MASS SPECTROMETRIC METHOD OF ANALYZING THE COMBINED ALKALOIDS OF *Aconitum leucostomum*

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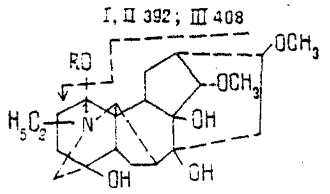
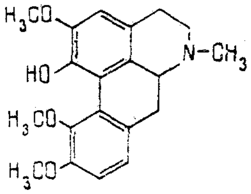
A quantitative determination of the main components of the saponified total alkaloids from the epigeal part of *Aconitum leucostomum* has been made by the integrated ion current mass-spectrometric technique. The minor components have been characterized by a combination of the method of metastable defocusing with measurements of the elementary compositions of the ions.

At the present time, the necessity for creating modern methods for the rapid evaluation of a plant raw material for the isolation of unknown components, and also of known components of practical importance, has come to a head. Purely chromatographic methods give incomplete qualitative characterization. The use of a mass-spectrometric detector in GC-MS combinations is an important step forward, but the efficacy of such a combination is limited by the complexity of selecting conditions for the satisfactory resolution of components with closely similar structures and by the long retention times of many compounds. The direct introduction of the sample expands the range of substances suitable for analysis. The complexity of the treatment of a spectrogram of a mixture introduced without preliminary separation is partially compensated in modern instruments by the possibility of checking the elementary composition of the ions and of extracting from the spectrum of a mixture a set of ions of an individual compound by recording metastable transitions. Also of no little importance is the fact that in this procedure the nanogram level of sensitivity is retained. The main requirement for such an approach is the presence of a characteristic fragment in the spectrum of each of the components being analyzed.

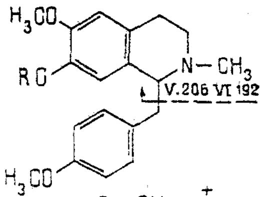
In the present paper we consider the possibility of the mass-spectrometric method for the qualitative and quantitative analysis of complex mixtures of substances isolated from plant materials using as example the previously saponified total alkaloids form the epigeal part of *Aconitum leucostomum* Worosch. collected in the fruit-bearing period in the Santash pass (Terskei-Alatau range).



I. R=H M^+ 409
II. R=CH_3 M^+ 423



IV. M^+ 341



V. R=CH_3 M^+ 327
VI. R=H M^+ 313

Quantitative determination was carried out for components (I-VI) which have previously been isolated from this material in the pure form [1]. The integrated ion current technique (IIC) [2, 3], consisting in measuring the area under the curve of the ion current of the characteristic ion with a determination of its elementary composition, was used.

A feature of the material studied is the presence in it of representatives of various groups of alkaloids: the diterpene, aporphine, and benzyltetrahydroisoquinoline groups, which differ sharply in their mass spectrometric behavior. Only in the case of the aporphines can a quantitative determination be made by measuring the peaks of the molecular ions,

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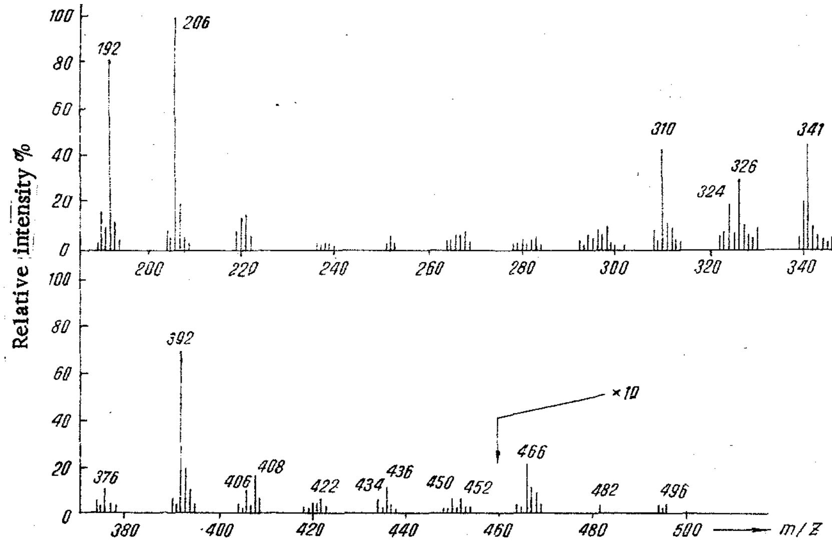

