Chemistry of Hermidin: Insights from Extraction Experiments with the Main Alkaloid of *Mercurialis perennis* L. Tracked by GC/MS and LC/MS^n

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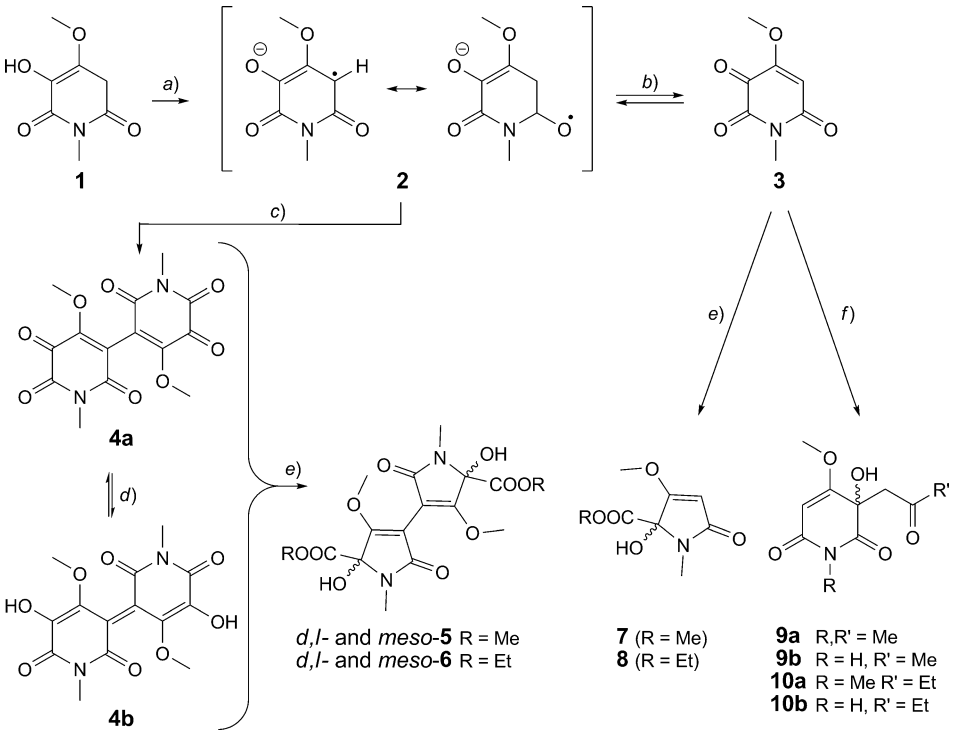
Hermidin (**1**), a piperidine-2,3-dione alkaloid, has been previously detected as a lipophilic constituent in *Mercurialis perennis* L. and other *Mercurialis* species. Because of strong electron-withdrawing effects of its carbonyl groups, an acidic H-atom is easily subtracted from **1**, whereas the latter shows high reactivity towards oxidation reactions or the attack of C-nucleophilic agents. To obtain a better understanding of possible chemical pathways upon extraction of root parts of *M. perennis*, the products obtained with different solvents from **1** were investigated. Extraction of *M. perennis* with aqueous MeOH or EtOH yielded a mixture of hermidin quinone (**3**), 5-hydroxy-4-methoxy-5-(alkoxycarbonyl)-1-methyl-3-pyrrolin-2-ones, **7** and **8**, and *d*,*l*- and *meso*-isochrysohermidins, **5** and **6**, all of them being investigated by GC/MS and LC/MS^n. The latter compounds were supposedly formed by free-radical reactions, followed by spontaneous benzilic acid rearrangement and esterification. Furthermore, extraction of *M. perennis* with aqueous Me_2CO produced an aldol condensation product, the known alkaloid speranskatine A (**9a**), which was identified by NMR after chromatographic purification. In a similar manner, a CH_2 homolog of speranskatine A (**10a**) was obtained as a novel compound when ethyl methyl ketone (= butan-2-one; EtCOMe) instead of Me_2CO was used for extraction. Consequently, **1** easily undergoes artefact formation upon extraction of plant material with polar or slightly polar solvents.

Introduction. – Extraction solvents play a crucial role in the isolation of natural products from plants and in bioanalytical chemistry [1], because side reactions may occur with the respective compounds. This has been reported in the literature for alkaloids [2][3], anthocyanins [4], or several other compounds [1][5][6]. The artefacts formed differ structurally from the genuine metabolites biosynthesized by the organisms. These novel constituents may exert biological or pharmacological properties different from those of the primary natural products. At the same time, extracts can reveal activity losses due to degradation of their active principles, or toxic compounds might be formed [1]. In the course of phytochemical investigations on different herbal plants, we identified dog's mercury (*Mercurialis perennis* L.) as a rich source of various natural constituents, such as terpenes, lipids, nitrogenous compounds, low-molecular phenolics, *n*-alkylresorcinols, depsides, flavonoids, *etc.* [7–10]. *M. perennis* is an old medicinal plant, belonging to the *Euphorbiaceae* family. Besides its use in ancient phytomedicine, it nowadays becomes increasingly important in complementary

medicine for the treatment of inflammations [11]. Comprehensive metabolomic studies of *M. perennis* [7][9][10] revealed a closer insight on the alkaloid content of this species. Hermidin (= 5-hydroxy-4-methoxy-1-methylpyridine-2,6(1*H*,3*H*)-dione, **1**), the main alkaloid of *M. perennis*, is an O_2-sensitive compound, entirely stable under reducing physiological conditions [12]. After extraction from the plant matrix, **1** is easily oxidized in solution, forming a semistable blue anionic radical, the so-called cyanohermidin (**2**), in a first step (*Scheme 1*) [7]. Compound **2** is stabilized *via* mesomeric effects and possibly by interaction with cations, and has been early characterized by redox titration [12] and ESR studies [13]. Downstream oxidation of **2** affords hermidin quinone (=4-methoxy-1-methylpyridine-2,3,6(1*H*)-*trione; **3**) or the dimeric quinone **4a** via* free-radical dimerization (*Scheme 1*). The latter is in a redox-equilibrium with **4b**, a dimeric analog of **1** [7][14]. We have recently shown that **1** and its oxidation products undergo metabolic conversion during fermentation, by *Lactobacteria* [15]. In the same line, the high reactivity of **1** towards C-nucleophilic agents such as MeCHO was demonstrated for the first time [15].

Scheme 1. *Proposed Pathways for the Reaction of Hermidin* (**1**) *under the Influence of Aqueous Organic Solvents during Extraction of* M. perennis



a) Deprotonation and oxidation ($-2\,H^+$; $-e^-$). *b*) Oxidation ($-e^-$). *c*) Dimerization and oxidation ($+2\,H$; $-2e^-$). *d*) Reduction ($+2\,H$). *e*) ROH (R = Me, Et)/H_2O, benzilic acid rearrangement and esterification. *f*) MeCOR (R = Me, Et)/H_2O, aldol condensation.

With the aim to achieve a standardization, we investigated the impact of different solvents on the secondary metabolite profile of the resulting extracts. In particular, potential artefact formation from **1**, which may occur upon the extraction of *M. perennis*, was considered in the present study. Two common solvent classes, alcohols and ketones, were used for extraction, and the resulting metabolite profiles were extensively analyzed by chromatographic and MS techniques.

