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Long-chain alkanediols from *Myricaria germanica* leaf cuticular waxes

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Abstract

In the leaf cuticular waxes of *Myricaria germanica* L. four different series of alkanediols were identified: (1) hentriacontanediol isomers with one functional group in the 12-position and a second group in positions ranging from 2 to 18, (2) C_{30}–C_{34} alkanediols carrying one hydroxyl function on a primary and one on a secondary carbon atom, (3) homologous series of C_{25}–C_{43} β-diols predominantly with 8,10- and 10,12-functionalities, and (4) homologous series of C_{39}–C_{43} γ-diols with a predominance of 8,11- and 10,13-isomers. Primary/secondary diols and γ-diols constituted only trace portions of the total wax mixture. The hentriacontanediols and the β-diols amounted to 3.5 and 0.6 μg per cm^2 of leaf surface area, corresponding to 9 and 2% of the wax mixture, respectively. Based on the different homolog and isomer patterns of respective diol fractions, two independent biosynthetic routes leading to the hentriacontanediols and the β-diols are proposed.

Keywords: *Myricaria germanica*; Tamaricaceae; Cuticular wax; Leaves; Alkanediols; Biosynthesis

1. Introduction

The genus *Myricaria* comprises 10 species occurring in temperate regions of Eurasia, especially in the Mediterranean area. The only species of the Tamaricaceae in Central Europe, *Myricaria germanica* L. is a pioneer shrub on the open spaces along the rivers flowing from the Alps. Other representatives of the same family, e.g. *Tamarix gallica* and *T. pentandra*, are frequently cultivated as ornamentals or planted along roadsides and dunes as protection against wind erosion.

The scale-like leaves of *M. germanica* are covered by an array of tubular wax crystals on the cuticle surface, which scatter visible light and give the plant a bluish-green appearance (Clark and Lister, 1975). On various plant species, epicuticular wax crystals have been described (Barthlott et al., 1998). Their presence enlarges the exposed hydrophobic surface, thereby rendering the leaf highly unwettable (Holloway, 1970). This has two important ecological functions: (1) by forcing water droplets to pearl off, dirt particles are washed away (Barthlott and Neinhuis, 1997) and (2) by preventing the formation of macroscopic water films, germination of pathogenic micro-organisms is inhibited (Deising et al., 1992).

In the course of ongoing studies on the chemical composition and molecular structure of epicuticular wax crystals of diverse plant taxa, the cuticular wax mixture of *M. germanica* leaves was analyzed. The predominant constituent, described as hentriacontan-12-ol (Jetter, 2000), was found associated with bifunctional compounds. As previous reports on plant wax constituents carrying two functional groups are scarce (Bianchi, 1995), the objective of the present work was to identify these *Myricaria* constituents by various chemical transformations and product structure assignment by GC–MS. Diverse alkanediol structures were elucidated and their isomer composition can supply information on their biosynthesis, e.g. on the sequence of the two involved hydroxylation steps.

2. Results and discussion

Leaf surface waxes of *Myricaria germanica* yielded 16 distinct bands after TLC on silica with $CHCl_3$. Three of them, designated as A (R_f 0.16), D (R_f 0.11) and E (R_f

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Table 1
MS of *Myricaria germanica* alkanediol derivatives. The mass and relative intensity [%] of selected ions are given, diagnostic α -fragments are highlighted in bold face

