Isolation of Diterpenoid Alkaloids from Herb and Flowers of *Aconitum napellus* ssp. *vulgare* and Electrospray Ion Trap Multiple MS Study of These Alkaloids

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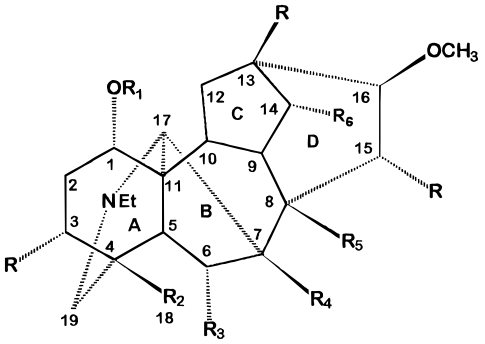
Chemical investigation of herb and flowers of *Aconitum napellus* L. ssp. *vulgare* led to the isolation of 12 diterpenoid alkaloids. Their chemical structures were identified on the basis of NMR and MS and of their complete ion trap multiple fragmentation mass spectrometry study.

Investigation of mass spectra of diterpenoid alkaloids has been reviewed by Pelletier[1] and Yunusov.[2] The presented fragmentation properties and patterns were based on electron ionization mass spectrometry (EIMS) analysis. So far, the use of ion trap multiple fragmentation mass spectrometry (MS^n) technique in the study of diterpenoid alkaloids has not been reported. MS^n is a rather new technique that allows consecutive fragmentation of compounds.[3] This permits a structure elucidation with quantities as little as a few nanograms when combined with high-resolution mass spectrometry.[3] We now describe the isolation and structure determination of 12 known diterpenoid alkaloids from the herb and flowers of *Aconitum napellus* L. ssp. *vulgare* (DC.) Rouy et Fouc. (Ranunculaceae). In addition, we report MS^n characteristics of these compounds for which a quadrupole ion trap system was used in the electrospray ionization mode (ESI). Positive ions were detected. For MS^2, a selected ion stored in the quadrupole ion trap is fragmented by transferring energy via the rf field. This process can be repeated by storing one of the generated fragment ions in the trap, which again will undergo fragmentation to give MS^3 and so on to MS^n spectra.

Results and Discussion

From the herb, six known C_{19} norditerpenoid alkaloids—aconitine (**1**), isotalatizidine (**2**), neoline (**3**), senbusine A (**4**), virescenine (**5**), and leroyine (**6**)—and five known C_{20} diterpenoid alkaloids—songorine (**7**), songoramine (**8**), 12-*epi*-napelline (**9**), 12-*epi*-dehydronapelline (**10**), and 12-*epi*-19-dehydrolucidusculine (**11**)—were obtained. From the flowers, we have isolated the known norditerpenoid alkaloid 14-*O*-acetylvirescenine (**12**) and the above-mentioned compounds **1**−**3**, **5**, **8**−**11**. Additionally, songorine (**7**) has been detected by TLC. Structure identification was carried out by NMR, comparing the obtained spectra with those reported in the literature.[4−7] 12-*epi*-19-Dehydrolucidusculine (**11**) and 14-*O*-acetylvirescenine (**12**) previously have been isolated from *Aconitum liangshanium* W. Z. Wang[7] and *Delphinium virescens* Nutt.,[1,8] respectively. Here is the first report of their occurrence in *Aconitum napellus*.

From a previous report, a novel norditerpenoid alkaloid, brachyaconitine characterized by a *trans*-3-hexenoyl substitutent at C-8 has been isolated[9] from aphids feeding on *A. napellus*. This compound has not been found in our previous chemical investigation of seeds and roots of *A. napellus*.[10,11] Likewise, in the present study no brachyaconitine could be detected by detailed TLC evaluation of the alkaloidal extracts from the herb and flowers of *A. napellus*. This suggests that brachyaconitine is a metabolite produced by the aphids, which replace the C-8-*O*-acetyl in the aconitine accumulated from the plant with the more lipophilic *trans*-3-hexenoyl group, thus facilitating the excretion of the toxic alkaloids with the waxy mass that envelopes the aphid and may serve as a repellent to predators.



