify respective diagnostic ions and, thus, facilitate structure elucidation of other volatiles representing this new type of compound in nature.

Results

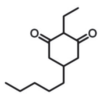
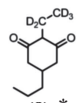
As a prerequisite for systematic investigations on mass spectra of 2,5-dialkylcyclohexan-1,3-diones, reference compounds were prepared (see Tables 1 and 2). The synthesis of chiloglottone1 (compound **1** in Fig. 2) followed the possible biomimetic way outlined above, involving ethyl 3-oxohexanoate and ethyl *E*-hex-2-enoate. Compounds **2** and **3** were prepared from known 5-alkylcyclohexan-1,3-diones through alkylation at position 2 (19) (see Fig. 2). Syntheses of compounds **4**-**7** following an entirely different way have been described earlier (20). In general, 2,5-dialkylcyclohexan-1,3-diones seem to be unusually unstable under normal conditions. In polar solvents, they tend to show an equilibrium between tautomers with a strong bias for the enol form (**2a** and **3a** in Fig. 2). Consequently, as a result of rapid degenerate rearrangements, some of the signals that would have been expected for positions 1/3 or 4/6 remained invisible in both ^{1}H and ^{13}C NMR spectra. We present the corresponding (incomplete) data of compounds **2** and **3**, and we fully characterize both compounds by the data of the corresponding ethyl enol ethers **2b** and **3b**.

In the fragmentation of 2,5-dialkylcyclohexan-1,3-diones three major routes may be distinguished. The first comprises retrocleavages and electron and hydrogen shifts as shown in Fig. 3. As supported by stable isotope labeling (see below), the primarily formed fragments **a**, **c**, and **e** may undergo further fragmentation to produce ions **b**, **d**, and **f**. As shown in Fig. 4, a second set of fragmentations starts with cleavage of the C-1/C-2 bond, giving rise to ions **g**–**m**. Finally, cleavage of bonds outside the 1,3-dicarbonyl moiety leading to fragments **n**–**p** is depicted in Fig. 5.

Atomic compositions of the fragments were determined upon high-resolution mass spectrometry (HR-MS). As a whole, structures of 16 fragments could be tentatively assigned; corresponding data are compiled in Tables 1 and 2.

Masses of characteristic fragments (*m*/*z*) and relative abundances in the 70 eV EI mass spectra (percentage) of seven 2,5-dialkylcyclohexan-1,3-diones and of a pentadeuterio-isotopomer are given in Table 1. In addition, Table 2 reflects the atomic composition of corresponding fragments as obtained upon HR-MS. In both tables, the first column refers to the designation used in the fragmentation schemes. As already mentioned by Vandewalle et al. (15, 16), relative abundances of the signals in the EI spectra of 2,5-dialkylcyclohexan-1,3-diones strongly depend on the substituents, and spectra may be different after excitation of either the diketo or the enol structures. According to our investigations, some signals observed in low resolution represent fragments of at least two different atomic compositions. In these cases, relative amounts of the species as obtained upon high resolution are provided. An example is the base peak at *m*/*z* 97 in the spectrum of compound **7** that consists of 92% C_6H_9O represented by fragment **m** and 8% $C_5H_5O_2$ represented by fragment **p**. Another example is the signal at *m*/*z* 84 in the spectrum of chiloglottone1: the peak with a relative abundance of 30% is made up of 6% $C_4H_4O_2$ represented by

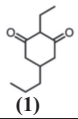
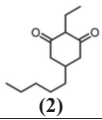
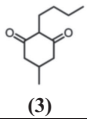
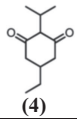
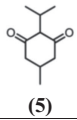
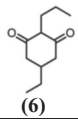
Table 1. Relative abundances [%] of molecular ions and fragments a–p (see Figs. 3–5) in 70 eV EI mass spectra of 2,5-dialkylcylcohexan-1,3-diones

