Pergamon

Phytochemistry, Vol. 46, No. 7, pp. 1169–1172, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

PII:S0031-9422(97)00428-7

HEADSPACE ANALYSIS OF VOLATILE FLOWER SCENT CONSTITUENTS OF BAT-POLLINATED PLANTS

HANS JÜRGEN BESTMANN,* LOTHAR WINKLER and OTTO VON HELVERSEN†

Institut für Organische Chemie 2 der Universität Erlangen-Nürnberg, Henkestrasse 42, 91054 Erlangen, Germany;
†Institut für Zoologie 2 der Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany

(*Received in revised form* 22 *April* 1997)

Key Word Index—Bat-pollinated plants; olfactory orientation; flower scent constituents; GC/MS analysis; sulphur compounds.

Abstract—The flower scents of 11 bat-pollinated plant species have been investigated using the 'closed-loop-stripping'-adsorption technique. GC and GC/MS analyses of the headspace samples resulted in the identification of 49 compounds, comprising amongst others sulphur compounds, terpenoids and aliphatic compounds. © 1997 Elsevier Science Ltd

INTRODUCTION

In the New World's tropics a considerable number of plants—more than 590 species from over 160 genera—are pollinated by bats (microchiroptera) [1]. The flowers of these plants show some characteristic traits, such as flowering at night, richness of pollen and nectar and dull colours; often the flowers are also situated at well exposed positions on the plant [2]. In most cases they also produce a typical and peculiar flower scent that is plainly different from the scents of flowers visited by bees, moths or flies. The scents of bat-pollinated flowers are commonly described as displeasing, e.g. reminiscent of fermenting fruits, cabbage-like, garlic-like, urine-like etc. [1]. While our knowledge about acoustical orientation of bats is already very extensive, practically no information exists about the importance of olfactory orientation for flower-visiting bats, nor is anything known about the special function of the peculiar scent of bat-pollinated flowers with respect to the animals' search for food. Thus, the major aim of this study was to identify the chemical compounds that comprise such scent bouquets, and which of these compounds might eventually be common and typical to the flower scents of bat-pollinated plants and thus perhaps responsible for the attractiveness of these scents to the animals.

RESULTS AND DISCUSSION

Floral volatiles were collected from 11 plant species from eight different genera reported or presumed from morphology to be bat-pollinated. In all cases sampling was done by dynamic headspace techniques ('closed-loop-stripping') from flowers *in situ* [3, 4].

The headspace samples were analysed by GC and GC/MS. Forty-nine compounds could be identified by their mass spectra as well as by their Kovàts retention indices. The chemical composition of the floral scents is shown in Table 1.

Several sulphur compounds were found in the flower headspace of *Bauhinia ungulata*, *Calyptrogyne ghiesbreghtiana*, *Cobaea scandens*, *Crescentia cujete*, *Parmentiera alata* and *Vriesea gladioliflora* (Fig. 1). In all of these scents except for *V. gladioliflora*, where only dimethyl disulphide was detected, a series of dimethyl sulphides containing two to four sulphur atoms were present. The headspace of *B. ungulata*, *C. ghiesbreghtiana* and *C. cujete* also contained some irregular, non-branched di- and trisulphides of chain lengths between five and ten. 2-thiapropane-1-thiol (*C. ghiesbreghtiana*) and dimethyltrithiocarbonate (*C. cujete*) to the best of our knowledge, are reported here to be constituents of floral scents for the first time.

A number of isoprenoid compounds were also trapped (Table 1). They occur in all of the flower scents investigated. Limonene and α-pinene were the most common compounds among the monoterpenes; the first was present in all 11 flower scents analysed. 1,8-cineole, which was identified in four flower scents, was the most abundant oxygenated terpene, while β-caryophyllene was found to be the most common of all the sesquiterpenes detected.

Only two aromatic compounds were found in the flower scents. *p*-cymene represents a minor headspace constituent of *Musa* sp. Benzaldehyde is present in the headspace of both *Weberocereus* species, where it is

---

* Author to whom correspondence should be addressed.

