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Chemical fingerprinting for the study of living systems

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Abstract

Results obtained in the studies of living systems using chemical fingerprinting methodology are presented. Gas chromatography – mass spectrometry and the tools of chemical fingerprinting were applied to obtain the chromatographic profiles and to determine the isomeric-homologous series of characteristic target metabolites of various classes of the objects of plant origin and cuticular lipids of insects at different stages of ontogenesis and living in different climatic conditions. The main target metabolites considered herein are hydrocarbons, ketones, alcohols, fatty acids and their hydroxy-derivatives, esters, alkylresorcinols, di- and triterpenoids, phenol acids, and lignans. The approach used in the work makes it possible to establish the composition of characteristic target metabolites in various living systems and their changes in the processes occurring under the influence of abiotic and biotic factors, targeted gene mutations, which can be used both in fundamental and applied research, including the improvement of approaches to biological control of economically important insect pests in agriculture.

Keywords: chemical fingerprinting, gas chromatography, mass spectrometry, characteristic target metabolites, chromatographic profiling, living systems, biologically active substances

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INTRODUCTION

Chemical fingerprinting is considered to be the basis of environmental forensics [1–3], with the goal to reveal chemical traces (characteristic compounds and their ratios) of any situations or processes, either of anthropogenic or natural origin.

In spite of the fact that fingerprinting (identification based on fingerprint pattern) is historically associated with forensic examination, in a wider sense this term is used in environmental science and protection, medicine, biology, phytochemistry, genetics, pharmaceutics and other sciences [4, 5]. It should be noted that the major

analytical approaches associated with chemical fingerprinting had been formed in such areas as petroleum chemistry and geochemistry [6–8].

Due to the versatility of chemical fingerprinting tools, they can be used e efficiently to establish chemical composition and structure of the characteristic target metabolites of living systems (plants, insects, animals, etc.) and their changes under the action of various factors.

In the area of applied tasks, a relevant issue is to reveal the promising sources of biologically active compounds, to standardise natural raw materials, to identify the marker components of biologically active products and preparations for quality control, to determine authenticity of preparations and products based on natural raw materials [9].

Instrumentation platforms that are most frequently used in chemical fingerprinting include gas chromatography – mass spectrometry (GC-MS), liquid chromatography – mass spectrometry (LC-MS), nuclear magnetic resonance. Gas chromatography – mass spectrometry with electron impact ionisation is considered to be the best standardised method because of high reproducibility, sensitivity, selectivity and availability of the information and databases on retention indices and mass spectra [10–14].

Application of chemical fingerprinting tools allows identifying not only the target metabolites but also isomeric-homologous groups in which the compounds with either high or low content can be determined, and the markers of systems and processes can be revealed [9, 15].

Chemical fingerprinting used to study living systems and processes that take place in living systems involves the following basin tools [1, 9]:

1) development of the informational-analytical legend of the objects under investigation;

2) detection and prediction of the target marker metabolites of an object or process under investigation on the basis of literature data;

3) selection of an instrumental method to study target metabolites;

4) analysis and selection of the procedures for targeted sample preparation;

5) experimental multi-vector chromatographic profiling (fingerprinting);

6) determination of marker compounds and their diagnostic characteristics;

7) utilisation of the algorithm to reveal characteristic ions in the mass spectra of metabolites for their identification in complex hard-to-separate mixtures;

8) analysis for biochemical feasibility imposing constraints on the structure of metabolites as following from the biochemical routes of the synthesis of these compounds, which is especially important for the analysis of isomeric compounds [16–18];

9) application of statistical methods for data processing.

Thus obtained results can be used to determine the composition of characteristic target metabolites of different objects related to living systems; to reveal the regularities of structure, content and dynamics of metabolites, their changes in the processes taking place under the effect of abiotic and biotic factors, directed gene mutations and selection; to establish chemotaxonomic features; to analyse and search for biologically active substances, to reveal the possibilities of application in medicine, agriculture, and other branches of the national economy.

An interesting direction of the studies in this area is environmental metabolomics, with its goal to analyse the target metabolites in the components of complex ecosystems (forest, aquatic, soil, etc.) under the conditions of changing environment [19–21], including anthropogenic pollution.

The goal of the present work is to demonstrate the potential of using a united chemical fingerprinting methodology based on chromatographic – mass spectrometric identification to establish the chemical composition and characteristic target metabolites of various living objects and their changes under the action of abiotic and biotic factors. This is one of the most important goals of environmental studies.

EXPERIMENTAL

The objects of investigation were the samples of plant origin and insects at different stages of ontogenesis living under different climatic conditions. The samples of plant origin were the leaves and stems of barleycorn (*Barley*) obtained as a result of targeted gene editing by the researchers at the Institute of Cytology and Genetics SB RAS (ICG SB RAS) in 2022; fresh wood of Siberian larch (*Larix sibirica*) sampled in 2007; needles of Siberian pine (*Pinus sibirica*) sampled in the Kargat District of the Novosibirsk Region by researchers from the Institute of Systematics and

Ecology of Animals SB RAS (ISEA SB RAS) in 2022; needles of Siberian larch and leaves of common birch (*Betula pendula*) collected by researchers from ISEA SB RAS in 2021 at the territory of West Siberia within the latitudinal transect extending for ~1000 km: at the Altai Territory (Karasi, Shelabolikha, Zavyalovo settlements), the Novosibirsk Region (Novosibirsk city, Ob settlement) and the Tomsk Region (Kargasok, Novokolomino, Gusevo, Melnikovo, Bazoy settlements), synchronously with the time of gypsy moth (*Lymantria dispar*) larvae eclosion. To study the individual-group composition of native triterpenoids of the leaves of common birch, sampling was carried out in the Karasuk District of the Novosibirsk Region at the gypsy moth outbreak area in May 2015. The insect samples for the studies of the composition of epicuticular lipids were:

1) bee moth (*Galleria mellonella*) of the laboratory population grown at ISEA SB RAS in 2019;

2) Colorado potato beetle (*Leptinotarsa decemlineata*) in ontogenesis, sampled in 2019 from the potato fields of the Karasuk Research station at the territory of the Novosibirsk Region (53°44′3.534″ N; 77°39′0.0576″ E) in mid-July, grown subsequently at the Laboratory of ISEA SB RAS to the required age or stage;

3) two locust species with different hygrothermal preferences – Italian locust (*Calliptamus italicus*, 43.518412° N; 76.830550° E) and migratory locust (*Locusta migratoria*, 45.385162° N; 75.249230° E), sampled in May 2019 at natural stations in South-eastern Kazakhstan (the Almaty Region) by researchers from the Novosibirsk Institute of Organic Chemistry SB RAS (NIOC SB RAS) and ISEA SB RAS.

To analyse the qualitative and quantitative composition of lipids in the epicuticular wax of barley leaves and stems, we prepared the extract by washing the plant raw material with trichloromethane for 30 s. To study the composition of nonpolar and low-polar metabolites of larch and pine needles, extraction of the plant material with isopropyl alcohol was carried out in Soxhlet apparatus, followed by re-extraction with petroleum ether and separation according to the standard scheme into the groups of neutral compounds and acids [22].

To study the native composition of triterpenoids in birch leaves, extraction with methanol was carried out at a temperature of 20–25 °C [23]. The CO_2 extract was obtained from freshly chopped larch wood [24]. The extracts of epicuticular lipids of wax moth and Colorado beetle were obtained by washing for 5 min with a mixture of *n*-hexane with methyl-*tert*-butyl ether (1 : 1, by volume) [25, 26], Italian locust and Asiatic locust – with a mixture of *n*-hexane and diethyl ether (1 : 1, by volume) [27].

For acid methanolysis of bound and free fatty, diterpenic, benzoic and cinnamic acids, a 1 % solution of H_2SO_4 in methanol was used [28], for free fatty acids – the solution of diazomethane (CH_2N_2) in diethyl ether was used [29].

The samples were analysed by GC-MS using a GC 6890N gas chromatograph with mass-selective detector MSD 5975N and automatic sampler 7683B (Agilent Technologies, USA) in the total ion current mode. The samples for GC-MS analysis were dissolved in acetone or in a mixture of acetone with hexane. The components under analysis were separated on a capillary quartz column HP-5 MS 30 m long, 0.25 mm in diameter, and phase thickness 0.25 μm under the following conditions: injector temperature 280 °C, ion source temperature 230 °C, quadrupole temperature 150 °C, carrier gas – helium, the volume of sample introduced into the chromatograph – 1 μL. Two temperature modes of the column thermostat were used: 1) at 50 °C (2 min), temperature rise from 50 to 280 °C (10 °C/min), 40 min at 280 °C, total time of analysis 65 min.; 2) at 50 °C (2 min), temperature rise from 50 to 310 °C (2 °C/min), 13 min at 310 °C, total time of analysis 145 min.