fragment	 (1)	 (2)	 (3)	 (4)	 (5)	 (6)	 (7)	 (8) *
M	182 [14]	210 [14]	182 [19]	182 [13]	168 [16]	182 [19]	182 [30]	187 [14]
a	112 [17]	112 [14] [2]	140 [36]	126 [2] *	126 [11]	126 [9]	112 [12] [13]	117 [14]
b	69 [12]	97 [8]	-	55 [17] [9]	-	55 [24] [11]	69 [28]	69 [23] +
c	70 [11]	70 [7] [3]	98 [11] [6]	84 [11]	84 [13]	84 [13]	70 [9] [14]	75 [13]
d	69 [33]	69 [12] [4]	69 [100]	69 [30] [10]	69 [100]	69 [15]	69 [32]	69 [23] +
e	-	-	126 [36]	140 [6]*	126 [11]	140 [13]	-	-
f	84 [6]	-	84 [57] [7]	84 [16]	84 [26]	84 [26]	84 [9]	84 [9]
g	84 [24]	84 [33]	112 [7]	98 [15]	98 [8]	98 [8] [12]	84 [26]	89 [19]
h	167 [2]	195 [2]	139 [22]	167 [26]	153 [25]	153 [26]	167 [3]	169 [2]
i	139 [6]	167 [7]	111 [37]	139 [27]	125 [26]	125 [26]	139 [12]	141 [6]
j	154 [5]	182 [3]	154 [3]*	154 [4]	140 [7]	-	154 [13]	159 [4]
k	125 [22]	153 [35]	97 [18] [8]	111 [24]	97 [14]	111 [10]	125 [25]	125 [30]
l	111 [13] [1]	111 [40] [5]	-	-	125 [26]	125 [26]	111 [25]	116 [25]
m	97 [80]	125 [72]	69 [100]	83 [100]	69 [100]	83 [100]	97 [92]	97 [89]
n	-	-	153 [26]	-	-	167 [16]	-	-
o	139 [4]	139 [31]	167 [4]	153 [4]	153 [25]	153 [26]	-	144 [4]
p	97 [4]	97 [9]	125 [14]	-	-	111 [13]	97 [8]	102 [6]

* = no high resolution - = fragment not present + = origin not defined

[1] + 13% $C_6H_7O_2$, [2] + 4% $C_7H_{12}O$, [3] +3% C_5H_{10}, [4] +24% C_4H_5O, [5] +5% $C_6H_7O_2$, [6] +20% $C_5H_6O_2$, [7] +9% C_5H_8O, [8] +9% $C_5H_5O_2$, [9] +5% C_3H_3O, [10] +9% C_5H_9, [11] +27% C_3H_3O, [12] +6% $C_5H_6O_2$, [13] +7% $C_7H_{12}O$, [14] +5% C_5H_{10}

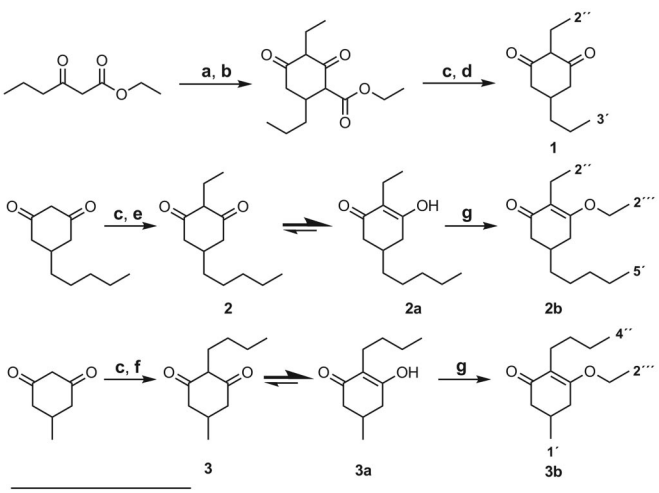
Table 2. Atomic compositions of molecular ions and fragments a-p (see Figs. 3–5) as determined by high-resolution mass spectrometry

fragment	 (1)	 (2)	 (3)	 (4)	 (5)	 (6)	 (7)
M	$C_{11}H_{18}O_2$	$C_{13}H_{22}O_2$	$C_{11}H_{18}O_2$	$C_{11}H_{18}O_2$	$C_{10}H_{16}O_2$	$C_{11}H_{18}O_2$	$C_{11}H_{18}O_2$
a	$C_6H_8O_2$	$C_6H_8O_2$, 82%	$C_8H_{12}O_2$	*	$C_7H_{10}O_2$	$C_7H_{10}O_2$	$C_6H_8O_2$, 61%
b	C_5H_9, 30%	C_7H_{13}, 46%	-	C_4H_7, 77%	-	C_4H_7, 46%	C_5H_9, 43%
c	C_4H_6O	C_4H_6O, 70%	$C_6H_{10}O$, 36%	C_5H_8O, 39%	C_5H_8O, 33%	C_5H_8O, 29%	C_4H_6O, 60%
d	C_4H_5O, 70%	C_5H_9, 30%	C_4H_5O	C_4H_5O, 76%	C_4H_5O	C_4H_5O	C_4H_5O, 57%
e	-	-	$C_7H_{10}O_2$	*	$C_7H_{10}O_2$	$C_8H_{12}O_2$	*
f	$C_4H_4O_2$, 20%	-	$C_4H_4O_2$, 86%	$C_4H_4O_2$, 61%	$C_4H_4O_2$, 67%	$C_4H_4O_2$, 71%	$C_4H_4O_2$, 23%
g	C_5H_8O, 80%	C_5H_8O	$C_7H_{12}O$	$C_6H_{10}O$	$C_6H_{10}O$	$C_6H_{10}O$, 58%	C_5H_8O, 77%
h	$C_{10}H_{15}O_2$	$C_{12}H_{19}O_2$	$C_8H_{11}O_2$	$C_{10}H_{15}O_2$	$C_9H_{13}O_2$	$C_9H_{13}O_2$	$C_{10}H_{15}O_2$
i	$C_9H_{15}O$, 63%	$C_{11}H_{19}O$	$C_7H_{11}O$	$C_9H_{15}O$	$C_8H_{13}O$	$C_8H_{13}O$	$C_9H_{15}O$
j	$C_{10}H_{18}O$	$C_{12}H_{22}O$	*	$C_{10}H_{18}O$	$C_9H_{16}O$	*	$C_{10}H_{18}O$
k	$C_8H_{13}O$	$C_{10}H_{17}O$	C_6H_9O, 67%	$C_7H_{11}O$	C_6H_9O	$C_7H_{11}O$, 42%	$C_8H_{13}O$
l	$C_7H_{11}O$, 49%	$C_7H_{11}O$, 87%	-	*	$C_8H_{13}O$	$C_8H_{13}O$	$C_7H_{11}O$
m	C_6H_9O, 94%	$C_8H_{13}O$	C_4H_5O	C_5H_7O	C_4H_5O	C_5H_7O	C_6H_9O, 92%
n	-	-	$C_9H_{13}O_2$	-	-	$C_{10}H_{15}O_2$	-
o	$C_8H_{11}O_2$, 37%	$C_8H_{11}O_2$	$C_{10}H_{15}O_2$	$C_9H_{13}O_2$	$C_9H_{13}O_2$	$C_9H_{13}O_2$	-
p	$C_5H_5O_2$, 6%	$C_5H_5O_2$, 54%	$C_7H_9O_2$	-	-	$C_6H_7O_2$, 58%	$C_5H_5O_2$, 8%

