FULL PAPER

1-Acetyl-3-[(3*R*)-hydroxyfatty acyl]glycerols: Lipid Compounds from *Euphrasia rostkoviana* Hayne and *E. tetraquetra* (Bréb.) Arrond.

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Dedicated to Prof. *Ulrike Lindequist* on the occasion of her 65th birthday.

Five homologous acetylated acylglycerols of 3-hydroxyfatty acids (chain lengths C(14) – C(18)), named euphrasianins A – E, were characterized for the first time in *Euphrasia rostkoviana* Hayne (Orobanchaceae) by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS^n). In addition to mass spectrometric data, structures of euphrasianins were verified *via* a three-step total synthesis of one representative homologue (euphrasianin A). The structure of the latter was confirmed by 1D- and 2D-NMR experiments as well as high-resolution electrospray ionization-mass spectrometry (HR-ESI-MS). The absolute configuration of the 3-hydroxyfatty acid moiety at C(3) was found to be *R* in the natural euphrasianins, which was determined by alkaline hydrolysis and methylation of a purified fraction, followed by chiral GC analysis. Furthermore, in extracts of *Euphrasia tetraquetra* (Bréb.) Arrond. euphrasianins C and E were detected exclusively, indicating that this subclass of lipid constituents is possibly valuable for fingerprinting methods.

Keywords: Eyebright, Orobanchaceae, Acetyl monoacylglycerols, Hydroxyfatty acids, Euphrasianins.

Introduction

Lipids are one of the most abundant classes of natural constituents found in higher plants. With regard to the chemical structure and physiological function, plant lipids may be divided into seven groups: fatty acids, acylglycerols, waxes, phospho- and sphingolipids, lipopolysaccharides, and isoprenoids (*e.g.*, steroids and carotenoids). Among these, triglycerides of long-chain fatty acids, besides mono- and diglycerides, are the most common storage lipids in plants (*e.g.*, in seed oils) [1]. Plant lipids comprise a multitude of biological functions, *e.g.*, they serve for energy production, as structure components of membranes, as signal molecules or pigments, etc. [1]. In plant cuticles, lipids represent a protective barrier against environmental impacts, such as desiccation, temperature extremes, microbial infection and exposure to intense UV radiation [2 – 4].

Within the scope of exploring biologically active lipid constituents from various medicinal plants, we took a closer look on the phytochemistry of European eyebright (*Euphrasia rostkoviana* Hayne). *Euphrasia* is a genus comprising about 350 species of green herbaceous, annual or perennial flowering plants [5], belonging to the Orobanchaceae family (formerly included in the Scrophulariaceae), with a cosmopolitan distribution. These hemiparasitic species [6][7] are mostly found in areas with temperate climate, with an emphasis on the mountains of the northern hemisphere [8]. Several *Euphrasia* species, mainly *E. stricta* D. Wolff ex. F. J. Lehm and *E. rostkoviana* Hayne, including their subspecies and hybrids, were synopsized by the term '*E. officinalis* L.' [9]. Hence, unambiguous taxonomic identification of *Euphrasia* species is mostly vague and presumably only possible based on DNA sequences [8].

The terms 'eyebright' or 'eyewort' indicate the long history of the plant for eye disease treatment. Eyebright has been stated to exert astringent, anticatarrhal, and anti-inflammatory activities [10]. Since the Middle Ages, herbal decoctions of *Euphrasia* have traditionally been applied to treat several diseases, such as nasal catarrh, hoarseness, cough, sinusitis, gastric disorders, skin problems and conjunctivitis [9][10]. To characterize the mode of action more precisely anti-oxidant, radical scavenging, antibacterial, antihyperglycemic, and cytokine inhibitory activities of *E. rostkoviana* extracts have been ascertained

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in vitro and *in vivo* [10 – 12]. Beside its use in folk medicine, preparations of *E. rostkoviana* are nowadays applied in phytotherapy, homeopathy, and anthroposophic medicine, *e.g.*, for the treatment of clotty and inflamed eyes, against conjunctivitis, stye, and blepharitis [9][13].

Numerous secondary metabolites have been found in *E. rostkoviana*, such as iridoids [14][15], lignans [16], phenylethanoids [17 – 19], phenolic acids [20], flavonoids, and tannins [21]. Furthermore, traces of alkaloids and essential oil compounds have been reported [9][22][23]. Since studies on *E. rostkoviana* lipid constituents are still rare [24], this study shall focus on their closer investigation. In addition to *E. rostkoviana*, a phylogenetically ulterior *Euphrasia* species, *E. tetraquetra* (seacliff eyebright), was included in this study for a phytochemical comparison.

Results and Discussion

For lipid constituent analysis, the fresh total plant material of *E. rostkoviana* was extracted with CH_2Cl_2. Based on GC/MS investigations, several known compounds were detected in the obtained extract. By comparing their mass spectra with those of the NIST database and reference compounds, a homologous series of *n*-alkanes (H–C(21) – C(31), odd-numbered representatives were dominating) was identified, with *n*-nonacosane (H–C(29)) being the predominant homologue (see GC/MS chromatogram, *Fig. 1a*). Moreover, free fatty acids, such as palmitic (C(16):0) and linolenic acid (C(18):3), as well as α-tocopherol (α-Toco) and β-sitosterol (β-Sito) were assigned. Also coumarin could be detected in the CH_2Cl_2 extract (data not shown). However, two compounds at t_R 45.4 and 49.2 min (**1** and **3**, respectively) could not be readily assigned. Exhibiting high conformity in their mass spectra, both **1** and **3** revealed signals at *m*/*z* 117 and 187 as most intense fragments (see *Table 1*), demonstrating that these constituents could be homologues. However, because of low intensity, the molecular ion signals of the compounds could not be allocated. Interestingly, three additional homologues of **1** and **3**, compounds **2**, **4,** and **5** were detected in the extracted ion mode (MS scan of *m*/*z* 117 or 187).

For further analysis, the crude CH_2Cl_2 extract was subjected to normal-phase vacuum liquid chromatography (VLC) purification on silica gel and subsequent *Chromatotron*® centrifugal radial preparative TLC (silica gel/gypsum) separation to enrich the target compounds **1** – **5** in a fraction. *Fig. 1b* exhibits a total ion chromatogram (TIC) indicating the presence of **1** – **5**, besides small amounts of monoacyl glycerols of unsaturated fatty acids (see insert, *Fig. 1b*). A gradual increase of the higher fragments (*m*/*z* > 187) in the mass spectra between **1** and **5** by 'CH_2' units indicated that these compounds to be members of a homologous series (*Table 1*).

To analyze the fatty acid composition, an aliquot of the purified fraction was saponified with 5% KOH in MeOH (*w*/*v*). After pH adjustment (pH 3.0), extraction with AcOEt, solvent removal and silylation with BSTFA/DMF GC/MS analyses were performed. Based on the mass spectra of their TMSi derivatives and by comparison with NIST spectral data and implementation of silylated reference compounds, five homologous 3-hydroxyfatty acids with chain lengths of C(14) – C(18) could be assigned as major constituents of the mixture (data not shown). Among these homologues, the even-numbered C(14) and C(16) representatives were predominant. Although 3-hydroxyfatty acids are an important class of microbial lipid constituents frequently found in the lipid A segment of bacterial lipopolysaccharides [25], reports on their occurrence in higher plants are still rare. 3-Hydroxyfatty acids have been identified in cuticular waxes of *Aloe arborescens* [26], in *Hypericum lysimachioides* [27] and *H. perforatum* [28]. Further, 3-acetoxy derivatives of 3-hydroxyfatty acids were found in floral nectars of the Krameriaceae and Scrophulariaceae families [29][30]. Glycerol esters of 3-hydroxyfatty acids were analyzed in glandular trichome exudates of *Verbascum blattaria* f. *erubescens* (Scrophulariaceae) [31].

To determine the stereochemistry of the 3-hydroxyfatty acids, the AcOEt extract of the saponified material from an *E. rostkoviana* CH_2Cl_2 extract was methylated by treatment with BF_3/MeOH. Me esters of the 3-hydroxyfatty acids (C(14) – C(18)) thus obtained, were separated on a chiral GC phase. By comparison with (3*R*)- and (3*R,S*)-hydroxymyristic acid methyl ester as references, the absolute configuration was found to be *R* (data not shown). Even the absolute configuration of the fatty acid moiety was only determined for compound **1**, we assume it to be the same for the other homologous, because of biogenetic considerations.

