off and, with cooling, the residue was dissolved in water and the solution was made alkaline with sodium carbonate and was extracted with chloroform. The reaction product was chromatographed on a column of deactivated alumina. Elution of the column with ether gave 26 mg of the homogeneous amorphous diacetate (X). IR spectrum: 1720 cm^{-1} (ester carbonyl).

SUMMARY

Structures have been put forward for the products of the reaction of dehydroeldelidine and dehydrodelcorine with sodium in liquid ammonia. Features of the fragmentation of these compounds under electron impact are discussed.

LITERATURE CITED

1. A. S. Narzullaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 498 (1972).
2. M. S. Yunusov, Ya. V. Rashkes, V. A. Tel'nov, and S. Yu. Yunusov, Khim. Prir. Soedin., 515 (1969).
3. M. S. Yunusov and S. Yu. Yunusov, Khim. Prir. Soedin., 90 (1970).
4. A. S. Narzullaev, M. S. Yunusov and S. Yu. Yunusov, Khim. Prir. Soedin., 497 (1973).
5. M. S. Yunusov and S. Yu. Yunusov, Khim. Prir. Soedin., 334 (1970).
6. M. S. Yunusov, V. A. Tel'nov, and S. Yu. Yunusov, Khim. Prir. Soedin., 774 (1970).
7. M. Takayama, K. Yamaguchi, T. Okazaki, N. Aimi, and S. Sakai, J. Chem. Soc. Chem. Commun., 11, 818 (1987).
8. Ya. V. Rashkes and M. S. Yunusov, Khim. Prir. Soedin., 481 (1984).

MASS-SPECTROMETRIC METHOD OF ANALYZING MIXTURES OF DITERPENE BASES FROM PLANTS OF THE GENUS Aconitum

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The qualitative composition of the mixture of diterpene alkaloids from the roots of *Aconitum septentrionale* from various growth sites has been studied. Twenty-one bases have been detected. The mass-spectrometric method of multipeak monitoring has been used for the rapid checking of the amounts of the main component and seven subsidiary components.

We have previously made a qualitative analysis of the saponified mixture of alkaloids from the epigeal part of *Aconitum leucostomum* gathered in the fruit-bearing period and a quantitative determination of the main components of this mixture by the ion-current integration (ICI) method [1].

In connection with the search for sources of lappaconitine — the base for the production of the antiarrhythmic drug allapinin — we have undertaken the analysis of the qualitative and quantitative compositions of *Aconitum septentrionale* growing in many regions of the USSR.

To solve this problem we propose to use the method of multipeak monitoring (MM) which consists in the repeated rapid recording of part of the spectrum containing all the characteristic ions up to the moment of the complete evaporation of a calibrated sample introduced through the direct introduction system. As compared with the ICI method, this is far more laborious, but with its aid it is possible to follow simultaneously the composition of the sample and its changes under the temperature conditions and to perform the quantitative determination of the components. A disadvantage of the MM method is that it is carried out in a low-resolution regime, where the peaks of the characteristic ions may be increased at the expense of the peaks of isobaric impurity ions.

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TABLE 1. Diterpene Alkaloids Found in Sample 3

Compound	Type	M+	Elementary composition of M+	m/z of characteristic ions	Metastable transitions
1. Songorine +	20-s	357	$C_{22}H_{31}NO_3$	357	—
2. Base A +	20-h	429	$C_{24}H_{31}NO_6$	429	—
3. N-Deacetyllappaconitine +	18-ℓ	542	$C_{30}H_{42}N_2O_7$	511, 405	$542^+ \to 511^+$
4. Sepaconitine +	18-ℓ	558	$C_{30}H_{42}N_2O_8$	527, 421	$558^+ \to 527^+$
5. Lappaconitine +	18-ℓ	584	$C_{32}H_{44}N_2O_8$	584, 553, 405	$584^+ \to 405^+$
6. Base B *	20-h	387	$C_{22}H_{29}NO_5$	387	—
7. Lappaconine *	18-ℓ	423	$C_{23}H_{37}NO_6$	423, 392	$423^+ \to 392^+$
8. 10-Hydroxylappaconine *	18-ℓ	439	$C_{23}H_{37}NO_7$	439, 408	$439^+ \to 408^+$
9. Lycoctonine *	19-ℓ	467	$C_{25}H_{41}NO_7$	467, 436	$467^+ \to 436^+$
10. Delphatine *	19-ℓ	481	$C_{26}H_{43}NO_7$	481, 466, 450	$481^+ \to 450^+$
11. Acetylsepaconitine *	18-ℓ	600	$C_{32}H_{44}N_2O_9$	569, 421	$600^+ \to 421^+$
12. Lycaconitine *	19-ℓ	668	$C_{36}H_{48}N_2O_{10}$	637	$668^+ \to 637^+$
13. Methyllycaconitine *	19-ℓ	682	$C_{37}H_{50}N_2O_{10}$	651	$682^+ \to 651^+$
14. Base C —	20-h	327	$C_{20}H_{25}NO_3$	327	—
15. Deoxylappaconine —	18-ℓ	407	$C_{23}H_{37}NO_5$	376	—
16. Base D —	19-ℓ	509	$C_{27}H_{43}NO_8$	509, 478	$509^+ \to 478^+$
17. Base E —	19-ℓ	525	$C_{27}H_{43}NO_9$	494	$525^+ \to 494^+$
18. Base F —	19-ℓ	465	$C_{25}H_{39}NO_7$	465, 434	$465^+ \to 434^+$
19. Base G —	19-ℓ	650	$C_{36}H_{46}N_2O_9$	650, 619	$650^+ \to 619^+$
20. Base H —	19-ℓ	696	$C_{37}H_{48}N_2O_{11}$	665	$696^+ \to 665^+$
21. Base I —	19-ℓ	698	$C_{37}H_{50}N_2O_{11}$	667	$698^+ \to 667^+$

