Isolation of the Pyrrolizidine Alkaloid Intermedine-*N*-oxide from *Cerinthe glabra* and *ab initio* Calculation of its ^{13}C NMR Shifts

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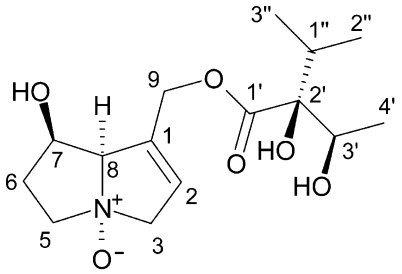
The pyrrolizidine alkaloid intermedine-*N*-oxide was isolated from *Cerinthe glabra* for the first time. Due to the questionable assignments of NMR signals of this compound in previous literature reports *ab initio* calculations of the ^{13}C NMR values based on the GIAO approach, using HF and B3LYP levels of theory and the four basis sets 6-31G(d), 6-311G(d), 6-31G(d,p), and 6-311G(d,p) were performed. The assignments obtained this way are in very good accordance with the experimental values.

Key words: Intermedine-*N*-oxide, *Cerinthe glabra*, Pyrrolizidine Alkaloids, NMR Data, *ab initio* Calculations

Introduction

Pyrrolizidine alkaloids (PA) comprise of a bicyclic heterocyclic core with a 1,2-double bond, which is functionalized by single esterification with a branched aliphatic carboxylic acid (necic acids) at the hydroxymethyl side chain at C-1, or by double esterfication at this position as well as of the hydroxy group at C-7. The latter group can occur as open-chain diesters or as macrocyclic 11- or 12-membered lactones [1, 2]. These alkaloids are mainly found in the plant families Asteraceae (Compositae), Boraginaceae and Leguminaceae (Fabaceae); the genera in which they were found frequently before are *Senecio*, *Heliotropium*, *Echium*, *Trichodesma*, and *Symphytum*. Meanwhile, more than 350 PA have been isolated from various plants [3]. Typically, the PA are accompanied by the corresponding, equally toxic *N*-oxides in plants, and frequently, the *N*-oxides predominate [4 – 7]. Pyrrolizidine alkaloids are highly toxic to humans and animals. Acute poisoning by PA causes severe damage to the liver, long-term exposition may cause cancer. Intoxications of humans may be caused by toxic grain, honey contaminated by the nectar from toxic plants, or by using medical plants that contain the alkaloids, *e.g. Symphytum* (comfrey) [4]. Consequently, the European Food Safety Authority (EFSA) has expressed the need for establishing sophisticated analytical methods for the determination of PA in plant materials [8]. Analytical methods for the determination of PA in plants have been reviewed by Röder [9] and updated by Crews [4]. Interestingly, some insects can accumulate pyrrolizidines from plants and use them directly, or after chemical modifications, as defense chemicals or as precursors of pheromones [4, 6]. The chemical ecology of pyrrolizidine alkaloids has been reviewed by Hartmann [1].

The identification of PA in plant material is generally performed by chromatographic methods like GC and HPLC, typically in combination with MS detection. But these techniques suffer from some shortcomings: Since PA *N*-oxides are quite unstable, they decompose under GC conditions, consequently GC analysis can only be performed on the free PA, obtained by reduction of the native *N*-oxides with zinc/sulfuric acid [10] or with new, milder reagents like sodium hydrosulfite [11] and indigocarmine-based redox resins [5, 12]. Further, isomeric PA like the alkaloids intermedine, lycopsamine, echinatine, rinderine, and indicine (and likewise the corresponding *N*-



1 (7*R*, 2'*S*, 3'*R*)

lycopsamine-*N*-oxide (**2**): (7*R*, 2'*S*, 3'*S*)
echinatine-*N*-oxide (**3**): (7*S*, 2'*S*, 3'*S*)
rinderine-*N*-oxide (**4**): (7*S*, 2'*S*, 3'*R*)
indicine-*N*-oxide (**5**): (7*R*, 2'*R*, 3'*S*)

Fig. 1. Structure of intermedine-*N*-oxide (**1**; relevant absolute configurations: 7*R*, 2′*S*, 3′*R*) and differing absolute configurations of isomeric pyrrolizidine alkaloids.

oxides) which have the same molecular masses, and differ only in the configurations at C-7, C-2′ and C-3′ (Fig. 1), show almost identical fragmentation patterns, and thus are indistinguishable solely on the basis of MS data [6, 10, 13]. Consequently, despite a large number of reports on the identification of PA and in an even higher gear PA *N*-oxides exclusively by GC-MS or HPLC-MS, a considerable part of structure correlations has been made only indirectly; even in a very recent publication on PA, Williams *et al.* [5] concede that "all other identifications remain tentative until studies result in sufficient isolation to allow for unambiguous NMR spectroscopy analysis."

