SUPPORTING INFORMATION

Article title: Diverse floral scents in Malagasy *Bulbophyllum* evolve in a bounded fashion

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The following Supporting Information is available for this article:

Fig. S1 Phylogeny of 32 species included in the scent analysis, generated by pruning the time-calibrated maximum clade credibility (MCC) tree of Malagasy *Bulbophyllum* (Gamisch *et al*., 2021), based on concatenated nuclear and plastid DNA sequence data.

Fig. S2 Nonmetric multidimensional scaling (NMDS) based on a Bray–Curtis dissimilarity matrix of relative abundances of floral volatile organic compounds (VOCs) between all 41 species of Malagasy *Bulbophyllum* of this study (i.e. with five outlier taxa included).

Table S1 Sample and voucher information for the 41 species and 50 accessions of Malagasy *Bulbophyllum* included in this study (nine sections; clades *A*, *B*, *D*; *sensu* Gamisch *et al*., 2021) plus details on the scent sampling per accession [date, duration, site, collector(s)].

Table S2 Evolutionary model fitting (BM, EB, OU) to each of the 59 floral volatile organic compounds (VOCs) shared among 32 species of Malagasy *Bulbophyllum*, using GEIGER.

Methods S1 Identification of a novel floral scent compound, N,N-dimethylleucine O-methyl ester, using Nuclear Magnetic Resonance (NMR) spectroscopy.

Dataset S1 (separate Excel file) List of all 297 volatile organic compounds (VOCs) detected in the 41 study species of Malagasy *Bulbophyllum*, including absolute amounts (peak areas, total ion currents). If more than one accession per species was analysed, absolute amounts were averaged across samples.

