

Volatile Constituents of Floral Scents from *Encyclia cordigera* (Kunth) Dressler and *E. randii* (Barb. Rodr.) Porto & Brade (Orchidaceae)

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Encyclia cordigera is native from Central America tropical region, and *Encyclia randii* occurs at Brazilian Amazon environment domains. Floral concentrates from both *Encyclia* species were extracted, analyzed by gas chromatography-mass spectrometry, and identified compounds belong to terpenoids, phenylpropanoids, and fatty acid derivatives classes. Primary constituents of the *E. cordigera* floral scent were β -ionol (45.4%), terpinen-4-ol (12.4%), benzyl benzoate (5.6%), and indole (5.0%). (2*E*,6*Z*)-Farnesol (24.9%), heptanal (11.8%), (*E*)-nerolidol (9.4%), nonanal (6.4%), *p*-vinyl-guaiacol (6.0%), and phenylethyl benzoate (5.3%) have predominated in the floral scent of *E. randii*. From the olfactory standpoint, *E. cordigera* scent was previously characterized as a blend of ionone-floral and aromatic-floral notes. Very likely, *E. randii* scent presents the woody fragrance due to sesquiterpenes (2*E*,6*Z*)-farnesol and (*E*)-nerolidol and the fruity note from the aldehydes heptanal and nonanal. Floral perfume plays a fundamental role in attracting pollinating insects, whose symbiosis contributes to the appearance of chemical variations in orchid species.

Keywords: Orchidaceae, floral scents, β -ionol, (2*E*,6*Z*)-farnesol, volatile concentrates

Introduction

Flowering plants and their volatile compounds attract birds, insects, and other animals, including mammals, as pollinators to their reproduction. More than 1,700 flower scent compounds, covering 990 taxa, have already been identified.¹⁻⁵ The perianth is primarily responsible for scent emission, though all floral organs might contribute to the emission of scents. Floral scents are stored in the oil glands such as the trichomes before it is released into the air as volatile compounds. In addition to flowers, volatile compounds emitted by other plant organs are involved in its defense mechanisms. As seen, floral volatiles play a significant role in the plant's reproductive process by attracting pollinators, but they also can act as repellents⁶ and physiological protectors against abiotic stress.⁵

Floral volatiles are expected to be used in the composition of perfumes, cosmetics, flavors, and therapeutic applications. However, the volatiles emitted by flowers are also the main signals captured by insects

to select gratifying flower species associated with the respective flower colors.⁷ Floral scents are composed by different types of volatile compounds, usually lipophilic and of low molecular weight. Based on their origin, function, and biosynthesis, floral scents are grouped into three main classes of compounds: terpenoids, phenylpropanoids, and derivatives of fatty acids. In the species of Orchidaceae, these have been the main constituents.⁸

Orchidaceae is the most prominent flowering plant family, with ca. 736 genera and ca. 28,000 species, showing a wide diversity of epiphytic and terrestrial specimens, which colonizes almost every Earth habitat.⁹ Associated with the significant number of Orchidaceae species, there is also an extraordinary floral diversification spread in the world. Orchids are renowned for their abundance of morphological types, with an unending number of beautiful variations and very well represented in the monocotyledons' floral evolution.¹⁰

As epiphytic, terrestrial, or rupicolous herbs, with branching sympodial, ovoid pseudobulbs, resupinate flowers, a free lip or one partially adnate to the column, the genus *Encyclia* is composed of about 150 species, all neotropical, distributed from Florida to Northern Argentina

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and in West Indies, of which approximately 40 species occur in Brazil. In the *Encyclia* species, some variations have been observed for the same individual, and their floral diversity seems to be linked to pollination by food deception and mainly mediated by different bee types. Also, the genus is very diverse in the various seasonal environments, from where new species and natural hybrids have been continually reported.¹¹⁻¹³

Encyclia cordigera (Kunth) Dressler (syn. *Cymbidium cordigerum* Kunth, *Encyclia atropurpurea* var. *leucantha* Schltr., *E. atropurpurea* var. *rosea* (Bateman) Summerh., *E. cordigera* var. *rosea* (Bateman) H.G. Jones, *E. doeringii* Hoehne, *E. macrochila* (Hook.) Neumann, *Epidendrum atropurpureum* var. *roseum* (Bateman) Rchb. f., *E. cordigerum* (Kunth) Foldats, among others) is native of the tropical and subtropical region of Central America and is currently cultivated as an ornamental plant in various parts of the world. In *E. cordigera*, the forms of its colors were not taken into account to establishing its synonymy. In the type variety of *E. cordigera*, the petals and sepals' predominant color is chocolate and the white lip. However, it is common to find plants with pink lips and chocolate sepals.^{14,15}

