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# FLORAL FRAGRANCE COMPONENTS OF *BRASSAVOLA* (ORCHIDACEAE: LAELIINAE)

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During the past several years the floral fragrance components of several genera of orchids have been identified (Hills, Williams & Dodson, 1968, 1972). In most of the genera in which floral fragrance components have been identified, the floral fragrance components have been important in the attraction of pollinators (Dodson & Hills, 1966; Hills, 1968; Hills, Williams & Dodson, 1968, 1972; Dodson *et al.*, 1969; Dodson, 1970; Williams & Dodson, 1972), especially in the attraction of male euglossine bees to various species of orchid flowers as pollinators. *Brassavola*, however, is not pollinated by male euglossine bees, but possesses the syndrome associated with moth pollination in the Orchidaceae: 1) odor production at night, 2) weakly zygomorphic flowers, 3) landing place curved backwards or turned upwards, 4) color white, cream or often greenish, 5) odor heavy-sweet, very strong, not fruity, 6) abundant nectar deeply hidden in narrow tubes, 7) colored nectar guides absent, 8) position of the flower rarely erect, usually horizontal or hanging (modified from van der Pijl & Dodson, 1966). Unfortunately, detailed observations on moth pollination in the group are scanty, being limited to one report by van der Pijl & Dodson (1966) of sphingid pollination in *B. digbyana* and a personal communication (from N. G. Smith) of moth pollination of *Brassavola nodosa* in Panama.

*Brassavola* has presented two taxonomic problems for several years: the species known as *B. digbyana* and *B. glauca*. Both of these species were described as brassavolas, *B. glauca* in 1839 and *B. digbyana* in 1846. Reichenbach (1861) united all of the species of *Brassavola* with *Bletia*, a move not accepted by recent workers (Dressler, 1968). Bentham (1880) transferred the two species to *Laelia*, a treatment followed by Williams (1951) and Ames & Correll (1953). Schlechter (1918) erected a new genus, *Rhyncholaelia*, for the two species and was followed by Williams (1956) and Hawkes (1964a, 1964b). Jones (1967) retained the two species in *Brassavola*. Oesterreich (1967) placed the two species in *Rhyncholaelia* on the basis of the number of pollinia, and also included *B. cucullata* in *Rhyncholaelia*, overlooking the fact that *B. cucullata* is the type species of *Brassavola*. Dressler (1959) presented evidence for including *B. digbyana* and *B. glauca* in *Brassavola*. With abundant controversy and little evidence on the status of these species in relation to the remainder of *Brassavola* in the literature, it seemed desirable to try a new line of investigation into the relationships of these species.

Since mono-terpenoids are often useful taxonomic characters in many plant groups (von Rudloff, 1969), it was felt that the floral terpenoids (and other volatile floral fragrance components) might be useful in providing evidence on the *Brassavola-Rhyncholaelia* question. The floral fragrances of *B. digbyana*, *B. glauca*, and six additional species of *Brassavola* were examined by gas chromatography.

## MATERIALS AND METHODS

Plants were grown in the greenhouse at the Department of Biology, University of Miami, Coral Gables, Florida. All were grown under as uniform conditions as possible. The floral fragrances were sampled from the second day after the flower opened until floral fragrance production stopped. In most cases the floral fragrances were strongest three to five days after

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anthesis. The inflorescence was placed in the sampling chamber at approximately 5:00 p.m. local time and fragrance production usually started approximately at dusk. Sampling was continued until dawn, when fragrance production ceased. The sampling chambers (necessary to concentrate the fragrance) were constructed of 1/4-inch clear plexiglas. The chambers were constructed so that the entire inflorescence was enclosed but was not cut from the plant. It was therefore possible to remove the intact inflorescence from the chamber the next day and return the plant to the greenhouse until further sampling was desired. An equilibration period of at least one hour was allowed for the concentration of the floral fragrances. After equilibration a ten milliliter sample was withdrawn from the sampling chamber by a gastight syringe and the sample was injected into the gas chromatograph for analysis.