Results and Discussion. – Hermidin (**1**) and its oxidation products are prone to benzilic acid rearrangements in the presence of aqueous alcohols. To investigate the solvent influence on the constituent profile, root parts of *M. perennis* were first extracted with aqueous MeOH. The obtained extract was evaporated to remove MeOH, and the remaining H_2O portion (pH 6.8) was extracted with AcOEt. GC/MS Analyses of this extract showed a complex compound pattern (*Fig. 1,a*). By alignment of mass spectra with the NIST database [16], several known compounds were assigned, such as 3,4-dimethoxyphenol (t_R 16.6 min), and simple fatty acids (palmitic, linoleic, and linolenic acids), as well as the corresponding methyl esters. Furthermore, nitrogenous compounds with odd-numbered molecular ion, M^+ peaks, were detected, like the formerly identified hermidin (**1**) and hermidin quinone (**3**) at t_R 16.5 and 20.3 min, respectively [7]. However, one N-containing compound at t_R 20.2 min (*Fig. 1,a*) could not readily be assigned. The M^+ peak at *m/z* 201, and the release of $COOMe^+$ and CO^+ fragments in EI-MS experiments (*Table 1*) revealed this compound to be an N-heterocyclic methyl ester. Comparison with literature MS data [17] disclosed the coincidence of this compound with methyl oxopyrrole carboxylate **7**. Compound **7** has been previously obtained by $^1O_2$-mediated oxidative decarboxylation of a pyrroledicarboxylate [17]. Furthermore, two peaks (t_R 43.8 and 44.5 min) with identical M^+ ions at *m/z* 400 and fragmentation patterns similar to those of **7** were found (*Fig. 1,a* and *Table 1*). Based on literature MS data [18] and comparison with synthesized reference material, these peaks could be assigned to a diastereoisomeric mixture of isochrysohermidin (*d,l*/*meso*-**5** 1.2 : 1.0 (*w*/*w*)). Interestingly, compound **5** was first isolated from the Asian *Mercurialis leiocarpa* [19], but obviously as an artefact, since the authors used MeOH for extraction. Later, a multistep total synthesis of *d,l*- and *meso*-**5** was accomplished by *Boger* and *Baldino*, and *Wasserman et al.* [17][18][20][21], and it has been even shown earlier that **5** could be obtained from chrysohermidin (**4a**) in one step *via* benzilic acid rearrangement in MeOH, catalyzed by alkoxides or tertiary amines [22][23] (see *Scheme 1*). In this context, it appears interesting that, in the current investigation, the reactivities of **3** and **4a** were high enough for a benzilic acid rearrangement, and that esterification already proceeded in MeOH/H_2O at pH 6.8. This finding was supported when the extraction of the plant material was performed with EtOH/H_2O. The GC/MS analysis of the resulting AcOEt extract exhibited a similar but more complex compound pattern (*Fig. 1,b*) as compared to the MeOH/H_2O extract. Among others, an unknown peak at t_R 21.5 min with an M^+ peak at *m/z* 215 was striking. Because of structural resemblance to *rac*-**7** (*Table 1*), the novel compound, **8**, could be readily assigned as a CH_2 homolog of **7**. Moreover, *rac*-**8** was independently synthesized from **3** by reaction with EtOH/EtONa, and its structure was determined by NMR. Furthermore, in the t_R range between 38 and 47 min of the EtOH/H_2O extracts, several peaks of dimeric nitrogenous compounds with even-

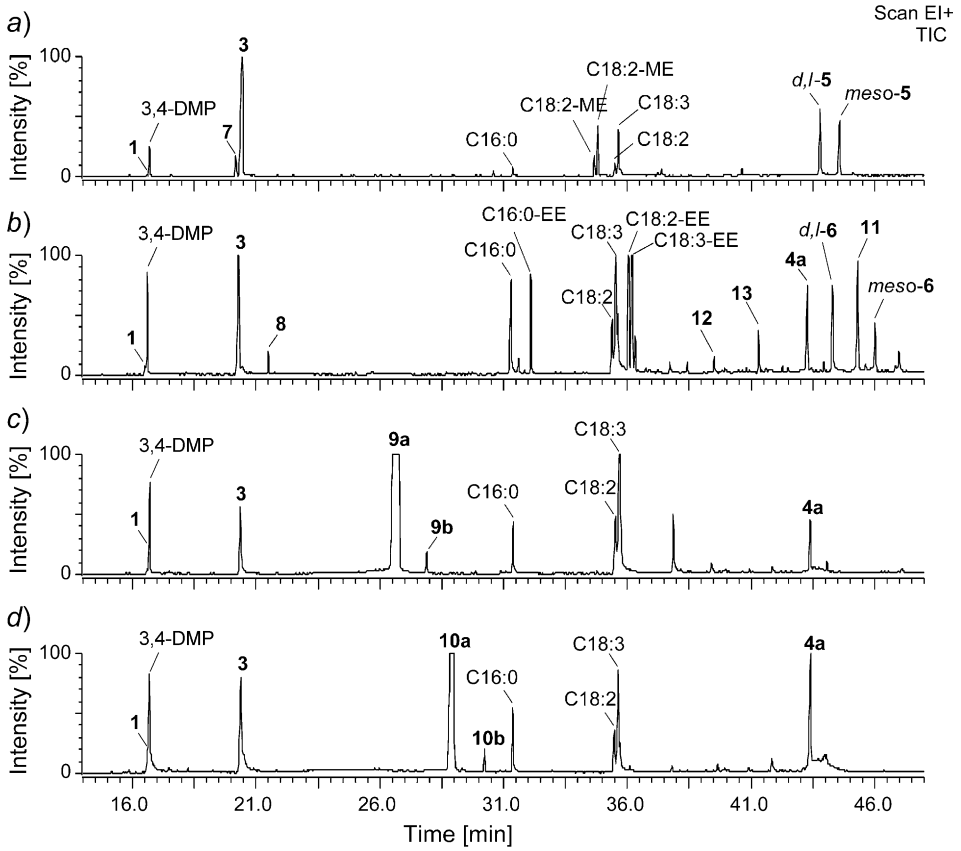


Fig. 1. *Sections of GC/MS profiles* (EI) *showing metabolites of AcOEt fractions from* M. perennis *root extracts obtained with different solvents. a*) MeOH/H_2O. *b*) EtOH/H_2O. *c*) Me_2CO/H_2O. *d*) EtCOMe/H_2O. Assignment: 3,4-DMP, 3,4-dimethoxyphenol; C16 : O, palmitic acid; C18 : 2, linoleic acid; C18 : 3, α-linolenic acid; and their corresponding methyl and ethyl esters (ME and EE, resp.)

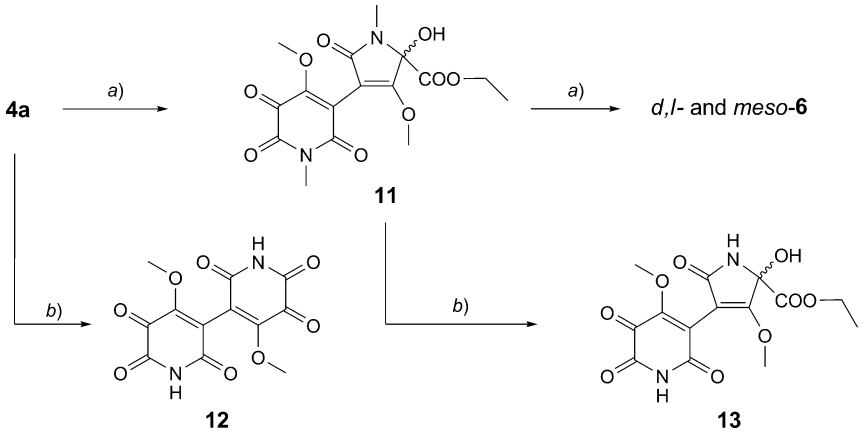
numbered M^+ ions were analyzed (*Fig. 1,b*). Besides chrysohermidin (**4a**; t_R 26.7 min), two peaks of the diastereoisomers *d,l*- and *meso*-**6** (*Scheme 1*) were detected at t_R 44.3 and 46.0 min, respectively (*Fig. 1,b*). The characterization of *d,l*- and *meso*-**6** was based again on comparison with literature MS data [23] and with a reference compound, obtained by reaction of **4a** with EtOH/Et_3N, as described in [23]. Interestingly, an unknown compound with a peak at t_R 45.3 min in GC/MS analyses attracted our attention. On the basis of an M^+ peak at *m/z* 382 and peaks of fragment-ions $[COOEt]^+$, $[CO]^+$, and $[NMe]^+$ in the EI mass spectrum, this compound was tentatively assigned as the half-benzilic acid rearrangement product **11** (see *Scheme 2*). However, complete structure elucidation only based on MS data was impossible. To support the proposed structure, a synthesis of **11** was attempted by treating **4a** with EtOH in the presence of low amounts of Et_3N. It was possible to trap the intermediate, and chromatographic purification on SiO_2 yielded **11** as a yellow solid, the structure of

Table 1. *GC/MS Data of Hermidin* (**1**) *Reaction Products, Identified in Alcohol- and Ketone/H_2O Extracts from the Root Parts of* M. perennis L.