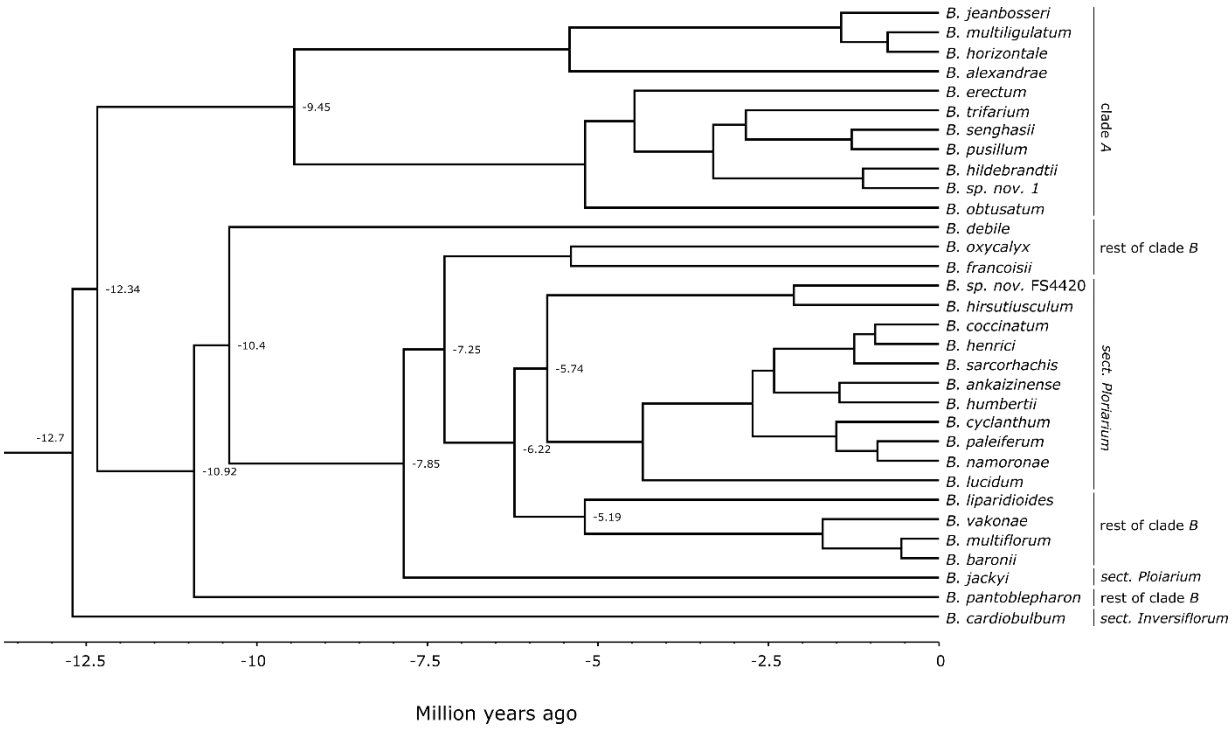


Fig. S1 Phylogeny of 32 species included in the scent analysis, generated by pruning the time-calibrated maximum clade credibility (MCC) tree of Malagasy *Bulbophyllum* (Gamisch *et al.*, 2021), based on concatenated DNA sequence data of three nuclear (nrITS, *PI, Xdh*) and five plastid gene regions (*atp*I–*atp*H, *ycf1*, *mat*K, *trn*D–*trn*E, *psb*A–*trn*H). This pruned 32 spp.-phylogeny was generated using the function *drop.tips* in the R package PHYTOOLS v.2.1-1.