Encyclia randii (Barb. Rodr.) Porto & Brade (syn. *Epidendrum randii* (L. Linden & Rodigas) Barb. Rodr., *Epidendrum atropurpureum* var. *randii* (Barb. Rodr.) L. Linden & Rodigas), an epiphyte with 65 cm tall, is characterized by ovoid-shaped pseudobulbs, almost always vinaceous, large and showy flowers, purple-green sepals and petals, entirely white lip with parallel pink stripes. It is distributed on the Amazonas, Pará, and Rondônia states, occurring at different Brazilian Amazon environment domains.^{16,17}

Continuing the chemical-biological survey of the aromatic flora of the Brazilian Amazon, being carried out by our research group, this work aimed to extract the volatile concentrates of the flowers of *Encyclia cordigera* and *E. randii*, identify their chemical constituents, and associate them with other species/specimens previously analyzed and taxonomically close.

Experimental

Plant materials

The orchids *Encyclia cordigera* and *E. randii* (Figures 1a and 1b), which provided the botanical material for this work, are live plants cultivated in pots containing charcoal and wood shavings, existing in the private nursery of Mr Luiz Otávio Teixeira, located in the Amazon Garden Condominium, BR-316, km 6, 67015-795 Ananindeua, PA,

Brazil (coordinates: 1° 22' 20.96" S/48° 23' 34.14 W). A sample of *E. randii*, native to the Amazon region, is already deposited in the Goeldi Museum Herbarium (MG 205037), Belém, PA, Brazil, while waiting for the subsequent flowering of *E. cordigera* to deposit an exsiccate in the Goeldi Museum Herbarium. *Encyclia cordigera* has its origins in Central America. The flowers of *E. cordigera* and *E. randii* were collected at 6 am to extract their volatile constituents.

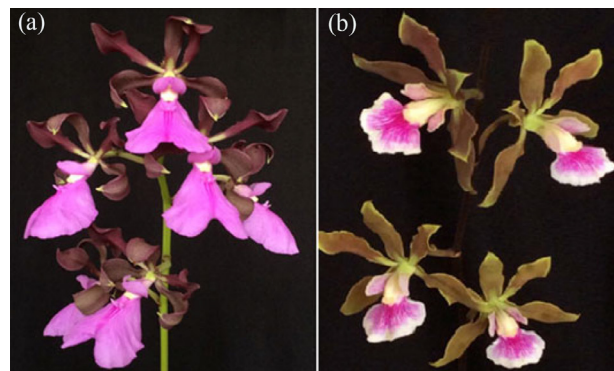


Figure 1. Floral morphology of studied species: *Encyclia cordigera* (a) and *Encyclia randii* (b).

Obtaining and analyzing volatile concentrates

The flowers of *E. cordigera* and *E. randii*, collected separately from the two specimens, were subjected to micro distillation-extraction in a Likens and Nickerson apparatus (3 flowers each, 15 g in total, 2 h, duplicate)¹⁸ to obtain their volatile concentrates, using *n*-pentane (99% high-performance liquid chromatography (HPLC) grade, 3 mL) (Sigma-Aldrich, São Paulo, Brazil) as the solvent.

The volatile concentrates of *Encyclia cordigera* and *E. randii* were submitted to gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. It was performed on a GCMS-QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan), equipped with an AOC-20i auto-injector and the GCMS-Solution software containing the Adams,¹⁹ NIST,²⁰ and FFNSC-2 libraries.²¹ A Rxi-5ms (30 m × 0.25 mm; 0.25 μm film thickness) silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used. The conditions of analysis were as follows: injector temperature: 250 °C; oven temperature programming: 60-240 °C (3 °C min⁻¹); helium as the carrier gas, adjusted to a linear velocity of 36.5 cm s⁻¹ (1.0 mL min⁻¹); split mode injection (split ratio 1:20) of 1.0 μL of the *n*-pentane solution; electron ionization at 70 eV; ionization source and transfer line temperatures of 200 and 250 °C, respectively. The mass spectra were obtained by automatic scanning every 0.3 s, with mass fragments in the range of 35-400 *m/z*. The retention index was calculated for all volatile components

using a homologous series of C8-C40 *n*-alkanes (Sigma-Aldrich, Milwaukee, WI, USA) according to the linear equation of van den Dool and Kratz.²² Individual components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GCMS-Solution system libraries.¹⁹⁻²¹ The quantitative data regarding the volatile constituents were obtained using a GC2010 series gas chromatograph, operated under similar conditions to those of the GC-MS system. The relative amounts of individual components were calculated by peak-area normalization using a flame ionization detector (GC-FID). Chromatographic analyses were performed in duplicate.