An F & M 810 dual flame ionization gas chromatograph equipped with a 1:1 effluent splitter was used for this study. The use of an effluent splitter allowed the operator to smell a compound as it eluted from the column. Thus it was possible to compare the odor of an unknown compound with the odors of known compounds. The floral fragrances were sampled at oven temperatures of 70, 130, 150, degrees Centigrade. The flow rates for the carrier gas (helium, 60 psi) were 70 ml/min at 70° C and 80 ml/min at 130°, and 150° C. The injection port temperature was 165° C and the detector temperature was 190° C. Standards for the calculation of relative retention times were beta-pinene at 70° C, ethyl benzoate at 130° C, and 2-phenyl ethanol at 150° C. The machine was equipped with an automatic disk integrator and the percentage of a compound in an odor was calculated from the integrator trace. Two columns were used throughout the study, with an additional two columns used near the end of the study. The best results were obtained using a 6-foot long, 1/4-inch O.D. stainless steel column packed with 3% carbowax 20M (polyethyleneglycol) on 80-100 mesh Diatoport S. Samples were also chromatographed on a 6-foot long, 1/4-inch O.D. stainless steel column packed with 10% Lac 446 (diethylene glycol adipate) on 80-100 Chromosorb W. In the latter parts of the study, two additional types of columns

TABLE 1

Species of *Brassavola* studied

| | Total number of compounds | Number of unique compounds | % Unique |
|---|---|---|---|
| Section *Grandiflorae* Rolfe | | | |
| *Brassavola digbyana* Lindl. | 9 | 0 | |
| *B. glauca* Lindl. | 8 | 0 | |
| Section *Brassavola* | | | |
| *B. martiana* Lindl. | 5 | 0 | |
| *B. perrinii* Lindl. | 8 | 2 | 25 |
| Section *Cuneilabia* Rolfe | | | |
| *B. acaulis* Lindl. | 11 | 1 | 9 |
| *B. cordata* Lindl. | 10 | 3 | 30 |
| *B. grandiflora* Lindl. | 9 | 1 | 11 |
| *B. nodosa* (L.) Lindl. | 3 | 0 | |

were used: a 16-foot long, 1/8-inch O.D. stainless steel column packed with 3% carbowax 20M on 60-80 mesh Gas Chrom P. and a 16-foot long, 1/8-inch O.D. stainless steel column packed with 3% Silicone Fluid SF-96 on 60-80 mesh Gas Chrom Q. The majority of the sampling was done with a 1/4-inch carbowax column at 70° and 130°C. Samples were also programmed from 50° to 200°C at 6 degrees/min to check for additional compounds which might have been missed at the isothermally sampled temperatures. No additional compounds were found by such programming; therefore, the relative retention times from isothermal temperatures of 70°C, 130°C, and 150°C on carbowax are the only ones reported in this study.

Some compounds have been tentatively identified by comparing the relative retention time of the unknown peak with the relative retention times of known compounds. If the relative retention times were in fairly close agreement on two different columns, the known compound was run as a mixture with the unknown compound at reduced temperatures. The temperature was decreased in five degree decrements until a double peak appeared (in which case the two were considered different compounds), or until the single peak became too spread to be sharply defined. If enrichment of an unknown compound with a known compound did not produce a double peak at reduced temperatures on two different columns, the compound was considered to be tentatively identified. The technique has been shown to be successful in identifying floral fragrance compounds by a bioassay for the attraction of pollinators to floral fragrance components in a number of field trials (Dodson *et al.*, 1969; Williams & Dodson, 1972). Although additional confirmation of the identifications would be desirable, such studies have not been done because of a lack of facilities.

Paired affinity indices were calculated for the floral fragrance components by the method of Alston and Turner (1963) using the following equation:

$$\text{paired affinity (PA)} = \frac{\text{Compounds in common for species A and B}}{\text{total compounds in A plus b}} \times 100$$

Compounds present in less than 0.1% are listed as trace (t).

## RESULTS

The species sampled, the number of compounds present per species, and the number of unique compounds per species are listed in Table 1.

The compounds found in the floral fragrances of the species sampled are listed in Table 2 and the identification is given where known or suspected. The percentage composition of the fragrance of each species sampled is given in Tables 3, 4 and 5. The percentage composition is calculated from the integrator trace and is calculated as the percentage of a compound in the injected sample. Table 6 lists the paired affinities of each species with every other species.

