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*Eda GRÜNER\**, *Aleksander-Mati MÜÜRISSEPP\**, and *Milana LIIV\**

## CHROMATO-MASS SPECTROMETRIC INVESTIGATION OF THE ETHANOL EXTRACT OF MINERAL WAX (MUMIE)

Mineral wax (mumie) has been used in Oriental folk medicine for centuries. However, very little is known about its chemical composition. In all the respective investigations (carried out mostly by biologists and pharmacists) attempts have been made to separate one or several compounds from the mixture and subsequently identify them<sup>[1-3]</sup>. Obviously such an approach is not justified in investigating a complex natural mixture. Moreover, low-concentration components, whose separation from the mixture is practically impossible, may be of great importance from a pharmacological point of view.

Chromato-mass spectrometry is a suitable method for the analysis of complex mixtures. There is no need for the separation of components and even trace concentration components may be identified.

This paper reports the results of chromato-mass spectrometric investigations of the ethanol extract of mineral wax. No speculations were made about its pharmacological qualities, only the chemical composition of mumie was identified.

### Experimental

The ethanol extract from the Altai mineral wax (mumie) was investigated. Preparative thin-layer chromatography (TLC) on silica gel L 100/160  $\mu$ , with *n*-hexane, ether, acetone, ethyl acetate, ethanol, and finally with a mixture water:ethanol (1:5) was used for fractionation.

The acetone fraction (9.1% of ethanol extract of mumie) was separated into two parts by TLC: (1) fraction A1 with  $R_f$  0.62—1.00 having an intensive radiation under UV, and (2) fraction A2 with  $R_f$  0.05—0.62.

The investigations were carried out using a Hitachi M-80B gas chromatograph double-focusing mass spectrometer. Quartz capillary columns 15 m  $\times$  0.52 mm with polar liquid phase SUPEROX and 30 m  $\times$  0.32 mm with nonpolar liquid phase RSL-150 were used. The temperature was programmed from 100 to 250 °C, rate 10 °C/min in case of the polar phase and from 100 to 280 °C, rate 10 °C/min for the nonpolar phase. The sample amount was 0.14  $\mu$ l on the column with RSL-150 and 1.4  $\mu$ l on the column with SUPEROX.

The mass range of mass spectra was 0—500 *m/z* and scanning speed 1.0 s. The techniques of electron impact, chemical ionisation with isobutane, and high resolution mass spectrometry were used.

\* Eesti Teaduste Akadeemia Keemia Instituut (Institute of Chemistry, Estonian Academy of Sciences). Akadeemia tee 15, EE0108 Tallinn, Estonia.

## Results and Discussion

Fig. 1 shows gas chromatograms of the fractions A1 and A2 on SUPEROX and RSL-150 capillary columns, respectively. Numbers of peaks indicate the substances in the list.

The peaks identification is illustrated on the example of peak 32. An electron impact spectrum served as a base spectrum for automatic library search (see Fig. 2a). Hitachi library containing 73 000 mass spectra was used. Five spectra having the highest similarity indexes (S. I.) were chosen from the library. In addition to the electron impact spectrum (Fig. 2b) a chemical ionisation spectrum was used to establish molecular mass (Fig. 2c). A quasi-molecular ion (MH<sup>+</sup>) having m/z 181 showed molecular mass of the unknown compound to be 180 amu. This allowed us to exclude the two first library spectra.

A high resolution spectrum (Fig. 2d) was used to establish the elemental composition of molecular and fragment ions. So, compound 32 was identified as mandelic acid ethyl ester.