Furthermore, silylation of the compounds in the unsaponified fraction yielded the corresponding TMSi derivatives of **1** – **5** (for GC/MS data see *Fig. 1c* and *Table 1*). The latter compounds were characterized by two intense fragments each, one at *m*/*z* 129 and another at *m*/*z* 189, as well as further characteristic fragments at higher *m*/*z* values which increased by each additional CH_2 unit (see *Scheme 1b* and *Table 1*). Furthermore, $[M-CH_3]^+$ fragments were formed as a result of the loss of a Me radical from the TMSi group, and these fragments were detected as highest *m*/*z* signals in the mass spectra (*Table 1*). By alignment with mass spectral data of similar structures reported [30], it was concluded that **1** – **5** may be assigned to 1-acetyl-3-[(3*R*)-hydroxyfatty acyl]-glycerol esters (*Fig. 2*). Additionally, isomers of the main constituents TMSi-**1** and -**3** (TMSi-**1*** and **3***, respectively, see *Fig. 1c*) were detected by GC/MS, which is presumably due to acetyl migration from the *sn*-1 to the *sn*-2 position of the glycerol unit upon derivatization.

To obtain additional structural information, compounds **1** – **5** were separately investigated by HPLC-DAD-MS^n (*Fig. 3*, *Table 2*). After HPLC separation, compound analysis was performed in the positive and negative

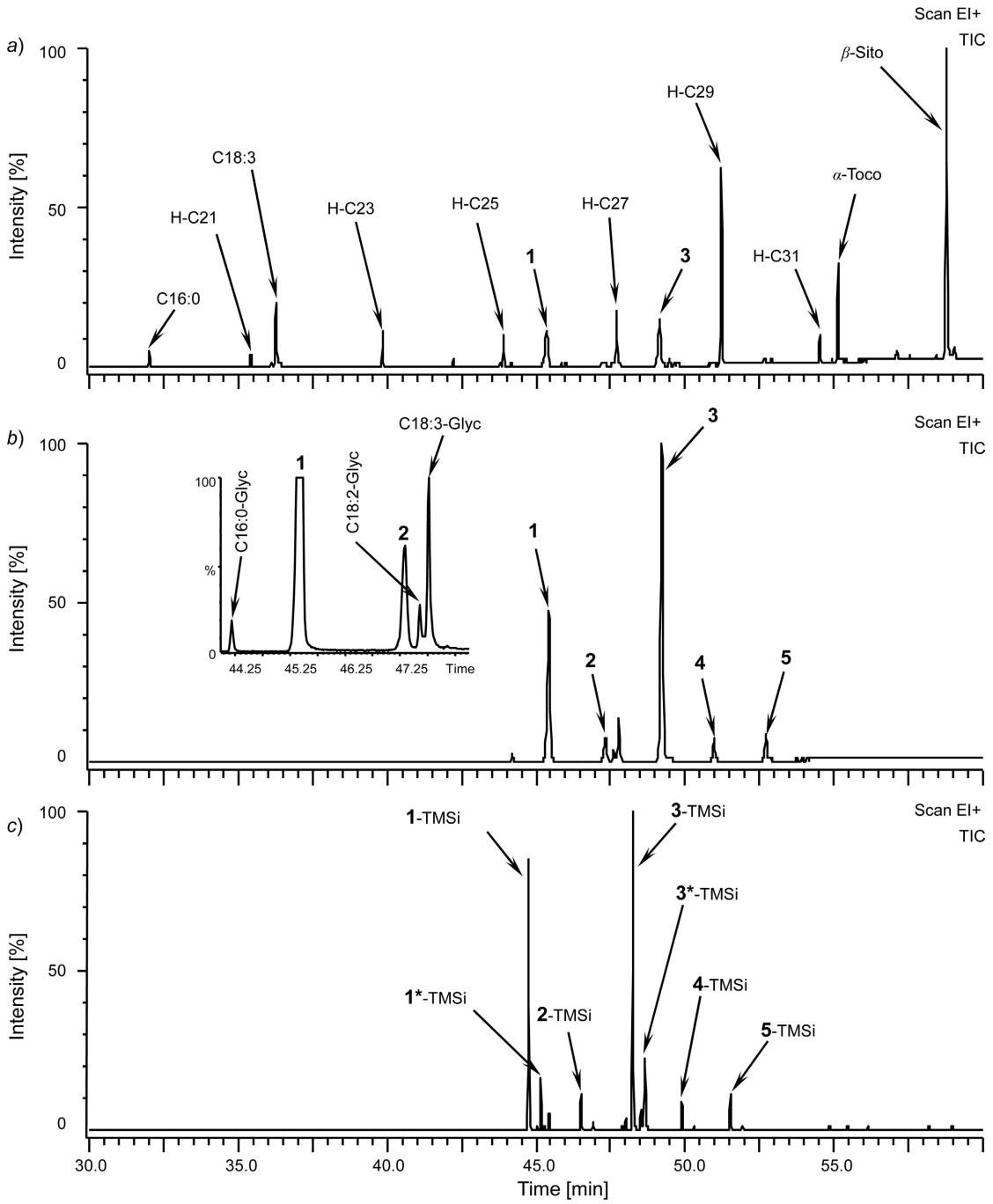


Fig. 1. Sections of GC/MS profiles (EI) showing metabolites of the total plant of *E. rostkoviana*. *a*) Crude CH_2Cl_2 extract. *b*) Fraction as obtained by chromatographic purification; the insert shows an expanded chromatogram (t_R 44 – 48.5 min). *c*) Fraction after TMSi derivatization. Assignment: C(16):0, hexadecanoic acid; C(18):3, α-linoleic acid; H–C(21) – H–C(31), *n*-alkanes C(21) – C(31); α-Toco, α-tocopherol; β-Sito, β-sitosterol; **1** – **5**, euphrasianins A – E; Glyc, Glycerol; **1** – **5**-TMSi, euphrasianin-TMSi derivatives; **1***, **3***-TMSi, isomers due to acyl migration.

APCI mode. In the $APCI^+$ mode (MS^1), base peak ions at *m*/*z* 343, 357, 371, 385, and 399 ($[M+H-H_2O]^+$) were detected from the five homologues **1** – **5**, respectively, generated by H_2O expulsion (see *Fig. 3* and *Table 2*). For a staggered illustration of extracted ion chromatograms, see *Fig. 3a*. Upon subsequent fragmentation of the $[M+H-H_2O]^+$ ions, daughter ions at *m*/*z* 159, 117, and 99 were detected in the MS^2 and MS^3 experiments (*Fig. 3b*). According to EI-MS studies on acetylated glycerol esters of 3-acetoxy fatty acids [30] and acetylated cyclopentane diols [32], three 1,3-dioxolenium cationic structures were postulated for the latter (*Fig. 3b*).

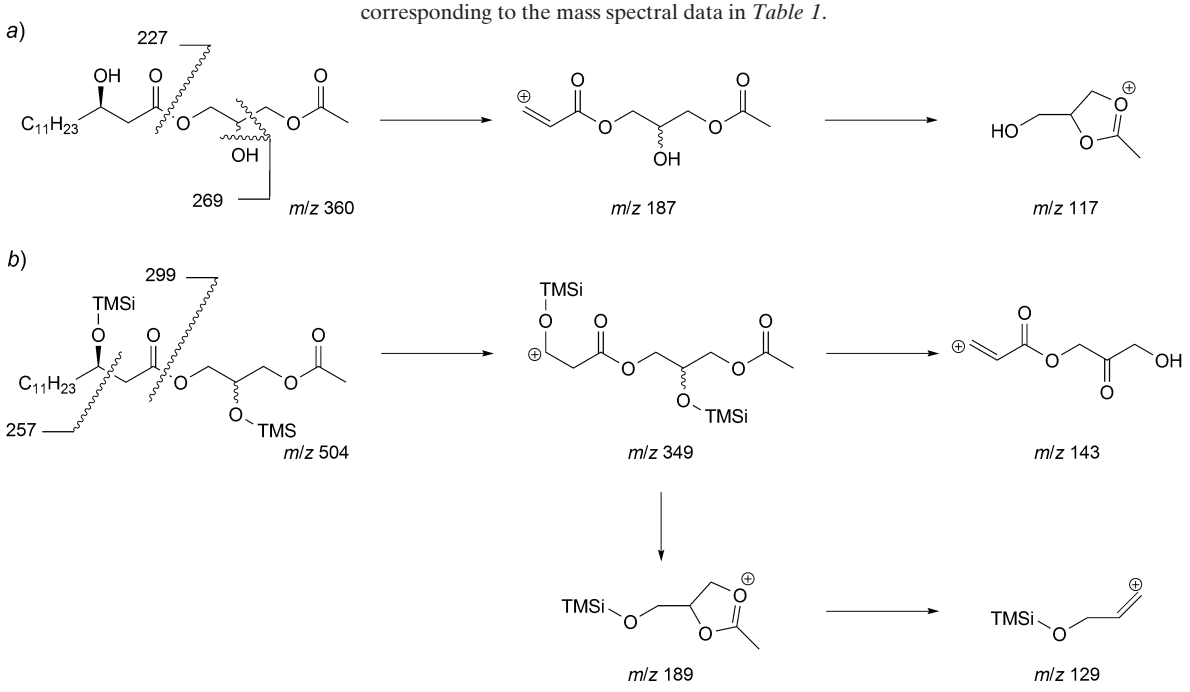
Interestingly, the occurrence of intense fragments at *m*/*z* 117 and 189 (base peaks) in the EI-MS spectra (see *Table 1*, *Scheme 1a,b*) of underivatized and silylated **1** – **5**, respectively, gave further evidence for this ion species. A