Solvent	Constituent	t_R [min]	M_r [Da]	Characteristic fragment-ion peaks, *m/z* [%BPI]	
				M^+	Other intrinsic ion peaks
MeOH	**7**	20.2	201.18	201 (4)	142 (100)[a]), 114 (9)[b]), 82 (18), 69 (14)[c])
	d,*l*-**5**	43.8	400.34	400 (12)	341 (54)[a]), 323 (100)[d]), 295 (14), 277 (39)[e]), 236 (24), 208 (8), 181 (9),
	meso-**5b**	44.5			154 (8), 123 (3), 95 (4)[f])
EtOH	**8**	21.5	215.20	215 (5)	142 (100)[g]), 114 (9)[h]), 82 (14), 69 (12)
	12	39.5	308.20	308 (46)	280 (34)[i]), 265 (20)[j]), 251 (29)[k]), 237 (14)[l]), 223(6), 208 (86), 195 (40),
					180 (100), 167(10), 152 (29), 123 (55), 95 (68), 80 (41)
	13	41.3	354.27	354 (2)	281 (100)[g]), 237 (2), 224 (7), 208 (6)[m]), 196 (27), 181 (18), 166 (3),
					153 (3), 138 (3), 123 (3), 95 (4)
	4a, **4b**[n])	43.3	336.25	336 (4)	321 (99)[o]), 308 (13)[i]), 280 (59)[p]), 265 (29)[q]), 251 (29)[r]), 236 (94), 208 (74),
			(338.27)	338 (2)	195 (29), 180 (100), 167 (12), 152 (35), 123 (45), 111 (13), 95 (71), 80 (47)[s])
	d,*l*-**6**	44.3	428.39	428 (7)	355 (41)[g]), 337 (100)[t]), 323(6)[u]), 281(12), 277 (37), 265(5), 236 (18), 224(5),
	meso-**6**	46.0			208 (7), 196 (8), 181 (8), 154 (9), 123 (2), 95 (3)
	11	45.3	382.32	382 (1)	309 (100)[g]), 281 (14)[h]), 252 (18), 237 (4), 224 (8), 208 (8), 196 (24), 181 (18),
					153 (4), 138(3), 123 (4), 95 (6)
Acetone	**9a**	26.7	227.21	227 (2)	199 (4)[i]), 194 (4)[v]), 170 (50)[k]), 142 (48)[r]), 127 (100), 109 (5), 99 (8), 84 (5),
					69 (22)[w])
	9b	27.9	213.19	213 (11)	185 (11)[i]), 180 (6)[v]), 170 (58)[j]), 156 (13), 142 (7)[l]), 127 (100), 109 (6),
					99 (11), 84 (6), 69 (26)[w])
EtCOMe	**10a**	29.0	241.24	241 (3)	212 (5)[x]), 194 (14)[y]), 184 (41)[z]), 170 (24), 142 (49), 127 (100), 109 (4),
					99 (6), 69 (18)
	10b	30.2	227.21	227 (16)	198 (28)[x]), 184 (47)[j]), 180 (33), 171 (21), 156 (40)[l]), 154 (18), 127 (100),
					109 (6), 100 (12), 84(5), 69 (25)

[a]) $[M - CO_2Me]^+$. [b]) $[M - CO_2Me - CO]^+$. [c]) The MS data correspond to those in [17]. [d]) $[M - CO_2Me - H_2O]^+$. [e]) $[M - CO_2Me - 2\,H_2O - CO]^+$. [f]) From [21]. [g]) $[M - CO_2Et]^+$. [h]) $[M - CO_2Et - CO]^+$. [i]) $[M - CO]^+$. [j]) $[M - CO - NH]^+$. [k]) $[M - CO - NMe]^+$. [l]) $[M - 2\,CO - NH]^+$. [m]) $[M - 2\,CO_2Et]^+$. [n]) Coelution. [o]) $[M - Me]^+$. [p]) $[M - 2\,CO]^+$.[q]) $[M - 2\,CO - Me]^+$. [r]) $[M - 2\,CO - NMe]^+$. [s]) From [7]. [t]) $[M - CO_2Et - H_2O]^+$. [u]) $[M - CO_2Et - MeOH]^+$. [v]) $[M - H_2O - Me]^+$. [w]) From [30]. [x]) $[M - Et]^+$. [y]) $[M - Et - H_2O]^+$. [z]) $[M - Et - CO]^+$.

Scheme 2. *Possible Pathways of the Reaction of Chrysohermidin* (**4a**) *with EtOH/H_2O*

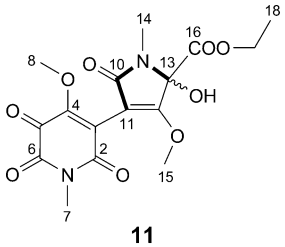


a) Benzilic acid rearrangement and esterification. *b*) Oxidative/reductive *N*-demethylation.

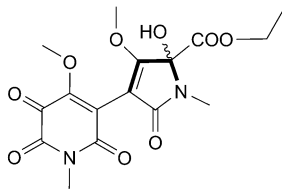
which was confirmed by comprehensive NMR investigations. The ^{1}H-NMR spectrum of the unknown compound (*Table 2*) exhibited a double set of seven different ^{1}H spin systems corresponding to one OH group (*s*), a CH_2 unit (2*dq*) coupled with a Me group (*t*), two MeO groups (2*s*), and two MeN groups (2*s*) (*Table 2*). A CH-edited gHSQC spectrum revealed the CH_2 group and enabled the assignment of all other protonated C-atoms (data not shown). Further NMR experiments involving ^{13}C and gHMBC allowed complete assignment of the NMR signals of **11**, but left the question regarding its configuration unanswered, because all H- and C-signals were doubled (*Table 2*). Since the synthesis of **11** was expected to yield a racemic mixture with respect to C(13), indistinguishable by NMR, the observation of a double signal set indicating, *e.g.*, diastereoisomers could be explained by the presence of a second chiral element in **11**. Obviously, free rotation of the C(3)–C(11) is hindered by steric strain energy barrier of neighboring substituents (MeO and C=O). Thus, the axial chirality produces atropisomers [24] which, in combination with the stereogenic center C(13), enables the detection of diastereoisomers by NMR; however, unfortunately, they are not separable by GC.

In addition to products resulting from the benzilic acid rearrangement, in the EtOH/H_2O extract, two further compounds were detected by GC/MS, at t_R 39.5 and 41.3 min, which were tentatively assigned to the *N*-demethylation products **12** and **13**, respectively (*Scheme 2*), based on their M^+ peaks and fragmentation behavior (*Table 1*). *N*-Demethylation of tertiary amines like alkaloids occurs in nature by means of oxidative enzymes, *e.g.*, cytochrome P 450, but it has been also achieved under oxidative/deoxidative laboratory conditions [25][26]. Since **1** and **3** form part of a common redox system, it is feasible that the latter may catalyze the *N*-demethylation of **4a** and **11** to yield **12** and **13**, respectively (*Scheme 2*). Remarkably, in a commercial EtOH/H_2O extract of *M. perennis* (tincture) the constituents *d,l*- and *meso*-**6**, and **13** were also detected as predominant N-containing artefacts by GC/MS (data not shown), thus confirming the aforementioned results.

Table 2. *^{1}H- and ^{13}C-NMR Data of the Novel Constituent* **11/11′** (diastereoisomeric atropisomer mixture)[a]). In $CDCl_3$; δ in ppm, J in Hz.



11



11'

Position	δ(H)	δ(C)	HMBC
1	–	–	–
2	–	162.63/162.71 (C=O)	–
3	–	119.47/119.85 (C)	–
4	–	157.00/157.16 (C)	–
5	–	171.80/171.91 (C=O)	–
6	–	155.43/155.58 (C=O)	–
7	3.32 (*s*)/3.33 (*s*)	27.65/27.73 (MeN)	C(6), C(2)
8	4.10 (*s*)/4.13 (*s*)	60.81/61.05 (MeO)	C(4)
9	–	–	–
10	–	168.34/168.49 (C=O)	–
11	–	98.62/98.79 (C)	–
12	–	167.81/167.88 (C)	–
13	–	86.92/86.94 (C)	–
14	2.78 (*s*)/2.79 (*s*)	24.12/24.20 (MeN)	C(13), C(10)
15	3.84 (*s*)/3.85 (*s*)	58.52/59.03 (MeO)	C(12)
16	–	168.51/168.94 (C=O)	–
17	4.38, 4.40 (2*dq*, J = 7.1, 10.8)/4.34, 4.35 (2*dq*, J = 7.1, 11.0)	63.91/64.31 (CH_2)	C(18), C(16)
18	1.31 (*t*, J = 7.1)/1.35 (*t*, J = 7.1)	14.08/14.09 (Me)	C(17)
OH	4.58/4.63	–	C(13), C(16)

[a]) Relative configurations of the diastereoisomers **11** and **11′**, together with arbitrary atom numbering.