It can be seen from Table 3 that two compounds (ocimene and cineole) form an important portion of the odor in every species. Also, ocimene and cineole are not found together in any significant concentrations. *Brassavola digbyana* is unique in producing large amounts of linalool in its floral fragrance. One of the most striking features of this study was the demonstration that the production of floral fragrance compounds did not follow any taxonomic lines in this group. Ocimene was produced by both *B. glauca* and *B. digbyana*, by *B. martiana* but not by its close relative *B. perrinii*, by *B.*

TABLE 2

Floral fragrance components of *Brassavola* species

| Temperature | Relative Retention Time on Carbowax* | Identification |
|---|---|---|
| 70° C | 0.37 | |
| | 0.55 | |
| | 0.66 | alpha-pinene |
| | 1.00 | beta-pinene |
| | 1.13 | |
| | 1.29 | myrcene ? |
| | 1.78 | 1-8 cineole |
| | 2.20 | ocimene |
| 130° C | 0.40 | citronellal ? |
| | 0.52 | linalool |
| | 0.80 | |
| | 0.87 | methyl benzoate ? |
| | 1.06 | alpha-terpeneol ? |
| | 1.18 | |
| | 1.34 | citronellol |
| | 1.58 | methyl salicylate ? |
| | 1.95 | |
| | 2.28 | |
| 150° C | 0.24 | |
| | 0.44 | |
| | 0.52 | |
| | 0.61 | |
| | 0.72 | |
| | 0.86 | |
| | 1.02 | |
| | 1.53 | |

* In some cases, indicated by a question mark, the identification is not certain.

*grandiflora* but not by its close relative *B. nodosa*. Conversely, cineole is produced by *B. perrinii* but not by its relative *B. martina*, and by *B. nodosa* but not by its close relative *B. grandiflora*. *Brassavola acaulis* and *B. cordata* produce mainly ocimene, but both also produce minute amounts of cineole.

## DISCUSSION

One of the major purposes of this study was to provide evidence on the systematic position of *Brassavola glauca* and *B. digbyana*. The data from gas chromatography of the floral fragrances support the treatment of these two species as members of the genus *Brassavola*, and not as a separate genus, *Rhyncholaelia*. The paired affinity indices which were calculated from the odor compositions show that *B. digbyana* and *B. glauca* were more closely related to each other than either was to any other species; however, this study also showed that *B. glauca* was quite similar to *B. cordata* in the production of floral fragrance components.

On the basis of the floral fragrance components it seems best at first thought to consider *B. digbyana* and *B. glauca* as members of the genus *Brassavola*. It should be remembered that the floral fragrances might be im-

TABLE 3

Percentage of fragrance compounds in the injected sample at 70° C

| | 0.37 | 0.55 | 0.66 | 1.00 | 1.13 | 1.29 | 1.78 | 2.20 |
|---|---|---|---|---|---|---|---|---|
| *B. digbyana* | 1.1 | 3.6 | 5.6 | - | 0.4 | 0.3 | - | 26.5 |
| *B. glauca* | - | 4.9 | 6.7 | - | 1.3 | - | - | 67.9 |
| *B. martiana* | 4.4 | - | 22.3 | - | 1.1 | - | - | 72.0 |
| *B. perrinii* | 0.1 | - | 17.6 | 0.8 | ? | 1.7 | 75.7 | - |
| *B. acaulis* | 0.6 | - | 1.5 | 0.5 | 0.2 | 0.3 | 0.8 | 58.9 |
| *B. cordata* | - | 0.6 | 0.6 | - | 0.7 | - | 0.3 | 53.2 |
| *B. grandiflora* | 0.3 | - | 2.5 | ? | - | 0.2 | | 83.1 |
| *B. nodosa* | - | ? | 24.9 | - | - | - | 35.5 | - |
| | | | alpha-pinene | beta-pinene | | myrcene ? | 1 - 8 cineole | ocimene |

TABLE 4

Percentage of fragrance compounds in the injected sample at 130° C

| | 0.40 | 0.52 | 0.80 | 0.87 | 1.06 | 1.18 | 1.34 | 1.58 | 1.95 | 2.28 |
|---|---|---|---|---|---|---|---|---|---|---|
| *B. digbyana* | 3.6 | 54.4 | - | - | - | - | 4.4 | - | - | - |
| *B. glauca* | 3.9 | t | - | - | - | - | 9.0 | 5.8 | - | - |
| *B. martiana* | - | t | - | - | - | - | - | - | - | - |
| *B. perrinii* | - | 0.4 | - | - | 0.8 | 2.6 | - | - | - | - |
| *B. acaulis* | - | 12.0 | - | 1.4 | - | - | 1.8 | - | - | 3.0 |
| *B. cordata* | - | 0.9 | - | - | - | - | 12.7 | 4.0 | 7.9 | 4.5 |
| *B. grandiflora* | - | 13.7 | t | - | - | - | t | t | t | - |
| *B. nodosa* | 39.4 | - | - | - | - | - | - | - | - | - |
| | citronellal ? | linalool | | methyl benzoate ? | alpha-terpeneol ? | | citronellol | methyl salicylate ? | | |

