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QUALITATIVE MASS SPECTROMETRIC ANALYSIS OF THE TOTAL DITERPENE BASES FROM THE ROOTS OF *Aconitum kusnezoffi*

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A quantitative analysis of the roots of Aconitum kusnezoffi *has been made by a group of mass-spectrometric methods. Twelve diterpene bases have been studied of which ten have been identified as beiwutine, aconitine. mesaconitine, 3-deoxyaconitine, hypaconitine, neoline, songorine, isotalatisidine, talatisidine, and 10-hydroxyneoline.*

In previous publications we have reported the use of various mass-spectral methods for the qualitative analysis of plant raw materials [1, 2]. Continuing these investigations, we have analyzed the total diterpene alkaloids from the roots of *A. kuznezoffi* gathered in the province of Shan-si (China) in July, 1994.

For the qualitative identification of the bases we used the method of multipeak monitoring [1], the spectra of metastable ions — metastable defocusing (MD) — and linked scanning, B/E = const, and we measured the elementary compositions of the key ions. In many cases, the use of secondary-ion mass spectrometry (LSIMS) enabled us to obtain additional information on the presence of alkaloids in the mixture being analyzed.

According to the literature, beiwutine, aconitine, mesaconitine, 3-deoxyaconitine, hypaconitine, neoline, and candicine, have been isolated from the roots of the plant growing in China [3, 4], and 3-deoxyaconitine, hypaconitine, mesaconitine, lepenine, and denudatine, from a Mongolian species of monkshood [5].

We have investigated three fractions of the total alkaloids of *A. kusnezoffi*. The information obtained is given in Table 1. In the mixture investigated we detected mass-spectrally six alkaloids isolated previously from *A. kuznezoffi*: bayvutine, aconitine, mesaconitine, 3-deoxyaconitine, hippaconitine, and neoline. The main ones were mesaconitine and hippaconitine, while 3-deoxyaconitine was present in trace amounts. In addition to this, in the EI and LSIMS spectra we recorded intense peaks of ions with *m/z* 357 and 358, respectively. Judging from the elementary composition of the ion with *m/z* 357 (see Table 1) and from the protonation of this ion in the LSIMS regime, the ion with *m/z* 357 was the molecular ion of either songorine or an isomer of it. The B/E = const spectrum of the ion with *m/z* 357 in the total alkaloids of *A. kusnezoffi* and a standard sample of songorine proved very similar (Table 2), which permitted us to assign the base with M^+ to songorine.

An intense peak of an ion with *m/z* 390 ($C_{23}H_{36}NO_4$) in the EI spectrum of the total bases of *A. kusnezoffi* could belong to either isotalatisidine $(M - 17)^+$ or talatisamine $(M - 31)^+$. The presence of the metastable transitions (MTs) $407^+ \to 390^+$ and $421^+ \to 390^+$, and the peaks of the MH^+ 408 and 422 ions in the LSIMS spectra confirmed the presence of both isotalatisidine and talatisamine in the mixture being analyzed.

In addition, we detected a base with MM 453, composition $C_{24}H_{39}NO_7$, the spectrum of which was characterized by the 100% peak of an ion with *m/z* 436. On the basis of these mass-spectral characteristics and biogenetic considerations, it was possible to assign this base to 10-hydroxyneoline. In the EI and LSIMS spectra of the mixture being analyzed we also detected the peaks of ions of another two unidentified bases with M^+ 485 and 589 having C_1-OCH_3 groups, to judge from the maximum peaks of the $(M - 31)^+$ ions (see Table 1).

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TABLE 1. Diterpene Alkaloids Found in the Total Bases from the Roots of *A. kusnezoffi*