To provide another analytical method to detect the assigned alkaloid reaction products, these were also independently investigated for the first time by HPLC-DAD/MS^n (*Table 3*). Sections of a total ion current (TIC) and UV chromatogram of an EtOH/H_2O extract are shown in *Fig. 2*, *a* and *b*, respectively. After HPLC separation, the compounds were analyzed in the ESI^+ mode, which provides optimum results for ionization of low-polar *N*-Me heterocycles, characterized in collision-induced dissociation (CID) experiments. All alkaloid homologs assigned by GC/MS (except **12**) could also be detected by LC/MS. As expected, differences in the EI-MS and ESI^+-MS fragmentation patterns were observed (*Table 3* and *Scheme 3*). Thus, MS^n spectra of the pyrrolidones **11** and *d*,*l*-**6** are shown, as examples, in *Fig. 2*, *c* and *d*, respectively. An initial H_2O loss from the *pseudo*-molecular ion $[M+H]^+$ was observed for both constituents resulting in fragment-ion peaks at *m*/*z* 365 and 411, respectively. While the peaks for the $[365-CO]^+$, $[337-CO_2]^+$, and $[293-CO]^+$ ions were detected for **11**

Table 3. *LC/MS Data of Hermidin* (**1**) *Reaction Products, Identified in Alcohol- and Ketone/H_2O Extracts from the Root Parts of* M. perennis *L.*

Solvent	Constituent	t_R [min]	M_r [Da]	UV_{max} [nm]	Intrinsic ion peaks in the MS^+ mode			
					MS^1 ($[M+H]$)	MS^2	MS^3	MS^4
MeOH	**7**	15.4	201.18	212, 258	202	170[a])	126[b]), 94	112, 98, 85, 66
	meso-**5**	26.0	400.34	212, 258 (sh)	401	383[c])	351[d]), 323, 294,	266, 238, 206
	d,l-**5**	37.1		212, 258 (sh)			282, 266	
EtOH	**8**	21.9	215.20	254	216	184[a])	156[e])	112, 94
	4a	26.7	336.25	220, 280, 350 (sh)	337	252[f])	237, 223, 210, 192	181, 153, 125, 96
	11	37.1	382.32	270, 350 (sh)	383	365[c])	337[g]), 321, 309, 293, 278, 265, 252	309, 293, 278, 265, 252
	12	n.d.	308.20	–	–	–	–	–
	13	50.0	354.27	308	355	337[c])	309[g]), 281, 265, 263	237, 224, 206, 166, 127
	meso-**6**	52.0	428.39	212, 258 (sh)	429	411[c])	379[d]), 322, 261, 248	248, 233, 204, 177,
	d,l-**6**	61.0		212, 256 (sh)				148, 132, 120, 92
Acetone	**9a**	17.6	227.21	228, 268	228	210[c]), 186, 168, 154	182[g]), 168[e]), 154, 150, 140, 125	151, 150, 140, 125
	9b	12.2	213.19	214, 262	214	197[h]), 196[c]), 169, 154[e]), 127	109, 93, 85	–
EtCOMe	**10a**	23.0	241.24	220, 268	242	224[c]), 186, 168	196[g]), 180, 168, 164, 139	164, 139, 111, 70
	10b	18.3	227.21	218, 262	228	211[h]), 210[c]), 193, 183, 167, 154, 127	109, 99, 67	–

[a]) $[M+H-MeOH]^+$; the corresponding ions which were further fragmented in the MS^n mode are underlined. [b]) $[M+H-MeOH-CO_2]^+$. [c]) $[M+H-H_2O]^+$. [d]) $[M+H-H_2O-MeOH]^+$. [e]) $[M+H-MeOH-CO]^+$. [f]) $[M+H-2\,CO-NMe]^+$. [g]) $[M+H-H_2O-CO]^+$. [h]) $[M+H-OH]^+$.

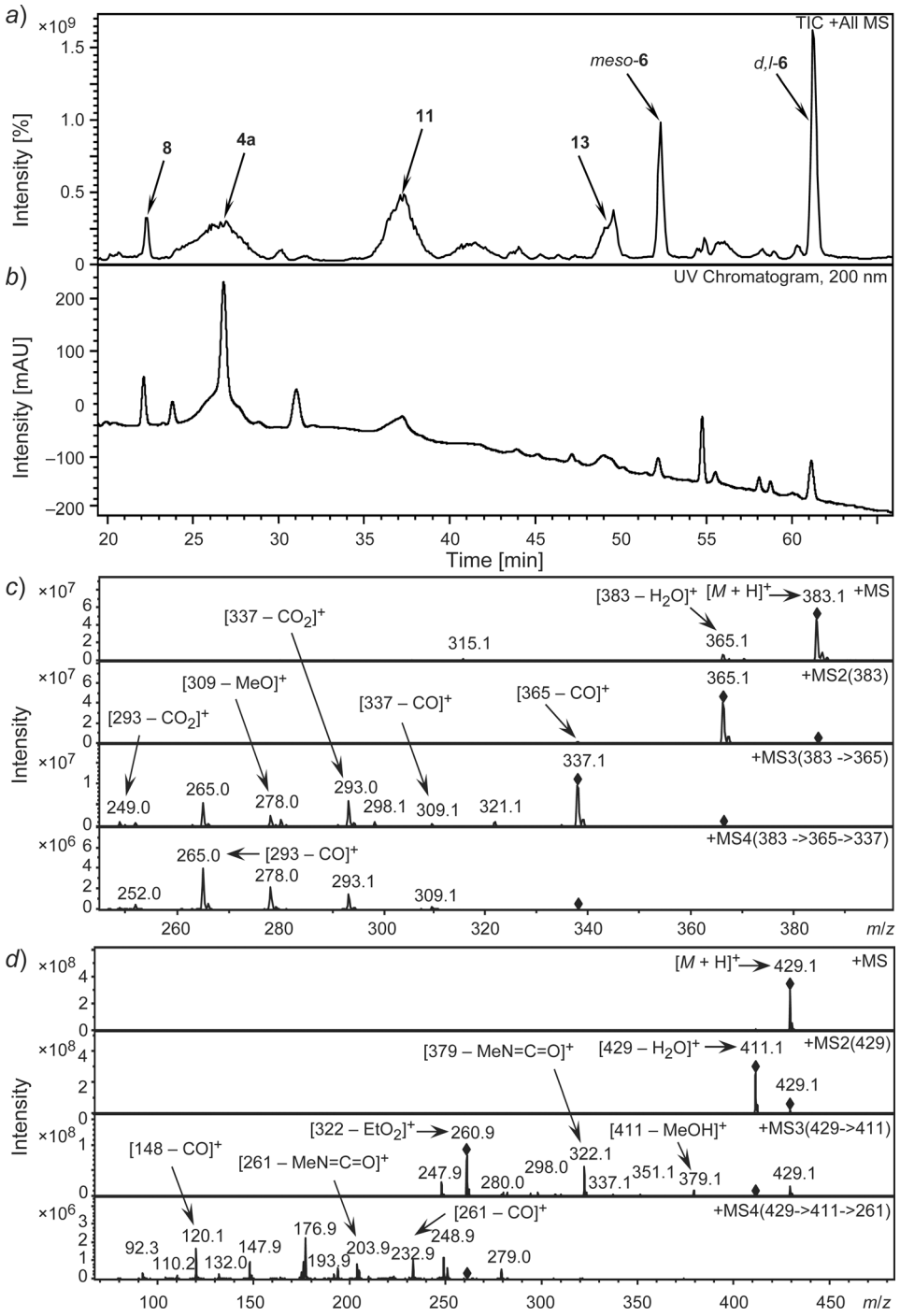
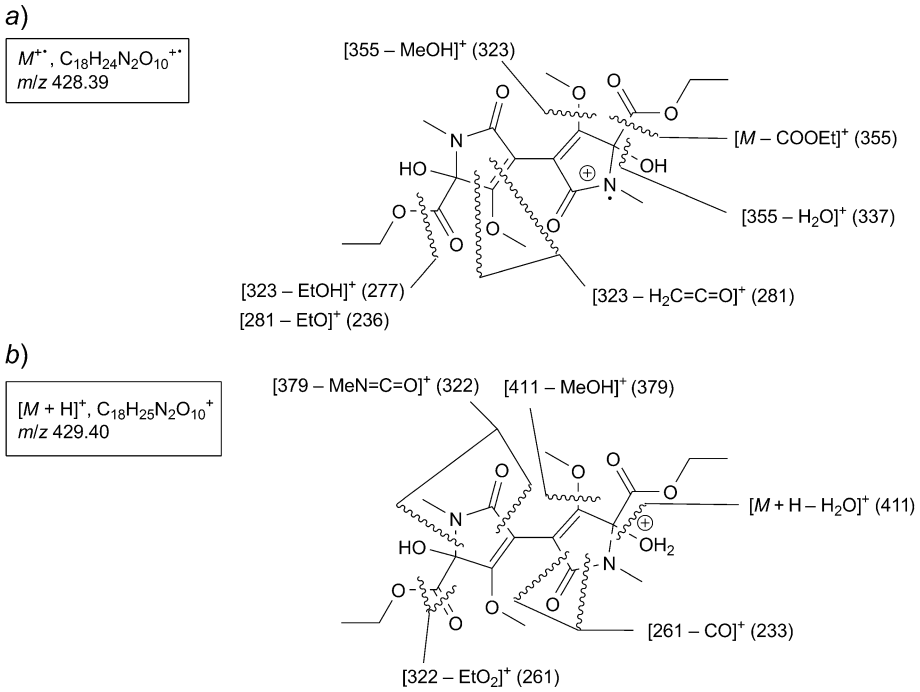


Fig. 2. *HPLC(DAD)/MS Sections of an AcOEt fraction obtained from an EtOH/H_2O extract of* M. perennis *roots. a*) Total ion chromatogram (TIC) recorded in the positive-ion mode (ESI^+). *b*) UV Chromatogram recorded at 200 nm. *c*) MS^n data of compound **11** ($C_{16}H_{18}N_2O_6$; M_r 382.32). *d*) MS^n data of compound *d,l*-**6** ($C_{18}H_{24}N_2O_{10}$; M_r 428.39).