Fig. 1. Mass spectrum of the combined alkaloids isolated from the epigeal part of *A. leucostomum*.

TABLE 1. Relative Sensitivities V_i, Calibration Constants k and b, Dispersions S_0, Minimum Detectable Amounts q_{min}, and Percentage Contents of the Integral Components of the Combined Alkaloids of the Epigeal Part of *Aconitum leucostomum*

Compound	M. в.	m/z	$V_{i\,rel}$	k	b	S_0	q_{min} ~ ng	C, % Σ
I. Lappaconidine	409	353	0,064	0,9	−6	1,9	49	0,5±0,1
I.*	409	392	0,440	6,0	32	—	14	—
II. Lappaconine	423	392	1,000	34,4	116	36	5	4,0±0,1
III. Hydroxylappaconine	439	408	0,449	3,3	2	8,6	16	1,9±0,1
IV. Corydine	341	341	0,429	5,7	−27	26	4	21±2
V. O-Methylarmepavine	327	206	0,173	1,9	13	3,2	33	28±0,3
VI. N-Demethylcolletine	313	192	0,946	14,5	95	18	10	8,0±0,7 Σ (63±2)

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*Calibration constants taking the contribution of (I) to the determination of (II) into account.

because of their high intensities [4]. The molecular ions of the benzyltetrahydroisoquinoline alkaloids are extremely unstable [5], which forces the selection of the analytical fragmentary ions of the products of the ejection of substituted benzyl radicals from M^+. This fact may lower the accuracy of the determination if the mixture contains appreciable amounts of other components decomposing with the formation of tetrahydroisoquinoline fragments with the same composition.

An almost identical situation arises in the analysis of the diterpene bases. Although in this case the peaks of the molecular ions possess a medium intensity, they are frequently overlapped by the peaks of the fragmentary ions of homologs. The analytical fragments in this series are even-electron fragments formed predominantly by the splitting out of the radical from C_1 [6]. The simultaneous presence in materials of molecules hydroxy- and methoxy-substituted at C_1, the breakdown of which leads in both cases to the $(M - OR)^+$ ion is extremely common. To determine such a pair of compounds it is necessary to have in the spectrum of at least one of these compounds an additional analytical fragment. Thus, the composition of lappaconidine (I) and of lappaconine (II) gives the $(M - OR)^+$ ion with m/z 392. There are no other fragments in the lappaconine spectrum that could be used for its independent determination. However, the lappaconidine spectrum has the medium-intensity peak of the $(M - 56)^+$ ion, which is characteristic for lycoctonine bases with an α-oriented OH group at C_1, as our investigations have shown.

TABLE 2. Elementary Compositions and Origins of the Fragmentary Ions in the Mass Spectrum of the Combined Alkaloids from the Epigeal Part of *Aconitum leucostomum*