Results and Discussion

The chemical constituents of the flower volatile concentrates of *Encyclia cordigera* and *E. randii*, analyzed by GC and GC-MS, are listed in Table 1. The yield of volatile concentrate was 0.2% for both orchids. In total, forty-four constituents were identified, comprising more than 99% of the volatile concentrates. Above 5%, the primary components of the flowers of *E. cordigera* were β -ionol (45.4%), terpinen-4-ol (12.4%), benzyl benzoate (5.6%), and indole (5.0%) (Figure 2). Likewise, the constituents (*2E,6Z*)-farnesol (24.9%), heptanal (11.8%), (*E*)-nerolidol (9.4%), nonanal (6.4%), *p*-vinyl-guaiacol (6.0%), linalool (5.5%), phenylethyl benzoate (5.3%), and phenylethyl alcohol (5.0%) predominated in the volatile concentrate of *E. randii* (Figure 3).

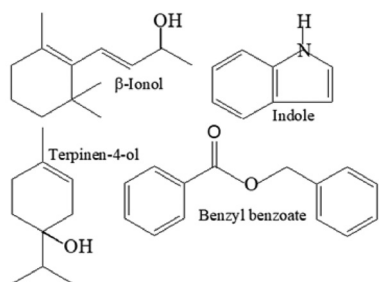


Figure 2. Primary constituents of volatile concentrate of *E. cordigera*.

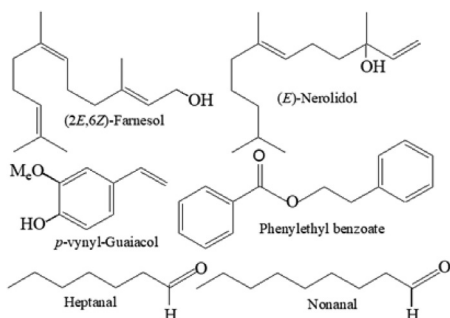


Figure 3. Primary constituents of volatile concentrate of *E. randii*.

Table 1. Constituents identified in the volatile concentrates of *Encyclia cordigera* and *E. randii*

Compound	RI _{Calc.}	RI _{Lit.}	Constituents / %	
			<i>E. cordigera</i>	<i>E. randii</i>
2-Methyl-3-pentanone	745	742 ^a	3.5	
(<i>2E</i>)-Penten-1-al	751	744 ^b	4.4	
1-Pentanol	756	759 ^a	1.3	
1-Hexen-3-ol	784	778 ^a	0.2	
(<i>3E</i>)-Hexen-1-ol	843	844 ^b		3.0
(<i>3Z</i>)-Hexen-1-ol	853	853 ^a		0.9
2,3-Heptanedione	884	885 ^a		0.7
Heptanal	898	901 ^b	1.4	11.8
Benzaldehyde	952	952 ^b	0.4	
<i>trans-m</i> -Mentha-2,8-diene	978	979 ^b		4.4
Benzene acetaldehyde	1038	1036 ^b	0.4	2.8
(<i>E</i>)- β -Ocimene	1047	1044 ^b	3.8	
(<i>2E,4E</i>)-Hexadienol acetate	1071	1070 ^a		1.3
<i>p</i> -Cresol	1075	1071 ^b		2.6
Linalool	1098	1095 ^b	0.2	5.5
Undecane	1102	1100 ^b	0.2	
<i>cis</i> -Rose oxide	1109	1106 ^b	1.9	
Nonanal	1113	1107 ^a		6.4
Phenylethyl alcohol	1118	1113 ^a		5.0
<i>trans</i> -Rose oxide	1125	1122 ^b	0.7	
Terpinen-4-ol	1174	1174 ^b	12.4	
2-Methoxy- <i>p</i> -cresol	1184	1188 ^b		1.5
Methyl salicylate	1190	1190 ^b	0.6	
Dodecane	1196	1200 ^b		0.2
Indole	1289	1290 ^b	5.0	
(<i>3E-6Z</i>)-Nonadienol acetate	1300	1296 ^a		0.5
Tridecane	1308	1300 ^b		0.4
<i>p</i>-Vinyl-guaiacol	1316	1309 ^b		6.0
α -Cubebene	1339	1345 ^b	0.1	
α -Longipinene	1356	1350 ^b	2.6	
(<i>E</i>)- β -Damascenone	1383	1383 ^b	0.3	
Tetradecane	1408	1400 ^b		0.4
Dodecanal	1415	1408 ^b		0.4
β-Ionol	1417	1412 ^b	45.4	
(<i>E</i>)-Caryophyllene	1422	1417 ^b	2.3	
(<i>E</i>)- β -Ionone	1485	1487 ^b	3.1	
(<i>E,E</i>)- α -Farnesene	1508	1505 ^b	1.6	
(<i>E</i>)-Nerolidol	1564	1561 ^b	1.7	9.4
<i>cis</i> - β -Elemenone	1585	1589 ^b		4.6
Dillapiol	1619	1620 ^b		0.9
(<i>2E,6Z</i>)-Farnesol	1719	1716 ^a		24.9
Benzyl benzoate	1758	1759 ^b	5.6	
Phenylethyl benzoate	1864	1856 ^a		5.3
<i>n</i> -Hexadecanol	1881	1874 ^b		0.8
Terpenoids (monoterpenes and sesquiterpenes)			76.1	48.8
Fatty acid derivatives			11.0	26.8
Phenylpropanoids/benzenoids			12.0	24.1
Total / %			99.1	99.7