* = no high resolution - = fragment not present

Percentages (%) refer to relative abundances when two fragments produce signals at the same mass number. In chiloglottone1 these are the pairs **b/d**, **f/g**, **i/o**, and **m/p**.

fragment **f** and 24% C_5H_8O represented by fragment **g**. Not in all cases could reasonable structures be attributed to signals: *m/z* 98 in compound **3** shows a relative abundance of 31%, which comprises 11% $C_6H_{10}O$ (fragment **c**) and 20% of $C_5H_6O_2$ to which no structure was assigned. In such a case, the composition of the unspecified ion and its abundance are given as a footnote in Table 1. In compounds **3** and **5** the base peaks appearing at *m/z* 69, represented by C_4H_5O, may be formed through two different ways yielding fragments **d** and/or **m**, which, in this case, show the same atomic composition. Similarly, in compound **5** the signals at *m/z* 125 and *m/z* 153 may each be represented (as a whole or in parts) by fragments of different origin. This uncertainty could be largely clarified by investigations using isotope-labeled compounds. Assignments of fragments **a**–**p** are supported upon inspection of the mass spectrum of 1″,1″,2″,2″,2″-pentadeuteriochiloglottone1, compound **8** in Table 1 (21). As expected, fragments including the alkyl substituent at position 2 (**a**, **c**, **g**, **j**, **l**, **o**, **p**) were found to be shifted upward by 5 mass units whereas the corresponding relative abundances were approximately the same as in undeuterated chiloglottone1. Consequently, masses of fragments produced after loss of the alkyl substituent at position 2 (**b**, **d**, **f**, **h**, **k**, and **m**) remained unchanged. In accord with the proposed fragmentation patterns, ions **i** and **h** were shifted by two mass units from *m/z* 139 and *m/z* 167 to *m/z* 141 and *m/z* 169, respectively. The latter data clearly show that formation of the fragment **h** (see Fig. 4), in chiloglottone1 loss of a methyl group, is exclusively because of cleavage of the C-1—C-2 bond.



a: KO^{t}Bu, THF, **b**: ethyl (2*E*)-hex-2-enoate, **c**: 20% NaOH, **d**: HCl, **e**: EtI, NaOH, H_2O
f: *n*-BuI, NaOH, H_2O, **g**: *p*-TsOH, EtOH

Fig. 2. Syntheses of 2,5-dialkylcyclohexan-1,3-diones.

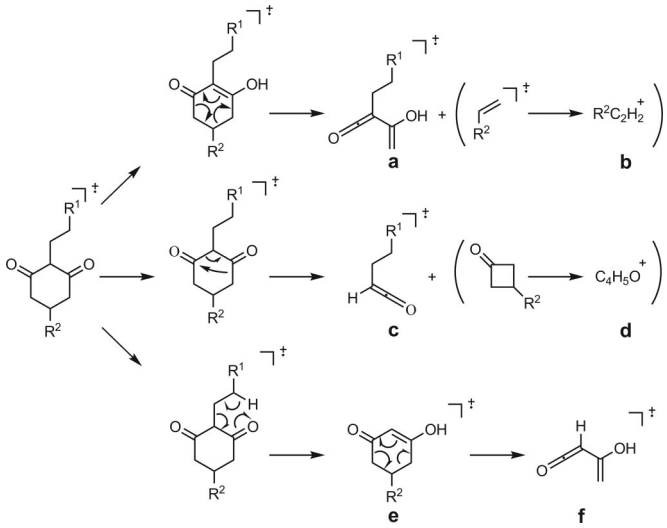


Fig. 3. Mass spectrometric fragmentation of 2,5-dialkylcyclohexan-1,3-diones induced by electron rearrangement.