To identify the compounds determined experimentally with respect to *n*-alkane series C_8–C_{40}, we used the mass spectral database NIST 14 MS, AMDIS software and linear retention indices (LRI) [30]. The data were processed using the standard data processing system ChemStation (Agilent Technologies, USA).

The internal and external standards were methyl stearate, 1-octaconasol and triacontane.

RESULTS AND DISCUSSION

In the present work, we demonstrated some possibilities to apply the tools of chemical fingerprinting using GC-MS for the studies of characteristic target metabolites in living systems under the action of abiotic and biotic factors, and directed gene mutations.

In the studies of variability of the composition of cuticular lipids in barley leaves and stems as a

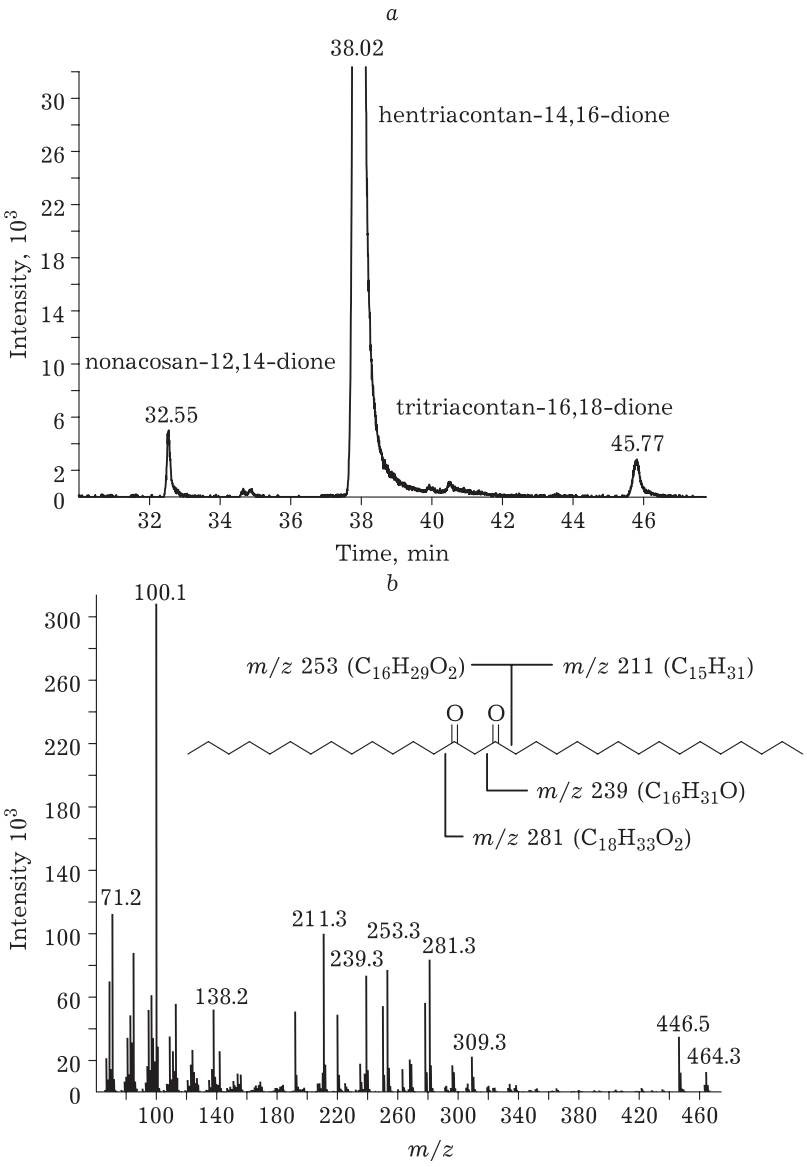

Fig. 1. Chromatographic profile of β-diketones from the extract of epicuticular lipids of barley stems (m/z 100) (*a*), experimental mass spectrum of hetriacontan-14,16-dione and the scheme of its fragmentation (*b*).

result of targeted gene editing, we identified saturated and unsaturated fatty acids C_{16}–C_{34}, aliphatic hydrocarbons, alcohols, aldehydes, β-diketones (C_{29}, C_{31} and C_{33}), two groups of alkylresorcinol (AR-1, AR-2), the esters of fatty acids and alkan-2-ols (C_{29}, C_{31}, C_{33} and C_{35}), and obtained their characteristic target chromatographic profiles over the corresponding characteristic ions.

In the group of β-diketones with the general formula R_1-CH_2-C(O)-CH_2-C(O)-CH_2-R_2, a simultaneous rupture of R_1–CH_2 and R_2–CH_2 bonds results in the formation of intense characteristic ion with m/z 100, corresponding to the fragment $[CH_3–C(O)–CH_2–C(O)–CH_3]^{+\cdot}$. These data were used to obtain a characteristic target chromatographic profile of β-diketones in the samples of barley stems (Fig. 1, *a*). One of the major compounds in the extract of the control sample of barley stems is β-diketone, namely hentriacontane-14,16-dione $C_{31}H_{60}O_2$ (molecular mass M = 464), for which the experimental (3395) and literature (3396) LRI coincide [31], and the mass spectrum (see Fig. 1, *b*) corresponds to that in NIST 14 MS database.

Keeping in mind the mass spectra and typical fragmentation in the homologous series of aliphatic β-diketones, as well as the data on literature and experimental LRI, we have additionally identified two minor β-diketones: nonacosane-

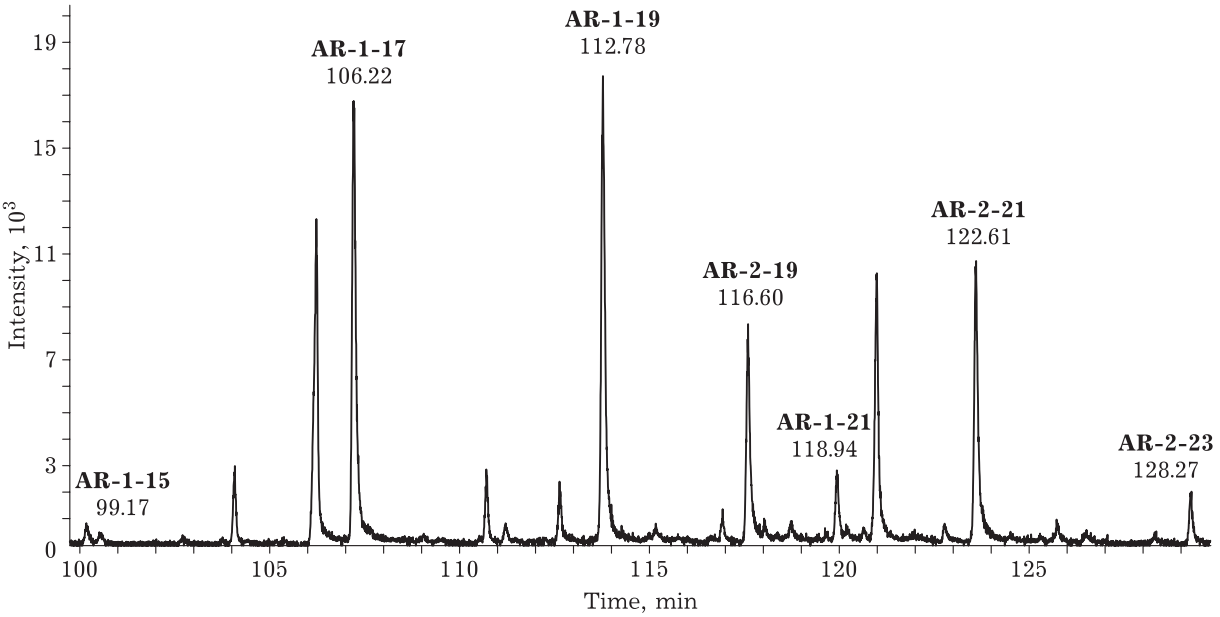

Fig. 2. Chromatographic profile of alkylresorcins of AR-1 and AR-2 groups, from the lipid extract of barley stem sample (m/z 138).