### Substances identified in the acetone fraction of the ethanol extract of Altai mineral wax

1. Benzoic acid
2. Salicylic acid
3. Trimethylbenzoic acid
4. Anisic acid
5. Cumic acid
6. *m*-Oxybenzoic acid
7. *p*-Oxybenzoic acid
8. Vanillic acid
9. Cyclohexanecarboxylic acid
10. Cyclohexanecarboxylic acid
11. Acetic acid
12. Phenylacetic acid
13. Mandelic acid
14. Methylmandelic acid
15. Morpholinoacetic acid
16. Hippuric acid
17. Phenylpropionic acid
18. Levulinic acid
19. Myristic acid
20. Pentadecanoic acid
21. Palmitic acid
22. Oleic acid
23. Stearic acid
24. Phthalic acid
25. Benzoic acid ethyl ester
26. Trimethylbenzoic acid ethyl ester
27. Cyclohexanecarboxylic acid ethyl ester
28. Cyclohexanecarboxylic acid ethyl ester
29. Phenylacetic acid ethyl ester
30. Phenylpropionic acid ethyl ester
31. Hippuric acid ethyl ester
32. Mandelic acid ethyl ester
33. Methylmandelic acid ethyl ester
34. Lactic acid ethyl ester
35. Levulinic acid ethyl ester
36. Myristic acid ethyl ester
37. Pentadecanoic acid ethyl ester
38. Palmitic acid ethyl ester
39. Heptadecanoic acid ethyl ester
40. Stearic acid ethyl ester
41. Oleic acid ethyl ester
42. Pimelic acid diethyl ester
43. Pentadecanoic acid methyl ester
44. Cyclohexylbenzoate
45. Phthalic acid dibutyl ester
46. Phthalic acid dioctyl ester
47.  $\delta$ -Laurolactone
48. 1,2-Cyclohexanediole
49. Diacetone alcohol
50. Mesityl oxide
51. 1,2-Isopropylidene glycerol
52. Phenylglycol
53. Phenol
54. *p*-Cresol
55. Cuminal
56. Lanol
57. *o*-Methoxy-cyclohexenylethylketone
58. Squalene
59. Tetracosane
60. Dimethylsulfone
61. Diethylsulfone
62. Acetamide
63. Butamide
64. Ethylcarbamate
65. Acetylpyrrolidine