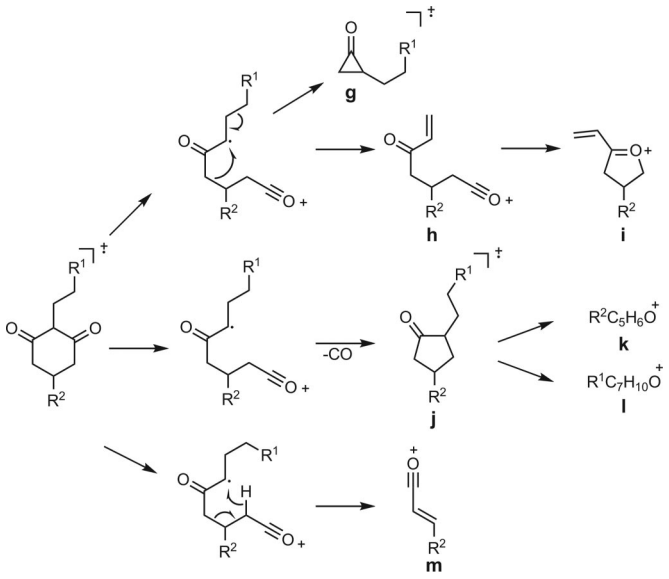


Fig. 4. Mass spectrometric fragmentation of 2,5-dialkylcyclohexan-1,3-diones after cleavage between C-1 and C-2.

Plotted 70 eV EI mass spectra of the three naturally occurring 2,5-dialkylcyclohexan-1,3-diones identified so far and the interpretation of their fragmentation according to Figs. 3–5 are given in Fig. 6.

Discussion

In addition to chiloglottone1, our systematic studies on the mass spectrometric fragmentation pattern of 2,5-dialkylcyclohexan-1,3-diones enabled us to identify two new natural products, 2-ethyl-5-pentylcyclohexan-1,3-dione (**2**) and 2-butyl-5-methylcyclohexan-1,3-dione (**3**), for which we suggest the common names chiloglottone2 and chiloglottone3, respectively.

As demonstrated by the data compiled in Tables 1 and 2, the pattern of mass spectra of volatile 2,5-dialkylcyclohexan-1,3-diones can be well interpreted by the fragmentation mechanisms depicted in Figs. 3–5. Nevertheless, some signals in the spectra cannot be explained by the presented fragmentation schemes: e.g., *m*/*z* 124 in chiloglottone2, *m*/*z* 127 in chiloglottone3, or 50% of *m*/*z* 111 in chiloglottone1 (see also Table 1). In such cases and in studies on spectra of compounds showing long-chain substituents or those carrying additional functional groups (e.g., double bonds), the application of tandem MS techniques are necessary. These elaborate and complex investigations will be subject of a separate article.

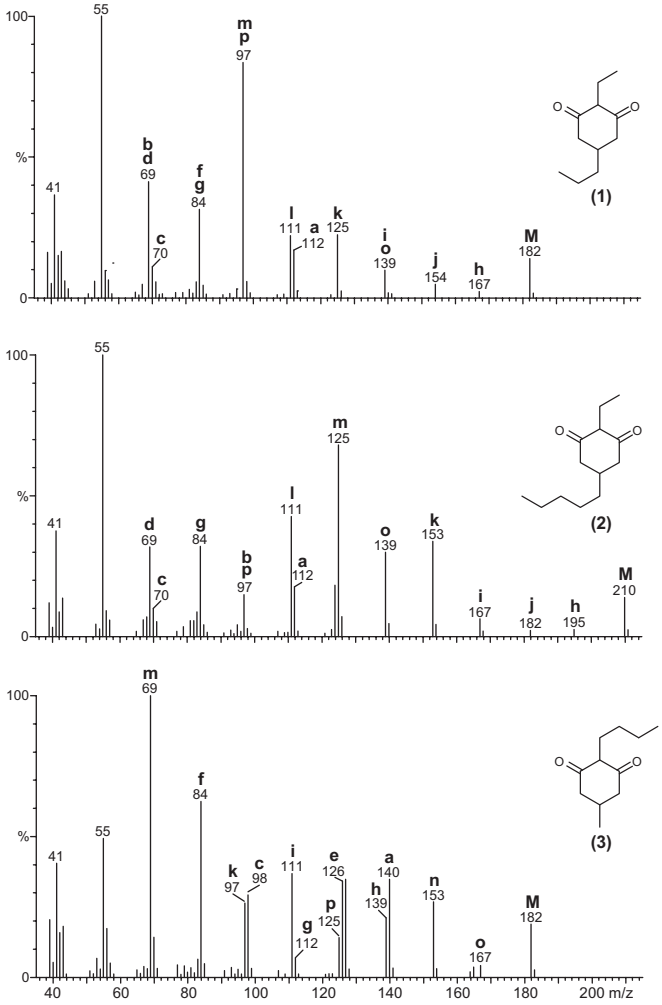


Fig. 6. 70 eV-EI mass spectra of chiloglottones1–3.