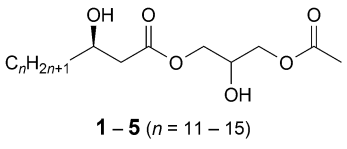
Table 1. GC/MS data of euphrasianins (**1** – **5**) and their TMSi derivatives (**1** – **5**-TMSi), detected in a fraction of a CH_2Cl_2 extract from *E. rostkoviana* L

Constituent	Underivatized		Characteristic fragment ion peaks, *m/z* [% BPI]	TMSi derivatives		Characteristic fragment ion peaks, *m/z* [% BPI]
	t_R [min]	M_r [Da]		t_R [min]	M_r [Da]	
1	45.4	360.5	342 (0.1)[a]), 324 (1), 287 (2), 269 (3), 227 (6), 209 (10)[b])	44.7	504.8	489 (2)[c]), 299 (7), 257 (18)[d])
2	47.3	374.5	356 (0.1)[a]), 338 (0.4), 301 (1), 283 (2), 241 (5), 223 (8)[b])	46.5	518.9	503 (2)[c]), 313 (7), 271 (16)[d])
3	49.2	388.5	370 (0.3)[a]), 352 (0.6), 315 (1), 297 (2), 255 (4), 237 (7)[b])	48.2	532.9	517 (2)[c]), 327 (6), 285 (14)[d])
4	50.9	402.6	384 (0.1)[a]), 366 (0.3), 329 (0.5), 311 (1), 269 (3), 251 (6)[b])	49.9	546.9	531 (1)[c]), 341 (5), 299 (12)[d])
5	52.7	416.6	398 (0.2)[a]), 380 (0.3), 343 (0.4), 325 (1), 283 (3), 265 (6)[b])	51.5	560.9	545 (1)[c]), 355 (5), 313 (11)[d])

[a]) $[M-H_2O]^+$, molecular ion signal not observed; [b]) In addition to characteristic fragments common to the compounds of one homologous series, fragments at *m/z* 187 (88), 176 (4), 158 (22), 145 (16), 135 (20), 117 (100), 103 (13), 97 (8), 83 (7), 71 (14), 57 (31), 55 (22) were also found (BPI corresponds to **1**); [c]) $[M-CH_3]^+$, molecular ion signal not observed; [d]) Additional fragments at *m/z* 349 (5), 189 (100), 145 (14), 143 (32), 129 (71), 117 (14), 101 (12), 73 (68) for the TMSi derivatives.

Scheme 1. Proposed mechanism for MS fragmentation (EI). *a*) Euphrasianin A (**1**). *b*) TMSi derivative of euphrasianin A (**1**-TMSi), corresponding to the mass spectral data in *Table 1*.





1 – **5** (n = 11 – 15)

Fig. 2. General structural formula of the euphrasianins A – E.

supposable mechanism leading to the formation of 1,3-dioxolenium cations *via* a homolytic cleavage of the fatty acyl moiety, with either subsequent or simultaneous cyclization, induced by EI, is illustrated in *Scheme 1* [30] [32]. This mechanism is also in line with the very high abundance (base peaks) of the proposed 1,3-dioxolenium cation fragments at *m/z* 117 and 189 (*Table 1*).

In addition, the presence of an acetyl group was indicated in the $APCI^+$ mode (MS^1) by an AcOH dissociation fragment ion $[M+H-AcOH]^+$ (*Fig. 3b*). Moreover, in the negative ionization mode a solvent cluster anion $[M + MeOH]^-$ was observed for compounds **1** – **5** as base peak in the MS^1 experiment (*Fig. 3c*). The dissociation of the corresponding ions afforded two fragment types, *i.e.*, $[\text{fatty acid}-H]^-$ and $[\text{fatty acid}–H–CO–H_2O]^-$ in the MS^2 and MS^3 experiments, respectively. In accordance with GC/MS data, these results supported the aforementioned structural proposal.

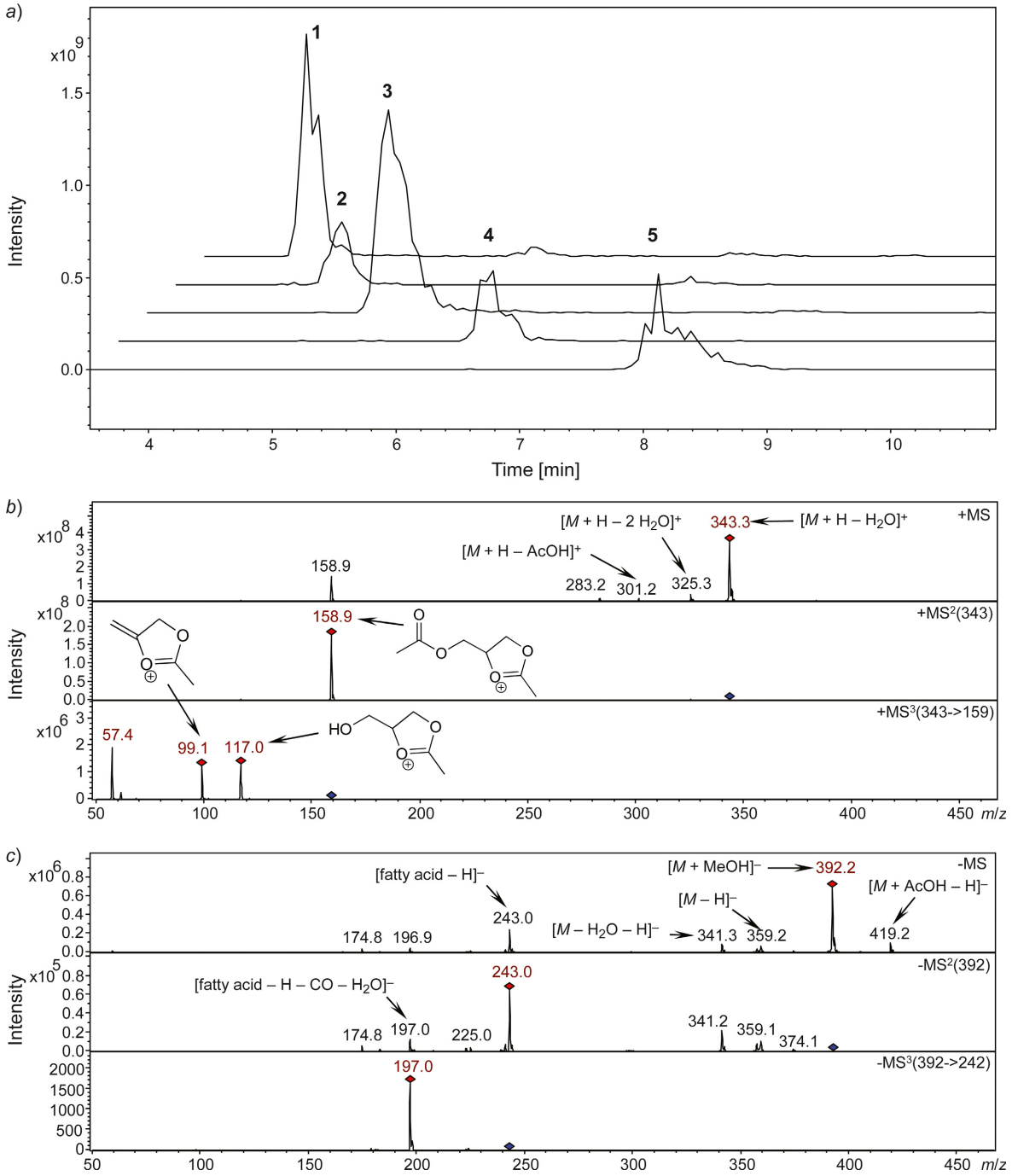


Fig. 3. HPLC/MS(APCI) sections of purified fraction of a CH_2Cl_2 extract of *E. rostkoviana*. *a*) Staggered illustration of extracted ion traces, recorded in the APCI$^+$ with a mass scan on most intense ions $[M+H-H_2O]^+$ of the homologous euphrasianins A – E: *m/z* 343 (**1**), 357 (**2**), 371 (**3**), 385 (**4**), and 399 (**5**). *b*) MS^n data of **1** ($C_{19}H_{36}O_6$, M_r = 360.5) as obtained in the APCI$^+$ mode. *c*) MS^n data of **1** as obtained in the APCI$^-$ mode.