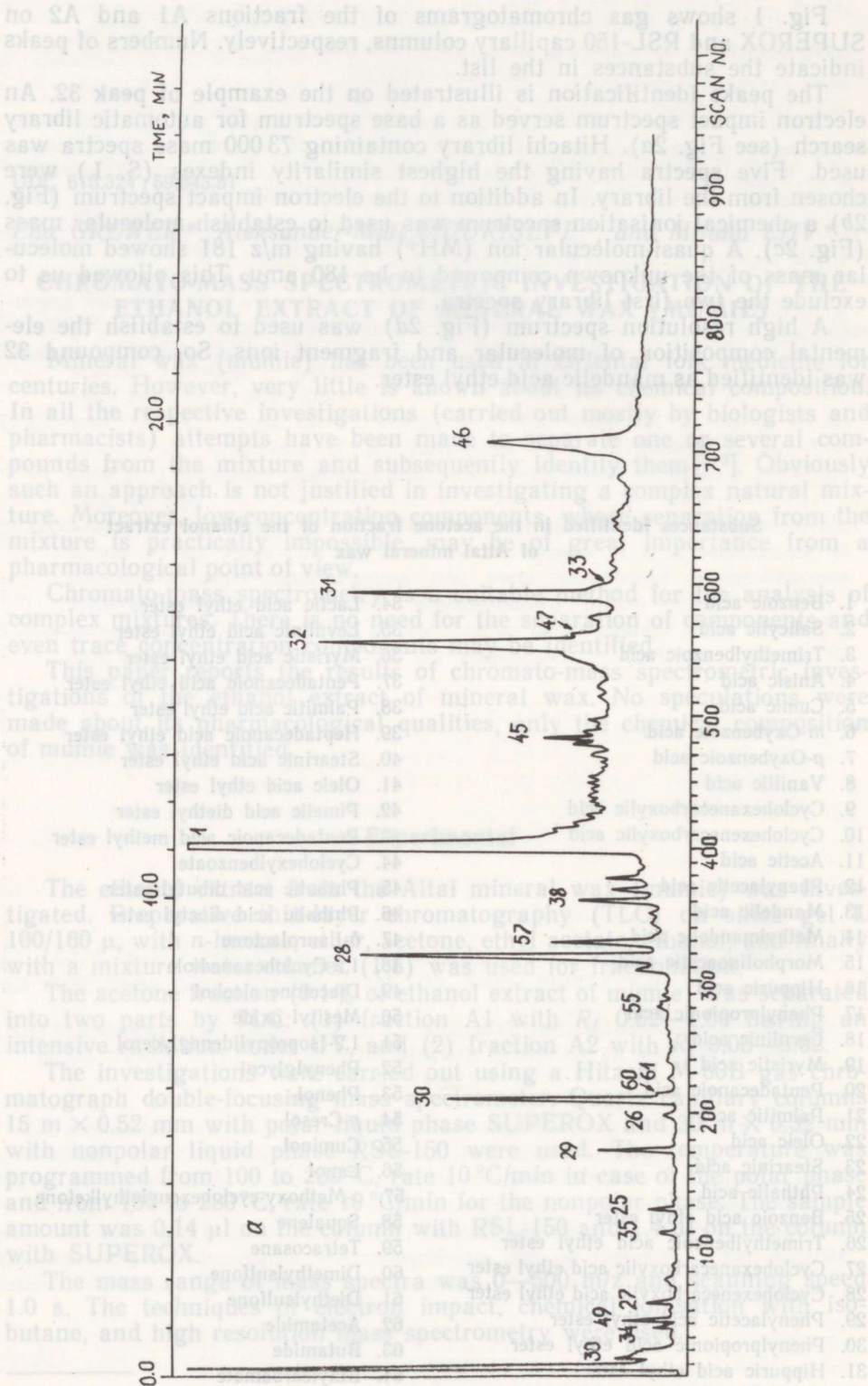


Fig. 1 shows gas chromatograms of the fractions A1 and A2 on SUPEROX RSL-150 capillary columns, respectively. Numbers of peaks indicate the substances in the list.

The peak identification is illustrated on the example peak 32. An electron impact spectrum served as a base spectrum for a systematic search (see Fig. 2). Hitachi library containing 73 000 mass spectra was used. Five compounds having the highest similarity index (2.1) were chosen from the library. In addition to the electron impact spectrum (Fig. 2b), a chemical ionization spectrum was used to establish molecular ions (Fig. 2c). A molecular ion (MH<sup>+</sup>) having m/z 181 showed molecular ions composed of m/z 180 ions. This ion was excluded by the library search. A high resolution mass spectrum (Fig. 2d) was used to establish the elemental composition of molecular ions. Fragment ions 20 compound 32 was usually a hydroxy acid ester. It still very interestingly and obviously to the most significant in the mixture of the fraction A2. A chromatogram of the fraction A2 is shown in Fig. 3. The chromatogram shows a complex mixture of compounds. The most significant peaks are labeled with numbers 1 through 61. The peak at 100 minutes is labeled with the Greek letter alpha (α). The chromatogram shows a complex mixture of compounds. The most significant peaks are labeled with numbers 1 through 61. The peak at 100 minutes is labeled with the Greek letter alpha (α).