	R_1	R_2	R_3	R_4	R_5	R_6	R
1	CH_3	CH_2OCH_3	OCH_3	H	OAc	OBz	OH
2	H	CH_2OCH_3	H	H	OH	OH	H
3	H	CH_2OCH_3	OCH_3	H	OH	OH	H
4	H	CH_2OCH_3	OH	H	OH	OH	H
5	H	CH_2OCH_3	H	OH	OH	OH	H
6	H	CH_3	H	OH	OH	OH	H
12	H	CH_2OCH_3	H	OH	OH	OAc	H

The detailed fragmentation pattern in the MS^n spectra of the C_{19} and C_{20} alkaloids are given in Tables 1 and 2, respectively. The characteristic and noteworthy features of the spectra are discussed below.

The main fragments in MS^n spectra of C_{19} norditerpenoid alkaloids (**1**−**6**, **12**) originate from the loss of H_2O (−18 u), MeOH (−32 u), and AcOH (−60 u). The relative abundance of these fragments is influenced by the nature and position of the substituents.

In the MS^n spectra of compounds **2**−**6** $[M - H_2O]^+$ is the base peak. The loss of water is caused by the elimination of the hydroxyl group at C-1 or C-8.

Senbusine A (**4**) and virescenine (**5**) have the same molecular weight and the same number of oxygenated

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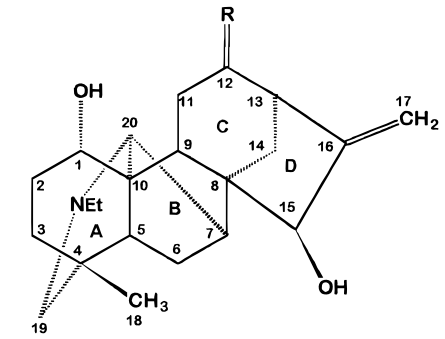
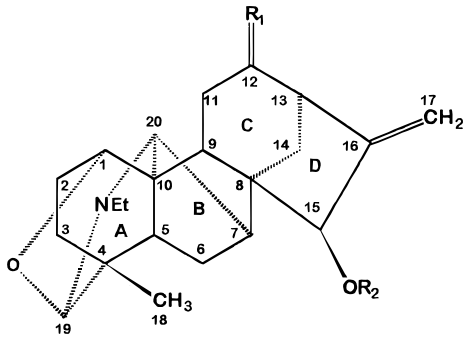


7 R = O

9 R = α-H, β-OH



8 R_1 = O; R_2 = H

10 R_1 = α-H, β-OH; R_2 = H

11 R_1 = α-H, β-OH; R_2 = Ac

groups. However, compound **4** possesses an α-OH at C-6, while compound **5** has a *β*-OH at C-7. In senbusine A (**4**), the relative intensity of the fragments at *m/z* 370 [P − $3H_2O$]$^+$ in the MS^2, as well as of the ions at *m/z* 388 [P_2 − H_2O]$^+$ and at *m/z* 370 [P_2 − $2H_2O$]$^+$ in the MS^3 is significantly higher than in virescenine (**5**) (Table 1). This suggests that the elimination of the hydroxyl group occurs more readily at C-6 than at C-7. This is confirmed by similar relative intensities of the corresponding masses in the MS^2 and MS^3 spectra of virescenine (**5**) and leroyine (**6**), both having a *β*-OH at C-7 and no oxygenation at C-6.

Isotalatizidine (**2**) and neoline (**3**) have no hydroxyl group at C-6 or C-7. Hence, the base peak in the MS^3 is caused by loss of MeOH from the base peak of the MS^2 record independent from the presence of a methoxyl at C-6. The MS^3 spectrum of neoline (**3**) gives an abundant [P_2 − 2MeOH]$^+$ ion and a recognizable [P_2 − 3MeOH]$^+$ ion, while only the [P_2 − 2MeOH]$^+$ fragment appears in the MS^3 spectrum of isotalatizidine (**2**) (Table 1). This is in agreement with the presence of three methoxyl groups in neoline and two in isotalatizidine. MS^2 and MS^3 spectra of virescenine (**5**) and leroyine (**6**) exhibit an identical fragmentation pattern with comparable abundances of corresponding peaks. The relative intensity of corresponding peaks show similarity. Accordingly, the MS^3 spectrum of virescenine (**5**) contains the [P_2 − 2MeOH]$^+$ and [P_2 − H_2O − 2MeOH]$^+$ ions, while no corresponding masses appear in the MS^3 spectrum of leroyine (**6**) (Table 1). This also concurs with the presence of two methoxyls in virescenine (**5**) and only one in leroyine (**6**).