Since further separation of the homologues **1** – **5** appeared difficult and structure elucidation exclusively based on MS data was ambiguous, a facile three-step total synthesis of one representative homologue was accomplished as racemic mixture, using an established protection group concept [33]. Accordingly, a *Steglich* esterification [34] of (3*R,S*)-**6** with *rac*-1,2-isopropyliden glycerol (**7**) yielded the intermediate condensation product *rac*-**8** as a diastereomeric mixture (*Scheme 2*). The protection group was removed by treatment with 1N HCl/MeOH to yield product *rac*-**9**. Finally, *rac*-**9** was regioselectively acetylated at the primary OH group with AcCl in the presence of *Hünig*'s base (DIPEA) at low temperature (−78°). After chromatographic purification, the obtained (3*R,S*)-**1** was consistent in its chromatographic and spectroscopic properties with the main

Table 2. LC/MS data of euphrasianins A – E (**1** – **5**), identified in a fraction of a CH_2Cl_2 extract obtained from *E. rostkoviana* L. ($APCI^{+/-}$ mode)

Constituent	t_R [min]	M_r [Da]	Intrinsic ion signals in the MS^+ Mode			Intrinsic ion signals in the MS^- Mode		
			MS^1	MS^2	MS^3	MS^1	MS^2	MS^3
1	4.5	360.5	343[a]), 325[b]), 301[c]), 283[d])	159[e])	117[f]), 99[g]), 57	419[h]), 392[i]), 359[j]), 341[k]), 243[l]), 225[m]), 197[n]), 183[o]), 175[p])	359[j]), 341[k]), 243[l]), 225[m]), 197[n]), 183[o]), 175[p])	197[n])
2	5.0	374.5	357[a]), 339[b]), 315[c]), 397(n.d.)[d])	159[e])	117[f]), 99[g]), 57	433[h]), 406[i]), 373[j]), 355[k]), 257[l]), 239[m]), 211[n]), 197[o]), 175[p])	373[j]), 355[k]), 257[l]), 239[m]), 211[n]), 197[o]), 175[p])	211[n])
3	5.6	388.5	371[a]), 353[b]), 329[c]), 311[d])	159[e])	117[f]), 99[g]), 57	447[h]), 420[i]), 387[j]), 369[k]), 271[l]), 253[m]), 225[n]), 211[o]) 175[p])	387[j]), 369[k]), 271[l]), 253[m]), 225[n]), 211[o]) 175[p])	225[n])
4	6.7	402.6	385[a]), 367[b]), 343[c]), 325[d])	159[e])	117[f]), 99[g]), 57	461(n.d.)[h]), 434[i]), 401[l]), 383[k]), 285[l]), 267[m]), 239[n]), 225[o]), 175[p])	401[l]), 383[k]), 285[l]), 267[m]), 239[n]), 225[o]), 175[p])	-
5	8.1	416.6	399[a]), 381[b]), 357[c]), 339[d])	159[e])	117[f]), 99[g]), 57	475 (n.d.)[h]), 448[i]), 415[j]), 397[k]), 299[l]), 281[m]), 255[n]), 239[o]), 175(n.d)[p]	n.d.	n.d.

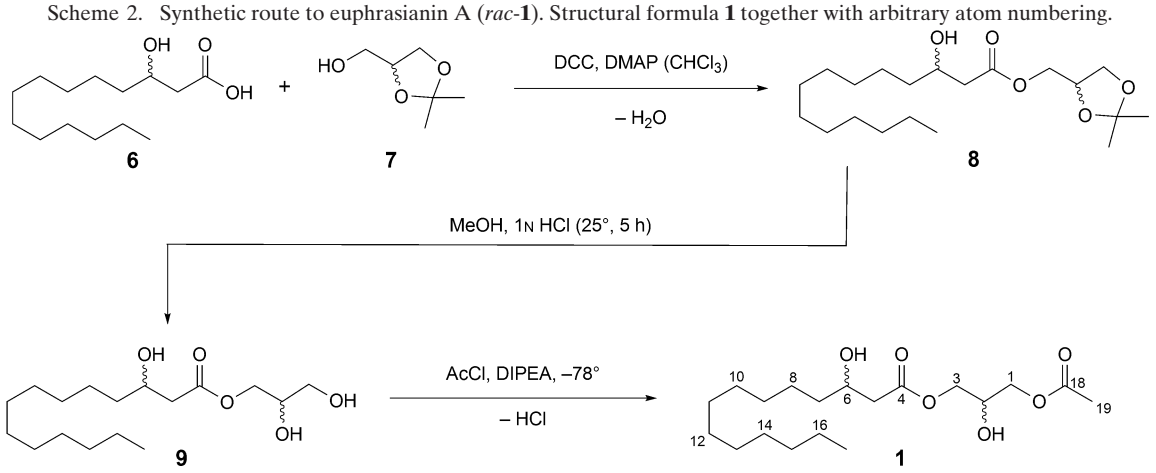
[a]) $[M+H-H_2O]^+$, the corresponding ions which were further fragmented in the MS^n mode are underlined; [b]) $[M+H-2H_2O]^+$; [c]) $[M+H-AcOH]^+$; [d]) $[M+H-AcOH-H_2O]^+$; [e]) $[C_7H_{11}O_4]^+$; [f]) $[C_5H_9O_3]^+$; [g]) $[C_5H_7O_2]^+$; [h]) $[M-H+AcOH]^-$; [i]) $[M+MeOH]^-$; [j]) $[M-H]^-$; [k]) $[M-H-H_2O]^-$; [l]) [fatty acid–H]$^-$; [m]) [fatty acid–H–H_2O]$^-$; [n]) [fatty acid–H–CO–H_2O]$^-$; [o]) [fatty acid–H–CO–H_2O–CH_2]$^-$; [p]) $[C_7H_{11}O_5]^-$.

constituent **1** in the *Euphrasia* fractions (see *Fig. 4a,c*). Thus, based on highly consistent mass spectrometric similarities, the other constituents were assigned to homologous acetylated acylglycerols of 3-hydroxy fatty acids (C(14) – C(18)), henceforth called euphrasianins A – E (**1** – **5**, *Fig. 2*).

Moreover, the positive-ion high-resolution electrospray ionization-mass spectral analysis of synthesized euphrasianin A ((3*R*,*S*)-**1**) revealed a molecular formula $C_{19}H_{36}O_6$, as deduced from the $[M+Na]^+$ ion at *m*/*z* 383.2387 (calcd. for $C_{19}H_{36}O_6Na$, 383.2404). Because of two stereogenic centers and performing the synthesis of **1** with racemic starting material, the ^1H- and ^{13}C-NMR spectra displayed two sets of signals, due to a diastereomeric mixture (the diastereomers could not even be separated by GC/MS). Structure and signal assignment was verified by assessing gHSQC and gHMBC spectra. The signals of CH_3 and CH_2 groups were allocated by gHSQC experiments (data not shown). However, several signals overlapped in the NMR spectra, and hence, interpretation of the data was not straightforward (*Table 3*). The ^1H-NMR spectrum of *rac*-**1** revealed numerous different ^1H-spin systems for the two diastereomers: a CH group at δ(H) 4.14 – 4.09 (2 × 1 H, *m*), coupled with two CH_2 groups at δ(H) 4.26, 4.25 (1 × 2 H, *dd*, *dd*, each *J* = 11.5, 4.5 Hz) and 4.20 – 4.15 (3 × 2 H, *m overlapped*); a CH_2 group at δ(H) 2.57, 2.56 (1 × 2 H, *dd*, *dd*, each *J* = 15.9, 3.1 Hz), 2.45, 2.44 (1 × 2 H, *dd*, *dd*, each *J* = 15.9, 9.2 Hz), coupled with a CH group at δ(H) 4.06 – 4.01 (2 × 1 H, *m*); two CH_3 groups at δ(H) 2.11 (2 × 3 H, *s*) and 0.88 (2 × 3 H, *t*, *J* = 6.8 Hz), besides several overlapped signal groups for the CH_2 groups of the aliphatic side chain (*Table 3*). Nevertheless, the 1D- and 2D-NMR data supported the structural assignment for the synthesized *rac*-**1**.

Finally, to demonstrate the occurrence of euphrasianins in further *Euphrasia* species, a phylogenetically different type, *i.e.*, *E. tetraquetra* [8], was investigated. *Euphrasia tetraquetra,* called sea cliff or maritime eyebright, is growing widespread on open, grassy, and turfy areas, and chalk cliffs at the western coasts of Britain and Ireland [35][36]. GC/MS screening of CH_2Cl_2 extracts of total plants of *E. tetraquetra* in comparison with *E. rostkoviana* revealed the appearance of euphrasianins in both species, however, exhibiting different profiles. While euphrasianins A and C (**1**, **3**) were predominant in *E. rostkoviana*, mainly euphrasianins C and E (**3**, **5**) were detected in *E. tetraquetra* (see extracted ion chromatograms, *Fig. 4a,b*). Thus, these compounds may serve as chemotaxonomic markers, or these can be used in fingerprinting methods for better identification on the species level. A recent report on the incidence of structurally similar compounds, *i.e.*, partially acetylated acylglycerols of (*3R*)-acetoxyfatty acids in floral oils of *Diascia* spp., belonging to the Scrophulariaceae [30], and our finding of **1** – **5** in the congeneric Orobanchaceae family (*Euphrasia*), might also suggest the significance of these compounds in a coevolutionary context. Further investigations

Scheme 2. Synthetic route to euphrasianin A (*rac*-**1**). Structural formula **1** together with arbitrary atom numbering.