TABLE 5

Percentage of fragrance compounds in the injected sample at 150° C*

| | 0.24 | 0.44 | 0.52 | 0.61 | 0.72 | 0.86 | 1.02 | 1.53 |
|---|---|---|---|---|---|---|---|---|
| *B. acaulis* | - | 4.2 | 7.9 | - | - | 4.4 | 1.8 | t |
| *B. cordata* | 1.3 | 7.4 | 1.3 | 2.5 | 1.3 | - | - | - |

* No compounds were found in the 150° C range in any of the other species sampled.

TABLE 6

Paired Affinity Indices of the Species of *Brassavola*

| | *B. digbyana* | *B. glauca* | *B. martiana* | *B. perrinii* | *B. acaulis* | *B. cordata* | *B. grandiflora* | *B. nodosa* |
|---|---|---|---|---|---|---|---|---|
| *B. digbyana* | - | 70.0 | 55.5 | 30.7 | 53.8 | 46.1 | 50.0 | 20.0 |
| *B. glauca* | | - | 44.4 | 14.2 | 35.7 | 63.6 | 41.6 | 22.2 |
| *B. martiana* | | | - | 30.0 | 45.4 | 36.3 | 40.0 | 14.2 |
| *B. perrinii* | | | | - | 46.1 | 21.4 | 30.7 | 22.2 |
| *B. acaulis* | | | | | - | 50.0 | 42.8 | 16.6 |
| *B. cordata* | | | | | | - | 6.1 | 18.1 |
| *B. grandiflora* | | | | | | | - | 9.0 |
| *B. nodosa* | | | | | | | | - |

portant mainly in attracting pollinators, and in that case it would be probable that any flower which was moth pollinated would produce the same general type of odor. If the floral fragrances are adaptive and function in attracting moths as pollinators, the common occurrence of the similar compounds in *B. digbyana* and *B. glauca* with the rest of *Brassavola* could be interpreted as an example of convergence of floral odors due to selection by pollinator pressure.

*Brassavola nodosa*, *B. cordata*, and *B. grandiflora* are essentially inseparable on the basis of morphological characters, but there are geographical differences and some slight morphological differences. *Brassavola cordata* is endemic to Jamaica and has numerous small flowers per inflorescence. *Brassavola grandiflora* is found on the Caribbean coast of Central America, San Andreas Island, and the Caribbean coast of northern South America. It generally has larger flowers, only two to five flowers per inflorescence, and open, flattened leaves. *Brassavola nodosa* is found on the Pacific coast of Central and South America, and on the Caribbean coast of Mexico north of Yucatan Peninsula. *Brassavola nodosa* usually has flowers intermediate in size between *B. cordata* and *B. grandiflora* and has terete leaves. The main differences between these three species are in the size of the flower, the number of flowers per inflorescence, and whether or not the leaves are open and flat or terete to semi-terete. It can be seen from Table 2 that there are also differences in the floral fragrance components. It is interesting that *B. cordata*, which has the most limited distribution and is most isolated from other species of the genus, has more unique compounds (3) than any other species sampled.

The actual role of these floral fragrance compounds in attracting the pollinators of these species is unknown. Information on pollination in this genus is essentially lacking. Field experiments with the identified (or suspected) floral fragrance components were conducted in Panama in 1968-1969 from dusk until midnight for several weeks with no positive results (presentations of the floral fragrances were as discussed in Williams and Dodson, 1972). Possibly a visual cue as well as an olfactory cue is also necessary for the complete attraction of moths to these flowers. Brantjes (1978) has discussed the relative importance of floral odors and visual cues in the attraction of some temperate zone moths to flowers, and found that floral odor was an impor-

tant part of the attraction of the moths to the flowers. Obviously, this subject needs further investigation.

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