Compound	M^+	$M\text{-}15^+$	α-And related fragments									Other fragments
Hentriacontanediol bis TMSi ethers												
Hentriacontane-2,12-diol	613 (0.4)	**598 (0.3)**	582 (0.2)		508 (1)	**369 (52)**	353 (1)	**345 (16)**	255 (25)	**117 (100)**	101 (3)	[a]
Hentriacontane-3,12-diol	Missing	Missing	**584 (0.1)**	522 (0.2)	494 (2)	**369 (37)**	353 (0.4)	**345 (9)**	255 (51)	**131 (100)**	115 (4)	[a]
Hentriacontane-4,12-diol	Missing	598 (0.5)	**570 (0.3)**		480 (6)	**369 (59)**	353 (0.2)	**345 (7)**	255 (53)	**145 (100)**		[a]
Hentriacontane-5,12-diol	613 (0.1)	Missing	**556 (0.3)**	522 (0.3)	466 (6)	**369 (41)**	353 (0.3)	**345 (14)**	255 (36)	**159 (100)**		[a]
Hentriacontane-6,12-diol	Missing	Missing	**542 (7)**	522 (0.2)	452 (4)	**369 (55)**	353 (0.4)	**345 (60)**	255 (13)	**173 (100)**	157 (4)	[a]
Hentriacontane-7,12-diol	Missing	Missing	**528 (4)**	522 (0.1)	438 (1)	**369 (38)**	353 (0.2)	**345 (37)**	255 (5)	**187 (100)**	171 (1)	[a]
Hentriacontane-8,12-diol	Missing	598 (0.1)	522 (0.1)	**514 (5)**	424 (2)	**369 (43)**	353 (0.2)	**345 (41)**	255 (5)	**201 (100)**	185 (0.2)	[a]
Hentriacontane-12,13-diol	Missing	598 (0.3)	522 (0.1)	**458 (0.2)**	367 (0.2)	**359 (1)**	**355 (56)**	339 (2)	269 (0.3)	**257 (100)**	241 (4)	[a]
Hentriacontane-12,15-diol	613 (0.1)	Missing	522 (0.1)	**458 (1)**	**387 (5)**	367 (36)	**327 (62)**	311 (0.3)	297 (77)	**257 (100)**	241 (1)	[a]
Hentriacontane-12,16-diol	Missing	598 (0.1)	522 (0.1)	**458 (16)**	**401 (31)**	367 (3)	**313 (79)**	311 (5)	297 (1)	**257 (100)**	241 (1)	[a]
Hentriacontane-12,17-diol	Missing	598 (0.1)	522 (0.1)	**458 (20)**	**415 (32)**	367 (4)	325 (4)	**299 (79)**	283 (1)	**257 (100)**	241 (1)	[a]
Hentriacontane-12,18-diol	Missing	598 (0.1)	522 (0.2)	**458 (19)**	**429 (23)**	367 (10)	339 (10)	**285 (89)**	269 (0.4)	**257 (100)**	241 (1)	[a]
Tritriacontane-8,14-diol	641 (0.3)	626 (1)	550 (0.1)	**542 (4)**	452 (4)	**373 (30)**	**369 (41)**	353 (0.4)	283 (13)	**201 (100)**	185 (2)	[a]
Primary/secondary alkanediol bis TMSi ethers												
Dotriacontane-1,9-diol	Missing	Missing			**426 (66)**			**303 (100)**	213 (1)			[b]
Dotriacontane-1,11-diol	**627 (1)**	612 (1)	537 (1)		**397 (100)**	381 (1)		**331 (57)**	241 (5)			[b]
Dotriacontane-1,13-diol	**627 (0.1)**	612 (0.2)	537 (0.3)		**369 (100)**	353 (0.4)		**359 (28)**	269 (1)			[b]
Tetratriacontane-1,11-diol	n.d.[c]	640 (0.2)	565 (0.2)		**426 (100)**	409 (0.2)		**331 (67)**	241 (3)			[b]
Hentriacontanediol isopropylidene bis ether												
Hentriacontane-12,13-diol	509 (0.1)	494 (97)	452 (13)	**353 (8)**	309 (5)	295 (2)	281 (2)	**255 (14)**	211 (12)	197 (7)	183 (4)	[d]

[a] Other fragments characterizing the compound class. Typical ranges for relative intensities [%] are given in brackets: 149 (2–15), 147 (6–19), 143 (1–7), 129 (12–60), 103 (6–18), 97 (6–19), 91 (1–13), 83 (7–23), 75 (13–41), 73 (33–84), 57 (4–23).

[b] Other fragments characterizing the compound class. Typical ranges for relative intensities [%] are given in brackets: 149 (26–44), 147 (9–19), 143 (1–4), 129 (13–29), 103 (9–19), 97 (11–28), 95 (6–41), 83 (14–34), 75 (32–44), 73 (41–67), 57 (20–31).

[c] n.d. Not detectable due to restricted MS scanning range.

[d] Other fragments characterizing the compound class. Relative intensities [%] are given in brackets: 125 (15), 111 (36), 97 (68), 83 (65), 71 (43), 69 (49), 59 (100), 57 (70).

0.10), migrated between primary alcohol and fatty acid standards. These three bands were analyzed by GC — MS together with two intermediate zones, designated as B (R_f 0.14) and C (R_f 0.12). Previously, similar TLC properties, and hence polarities, had been reported only for polyfunctional plant wax constituents, e. g. hydroxy-β-diketones (Tulloch, 1975), ketols (Jetter and Riederer, 1999) and alkanediols (Jetter and Riederer, 1996).

2.1. Hentriacontanediol isomers

The predominating compounds in fractions B–E were identified as hentriacontanediol isomers with one hydroxyl group in 12-position and a second group at varying positions between carbon 2 and 18 (Table 2). The GC retention order of the positional isomers was dictated first by the asymmetric position of both functional groups on the carbon chain and second by the distance between both hydroxyl functions. Within TLC fractions, baseline separation was achieved for most hentriacontanediol isomers. For all of these compounds full MS of the TMSi ether derivatives could be directly acquired (Table 2a, MS Quality "f"). Mixed isomers with low concentrations and/or poor separation were identified using differential MS (Table 2, MS Quality "m"). In the resulting spectra the characteristic α-fragments showed constant abundances, while the less specific low-mass fragments had slightly varying intensities. Other alkanediol homologs were identified in small percentages in fraction C, in some cases only according to their GC-retention behavior and their MS α-fragments (Table 2, MS Quality "α"). To date, only the 6,12- and the 10,13-isomer of hentriacontanediol had been reported as constituents of *Papaver alpinum* (Jetter and Riederer, 1996) and *Pinus radiata* (Franich et al., 1979) leaf waxes, respectively.