Table 1. Chemical composition of the floral scent of 11 species of bat-pollinated plants*

Plant species†	*B. ung.*	*C. ghi.*	*C. sca.*	*C. cuj.*	*H. cal.*	*Musa*	*P. acu.*	*P. ala.*	*V. gla.*	*W. bio.*	*W. tun.*
Number of samples collected	3	3	3	2	3	2	1	3	3	2	2
Sulphur containing compounds											
Thiols											
2-Thiapropane-1-thiol[°]		3.6									
Polysulphides											
Dimethyldisulphide	4.3	0.9	21.3	10.8				0.9	8.7		
Dimethyltrisulphide[°]	0.8	20.4	13	28.5				tr			
Dimethyltetrasulphide[°]	tr	33.2	3	36.1				0.7			
Irregular sulphides											
2,4-Dithiapentane	0.6	4.2		tr							
2,3,5-Trithiahexane[°]	1	7.3		6.7							
2,3,4,6-Tetrathiaheptane		14.7		tr							
2,4,5,7-Tetrathiaoctane[°]		10		tr							
2,3,5,6,8-Pentathianonane		5.5		tr							
($M = 232$)		tr									
Thioesters											
Dimethyltrithiocarbonate				1.4							
Aliphatic compounds											
3-Hexanone		0.1			7.9		31.4		29.1		
Butyl acetate						1.1			tr		
(*E*)-2-Hexenal										72.7	12.2
1-Methylbutyl acetate					tr		tr				
1-Octen-3-ol			tr				tr			4.2	1.4
Hexyl acetate										tr	1.3
1,3(*E*),5(*Z*)-Undecatriene										5.8	37.2
1,3(*E*),5(*E*)-Undecatriene										1.2	1.71
Tetradecene						5.5					
1-Hexadecene						3.9					
unknown (sum)										2.1	12.4
Alicyclic compounds											
Cyclohexanone		tr				8.6			8.5		
Aromatic compounds											
Benzaldehyde										11	33.5
p-Cymene						0.9					
Isoprenoid compounds											
Irregular terpenes											
6-Methyl-5-hepten-2-one			tr		tr						
4,8-Dimethyl-1,3(*E*),7-nonatriene									tr	tr	
Geranylacetone				tr	tr						
Monoterpenes											
Tricyclene	0.6										
α-Thujene	tr					0.5		tr			
α-Pinene	24.5	tr	tr		2.8	35.2	tr	6.5	21.3		
Camphene	tr					0.4					
Sabinene					3.7						
β-Pinene	9.9		tr			19.8	tr	7.3	8.8		
β-Myrcene	8		24.4		8.6	7.2	23.4	41.7	tr		
δ-3-Carene					2.6						
Limonene	7.9	0.2	3	16.5	27.5	12.7	38.8	42.6	17.7	tr	tr
(*Z*)-Ocimene	tr		1.9								
(*E*)-Ocimene	21.4		25.4					tr			
γ-Terpinene	tr				10.2	0.3	tr				
Terpinolene	tr										

Table 1—*Continued.*

Plant species†	*B. ung.*	*C. ghi.*	*C. sca.*	*C. cuj.*	*H. cal.*	*Musa*	*P. acu.*	*P. ala.*	*V. gla.*	*W. bio.*	*W. tun.*
Number of samples collected	3	3	3	2	3	2	1	3	3	2	2
Oxygenated terpenes											
1,8-Cineole				tr	18.3		6.4		5.9		
Linalool					2.4					3	0.2
Perillene	0.6				13.1		tr				
Camphor		tr			2.9	0.5			tr		
Linalyl acetate			5.9			tr					
Bornyl acetate						tr		tr			
Sesquiterpenes											
α-Copaene		tr				tr		tr	tr		
Longifolene					tr	2.1					
β-Caryophyllene	14.6		2.1				tr	tr			
α-Caryophyllene	3.7										
δ-Cadinene								tr			
unknown (sum)	2.1					0.4					

*The identification of each compound has been confirmed by Kovàts-index (SE 52 capillary column) and mass spectrum of an authentic sample. ° behind a compound name indicates that the structure has additionally been confirmed by analysis of the synthesised compound. The compounds are listed in order of retention indices in each compound class. Average relative amounts (in % FID) of floral volatiles collected from the respective plant species; tr, trace amounts (less than 0.01% FID).