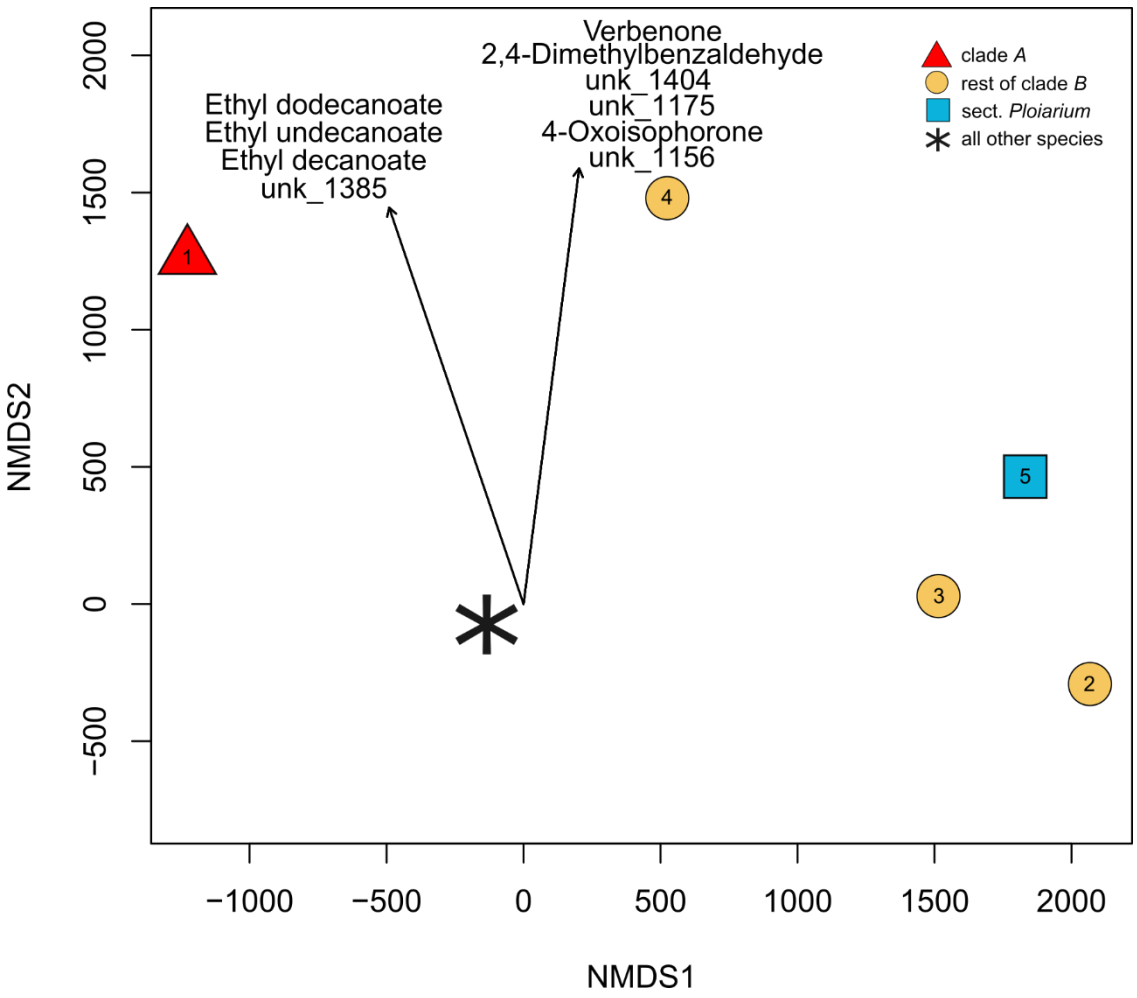


Fig. S2 Nonmetric multidimensional scaling (NMDS) based on a Bray–Curtis dissimilarity matrix of relative abundances of floral volatile organic compounds (VOCs) between all 41 Malagasy *Bulbophyllum* species of this study. Sample symbols and colours represent clade/section memberships of five outlier species [i.e. clade *A*, rest of clade *B* (without sect. *Ploiarium*), sect. *Ploiarium*] with highly distinct scent profiles, and which are separated in the graph; all other 36 species, including one of clade *D*) clustered in the center (marked by an asterisk) and thus remained indistinguishable. Arrows indicate the vectors of significantly ($P < 0.05$) associated scent compounds. Code number identification of outlier species: 1*, B. obtusatum;* 2*, B. debile*; 3*, B. melleum*; 4*, B. sp.* 7664; 5*, B. sp.* FS 1021.

Table S1 Sample and voucher information for the 41 species and 50 accessions of Malagasy *Bulbophyllum* included in this study (nine sections; clades *A*, *B*, *D*; *sensu* Gamisch *et al*., 2021) plus details on the scent sampling per accession [date, duration, site, collector(s)]. The 32 species included in the phylogenetic comparative analysis of scent variation (Fig. S1; Table 2) are marked by an asterisk.

	Species	Section (clade)	Voucher no.	Scent sampling			
				Date	Duration (min)	Site [a]	Collector(s) [b]
*	*B. alexandrae* Schltr.	*Kainochilus* (*A*)	7767	01/03/2018	20	HBV	AS
*	*B. ankaizinense* Schltr.	*Ploiarium* (*B*)	S 7143	17/07/2017	15	HBV	AS
*	*B. baronii* Ridl.	*Loxosepalum* (*B*)	7433	23/01/2018	5	M	AG, AS, DP
*	*B. cardiobulbum* Bosser	*Inversiflora* (*D*)	7439	23/01/2018	15	M	AG, AS, DP
*	*B. coccinatum* H.Perrier	*Ploiarium* (*B*)	FS 5961	21/07/2018	20	HBV	AS
*	*B. cyclanthum* Schltr.	*Ploiarium* (*B*)	7746	13/06/2019	150	HBV	AS
*	*B. debile* Bosser	*Lichenophylax* (*B*)	7661	05/02/2018	30	M	AG, AS, DP
*	*B. erectum* Thouars	*Ploiarium* (*B*)	FS 1021	31/07/2018	20	HBV	AS
*	*B. francoisii* H.Perrier	*Loxosepalum* (*B*)	7718	25/01/2019	150	HBV	AS
*	*B. henrici* Schltr. (1)	*Ploiarium* (*B*)	ORCH940162	29/06/2017	10	HBV	AS
	– (2)		3814	02/04/2018	20	HBV	AS
*	*B. hildebrandtii* Rchb.f. (1)	*Calamaria* (*A*)	FS 7110	29/06/2017	10	HBV	AS
	– (2)		7110	27/02/2019	150	HBV	AS
*	*B. hirsutiusculum* H.Perrier	*Ploiarium* (*B*)	7691	12/04/2019	150	HBV	AS
*	*B. horizontale* Bosser	*Kainochilus* (*A*)	7765	08/02/2018	15	M	AG, AS, DP
*	*B. humbertii* Schltr.	*Ploiarium* (*B*)	ORCH000463	29/06/2017	10	HBV	AS
*	*B. jackyi* G.A.Fischer, Sieder & P.J. Cribb	*Ploiarium* (*B*)	7051	01/03/2018	15	HBV	AS
*	*B. jeanbosseri* Gamisch & Hermans	*Kainochilus* (*A*)	7342	02/08/2018	30	HBV	AS
*	*B. liparidioides* Schltr.	*Pachyclamys* (*B*)	ORCH000359	03/08/2017	15	HBV	AS
*	*B. lucidum* Schltr.	*Ploiarium* (*B*)	S 2704	17/07/2017	15	HBV	AS
*	*B. multiflorum* Ridl.	*Loxosepalum* (*B*)	7378	19/01/2018	30	M	AG, AS, DP
*	*B. multiligulatum* H.Perrier	*Kainochilus* (*A*)	5612	15/01/2018	10	M	AG, AS, DP
*	*B. namoronae* Bosser (1)	*Ploiarium* (*B*)	FS 6600	18/08/2017	20	HBV	AS
	– (2)		FS 6600	27/08/2019	150	HBV	AS
*	*B. obtusatum* (Jum. & H.Perrier) Schltr. (1)	*Calamaria* (*A*)	FS 7137	18/04/2017	5	HBV	AS
	– (2)		FS 7137	18/04/2017	20	HBV	AS