In fraction A (and in the adjacent area B), a pair of GC-separable compounds were present in a ratio of ca. 4:6. They showed identical TMSi ether MS with characteristic alkanediol patterns including two α-fragments $[C_nH_{2n}OTMSi]^+$ with $m/z = 257$, 355 and an ion $[M\text{-}15]^+$ with $m/z = 598$ (Table 1). Accordingly, both compounds were identified as hentriacontane-12,13-diol isomers, possibly *threo* and *erythro* forms of this vicinal diol. Because other α-fragments $[C_mH_{2m-1}(OTMSi)2]^+$ with $m/z = 458$, 359 and the corresponding secondary fragments $\Delta m/z = 90$ were detectable only with very small abundances, they could not be used to confirm the structure assignment. Instead, the vicinal diol geometry of both isomers was verified by formation of cyclic isopropylidene

bis ethers. The MS of the resulting derivatives showed ions $[M]^+$ with $m/z = 509$ and a corresponding $[M\text{-}15]^+$, two α-fragments $[C_nH_{2n+1}\ (C_5H_8O_2)]^+$ with $m/z = 353$, 255 together with corresponding secondary fragments $\Delta m/z = 44$, 58 (loss of acetone), 72, and a series of low-mass fragments typical for alkyl compounds with $m/z = 57$, 59, 69, 71, 83, 97. Thus, the hydroxyl positions and the overall chain length could unambiguously be confirmed for both hentriacontane-12,13-diols. It should be noted that the cyclic derivatives showed better GC separation than the OTMSi ethers. This might reflect the *trans*- vs. *cis*-configuration of the alkyl substituents at the 5-membered ring in both isomers and would hence support the presence of *threo*- vs. *erythro*-forms in the original diols. In accordance with the present findings GC separation of vicinal diol isomers had previously been reported for synthetic (i.e. racemic) nonacosane-10,11-diols (Jetter and Riederer, 2000). The novel hentriacontane-12,13-diol isomers were the only α-diols identified in *Myricaria* leaf waxes. Series of other vicinal diols, e.g. of C_{25}–C_{29} 10,11-diols, C_{29} 12,13-diol and C_{31} 14,15-diol, had previously been identified in *Papaver* wax mixtures (Jetter and Riederer, 1996). Interestingly, compounds with similar structures, e.g. heptacosane- and nonacosane-8,9-diol, were also reported for insect cuticles (Espelie and Bernays, 1989).

It is noteworthy that TLC separation based upon rising polarity (Table 2) was in the order of: α- and β-diols (fraction A), γ- and ϕ- through ω-diols (fraction C), γ- and ε-diols (fraction D), and δ-diols (fraction E). This pattern can be explained by the combination of two effects: (1) α-, β-, and possibly ϕ- through ω-diols can form intramolecular hydrogen bonds and will therefore have decreased polarity, and (2) if both functional groups are located on even-numbered carbons, they will be oriented to the same side of the alkyl backbone, thus increasing polarity.

Leaf surface coverages of hentriacontanediols, assessed by GC-FID comparison with an internal standard in the total wax mixture, added up to 3.5 μg/cm^2 (9% of the wax mixture). Hentriacontane-7,12-diol and -12,16-diol were found to be the most abundant isomers (Table 2) with 1.1 and 0.8 μg/cm^2, respectively. Besides, 0.1 μg/cm^2 of the 6,12-isomer and two unresolved GC peaks containing six further diols were detected. From their relative abundances within TLC fractions it could be estimated that hentriacontane-12,15-diol,-12,17-diol and -8,12-diol were each represented with coverages between 0.3 and 0.4 μg/cm^2. The two hentriacontane-12,13-diol isomers accounted for ca. 0.1 and 0.2 μg/cm^2, respectively.

Table 2
Composition of *Myricaria germanica* hentriacontanediol isomers and their homologs, arranged in the order of GC retention times [min][a]

Compound	GC retention [min]	Coverage[a] [μg/cm^2]	TLC fraction A	B	C	D	E	MS quality
Nonacosane-3,10-diol	25.7	–	–	–	2	–	–	f
Hentriacontane-10,12-diol	26.7	–	–	tr	–	–	–	m
Hentriacontane-12,13-diols	26.8 + 27.0		100	74	–	–	–	f
Hentriacontane-12,14-diol	26.9	0.7	–	tr	–	–	–	m
Hentriacontane-12,15-diol	26.9		–	–	6	15	2	f
Hentriacontane-12,16-diol	27.1	0.8	–	–	–	8	53	f
Hentriacontane-12,17-diol	27.2		–	–	6	17	–	f
Hentriacontane-12,18-diol	27.3	0.8	–	–	24	–	–	f
Hentriacontane-8,12-diol	27.3		–	–	–	–	35	f
Hentriacontane-7,12-diol	27.6	1.1	–	–	7	55	10	f
Hentriacontane-6,12-diol	27.8	0.1	–	–	32	5	tr	f
Hentriacontane-5,12-diol	27.9	–	–	4	5	–	–	f
Hentriacontane-4,12-diol	28.2	–	–	9	3	–	–	f
Hentriacontane-3,12-diol	28.6	–	–	5	2	–	–	m
Hentriacontane-2,12-diol	28.8	–	–	9	6	–	–	m
Tritriacontane-6,12-diol	30.5	–	–	–	1	–	–	α
Tritriacontane-8,14-diol	30.3	–	–	–	3	–	–	f
Tritriacontane-10,14-diol	30.1	–	–	–	1	–	–	α
Tritriacontane-12,16-diol	30.1	–	–	–	2	–	–	α
Tritriacontane-14,18-diol	30.1	–	–	–	1	–	–	α