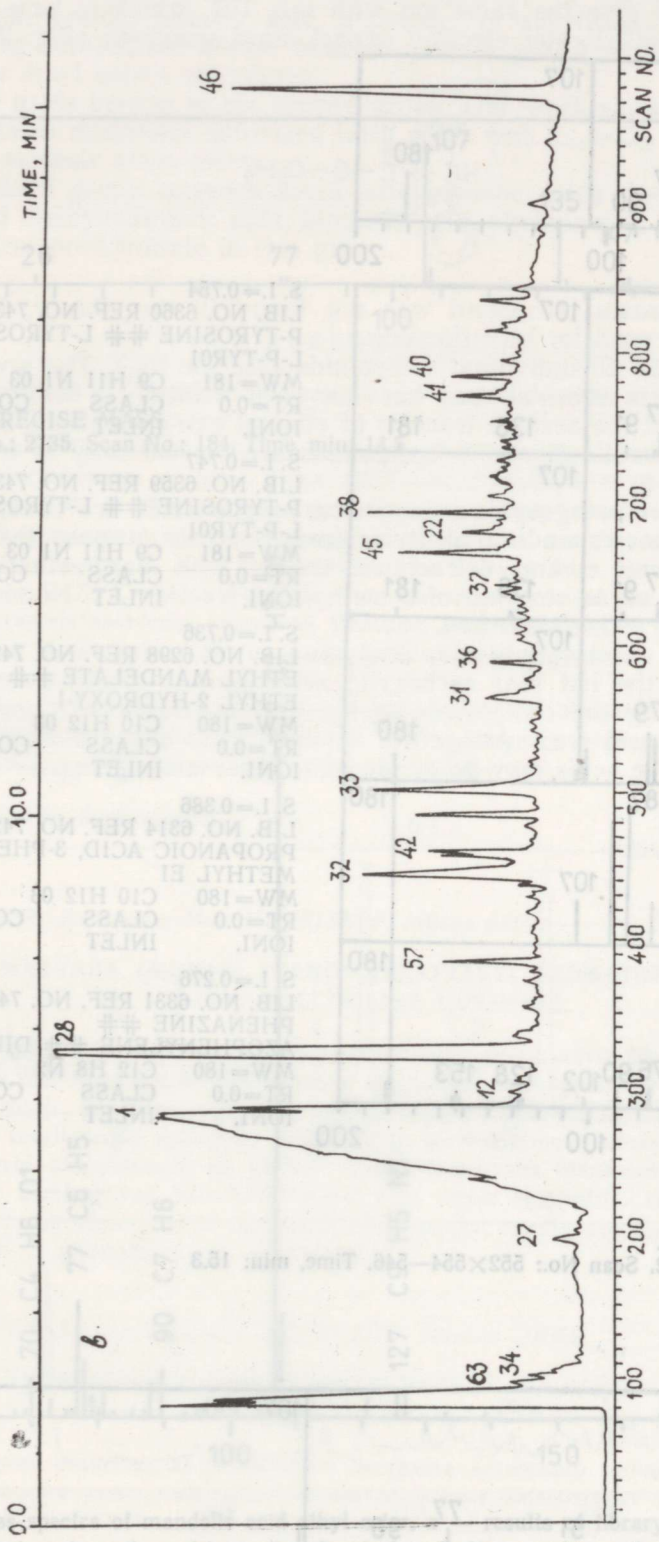
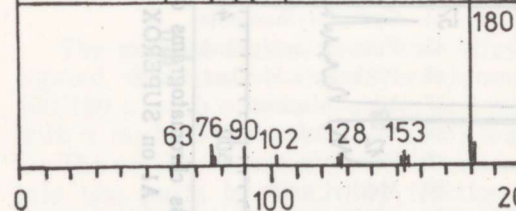
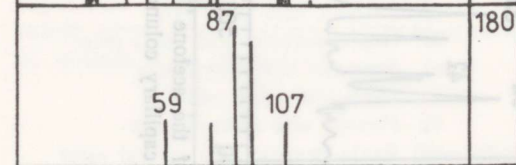
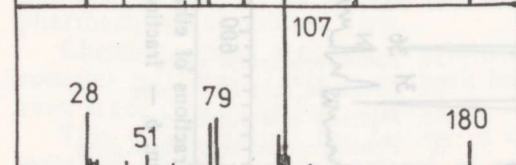
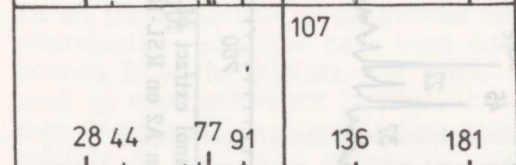
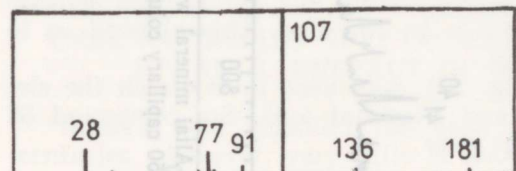
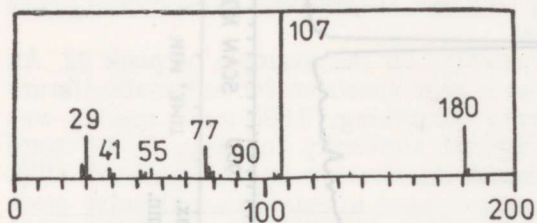