Scheme 3. *Proposed MS Fragmentation of d,l-/meso-***6**. *a*) Electron-impact mode (EI-MS; 70 eV). *b*) Positive-ionization mode (ESI^+).



(*Fig. 2*,*c*) upon CID, the 2,2′-dioxo-3,3′-bipyrrole-dicarboxylate **6** displayed [411 – MeOH]$^+$ and [379 – MeN=C=O]$^+$ fragment-ion peaks (*Fig. 2*,*d*, and *Scheme 3*,*b*). The latter neutral loss of MeN=C=O (57 Da; see *Fig. 2*,*d*) has been formerly reported for the fragmentation of *N*-Me carbamates, analyzed in the ESI^+ mode [27–29], and appears to be a key step in the fragmentation of bipyrrole-dicarboxylates. Hence, by applying a combination of GC/MS and LC/MS techniques, it was possible to obtain a clear picture of the respective chemical structures.

Formation of Aldol Condensation Products upon Extraction with Aqueous Ketones. Besides alcohols also ketones are occasionally used for the extraction of medicinal plants, even though ketones are capable of artefact formation [2][4]. The extraction of *M. perennis* roots with a Me_2CO/H_2O mixture yielded an extract which was further purified by AcOEt extraction as mentioned before. While TLC analysis (SiO_2; AcOEt/hexane 5:1) of the latter gave an orange spot with anisaldehyde/H_2SO_4 spraying reagent, GC/MS chromatograms exhibited an intense peak at t_R 26.7 min (*Fig. 1*,*c*). MS Experiments revealed an M^+ ion peak at *m*/*z* 227, and peaks of [*M* – CO]$^+$ and [*M* – CO – NMe]$^+$ fragment ions (*Table 1*). A pure crystalline compound was isolated from the crude AcOEt extract *via* preparative TLC (yield, 0.1% of the plant material). By comparison of its ^{1}H- and ^{13}C-NMR, UV/VIS, and MS data, as well as melting point, with literature data, the known pyridine-2,6-(1*H*,3*H*)-dione alkaloid speranskatine A (**9a**) was identified. Compound (+)-(*R*)-**9a** has previously been isolated from the Asian

plant *Speranskia tuberculata* (Euphorbiaceae) [30][31]. Recently, a stereoselective synthesis of (+)-(*R*)-**9a** from **3** and Me_2CO in the presence of the chiral amine *l*-leucinol has been achieved [32]. However, in the present investigation no optical rotation ($[\alpha]_D^{22} = \pm 0.00$; $c = 0.25$, MeOH) could be observed. Consequently, the isolated compound was a racemate (*rac*-**9a**). Surprisingly, aldol condensation products of the dimer **4a** were not detected.

The formation of **9a** by condensation of Me_2CO with **1** or **3** *via* an aldol-type oxidative addition reaction appears plausible. An aldol reaction is generally defined as the nucleophilic addition of a carbonyl compound, in form of its enol or enolate, to another C=O compound acting as an electrophile; the initial enolization is promoted by an acid or a base. However, aldol condensations proceed also in neutral media, *e.g.*, under phase-transfer conditions [33], catalyzed by amino acids in salt-buffered solutions [34], enzymatically [35], or spontaneously, such as the condensation of Me_2CO with the alkaloid berberine [2]. Hence, when **1** was stirred under N_2 in Me_2CO/H_2O (phosphate-buffered saline (PBS); pH 7.4) for 24 h, the GC/MS analysis revealed a complete conversion to **9a** (data not shown). However, intermediate formation of **3** from **1** cannot be ruled out, since AcOEt extraction and further sample preparation was performed in the presence of O_2.

Interestingly, low amounts of **9a** were likewise detected in AcOEt fractions of fermented aqueous extracts of *M. perennis* [15] when investigated by GC/MS. This example also demonstrates that **9a** is readily formed from **1** or **3** in the presence of Me_2CO, with the latter being presumably produced as a microbial side fermentation product (data not shown).

Moreover, when the extraction of *M. perennis* was performed with ethyl methyl ketone (EtCOMe)/H_2O, and subsequent partitioning was conducted with AcOEt, an indigo-blue spot on the TLC of this extract was observed, when sprayed with anisaldehyde/H_2SO_4. Chromatographic purification of the crude extract yielded a yellowish semicrystalline compound (0.15% of the plant material). EI-MS Fragmentation (GC/MS; *Fig. 1*,*d*, and *Scheme 4*,*a*) of the compound was similar to that of **9a**, but the M^+ ion peak indicated elongation by a CH_2 unit (*m*/*z* 241). Fragmentation of the unknown compound in the EI-MS mode exhibited the formation of $[M - Et]^+$, $[M - Et - H_2O]^+$, and $[M - Et - CO]^+$ ions (*Table 1* and *Scheme 4*,*a*). In contrast, collision-induced dissociation (CID) in LC/MS experiments showed the predominance of the following fragments: $[M + H - H_2O]^+$, $[M + H - H_2O - CO]^+$, $[M + H - H_2O - CO - MeOH]^+$ (*Table 3* and *Scheme 4*,*b*). Moreover, in ESI^+-MS experiments the release of the MeN=C=O fragment (57 Da) was observed, similar to the fragmentation of bipyrrole-dicarboxylates **5** and **6** (*Scheme 3*,*b*). Based on 1D- and 2D-NMR experiments, *i.e.*, ^1H- and ^{13}C-NMR, gHSQC, gHMBC (see *Table 4*), the novel compound was identified as the CH_2 homolog, *rac*-**10**, of speranskatine A (*rac*-**9a**).

Interestingly, GC/MS and LC/MS investigations revealed also *N*-demethylated side-products of **9a** and **10a**, the piperidine-2,3-diones **9b** and **10b**, respectively (*Fig. 1*,*c* and *d*, and *Tables 1* and *3*). *Fig. 3*,*a*, displays the fragmentation pattern of **10b**, obtained by LC/MS^n. The peak at *m*/*z* 210 indicated an initial loss of a H_2O moiety $[M + H - H_2O]^+$ from the *pseudo*-molecular ion $[M + H]^+$. However, in the MS^2 experiment, **10b** exhibited fragment-ion peaks at *m*/*z* 210 and 211, evidencing both a homolytic, as well as a heterolytic cleavage of the C(3)–O bond. Thus, upon CID [36], $[M - OH]^{+\bullet}$ and

Scheme 4. *Proposed MS Fragmentation of* **10a**. *a*) Electron-impact mode (EI-MS; 70 eV). *b*) Positive-ionization mode (ESI^+).