In this communication we report on the isolation and identification of the PA, intermedine *N*-oxide (**1**; Fig. 1) from *Cerinthe glabra* Miller (Boraginaceae), a plant known as "smooth honeywort" and (in German) "Alpenwachsblume". To the best of our knowledge no detailed phytochemical analysis of this plant has been published yet, except an investigation of the fatty acid pattern of the oil obtained from the seeds [14]. Other *Cerinthe* species have been shown to contain flavonoids [15], and in 1990 Röder [16] reported on the first isolation and full spectroscopic characterisation of a PA, intermedine, from *Cerinthe minor*. Later in 2004, Mrozcek [17] identified three other PA from this plant based on HPLC-MS and DAD data. These reports prompted us to investigate *Cerinthe glabra* for its alkaloid content.

Results and Discussion

The powdered aerial parts of *Cerinthe glabra* were extracted with ethanol, the crude extract was evaporated to dryness, dispersed in water, defatted with hexane and then extracted with ethyl acetate. The ethyl acetate extract contained some well known flavonoids (data not shown). Investigation of the aqueous layer showed a spot which gave a positive Dragendorff reaction. After evaporation to dryness, this fraction was chromatographed on silica gel, and finally purified by Sephadex chromatography to give the alkaloid intermedine-*N*-oxide (**1**).

The CI mass spectrum of the alkaloid showed $[M+H]^+$ at $m/z = 316$ for the formula $[C_{15}H_{25}NO_6+H]^+$, the fragment $[M+H-16]^+$ at 300 indicating the loss of an oxygen atom, a typical behavior of alkaloid *N*-oxides [4, 7]. Furthermore, we found prominent peaks at $m/z = 156$ and 138, which are characteristic of the 1,2-unsaturated pyrrolizidine unit [5]. By comparing the ^{1}H and ^{13}C NMR data of the alkaloid with those available for the five presently known pyrrolizidine-*N*-oxides of the formula $C_{15}H_{25}NO_6$ (intermedine-*N*-oxide **1** [18, 19], lycopsamine-*N*-oxide **2** [20, 21], echinatine-*N*-oxide **3** [21, 22], rinderine-*N*-oxide **4**, and indicine-*N*-oxide 5 [18, 21]), we found a very good accordance only with the data of **1**, which had previously been obtained by unambiguous total synthesis [18] (Table 1). Moreover, the optical rotation was in good accordance with the reported literature value [18], so this alkaloid was identified as intermedine-*N*-oxide (**1**).

Closer inspection of the NMR data reported for this alkaloid [18] revealed some unclear and some obviously wrong correlations of resonances. Our data from 1D and 2D NMR experiments (see Supporting Information) suggested new assignments for the chemical shifts of C-5, C-7, C-8, and C-9. For further confirmation of our results we performed a series of *ab initio* calculations. Quantum-chemical methods for the calculation of NMR chemical shift values have been found to be of exceptional value for the structural assignment of complex natural products like firefly luciferin, 4′-epiadriamycin, viridol, and samoquasine A [23]. In an extension of this methodology to the pyrrolizidine alkaloids we calculated the ^{13}C NMR shifts of intermedine-*N*-oxide (**1**) based on the gauge including atomic orbitals (GIAO), using the Hartree-Fock (HF) theory and the