*	*B. oxycalyx* Schltr.	*Elasmotopus* (*B*)	7619	03/02/2018	10	M	AG, AS, DP
*	*B. paleiferum* Schltr. (1)	*Ploiarium* (*B*)	FS 5063	31/07/2018	20	HBV	AS
	– (2)		5063	28/08/2019	150	HBV	AS
*	*B. pantoblepharon* Schltr.	*Trichopus* (*B*)	7526	31/01/2018	15	M	AG, AS, DP
*	*B. pusillum* Thouars (1)	*Calamaria* (*A*)	S 6794	17/07/2017	15	HBV	AS
	– (2)		7520	09/03/2018	20	HBV	AS
*	*B. sarcorhachis* Schltr.	*Ploiarium* (*B*)	FS 1610	19/02/2019	150	HBV	AS
*	*B. senghasii* G.A.Fischer & Sieder (1)	*Calamaria* (*A*)	FS 7210	29/06/2017	15	HBV	AS
	– (2)		ORCH110820	30/01/2019	150	HBV	AS
*	*B. trifarium* Rolfe	*Calamaria* (*A*)	FS 6310	17/05/2017	30	HBV	AS
*	*B. vakonae* Hermans	*Loxosepalum* (*B*)	7664	05/02/2018	5	M	AG, AS, DP
*	*B. sp. nov.* 1	*Calamaria* (*A*)	FS 5383	05/03/2018	30	HBS	SA
	B. sp.	*Calamaria* (*A*)	20792	29/06/2017	15	HBV	AS
	B. sp. 8013-1	*Calamaria* (*A*)	8013-1	16/08/2019	150	HBV	AS
	B. sp. 1525	*Elasmotopus* (*B*)	1525	27/08/2019	150	HBV	AS
	B. sp. FS 4397	*Ploiarium* (*B*)	FS 4397	18/05/2017	15	HBV	AS
*	*B. sp.* FS 4420 (1)	*Ploiarium* (*B*)	FS 4420	29/06/2017	15	HBV	AS
	– (2)		FS 4420	26/06/2019	150	HBV	AS
	B. sp. FS 7491	*Ploiarium* (*B*)	FS 7491	10/05/2018	30	HBV	AS
	B. sp. 6755	*Ploiarium* (*B*)	6755	29/01/2019	150	HBV	AS
	B. sp. 7674	*Ploiarium* (*B*)	7674	11/03/2019	150	HBV	AS
	B. sp. OR 36_98	*Ploiarium* (*B*)	OR 36_98	Unknown	30	HBS	SA
	B. sp. OR 83_2003	*Ploiarium* (*B*)	OR 83_2003	Unknown	30	HBS	SA

[a] Site codes: HBS, Botanical Garden of Salzburg University; HBV, Botanical Garden of Vienna University; M, Madagascar.

[b] Collector abbreviations: AG, Alexander Gamisch; AS, Anton Sieder; DP, David Prehsler; SA, Silvia Artuso.