Fig. 1. Mass chromatograms of the acetone fractions of ethanol extract of Altai mineral wax. *a* — fraction A1 on SUPEROX capillary column; *b* — fraction A2 on RSL-150 capillary column.

M10-N2. 1.4MKL.

Sample No.: 2572. Scan No.: 554-546. Time, min: 15.4

**a**



S. I.=0.754

LIB. NO. 6360 REF. NO. 74325

P-TYROSINE ## L-TYROSINE ##  
L-P-TYR01

MW=181 C9 H11 N1 O3

RT=0.0 CLASS CON. RMSD  
IONI. INLET

S. I.=0.747

LIB. NO. 6359 REF. NO. 74324

P-TYROSINE ## L-TYROSINE ##  
L-P-TYR01

MW=181 C9 H11 N1 O3

RT=0.0 CLASS CON. RMSD  
IONI. INLET

S. I.=0.736

LIB. NO. 6298 REF. NO. 74262

ETHYL MANDELATE ##  
ETHYL 2-HYDROXY-I

MW=180 C10 H12 O3

RT=0.0 CLASS CON. RMSD  
IONI. INLET

S. I.=0.386

LIB. NO. 6314 REF. NO. 74278

PROPANOIC ACID, 3-PHENOXY-,  
METHYL EI

MW=180 C10 H12 O3

RT=0.0 CLASS CON. RMSD  
IONI. INLET

S. I.=0.276

LIB. NO. 6331 REF. NO. 74296

PHENAZINE ##  
AZOPHENYLENE ## DIBENZ

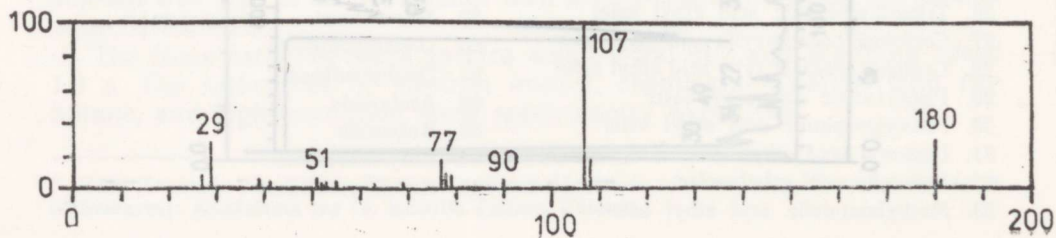
MW=180 C12 H8 N2

RT=0.0 CLASS CON. RMSD  
IONI. INLET

M10-N2. 1.4MKL.