12,14-dione $C_{29}H_{56}O_2$ (M = 436) with experimental LRI 3182 and tritriacontane-16,18-dione $C_{33}H_{64}O_2$ (M = 492), with the experimental and literature LRI 3597 and 3598 [31], with the mass spectra corresponding to the literature data [31].

In the group of low-polar polyphenol compounds in the samples of barley leaves and stems, two groups of minor metabolites belonging to alkylresorcins. These compounds are synthesised by bacterial, fungi and higher plants and are considered as promising bioregulators of metabolic and immune processes. These compounds can scarcely be identified by the analysis of chromatograms over the full ion current. However, using the tools of chemical fingerprinting, we succeeded in obtaining the chromatographic profile of alkylresorcins of two isomeric-homologous groups AR-1 and AR-2 relying on the characteristic ion with m/z 138 $C_8H_{10}O_2$ (Fig. 2).

These groups of alkylresorcins differ from each other by the substituents in the benzene ring and by the length of the hydrocarbon fragment. For AR-1 group, the substituents in the benzene ring are hydroxy and methoxy fragments in *meta*-position with respect to the aliphatic hydrocarbon chain C_{15}, C_{17}, C_{19} and C_{21}; for AR-2 group, the substituents are two hydroxyl groups in *meta*-position and methyl group in *para*-position with respect to the aliphatic hydrocarbon chain C_{19}, C_{21} and C_{23} in the benzene ring (Fig. 3). Experimental LRI and those reported in the literature almost coincide for each compound of alkylresorcin groups AR-1 and AR-2. For instance, for compound AR-1-19, the experimental and literature LRI values are 3144 and 3145, respectively; for compound AR-2-19 – 3268 and 3275, respectively [32].

Detailed analysis of the chromatogram recorded in the full ion current mode allowed us to reveal a group of compounds in the region of high-molecular metabolites for which we observed fragmentation corresponding to plant wax – the esters of long-chain fatty acids (FA) R_1COOH and alcohols R_2OH [33]. Using the corresponding characteristic ions, we obtained a chromatographic profile of plant wax of the lipid extract of barley stems (Fig. 4, *a*). For compounds 1, 3–5 the fatty acid part is the fragment of stearic acid C18:0 with characteristic ions m/z 267 $[C_{17}H_{35}CO]^+$, 284 $[C_{17}H_{35}COOH]^{+\cdot}$ and 285 $[C_{17}H_{35}COOH_2]^+$; for compound 4 – the fragment of eicosanoic acid C20:0 with characteristic ions m/z 295 $[C_{19}H_{39}CO]^+$, 312 $[C_{19}H_{39}COOH]^{+\cdot}$ and 313 $[C_{19}H_{39}COOH_2]^+$. As the alcoholic component, the fragments with the general formula (R_2OH-H_2O) were identified, with the peaks of characteristic ions m/z 126 (C_9H_{18}) for compound 1; m/z 154 ($C_{11}H_{22}$) for compound 3; m/z 182 ($C_{13}H_{26}$) for compounds 2 and 4; m/z 210 ($C_{15}H_{30}$) for compound 5. So, five esters have been identified in the sample of plant wax of the lipid extract of barley stems: $C_{29}H_{58}O_2$ (FA C20:0 and alkan-2-ol C_9) – 1, $C_{31}H_{62}O_2$ (FA C18:0 and alkan-

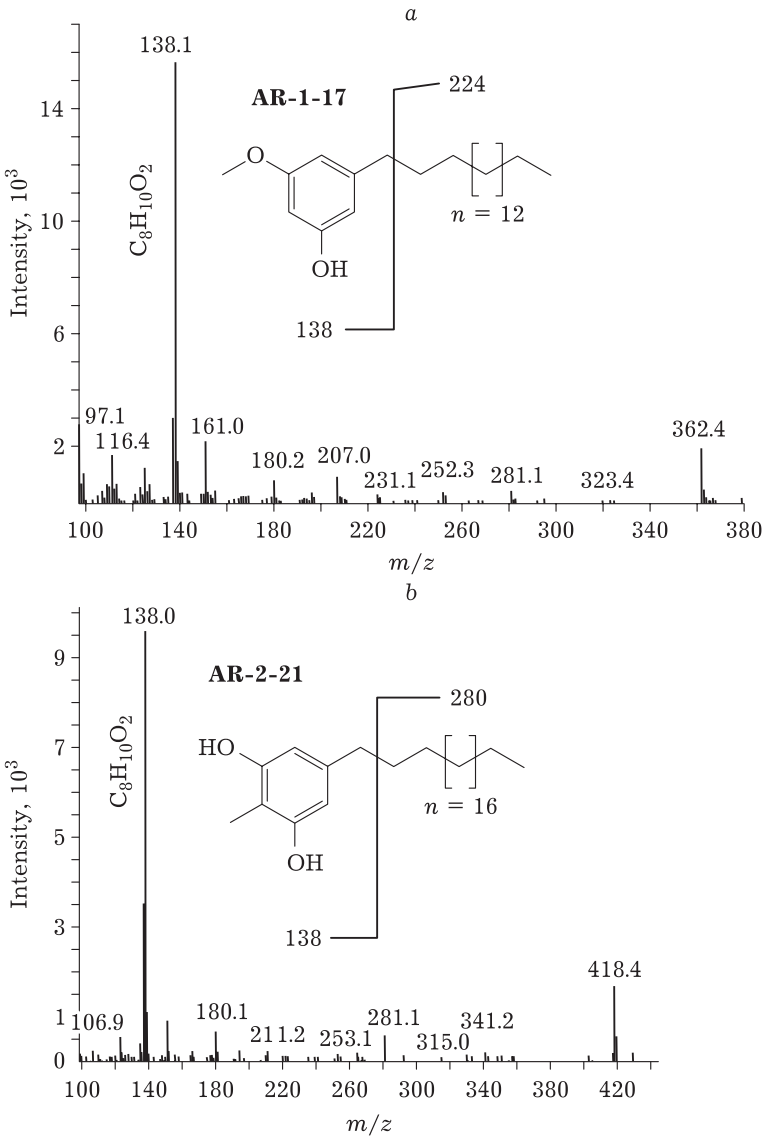

Fig. 3. Mass spectra of alkylresorcin of AR-1 type with the hydrocarbon fragment C_{17} (*a*) and AR-2 with hydrocarbon fragment C_{21} (*b*).

2-ol C_{13}) – 2, $C_{31}H_{62}O_2$ (FA C20:0 and alkan-2-ol C_{11}) – 3, $C_{33}H_{66}O_2$ (FA C20:0 and alkan-2-ol C_{13}) – 4, $C_{35}H_{70}O_2$ (FA C20:0 and alkan-2-ol C_{15}) – 5 (see Fig. 4, *a*). The mass spectrum of compound 4 is shown as example: according to the fragment ions with m/z 182, 295 and 312, this compound was identified as an ester with the composition $C_{33}H_{66}O_2$ (see Fig. 4, *b*). The peaks of molecular ions are not observed in the spectra of esters.

The data obtained were used to study the variability of the lipid components of barley leaves and stems during targeted genome editing [34].

The chromatographic profiles of lignans, hydroxybenzoic and hydroxycinnamic acids were obtained in the studies of the individual-group composition of phenol compounds of the carbon dioxide extract of Siberian larch wood. Phenol compounds are one of the main classes of polar secondary plant metabolites exhibiting a broad range of biological activity. It is known that phenol metabolites present in larch wood exhibit antioxidant, anti-inflammatory, antimicrobial and other kinds of biological activity [35], participate in the mechanisms of plant protection from diseases and pests, and are characterised by the growth regulating properties [36].