Aconitine (**1**) possesses an acetoxyl group at C-8. Consequently, the base peak at *m/z* 586 in the MS^2 spectrum is formed by the loss of acetic acid from the [M + H]$^+$ ion. Maier[12] suggests that the formation of the base peak [P_2 − MeOH − CO]$^+$ at *m/z* 526 in the MS^3 spectrum of aconitine (**1**) is related to the well-known loss of AcOH from C-8 leading to the formation of a keto functionality at C-15[13,14] (Table 1).

The flowers of *A. napellus* contain compound **12** in micro amounts only. The MS showed the molecular ion [M + H]$^+$ at *m/z* 466. The fragments in the MS^2 and MS^3 spectra indicate the presence of an acetoxyl, two methoxyls, and three hydroxyls. Due to the similar characteristic fragmentation patterns and relative abundances in MS^2 and MS^3 spectra of compound **12** and virescenine (**5**), **12** was identified as 14-*O*-acetylvirescenine by MS^n. This was confirmed by comparison of the ^{1}H NMR spectrum with those reported in the literature.[1]

The C_{20} diterpenoid alkaloids have fewer oxygenation centers than C_{19} skeleton alkaloids. Hence, their MS^n

Table 1. MS^n Data *m/z* of C_{19}-Diterpenoid Alkaloids **1**−**6** and **12**[a]

		1	**2**	**3**	**4**	**5**	**6**	**12**
MS	[M + H]$^+$	646 (100)	408 (100)	438 (100)	424 (100)	424 (100)	394 (100)	466 (100)
	[M + Na]$^+$			460 (33)	446 (2)	446 (5)		488 (14)
	[2M + Na]$^+$		837 (18)	897 (64)	869 (70)	869 (70)	809 (4)	953 (3)
MS^2	[P − H_2O]$^+$		390 (100)	420 (100)	406 (100)	406 (100)	376 (100)	448 (100)
	[P − $2H_2O$]$^+$		372 (8)	402 (4.4)	388 (65)	388 (51)	358 (49)	430 (48)
	[P − $3H_2O$]$^+$				370 (21)	370 (3)	340 (3)	
	[P − MeOH]$^+$	614 (2)		406 (1.1)				
	[P − AcOH]$^+$	586 (100)						
	[P − H_2O − MeOH]$^+$	596 (3)	358 (4)	388 (37)	374 (5)	374 (11)	344 (9.5)	416 (10)
	[P − $2H_2O$ − MeOH]$^+$		340 (2)	370 (10)	356 (28)	356 (11)	326 (13)	398 (12)
	[P − H_2O − 2MeOH]$^+$			356 (10.4)				
MS^3	[P_2 − H_2O]$^+$	568 (2)	372 (52.5)	402 (11.4)	388 (79)	388 (100)	358 (100)	430 (100)
	[P_2 − $2H_2O$]$^+$				370 (100)	370 (6.5)	340 (7)	412 (10)
	[P_2 − MeOH]$^+$	554 (38)	358 (100)	388 (100)	374 (38)	374 (42)	344 (31)	416 (14)
	[P_2 − 2MeOH]$^+$	522 (5)	326 (13)	356 (31.5)		342 ((7)		
	[P_2 − 3MeOH]$^+$			324 (4.7)				
	[P_2 − AcOH]$^+$							388 (13)
	[P_2 − H_2O − MeOH]$^+$	536 (14.2)	340 (30)	370 (37.8)	356 (83)	356 (31)	326 (32)	398 (22)
	[P_2 − H_2O − 2MeOH]$^+$	504 (3)	308 (7)	338 (13.2)	324 (38)	324 (7)		366 (<1)
	[P_2 − $2H_2O$ − MeOH]$^+$		322 (5)			338 (6)	308 (8)	
	[P_2 − C_2H_4]$^+$		362 (31)	392 (3)				
	[P_2 − H_2O − C_2H_4]$^+$		344 (11)		360 (23)	360 (10)	330 (8)	
	[P_2 − MeOH − CO]$^+$	526 (100)						
	[P_2 − H_2O − MeOH − C_2H_4]$^+$					328 (6)	298 (6)	
	[P_2 − H_2O − AcOH]$^+$							370 (12)

[a] P = Parent ion ([M + H]$^+$), P_2 = base ion of MS^2, relative abundances in parentheses.