Position[a]	Ref. [18] δ_C[b, c]	This work δ_C[b, d]	Ref. [18] δ_H[b, e] (mult., *J*)	This work δ_H[b, f] (mult., *J*)
1	132.811	132.88	–	–
2	122.125	122.83	5.81 (s, 1H)	5.84 (br s, 1H)
3	77.993	77.99	4.44 (br t, $J = 18$ Hz, 2H)[h]	4.47 (m, 2H)
5	61.292	69.45	3.75–3.85 (m, 2H)	3.72–3.80 (m, 2H)
6	34.701	34.46	2.01 (br d, $J = 11$ Hz, 1H)	2.02 (br d, $J = 12.6$ Hz, 1H)
			2.45–2.65 (m, 1H)	2.54–2.64 (m, 1H)
7	95.883	69.73[g]	4.75 (br s, 1H)[h]	4.70 (br s, 1H)
8	69.908[h]	95.73	4.67 (br s, 1H)[h]	4.91 (m, 1H)
			4.85 (br t, $J = 14$ Hz, 2H)[h]	4.84 (d, $J = 12.5$ Hz, 1H)
9	69.223[h]	61.31		4.97 (m, 1H)
1′ (10)	174.802	174.97	–	–
2′ (11)	84.064	84.18	–	–
3′ (12)	69.559[h]	70.17[g]	4.11 (q, $J = 6.7$ Hz, 1H)	4.15 (m, 1H)
4′ (13)	16.741[h]	16.67	1.16 (d, $J = 7$ Hz, 3H)	1.21 (m, 1H)
1″ (14)	33.722	33.92	1.85 (q, $J = 6.7$ Hz, 1H)	1.87 (sept, $J = 7.0$ Hz, 1H)
2″ (CH_3)	17.174[h]	17.13	0.97 (d, $J = 7$ Hz, 3H)	0.99 (d, $J = 7$ Hz, 3H)
3″ (CH_3)	17.636[h]	17.63	0.91 (d, $J = 7$ Hz, 3H)	0.93 (d, $J = 7$ Hz, 3H)

Table 1. ^{13}C and ^{1}H NMR chemical shift values (δ in ppm) and coupling constants J of **1** in $CDCl_3$ from this work compared to previously reported values [18].

[a] In parentheses: differing numbering in Ref. [18]; [b] chemical shift values in ppm relative to TMS; [c] ^{13}C NMR data recorded at 100.4 MHz; [d] ^{13}C NMR data recorded at 125.7 MHz; [e] ^{1}H NMR data recorded at 400 MHz; [f] ^{1}H NMR data recorded at 500 MHz. [g] no exact allocation of measured chemical shift values for C-7 and C-3′; [h] chemical shift values are not correlated exactly in Ref. [18].

Table 2. Average of standard Free Energies calculated for fully optimized structure **1** (in a. u.).

Basis set\Theory	HF	B3LYP
6-31G(d)	–1086.0815	–1092.6203
6-311G(d)	–1086.3142	–1092.8904
6-31G(d,p)	–1086.1306	–1092.6686
6-311G(d,p)	–1086.3663	–1092.9384

density functional theory (DFT) Becke, Lee-Yang-Parr (B3LYP), and the following basis sets 6-31G(d), 6-311G(d), 6-31G(d,p) and 6-311G(d,p) as standard implemented methods in the GAUSSIAN03 software package [24].

In an initial step several optimization cycles for the full geometry optimization of **1** had to be performed [25, 26]. The calculated average energies of each series of optimizations steps of each used level of theory and the employed basis set are presented in Table 2.

Subsequently, the ^{13}C NMR shift values relative to tetramethylsilane (TMS) were calculated for each optimized structure by the GIAO method [25, 26]. The results are shown in Table 3.

Correlation plots have been described as convenient parameters for comparing theoretical with experimental chemical shift values of aliphatic and aromatic sys-