The chromatographic profiles of hydroxybenzoic and hydroxycinnamic acids after acid methanolysis, as well as the profiles of lignans, were obtained through reconstruction of initial chromatograms on the basis of characteristic ions for

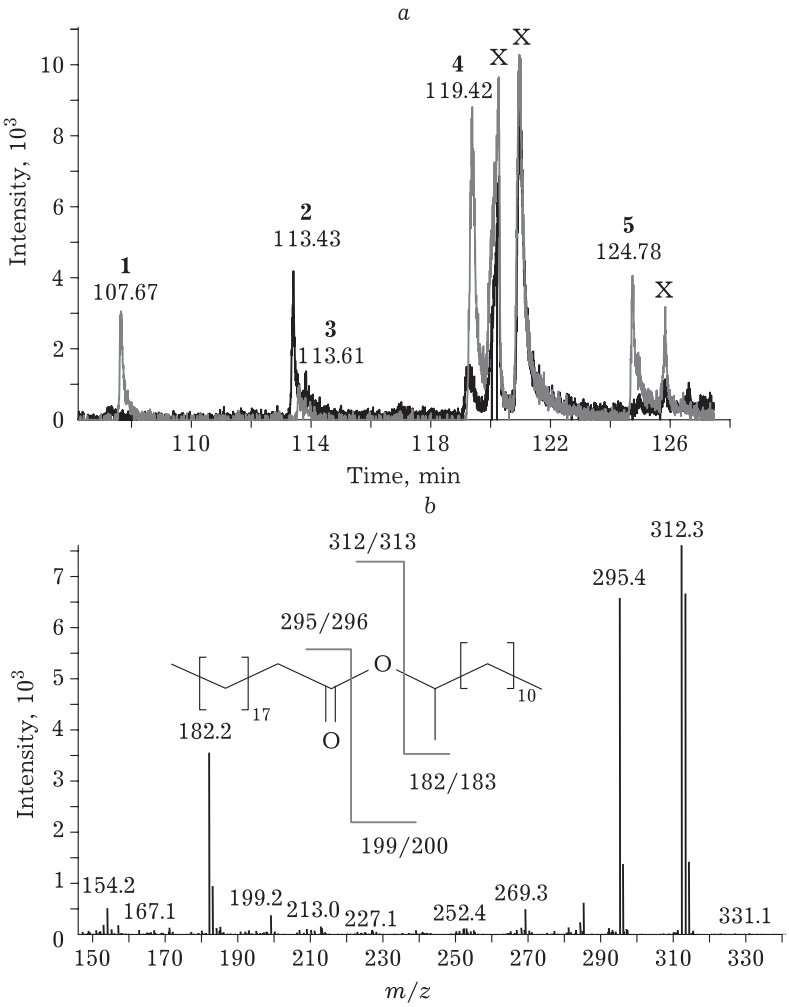

Fig. 4. Chromatographic profile of esters from barley stems, built up over the characteristic ions with m/z 267 and 295 for the fragments of fatty acids C18:0 and C20:0, respectively (*a*), mass spectrum of compound 4 corresponding to the ester of eicosanoic acid C20:0 (m/z 295 and 312) and tridecan-2-ol (m/z 182) (*b*).

each group of compounds: for methyl esters of hydroxycinnamic acids – from m/z 147, 177, 191; for methyl esters of hydroxybenzoic acids – from m/z 182, 196, 212, 226; for lignans – from m/z 137 and 151. The corresponding chromatographic profiles and mass spectra of the target compounds are shown in Fig. 5–7.

The data obtained were used to develop extraction-based methods to isolate biologically active complexes from the biomass of coniferous trees of Siberia for the purpose of making low-dose new-generation agents possessing fungicidal and antistress activity to stimulate immunity, plant growth and development [24, 37, 38].

The methodology of chemical fingerprinting was successfully used to study the composition of target non-polar and low-polar metabolites in the needles of Siberian larch and the leaves of common birch, which form the nutritive base of one of the most dangerous forest pests in West Siberia – gypsy moth, which is especially relevant in connection with pest expansion to the north under the conditions of global climate change. The study was carried out at the territory of West Siberia along the transect extending for ~1000 km from south to north, including the Altai Territory, Novosibirsk and Tomsk Regions.

For nonpolar and low-polar compounds in the lipid extract of larch needles after acid methanolysis, the chromatographic target metabolomic profiles were obtained for the methyl esters of fatty acids (MEFA) and diterpenic acids, acyclic diterpenoids and the group of triterpenoids; the compounds identified among the latter are α-toco-

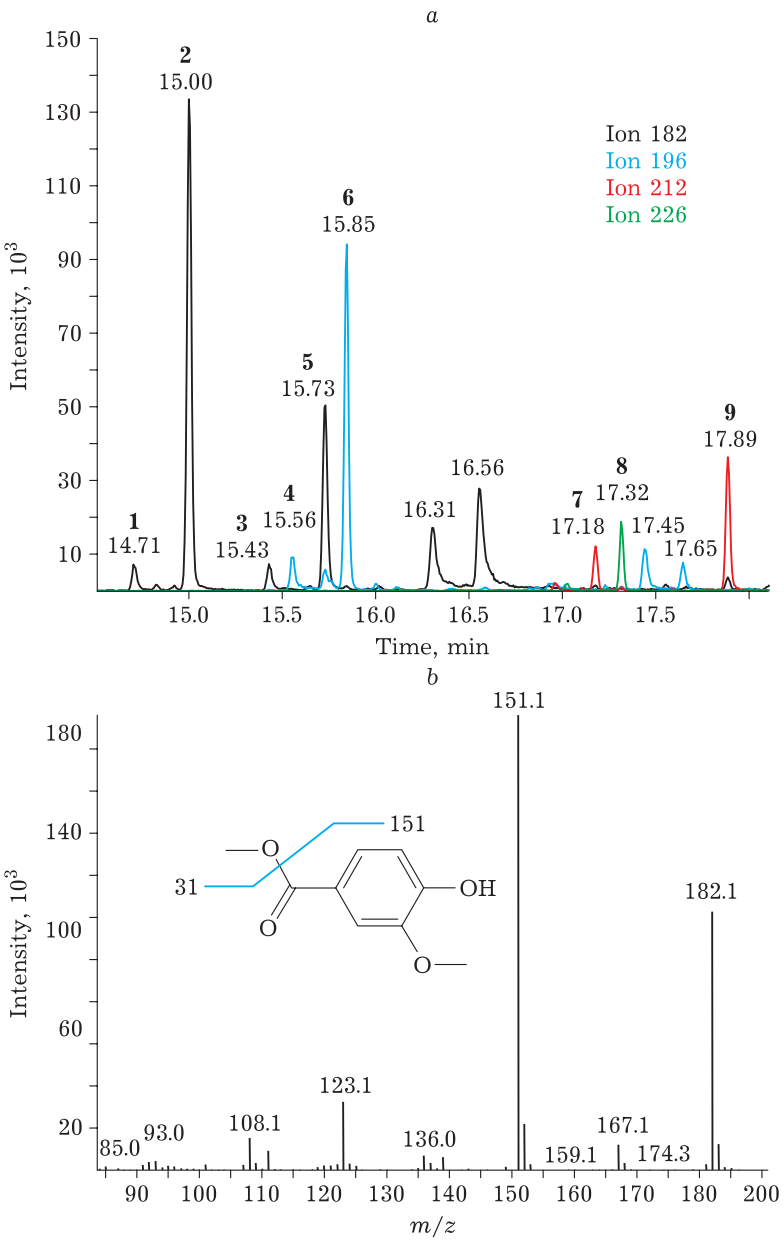

Fig. 5. Chromatographic profile of the methyl esters of hydroxybenzoic acids over characteristic ions with m/z 182, 196, 212 and 226: 2 – methyl ester of 4-hydroxy-3-methoxybenzoic acid; 1, 3, 5 – isomers of 2; 4, 6 – methyl esters of 3,4-dimethoxybenzoic acid; 7, 9 – isomers of methyl esters of hydroxy-dimethoxybenzoic acid; 8 – methyl ester of trimethoxybenzoic acid (*a*); mass spectrum of the methyl ester of 4-hydroxy-3-methoxybenzoic acid and the scheme of its fragmentation (*b*).

pherol, γ-tocopherol, β-sitosterol, γ-stigmastenone and campesterol. In addition, the compounds characteristic of larch needles were identified in the samples under investigation: nonacosan-10-ol and nonacosan-10-one at a ratio of about 35 : 1, as well as heptadecan-10-ol.

The chromatographic profile of acyclic diterpenoids of larch needles (neophytadiene and its isomers, phytol and isophytol) built on the characteristic ions with m/z 71, 123 и 278 is shown as an example in Fig. 8; the mass spectra of neophytadiene and phytol are shown in Fig. 9.