Table 2. MS^n Data *m/z* of C_{20}-Diterpenoid Alkaloids **7**−**11**[a]

		7	8	9	10	11
MS	$[M + H]^+$	358 (100)	356 (100)	360 (100)	358 (100)	400 (100)
	$[2M + Na]^+$	737 (16)		741 (35)		
MS^2	$[P - O]^+$		340 (6)			
	$[P - H_2O]^+$	340 (100)	338 (62)	342 (100)	340 (100)	382 (30)
	$[P - 2H_2O]^+$	322 (0.35)			322 (3)	
	$[P - C_2H_4/CO]^+$		328 (17)			
	$[P - AcOH]^+$					340 (100)
	$[P - AcOH - H_2O]^+$					322 (23)
	$[P - C_3H_8]^+$		312 (24)		314 (4.2)	
	$[P - H_2O - C_2H_4/CO]^+$		310 (14.2)		312 (2)	
			298 (56)		298 (2)	296 (12)
			296 (100)		296 (16)	
MS^3	$[P_2 - H_2O]^+$			324 (100)	322 (50)	322 (100)
	$[P_2 - C_2H_4/CO]^+$			314 (5.2)	312 (80)	312 (15)
				298 (7.5)	297 (52)	298 (41)
				296 (35)	295 (100)	296 (13)

[a] P = Parent ion ($[M + H]^+$), P_2 = base ion of MS^2, relative abundances in parentheses.

spectra show lower abundance of the losses of 18 u, 32 u, and 60 u fragments. However, some complicated peaks due to cleavage and loss of rings cannot be interpreted.

Compounds **7**, **9**, and **10** give $[MH - H_2O]^+$ as base peak in the MS^2 spectra, while the relative intensity of this ion in the MS^2 of songoramine (**8**) is only 62% (Table 2). This indicates that elimination of H_2O from the hydroxyl at C-15 is not as easy as at C-1 or C-12. Compound **11**, possessing an acetoxyl at C-15, gives $[MH-AcOH]^+$ as base peak in the MS^2 spectrum.

Both songoramine (**8**) and 12-*epi*-dehydronapelline (**10**) show a peak at *m/z* 296 in their MS^2 spectra, which is formed by loss of C_3H_8 and O from the molecular ion $[M + H]^+$ at *m/z* 356 in compound **8** and loss of C_3H_8 and H_2O from $[M + H]^+$ at *m/z* 358 in compound **10**, respectively. Comparing both structures, we deduce that the fragment in common at *m/z* 296 arises from loss of the substituent at C-12 and further cleavage and partial loss of rings C and D. As to compound **11**, the same fragment at *m/z* 296 in the MS^2 spectrum may originate from loss of acetic acid from C-15 followed by cleavage and loss of ring D. The MS^3 spectra of compound **9** also contains an abundant ion at *m/z* 296, which results from loss of H_2O and C_2H_4 from the base ion in the MS^2 at *m/z* 342.

MS^n was first applied for the structure elucidation of diterpenoid alkaloids by Maier[12] in these laboratories. In this paper, we first report the complete MS^n analysis of 12 alkaloids from herb and flowers of *A. napellus*. The application of MS^n spectra not only proves purity and provides the molecular weight, but also demonstrates the kind and number of oxygenated groups and displays differences in chemical structures. Above all, a 100-ng sample is sufficient to obtain satisfactory spectroscopic data. This technique proves to be useful in solving structural problems of diterpenoid alkaloids, especially when only micro amounts are available.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ on a Bruker 200 spectrometer with TMS as internal standard. MS^n spectra were obtained on a Finnigan LCQ-G2 spectrometer. Positive ions were detected by ESI. Sample introduction: syringe pump (2−5 μL/min injected). Sheath gas flow: 20 arbitrary units; spray voltage: 4 kV; heated capillary temperature: 150 °C; no optimization. Scan range: 300−1500 *m/z*. Samples, 10 μg each, were dissolved in 500 μL of H_2O and 500 μL of CH_3OH, injection of <10 μL for recording of a MS^n spectrum. Chromatographic separations were carried out by TLC on Merck TLC plates Si gel 60 F_{254} (0.25 mm) and neutral Al_2O_3 60 F_{254} (0.25 mm).

Plant Material. The plant material (herb and flower) was collected by A. Katz in the Engadin Valley, Switzerland, in August 1997. Voucher specimens are deposited in his herbarium.