†Abbreviations: *Bauhinia ungulata* (*B. ung.*), *Calyptrogyne ghiesbreghtiana* (*C. ghi.*), *Cobaea scandens* (*C. sca.*), *Crescentia cujete* (*C. cuj.*), *Hippeastrum calyptratum* (*H. cal.*), *Musa* sp. (*Musa*), *Parmentiera aculeata* (*P. acu.*), *Parmentiera alata* (*P. ala.*), *Vriesea gladioliflora* (*V. gla.*), *Weberocereus biolleyi* (*W. bio.*) and *Weberocereus tunilla* (*W. tun.*).

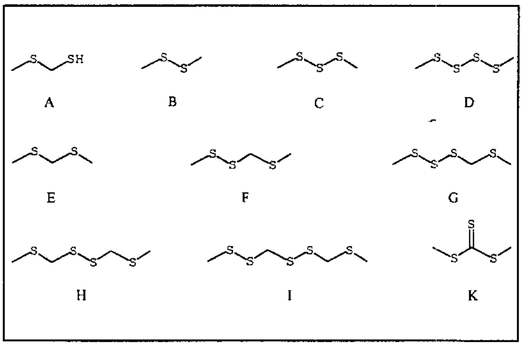

Fig. 1. Structural formulae of the sulphur compounds found in the headspace of bat-pollinated species. The Kovàts indices (SE 52 capillary column) are given in brackets. A, 2-thiapropane-1-thiol (804); B, dimethyldisulphide (736); C, dimethyltrisulphide (969); D, dimethyltetrasulphide (1216); E, 2,4-dithiapentane (888); F, 2,3,5-trithiahexane (1126); G, 2,3,4,6-tetrathiaheptane (1359); H, 2,4,5,7-tetrathiaoctane (1518); I, 2,3,5,6,8-pentathianonane (1763); K, dimethyltrithiocarbonate (1190).

one of the dominant scent compounds. According to their FID-signal areas, 3-hexanone in the scents of *Hippeastrum calyptratum* and *Parmentiera aculeata*, and (*E*)-2-hexenal in the headspaces of *Weberocereus biolleyi* and *Weberocereus tunilla* are the dominant aliphatic compounds. In the flower scent of *Musa* sp. traces of two nitrogen containing heterocyclic isomers were also detected, but have not yet been identified.

Although only 11 plant species were investigated, sulphur compounds were found in the scent bouquets of no less than six of them. This is extraordinary since until now sulphur compounds have only been reported in very few individual cases to be constituents of floral scents [5–7]. They noticeably influence even the odour of relatively weak smelling flowers like *V. gladioliflora* and *B. ungulata*. In higher concentrations however, flower scents are clearly dominated by these sulphides, e.g. *C. ghiesbreghtiana* and *C. cujete*, which emit a strong leek- or garlic-like odour. Unfortunately, nothing is known as yet about the pathways and control mechanisms of the biogenesis of these exceptional floral scent constituents.

Table 1 shows that the plants studied can to some degree be divided into two fundamental groups according to their floral scent chemistry. The first group consists of species emitting sulphur compounds and thus a typical unpleasant 'garlic-like' odour (*B. ungulata*, *C. ghiesbreghtiana*, *C. scandens*, *C. cujete*, *P. alata*, *V. gladioliflora*). In comparison to these plants, the flower scents of species in the second group do not contain any sulphur compounds (*H. calyptratum*, *Musa* sp., *P. aculeata*, *W. biolleyi*. *W. tunilla*). According to our analytical results, it is obvious that no chemical substance characteristic for all bat-pollinated plants exists, although the emission of sulphur compounds seems to be a very typical trait for many of them [6, 7].

EXPERIMENTAL

General sample preparation. An opening flower (or the complete inflorescence) was placed into a container adapted to its size and shape (e.g. Erlenmeyer glass vessel or polyethylene bag). In no case were the flowers cut from the plant. An adsorption filter containing a thin layer of 1.5 mg of activated charcoal embedded between two grids fused into the wall of a glass tube (65 mm, 3 mm i.d.; Brechbühler AG, CH-8952 Schlieren, Switzerland) was placed as close as possible to the opening of the bloom. Transport of volatiles from the so-called headspace of the flower to the charcoal trap was achieved by sucking the fragrant air by means of a small battery-operated pump (flow rate 100–120 ml min^{-1}). The air re-entering the bloom container was filtered using two combined charcoal filters to avoid contamination of the flower headspace by pump emissions. The adsorbed volatiles were recovered by extraction with CS_2 (2×10 μl). The filter eluates were stored in small glass capillaries at $-18°$.