Sample No.: 2572. Scan No.: 552×554-546. Time, min: 15.3

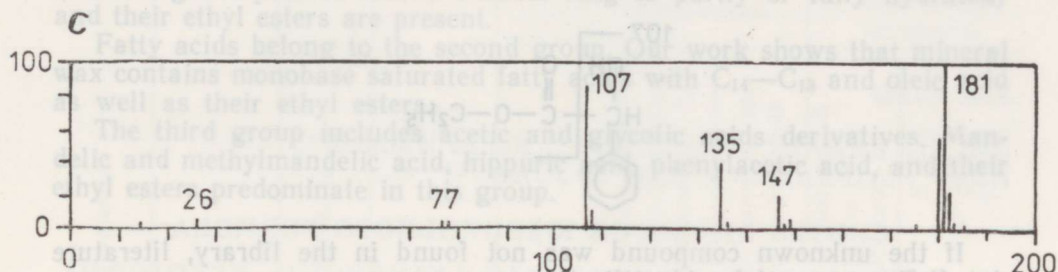
**b**





M10-N2 CI SUPEROX 15M×0.53

Sample No.: 2679. Scan No.: 719-714. Time, min: 14.9



M10-N2 PRECISE MASS

Sample No.: 2735. Scan No.: 184. Time, min: 14.5

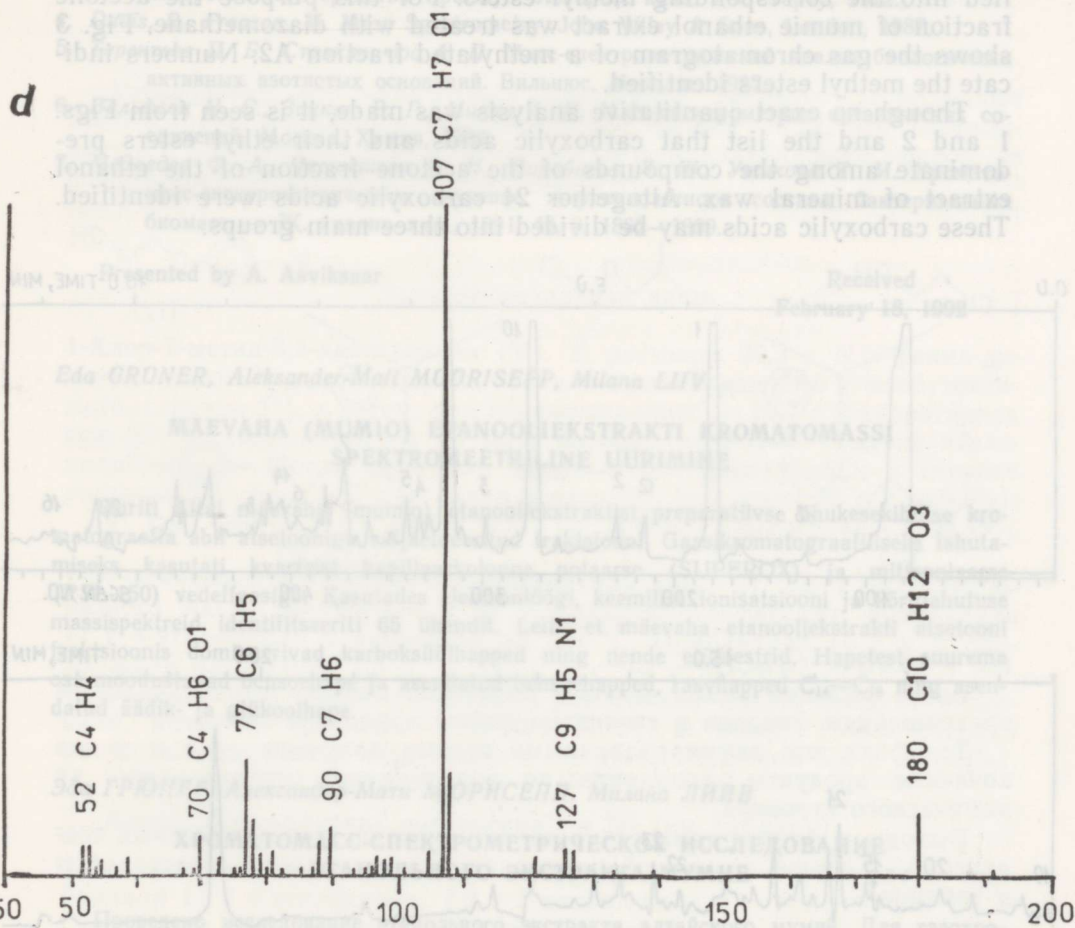
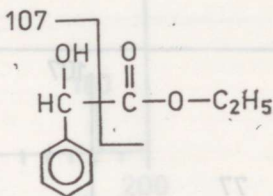


Fig. 2. Mass spectra of mandelic acid ethyl ester. *a* — results of library search for peak 32; *b* — electron impact spectrum; *c* — chemical ionisation spectrum; *d* — high resolution mass spectrum.