The chromatographic profiles of the methyl esters of diterpene acids (abietic, dehydroabietic, and isopimaric) were obtained over the characteristic molecular ions with m/z 314 for isopimaric and abietic acids, m/z 316 for dehydroabietic acid (Fig. 10); the profiles of MEFA with the com-

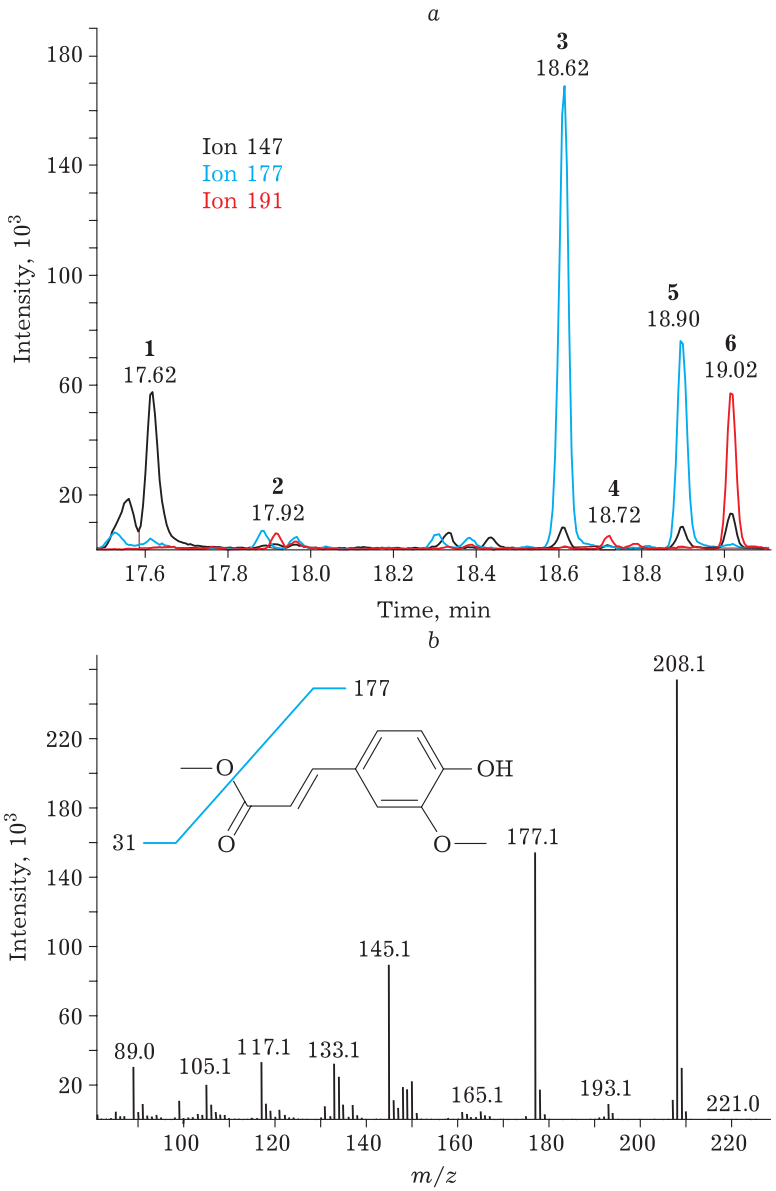

Fig. 6. Chromatographic profile of the methyl esters of hydroxycinnamic acids over the characteristic ions with m/z 147, 177 and 191: 1 – methyl ester of 4-hydroxycinnamic acid; 2, 4, 6 – isomers of the methyl esters of trimethoxycinnamic acid; 3, 5 – isomers of the methyl esters of hydroxy-methoxycinnamic acid (*a*); mass spectrum of 4-hydroxy-3-methoxycinnamic acid and the scheme of its fragmentation (*b*).

position C_{14}–C_{32} – over the characteristic ion with m/z 87 (Fig. 11). The major fatty acids are palmitic acid (C16:0) and two polyunsaturated acids: linoleic (C18:2) and linolenic (C18:3).

Common birch is the main feeding plant for gypsy moth, one of the dangerous forest pests. To study the individual-group composition of native triterpenoids of birch leaves collected in the Karasuk District of the Novosibirsk Region in the site affected by gypsy moth at the stage of the early development of leaves, we prepared the methanol extracts and investigated them using the tools of chemical fingerprinting to obtain characteristic target chromatographic profiles of metabolites: triterpenoids, hydrocarbons and phytosterols. It is known that triterpenoids with the dammarane backbone are antifeeding substances, that is, agents causing direct toxic action on insect pests [23, 39].

Triterpenoids of birch leaves have the backbones of lupine, oleonane and dammarane types [40], and their mass spectra contain the peaks of characteristic ions with m/z 135 and 189,

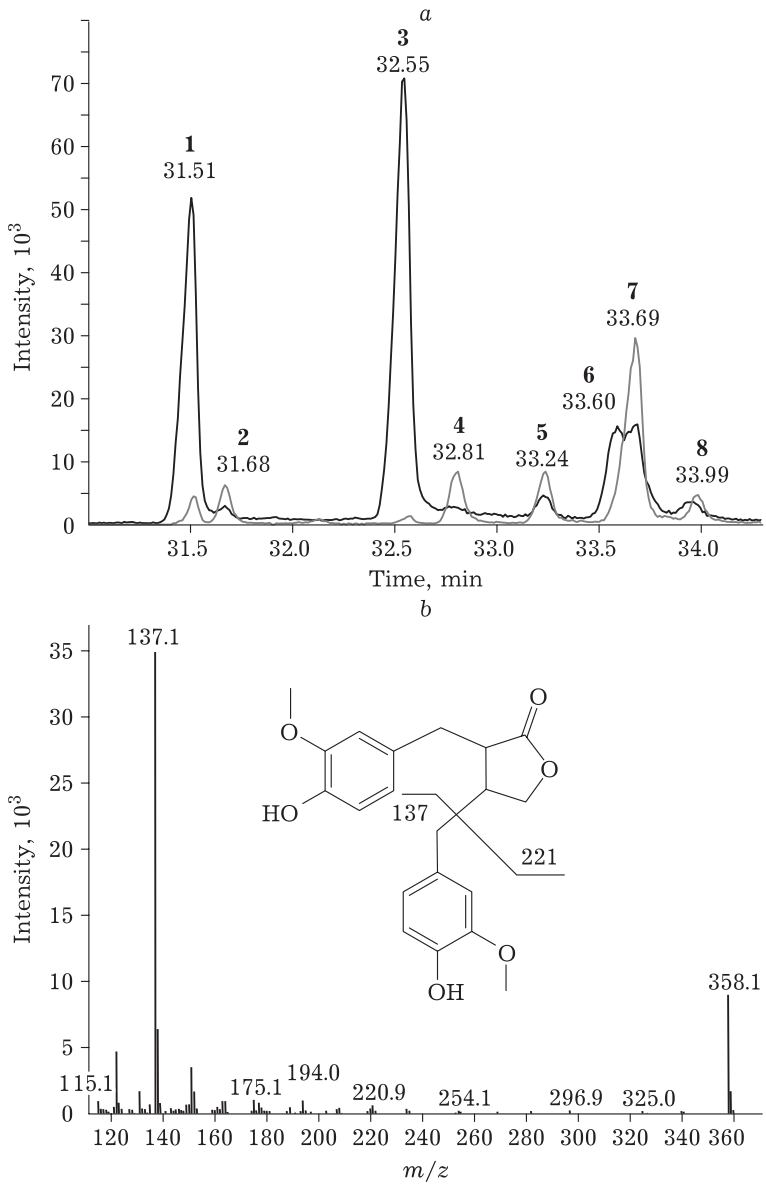

Fig. 7. Chromatographic profile of lignans over characteristic ions with m/z 137 and 151: 1 – matairesinol; 2 – ketomatairesinol; 3 – nortrachelogenine, 4 – medioresinol, 5 – pinoresinol, 6 – secoisolaricyresinol, 7 – dehydrodiconiferyl alcohol (apparently), 8 – ketopinoresinol (*a*); mass spectrum of matairesinol and the scheme of its fragmentation (*b*).

while for dammarane-type triterpenoids with the open side chain at C_{17} atoms, the characteristic ion peak is that of medium intensity with m/z 341; an intense ion with m/z 143 is characteristic for triterpenoids with the furan fragment at C_{17} atom. The chromatographic profiles of birch leaf triterpenoids plotted over the characteristic ions with m/z 135, 143 and 341 are shown in Fig. 12.

Taking into account the characteristics obtained by gas chromatography – mass spectrometry and literature data, we identified papyriferic acid and its deacylated derivative (peaks with retention times 58.31 and 57.81 min (see Fig. 12, *c*)), which are the marker compounds of birch leaves [41, 42].

