Floral Fragrance Components of *Zygopetalum mackayi (Orchidaceae)*

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Zygopetalum which belongs to *Orchidaceae* (subtribe *Zygopetalinae*) is a genus of about 20 species, native to Brazil. *Zygopetalum mackayi* is common in cultivation. The flower spike has several flowers (5cm), green, blotched with purple or brown. The flower opens at the beginning of winter (November ~ December) in greenhouses in Japan, and is long-lasting and fragrant. The greenhouse is very fragrant with the flowers.

Orchid floral fragrances have been studied in connection with species-specific attraction of pollinators. All species of the Neotropical subtribes *Stanhopeinae* and *Catasetinae* (*Orchidaceae*) are pollinated exclusively by male euglossine bees, *Stanhopea* species, which are attracted to and collect the floral fragrances. The flower of a given species may attract males of only one or a few species out of dozens on euglossine species. The methods and advances in the studies of the orchid-euglossine bee relationship in the last sesquidecade has been summarized by Williams and Whitten.[1] The other orchids adapted to euglossine bees include the *Lycastinae*, *Zygopetalinae*, and a number of *Oncidium* allies.[2] The advances and trends in orchid floral fragrance analysis in the past 30 years have been reviewed by Williams and Whitten.[3] The male melon fly (*Dacus cucurbitae*) is attracted to the blossoms of *Dendrobium superbum* (*Orchidaceae*). The volatile components of the flower were identified by GC-MS[4]. The relationship between the volatile components of the flower of genus *Ophrys* (*Orchidaceae*) and their pollinator species among the solitary bee genus *Andrena* has been studied by Borg-Karlson *et al.*[5~7] So far as we searched with the Lockheed DIALOG System, no investigations have been made of floral fragrance components of the genus *Zygopetalum*.

The Tenax GC trapping technique described in our previous paper[8] was used. The flowers of *Z. mackayi* give out a strong fragrance around eleven o clock a.m. in the

TABLE I. FLORAL FRAGRANCE COMPOUNDS IDENTIFIED IN *Zygopetalum mackayi*

Peak No.	Compound	Relative count %[a]	Identification means	Attractiveness[b,2] to male bees
1	2-Butanone	1.4	MS, Rt	
2	Myrcene	21.3	MS, Rt	*
3	(*Z*)-β-Ocimene	4.5	MS, Rt	*
4	(*E*)-β-Ocimene	49.5	MS, Rt	*
5	Benzaldehyde	1.2	MS, Rt	*
6	Unidentified	0.2		
7	Phenylacetaldehyde	1.2	MS, Rt	
8	Benzyl acetate	7.2	MS, Rt	**
9	α-Farnesene	1.2	MS, Rt	
10	Methyl salicylate	1.4	MS, Rt	**
11	Unidentified	0.2		
12	2-Phenylethyl acetate	9.7	MS, Rt	**
13	Cinnamyl acetate	1.0	MS, Rt	*

[a] Total ion intensity.
[b] Attractiveness for euglossine bees: *=moderate attractant; **=good attractant.

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greenhouse. Eleven flowers are picked from two flowe. stalks, then placed in a 500-ml flask and heated at 27°C in a water bath. Purified air (50 ml/min passed through a Molecular Sieve 5A column) was led into the flask *via* a Teflon tube and passed over the flower and out of the flask through a column of Tenax GC (9-cm length × 0.5-cm i.d., 0.3 g of 60/80 mesh) for 30 min. After collection of volatiles the Tenax column was purged with a stream of purified nitrogen (50 ml/min for 30 min) to remove water. The column was placed in a desorber (Flash Sampler FLS-3, Shimadzu Corp. Kyoto, Japan) attached to a GC/MS inlet. Helium carrier gas was passed through the column for 5 min to remove nitrogen, and then the column was heated at 250°C within one minute with carrier flow. The volatile components were thermally desorbed from Tenax GC and directly transferred to a capillary column *via* a splitter.