The principle cleavage of carbonyl compound is  $\alpha$ -to CO group and, in another way, preferential cleavage occurs  $\beta$ -to the benzene ring. Both fragmentations give the same ion with  $m/z$  107, which is base peak in mandelic acid ethyl ester electron impact mass spectrum (Fig. 2b).



If the unknown compound was not found in the library, literature data [4-7] were used for identification.

We identified 65 individual compounds (see the list). The presence of most of them in mineral wax has not been mentioned before. It was established that the acetone fraction of ethanol extract consists of mainly O compounds, while N and S compounds are present only in trace concentrations.

For gas chromatographic analysis carboxylic acids are usually modified into the corresponding methyl esters. For this purpose the acetone fraction of mumie ethanol extract was treated with diazomethane. Fig. 3 shows the gas chromatogram of a methylated fraction A2. Numbers indicate the methyl esters identified.

Though no exact quantitative analysis was made, it is seen from Figs. 1 and 2 and the list that carboxylic acids and their ethyl esters predominate among the compounds of the acetone fraction of the ethanol extract of mineral wax. Altogether 24 carboxylic acids were identified. These carboxylic acids may be divided into three main groups.

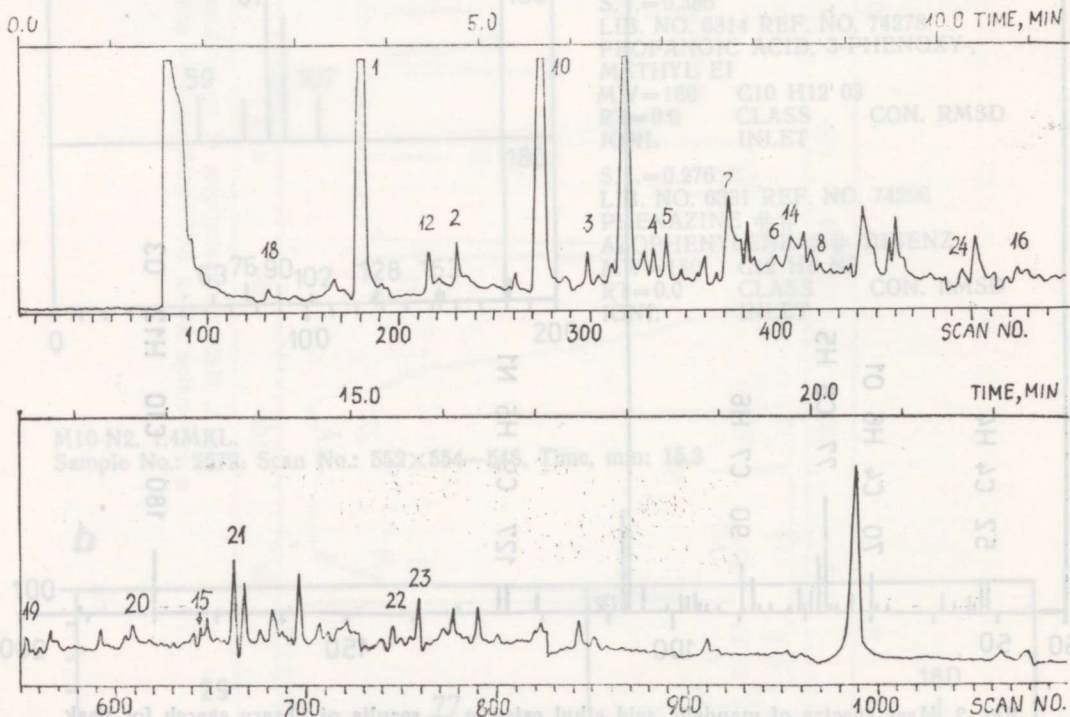


Fig. 3. Mass chromatogram of the methylated fraction A2 on RSL-150 capillary column,