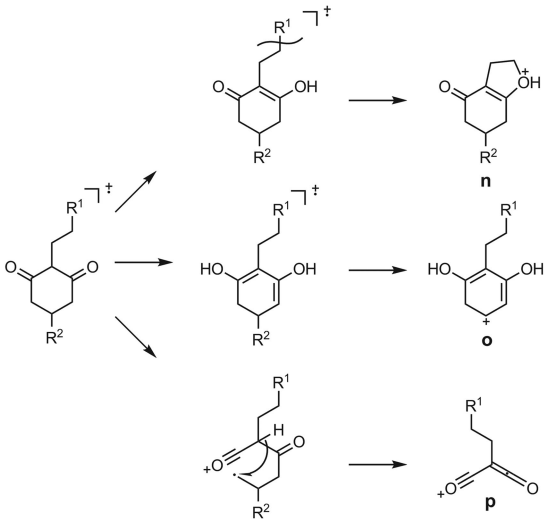


Fig. 5. Mass spectrometric fragmentation of 2,5-dialkylcyclohexan-1,3-diones upon fissions outside the 1,3-diketone moiety.

Chiloglottone2, possibly formed from 2-octenoate and 3-oxohexanoate (Fig. 1: R^2 = Et, R^1 = *n*Pent), has been found in *Chiloglottis grammata*, which is pollinated by *Eirone leai.* Interestingly, the wasp genus *Eirone* is distantly related to the genus *Neozeleboria*, which are the usual pollinators of *Chiloglottis* orchids (22). Chiloglottone3, possibly formed from crotonate and 3-oxooctanoate (Fig. 1: R^2 = *n*But, R^1 = Me), has been found in the orchid *Chiloglottis chlorantha*, which is pollinated by an undescribed species of *Neozeleboria.* This compound was both GC/EAD active and attractive in field bioassays to the pollinator of *C. chlorantha*. In contrast to *C. valida* (10), *C. trapeziformis* (13), *C. grammata*, and *C. chlorantha* that appear to rely on a single compound, in *Chiloglottis pluricallata*, chiloglottone1 and chiloglottone2 are GC/EAD active and form an ≈1:5 blend. Similar mixtures proved to be attractive to the *Neozeleboria* pollinator in the field. Although differences in the chemical composition of sexual attractants can be expected between co-occurring wasps–pollinator pairs, chemical signals may be the same among allopatric orchids and wasps. Indeed, sharing of one or more active components may be common. For example, GC/EAD analysis has indicated sharing of some volatiles among *Chiloglottis*

trilabra, *Chiloglottis seminuda*, and *Arthrochilus huntianus*, although each species has a distinct blend of two compounds (23). Results obtained so far support the following statements: (*i*) Single compounds or combinations of volatiles are involved. (*ii*) When there is more than one active component, a specific ratio is required for biological activity. (*iii*) Although some allopatric orchids may share the same attractant, chemical signals of coflowering sympatric species are always characterized by quantitative or qualitative differences to attract their specific pollinators.

Although chiloglottone1 has been identified in both orchid and its wasp pollinator (13), it remains to be confirmed that chiloglottone2 and chiloglottone3 are also present in the wasp pollinators of the respective orchids. As mentioned above, female thynnine wasps spend most of their lives underground, only appearing above ground to mate. Consequently they are extremely difficult to find. Nonetheless, we predict that the wasp pheromones will include compounds identical to those produced by the orchids that mimic the wasps.

Chiloglottones1–3 play a key role in pollinator attraction in *Chiloglottis* orchid species and attract pollinators from at least two phylogenetically diverse genera of thynnine wasps. This suggests that this type of compound may be widely used as a key constituent of thynnine wasp pheromone systems in general. Given the sheer diversity of both terrestrial orchids and thynnine wasps in Australia (24–26), we predict that 2,5-dialkylcyclohexan-1,3-diones will be widely involved in both intra- and interspecies communication and have been decisive in the evolution of both orchids and wasps.

Because of the variability of the alkyl chains at either side of the carboxylic moiety, wax-type esters are versatile (components of) chemical signals (27). Similarly, substituents at positions 2 and 5 of the cyclohexan-1,3-dione nucleus are suitable to contribute to specificity of these compounds (or mixtures thereof) in chemical communication. Because of their likely biogenesis from ubiquitous fatty acid precursors, 2,5-dialkylcyclohexan-1,3-diones may not only occur in orchids and their pollinators, but may be found also in other living organisms, forming a new and widespread class of natural products.