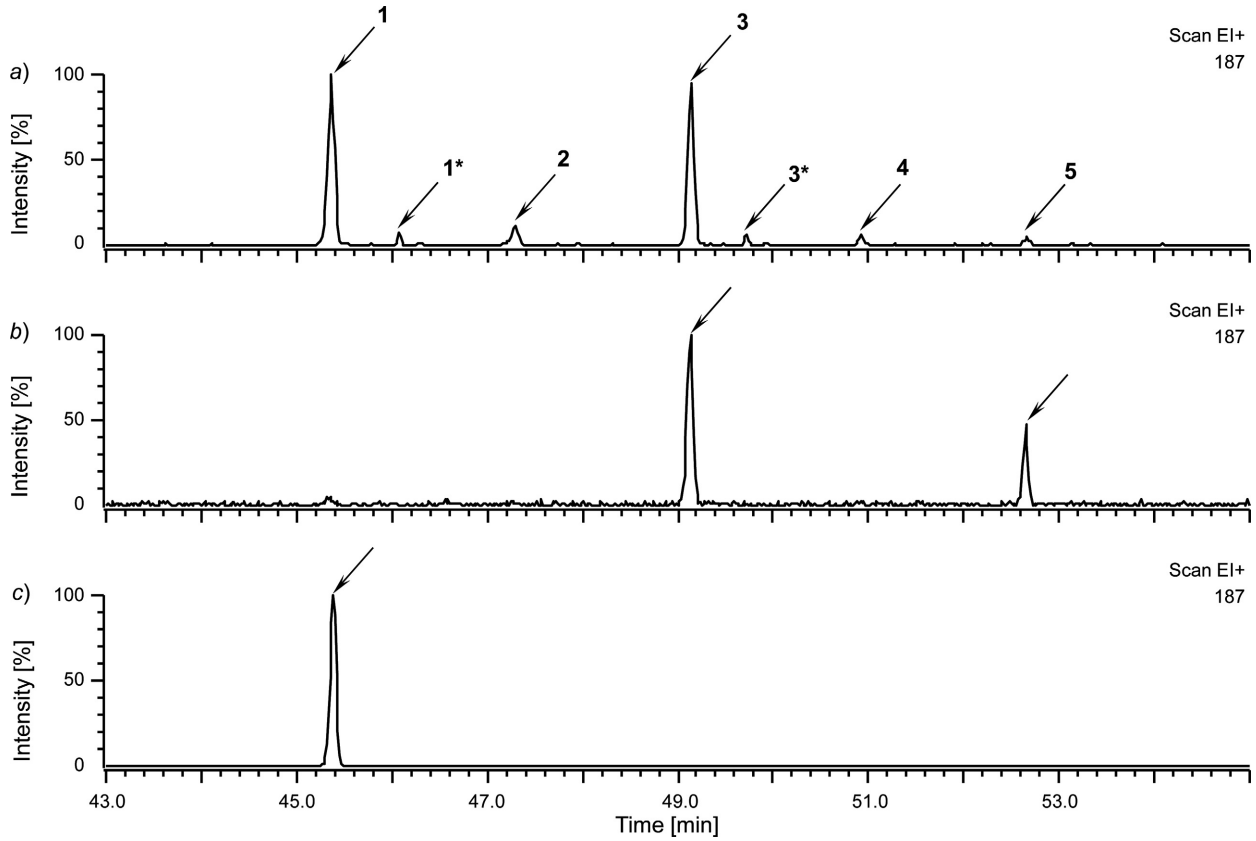


Fig. 4. GC/MS extracted ion chromatograms (mass scan at *m*/*z* 187). *a*) CH_2Cl_2 extract of dried total plants of *E. rostkoviana*. Assignment: **1** – **5**, euphrasianins A – E; **1***,**3***, isomers due to acyl migration. *b*) CH_2Cl_2 extract of dried total plants of *E. tetraquetra*. *c*) synthetic (3*R*,*S*)-**1** as a reference.

on a higher number of other *Euphrasia* species are warranted to verify this first monitoring.

Conclusions

The detection of euphrasianins A – E (**1** – **5**) as a novel class of lipophilic constituents in *Euphrasia* sheds new light on the lipid composition of this plant family. Because of the amphiphilic character of these molecules, a membrane stabilizing function as structural lipids may be possible [37][38]. However, the biological function of euphrasianins still appears obscure. Since diacylglycerols are important intermediates in the biosynthesis of triacylglycerols and phospholipids [39], and play a role in cellular signaling [40], it is assumed that the structurally related euphrasianins could be also involved in such

Table 3. ^{1}H- and ^{13}C-NMR data of *rac*-**1** (diasteromeric mixture[a]), in $CDCl_3$). δ in ppm, *J* in Hz (ov, overlapping signals)

Position[b])[c])	δ(H)[d])	δ(C)[e])	HMBC
1, 1′	4.20 – 4.15 (*m*, ov)	65.05 (CH_2)	C(2), C(3), C(18)
2, 2′	4.14 – 4.09 (*m*)	68.00, 67.97 (CH)	C(1), C(3)
3, 3′	4.26 (*dd*, *J* = 11.5, 4.5), 4.25 (*dd*, *J* = 11.5, 4.5), 4.20 – 4.15 (*m*, ov)	65.11, 65.07 (CH_2)	C(1), C(2), C(4)
4, 4′	–	172.73, 172.67 (C=O)	–
5, 5′	2.57 (*dd*, *J* = 15.9, 3.1), 2.56 (*dd*, *J* = 15.9, 3.1), 2.45 (*dd*, *J* = 15.9, 9.2), 2.44 (*dd*, *J* = 15.9, 9.2)	41.56, 41.52 (CH_2)	C(4), C(6), C(7)
6, 6′	4.06 – 4.01 (*m*)	68.30, 68.28 (CH)	C(4), C(7), C(8)
7, 7′	1.56 – 1.50 (*m*), 1.47 – 1.42 (*m*, ov)	36.70 (CH_2)	C(5), C(6), C(8)[f])
8, 8′	1.47 – 1.42 (*m*, ov), 1.33 – 1.28 (*m*, ov)	25.46 (CH_2)	C(7)[f])
9, 9′ – 14, 14′	1.26 (br. *s*, ov)	29.62, 29.60, 29.55, 29.54, 29.47, 29.32 (CH_2's)	C(15), C(16), C(17)[f])
15, 15′	1.26 (br. *s*, ov)	31.89 (CH_2)	
16, 16′	1.33 – 1.28 (*m*, ov), 1.26 (br. *s*, ov)	22.66 (CH_2)	C(15), C(17)[f])
17, 17′	0.88 (*t*, *J* = 6.8)	14.09 (Me)	C(15), C(16)
18, 18′	–	171.08, 171.07 (C=O)	–
19, 19′	2.11 (*s*)	20.77 (Me)	C(1), C(18)

[a]) Obtained by synthesis; [b]) For atom numbering, see *Scheme 2*; [c]) Primed numbers refer to the other diastereoisomer; [d]) Recorded at 600 MHz; [e]) Recorded at 150 MHz, 13 signals overlapped; [f]) Due to overlapping signals HMBC correlations were not completely assigned.

functions. However, further investigations are required to investigate this hypothesis in more detail.

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Experimental Part

General

Source of the standards for chiral GC analysis: (3*R*)- and (3*R,S*)-*hydroxymyristic acid* ((3*R*)- and (3*R,S*)-**6**) were purchased from *Santa Cruz Biotechnology*, DE-Heidelberg, Germany and *ABCR GmbH*, DE-Karlsruhe, Germany, respectively. Liquid chromatographic analyses were carried out on an *Agilent 1200* HPLC system (*Agilent Technologies Inc.*, DE-Waldbronn, Germany) coupled to an *HCTultra Ion Trap* mass spectrometer (*Bruker Daltonik GmbH*, DE-Bremen, Germany) with an APCI interface. HR-ESI-MS was performed on a *Thermo Scientific Exactive Orbitrap* mass spectrometer (*Thermo Fisher Scientific*, DE-Dreieich, Germany), by direct admittance of the samples dissolved in MeOH.

GC/MS Analyses

GC/MS of all analytes was performed with a *Clarus 500* (*PerkinElmer, Inc.*, Shelton, CT, USA) gas chromatograph with split injection (split ratio 30 : 1, injection volume 1.0 µl) coupled to a mass detector. The column used was a *Zebron ZB-5ms* cap. column (60 m × 0.25 mm i. d. × 0.25 µm film thickness, 5% phenylpolysiloxane and 95% dimethylpolysiloxane coating; *Phenomenex*, Torrance, CA, USA). Carrier gas: Helium at a flow rate of 1 ml/min. The injector used was a PSS (programmed-temperature split/splitless injector; temp.: 250°). The temperature program for the column oven was 100 – 320° with a linear gradient of 4°/min and a final hold time of 30 min. The mass spectrometer was run in electron ionization mode (70 eV).