^aMondello;²¹ ^bAdams.¹⁹ RI_{Calc.}: calculated retention index (Rxi-5ms column); RI_{Lit.}: literature retention index; in bold: main constituents above 5%.

From an olfactory point of view, Kaiser²³ highlighted the “*Encyclia* perfume”, characterized as a very diffuse “ionone-floral” perfume, accompanied by distinct “aromatic floral” and “spicy floral” notes, that was exemplified by the analysis of smell from *E. adenocarpa* flowers, containing about 10% β -ionone, together with a significant content of aliphatic and aromatic esters. As can be seen, the content of β -ionol and β -ionone is 48.5% in the volatile concentrate of the *E. cordigera* flowers. Although the chemical analysis does not substitute the olfactory evaluation, due to qualitative and quantitative variations that lead to distinct odors, it is assumed that the volatile concentrate of *E. cordigera* presents an “*Encyclia* perfume”, as described by Kaiser²³ for the smell of *E. adenocarpa* (Lex.) Schltr. flowers, in association with the significant amounts of indole and benzyl benzoate present in the floral scents of both species. The volatile concentrate of the flowers of another specimen of *E. cordigera*, existing in Southeast Mexico, exhibited a different chemical composition, with *cis*-geraniol (nerol), benzyl benzoate, and indole as their primary constituents.²⁴ In turn, the scent of *E. randii* presented a floral-woody note, probably due to the high amounts of the sesquiterpenes (2*E*,6*Z*)-farnesol and (*E*)-nerolidol, associated with a fruity note from the aldehydes heptanal and nonanal, in a refreshing and pleasant combination. No previous analysis of the volatile composition of *E. randii* flowers has been found in the literature.

Some floral scents of other *Encyclia* species have been previously reported. *Encyclia adenocarpa* and *E. parviflora* from Mexico showed lightly fragrant flowers, with hexadecene, (*E*)-nerolidol, indole, and (*E*)- β -ocimene as the more significant components in the volatile concentrate of the first species, and methyl 3,4,5-trimethoxybenzoate and *p*-vinylanisole in the volatile concentrate of the second species.²⁴ The volatile constituents of the flowers of *Encyclia vespa* (Vell.) Dressler and *E. fragrans* (Sw.) Lemée, sampled in Belém, Brazil, were identified. Terpinen-4-ol, verbenone, *trans*-verbenol, and α -pinene were the significant components of *E. vespa* flowers, while the compounds terpinen-4-ol, (2*Z*-6*E*)-farnesol and *trans*-verbenol have predominated in the *E. fragrans* flowers.²⁵ The floral scent of another *E. fragrans* specimen, distributed from Mexico across Central and South America and West Indies, was also reported by Kaiser,²³ where (*E*)- β -ocimene, 3,5-dimethoxy-toluene, (*E*)-methylcinnamate, hydroquinone dimethyl ether, and hexyl butyrate were the main constituents. The floral scents of three other *Encyclia* species were also described in the book by Kaiser.²³ *Encyclia baculus* (Rchb. f.) Dressler & G.E. Pollard, distributed from Mexico to Colombia, was rich in (*E*)- β -ocimene, 2,2,6-trimethyl-cyclohex-2-ene-1,4-dione,

and benzaldehyde; *Encyclia citrina* (Lex.) Dressler, occurring in Mexico, was rich in ipsdienol, ipsdienone, myrcene, and geraniol; and *Encyclia glumacea* (Lindl.) Pabst, existing in Brazil, was rich in linalool and (*E*)- β -ocimene.