Position	6-31G(d)		6-311G(d)		6-31G(d,p)		6-311G(d,p)		Exp.
1	*130.06*	**127.39**	*136.93*	**140.18**	*131.47*	**129.47**	*137.38*	**140.00**	132.88
2	*128.21*	**125.11**	*135.09*	**136.61**	*129.73*	**126.38**	*135.84*	**138.37**	122.97
3	*67.74*	**77.32**	*70.35*	**84.63**	*67.78*	**79.18**	*71.84*	**86.13**	78.15
5	*61.29*	**70.58**	*63.09*	**75.17**	*61.23*	**71.44**	*64.27*	**75.88**	69.59
6	*32.31*	**34.91**	*31.60*	**37.69**	*32.37*	**35.61**	*32.27*	**38.47**	34.60
7	*60.98*	**69.71**	*63.44*	**75.72**	*62.34*	**71.21**	*64.13*	**76.07**	69.87
8	*89.16*	**102.48**	*91.78*	**111.37**	*89.12*	**103.86**	*92.80*	**112.84**	95.87
9	*55.90*	**60.96**	*58.26*	**64.74**	*55.88*	**61.80**	*59.72*	**64.65**	61.44
1′	*171.55*	**170.68**	*177.35*	**184.31**	*172.30*	**172.91**	*178.98*	**183.29**	174.96
2′	*72.79*	**84.49**	*71.08*	**90.31**	*73.65*	**86.38**	*71.03*	**90.84**	84.16
3′	*58.74*	**67.94**	*61.81*	**73.21**	*60.22*	**69.19**	*61.66*	**73.23**	70.29
4′	*18.38*	**20.11**	*19.23*	**20.99**	*19.11*	**20.39**	*19.88*	**21.47**	16.81
1″	*29.45*	**35.85**	*31.82*	**40.15**	*30.22*	**37.13**	*33.54*	**40.81**	34.05
2″	*16.75*	**17.87**	*17.50*	**18.70**	*17.29*	**17.96**	*17.99*	**18.69**	17.12
3″	*16.13*	**17.88**	*17.35*	**18.68**	*16.84*	**18.08**	*17.93*	**19.00**	17.61

Table 3. Calculated average ^{13}C NMR shift values (relative to TMS) of **1** at *ab initio* HF (written italic type) and B3LYP (written bold type) levels using 6-31G(d), 6-311G(d), 6-31G(d,p), and 6-311G(d,p) basis sets, compared to experimental values (Exp.).

Theory/Basis set	Slope		Intercept		*r*	
	This work[a]	Ref. [18]	This work[a]	Ref. [18]	This work[a]	Ref. [18]
HF/6-31G(d)	0.9919	0.9679	−4.0784	−2.2408	0.994(3)	0.968(0)
HF/6-311G(d)	1.0312	1.0072	−4.4233	−2.5822	0.992(2)	0.966(9)
HF/6-31G(d,p)	0.9955	0.9727	−3.6605	−1.9102	0.994(0)	0.969(0)
HF/6-311G(d,p)	1.0333	1.0091	−3.7326	−1.9768	0.992(0)	0.966(5)
B3LYP/6-31G(d)	0.9718	0.9426	2.2920	4.4964	0.998(2)	0.966(1)
B3LYP/6-311G(d)	1.0600	1.0280	1.8923	4.2900	0.998(1)	0.966(0)
B3LYP/6-31G(d,p)	0.9841	0.9551	2.5905	4.7861	0.998(3)	0.966(7)
B3LYP/6-311G(d,p)	1.0575	1.0245	2.5577	5.0434	0.997(3)	0.964(0)

[a] Values obtained using ^{13}C chemical shift values of 69.73 ppm for C-7 and 70.17 ppm for C-3′ (see Table 1).

Table 4. Slopes, intercepts, and correlation coefficients (*r*) obtained by linear fitting of calculated *versus* experimental ^{13}C NMR chemical shifts for **1**.

tems [26]. On this account the calculated results were plotted against the reported [18] and our ^{13}C NMR correlations. By employing the newly assigned chemical shifts of C-5, C-7, C-8, and C-9 (see Table 1), our correlations showed, independently from the used levels of theory and basis sets, a better linear trend than the previous correlations. This is substantiated by the improvement of all values of slopes, intercepts, and correlation coefficients *r*, as shown in Table 4. As an example, Fig. 2 presents correlation plots of theoretical shift values calculated at B3LYP/6-31G(d,p) level *versus* reported and newly assigned shift values from Table 1.