Table S2 Evolutionary model fitting to each of the shared 59 floral volatile organic compounds (VOCs) of 32 Malagasy *Bulbophyllum*, using GEIGER. Each model (i.e. BM, Brownian motion; EB, early burst; OU, single-optimum Ornstein–Uhlenbeck) was evaluated over the time-calibrated maximum clade credibility (MCC) tree of Malagasy *Bulbophyllum* (Gamisch *et al*., 2021) pruned to these 32 species for which shared VOC data could be obtained in the present study (see also Fig. S1).

VOC	AICc			ΔAICc			Best model(s)	Best model parameter(s)
	BM	OU	EB	BM	OU	EB		
3-Methylbutanoic acid*	180.73	159.26	160.12	21.48	0.00	0.86	OU	$\alpha = 1.09$
2-Methylbutanoic acid*	-38.89	-62.85	-61.23	23.95	0.00	1.61	OU	$\alpha = 1.49$
1-Octen-3-ol*	208.69	210.13	210.13	0.00	1.44	1.44	BM	$\sigma^2 = 7.36$
6-Methyl-5-hepten-2-one*	241.49	242.61	242.61	0.00	1.12	1.12	BM	$\sigma^2 = 20.52$
(*E*)-β-Ocimene*	117.86	116.53	116.53	1.32	0.00	0.00	OU/EB	$\alpha = 0.21/r = 0.43$
Undecane*	329.57	298.81	302.47	30.76	0.00	3.66	OU	$\alpha = 1.67$
Linalool*	321.20	280.04	289.70	41.16	0.00	9.65	OU	$\alpha = 10.0$
2-Phenylethanol*	192.08	167.04	169.77	25.04	0.00	2.73	OU	$\alpha = 3.00$
Methyl octanoate*	-35.56	-34.32	-34.32	0.00	1.24	1.24	BM	$\sigma^2 = 0.003$
(*E*,*E*)-2.6-Dimethyl-1,3,5,7-octatetraene	271.73	235.20	241.15	36.53	0.00	5.95	OU	$\alpha = 4.74$
Octanoic acid*	289.44	282.54	282.57	6.90	0.00	0.03	OU	$\alpha = 0.60$
Methyl salicylate*	184.26	154.79	158.26	29.48	0.00	3.47	OU	$\alpha = 2.66$
2-Aminobenzaldehyde*	69.21	53.26	53.26	15.95	0.00	0.00	OU/EB	$\alpha = 0.45/r = 0.89$
unk_1245	171.60	149.36	150.56	22.24	0.00	1.20	OU	$\alpha = 1.11$
Geraniol*	274.88	273.92	273.92	0.96	0.00	0.00	OU/EB	$\alpha = 0.19/ r = 0.38$
3,5-Dimethoxytoluene*	149.98	122.27	124.88	27.71	0.00	2.61	OU	$\alpha = 3.48$
unk_1281	201.37	179.17	180.35	22.20	0.00	1.18	OU	$\alpha = 1.11$
Tridecene isomer 1	-113.11	-129.86	-129.49	16.75	0.00	0.38	OU	$\alpha = 1.28$
Tridecadiene isomer	-39.11	-56.51	-56.02	17.41	0.00	0.49	OU	$\alpha = 1.38$
Tridecene isomer 2	160.49	143.58	143.64	16.92	0.00	0.06	OU	$\alpha = 1.66$
Tridecane*	322.96	307.47	309.29	15.48	0.00	1.82	OU	$\alpha = 6.10$
Indole*	282.28	256.92	257.90	25.36	0.00	0.98	OU	$\alpha = 0.76$
Methyl 2-hydroxy-4/5/or 6-methylbenzoate	215.53	197.94	198.77	17.60	0.00	0.83	OU	$\alpha = 1.08$
Methyl anthranilate*	93.00	77.92	77.92	15.08	0.00	0.00	OU/EB	$\alpha = 0.43/ r = 0.86$
Decanoic acid*	76.10	42.79	48.01	33.31	0.00	5.22	OU	$\alpha = 4.73$
unk_1376	201.55	186.28	186.73	15.27	0.00	0.45	OU	$\alpha = 0.87$
Geranyl acetate*	86.98	56.51	60.59	30.47	0.00	4.08	OU	$\alpha = 10.0$
Tetradecene isomer 1	-66.44	-64.00	-74.83	8.38	10.83	0.00	EB	$r = -0.27$
Tetradecene isomer 2	-24.33	-43.60	-42.07	19.27	0.00	1.53	OU	$\alpha = 1.75$
α-Copaene*	-3.94	-40.04	-34.05	36.10	0.00	5.99	OU	$\alpha = 4.46$
N-Formyl-2-aminobenzaldehyde	211.22	188.25	188.69	22.97	0.00	0.44	OU	$\alpha = 0.65$
unk_1430	-33.43	-69.60	-63.78	36.16	0.00	5.81	OU	$\alpha = 5.21$
β-Caryophyllene	252.30	241.39	241.39	10.92	0.00	0.00	OU/EB	$\alpha = 0.50/ r = 0.99$
unk_1450	219.29	213.32	213.32	5.96	0.00	0.00	OU/EB	$\alpha = 0.28/ r = 0.56$
(*E*)-β-Farnesene*	184.48	153.40	157.99	31.08	0.00	4.59	OU	$\alpha = 4.54$
Pentadecadiene isomer	273.77	246.99	249.50	26.78	0.00	2.51	OU	$\alpha = 2.23$
α-Caryophyllene*	183.85	156.98	160.50	26.86	0.00	3.52	OU	$\alpha = 4.17$
Pentadecene isomer 1	214.05	216.50	64.62	149.43	151.88	0.00	EB	$r = -1.0$