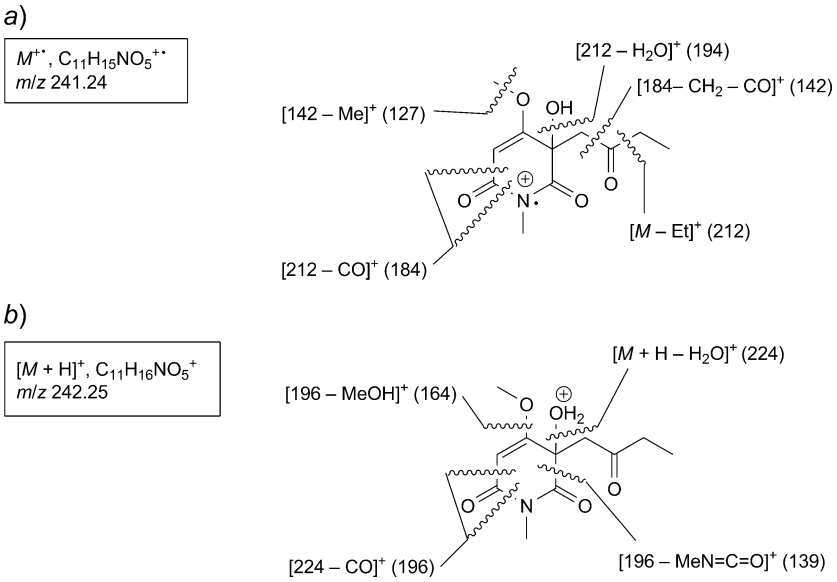
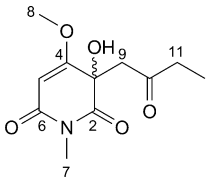


Table 4. *^{1}H- and ^{13}C-NMR Data of the Novel Compound* rac-**10a**[a]). In (D_6)DMSO, δ in ppm, J in Hz.



10a

Position	δ(H)	δ(C)	HMBC
2	–	172.38	
3	–	69.22	
4	–	169.87	
5	5.47 (*s*)	93.53	C(3), C(4)
6	–	164.80	–
7	3.05 (*s*)	25.97	C(2), C(6)
8	3.68 (*s*)	56.70	C(4)
9	3.35 (*d*, J = 17.4, H_a), 3.24 (*d*, J = 17.4, H_b)	48.16	C(2), C(3), C(4), C(10)
10	–	208.49	–
11	2.43 (*dq*, J = 7.3, 17.9, H_a), 2.36 (*dq*, J = 7.2, 17.9, H_b)	34.94	C(10), C(12)
12	0.84 (*t*, J = 7.3)	7.30	C(11), C(10)
OH	6.64 (*s*)	–	C(9)

[a]) Arbitrary atom numbering as shown below.

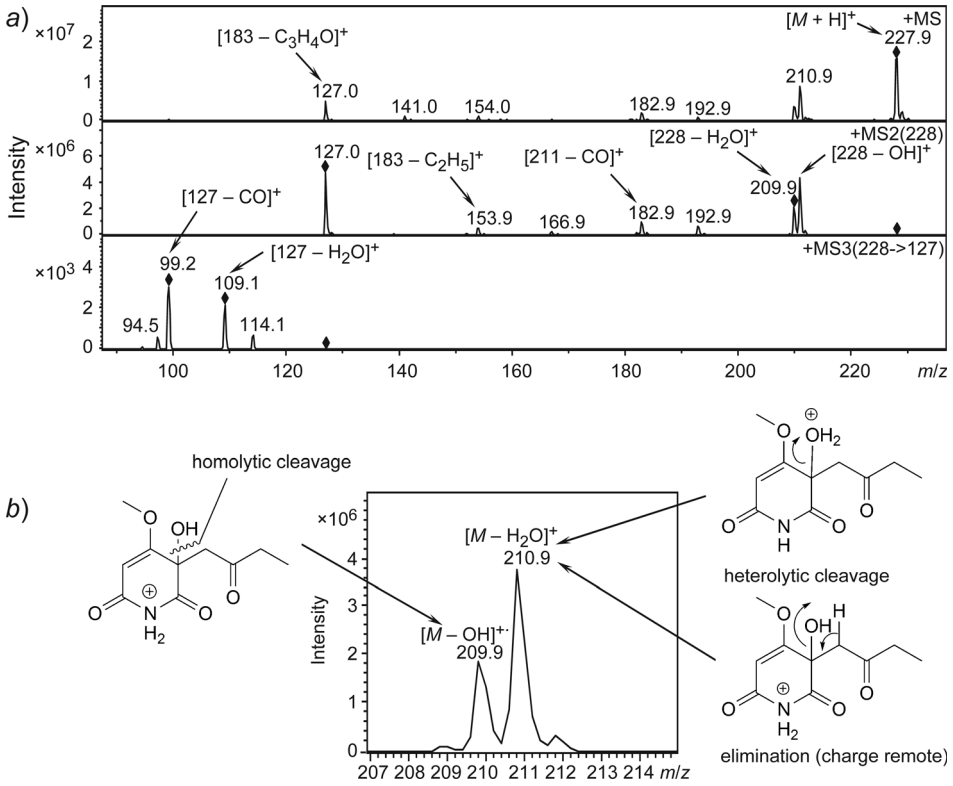


Fig. 3. a) *MS, MS², and MS³ data of* **10b** *as obtained in the ESI⁺ mode.* b) *Section of MS² data showing fragment ions at* m/z *210 and 211, suggesting homo- and heterolytic dissociation of HO• and/or H_2O from the* pseudo-*molecular ion* ($[M+H]^+$).

$[M - H_2O]^+$ ions are produced (*Fig. 3,b*), depending on the localization of the positive charge. Moreover, a neutral loss of a H_2O moiety appears possible. Furthermore, fragment ions such as CO^+, Et^+, and $C_3H_4O^+$ confirm the proposed structure **10b**.

Conclusions. – The results of the present study have revealed the high sensitivity of *M. perennis* constituents towards extraction solvents. Hermidin (**1**) and its oxidation products **3** and **4a** react with aqueous alcohols to yield mono- and bipyrrole-carboxylates *via* benzilic acid rearrangement and esterification. Furthermore, the extraction of the plant material with aqueous ketones was shown to yield highly selective aldol condensation products *via* oxidative condensation. The spontaneous formation of these products under neutral conditions is unusual, since such reactions are generally proceed under strongly basic or acidic conditions. The current investigations form a basis for the development of standardized *Mercurialis* extracts with defined compound signatures. This approach of pinpointing artefact chemistry may be applied to other plant secondary metabolites. In this way, it appears worthwhile to study extraction chemistry by using specific solvents to obtain tailor-made extracts.

Experimental Part

General. Fluorescence indicator green, 254 nm, for chromatographic purifications with a *Chromatotron®*, was purchased from *Sigma–Aldrich* (Saint Louis, MO, USA). *4-Methoxy-1-methylpyridine-2,6(1*H,*3*H*)-dione* (**14**; m.p. 111–112°) was synthesized as described in [7]. *3-(Hydroxyimino)-4-methoxy-1-methylpyridine-2,6-(1*H,*3*H*)-dione* (**15**; m.p. 195–196°) and *hermidin* (**1**) were obtained as described by *Swan* [7][14].

GC/MS Analyses. GC/MS Analyses were performed with a *PerkinElmer Clarus 500* gas chromatograph by 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, USA). Carrier gas, He at a flow rate of 1 ml/min. The injector used was a PSS (programmed-temp. split/splitless injector; temp., 250°). The temp. program for the column oven was 100 to 320° with a linear gradient of 4°/min and a final hold time of 30 min. The mass spectrometer was run in electron ionization (EI) mode (70 eV).

RP-HPLC-ESI-MS^n Analyses. Chromatographic analyses were carried out on an *Agilent 1200* HPLC system (*Agilent Technologies Inc.*, Palo Alto, USA), equipped with a binary pump, a micro-vacuum degasser, an autosampler, a thermostatic column compartment, and a UV/VIS diode array detector. A *Sunfire® C18*-reversed phase (RP) column (5 µm particle size, 150 × 2.1 mm i.d.; *Waters Corporation*, Milford, MA, USA) was used for chromatographic separation at 25° and a flow rate of 0.21 ml/min. The UV detection of heterocyclic compounds (*Fig. 2*,*a* and *Table 3*) was performed at 200 nm. The mobile phase consisted of HCOOH/H_2O 0.2 : 99.8 (mobile phase *A*) and MeCN (100%; mobile phase *B*). Starting with 0% *B* for 10 min, a linear gradient was followed to 10% *B* at 10 min, then increasing to 23% *B* at 60 min, further increasing to 100% *B* at 65 min, continuing for 5 min, before re-equilibration to starting conditions. The injection volume of each sample was 10 µl. The LC system was coupled to an *HCTultra* ion trap (*Bruker Daltonik GmbH*, D-Bremen) with an ESI source operating in the positive-ionization mode by applying the following parameters: HV voltage, −4000 V; dry gas, N_2; 9.00 l/min with a dry gas temp. of 365°; nebulizer, 35 psi. Full-scan mass spectra (mass range, *m*/*z* 50–2000) of the HPLC eluates were recorded during chromatographic separation to yield $[M+H]^+$ ions. To obtain further structural information, CID experiments were performed. MS^n data were acquired in the auto MS/MS mode. The instruments were controlled by *Agilent* Chemstation and EsquireControl Software (V6.1).