Out of three similar ^{13}C NMR resonances (69.45, 69.73 and 70.17 ppm), the one at 69.45 ppm was clearly assigned as a methylene carbon (C-5) by a DEPT experiment. The shift values of the methine carbons at 69.73 and 70.17 ppm could, however not be assigned unambiguously to C-7 and C-3′ by HMQC and HMBC experiments. So we tried to achieve an unequivocal assignment by quantum chemical calculations. In a first step we simply interchanged the correlations for these two carbon resonances, but recalculation of correlation plots resulted in virtually unchanged correlation coefficients for each used levels of theory and basis sets. In a next step we employed the scaled theoretical chemical shift value (CS_{SX}) of any carbon atom at position X of **1**. This value describes the correlation between theoretical chemical shift and correlation plot quite well, and is obtained as:

$$CS_{SX} = (\text{theoretical CSX} - \text{intercept})/\text{slope}\ [26b].$$

As an example, Fig. 3 presents the differences between scaled and experimental ^{13}C NMR chemical shift values for each atom. Both $\Delta\delta$ values of C-7 and C-3′ showed deviations from around |0.4| independent from the used level of theory and basis sets.

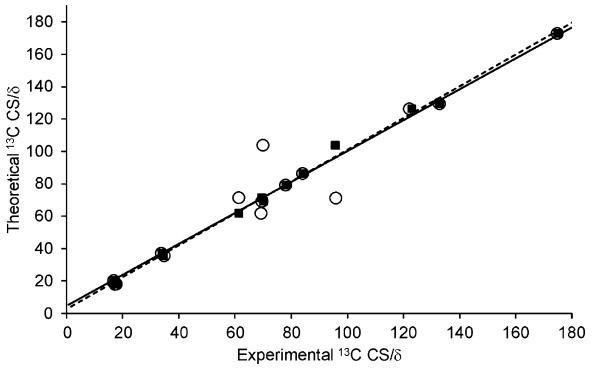


Fig. 2. Correlation plots of theoretical *versus* experimental ^{13}C NMR chemical shift (CS) values of **1**. Experimental data plotted against the corresponding theoretical data at the B3LYP/6-31G(d,p) level are drawn as solid line and ring symbols for reported values [18], and as dotted line and filled boxed symbols for new correlations.

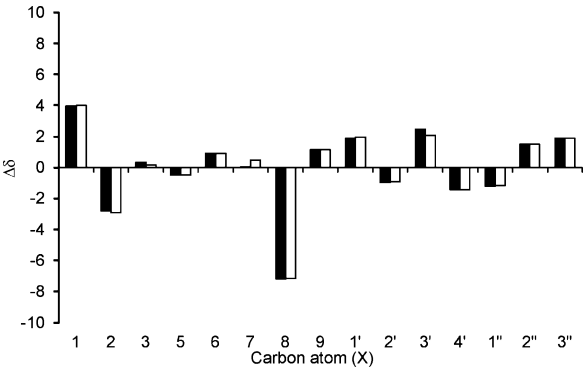


Fig. 3. Differences between scaled and experimental ^{13}C NMR chemical shift values at B3LYP level of theory, using 6-31G(d,p) as basis set. Black columns define an arbitrary allocation of C-7 at 69.73 ppm and C-3′ at 70.17 ppm, white columns define the opposite allocation.

Due to these small values even the *ab initio* calculations do not allow for unequivocal assignments of the resonances of C-7 and C-3′. This discloses the limit of quantum-chemical calculations of ^{13}C NMR chemical shift values.

Conclusion

With this study we were able to correct the chemical shift values of C-5, C-8, and C-9 of intermedine-*N*-oxide (**1**), using ^{1}H, ^{13}C, DEPT, HMQC and HMBC NMR experiments. Furthermore we calculated ^{13}C NMR shift values of **1** based on the GIAO approach, using HF and B3LYP levels of theory and the four basis sets 6-31G(d), 6-311G(d), 6-31G(d,p), and 6-311G(d,p). Independently from the used theories and the basis sets all calculations showed very good correlation coefficients (0.992 or higher). Among the theories and basis sets used here, B3LYP/6-31G(d) and B3LYP/6-31G(d,p) gave the best results. Taking all experimental and theoretical results together, all ^{13}C NMR resonances of **1**, except the critical pair C-7/C-3′ could be allocated unambiguously. The results presented here indicate the value of quantum-mechanical calculations also for the large class of closely related pyrrolizidine alkaloids.