Accurate mass of the daughter ion	Relative intensities in the doublet, %	Composition				Type of skeleton*	Parental ions determined by the MD method
		C	H	N	O		
496, 2879	50	26	42	1	8	a	
496, 2529	50	25	38	1	9	b	
494, 2726	100	26	40	1	8	b	
482, 2755	100	25	40	1	8	a	
480, 2637	100	25	38	1	8	b	
464, 2708	80	25	38	1	7	b	
464, 2321	20	24	34	1	8	c	
452, 2652	100	24	38	1	7	a	467, 483
450, 2854	66	25	40	1	6	a	467, 481
450, 2520	33	24	36	1	7	b	
466, 2795	100	25	40	1	7	a	
436, 2700	100	24	38	1	6	a	451, 467
434, 2181	33	23	32	1	7	c	
434, 2569	66	24	36	1	6	b	
422, 2175	20	22	32	1	7	b	439, 453
422, 2508	80	23	36	1	6	a	
420, 2368	50	23	34	1	6	b	437, 451
420, 2743	50	24	38	1	5	a	
418, 2593	33	24	36	1	5	b	
418, 2201	66	23	32	1	6	c	
408, 2402	100	22	34	1	6	a	423, 439
406, 2242	66	22	32	1	6	b	423, 437
406, 2578	33	23	36	1	5	a	
390, 2289	100	22	32	1	5	b	407, 421, 437
378, 2602	20	22	36	1	4		
378, 2282	80	21	32	1	5	a	
376, 2493	60	22	34	1	4	a	393, 409
376, 2121	40	21	30	1	5	b	
353, 2202	100	18	31	1	5		
		Tetrahydroisoquinolines					
221, 1032	66	12	15	1	3		287, 305, 319, 407
221, 136	33	13	19	1	3		
220, 0970	60	12	14	1	3		
220, 1327	40	13	18	1	2		
206, 0794	33	11	12	1	3		221, 235, 249, 263, 327, 343
206, 1179	66	12	16	1	2		
204, 1034	100	12	14	1	2		207, 221, 235, 249, 313, 327, 341
192, 1019	100	11	14	1	2		
190, 1237	33	12	16	1	1		
190, 0862	66	11	12	1	2		
178, 0853	10	10	12	1	2		193, 207
178, 0462	90	9	8	1	3		

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*For the interpretation of the type of skeleton, a, b, c, see text.

Having determined the amount of lappaconidine present by means of this ion and having measured the area under the curve of the ion current of the fragment with m/z 392, the contribution made to this magnitude by lappaconidine (I) was deducted and the amount of lappaconine (II) was determined by difference.

Lappaconine (II) and hydroxylappaconine (III) are the products of the saponification of lappaconitine and hydroxylappaconitine, respectively. The latter has the developed formula $C_{18}H_{18}(OCH_3)_3(OH)_3N{-}C_2H_5(OCOC_6H_5NHCOCH_3)$, coinciding with the formula of the alkaloid ramaconitine [9] but with a ^{13}C NMR spectrum differing from that of this base. According to mass spectroscopy, hydroxylappaconitine contains a methoxy group at C_1, because of which the quantitative determination of hydroxylappaconine (III) (M^+ 439) was performed from the peak of the $(M - 31)^+$ ion with m/z 408, with a deduction of the contribution made to this mass by the $(M - 15)^+$ ions of lappaconine.

The aporphine base corydine (IV) was determined from its molecular peak with m/z 341, and the benzyltetrahydroquinolines O-methylarmepavine (V) and N-demethylcolletine (VI) from the fragments with m/z 206 and 192, respectively. As can be seen from the spectrum of the combined alkaloids (Table 1), all the analytical peaks apart from the peak of the ion with m/z 353 $(M - 56)^+$ occupy commanding positions in it. The results of a determination of the quantitative composition of the combined alkaloids are given in Table 1. The relative sensitivities for the components of the combined material were calculated from a formula given in the literature [7]. The maximum sensitivity was taken as 1.000 (II). As can be seen from Table 1, the range of relative sensitivities for the components being analyzed is fairly wide. The minimum detectable amount of the individual components also varies widely (see Table 1).

The equation of the calibration functions are satisfactorily approximated by the straigh line

$$S=b+kq, \tag{1}$$

where S is the area under the curve of the ion current for the sample determined; and q is the weight of the substance, ng.

The coefficients b and k were obtained by the method of regression analysis [8]. The accuracy of the calibration equations is characterized by the dispersion S_0 (Table 1).