Experimental Procedures

GC/MS was carried out with a GC 8000 series/MD800 (Fisons Instruments). Mass spectra were taken in EI mode at 70 eV and a mass range of 35–500. Separations were performed by using a 30-m, 0.25-mm inner diameter fused silica capillary VF-5m (Varian) under the following conditions: 80 °C for 5 min and then programmed to 310 °C at a rate of 10 °C/min. High-resolution GC/MS was carried out with an HP5890 gas chromatograph (Hewlett–Packard) linked to a VG70–250SE (Vacuum Generators/Waters Micromass) sector field mass spectrometer (EI, 70 eV, mass range 35–300, RP10000, reference PFK, source temperature 200 °C). The gas chromatograph was equipped with a 25-m, 0.15-mm inner diameter fused silica capillary BPX5 (SGE Analytical Science) that was operated at 60 °C for 5 min and then programmed to 270 °C at a rate of 10 °C/min. All analyses were performed by using helium as carrier gas; injections were made splitless for 1 min. Chromatographic purifications were carried out by using Merck silica 40, 70–230 mesh ASTM.

2-Ethyl-5-propylcyclohexan-1,3-dione, Chiloglottone1. To a stirred solution of 449 mg (4 mmol) of potassium *tert*-butoxide in 40 mL of anhydrous tetrahydrofuran a solution of 633 mg (4 mmol) of ethyl 3-oxohexanoate in 5 mL of anhydrous tetrahydrofuran was added dropwise. After stirring for 24 h at room temperature, 569 mg (4 mmol) of ethyl *E*-hex-2-enoate was added. The reaction mixture was refluxed for 6 h and then cooled to room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in 5 mL of 20% sodium hydroxide. The mixture was refluxed for 1 h, cooled, and then acidified dropwise to pH 2 with concentrated hydrochloric acid and subsequently refluxed for 1 h, cooled again, and extracted three times with 10-mL portions of diethylether. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography (gradient elution 5–20% ethyl acetate in hexane) to afford 326 mg (1.8 mmol; 45%) chiloglottone1 as a pale yellow solid; m.p. (uncorrected) 126 °C. NMR data perfectly matched those of literature data (20).

2-Ethyl-5-pentylcyclohexan-1,3-dione, Chiloglottone2. To a solution of 500 mg (2.7 mmol) of 5-pentylcyclohexan-1,3-dione (28) in 5 mL of water containing 1.35 mL of 2 N sodium hydroxide (2.7 mmol) was added 858 mg (5.5 mmol) of ethyliodide. The mixture was refluxed for 36 h while an orange oil was formed. The mixture was cooled, acidified with dilute hydrochloric acid, and extracted three times with dichloromethane. The combined extracts were washed with brine and dried over anhydrous sodium sulfate. The residue was purified by column chromatography (gradient elution 5–10% ethyl acetate in hexane) to afford 165 mg (0.8 mmol; 32%) of chiloglotton2 as a pale yellow solid.

^{1}H NMR (400 MHz, CD_3OD). δ 2.45 (dd, 2*H*, ^2J = 16.7 Hz, ^3J = 4.2 Hz, H-4$_{eq.}$/H-6$_{eq.}$), 2.24 (q, 2*H*, ^3J = 7.5 Hz, H-1″), 2.13 (dd, 2*H*, ^2J = 16.7 Hz, ^3J = 11.3 Hz, H-4$_{ax.}$/H-6$_{ax.}$), 2.05–1.96 (m, 1*H*, H-5), 1.42–1.26 (m, 8*H*, H-1′-H-4′), 0.91 (t, 3*H*, ^3J = 7.1 Hz, H-5′), 0.90 (t, 3*H*, ^3J = 7.5 Hz, H-2″).

^{13}C NMR (101 MHz, CD_3OD). δ 118.24 (s, C-2), 44.03 (t, C-4/C-6), 36.61 (t, C-1′), 34.79 (d, C-5), 33.08 (t, C-3′), 27.39 (t, C-2′), 23.69 (t, C-4′), 16.01 (t, C-1″), 14.42 (q, C-5′), 13.59 (q, C-2″).

3-Ethoxy-2-ethyl-5-pentylcyclohex-2-enone (2b). A solution of 160 mg (0.76 mmol) of chiloglotton2 in 2 mL of dry ethanol and a catalytic amount of *p*-TsOH was refluxed for 2 h. After cooling, 30 mL of diethylether was added, and the mixture was washed three times with 10-mL portions of saturated aqueous sodium hydrogen carbonate. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The residue was purified by column chromatography (pentane:diethylether 4:1) to afford 5 mg (0.02 mmol; 2.6%) of **2b**.