Interestingly, it has been detected for birch leaves collected in the Karasuk District of the Novosibirsk Region at the stages of early development of leaves and intense development of gypsy moth caterpillars that the production of dammarane triterpenoids by birch leaves increases in the affected sites, which can be considered as one of the possible adaptation mechanisms of plant protection from gypsy moth (the content of

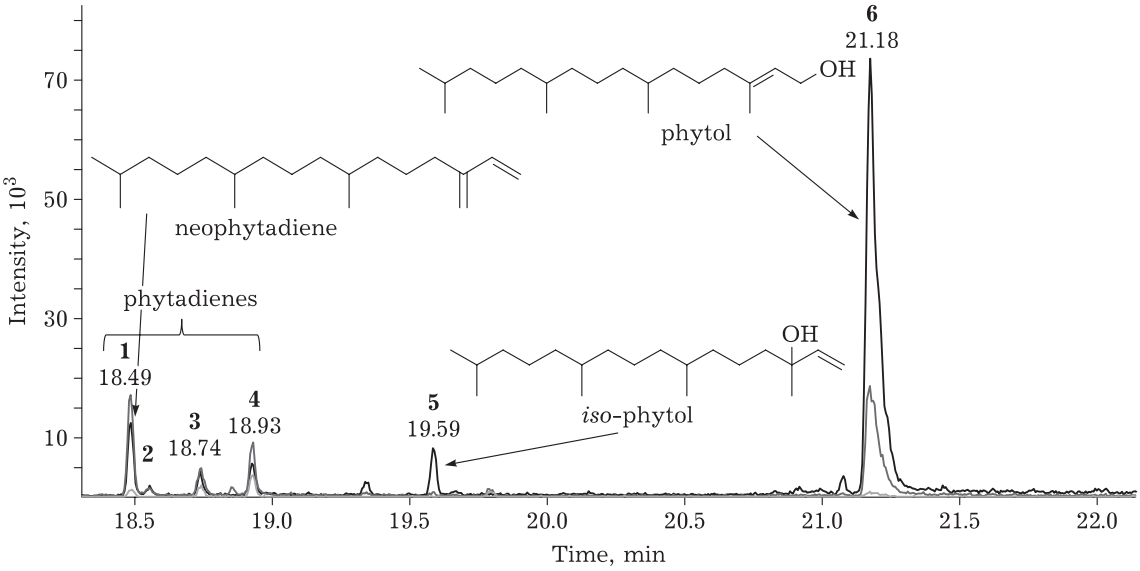

Fig. 8. Chromatographic profile of acyclic diterpenoids of Siberian larch needles, plotted over characteristic ions with m/z 71, 123 and 278.

triterpenoids in birch leaves in the affected site corresponds to the dynamics of caterpillar mortality within the time interval under investigation) [23, 39].

As a result of the studies of target metabolites in the needles of Siberian larch and in the leaves of common birch, carried out over the latitudinal gradient of about 1000 km at the Altai Territory, Novosibirsk and Tomsk Regions, it has been shown that the variability of composition and profiles of metabolites, including the determinants of host plant toxicity, would not prevent gypsy moth migration to the north and the formation of forest affection sites under the conditions of changing climate [43].

The composition of lipid extracts of the young and adult needles of Siberian pine was investigated. After acid methanolysis of the samples, chromatographic profiles of the characteristic target metabolites MEFA and their hydhroxy-substituted derivatives, tricyclic diterpenic acids, acyclic and bicyclic diterpenoids and sterols were obtained. The chromatographic profile of MEFA C12:0, C14:0 and C16:0 with terminal OH-group (ω-OH) by characteristic ions [M-30], namely m/z 200, 228 and 256, corresponding to detachment of CH_2O fragment [44], is shown as an example in Fig. 13, and the mass spectrum of the methyl ester of ω-OH tetradecanoic acid is presented in Fig. 14.

It should be stressed that a substantial increase in the intensity of ion with m/z 98 was observed in the mass spectra of methyl esters of ω-OH-substituted fatty acids in comparison with unsubstituted analogues, for which the characteristic ion is m/z 87. So, a characteristic mass spectrometric feature for ω-OH-substituted fatty acids is the ratio of intensities (I) of the characteristic ions $I(m/z\ 98)/I(m/z\ 87)$, which is within the range of 0.7–1 for ω-OH-substituted fatty acids and about 0.1 for unsubstituted fatty acids.

To understand the fundamental chemical mechanisms of insect relationships with the environment, and to develop biological methods for insect pest control, investigation of the composition of epicuticular lipids of economically essential agricultural insect pests was carried out for wax moth, Colorado potato beetle in ontogenesis, and two locust species with different hygrothermal preferences – Italian locust and migratory locust. The studies of insect cuticular lipids, similarly to the objects of plant origin, are based on chemical fingerprinting tools. Insect epicuticle is the first protective barrier from the action of abiotic and biotic environmental factors, and its major function is protection from dehydration. The composition of epicuticular lipids is closely associated both with the climatic conditions of the habitat and with susceptibility to entomopathogenic fungi, which are natural enemies of insects [45–48].

The chromatographic profiles of characteristic target metabolites of epicuticular lipids of wax moth larvae after acid methanolysis were obtained on the corresponding characteristic ions

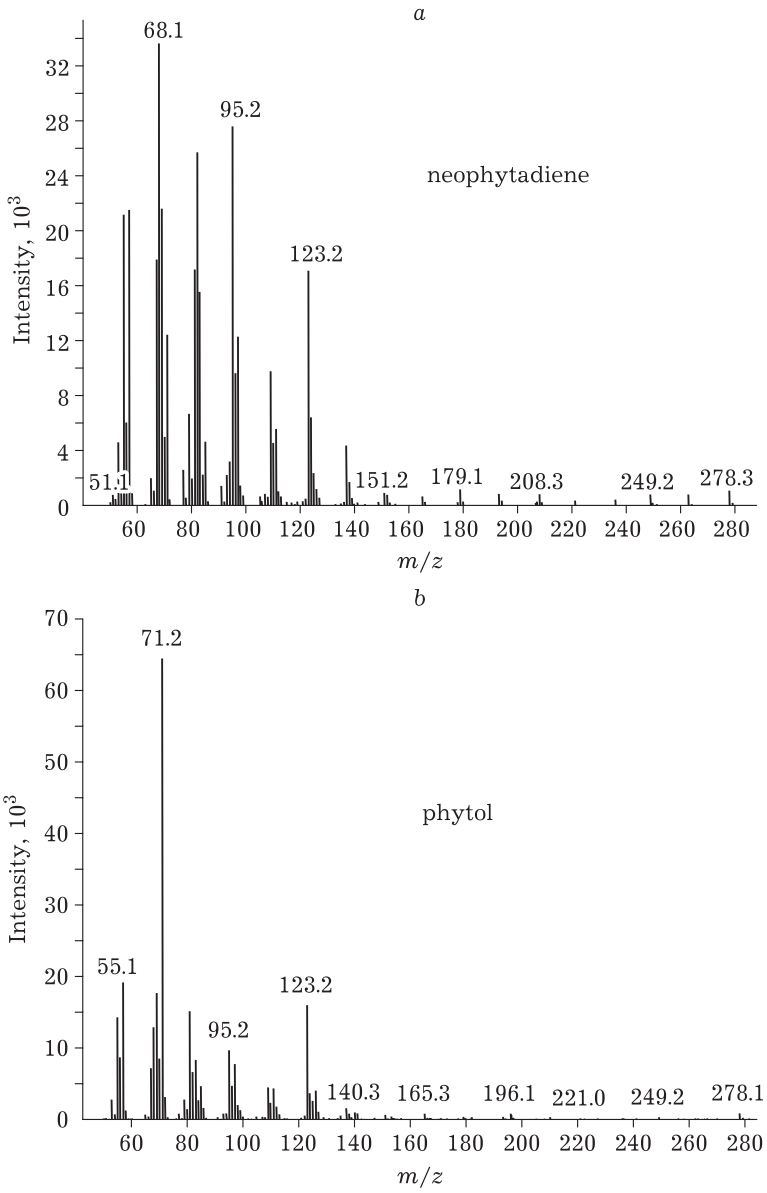

Fig. 9. Mass spectra of acyclic diterpenoids of Siberian larch needles: neophytadiene (*a*); phytol (*b*).

for saturated and unsaturated C_{16}–C_{33} hydrocarbons, saturated and unsaturated fatty acids C_{12}–C_{34}, (ω-1)- and β-hydroxy-substituted fatty acids. In addition, cholesterol and 15-oxohexadecanoic acid were identified in the samples. A fragment of the chromatographic profile of MEFA C_{14}–C_{28} epicuticular layer of wax moth larcae, obtained by the chromatographic profiling on the characteristic ion with m/z 74, is shown in Fig. 15.