Experimental Section

General information

IR spectra were recorded on a Perkin-Elmer FT-IR Paragon-1000 spectrometer, NMR spectra on a Jeol JNMR-GX 500 (^{1}H: 500 MHz, ^{13}C: 125.7 MHz) spectrometer. Mass spectra were recorded on a Hewlett Packard MS-Engine, electron ionization (EI) at 70 eV, chemical ionization (CI) with CH_4 (300 eV), high-resolution EIMS on a Jeol M Station JMS 700. Optical rotations were determined on a Perkin Elmer polarimeter 241 MC. Flash column chromatography was performed using silica gel 60 (230 – 400 mesh, E. Merck, Darmstadt). TLC was performed on Merck silica gel F_{254} plates (40×80 mm^2). Calculations were carried out on the cluster of the Faculty of Chemistry and Pharmacy at the Ludwig-Maximilians University of Munich. The computers are endowed with two Opteron Dualcore 2.4-GHz processors, using Debian GNU/Linux 4.0 (64-Bit) as operating system and the GAUSSIAN03 program package [24] for all calculations.

Plant material

Cerinthe glabra Miller was collected in August 2008 from the Botanical Garden in Munich (Germany), and kindly identified by Dr. A. Gröger.

Extraction and isolation

250 g of the air-dried, ground aerial parts of *C. glabra* were exhaustively extracted with ethanol (70%) (4×3 L), and the combined extracts were evaporated under reduced pressure at 40 °C to obtain 25 g crude extract. This extract was suspended in 300 mL distilled H_2O, filtered, and the filtrate evaporated to dryness again. The residue (15 g) was suspended in 500 mL distilled water and defatted with hexane (3×500 mL). The defatted aqueous layer was extracted with ethyl acetate (3×500 mL). The organic layer was discarded, the aqueous layer was evaporated under reduced pressure at 40 °C. The residue (4.0 g) was submitted to flash column chromatography using CH_2Cl_2-EtOH mixtures of increasing polarity (9 : 1 to 6 : 4). Upon elution with a 6 : 4 mixture a fraction containing a Dragendorff-positive spot (TLC control) was eluted. This fraction was evaporated and re-chromatographed on Sephadex LH-20 (eluent: methanol) for final purification to give 25 mg (0.01% dry weight) of the alkaloid intermedine-*N*-oxide (**1**). Colorless viscous oil. – ^{1}H and ^{13}C NMR data: see Table 1. – $[\alpha]_D^{22} = +12.0$ ($c = 0.01$, methanol). – IR (film): $\nu = 2970$, 1733, 1454, 1374, 1307, 1226, 1177, 1138, 1086, 1005 cm^{-1}. – MS (CI): m/z (%) = 316 (2) $[M+H]^+$, 300 (5), 172 (6) 156 (38), 154 (18), 138 (56), 136 (85), 127 (49), 117 (100), 103 (36). – HRMS (EI): $m/z = 299.1712$ (calcd. 299.1733 for $C_{15}H_{25}NO_5$, $[M-16]^+$).

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Supporting Information

General procedures of full geometry optimization and ^{13}C NMR chemical shift calculations, all calculated coordinates, Free Energies, ^{13}C NMR chemical shifts, scaled theoretical chemical values and ^{1}H, ^{13}C, DEPT, HMQC and HMBC spectra of intermedine-*N*-oxide (**1**) are provided as Supporting Information online only (http://www.znaturforsch.com/ab/v67b/c67b.htm).