^{1}H NMR (400 MHz, C_6D_6). δ 3.89 (q, 2*H*, ^3J = 7.1 Hz, H-1‴), 2.71 (q, 2*H*, ^3J = 7.4 Hz, H-1″), 2.48 (dd, 1*H*, ^2J = 16.1 Hz, ^3J = 4.1 Hz, H-6$_{eq.}$), 2.02 (dd, 1*H*, ^2J = 16.7 Hz, ^3J = 4.4 Hz, H-4$_{eq.}$), 1.84 (dd, 1*H*, ^2J = 16.1 Hz, ^3J = 12.6 Hz, H-6$_{ax.}$), 1.64–1.57 (m, 1*H*, H-5), 1.51 (dd, 1*H*, ^2J = 16.7 Hz, ^3J = 10.4 Hz, H-4$_{ax.}$), 1.26 (t, 3*H*, ^3J = 7.4 Hz, H-2″), 1.34–1.05 (m, 8*H*, H-1′-H-4′), 0.92 (t, 3*H*, ^3J = 7.1 Hz, H-2‴), 0.89 (t, 3*H*, ^3J = 7.4 Hz, H-5′).

^{13}C NMR (101 MHz, C_6D_6). δ 196.79 (s, C-1), 169.50 (s, C-3), 121.90 (s, C-2), 63.06 (t, C-1‴), 43.64 (t, C-6), 33.80 (d, C-5), 32.04 (t, C-4), 30.46/30.08/26.72/23.20 (4 × t, C-1′–C-4′), 16.44 (t, C-1″), 15.44 (q, C-2‴), 14.52 (q, C-2″), 14.15 (q, C-5′).

2-Butyl-5-methylcyclohexan-1,3-dione, Chiloglottone3. To a stirred solution of 2 g (16 mmol) of commercially available (Aldrich) 5-methylcyclohexan-1,3-dione, 8 mL of 2 N sodium hydroxide (16 mmol) was dropwise added followed by 1.84 g (10 mmol) of *n*-butyliodide. The mixture was refluxed for 48 h while a white precipitate was formed. Subsequently it was cooled, and the precipitate was filtered off and dried in vacuo overnight. The residue was purified by column chromatography (gradient elution 5–20% ethyl acetate in hexane) to afford 832 mg (4.6 mmol; 46%) of chiloglotton3 as a white solid.

^{1}H NMR (500 MHz, CD_3OD). δ 2.42 (dd, 2*H*, ^2J = 16.7 Hz, ^3J = 4.1 Hz, H-4$_{eq.}$/H-6$_{eq.}$), 2.23 (t, 2*H*, ^3J = 7.2 Hz, H-1″), 2.18–2.08 (m, 3*H*, H-4$_{ax.}$/H-5/H-6$_{ax.}$), 1.33–1.24 (m, 4*H*, H-2″/H-3″), 1.06 (d, 3*H*, ^3J = 5.8 Hz, H-1′), 0.89 (t, 3*H*, ^3J = 7.0 Hz, H-4″).

^{13}C NMR (101 MHz, CD_3OD). δ 176.77 (s, C-1/C-3), 116.76 (s, C-2), 32.05 (t, C-2″), 29.88 (d, C-5), 23.77 (t, C-3″), 22.45 (t, C-1″), 21.18 (q, C-1′), 14.46 (q, C-4″).

3-Ethoxy-2-butyl-5-methylcyclohex-2-enone (3b). In a procedure similar to that which furnished (**2b**) from chiloglottone2, 150 mg (0.82 mmol) chiloglottone3 yielded 50 mg (0.24 mmol, 29%) of **3b**.

^{1}H NMR (400 MHz, C_6D_6). δ 3.89 (q, 2*H*, ^3J = 7.1 Hz, H-1‴), 2.70 (t, 2*H*, ^3J = 7.4 Hz, H-1″), 2.39 (dd, 1*H*, ^2J = 15.8 Hz, ^3J = 3.1 Hz, H-6$_{eq.}$), 1.88 (dd, 1*H*, ^2J = 16.8 Hz, ^3J = 4.6 Hz, H-4$_{eq.}$), 1.80 (dd, 1*H*, ^2J = 15.8 Hz, ^3J = 12.2 Hz, H-6$_{ax.}$), 1.70–1.64 (m, 3*H*, H-5/H-2″), 1.48 (dd, 1*H*, ^2J = 16.8 Hz, ^3J = 7.6 Hz, H-4$_{ax.}$), 1.43–1.38 (m, 2*H*, H-3″), 1.01 (t, 3*H*, ^3J = 7.1 Hz, H-4″), 0.92 (t, 3*H*, ^3J = 7.1 Hz, H-2‴), 0.64 (d, 3*H*, ^3J = 6.4 Hz, H-1′).

^{13}C NMR (101 MHz, C_6D_6). δ 196.93 (s, C-1), 169.63 (s, C-3), 120.24 (s, C-2), 60.32 (t, C-1‴), 45.33 (t, C-6), 33.61 (t, C-4), 28.89 (d, C-5), 30.46/23.29 (2 × t, C-2″/C-3″), 22.40 (t, C-1″), 20.79 (q, C-1′), 15.41 (q, C-2‴), 14.68 (q, C-4″).