Pentadecene isomer 2	-28.58	-26.14	-65.04	36.46	38.90	0.00	EB	r = -0.48
unk_1496	93.03	63.76	67.11	29.27	0.00	3.35	OU	α = 2.41
Pentadecane*	195.34	186.07	187.31	9.27	0.00	1.24	OU	α = 3.01
Germacrene D*	264.35	266.80	253.19	11.16	13.61	0.00	EB	r = -1.0
unk_1507	249.13	224.11	224.89	25.02	0.00	0.78	OU	α = 0.73
α-Selinene*	74.65	77.09	75.74	0.00	2.44	1.10	BM	σ^2 = 0.11
(*E,E*)-α-Farnesene	223.90	205.55	206.85	18.35	0.00	1.29	OU	α = 1.91
β-Selinene*	-10.22	-7.77	-33.11	22.90	25.34	0.00	EB	r = -0.33
β-Bisabolene	56.06	31.24	32.07	24.82	0.00	0.84	OU	α = 0.73
unk_1522	42.10	21.40	23.68	20.71	0.00	2.29	OU	α = 2.11
unk_1528	82.72	46.30	52.32	36.42	0.00	6.01	OU	α = 5.07
unk_1545	187.91	164.61	165.29	23.29	0.00	0.67	OU	α = 0.72
unk_1570	259.72	238.93	240.25	20.79	0.00	1.32	OU	α = 1.33
(*E*)-Nerolidol*	77.88	58.39	58.56	19.49	0.00	0.17	OU	α = 0.61
unk_1577	171.07	148.80	150.27	22.27	0.00	1.47	OU	α = 1.29
Caryophyllene oxide*	167.24	150.54	151.14	16.71	0.00	0.61	OU	α = 0.99
Humulene epoxide isomer	45.51	26.41	27.83	19.10	0.00	1.42	OU	α = 3.39
Heptadecadiene isomer	-103.80	-101.35	-105.72	1.92	4.36	0.00	EB	r = -0.18
Heptadecene isomer	114.02	116.47	13.36	100.66	103.10	0.00	EB	r = -0.84
unk_1707	138.93	116.06	117.58	22.88	0.00	1.52	OU	α = 1.28
Hexahydrofarnesylacetone	160.79	152.30	152.30	8.48	0.00	0.00	OU/EB	α = 0.37/ r = 0.75

AICc, Akaike Information Criterion, corrected for small sample data; ΔAICc, difference in AICc value between the best model and the model being compared. Model parameters: α, rate of adaptation parameter for OU; r, EB parameter of exponential rate decrease; σ^2, diffusion parameter of the univariate BM model. * indicates compounds indentified based on synthetic compounds.