[1] T. Hartmann, L. Witte in *Alkaloids: Chemical and biological perspectives*, Vol. 9. (Ed.: S. W. Pelletier), Pergamon Press, Oxford, **1995**, pp. 155 – 233.
[2] C. Frölich, D. Ober, T. Hartmann, *Phytochemistry* **2007**, *68*, 1026 – 1037.
[3] B. L. Stegelmeier, J. A. Edgar, S. M. Colegate, D. R. Gardner, T. K. Schoch, R. A. Coulombe, R. J. Molyneux, *J. Nat. Toxins* **1999**, *8*, 95 – 116.
[4] C. Crews, F. Berthiller, R. Krska, *Anal. Bioanal. Chem.* **2010**, *396*, 327 – 338.
[5] M. T. Williams, B. J. Warnock, J. M. Betz, J. J. Beck, D. R. Gardner, S. T. Lee, R. J. Molyneux, S. M. Colegate, *Phytochem. Anal.* **2011**, *22*, 532 – 540.
[6] A. Biller, M. Boppré, L. Witte, T. Hartmann, *Phytochemistry* **1994**, *35*, 615 – 619.
[7] X. Qi, B. Wu, Y. Cheng, H. Qu, *Rapid Commun. Mass Spectrom.* **2009**, *23*, 291 – 302.
[8] European Food Safety Authority (EFSA), Opinion of the scientific panel on contaminants in the food chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed, *EFSA J.* **2007**, *447*, 1 – 51.
[9] E. Röder, *Curr. Org. Chem*. **1999**, *3*, 557 – 576.
[10] L. Witte, P. Rubiolo, C. Bicchi, T. Hartmann, *Phytochemistry* **1993**, *32*, 187 – 196.
[11] T. Mroczek, K. Ndjoko-Ioset, K. Głowniak, A. Miętkiewicz-Capała, K. Hostettmann, *Anal. Chim. Acta* **2006**, *566*, 157 – 166.
[12] S. M. Colegate, J. A. Edgar, A. M. Knill, S. T. Lee, *Phytochem. Anal.* **2005**, *16*, 108 – 119.
[13] J. C. A. Wuilloud, S. R. Gratz, B. M. Gamble, K. A. Wolnik, *Analyst* **2004**, *129*, 150 – 156.
[14] W. Cisowski, M. Zielińska-Stasiek, A. Stołyhwo, P. Migas, A. Kamieniec, *Acta Chromatogr.* **2001**, *11*, 215 – 223.
[15] E. Wollenweber, R. Wehde, M. Dörr, J. F. Stevens, *Z. Naturforsch.* **2002**, *57c*, 445 – 448.
[16] E. Röder, H. Wiedenfeld, K.-J. Kabus, *Sci. Pharm.* **1990**, *58*, 9 – 13.
[17] T. Mroczek, S. Baj, A. Chrobok, K. Glowniak, *Biomed. Chromatogr.* **2004**, *18*, 745 – 751.
[18] Y. Nishimura, S. Kondo, T. Takeuchi, H. Umezawa, *Bull. Chem. Soc. Jpn*. **1987**, *60*, 4107 – 4113.
[19] L. H. Zalkow, J. A. Glinski, L. T. Gelbaum, T. J. Fleischmann, L. S. McGowan, M. M. Gordon, *J. Med. Chem.* **1985**, *28*, 687 – 694.
[20] E. Röder, T. Bourauel, *Phytochemistry* **1992**, *31*, 3613 – 3615.
[21] C. G. Logie, M. R. Grue, J. R. Liddell, *Phytochemistry* **1994**, *37*, 43 – 109.
[22] C. F. Asibal, J. A. Glinski, L. T. Gelbaum, L. H. Zalkow, *J. Nat. Prod.* **1989**, *52*, 109 – 118.
[23] a) T. Helgaker, M. Jaszunski, *Chem. Rev.* **1999**, *99*, 293 – 352; b) K. Odai, S. Nishiyama, Y. Yoshida, N. Wada, *J. Mol. Struc. Theochem.* **2009**, *901*, 60 – 65; c) R. Barthwal, P. Agrawal, A. N. Tripathi, U. Sharma, N. R. Jagannathan, G. Govil, *Arch. Biochem. Biophys.* **2008**, *474*, 48 – 64; d) P. Wipf, A. D. Kerekes, *J. Nat. Prod.* **2003**, *66*, 716 – 718; e) C. Timmons, P. Wipf, *J. Org. Chem.* **2008**, *73*, 9168 – 9170.
[24] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J.-A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, GAUSSIAN 03, (revision D.01), Gaussian, Inc., Wallingford CT (USA) **2004**.
[25] T. Zuschneid, H. Fischer, T. Handel, K. Albert, G. Häfelinger, *Z. Naturforsch.*, **2004**, *59b*, 1153 – 1176.
[26] a) G. Barone, D. Duca, A. Silvestri, L. Gomez-Paloma, R. Riccio, G. Bifulco, *Chem. Eur. J.* **2002**, *8*, 3240 – 3245; b) G. Barone, L. Gomez-Paloma, D. Duca, A. Silvestri, R. Riccio, G. Bifulco, *Chem. Eur. J*. **2002**, *8*, 3233 – 3239.