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1. Schiestl FP (2005) On the success of a swindle: Pollination by deception in orchids. *Naturwissenschaften* 92:255–264.
2. Peakall R (2007) Speciation in the Orchidaceae: Confronting the challenges. *Mol Ecol* 16:2834–2837.
3. Cozzolino S, Widmer A (2005) Orchid diversity: An evolutionary consequence of deception? *Trends Ecol Evol* 20:487–494.
4. Kullenberg B (1961) Studies in Ophrys pollination. *Zool Bidrag från Uppsala* 34:1–340, plates 1–51.
5. Peakall R (1989) A new technique for monitoring pollen flow in orchids. *Oecologia* 79:361–365.
6. Peakall R, Beattie AJ (1996) Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentaculata*. *Evolution* 50:2207–2220.
7. Peakall R (1990) Responses of male *Zaspilothynnus trilobatus* wasps to females and the sexually deceptive orchid it pollinates. *Funct Ecol* 4:159–167.
8. Correvon H, Pouyanne A (1916) A curious case of mimicry in Ophrys. *J Soc Nat Horticult France* 17:29–47.
9. Coleman E (1927) Pollination of the orchid *Cryptostylis leptochila*. *Vict Nat* 44:20–22.
10. Schiestl FP, Peakall R (2005) Two orchids attract different pollinators with the same floral odour compound: Ecological and evolutionary implications. *Funct Ecol* 19:674–680.
11. Schiestl FP, et al. (1999) Orchid pollination by sexual swindle. *Nature* 399:421–422.
12. Ayasse M, Schiestl FP, Paulus HF, Ibarra F, Francke W (2003) Pollinator attraction in a sexually deceptive orchid by means of unconventional chemicals. *Proc R Soc London Ser B* 270:517–522.
13. Schiestl FP, et al. (2003) The chemistry of sexual deception in an orchid–wasp pollination system. *Science* 302:437–438.
14. Peakall R, Jones L, Bower CC, Mackey BG (2002) Bioclimatic assessment of the geographic and climatic limits to hybridisation in a sexually deceptive orchid system. *Aust J Bot* 50:21–30.
15. Vandewalle M, Schamp N, De Wilde H (1967) Studies in organic mass spectrometry. III. 1,3-Cyclohexanediones. *Bull Soc Chim Belges* 76:111–122.
16. Vandewalle M, Schamp N, De Wilde H (1967) Studies in organic mass spectrometry. IV. Fragmentation of 2-alkyl-1,3-cyclohexanediones. *Bull Soc Chim Belges* 76:123–132.
17. Goto T, Tatematsu A, Nakajima Y, Tanyama H (1965) Organic mass spectrometry. II. The electron impact fragmentation of dimedone and 2-ethyldimedone. *Tetrahedron Lett* 757–762.
18. Maquestiau A, Lejeune P (1967) Spectres de masse de quelques dérivés de la dimédone. *Bull Soc Chim Belges* 76:133–144.
19. Clark RD, Ellis JE, Heathcock H (1973) Methylation of dimedone. *Synth Commun* 3:347–354.
20. Poldy J, Peakall R, Barrow RA (2008) Pheromones and analogs from *Neozeleboria* wasps and the orchids that seduce them: A versatile synthesis of 2,5-dialkylated 1,3-cyclohexanediones. *Tetrahedron Lett* 49:2446–2449.
21. Ibarra F (2002) Intra- und interspezifische chemische Kommunikation von Insekten: Identifizierung und Synthese flüchtiger Signalstoffe. PhD dissertation (University of Hamburg, Hamburg, Germany).
22. Mant J, Peakall R, Weston PH (2005) Specific pollinator attraction and the diversification of sexually deceptive *Chiloglottis* (Orchidaceae). *Plant Syst Evol* 253:185–200.
23. Mant J, Schiestl FP, Peakall R, Weston PH (2002) A phylogenetic study of pollinator conservatism among sexually deceptive orchids. *Evolution* 56:888–889.
24. Phillips RD, Faast R, Bower CC, Brown GR, Peakall R (2009) *Austr J Bot*, in press.
25. Naumann ID (1991) in *The Insects of Australia* (Melbourne Univ Press, Carlton, Victoria), Vol 2, pp 916–1000.
26. Jones DL (2006) *A Complete Guide to Native Orchids of Australia Including the Island Territories* (Reed New Holland, Sydney).
27. Jarau S, et al. (2006) Hexyl decanoate, the first trail pheromone compound identified in a stingless bee, *Trigona recursa*. *J Chem Ecol* 32:1555–1564.
28. Focella A, Teitel S, Brossi A (1977) A simple and practical synthesis of olivetol. *J Org Chem* 42:3456–3457.