The first group consists of derivatives of benzoic acid. Benzoic acid has been identified by almost all investigators [1-3]; our work, however, shows that besides benzoic acid, a number of substituted benzoic acids (including compounds whose benzoic ring is partly or fully hydrated) and their ethyl esters are present.

Fatty acids belong to the second group. Our work shows that mineral wax contains monobase saturated fatty acids with  $C_{14}$ — $C_{18}$  and oleic acid as well as their ethyl esters.

The third group includes acetic and glycolic acids derivatives. Mandelic and methylmandelic acid, hippuric acid, phenylacetic acid, and their ethyl esters predominate in this group.

#### REFERENCES

1. Корчубеков Б. К. Мумиё «Архар-таш» и его физиологическая активность. Фрунзе, Илим, 1987.
2. Порошин К. Т., Давидянц С. Б., Кириченко Л. Н. Химическое исследование органической части мумиё. — Докл. АН ТаджССР, 1964, 7, 7, 18—20.
3. Сыровежко И. В. Материалы к фармакогностическому исследованию тибетского лекарственного средства Брагшун. Автореф. канд. дис. Тарту, 1972.
4. Davis, R., Freason, M. Mass Spectrometry. John Wiley & Sons, London, 1987.
5. Терентьев П. Б., Станкявичюс А. П. Масс-спектрометрический анализ биологически активных азотистых оснований. Вильнюс, Моклас, 1987.
6. Вульфсон Н. С., Заикин В. Г., Микая А. И. Масс-спектрометрия органических соединений. Москва, Химия, 1986.
7. Медведев Ф. А., Чернышева О. Н., Воробьева Л. Ш., Ушакова Т. М. Хромато-масс-спектрометрическое изучение жирнокислотного состава бактериальных биомасс. — Ж. аналит. хим., 1991, 46, 9, 1862—1869.

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Eda GRÜNER, Aleksander-Mati MÕURISEPP, Milana LIIV

#### МÄЕВАНА (MUMIO) ETANOLIEKSTRAKTI KROMATOMASSI SPEKTROMEETRILINE UURIMINE

Uuriti Altai mäevaha (mumio) etanooliekstraktist preparatiivse õhukesekihilise kromatograafia abil atsetooniga väljaelueeritud fraktsiooni. Gaasikromatograafiliseks lahutamiseks kasutati kvartsist kapillaarkolonne polaarse (SUPEROX) ja mittepolaarse (RSL-150) vedelfaasiga. Kasutades elektronloogi, keemilise ionisatsiooni ja kõrglahutuse massispektreid identifitseeriti 65 ühendit. Leiti, et mäevaha etanooliekstrakti atsetooni fraktsioonis domineerivad karboksüülhapped ning nende etüülestrid. Hapetest suurema osa moodustavad bensoehape ja asendatud bensoehapped, rasvhapped  $C_{14}$ — $C_{18}$  ning asendatud äädik- ja glükoolhape.

Эда ГРЮНЕР, Александер-Мати МЮРИСЕПП, Милана ЛИИВ

#### ХРОМАТОМАСС-СПЕКТРОМЕТРИЧЕСКОЕ ИССЛЕДОВАНИЕ ЭТАНОЛЬНОГО ЭКСТРАКТА МУМИЕ

Проведено исследование этанольного экстракта алтайского мумиё. Для газохроматографического разделения использованы кварцевые капиллярные колонки с жидкими фазами SUPEROX и RSL-150. На основании масс-спектров электронного удара, химической ионизации и высокого разрешения идентифицировано 65 соединений. Среди них доминируют карбоксильные кислоты и их этиловые эфиры.