Compound	M^+	Elementary composition of M^+	*m/z* of characteristic ions	Metastable transitions	*m/z* of ions in LSIMS
1. Beiwutine*	647	$C_{33}H_{45}NO_{12}$	647, 616, 556	$647^+ \rightarrow 556^+$	$648(MH^+)$
2. Aconitine*	645	$C_{34}H_{47}NO_{11}$	645, 614, 554	$645^+ \rightarrow 554^+$	$646(MH^+)$
3. Mesaconitine*	631	$C_{33}H_{45}NO_{11}$	631, 600, 540	$631^+ \rightarrow 540^+$	$632(MH^+)$
4. 3-Deoxyaconitine*	629	$C_{34}H_{47}NO_{10}$	598, 538	$629^+ \rightarrow 538^+$	$630(MH^+)$
5. Hippaconitine*	615	$C_{33}H_{45}NO_{10}$	615, 584, 524	$615^+ \rightarrow 524^+$	$616(MH^+)$
6. Neoline*	437	$C_{24}H_{39}NO_6$	437, 420	$437^+ \rightarrow 420^+$	$438(MH^+)$
7. Songorine	357	$C_{22}H_{31}NO_3$	357, 340, 328	–	$358(MH^+)$
8. Isotalatisidine	407	$C_{23}H_{37}NO_5$	407, 390	$407^+ \rightarrow 390^+$	$408(MH^+)$
9. Talatisamine	421	$C_{24}H_{39}NO_5$	421, 406, 390	$421^+ \rightarrow 390^+$	$422(MH^+)$
10. 10-Hydroxyneoline	453	$C_{24}H_{39}NO_7$	453, 436	$453^+ \rightarrow 436^+$	$454(MH^+)$
11. Base with M^+ 485	485	$C_{24}H_{39}NO_9$	485, 434	$485^+ \rightarrow 454^+$	$486(MH^+)$
12. Base with M^+ 589	589	$C_{31}H_{43}NO_{10}$	589, 558	$589^+ \rightarrow 558^+$	$590(MH^+)$

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*Alkaloids isolated previously from *A. kusnezoffi*.

TABLE 2. Mass Numbers and Relative Intensities of the Metastable Daughter Ions in the B/E = const Spectra of Ions with m/z 357 from Songorine and the Total Bases of *A. kusnezoffi*

Sample	Parental ion	Daughter ions
Songorine	357	340(100). 328(70). 314(50). 298(65). 284(30). 246(35)
Total bases of *A. kusnezoffi*	357	340(100). 328(65). 314(46). 298(63). 284(24). 246(35)

In the LSIMS spectra of the chloroform fraction of the total material we recorded the peak of an ion with MH^+ 600 which shifted by 22 a.m.u. on the addition of NaCl to the glycerol matrix [m/z 622 $(M + Na)^+$].

EXPERIMENTAL

Isolation of the Total Alkaloids. The air-dry comminuted roots of the plant (1365 g) were moistened with a 5% solution of Na_2CO_3 and, after 2 h, were covered with chloroform. The chloroform was decanted off after 24 h. Extraction was repeated for four times. The combined chloroform extracts were evaporated to a volume of 1.5 liter and were shaken out three times with a 5% solution of H_2SO_4 (400 ml). The acid solution was filtered and was washed twice with chloroform, after which, with cooling, it was alkalinized with sodium carbonate and extracted first with ether and then, exhaustively, with chloroform. Evaporation and elimination of the solvents yielded 0.09 g of washing fraction, 3.83 g of ether fraction, and 0.53 g of chloroform fraction. When the ether fraction was treated with acetone, 0.39 g of a crystalline mixture separated out. The crystals, the mother solution of the ether fraction, and the chloroform fractions were investigated by mass-spectrometric methods.

MKh 1310 mass spectrometer with SVP 5 double focusing, direct sample injection, temperature of the ionization chamber 170°C, temperature of the heater bulb 80-160°, ionizing potential 70 V, collector current 60 μA. For the conditions of obtaining the MD spectra, see [6] and for the B/E = const spectra [7]. To record the LSIMS spectra we used a LSIMS ion source made in the Institute of Analytical Instrument Construction of the Russian Academy of Sciences, St. Petersburg. Ionization was achieved with accelerated Cs^+ ions having an energy of 7 keV, the accelerating potential being 5 kV.

REFERENCES

1. E. G. Sirotenko, Ya. V. Rashkes, and S. K. Usmanova, Khim. Prir. Soedin., 532 (1989).
2. E. G. Mil'grom, V. N. Plugar', U. A. Abdullaev, N. Kirimer, K. Kh. N. Basher, and B. T. Salimov, Khim. Prir. Soedin., 828 (1994).
3. Y. G. Wang, Y. L. Zhu, and R. H. Zhu, Acta Pharmaceutica Sinica, No. 15, 526 (1980).
4. Chang Qi and Chen Oihua, Zhongcaoyao, **23**, No. 8, 401; Chem. Abstr., **118**, 19236 (1993).
5. D. Urhin, B. Proksa, and J. Zhamiansan, Planta Med., **57**, No. 4, 390 (1991).
6. Ya. V. Rashkes and M. S. Yunusov, Khim. Prir. Soedin., 481 (1984).
7. Yu. M. Mil'grom, Ya. V. Rashkes, G. V. Fridlyanskii, and B. M. Voronin, Khim. Prir. Soedin., 489 (1990).