Thus, quantitatively, the main components of the combined material are the benzyltetrahydroquinoline bases (V) and (VI) and the aporphine alkaloid corydine (IV) (Table 1). The diterpene bases (I), (II), and (III) were detected in an amount of only 6.4%. The components analyzed amounted in total to 63 ± 2% of the amount taken for analysis (≈ 3 mg). However, in the mass spectrum of the combined material in the region of higher mass numbers there is a series of peaks of ions of medium and low intensities which may relate to diterpene bases with a higher degree of oxidation not detected on chemical separation. We confirmed the soundness of this suggestion by two methods. In the first place, by measurements of the elementary compositions of the ions with even mass numbers in the interval of 376-496 a.m.u. All the compositions shown correspond to homologous series of ions $(M - OR)^+$, $(M - CH_3)^+$, and $(M - H_2O - CH_3)^+$ of C_{18} and C_{19} diterpene bases. These compositions can be divided into three groups according to their degree of unsaturation:

a) $C_nH_{2n-10}O_mN$ (a C_{18} of the type of lappaconine and a C_{19} base of the type of lycoctonine);

b) $C_nH_{2n-12}O_mN$ (a C_{18} base of the type of excelsine and a C_{19} base of the type of eldeline, and also alkaloids with a carbonyl group or a double bond); and

c) $C_nH_{2n-14}O_mN$ (C_{18} bases with additional ether bridges and a methylenedioxy group) (Table 2).

The second method, confirming the fact that these ions belong to diterpene bases, consists in a search for the parental ions corresponding to them, which was made by the method of defocusing the ion beam in the first field-free space of a high-resolution mass spectrometer. For each of the daughter ions with an even mass and sufficient intensity we found two precursor ions. The differences between the parental and the daughter ions amounted, as a rule, to 17 and 31 a.m.u., which is most characteristic for these type of diterpene bases. Examples of the recording of the metastable transitions are given in Fig. 2. Because of the low resolving capacity of the defocusing method, the $M^+ \to (M - 17)^+$ and $M^+ \to (M - 31)^+$ transitions may be marked by the $M^+ \to (M - 15)^+$ and the $M^+ \to (M - 33)^+$ transitions which are also characteristic of diterpene alkaloids. Table 2 gives the mass numbers of the probable molecular ions determined by this method.

So far as concerns the minor components of the benzyltetrahydroisoquinoline series, their presence was confirmed by the following facts. One third of the total intensity of the peaks of ions with m/z 206 is due to ions with the composition $C_{11}H_{12}NO_3$ corresponding to compounds containing methylenedioxy and hydroxy groups in the tetrahydroisoquinoline nucleus.

Two thirds of the total intensity of the peak with m/z is due to $C_{12}H_{16}NO_2$ ions from the analogous part of the O-methylarmepavine molecule.

As mentioned above, the presence of ions with a different elementary composition does not interfere with the determination. On the other hand, as a precursor of the ion with m/z 206 we detected, in addition to the ion with m/z 327 from O-methylarmepavine, an ion with m/z 343, which may be the molecular ion of a compound with a hydroxy and a methoxy group in the benzyl moiety of the alkaloid.