Plants and sampling conditions. The scent samples were taken at the Botanischer Garten der Universität Erlangen-Nürnberg (BGE), at the Botanischer Garten der Universität Mainz/Germany (BGM, October 1994), at La Selva/Costa Rica (LS, November 1995) and at Santa Rosa national park/Costa Rica (SR, November 1995); total number of samples/sampling durations are given. Before scent samples of a new plant species were collected, a blank sample collection was done. *Bauhinia ungulata* (Caesalpiniaceae): SR, 3/13 hr; *Calyptrogyne ghiesbreghtiana* (Arecaceae): LS, 3/13.5 hr; *Cobaea scandens* (Polemoniaceae): BGE, Oct. 1993, 3/12 hr; *Crescentia cujete* (Bignoniaceae): BGM, 2/12.5 hr; *Hippeastrum calyptratum* (Amaryllidaceae): BGE, Oct. 1994, 3/14 hr; *Musa* sp. (Musaceae): LS, 2/17 hr; *Parmentiera aculeata* (Bignoniaceae): BGM, 1/12 hr; *Parmentiera alata* (Bignoniaceae): SR, 3/11.5 hr; *Vriesea gladioliflora* (Bromeliaceae): LS, 3/13 hr; *Weberocereus biolleyi* (Cactaceae): BGE, Sept. 1994, 2/9.5 hr; *Weberocereus tunilla* (Cactaceae): BGE, June 1994, 2/13 hr.

GC. Capillary GC was performed on a Hewlett Packard 5890 equipped with a Shimadzu Chromatopac C-R3A data system. Injector 220°, FID 260°; SE 52 FSCC (25 m × 0.25 mm), N_2 as carrier gas (2 ml min^{-1}); temp. programming: 40° for 4 min, then 5° min^{-1} to 220°, then 220° for 1 min. Splitless injection of 1 μl of a not concd scent eluate. R_Is were determined by calculating the sample values by reference to a co-injected series of *n*-alkanes.

GC/MS. A Varian 3400 gas chromatograph (Injector: 150°; column as above, He as carrier gas (2 ml min^{-1}); temp. programming: 40° for 4 min, then 4° min^{-1} to 220°, then 220° for 1 min) linked to a Finnigan MAT 90 mass spectrometer (EI 70 eV) with a Data System 6000 was used. All eluates (20 μl) were carefully concd at room temp.; the resulting concentrates (1–2 μl) were injected. Mass spectra were compared with those from authentic samples.

Acknowledgements—This study has been financially supported by the Deutsche Forschungsgemeinschaft (DFG). We thank the Botanic Gardens of Erlangen and Mainz, Dr R. Grünmeier (Mainz) and Dr G. Schmaus (Fa. Dragoco Ag, Holzminden) for their kind support.

REFERENCES

1. Dobat, K. and Peikert-Holle, Th., *Blüten und Fledermäuse*. Waldemar Kramer, Frankfurt am Main, 1985.
2. von Helversen, O., In *Plant–Animal Interactions in Tropical Environments*, ed. W. Barthlott. Museum Alexander Koenig, Bonn, 1993, p. 48.
3. Grob, K. and Zürcher, F., *Journal of Chromatography*, 1976, **117**, 285.
4. Brunke, E.-J., Hammerschmidt, F.-J. and Schmaus, G., *Dragoco Report*, 1992, **39**, 3.
5. Knudsen, J. T., Tollsten, L. and Bergström, L. G., *Phytochemistry*, 1993, **33**, 253.
6. Knudsen, J. T. and Tollsten, L., *Botanical Journal of the Linnean Society*, 1995, **119**, 45.
7. Kaiser, R. and Tollsten, L., *Flavour and Fragrance Journal*, 1995, **10**, 153.