Identification of the methyl esters of hydroxy-substituted fatty acids was performed taking into account LRI of compounds, characteristic ions and their ratios, and analysis of literature data and special mass spectrometric databases [44]. On the basis of $[M\text{-}C_2H_4O]^{+\cdot}$ fragmentation characteristic of the methyl esters of (ω-1)-hydroxy-substituted fatty acids, (ω-1)-hydroxy-substituted fatty acids C_{12}, C_{14}, C_{16} and C_{18} were identified in the extract of the epicuticular lipids of wax moth larvae over characteristic ions with m/z 186, 214, 242 and 270, respectively (see Fig. 15, 16).

In addition to (ω-1)-hydroxy-substituted fatty acids, the compounds identified in the extract of epicuticular lipids of wax moth larvae included β-hydroxy-substituted fatty acids. Their chromatographic profile over the characteristic ion with m/z 103, corresponding to fragment $[C_4H_7O_3]^+$, is shown in Fig. 17.

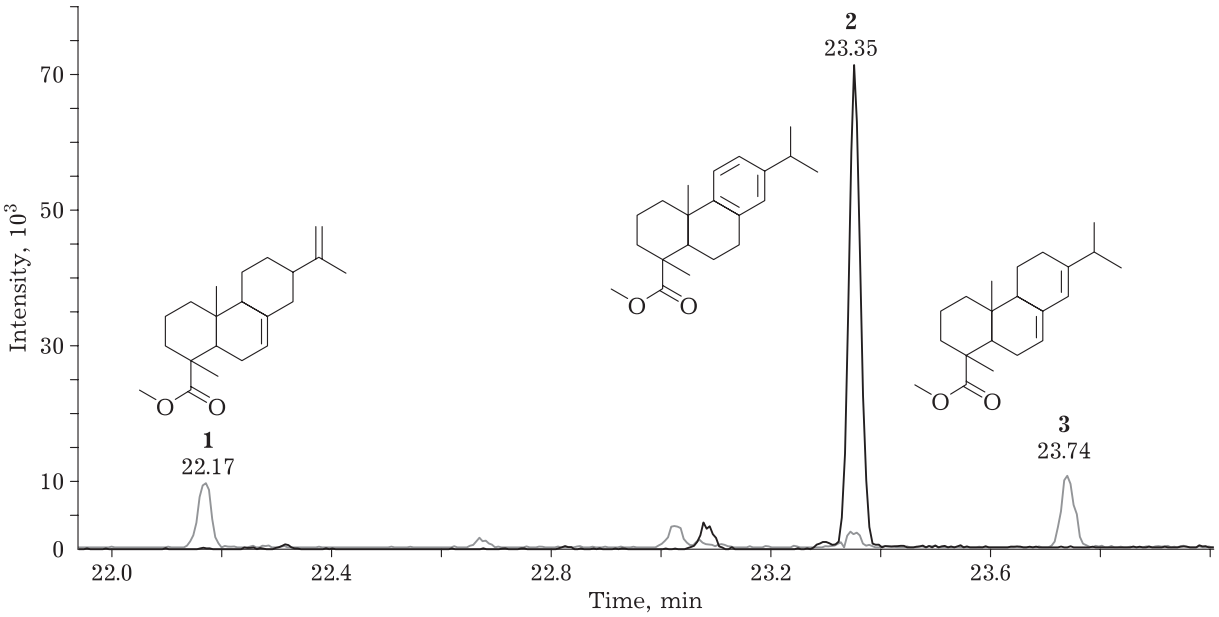

Fig. 10. Chromatographic profile of methyl esters of diterpene acids of Siberian larch needles over characteristic ions with m/z 314 and 316: 1 – methyl ester of isopimaric acid; 2 – methyl ester of dehydroabietic acid; 3 – methyl ester of abietic acid.

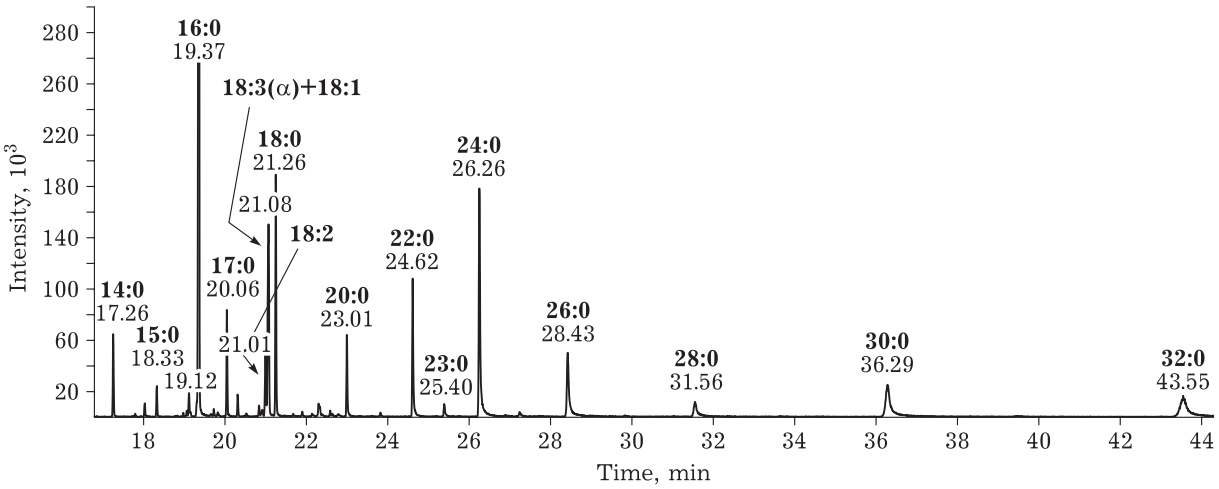

Fig. 11. Chhromatographic profile of MEFA C_{14}–C_{32} over characteristic ion with m/z 87.

It is known that hydroxy-substituted fatty acids are produced by higher plants, fungi, insects, and bacteria [49].

The data obtained in the studies of the composition of characteristic target metabolites of wax moth larvae epicuticular lipids were used to study susceptibility of wax moth larvae, paralised by the parasitoid poison, to entomopathogenic fungi [25].

The composition and chemical structure of epicuticular lipids of Colorado potato beetle larvae, imago, and swarming locust nymphs with different hygrothermal preferences were studied by GC-MS. The major components of epicuticular lipids of the insects duner study are hydrocarbons, free and bound fatty acids. The percentage of hydrocarbons in epicuticle is more than 90 %.

The compounds identified in the cuticular lipids of Colorado potato beetle larvae, pupae, and imago, the nymphs of migratory locusts and Italian locust were saturated normal, mono-, di-, and trimethyl-branched hydrocarbons with the composition C_{25}–C_{41}, free and bound fatty acids with the composition C_{12}–C_{31}.

Comparison between the chromatographic profiles of hydrocarbons from epicuticles of the nymphs of Italian locusts and migratory locusts over the