Note: +) bases isolated previously from A. septentrionale; *) bases isolated previously from other species of Aconitum; —) new bases.

We have compared the qualitative and quantitative compositions of the mixture of alkaloids from the epigeal part of A. leucostomum plants collected in June in the Issyk-Kul' oblast in the period before budding (sample 1) and of A. septentrionale collected in May in the Novosibirsk oblast in the period of withering of the stem (sample 2), and also the combined alkaloids from the roots of A. septentrionale collected in August in the Vladimir oblast in the fruit-bearing phase (sample 3), in October in the environs of Tomsk in the period of the withering of the epigeal part (sample 4), and in August in the Arkhangel'sk oblast in the fruit-bearing phase (sample 5).

Table 1 gives a list of the bases detected in sample 3. Seven of them belong to the C_{18}-(18-ℓ) and ten to the C_{19}-lycoctonine (19-ℓ) series. Four bases of the C_{20} series were revealed, of which three had the hetisine skeleton (20-h) and four the songorine skeleton (20-s). Thirteen of the twenty-one detected were known bases that had been isolated from other Aconitum species, including five alkaloids found previously in A. septentrionale.

TABLE 2. Relative Percentage Amounts of Eight Diterpene Bases

Sample		Σ, % on the weight of the air-dry mass	Songorine	Lappaconine	10-Hydroxylappaconine
1	I	0.45	0,3	3,4±0,3	0,38±0.06
	II		0,001	0,015±0,002	0,0020±0,0005
2	I	0,35	1,8±0,2	5,0	2,41±0,02
	II		0.0063±0,0007	0,017	0,0084±0,0001
3	I	3,0	1,52±0,04	2,4±0,3	0,80±0,08
	II		0,046±0,003	0,07±0,01	0,024±0,002
4	I	2.4	3,4	2,9±0,4	0,6±0,1
	II		0,08	0,070±0,009	0,015±0,002
5	I	3,9	1,10±0,05	2,7	0,70±0,07
	II		0,041±0,002	0,1	0,028±0,003

The determination of the nature of the bases was helped by measuring the elementary compositions and analyzing the distribution of the intensities of the peaks of the characteristic ions and also by a calculation of the mass numbers of the parental ions by the method of metastable defocusing (MD). Thus, the elementary composition of the ion with m/z 478 ($C_{26}H_{40}NO_7$) and the presence in the MD spectrum of a parental ion with m/z 509 showed that the mixture contained the monoacetate of a base of lycoctonine series (lycoctonine or an isomer) — base D. Base E, judging from its composition, was a hydroxy analog of this alkaloid. Base F most probably correspond to eldelidine, judging from the composition and intensity of the peak of the $(M - OCH_3)^+$ ion with m/z 434. The nature of bases G, H, and I can be predicted by correlating their empirical formulas with methylycaconitine in relation to which they may represent products of dehydromethoxylation (G) and oxidation (H, I).

As can be seen from Table 2, the amount of lappaconitine in the mixture of bases from the epigeal part of A. leucostomum exceeded 60%, although in relation to the air-dry mass it was 0.27%. The proportion of this alkaloid in the total samples from the roots of A. septentrionale was considerably smaller (33-45%), but its amount in the air-dry mass was, on average, 4.5 times greater (1.1-1.3%). These results agree satisfactorily with those of the direct isolation of the main component from the same plant materials.

The epigeal part of A. septentrionale (sample 2) contained 5.5 times less lappaconitine than that of A. leucostomum (sample 1), but it contained a larger amount of subsidiary components such as songorine, 10-hydroxylappaconine, and N-deacetyllappaconitine, while the amount of sepaconitine was far greater than in the other samples. Account must also be taken of the fact that the amount of all eight components that were determined in sample 1 was about 74%, and in sample 2 only about 47%.