NMR Spectroscopy

NMR spectra were recorded in $CDCl_3$ with a *Varian Unity Inova* 500 MHz spectrometer and a *Bruker Avance III* 600 MHz spectrometer. The measurement temperature was 298 K. Chemical shifts were expressed in δ (ppm) and referenced to residual (non-deuterated) solvent signals of $CDCl_3$ (^{1}H: δ(H) 7.27; ^{13}C: δ(C) 77.00). ^{13}C-NMR signal assignment of the novel compounds **1**, **8**, and **9** was based on two-dimensional heteronuclear NMR experiments (gHMBC and gHSQC). The assignment of the diastereotopic H-atom signals was supported by ^{1}H-decoupling experiments (data not shown). For evaluation of NMR spectra the program, *SpinWorks 3.1.7.* (Copyright® 2010, K. Marat, University of Manitoba, USA) was used.

Plant Material

E. rostkoviana Hayne (aerial parts with roots) was collected in August 2012 and 2014 near Markstein, in the southern Vosges Mountains (France), mechanically

cleaned from impurities and stored at −80° until investigation. A separate sample of *E. rostkoviana* was air-dried. *E. tetraquetra* (Bréb.) Arrond. (seacliff eyebright) was collected close to Dunlough Castle, at the northern tip of the Mizen Peninsula (Ireland), in August 2014, air-dried and stored frozen at −80° until extraction. Voucher specimens of *E. rostkoviana* and *E. tetraquetra* (voucher Nos. HOH-010792 – 010795 and HOH-014239, respectively) were deposited in the herbarium of the Institute of Botany, Hohenheim University (Germany).

Extraction and Chromatographic Purification of Euphrasianins A – E

Frozen plant material of *E. rostkoviana* was extracted in two batches (2 × 100 g) with CH_2Cl_2 (500 ml each) by *Ultra-turrax*® treatment (3 min at 12,400 rpm; *IKA-Werke GmbH & Co. KG*, D-Staufen). After maceration for 24 h at +4°, insoluble plant material was filtered by vacuum suction over *Celite* through a *Büchner* funnel, followed by a second extraction in the same manner. Subsequently, the pooled extracts were dried (Na_2SO_4), and the solvent was removed by rotary evaporation *in vacuo* to yield a dark green viscous extract (0.84 g). The extract was loaded onto a silica gel VLC column (100 g TLC grade silica gel 60 G, column dimension: 100 × 60 mm i.d., preconditioned with petroleum ether (PE), b.p. 40 – 60°) and subsequently flushed with PE, CH_2Cl_2, and AcOEt (500 ml each). After TLC control and solvent evaporation a PE (0.070 g), a CH_2Cl_2 (0.188 g), and three AcOEt (0.249, 0.105, and 0.178 g) fractions were obtained. All fractions were analyzed by GC/MS revealing the second AcOEt fraction to contain euphrasianins **1** – **5**. This fraction (0.105 g) was further purified applying a *Chromatotron*® centrifugal radial preparative TLC (2 mm layer, silica gel/gypsum/fluorescence indicator 254 nm, 45:18:1.2, *w/w/w*; preconditioned with CH_2Cl_2). Elution was performed with a CH_2Cl_2/MeOH linear gradient (100:0 – 90:10, *v/v*). One fraction (CH_2Cl_2/MeOH, 95:5, *v/v*) was separated, and the solvent was removed *in vacuo* to yield 27 mg of a beige residue, containing the highly enriched target compounds **1** – **5**.

For the screening of euphrasianins in air-dried samples, 10.0 g of total plants of *E. rostkoviana* and *E. tetraquetra,* respectively, were minced in CH_2Cl_2 (200 ml) by *Ultra-turrax*® treatment (see above). Subsequently the mixture was macerated for 24 h (+4°) and then filtered over *Celite* by vacuum suction. The solvent was removed *in vacuo* from the obtained extract to yield a dark green residue (0.2 g). A solution of the residue (1 µl) in $CHCl_3$ (0.014 g/1 ml) was directly subjected to GC/MS analysis.

Silylation of Samples for GC/MS Analyses

For GC/MS analysis, fractions or purified compounds (4 mg) were dissolved in DMF (0.5 ml) and treated with BSTFA (300 µl) at 105° for 15 min. From the obtained solution, 1 µl was injected into the GC/MS system.

RP-HPLC/APCI (pos/neg)-MS^n Analyses

For chromatographic separation a *YMC™ carotenoid S-5* C30 reversed-phase column (5 µm particle size, 250 × 3.0 mm i.d., *Waters Corporation*, Milford, MA, USA) was used at 25 °C and a flow rate of 0.42 ml/min. The mobile phase consisted of MeOH/MTBE/H_2O (90:5:5, *v/v/v*; mobile phase A) and MeOH/MTBE (8:92, *v/v*; mobile phase B). Elution started with 0% B for 5 min, followed by a linear gradient to 30% B at 20 min, then increasing to 100% B at 25 min, continuing for 5 min, before re-equilibration to starting conditions. The injection volume of euphrasianin samples (1 mg of the fraction dissolved in 1 ml MeOH) was 10 µl. The detection of euphrasianins (*Fig. 3a*) was performed by mass spectrometry with an APCI interface operating in the positive and negative ionization mode applying the following parameters: HV capillary: ±4000 V; dry gas N_2: 5.00 l/min with a dry gas temperature of 350°; nebulizer: 50 psi; vaporizer temperature: 400 °C. Full scan mass spectra (mass range *m*/*z* from 50 to 1000) of the HPLC eluates were recorded during chromatographic separation. To obtain further structural information, collision-induced dissociation (CID) experiments were performed. MS^n data were acquired in the auto-MS/MS mode. The instruments were controlled by *Agilent ChemStation* (Rev. B.01.03 SR1) and *Bruker Daltonik EsquireControl* Software (V6.1).

Configuration Analysis of the 3-Hydroxyfatty Acids

Configuration analysis was performed according to a modified literature protocol [41]. In brief, a purified euphrasianin fraction (9.3 mg) was treated with 1N NaOH (10 ml) and stirred at room temperature (24 h). Subsequently, the slurry was acidified with 1N HCl to pH 1.1 and extracted with AcOEt (2 × 20 ml). The combined AcOEt extracts were dried (Na_2SO_4), the solvent removed by evaporation, and the residue obtained was treated with 20% BF_3 in MeOH (*w/v*; 1 ml) for 45 min at 105°. Subsequently, the mixture was transferred into satd. aq. NaCl solution (5 ml) and extracted with hexane (2 × 5 ml). The hexane extract was dried (Na_2SO_4) and evaporated to dryness by a vigorous stream of N_2. From the residue thus obtained, 1 µl of an hexane solution (1 mg/ml) was injected into a GC-FID system (*PerkinElmer, Autosystem XL*), equipped with a chiral capillary column (*IVADEX-7*, 25 m × 0.25 mm i. d. × 0.25 µm film thickness, diethyl *tert*-butylsilyl *β*-cyclodextrine coating; *IVA Analysentechnik*, DE-Meerbusch). Helium was used as carrier gas at a flow rate of 1.3 ml/min. The temperature program for the column oven was 130 – 170 °C at 0.5 °C/min with a total run time of 80 min. For comparison (3*R*)- and (3*R,S*)-hydroxymyristic acid methylesters,

obtained by methylation of (3*R*)- and (3*R,S*)-**6**, respectively, were used as reference compounds.