[a] Leaf coverages [μg/cm^2] were calculated from a GC–FID analysis of the total wax mixture. Relative amounts [%] of individual compounds, assessed from GC–FID signals in the fractions, show their partial TLC separation. Depending on this separation, the MS of a diol TMSi ether could be acquired with full information (f), had to be calculated from a mixed spectrum (m), or was inferred only from the presence of characteristic α-fragments (α).

2.2. β-Alkanediols

A homologous series of compounds was found associated with the α-diols in fraction A (and in smaller amounts in fraction B). Again, the TMSi ether MS showed the characteristics for alkanediols, but also an additional diagnostic fragment $m/z = 130$ (Table 3). While two α-fragments $[C_nH_{2n}OTMSi]^+$ were detected for each compound, the corresponding α-fragments $[C_mH_{2m-1}(OTMSi)_2]^+$ were missing. Instead, the corresponding secondary ions $\Delta m/z = 90$ could be used to infer homolog chain lengths and isomer geometries. For the smaller representatives of this compound class, $[M]^+$ and/or $[M\text{-}15]^+$ were detected, thus independently confirming the structure assignments. Respective *Myricaria* wax constituents were identified as β-alkanediols with chain lengths C_{25}–C_{43}. A total of 27 novel compounds were identified by acquisition of full TMSi ether MS (Table 3). The presence of 56 other isomers of the C_{33}–C_{43} homologs could be inferred from their characteristic α-fragments in respective GC-MS peaks (Table 4). The β-alkanediol structure of this compound class was finally verified by formation of isopropylidene bis ether derivatives and inspection of their MS

Table 3
MS of *Myricaria germanica* β- and γ-alkanediol derivatives. The mass and relative intensity [%] of selected ions are given, diagnostic α-fragments are highlighted in bold face