NMR Spectroscopy. NMR Spectra were recorded in (D_6)DMSO or $CDCl_3$ at 500 (^{1}H) and 125 MHz (^{13}C), resp., with a *Varian Unity Inova* NMR spectrometer (D-Darmstadt); δ in ppm rel. to residual solvent signals of (D_6)DMSO (^{1}H: δ(H) 2.50; ^{13}C: δ(C) 39.51) and $CHCl_3$ (^{1}H: δ(H) 7.27; ^{13}C: δ(H) 77.00) as internal standard; *J* in Hz. ^{13}C-NMR Signal assignments of the novel compounds **8**, **10a**, and **11** were based on 2D-heteronuclear NMR experiments (gHMBC and gHSQC). For evaluation of NMR spectra, the program SpinWorks 3.1.7. (Copyright® 2010, *K. Marat*, University of Manitoba, USA) was used.

Plant Material. Extraction (*General Procedure* (*GP*)) *and Sampling.* Root parts of *M. perennis* were collected in September 2008 and 2013 in the forest close to Bad Boll/Eckwaelden (Germany), cleaned by rinsing with tap H_2O, and stored at −80° until investigation. Voucher specimens of *M. perennis* were deposited with the herbarium of the Department of Botany, Hohenheim University (Germany), and the plant material was identified by Prof. *O. Spring* (voucher Nos. HOH-011281, HOH-011282, and HOH-011283). For extraction, root parts of *M. perennis* (60.0 g) were immersed in an organic solvent/H_2O mixture (600 ml) and bubbled with N_2 (15 min). Subsequently, the plant material was minced for 3 min using an *Ultra-turrax®* (15.000 rpm; *IKA-Werke GmbH & Co. KG*, D-Staufen), and the slurry was allowed to stand for 24 h at 4°. The sediment was recovered by vacuum suction over *Celite*, and the filter cake was re-extracted in the same manner. The org. solvent was removed from the combined filtrates by vacuum roto-evaporation, and the remaining aq. layer was saturated with NaCl and extracted with AcOEt (200 ml and 2 × 100 ml). After drying (Na_2SO_4), the solvent was evaporated *in vacuo* to yield a crude extract. For GC/MS analysis, the crude extract was re-dissolved in AcOEt (10 ml), and 1 µl injected into the GC/MS. For LC/MS investigations, samples (20 mg each) were dissolved in MeCN/H_2O 1 : 9 and centrifuged for 5 min (14.000 rpm) before injection. The reaction products of **1** were isolated from the crude extracts prior to compound characterization (see below).

Isolation of rac-*Speranskatine A* (= *3-Hydroxy-4-methoxy-1-methyl-3-(2-oxopropyl)pyridine-2,6(1*H,*3*H*)-dione*; **9a**). Compound **9a** was isolated from the crude AcOEt extract (0.16 g), obtained by Me_2CO/H_2O extraction of the roots (see *GP*) using a *Chromatotron*® (2-mm layer, SiO_2/gypsum/fluorescence indicator 254 nm 45 : 18 : 1.2 (*w*/*w*/*w*); preconditioned with hexane). Elution of **9a** was performed with hexane/AcOEt 100 : 0 to 80 : 20, and the corresponding compound bands were monitored by fluorescence extinction (λ_{max} 254 nm). The fraction containing **9a** was evaporated to dryness using a vacuum rotoevaporator. Yield: 0.062 g (0.1% of the plant material). Colorless crystals. TLC (SiO_2; AcOEt/hexane 5 : 1): R_f 0.22; GC/MS purity: > 99% at t_R 26.6 min. The m.p. (158 – 160°), UV/VIS, and MS data were in agreement with those reported in [30]. The NMR data of **9a** (recorded in (D_6)DMSO); corresponded to those recorded in $CDCl_3$ [30]. $[\alpha]_D^{22} = \pm 0.00$ ($c = 0.25$, MeOH). ^{1}H-NMR ((D_6)DMSO, 500 MHz): 6.63 (br., OH); 5.47 (*s*, H–C(5)); 3.03 (*s*, H–C(7)); 3.68 (*s*, H–C(8); 3.36 (*d*, $J = 17.5$, H_a–C(9); 3.25 (*d*, $J = 17.5$, H_b–C(9)); 2.05 (*s*, H–C(11)). ^{13}C-NMR ((D_6)DMSO, 125 MHz): 206.04 (C(10)); 172.34 (C(2)); 169.88 (C(4)); 164.80 (C(6)); 93.51 (C(5)); 69.13 (C(3)); 56.71 (C(8)); 49.30 (C(9)); 29.68 (C(11)); 25.95 (C(7)).

Detection of **9a** *in Fermented Aq. Extracts.* Fermented aq. extracts of *M. perennis* were obtained according to an official procedure (German Homoeopathic Pharmacopoeia (GHP), 2008) [37] described in [15]. Samples of three batches were taken after 2 years of storage. Aliquots of 4 ml were extracted with AcOEt (2 × 10 ml), dried (Na_2SO_4), and the solvent was removed by vacuum rotoevaporation. The residues were re-dissolved in AcOEt (1 ml) and analyzed by GC/MS. Isolated **9a** (1 mg/10 ml AcOEt) was used as a reference.

Isolation of rac-*3-Hydroxy-4-methoxy-1-methyl-3-(2-oxobutyl)pyridine-2,6(1*H,*3*H*)-dione* (**10a**). After extraction of *M. perennis* roots with $EtCOMe/H_2O$ and partitioning of the corresponding H_2O phase with AcOEt (see *GP*), the obtained extract (0.37 g) was subjected to a *Chromatotron*® separation (for conditions, see above) to afford **10a**. Yield: 0.093 g (0.15% of the plant material). Yellowish semicrystalline solid; purity: > 95% (GC/MS). For NMR characterization, the material was further purified *via* a second *Chromatotron*® separation. TLC (SiO_2; AcOEt/hexane 5 : 1): R_f 0.28. UV/VIS (MeCN): 219 (4.12), 260 (3.73). GC/MS (70 eV) purity: 98% (at t_R 29.0 min). For ^{1}H- and ^{13}C-NMR: see *Table 4*.

Hermidin Quinone (= *4-Methoxy-1-methylpyridine-2,3,6(1*H*)-trione*; **3**). Compound **15** (1.00 g, 5.43 mmol) was dissolved in a mixture of $SnCl_2 \cdot 2\,H_2O$ (2.00 g, 8.86 mmol) and HCl (37% (*w*/*w*), 100 ml). After stirring (22 h), H_2O (100 ml) was added, the mixture was neutralized (pH 7.0) by addition of NaOH (16% in H_2O (*w*/*w*)), and extracted with $CHCl_3$ (3 × 100 ml). After drying (Na_2SO_4), the solvent was evaporated *in vacuo* to yield **3** (0.23 g; GC/MS purity: 87% at t_R 20.3 min). Yield: 22% of the theory. A *Chromatotron*® purification yielded **3** in higher purity (> 97%). UV/VIS (MeCN): 274 (3.96), 326 (3.16). MS (GC/MS, 70 eV; at t_R 20.2 min): data in agreement with those reported in [7].

rac-*Methyl 2,5-Dihydro-2-hydroxy-3-methoxy-1-methyl-5-oxo-1*H-*pyrrole-2-carboxylate* (**7**). Compound **3** (0.23 g; GC/MS purity: 87%; 1.183 mmol) was dissolved in MeOH (65 ml), and MeONa (3.2 ml; 30% in MeOH (*w*/*w*)) was added under N_2 to yield a blue soln. After stirring (19 h), the reaction was quenched by adding sat. NH_4Cl/H_2O soln. (25% (*w*/*w*); 240 g), and the mixture was extracted with $CHCl_3$ (3 × 100 ml). Subsequently, the $CHCl_3$ extract was dried (Na_2SO_4), and the solvent was evaporated *in vacuo*. Compound **7** was isolated from the crude residue (0.185 g) by applying a *Chromatotron*® separation (2-mm layer; SiO_2/gypsum/fluorescence indicator 254 nm 45 : 18 : 1.2 (*w*/*w*/*w*); preconditioned with hexane). Elution of **7** was performed with hexane/AcOEt 70 : 30 to 0 : 100. Two fractions containing **7** (at 100% AcOEt) were separated, and the solvent was removed *in vacuo* to yield white crystals (0.084 and 0.046 g; GC/MS (at t_R 20.2 min) purity: 86 and > 97%, resp.; total yield: 49% of the theory). M.p. 132 – 133°. TLC (SiO_2; $CHCl_3$/MeOH 4 : 1): R_f 0.59. UV/VIS (MeCN): 207 (4.10), 250 (sh). The MS, ^{1}H- and ^{13}C-NMR data were in agreement with those reported in [17].