To survive and reproduce, plants interact with various organisms in their environment to defend themselves against herbivores and pathogens and, for sexual reproduction, they need to attract insect pollinators. Therefore, the plants have evolved defense signals, such as herbivore-induced volatile signals and visual and olfactory floral signals, to attract pollinators. The floral scent stands out for its chemical variation between floral signals, both among and within taxa.²⁶ In a complementary way,²⁷ hypothesized that in the plant-pollinator relationship, floral scent plays a fundamental role in attracting pollinating insects, whose symbiosis contributes to the plant evolution, resulting in the appearance of intra- and interspecific variations which will be more related to the volatile composition of the floral scent than the proper floral morphology.

The floral chemical diversity mediates various functions to attract pollinators or deter herbivores from consuming the plant's reproductive structures.²⁸ As already mentioned, more than 1700 constituents of the scent of flowers, at about 1000 taxa, have been reported. The floral scent comprises different secondary metabolism compounds in plants: terpenoids, phenylpropanoids, and fatty acid derivatives.⁵ Terpenes are produced by both the classical mevalonate and the methylerythritol phosphate pathways,²⁹ and they represent the largest group of plant floral volatiles, including those emitted by orchids. Phenylpropanoids are the second most dominant group of volatile compounds in plant floral scents, produced from the aromatic amino acid phenylalanine via the shikimate pathway and some enzymatic steps. Among the volatile floral compounds, fatty acid derivatives are the smallest group, consisting mainly of floral fatty acids derived from C18 polyunsaturated fatty acids and linolenic and linoleic acids.³⁰ In the floral scents of *E. cordigera* and *E. randii*, the terpenic compounds (mono- and sesquiterpenes) were the major constituents (76.1 and 48.8%), followed by benzenoids/phenylpropanoids (12.0 and 24.1%) and fatty acid derivatives (11.0 and 26.8%), respectively.

The monoterpenes and sesquiterpenes, linalool, terpinen-4-ol, (*E*)- β -ocimene, farnesene, (*E*)-nerolidol, farnesol, and (*E*)-caryophyllene, identified in the volatile concentrates of *E. cordigera* and *E. randii*, are common constituents found in other plant floral scents, particularly in orchids.³ The ionones originate from the oxidative cleavage of carotenoids, resulting in low molecular weight volatile compounds,³¹ such as β -ionol and (*E*)- β -ionone found in the *E. cordigera* and *E. randii* floral scents.

Lipoxygenases and hydroperoxide lyases are involved in the production of short-chain alcohols, aldehydes, and esters,³² such as 2-methyl-3-pentanone, (2*E*)-penten-1-ol, 1-pentanol, 1-hexen-3-ol, (3*E*)- and (3*Z*)-hexen-1-ol, heptanal, nonanal, (3*E*,6*Z*)-nonadienol acetate, and dodecanal, all of them produced by the degradation of C-18 fatty acids and identified in the *E. cordigera* and *E. randii* volatile concentrates. Phenylpropanoids belong to another class highly distributed among aromatic flower compounds. More than 300 compounds of this class have been identified, which includes benzaldehyde, benzene acetaldehyde, *p*-vinyl-guaiacol, *p*-cresol, 2-methoxy-*p*-cresol, phenylethyl alcohol, phenylethyl benzoate, benzyl benzoate, methyl salicylate, indole, and dillapiolol,⁵ all of them found in the floral scents of *E. cordigera* and *E. randii*.

Conclusions

The floral scents of *E. cordigera* and *E. randii* exhibited volatile compounds from the classes of terpenoids, phenylpropanoids, and fatty acid derivatives. The main constituents of the floral scent of *E. cordigera* were β -ionol, terpinen-4-ol, benzyl benzoate, and indole, while (2*E*,6*Z*)-farnesol, heptanal (*E*)-nerolidol, nonanal, and *p*-vinyl-guaiacol have predominated in the floral scent of *E. randii*. From the olfactory standpoint, the scent of *E. cordigera* can be characterized as a blend of ionone-floral and aromatic-floral notes. In contrast, the scent of *E. randii* presented a floral-woody note due to the sesquiterpenes (2*E*,6*Z*)-farnesol and (*E*)-nerolidol with a fruity note from the aldehydes heptanal and nonanal, in a refreshing and pleasant combination. It has been considered that floral perfume plays a fundamental role in attracting pollinating insects, whose symbiosis contributes to the evolution of the plant and the appearance of specific variations related to floral perfume's volatile composition than floral morphology.

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