Compound	M^+	$M\text{-}15^+$	α- And related fragments									Other fragments
β-Alkanediol bis TMSi ethers												
Pentacosane-8,10-diol	529 (0.2)	514 (0.4)	439 (0.1)	387 (0.1)	355 (1)	339 (2)	**313 (41)**	275 (3)	242 (5)	227 (10)	**201 (100)**	[a]
Heptacosane-6,8-diol	557 (0.3)	542 (0.1)	467 (0.4)	444 (0.4)	411 (0.3)	395 (1)	**369 (16)**	247 (3)	214 (2)	199 (8)	**173 (100)**	[a]
Heptacosane-8,10-diol	557 (0.2)	542 (1)	467 (1)	416 (0.1)	383 (0.4)	367 (2)	**341 (21)**	275 (2)	242 (7)	227 (6)	**201 (100)**	[a]
Heptacosane-10,12-diol	557 (0.1)	Missing	467 (0.2)	387 (1)	355 (1)	339 (3)	**313 (43)**	303 (2)	270 (3)	255 (6)	**229 (100)**	[a]
Nonacosane-8,10-diol	Missing	Missing	495 (0.2)	444 (0.2)	411 (0.1)	395 (1)	**369 (18)**	275 (2)	242 (4)	227 (6)	**201 (100)**	[a]
Nonacosane-10,12-diol	Missing	Missing	495 (0.2)	416 (0.2)	383 (0.4)	367 (2)	**341 (34)**	303 (2)	270 (3)	255 (10)	**229 (100)**	[a]
Hentriacontane-10,12-diol	Missing	598 (0.1)	523 (0.1)	444 (0.2)	411 (1)	395 (2)	**369 (23)**	303 (1)	270 (3)	255 (6)	**229 (100)**	[a]
Hentriacontane-12,14-diol	Missing	Missing	523 (0.1)	**458 (0.2)**	**373 (0.1)**	367 (1)	**341 (19)**	325 (0.1)	283 (4)	**257 (100)**	241 (3)	[a]
Tritriacontane-8,10-diol	Missing	626 (0.1)	551 (0.1)	500 (0.1)	467 (1)	452 (1)	**426 (16)**	275 (1)	242 (4)	227 (7)	**201 (100)**	[a]
Tritriacontane-10,12-diol	Missing	Missing	523 (0.1)	472 (0.2)	439 (0.2)	423 (2)	**397 (28)**	303 (2)	270 (4)	255 (9)	**229 (100)**	[a]
Pentatriacontane-8,10-diol	n.d.	n.d.	579 (0.2)	528 (0.2)	495 (1)	480 (1)	**454 (14)**	275 (2)	242 (3)	227 (7)	**201 (100)**	[a]
Pentatriacontane-10,12-diol	n.d.	n.d.	579 (0.1)	500 (0.1)	467 (1)	452 (2)	**426 (20)**	303 (2)	270 (3)	255 (7)	**229 (100)**	[a]
Hexatriacontane-8,10-diol	n.d.	n.d.	593 (0.1)	542 (0.1)	509 (0.3)	494 (1)	**468 (12)**	275 (2)	242 (3)	227 (7)	**201 (100)**	[a]
Hexatriacontane-9,11-diol	n.d.	n.d.	593 (0.3)	528 (0.2)	495 (1)	480 (0.3)	**454 (17)**	289 (2)	256 (3)	241 (8)	**215 (100)**	[a]
Heptatriacontane-8,10-diol	n.d.	n.d.	607 (0.2)	556 (0.2)	523 (0.4)	508 (1)	**482 (13)**	275 (2)	242 (3)	227 (6)	**201 (100)**	[a]
Heptatriacontane-10,12-diol	n.d.	n.d.	607 (0.1)	528 (0.3)	495 (0.2)	480 (1)	**454 (18)**	303 (2)	270 (3)	255 (6)	229 (100)	[a]
Octatriacontane-8,10-diol	n.d.	n.d.	621 (0.1)	570 (0.3)	537 (0.4)	522 (1)	**496 (10)**	275 (3)	242 (2)	227 (8)	**201 (100)**	[a]
Octatriacontane-9,11-diol	n.d.	n.d.	621 (0.1)	556 (0.2)	523 (0.2)	508 (1)	**482 (13)**	289 (3)	256 (4)	241 (6)	**215 (100)**	[a]
Octatriacontane-10,12-diol	n.d.	n.d.	621 (0.1)	542 (0.1)	509 (0.2)	494 (0.4)	**468 (16)**	303 (3)	270 (4)	255 (8)	**229 (100)**	[a]
Nonatriacontane-8,10-diol	n.d.	n.d.	635 (0.1)	584 (0.1)	551 (0.3)	536 (1)	**510 (9)**	275 (2)	242 (3)	227 (6)	**201 (100)**	[a]
Nonatriacontane-10,12-diol	n.d.	n.d.	635 (0.1)	556 (0.2)	523 (0.4)	508 (1)	**482 (14)**	303 (2)	270 (3)	255 (7)	**229 (100)**	[a]
Tetracontane-8,10-diol	n.d.	n.d.	649 (0.1)	598 (0.2)	565 (1)	550 (1)	**524 (9)**	275 (2)	242 (2)	227 (10)	**201 (100)**	[a]
Tetracontane-9,11-diol	n.d.	n.d.	649 (0.3)	584 (0.1)	551 (1)	536 (1)	**510 (9)**	289 (2)	256 (2)	241 (7)	**215 (100)**	[a]
Tetracontane-10,12-diol	n.d.	n.d.	649 (0.1)	570 (0.1)	537 (0.4)	522 (1)	**496 (10)**	303 (2)	270 (3)	255 (8)	**229 (100)**	[a]
Hentetracontane-8,10-diol	n.d.	n.d.	n.d.	612 (0.1)	579 (0.1)	564 (0.3)	**538 (6)**	275 (2)	242 (3)	227 (6)	**201 (100)**	[a]
Hentetracontane-10,12-diol	n.d.	n.d.	n.d.	584 (0.1)	551 (0.3)	536 (1)	**510 (9)**	303 (2)	270 (3)	255 (7)	**229 (100)**	[a]
Tritetracontane-10,12-diol	n.d.	n.d.	n.d.	612 (0.1)	579 (0.1)	564 (1)	**538 (4)**	303 (1)	270 (2)	255 (3)	**229 (100)**	[a]
β-Alkanediol isopropylidene bis ethers												
Pentatriacontane-8,10-diol	565 (1)	550 (39)		506 (0.4)	488 (0.4)		**466 (0.4)**	407 (3)		**213 (6)**	155 (18)	[b]
Heptatriacontane-8,10-diol	Missing	578 (26)	554 (0.1)	534 (0.3)	516 (0.1)		**494 (0.2)**	436 (2)		**213 (6)**	155 (18)	[b]
Nonatriacontane-10,12-diol	Missing	606 (19)	582 (0.1)	562 (0.3)	544 (0.1)		**494 (0.4)**	436 (3)		**241 (5)**	183 (13)	[b]
Hentetracontane-10,12-diol	Missing	634 (11)	610 (0.4)	590 (0.3)	572 (1)		**522 (0.3)**	464 (1)		**241 (8)**	183 (13)	[b]
γ-Alkanediol bis TMSi ethers												
Nonatriacontane-8,11-diol	n.d.	n.d.	635 (0.1)	570 (0.2)	537 (0.1)	522 (0.2)	**496 (10)**	275 (3)	242 (3)	227 (8)	**201 (100)**	[c]
Hentetracontane-8,11-diol	n.d.	n.d.	n.d.	598 (0.1)	565 (0.1)	550 (0.2)	**524 (7)**	275 (3)	242 (7)	227 (9)	**201 (100)**	[c]
Hentetracontane-10,13-diol	n.d.	n.d.	n.d.	570 (0.1)	537 (0.1)	522 (0.2)	**496 (14)**	303 (3)	270 (3)	255 (10)	**229 (100)**	[c]
Tritetracontane-8,11-diol	n.d.	n.d.	n.d.	626 (0.1)		578 (0.3)	**552 (4)**	275 (2)	242 (11)	227 (12)	**201 (100)**	[c]
Tritetracontane-10,13-diol	n.d.	n.d.	n.d.	598 (0.1)		550 (0.1)	**524 (10)**	303 (3)	270 (3)	255 (8)	**229 (100)**	[c]