Extraction and Isolation. The air-dried herb, that is, stalks, leaves, and flower buds (57.4 g) of *A. napellus* L. ssp. *vulgare*, was extracted as described earlier[15] to give 45 mg of pH 9 alkaloidal extract and 36 mg of pH 12 alkaloidal extract. The extracts showed orange-red spots on TLC with Dragendorff reagent. Purification of each part was accomplished by repeated preparative TLC on Al_2O_3 (cyclohexane−EtOAc−EtOH, 6:3.5:0.5; cyclohexane−$CHCl_3$−EtOH, 2.2:7.5:0.3) and Si gel ($CHCl_3$−CH_3OH, 8.5:1.5). Eleven diterpenoidal alkaloids—aconitine (**1**) (23.3 mg), isotalatizidine (**2**) (4.6 mg), neoline (**3**) (9.6 mg), senbusine A (**4**) (0.3 mg), virescenine (**5**) (1.9 mg), leroyine (**6**) (1.4 mg), songorine (**7**) (0.35 mg), songoramine (**8**) (0.5 mg), 12-*epi*-napelline (**9**) (5.0 mg), 12-*epi*-dehydronapelline (**10**) (1.8 mg), and 12-*epi*-19-dehydrolucidusculine (**11**) (0.4 mg)—were finally obtained. For MS^n data of these compounds, see Tables 1 and 2.

Air-dried flowers (9.7 g) were extracted as described above to afford 3.54 mg of **1**, 0.12 mg of **2**, 0.14 mg of **3**, 0.82 mg of **5**, 0.40 mg of **8**, 0.30 mg of **9**, 0.33 mg of **10**, 0.30 mg of **11**, and 0.24 mg of **12**. Identification of alkaloids **1**−**3**, **5**, and **8**−**11** was based on the comparison of their TLC R_f value with authentic compounds. MS^n data of 14-*O*-acetylvirescenine (**12**), see Table 1.

Copies of the original spectra are obtainable from the corresponding author.

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References and Notes

(1) Pelletier, S. W.; Mody, N. V. In *The Alkaloids*; Rodrigo, R. G. A., Ed.; Academic Press: New York, 1981; Vol. 18, Chapter 2, pp 100−216.
(2) Yunusov, M. S. *Nat. Prod. Rep*. **1991**, *8*, 499−526.
(3) Koelliker, S.; Oehme, M.; Dye, Ch. *Anal. Chem*. **1998**, *70*, 1979−1985.
(4) Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. In *Alkaloids*; Pelletier, S. W., Ed.; Wiley: New York, 1984; Vol. 2, Chapter 5, pp 205−462.
(5) Pelletier, S. W.; Joshi, B. S. In *Alkaloids*; Pelletier, S. W., Ed.; Springer-Verlag: New York, 1991; Vol. 7, Chapter 3, pp 297−564.
(6) Atta-ur-Rahman. In *Handbook of Natural Products Data*; Elsevier Science: New York, 1990; Vol. 1, pp 1−343.
(7) Takayama, H.; Wu, F. E.; Eda, H.; Oda, K.; Aimi, N.; Sakai, S. *Chem. Pharm. Bull*. **1991**, *39*, 1644−1646.

(8) Pelletier, S. W.; Mody, N. V.; Venkov, A. P.; Jones, S. B., Jr. *Heterocycles* **1979**, *12*, 779−782.
(9) Liu, H.; Katz, A. *J. Nat. Prod*. **1996**, *59*, 135−138.
(10) Liu, H.; Katz, A. *Nat. Prod. Lett*. **1994**, *5*, 147−151.
(11) Gauch, U. Über die Diterpenalkaloide der Samen und Wurzeln von *Aconitum napellus* L. verschiedener schweizerischer Standorte. Ph.D. Thesis, Universität Basel, Basel, Switzerland, 1992.
(12) Maier, J. Ph.D. Thesis, Universität Basel, Switzerland, 1999 (in preparation).
(13) Pelletier, S. W.; Mody, N. V. In *The Alkaloids*; Manske, R. H. F., Rodrigo, R. G. A. Ed.; Academic Press: New York, 1979; Vol. 17, Chapter 1, pp 1−103.
(14) Wang, F. P.; Liang, X. T. In *Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 1992; Vol. 42, Chapter 3, pp 151−247.
(15) Hanuman, J. B.; Katz, A. *J. Nat. Prod*. **1993**, *56*, 801−809.

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