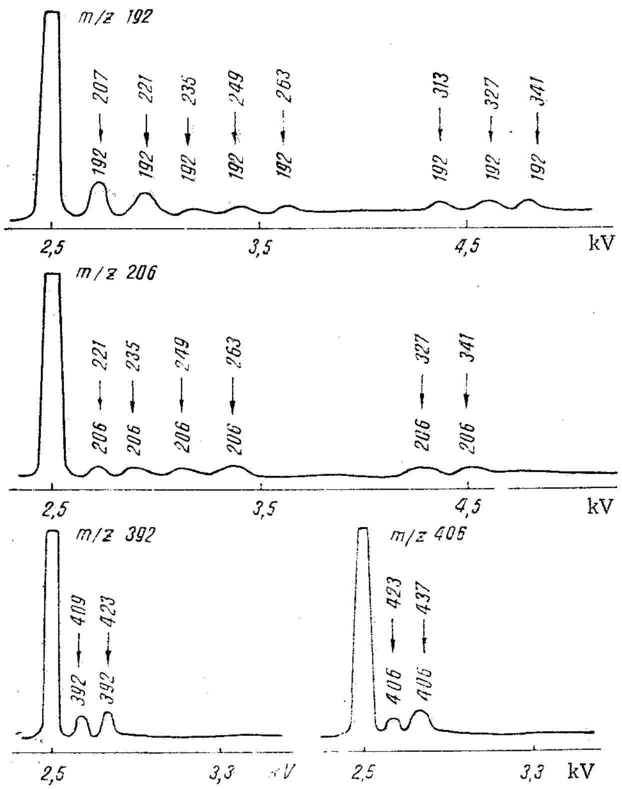

Fig. 2. MD spectra of some daughter ions of the combined alkaloids of the epigeal part of *A. leucostomum*.

An ion with m/z 192 in the spectrum of the combined material agrees completely with the composition $C_{11}H_{14}NO_2$, corresponding to N-demethylcolletine. However, in this case the defocusing method showed, together with a precursor ion having m/z 313 (M^+, VI), the presence of parental ions with mass numbers of 327 and 341, which, judging from their masses, may belong, for example, to oxybenzyl- or phenyltetrahydroisoquinoline alkaloids.

The analytical fragments of the benzyltetrahydroisoquinolines (m/z 178, 192, 206) also have precursors that can be characterized on the basis of measured metastable transitions as molecular ions of alkyltetrahydroisoquinolines (for example, the transitions 263, 249, 235, 221 → 206; see Table 2). Thus, the total material taken for analysis has shown a complex qualitative composition. In addition to the main components isolated chemically (I-VI), a considerable amount of minor components has been found for which, on the basis of measurements of the elementary compositions of the ions and of metastable defocusing spectra preliminary structural suggestions have been made.

The results of our work do not contradict those of a determination of the relative amounts of diterpene and isoquinoline alkaloids in the same material of the GLC method [1].

EXPERIMENTAL

The analysis of the combined material was carried out on a MKh 1310 instrument with a SVP-5 system of direct introduction. The temperature of the ionizing chamber was 150-170°C, the collector current 60 μA, and the ionizing voltage 50 V. The resolving power of the instrument in measurements of elementary composition was 10,000, and in the recording of the total material and in the use of the method of metastable defocusing (MD) it was 2000. The MD spectra were recorded with the scanning of the accelerating voltage from 2.5 to 5 kV.

Quantitative Analysis. The calibration of the instrument with respect to the analytical peaks of substances (I-VI) was carried out as described previously [3]. Within the range of samples weighing $8.4 \cdot 10^{-7}$ to $8.4 \cdot 10^{-9}$ g the calibration factors were calculated by this method [3] and by the method of regression analysis [8]. The results proved to be close. Calibration graphs were plotted in the coordinates S (mm^2) versus q (ng). The amount of the substance being analyzed was calculated, taking the sensitivity of the instrument into account, from the formula

$$q_{\Sigma} = q_{\text{st}} - \frac{S_{\text{st}} - S_{\Sigma}}{k}, \quad (2)$$

where q_{Σ} and q_{st} are the amounts of components being analyzed in aliquots of solutions of the combined alkaloids and of a standard, respectively; S_{Σ} and S_{st} are the areas of the curves of the ion current of the total material and of the standard, respectively; and k is a calibration parameter (see Table 1).

CONCLUSION

A quantitative determination of the main components of the combined alkaloids from the epigeal part of *Aconitum leucostomum* (in the fruit-bearing period), amounting to about 63% of the total, has been made by the integrated ion current mass-spectrometric technique. Measurements of elementary compositions and the study of metastable transitions have revealed the existence of a number of minor components.

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