[a] Other fragments characterizing the compound class. Typical ranges for relative intensities [%] are given in brackets: 149 (2–7), 147 (11–24), 143 (3–15), 130 (12–29), 129 (5–14), 103 (3–13), 97 (3–13), 83 (2–17), 75 (5–17), 73 (20–50), 57 (2–27).

[b] Other fragments characterizing the compound class. Typical ranges for relative intensities [%] are given in brackets: 125 (25–26), 111 (52–61), 97 (100), 83 (85–88), 71 (56–58), 69 (49–64), 57 (82–91).

[c] Other fragments characterizing the compound class. Typical ranges for relative intensities [%] are given in brackets: 149 (5–8), 147 (16–20), 143 (9–18), 130 (27–37), 129 (8–14), 103 (4–6), 97 (5–13), 83 (4–19), 75 (6–11), 73 (26–43), 57 (9–14).

Table 4
Composition of the β-alkanediol fraction A from *Myricaria germanica* leaf wax[a]

Carbon number	Coverage 8,10 isomers [μg/cm²]	Coverage 10,12 isomers [μg/cm²]	Isomer percentage 6,8	7,9	8,10	9,11	10,12	11,13	12,14	13,15	Totals
33	–	–	–	tr[b]	0.6±0.46	tr	0.2±0.04	tr	tr	tr	1.0
34	–	–	tr	tr	tr	tr	tr	tr	tr	tr	0.1
35	0.1	–	tr	tr	8.4±1.63	tr	1.0±0.21	0.1±0.03	tr	tr	9.6
36	–	–	tr	tr	0.4±0.16	0.2±0.03	tr	tr	tr	tr	0.7
37	0.1	0.1	tr	0.1±0.04	24.7±0.68	0.3±0.04	12.4±0.16	0.4±0.06	tr	0.2±0.02	38.1
38	–	–	tr	tr	0.5±0.09	0.5±0.04	0.6±0.03	tr	tr	tr	1.7
39	0.1	0.1	tr	tr	7.7±0.55	0.2±0.03	30.1±2.72	0.4±0.03	0.3±0.11	0.4±0.04	39.1
40	–	–	tr	tr	0.1±0.05	0.1±0.01	0.5±0.03	tr	tr	tr	0.8
41	–	0.05	tr	tr	0.5±0.37	tr	7.9±0.60	0.1±0.03	0.1±0.05	0.1±0.02	8.7
42	–	–	–	–	–	–	–	–	–	–	–
43	–	–	–	–	tr	–	0.2±0.01	tr	tr	–	0.2

[a] Leaf surface coverages [μg/cm²] of the six most abundant compounds were quantified within the total wax mixture. Relative amounts [%] of individual isomers and the resulting homolog totals were further quantified using abundances of corresponding α-fragments of OTMSi derivatives. Mean values and standard deviations of four independent experiments are given.
[b] tr: 0.01–0.1% detectable.

(Table 3). The four prevailing homologs/isomers showed the expected $[M]^+$, $[M\text{-}15]^+$, two α-fragments $[C_nH_{2n+1}(C_6H_{10}O_2)]^+$, their secondary fragments $\Delta m/z = 58$ (loss of acetone), and ions $m/z = 57, 69, 71, 83, 97$.

While C_{25}–C_{31} β-alkanediols were present only as minor components in fraction B (and could not be accurately quantified), the C_{35}–C_{43} homologues were detected in higher amounts in fraction A. Not all the isomers in this fraction were resolved by GC and consequently the relative percentages of all individual compounds had to be calculated from the abundances of α-fragments $[C_nH_{2n}OTMSi]^+$ within GC–MS peaks (Table 4). Odd-numbered homologs, especially the chain lengths C_{37} and C_{39}, and 8,10- and 10,12-isomers were found to prevail. Hence, heptatriacontane-8,10-diol and nonatriacontane-10,12-diol were the dominating constituents of the β-diol fraction. Six β-alkanediols could also be detected in the total wax mixture of *M. germanica* and hence quantified against the internal standard. They individually amounted to ca. 0.1 μg/cm² (0.1–0.4% of the extract) and added up to a leaf surface coverage of 0.6 μg/cm² (2% of the extract). A similar series of secondary β-alkanediol homologs, but with 4,6- and 6,8-configuration of functional groups, had previously been reported for the pollen lipids of *Helianthus annuus* (Schulz et al., 2000) and the petal wax of *Carthamus tinctorius*, respectively (Akihisa et al., 1994).