Synthesis of the Reference Compound *rac*-1-Acetyl-3-(3-hydroxymyristoyl)glycerol (= ***rac*-Euphrasianin A**; ***rac*-3-(Acetyloxy)-2-hydroxypropyl 3-hydroxytetradecanoate**; ***rac*-1**). ***rac*-1,2-Isopropyliden-3-(3-hydroxymyristoyl)glycerol** (= ***rac*-(2,2-Dimethyl-1,3-dioxolan-4-yl) methyl 3-Hydroxytetradecanoate**; ***rac*-8**). A mixture of (3*R,S*)-**6** (1.00 g, 4.10 mmol), *rac*-1,2-isopropyliden glycerol (**7**, 0.65 g, 4.92 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.50 g, 4.10 mmol) in dried $CHCl_3$ (20.5 ml) was cooled in an ice/NaCl bath, while a solution of *N,N*′-dicyclohexylcarbodiimide (DCC, 0.84 g, 4.00 mmol) in 10.5 ml $CHCl_3$ was added under stirring. Then the mixture was stirred for 21 h at room temperature, and afterwards the solvent was removed by vacuum rotoevaporation. The target product **8** was isolated from the crude residue, thus obtained, by VLC (110.0 g TLC grade silica gel 60 G, preconditioned with *n*-hexane). Elution of **8** was performed with an *n*-hexane/AcOEt linear gradient (100:0 – 50:50, *v/v*), and the corresponding compound was monitored by GC/MS. Two fractions, containing **8** were evaporated to dryness *in vacuo*; total yield: 1.24 g, 84.5% of the theory; wax-like compound. GC/MS purity (70 eV): 98 and 93%, respectively (t_R 42.8 min, diastereomers were not separated by GC) *m/z* 343 (24, $[M-CH_3]^+$)*, 325 (6, $[M-CH_3-H_2O]^+$), 243 (4), 203 (28, $[C_9H_{15}O_5]^+$), 183 (5), 159 (56, $[C_7H_{11}O_4]^+$), 145 (100, $[C_7H_{13}O_3]^+$), 116 (42), 101 (51), 83 (7), 71 (21), 57 (42); *molecular ion not observed. ^{1}H-NMR ($CDCl_3$, 500 MHz): 4.36 – 4.31 (2 H, *m*, H–C(2, 2′)); 4.22 (1 H, *dd*, *J* = 11.5, 4.5, H_a–C(3)); 4.18 – 4.17 (2 H, *m*, $H_{a,b}$–C(3′)); 4.13 (1 H, *dd*, *J* = 11.5, 6.0, H_b–C(3)); 4.08 (2 H, *dd*, *J* = 8.5, 6.5, H_a–C(1, 1′)); 4.04 – 3.98 (2 H, *m*, H–C(6, 6′)); 3.75 (2 H, *dd*, *dd*, *J* = 8.6, 5.8, 8.6, 5.8, H_b–C(1, 1′)); 2.56 (1 H, *dd*, *J* = 16.3, 5.6, H_a–C(5)), 2.55 (1 H, *dd*, *J* = 16.3, 5.6, H_a–(5′)); 2.45 (1 H, *dd*, *J* = 16.3, 9.1, H_b–C(5)), 2.44 (1 H, *dd*, *J* = 16.3, 9.2, H_b–C(5′)); 1.55 – 1.50 (2 H, *m*, H–C(7)); 1.44 – 1.43 (6 H, *m*, H–C(20, 20′)); 1.43 – 1.41 (4 H, *m overlapped*, H–C(7′, 8)); 1.37 – 1.36 (6 H, *m*, H–C(19, 19′)); 1.31 – 1.27 (4 H, *m overlapped*, H–C(8′, 16)); 1.26 (30 H, br. *s*, H–C(9,9′ – 15, 15′, 16′)); 0.88 (6 H, *t*, *J* = 7.0, H–C(17, 17′)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.68, 172.63 (C(4, 4′)); 109.92, 109.89 (C(18, 18′)); 73.47, 73.42 (C(2, 2′)); 68.02, 67.92 (C(6, 6′)); 66.15, 66.12 (C(1, 1′)); 64.86, 64.77 (C(3, 3′)); 41.42, 41.37 (C(5, 5′)); 36.54, 36.51 (C(7, 7′)); 31.88 (C(15, 15′)); 29.61, 29.59, 29.54, 29.53, 29.48, 29.30 (C(9, 9′)–C(14, 14′)); 26.63 (C(20, 20′)); 25.45, 25.44 (C(8, 8′)); 25.25, 25.22 (C(19, 19′)); 22.65 (C(16, 16′)); 14.07 (C(17, 17′)), 10 signals overlapped. Primed numbers refer to the other diastereoisomer. APCI$^+$-MS *m/z* 376 $[M+NH_4]^+$, 359* $[M+H]^+$ (MS^1); 301* $[M+H-OC(CH_3)_2]^+$ (MS^2); 283, 227, 209, 191, 117* $[C_5H_9O_3]^+$ (MS^3); 57 (MS^4). APCI$^-$-MS *m/z* 390* $[M+MeOH]^-$ (MS^1); 339* $[M-H-H_2O]^-$ (MS^2); 281, 251, 207* (MS^3); 189, 80 (MS^4); *fragment ions. HR-MS-ESI$^+$ *m/z* 381.2601 $[M+Na]^+$ (calcd. for $C_{20}H_{38}NaO_5$, 381.2617). Further characterization was performed by TMSi-derivatization. 1,2-Isopropyliden-3-(3-trimethylsiloxymyristoyl)glycerol (=(2,2-dimethyl-1,3-dioxolan-4-yl) methyl 3-[(trimethylsilyl)oxy]tetradecanoate; TMSi-**8**). GC/MS (70 eV) at t_R 42.6 min: *m/z* 415 (10, $[M-CH_3]^+$)*, 357 (2, $[M-TMSi]^+$), 275 (7), 257 (26), 217 (20), 143 (9), 115 (100), 101 (19), 73 (28); *molecular ion not observed.

***rac*-1-(3-Hydroxymyristoyl)glycerol** (= ***rac*-2,3-Dihydroxypropyl 3-Hydroxytetradecanoate**; ***rac*-9**). A quantity of 1N HCl (4.35 ml) was added to a soln. of **8** (0.83 g, 2.32 mmol) in MeOH (22 ml), and the mixture was stirred under N_2 (5 h). Afterwards, the reaction was quenched by adding satd. $NaHCO_3$ solution (45 ml) and the aq. phase was extracted with Et_2O (3 × 80 ml). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo* to yield the crude product (0.91 g). Purification by VLC (conditions see above) gave pure **9**. Wax-like crystals; yield: 0.65 g (89% of the theory). GC/MS purity (70 eV): 99% (t_R 43.9 min, diastereomers were not separated by GC) *m/z* 300 (0.1, $[M-H_2O]^+$)*, 287 (1, $[M-CH_2OH]^+$), 269 (2, $[M-CH_2OH-H_2O]^+$), 227 (6), 209 (7), 163 (7, $[C_6H_{11}O_5]^+$), 145 (100, $[C_6H_9O_4]^+$), 134 (14, $[C_5H_{10}O_4]^+$), 116 (6), 97 (10), 83 (10), 75 (23), 71 (27), 57 (38); *molecular ion not observed. ^{1}H-NMR ($CDCl_3$, 500 MHz): 4.23 (1 H, *dd*, *J* = 11.5, 4.3, H_a–C(3)); 4.22 (1 H, *dd*, *J* = 11.5, 6.5, H_a–C(3′)); 4.17 (1 H, *dd*, *J* = 11.5, 4.2, H_b–C(3′)); 4.15 (1 H, *dd*, *J* = 11.5, 6.4, H_b–C(3)); 4.06 – 4.00 (2 H, *m*, H–C(6, 6′)); 3.95 – 3.91 (2 H, *m*, H–C(2, 2′)); 3.68 (1 H, *dd*, *J* = 11.6, 3.9, H_a–C(1)); 3.67 (1 H, *dd*, *J* = 11.6, 3.9, H_a–C(1′)); 3.59 (1 H, *dd*, *J* = 11.6, 6.0, H_b–C(1)); 3.58 (1 H, *dd*, *J* = 11.6, 6.0, H_b–C(1′)); 2.54 (2 H, *dd*, *dd*, *J* = 15.4, 3.0, 15.5, 3.0, H_a–C(5, 5′)); 2.43 (2 H, *dd*, *dd*, *J* = 15.6, 9.6, 15.6, 9.6, H_b–C(5, 5′)); 1.55 – 1.50 (2 H, *m*, H–C(7)); 1.48 – 1.39 (4 H, *m overlapped*, H–C(7′, 8)); 1.31 – 1.27 (4 H, *m overlapped*, H–C(8′,16)); 1.26 (30 H, br. *s*, H–C(9,9′ – 15, 15′, 16)); 0.88 (6 H, *t*, *J* = 7.0 Hz, H-–C(17, 17′)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.82 (C(4, 4′)); 69.99, 69.97 (C(2, 2′)); 68.47, 68.45 (C(6, 6′)); 65.40, 65.39 (C(3, 3′)); 63.26, 63.25 (C(1, 1′)); 41.94, 41.91 (C(5, 5′)); 36.88, 36.87 (C(7, 7′)); 31.88 (C(15, 15′)); 29.65, 29.62, 29.60, 29.58, 29.52, 29.32 (C(9, 9′-14, 14′)); 25.54 (C(8, 8′)); 22.65 (C(16, 16′)); 14.08 (C(17, 17′)), 11 signals overlapped. Primed numbers refer to the other diastereoisomer. APCI$^+$-MS *m/z* 336* $[M+NH_4]^+$, 319 $[M+H]^+$, 283 $[M+H-H_2O]^+$ (MS^1); 301* $[M+H-H_2O]^+$ (MS^2); 117*$[C_5H_9O_3]^+$ (MS^3); 57 (MS^4). APCI$^-$-MS *m/z* 350* $[M+MeOH]^-$ (MS^1); 317 $[M-H]^+$, 299 $[M-H-H_2O]^+$, 243 [fatty acid − H]$^+$, 225 [fatty acid−H−H_2O]$^+$, 197 [fatty acid−2H−COOH]$^+$, 133 (MS^2); *fragment ions. HR-MS-ESI$^+$ *m/z* 341.2289 $[M+Na]^+$ (calcd. for $C_{17}H_{34}O_5Na$, 341.2298). Further characterization was performed by TMSi-derivatisation. 1,2-Bis(trimethylsilyl)-3-(3-trimethylsiloxymyristoyl)glycerol (=2,3-bis[(trimethylsilyl)oxy]propyl 3-[(trimethylsilyl)oxy]tetradecanoate; TMSi-**9**). GC/MS (70 eV) at t_R 44.2 min: *m/z* 519 (1, $[M-CH_3]^+$)*, 431 (13, $[M-CH_3-TMSi]^+$), 379 (5), 373 (9), 309 (5), 257 (32), 219 (11), 147 (19), 143 (26), 129 (16), 116 (8), 103 (40), 73 (100); *molecular ion not observed.