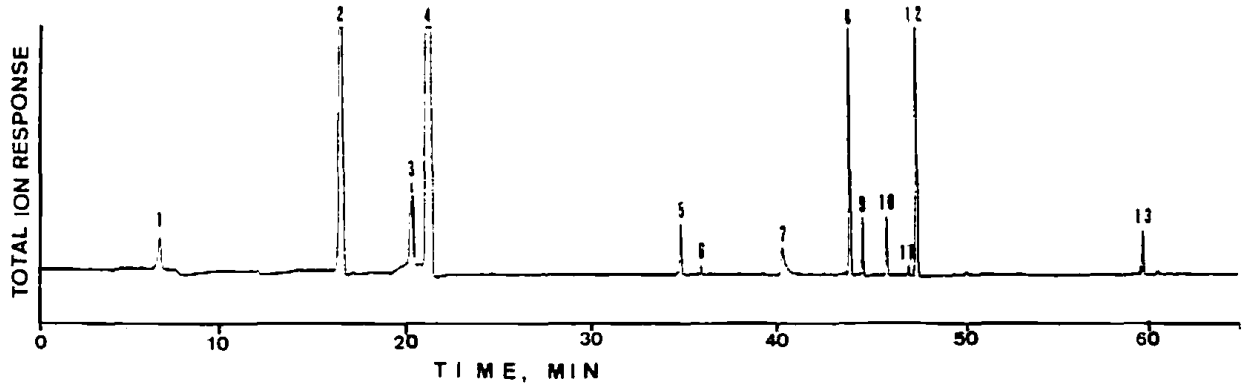

FIG. 1. Total Ion Chromatogram of Headspace Volatile Components Obtained by Tenax GC Trapping Technique and Capillary Column (Quadrex 007 CW-20M) GC/MS.

Peak numbers refer to numbers in Table I.

The capillary column used for the study was a 50 m × 0.25 mm (i.d.) fused silica Quadrex 007 CW-20M wall coated column. The carrier gas was helium at a flow velocity of 22 cm/sec. The oven was held for 5 min at 40°C after thermal desorption of volatiles, then programmed at 3°C/min from 40 to 180°C and then held at the upper limit. The split ratio was about 40:1. The outlet of the column was directly coupled to the ion souce of a quadrapole Mass Spectrometer (GCMS QP-1000, Shimadzu Corp. Kyoto, Japan) equipped with a data system. Conditions for EI spectra runs were: ionization potential 70 eV; ionzation current 60 μA; ion source temperature 260°C; scan rate 1-sec interval. Identification of compounds was based on computer matching of mass spectra of compounds published in "EPA/NIH Mass Spectra Data Base (1975, 1980, and 1983)" and coincidence for MS pattern of authentic compounds as well as coincidence for Kovàts retention indices.

The floral fragrance compounds identified in *Z. mackayi* are listed in Table I, and a corresponding total ion chromatogram is shown in Fig. 1. The capillary column GC/MS of Tenax GC concentrates indicated 13 peaks, of which 11 peaks, not including two weak peaks, were identified. The identified compounds are 4 hydrocarbons (3 terpenes and 1 sesquiterpene), 1 ketone, 2 aldehydes, 4 esters. The eight compounds of peaks No. 2 ~ 5, 8, 10, 12, and 13 marked with * and ** in Table I are found in several genera of Neotropical orchids, and attract male euglossine bees.[1] α-Farnesene and phenylacetaldehyde are found in the floral fragrance of the genus *Ophrys*.[5~7] The oxygenated compounds mainly account for the characteristic fragrance of the flower. Sensory evaluations were done by the sniff test for the pentane solution of each authentic oxygenated compound, and for the pentane solution mixed with the ratio of relative count percent of oxygenated compounds. The odor of the mixture is very similar to the floral fragrance of the species. According to human olfactory perception, β-phenylethyl acetate is the most significant volatile compound which characterized the froral fragrance. Phenylacetaldehyde and cinnamyl acetate are secondary important compounds.

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