2.3. γ-Alkanediols

Additionally, a series of γ-alkanediols was identified in fraction C based on their TMSi ether MS (Table 3). Spectral characteristics, including the diagnostic fragment $m/z = 130$ were similar to those of β-diols. But compounds in both fractions differed in GC retention behavior, thus forming consecutive peaks for 10,13-, 8,11-, 10,12- and 8,10-isomers of a given homolog. Five γ-diol structures could be assigned directly from complete MS information, while 18 other compounds in this class were detected only by inspection of α-fragments and GC retention times. The α-fragments were also used to calculate percentages of individual compounds (Table 5). Thus, predominantly 8,11- and 10,13-isomers of the odd-numbered homologs with chain lengths C_{37}–C_{43} were found. The most abundant compounds were hentetracontane-8,11-diol, nonatriacontane-8,11-diol and hentetracontane-10,13-diol. By comparison with other diols within their TLC fraction, the added leaf surface coverage of respective γ-diols was estimated to be ca. 0.1 μg/cm². All the identified γ-diols represent novel

Table 5
Composition of the γ-alkanediol fraction C from *Myricaria germanica* leaf wax[a]

Carbon number	Isomer percentage 7,10	8,11	9,12	10,13	11,14	12,15	Totals
37	tr	6.9	0.2	0.8	0.1	tr	8.0
39	–	20.4	0.4	0.4	0.6	0.3	22.1
41	0.4	43.7	0.7	14.2	1.5	0.4	60.9
43	tr	2.0	tr	6.1	0.3	0.5	9.0

[a] Relative amounts [%] of individual isomers and the resulting homolog totals were quantified using abundances of corresponding α-fragments of OTMSi derivatives.

compounds. Previously, only C_{17}–C_{33} γ-diols with 5,8-substitution had been described for the surface wax from flowers of *Rosa damascena* (Stoianova-Ivanova et al., 1974).

2.4. Primary/secondary alkanediols

In fractions B, C and D small amounts of five more alkanediols were detected. Their TMSi ether derivatives showed MS characteristics, especially an abundant fragment $m/z = 149$ (Table 1), that had previously been described as diagnostic features for diols carrying one primary and one secondary hydroxyl function (Jetter et al., 1996). One of the *Myricaria* wax constituents, triacontane-1,11-diol, was identified by direct comparison with the TMSi ether spectrum of the authentic compound from *Papaver* surface waxes (Jetter et al., 1996). Dotriacontane-1,9-diol,-1,11-diol,-1,13-diol and tetratriacontane-1,11-diol could then be identified according to analogous GC retention and MS data. Interestingly, the alkane-1,13-diol, the-1,11-diols and the-1,9-diol were prevailing in fractions B, C and D, respectively. The primary/secondary diols are hence co-migrating with secondary/secondary diols of widely differing molecular geometries (see above). The (partial) TLC separation of the primary/secondary alkanediol isomers is probably due to the increasing polarities of the molecules that result from the reduced distances between both functional groups near one alkyl terminus. Comparable TLC separation had previously been reported for isomeric primary/secondary ketols and ketoaldehydes (Jetter and Riederer, 1999).

2.5. Biosynthesis of alkanediols

The alkanediol fractions from *Myricaria* leaves were largely dominated by C_{31} isomers with one hydroxyl group on carbon 12. Hence, their molecular geometry closely resembled that of hentriacontan-12-ol, the prevalent secondary alcohol in the wax mixture of this species (Jetter, 2000). This finding is analogous to diverse plant taxa (e.g. moss sporophytes, Gymnosperms, Papaveraceae, Anacardiaceae) where wax mixtures are dominated by nonacosan-10-ol and its nonacosanediol derivatives (Franich et al., 1979; Hunt and Baker, 1979; Jetter and Riederer, 1996; Neinhuis and Jetter, 1995). It has been suggested that the nonacosanediols are biosynthesized from nonacosan-10-ol by relatively unspecific hydroxylation reactions (Franich et al., 1979). Analogously, the hentriacontanediols in *Myricaria* could be generated from hentriacontan-12-ol by similar hydroxylations. This hypothesis implies that a first hydroxylation is directed toward the methylene at the α + 11 and ω-19 position. It is supported by the fact that the functional groups in the other *Myricaria* diol homologs are located in corresponding positions, i.e. 10-OH in C_{29} (ω-19), 12-OH in C_{33} (α + 11) and 14-OH in C_{33} (ω-19).

The primary/secondary alkanediols identified in *Myricaria* waxes can be regarded as a series of 1,11-homologues and another series of dotriacontanediol isomers. The one compound that is shared by both series, dotriacontane-1,11-diol, is not prevailing in the fraction. Hence, the secondary hydroxyl function seems to be introduced in a reaction that is unspecific either for the chain length or the methylene position of the substrate. This finding is in contrast to the primary/secondary diols in *Papaver* waxes that are apparently generated under the directing control of the ω-alkyl terminus (Jetter et al., 1996). At present, it can only be inferred that the two hydroxyl functions of the primary/secondary diols are generated in independent reactions but the exact nature and order of biosynthetic steps cannot be predicted.