Methods S1 Identification of a novel floral scent compound, N,N-dimethylleucine O-methyl ester, using Nuclear Magnetic Resonance (NMR) spectroscopy.

A previously unknown floral scent compound [mass-to-charge (m/z) values (relative intensity, %): 42(6), 58(12), 72(34), 114(100), 115(8), 116(12)] dominating the field-collected scent of a *Bulbophyllum cardiobulbum* (clade *D*) specimen (voucher 7439; see Table S1) was analysed by Nuclear Magnetic Resonance (NMR) spectroscopy. To this aim, the floral scent of *B. cardiobulbum* from the same specimen plant cultivated at the Botanical Garden of Vienna University (HBV), was sampled again as described in the main text, but with a larger adsorbent tube (glass capillary, length = 8 cm, inner diameter = 2.5 mm) filled with 15 mg Tenax-TA (mesh 60-80) and 15 mg Carbotrap B (mesh 20-40). Scent was collected in this tube eight times for four hours each from a single flower. The volatiles trapped in the adsorbent tube were eluted with 750 µl of deuterated aceton-d6 (Armar Europe, Cunnersdorf, Germany) and measured in a standard 5 mm NMR tube, TA quality (Armar), on a 600 MHz Avance III HD spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a QXI ($^{1}H/^{13}C/^{15}N/^{31}P$) probe at 298 K. Chemical shift assignment (Table S3) was achieved using standard one-dimensional ^{1}H and two-dimensional ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra (Fig. S3). Spectra were processed by TOPSPIN v.3.2 (Bruker Biospin) and referenced to tetramethylsilane (TMS). Spectra were analyzed using the software SPARKY v.3.114 (Goodard & Keller, 2007).

Table S3 Observed chemical shifts of N,N-dimethylleucine O-methyl ester observed in acetone-d6 at 298 K, referenced to tetramethylsilane (TMS) in comparison with values of similar or identical molecules from the literature. Atom numbering is given in Figure S3.

Chemical shift assignments																Reference
H2	H3	H3'	H4	H5	H5'	H6/H6'	H7	C1	C2	C3	C4	C5	C5'	C6	C7	
3.24	1.53	1.48	1.64	0.92	0.88	2.28	3.64	172.9	65.4	39.1	25.3	23.1	22.2	41.2	n.d.	This study
3.23	1.60	1.60	1.50	0.92	0.90	2.32	3.70	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	Kaiser (1986) [a]
3.15	1.52*	1.41*	1.71*	0.88	0.88	2.48	–	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	–	Tajbakhsh *et al.* (2011) [b]
4.65	1.55	1.08	0.94	0.63	0.55	2.88	–	170.1	52.6	38.2	24.7	23.0	22.9	30.9	–	Cruz *et al.* (2006) [c]
4.34	1.62	1.62	1.59	0.92	0.87	–	–	174.9	52.4	39.7	24.4	22.2	20.6	–	–	Wishart *et al.* (1995) [d]

n.r., not reported values; n.d., not determined values; –, this atom does not exist in the entity.