rac-*Ethyl 2-Hydroxy-3-methoxy-1-methyl-5-oxo-2,5-dihydro-1*H-*pyrrole-2-carboxylate* (**8**). Synthesis and purification of **8** were performed as described for **7**, by treatment of **3** (0.14 g; GC/MS purity: 85%, 7.036 mmol) with EtOH (100 vol-%, 50 ml) and EtONa (1.0 ml, 20% in EtOH (*w*/*w*) under N_2. After stirring (23 h), **8** was isolated from the mixture as described above. By *Chromatotron*® purification (for conditions, see above), three fractions (46, 18, and 20 mg) were obtained from the crude extract (0.165 g); GC/MS purity (at t_R 21.5 min): 91, 98 and 94%, resp. Overall yield: 49% of the theory. TLC

(SiO_2; $CHCl_3$/MeOH 4:1); R_f 0.61. UV/VIS (MeCN): 207 (4.14), 250 (sh). ^{1}H-NMR ($CDCl_3$, 500 MHz): 5.08 (*s*, H–C(3)); 4.60 (*s*, OH); 4.33 (*dq*, *J* = 7.1, 10.7, H_a–C(9); 4.27 (*dq*, *J* = 7.1, 10.7, H_b–C(9)); 3.81 (*s*, H–C(7)); 2.73 (*s*, H–C(6)); 1.27 (*t*, *J* = 7.1, H–C(10)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 171.80 (C(8)); 170.59 (C(2)); 168.97 (C(4)); 94.11 (C(3)); 87.09 (C(5)); 63.83 (C(9)); 58.71 (C(7)); 23.46 (C(6)); 14.04 (C(10)). MS: see *Tables 1* and *3*.

Mixture of Chrysohermidin (*=4,4′-Dimethoxy-1,1′-dimethyl-3,3′-bipyridine-2,2′,5,5′,6,6′(1*H*,1′*H*)-hexone*; **4a**) *and* **3**. Compound **14** (1.00 g, 6.45 mmol) was dissolved in $CHCl_3$ (50 ml) containing 1 drop of HCl (37% (*w*/*w*)). Subsequently, SeO_2 (1.20 g, 10.82 mmol) was added, and the mixture was stirred 24 h at r.t. Then, precipitated Se was filtered off by vacuum suction over *Celite* and washed with $CHCl_3$ (3 × 25 ml). The solvent was removed *in vacuo* to yield a crude product (2.71 g) containing high amounts of Se. Purification was achieved by vacuum liquid chromatography (VLC) on silica *60 G* (60 g), preconditioned with hexane. Elution was performed with hexane/AcOEt 100:0 to 20:80. The corresponding fractions containing **4a** (analyzed by GC/MS) were combined, and the solvent was distilled off *in vacuo* to yield an orange residue (0.21 g). GC/MS revealed a **3**/**4a** ratio of 54/46% (*w*/*w*; corrected by M_r; at t_R 20.2 and 43.3 min, resp.). This mixture was used without further purification.

Isochrysohermidin (*=d,l-* and meso-*Dimethyl 2,2′,5,5′-Tetrahydro-5,5′-dihydroxy-4,4′-dimethoxy-1,1′-dimethyl-2,2′-dioxo-1*H,*1′*H*-3,3′-bipyrrole-5,5′-dicarboxylate*; *d,l*-**5** and *meso*-**5**, resp.). Compound **5** was synthesized as described by *Abe et al.* in [22]. In brief, **3**/**4a** (0.25 g; see above) was dissolved in MeOH (75 ml) and treated under N_2 with a MeONa soln. (3.6 ml; 30% in MeOH (*w*/*w*)). After stirring (19 h), the reaction was quenched with NH_4Cl soln. (240 g, 25% in H_2O (*w*/*w*)), and the mixture was extracted with $CHCl_3$ (3 × 100 ml). Then, the extract was dried (Na_2SO_4), and the solvent was evaporated *in vacuo* to yield a crude product (0.21 g). The mixture *d,l*-**5**/*meso*-**5** 1.2:1.0 (*w*/*w*) and small amounts of **7** were isolated from this crude material by using *Chromatotron*® (2 mm layer; SiO_2/gypsum/fluorescence indicator 254 nm 45:18:1.2 (*w*/*w*/*w*); preconditioned with hexane). The elution of **7** was performed with hexane/AcOEt 100:0 to 0:100. Yield: 0.045 g (GC/MS purity: 96% (at t_R 20.2 min); 2% of the theory. Subsequently, *d,l*-**5** and *meso*-**5** were eluted with $CHCl_3$/MeOH 90:30. Yield: 0.108 g (2.5% of the theory; calc. on **14**); GC/MS purity: 94% (at t_R 43.8 and 44.5 min, resp.). Further purification of **5** was achieved by applying a second *Chromatotron*®. The MS, and ^{1}H- and ^{13}C-NMR data of *d,l*-**5** and *meso*-**5** were in agreement with those in [17].

d,l- and meso-*Diethyl 2,2′,5,5′-Tetrahydro-5,5′-dihydroxy-4,4′-dimethoxy-1,1′-dimethyl-2,2′-dioxo-1*H,*1′*H*-3,3′-bipyrrole-5,5′-dicarboxylate* (*d,l*-**6** and *meso*-**6**, resp.). Compound **6** was synthesized according to a modified procedure [23]. In brief, the mixture **3**/**4a** (0.21 g; see above) was dissolved in EtOH (50 ml, 96 vol-%) under N_2, treated with Et_3N (1 ml), and stirred 20 h at r.t. The reaction was quenched with a NH_4Cl/H_2O soln. (see above), and the mixture was extracted with $CHCl_3$ (3 × 100 ml). After drying (Na_2SO_4), the solvent was distilled off *in vacuo* to yield a black residue (0.336 g). Purification was performed by using *Chromatotron*® (2-mm layer; SiO_2/gypsum/fluorescence indicator 254 nm 45:18:1.2 (*w*/*w*/*w*); preconditioned with $CHCl_3$). Elution of **6** was performed with $CHCl_3$/MeCN 100:0 to 60:40. A fraction at a $CHCl_3$/MeCN ratio of 60:40 yielded *d,l*-**6**/*meso*-**6** as 1:1 (white solid, 0.059 g; yield: 2.1% calculated on **14**; GC/MS purity: > 99%, at t_R and 44.3 and 46.0 min, resp.). The ^{1}H-NMR data of the product were in accordance with those reported in [23]. ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.77 (C(8,8′), *d,l*-**6**/*meso*-**6**, overlapped); 169.65 (C(2,2′), *d,l*-**6**/*meso*-**6**, ov); 166.63 (C(4,4′), *d,l*-**6**/*meso*-**6**, ov); 97.11 (C(3,3′), *d,l*-**6**); 95.96 (C(3,3′), *meso*-**6**); 87.46 (C(5,5′), *d,l*-**6**); 86.86 (C(5,5′), *meso*-**6**); 62.62 (C(9,9′), *d,l*-**6**/*meso*-**6**, ov); 59.08 (C(7,7′), *meso*-**6**); 58.87 (C(7,7′), *d,l*-**6**); 24.58 (C(6,6′), *d,l*-**6**); 24.15 (C(6,6′), *meso*-**6**); 14.09 (C(10,10′), *d,l*-**6**); 14.07 (C(10,10′), *meso*-**6**).

*Ethyl 2-Hydroxy-3-methoxy-4-(4-methoxy-1-methyl-2,5,6-trioxo-1,2,5,6-tetrahydropyridin-3-yl)-1-methyl-5-oxo-2,5-dihydro-1*H*-pyrrole-2-carboxylate* (**11**). The mixture **3**/**4a** (0.30 g) was dissolved in EtOH (100 ml, 96 vol-%) and treated under N_2 with Et_3N (4 drops). After stirring (6 h), the reaction was quenched by addition of HCl (37% (*w*/*w*); 5 drops), and EtOH was removed by vacuum rotoevaporation. Subsequently, the residue was dissolved in AcOEt (50 ml), ammonium salts were filtered off by vacuum suction, washed with AcOEt (50 ml), and the solvent was removed again *in vacuo*. The novel compound **11** (0.017 g) was isolated from the crude (0.35 g) as a yellow solid by *Chromatotron*® separation (for conditions, see above); GC/MS purity: > 95%, at t_R 45.3 min. NMR Data: see *Table 2*.

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