β-Alkanediol homologs in *Myricaria* waxes were characterized by extremely long carbon chains and by two alkyl chains with odd carbon numbers on both sides of the 1,3-diol group. Both features could be explained by two alternative biosynthetic pathways: (1) the compounds could be regarded as polyketides, i.e. products of a specialized elongation-decarboxylation pathway that would generate the hydroxyl groups in two consecutive elongation cycles, would then synthesize the long carbon chains in additional elongation cycles and would finally yield the products in a reduction/decarbonylation. It is widely accepted that a similar mechanism is involved in the formation of other 1,3-bifunctional wax constituents, e.g. β-diketones (von Wettstein-Knowles, 1995; Schulz et al., 2000). (2) On the other hand, the *Myricaria* β-diols could also be regarded as condensation products of a Claisen-type reaction between a β-keto-acid (derivative) and an ester. Although this pathway could explain the extraordinary chain lengths of the compounds through the condensation of C_8–C_{14} and C_{24}–C_{32} compounds, analogous wax biosynthetic reactions have not been reported previously. Unfortunately, none of these hypotheses can account for the presence of γ-alkanediols with similar homolog and isomer patterns and alternative biosynthetic pathways leading to these trace compounds cannot be conjectured on the basis of the present phytochemical data set.

In conclusion, *Myricaria* leaf surface diols are largely dominated by a series of hentriacontanediol isomers and a series of β-alkanediol homologs. As these series share one compound, hentriacontane-10,12-diol, that is present only in trace amounts, they are probably generated via two independent routes. This inference is manifest in the pathways hypothesized above: while the hentriacontanediols are produced in two consecutive, independent hydroxylation steps, the β-diols are probably formed in concerted reactions.

3. Experimental

3.1. Plant waxes

Plants were grown continuously in the Botanical Garden of the University of Würzburg. Whole twigs (ca. 20 cm) were harvested in September 1998 and September 1999 and immediately immersed twice for 30 s in $CHCl_3$ at room temp. For absolute quantification of alkanediols within the whole wax mixture, a defined amount of tetracosane was added as internal standard. The resulting solns of cuticular waxes were dried, filtered and the solvent removed under reduced pressure. Extracted leaf surface areas were assessed by multiplying average leaf numbers per twig with the average (projected) leaf surface area and the number of twigs. Wax mixtures from both collection dates were stored at 4°; and later analyzed in parallel using GC–FID and GC–MS. Leaf surface coverages of individual constituents were calculated as mean values of these two independent analyses, values for the separate analyses differing by less than 2%.

3.2. Gas chromatography

Compounds containing hydroxyl groups were transformed (with bis-*N*,*N*-trimethylsilyltrifluoroacetamide in pyridine 30 min at 70°C) to TMSi derivatives. Qualitative analyses were carried out by GC (30 m DB-1 WCOT i.d. 320 μm, on-column-injection at 50°C, oven 2 min at 50°C, 40°C min^{-1} to 200°C, 2 min at 200°C, 3°C min^{-1} to 320°C, 30 min at 320°C and He carrier gas inlet pressures 1 min at 5 kPa, 4 kPa min^{-1} to 18 kPa, 0.6 kPa min^{-1} to 40 kPa, 37 min at 40 kPa) with MS detection (70 eV, m/z 50–650), quantification of individual compounds by GC–FID (as above, but H_2 carrier gas inlet pressures 41 min at 50 kPa, 10 kPa min^{-1} to 150 kPa, 30 min at 150 kPa).

3.3. Alkanediol analysis

Substance classes were separated by TLC (sandwich technique, silica gel, mobile phase $CHCl_3$–EtOH (99:1), staining with primuline and UV-light). The alkanediol bands were removed from the plates and eluted with $CHCl_3$. The resulting solns were subjected to TMSi ether derivatization and relative quantities of individual compounds in the fractions were assessed by integration of relevant GC–FID peaks. Combined TLC and GC analyses of fractions A, D and E were repeated at least twice with waxes from both collection dates. Compound percentages were calculated as mean values of these two independent analyses, values for the separate analyses differing by less than 5%.

Alternatively, diols in fraction A were transformed into isopropylidene bis ether derivatives instead of TMSi ethers. To this end, ca. 50 μg of the fraction were isolated from parallel TLC plates and added to 200 μl of 2,2-dimethoxypropane with ca. 5% *p*-toluenesulfonic acid and the mixture was allowed to stand at RT for two days. Then ca. 100 μg of Amberlite 400 were added, the soln. filtered and the solvent removed in a stream of N_2. The residue was taken up in $CHCl_3$ and analyzed by GC–MS.

Relative portions of β-alkanediol isomers in fraction A were calculated using abundances of corresponding α-fragments $[C_nH_{2n}OTMSi]^+$. Four independent analyses of this fraction were carried out and hence mean values and standard deviations for all individual compounds could be calculated. Similarly, the percentages of γ-alkanediol isomers in fraction C were assessed from respective TMSi ether MS. In this case only one experiment was carried out.

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