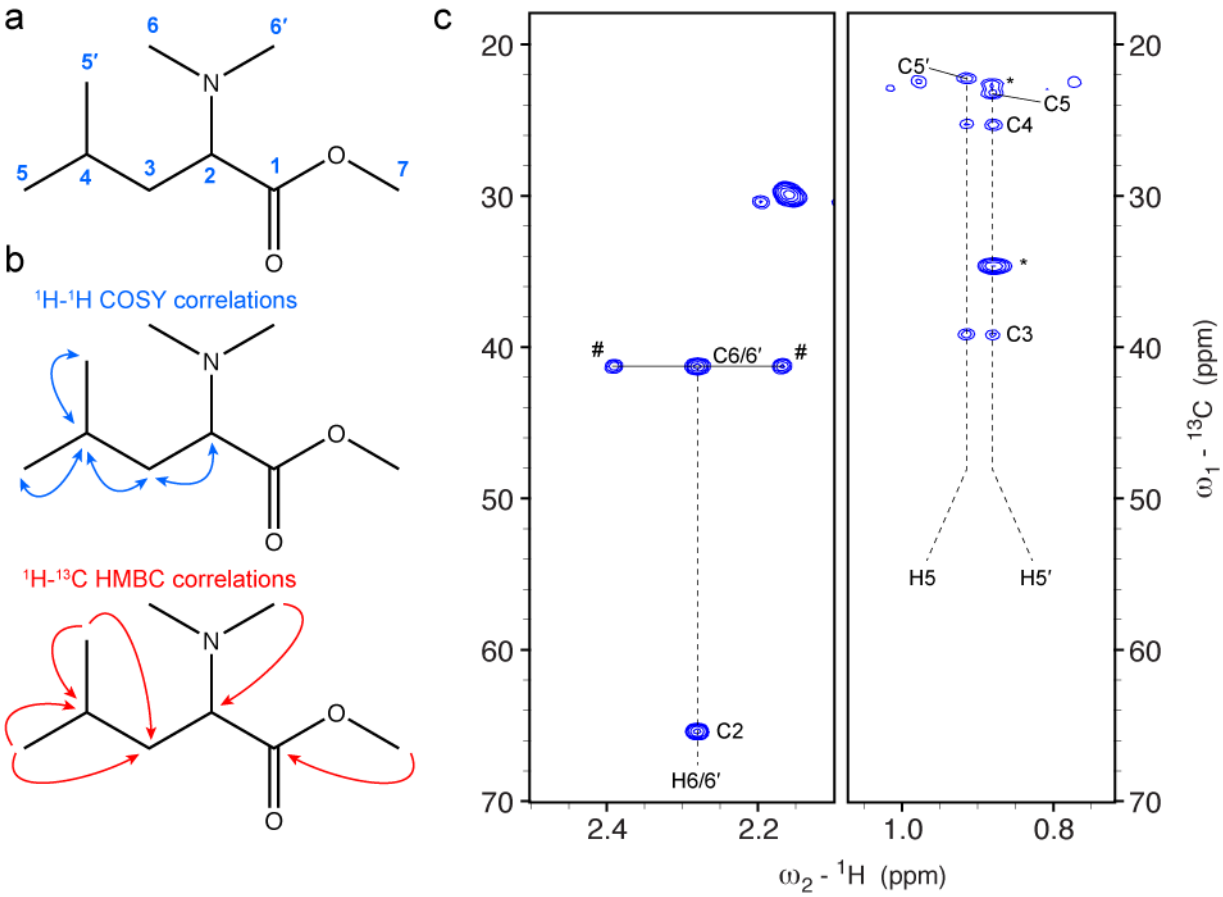
[a] Data of N,N-dimethylleucine O-methyl ester; the solvent was not mentioned in Kaiser (1986).

[b] Data of N,N-dimethylleucine measured (lacking the ester) in DMSO-d6; no assignments were given in Tajbakhsh *et al.* (2011); here we assigned the reported values to the best fitting observed values; values with an asterisk were given as a range, e.g. 1.74–1.67 ppm, and for simplicity we report in the table the average value of both limits.

[c] Data of N,N-dimethylleucine as the N-terminal residue of a cyclodepsipeptide measured in $CDCl_3$.

[d] Random coil chemical shifts of leucine as part of a peptide (lacking N- and O-methylation) measured at 25 °C, in D_2O in the presence of 1 M urea-d4 and 50 mM phosphate pH 5.1; ^{13}C values originally referenced to disuccinimidyl suberate (DSS) were recalibrated to TMS by subtracting 2.66 ppm.

Fig. S3 Identification of N,N-dimethylleucine O-methyl ester by NMR spectroscopy. (a) Chemical structure and used nomenclature of N,N-dimethylleucine O-methyl ester. (b) The most important observed correlations in 2D ^{1}H-^{1}H COSY and 2D ^{1}H-^{13}C HMBC spectra, as illustrated by arrows on the chemical structure. (c) Selected regions of a 2D ^{1}H-^{13}C HMBC spectrum showing key correlations.



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