***rac*-Euphrasianin A (= *rac*-1-Acetyl-3-(3-hydroxymyristoyl)glycerol; *rac*-3-(Acetyloxy)-2-hydroxypropyl 3-hydroxytetradecanoate; (3*R*,*S*)-1)**. A solution of **9** (0.23 g, 0.72 mmol) and DMAP (0.15 ml, 0.19 g, 1.47 mmol) in dry CH_2Cl_2 (4.6 ml) was externally cooled to −78° in a Me_2CO/dry ice mixture. Subsequently, AcCl (52 µl, 0.0572 g, 0.73 mmol) was added, the mixture was slowly warmed up to room temperature, and stirred for 1 h. Subsequently, after addition of Et_2O (100 ml) the ppt. ammonium salts were filtered off. Finally, the solvent was removed by vacuum evaporation, and the crude product (0.40 g) was purified by VLC (conditions see above) to yield **1** (0.15 g, 60.0% of the theory) as a colorless syrup; GC/MS purity (70 eV): > 99% (at t_R 45.4 min). For MS and NMR data, see *Tables 1 and 3*, respectively. HR-ESI$^+$-MS *m*/*z* 383.2387 $[M+Na]^+$ (calcd. for $C_{19}H_{36}O_6Na$, 383.2404). In solution (AcOEt) **1** slowly isomerised at room temperature by acyl migration (data not shown).

REFERENCES

[1] C. M. Scrimgeour, J. L. Harwood, 'Fatty Acid and Lipid Structure', in 'The Lipid Handbook', 3rd Edn, Eds. F. D. Gunstone, J. L. Harwood and A. J. Dijkstra, CRC Press, Taylor & Francis Group, LLC, Boca Raton, USA, 2007, p. 1.

[2] P. E. Kolattukudy, 'Biosynthetic pathways of cutin and waxes, and their sensitivity to environmental stresses', in: 'Plant Cuticles – An Integrated Functional Approach', Ed. G. Kerstiens, BIOS Scientific Publishers, Oxford, UK, 1996, p. 83.

[3] C. Buschhaus, R. Jetter, *J. Exp. Bot.* **2011**, *62*, 841.

[4] T. H. Yeats, J. K. C. Rose, *Plant Physiol.* **2013**, *163*, 5.

[5] E. Fisher, 'Scrophulariaceae', in 'The Families and Genera of Vascular Plants, Vol. 7. Flowering Plants Dicotyledons: Lamiales (except Acanthaceae including Avicenniaceae)', Ed. J. W. Kadereit, Springer-Verlag, Berlin, 2004, p. 333.

[6] H. C. Weber, *Plant Syst. Evol.* **1980**, *136*, 217.

[7] J. Těšitel, L. Plavcová, D. D. Cameron, *Planta* **2010**, *231*, 1137.

[8] G. Gussarova, M. Popp, E. Vitek, C. Brochmann, *Mol. Phylogenet. Evol.* **2008**, *48*, 444.

[9] K. Hiller, D. Loew, 'Euphrasia herba – Augentrostkraut', in 'Teedrogen und Phytopharmaka – Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage', 5th Edn, Ed. M. Wichtl, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 2009, p. 252.

[10] R. Teixeira, L. R. Silva, *Ind. Crops Prod.* **2013**, *50*, 680.

[11] E. Porchezhian, S. H. Ansari, N. K. K. Shreedharan, *Fitoterapia* **2000**, *71*, 522.

[12] R. Paduch, A. Woźniak, P. Niedziela, R. Rejdak, *Balkan Med. J.* **2014**, *31*, 29.

[13] N. Wszelaki, M. F. Melzig, *Z. Phytother.* **2011**, *32*, 40.

[14] O. Salama, O. Sticher, *Planta Med.* **1983**, *47*, 90.

[15] S. Strahler, H. Rauwald, *Planta Med.* **2010**, *76*, P201.

[16] O. Salama, R. K. Chaudhuri, O. Sticher, *Phytochemistry* **1981**, *20*, 2603.

[17] O. Sticher, O. Salama, R. K. Chaudhuri, T. Winkler, *Helv. Chim. Acta* **1982**, *65*, 1538.

[18] B. Blazics, A. Alberti, L. Kursinszki, A. Kéry, S. Béni, L. Tölgyesi, *J. Chromatogr. Sci.* **2011**, *49*, 203.

[19] G. Tóth, A. Sólyomváry, I. Boldizsár, B. Noszál, *Proc. Biochem.* **2014**, *49*, 1533.

[20] S. Łuczak, L. Świątek, *Plantes Méd. Phytothér.* **1990**, *24*, 66.

[21] I. Matlawska, M. Sikorska, Z. Kowalewski, *Herba Pol.* **1985**, *31*, 119.

[22] K. J. Harkiss, P. Timmins, *Planta Med.* **1973**, *23*, 342.

[23] P. Novy, H. Davidova, C. S. Serrano-Rojero, J. Rondevaldova, J. Pulkrabek, L. Kokoska, *Evid.-Based Complementary Altern. Med.* **2015**, Article ID 734101. DOI: 10.1155/2015/734101.

[24] B. Blazics, K. Ludanyi, S. Szarka, A. Kery, *Chromatographia* **2008**, *68*, 119.

[25] N. Binding, S. Jaschinski, S. Werlich, S. Bletz, U. Witting, *J. Environ. Monit.* **2004**, *6*, 65.

[26] R. C. Racovita, C. Peng, T. Awakawa, I. Abe, R. Jetter, *Phytochemistry* **2015**, *113*, 183.

[27] H. C. Özen, M. Bashan, Z. Toker, C. Keskin, *Turk. J. Chem.* **2004**, *28*, 223.

[28] W. Maury, J. P. Price, M. A. Brindley, C. S. Oh, J. D. Neighbors, D. F. Wiemer, N. Wills, S. Carpenter, C. Hauck, P. Murphy, M. P. Widrlechner, K. Delate, G. Kumar, G. A. Kraus, L. Rizshsky, B. Nikolau, *Virol. J.* **2009**, *6*, 101.

[29] S. L. Buchmann, *Ann. Rev. Ecol. Syst.* **1987**, *18*, 343.

[30] K. Dumri, L. Seipold, J. Schmidt, G. Gerlach, S. Dötterl, A. G. Ellis, L. A. Wessjohann, *Phytochemistry* **2008**, *69*, 1372.

[31] A. Okawa, K. Ohyama, Y. Fujimoto, *Nat. Prod. Res.* **2013**, *27*, 1372.

[32] C. C. Van de Sande, F. Van Gaever, R. Hanselaer, M. Vandewalle, *Z. Naturforsch B* **1977**, *32*, 810.

[33] D. R. Kodali, *J. Lipid Res.* **1987**, *28*, 464.

[34] B. Neises, W. Steglich, *Angew. Chem.* **1978**, *90*, 556.

[35] C. Stace, 'New Flora of the British Isles', 3rd edn, Cambridge University Press, The Bath Press, UK, 2010, p. 651.

[36] A. Haines, 'New England Wildflower Society's Flora Novae Anglia: A Manual for the Identification of Native and Naturalized Higher Vascular Plants of New England', Yale University Press, 2011, p. 693.

[37] F. M. Goñi, A. Alonso, *Progr. Lipid Res.* **1999**, *38*, 1.

[38] J. C. Gómez-Fernández, S. Corbalán-García, *Chem. Phys. Lipids* **2007**, *148*, 1.

[39] J.-M. Routaboul, C. Benning, N. Bechtold, M. Caboche, I. Lepiniec, *Plant Physiol. Biochem.* **1999**, *37*, 831.

[40] F. Chevalier, E. Maréchal, *Med. Sci.* **2015**, *31*, 320.

[41] P. Lorenz, S. Duckstein, J. Conrad, M. Knödler, U. Meyer, F. C. Stintzing, *Chem. Biodiversity* **2012**, *9*, 282.

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