So far as concerns the amounts of the subsidiary components in the roots (samples 3-5), in spite of the difference in the climatic conditions of the growth regions they were of the same order, with the exception of sepaconitine.

EXPERIMENTAL

A MKh 1310 mass spectrometer with a SVP 5 device for the direct introduction of the sample, an 80-micron slit, a resolving capacity of 1200, an ionizing voltage of 50 V, and a collector current of 14 μA was used.

The following solutions in $CHCl_3$ were used for quantitative determination by the MM method: 1) solutions of samples 1-5 at a concentration of 1 mg/ml, volume introduced 1-2 μl; 2) standard solution A containing all eight components to be determined in the same concentration, $1.25 \cdot 10^{-4}$ M; volume introduced 4 μl; 3) standard solution B containing $2.5 \cdot 10^{-4}$ M each of N-deacetyllappaconitine and lappaconitine and $1.25 \cdot 10^{-4}$ M sepaconitine, volume introduced 1 μl; and 4) standard solution C containing $5 \cdot 10^{-4}$ M lappaconitine, $1.25 \cdot 10^{-4}$ M N-deacetyllappaconitine; and $6.25 \cdot 10^{-5}$ M sepaconitine, volume introduced 1 μl.

After evaporation of the chloroform, the evaporator ampul was gradually introduced into the ion source, the temprature of the ionization chamber being maintained between 150 and 200°C. After the introduction of the ampul, this temperature was raised smoothly to 250°C. During this period, 11-12 "scans" in the m/z interval of 300-600 (with standard A) or with m/z 500-600 (standards B and C) were recorded at the rate of 4 sec for each 100 m.u. at chart speed of 25 mm/sec.

in the Mixture of Alkaloids (I) and in the Raw Material (II)

Lycoctonine	Delphatine	N-Deacetyl-lappaconitine	Sepaconitine	Lappaconitine
1,7±0,1 0,0075±0,0003	1,75±0.02 0,0078±0.0001	5,5±0.2 0,025±0,001	Tr.	61,0±1,3 0,27±0,01
2,0±0.2 0.0070±0,0007	1.8±0,2 0,0063±0,0007	12,8±1,1 0,045±0,004	6,7±0,5 0,023±0,002	14.0±0.2 0,049±0,001
0,92±0,06 0.028±0,002	1,4 0,04	3 6±0,1 0,11±0,01	Tr.	41.0±1 0 1.23±0,03
0.8±0,2 0,019±0,005	0 6 0,01	3,6±0,8 0,09±0,02	Tr.	45.0±6.0 1,09±0,14
1,3±0,1 0,050±0.006	1.50±0,15 0,053±0,005	4,1 0,2	0,23±0,06 0,009±0,003	35,0±3.5 1,3±0,1

TABLE 3. Percentages of the Ester-Type Bases in Samples 3 and 5 Calculated by the Method of Additives

Sample	Σ, % on the weight of the air-dry mass	N-Deacetyllappaconitine	Sepaconitine	Lappaconitine
3 I	3,0	3,1±0,1	Tr.	36,1±0,1
II		0,093±0,003		1,08±0,03
5 I	3,9	4.4±0,8	0,21±0,06	33,0±6,0
II		0.17±0,03	0,008±0,002	1,3±0,2

The results were obtained in two ways: A and B.

A. Successive Two- or Threefold Introduction of the Samples and the Standards. The heights of each characteristic peak were summed over all the scans in the spectra of the sample and of the standard. The amount of each component was determined in the light of the proportionality of this magnitude to the total peak height. The existence of a linear relationship between the height of the peaks and of the amount of sample in the required range was established. The results obtained by this method were corrected by determining the relative sensitivities of the mass-spectral detector for all the samples (mass of four determinations of standard A, two determinations of standard B, and three determinations of standard C) and a calculation of the amounts of unesterified bases relative to lappaconine (standard A, samples 1-5) and of the ester-type bases relative to lappaconitine (standard B, sample 1; standard C, samples 2-5).

B. Successive Twofold Introduction of Samples 3 and 5, Standard C, and Their Sums (Method of Additives) in Order to Check the Amount of Ester-Type Bases (Table 3). Amounts were calculated by an additive scheme. As the analytical peaks we used the peaks of ions with the following m/z values: 357 (songorine), 392 (lappaconine), 408 (10-hydroxylappaconine), 436 (lycoctonine), 450 (delphatine), 511 (N-deacetyllappaconitine), 527 (sepaconitine), 553 (lappaconitine).

SUMMARY

The qualitative compositions of the mixtures of diterpene alkaloids from the roots of *A. septentrionale* from various growth sites have been studied. The number of bases detected was 21. The mass-spectrometric method of multicomponent monitoring had been used for the rapid checking of the amounts of the main components and seven subsidiary components in the mixture.

LITERATURE CITED

1. V. N. Plugar', Ya. V. Rashkes, M. G. Zhamierashvili, V. A. Tel'nov, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 80 (1982).