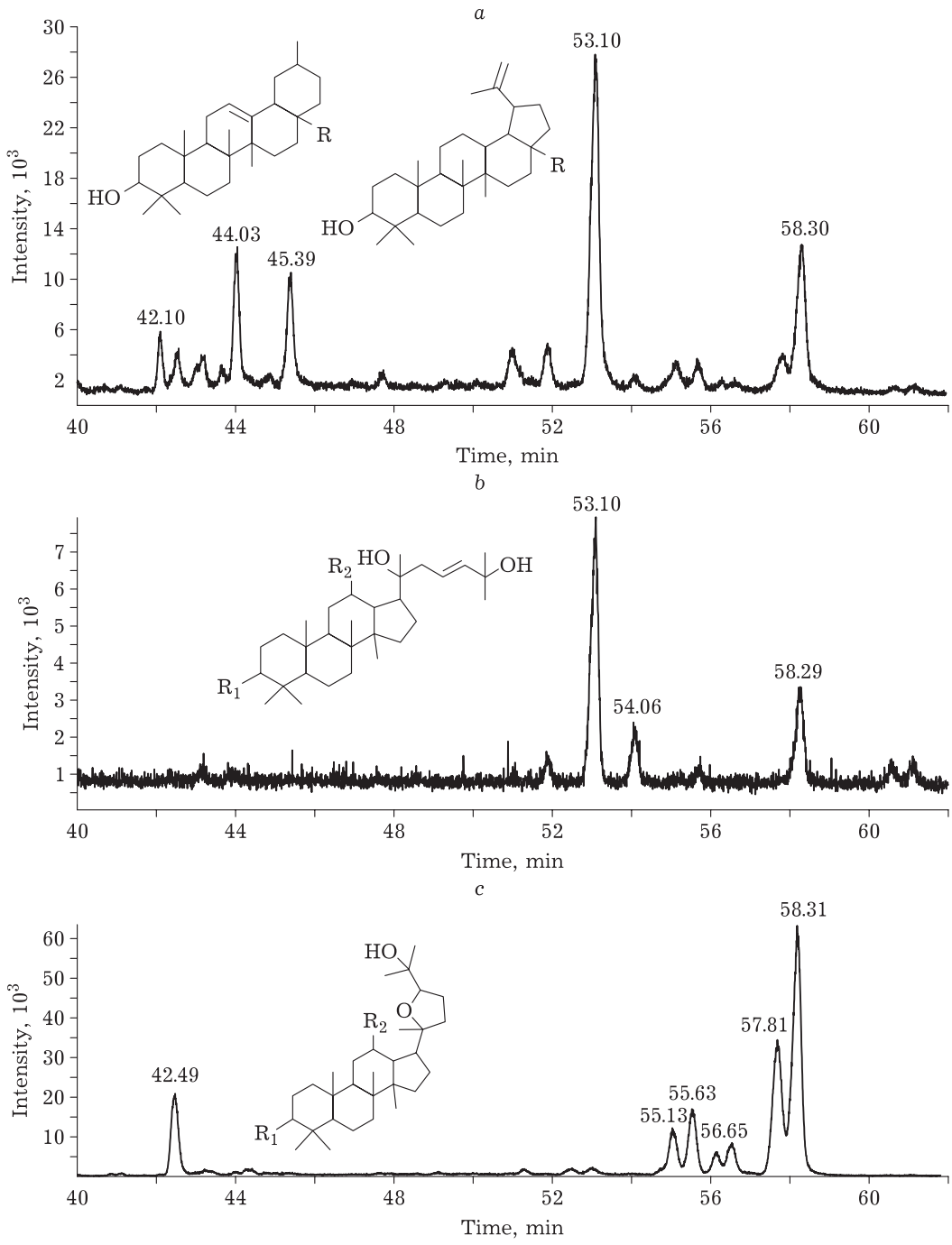

Fig. 12. Chromatographic profiles of triterpenoids from birch leaves over characteristic ions: m/z 135 – pentacyclic triterpenoids with oleonane and lupine backbone types (*a*); m/z 341 – tetracyclic triterpenoids with dammarane backbone and open side chain at C_{17} atom (*b*); m/z 143 – tetracyclic triterpenoids with dammarane backbone and furan fragment at C_{17} atom (*c*). R_1 – malonate fragment, R_2 – acetate fragment.

characteristic ion with m/z 85 shows that they substantially differ from each other in composition (Fig. 18), which is due to different habitats: migratory locust is a mesoxerophilous species preferring humid sites, while Italian locust is a xerophi9le species preferring arid sites.

To determine the composition and chemical structure of epicuticular methyl-branched alkanes, the scheme described in [18] was used, which included a comparison of experimental LRI with the available literature data [26, 27, 50], NIST 14 MS; analysis of the characteristic ions in mass spectra,

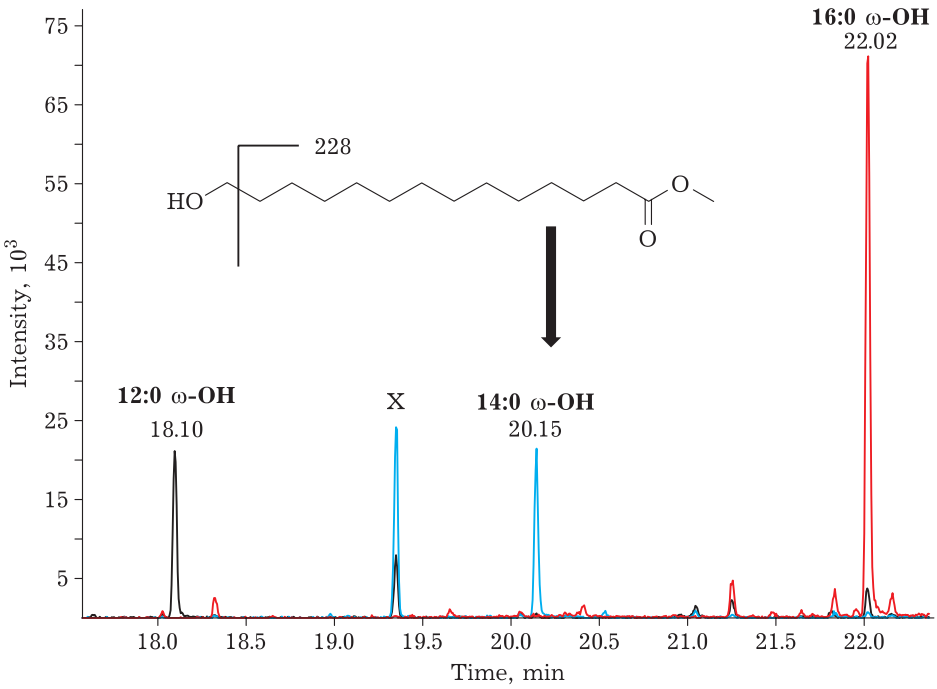

Fig. 13. Chromatographic profile of MEFA C12:0, C14:0 and C16:0 with ω-OH group over characteristic ions with m/z 200, 228 and 256.

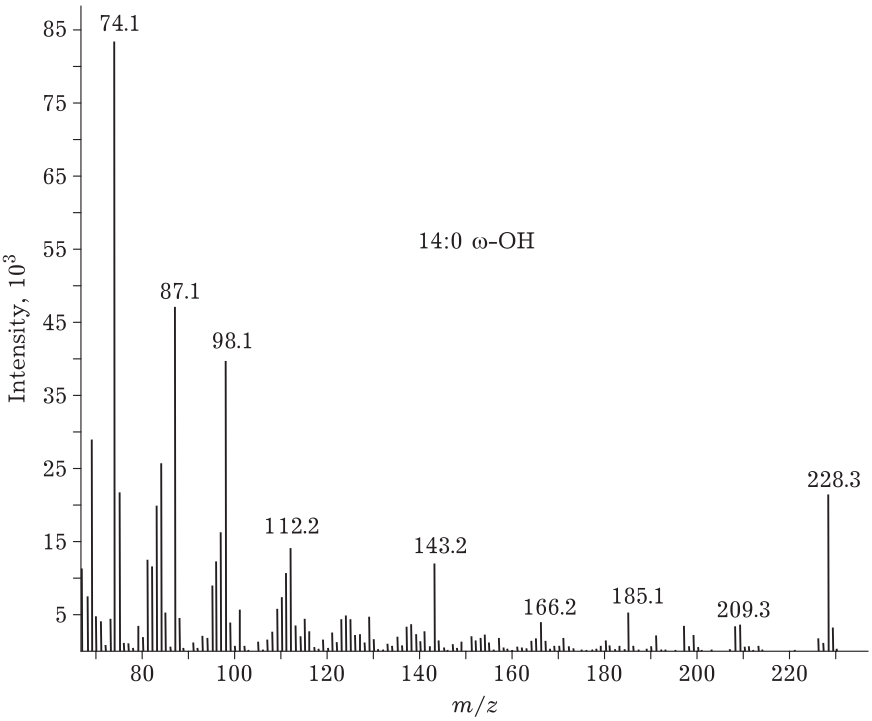

Fig. 14. Mass spectrum of methyl ester of ω-OH-substituted tetradecanoic acid (C14:0).

formed as a result of molecule decomposition at the sites of carbon chain branching; tests of the assumed structure for biochemical feasibility taking into account the data on biosynthesis of methyl-branched hydrocarbons of insects. According to the scheme of biosynthesis of methyl-branched alkanes, the number of carbon atoms in the end part of a molecule should be even for all structures; for internally branched structures between methyl groups – an odd number of carbon atoms;

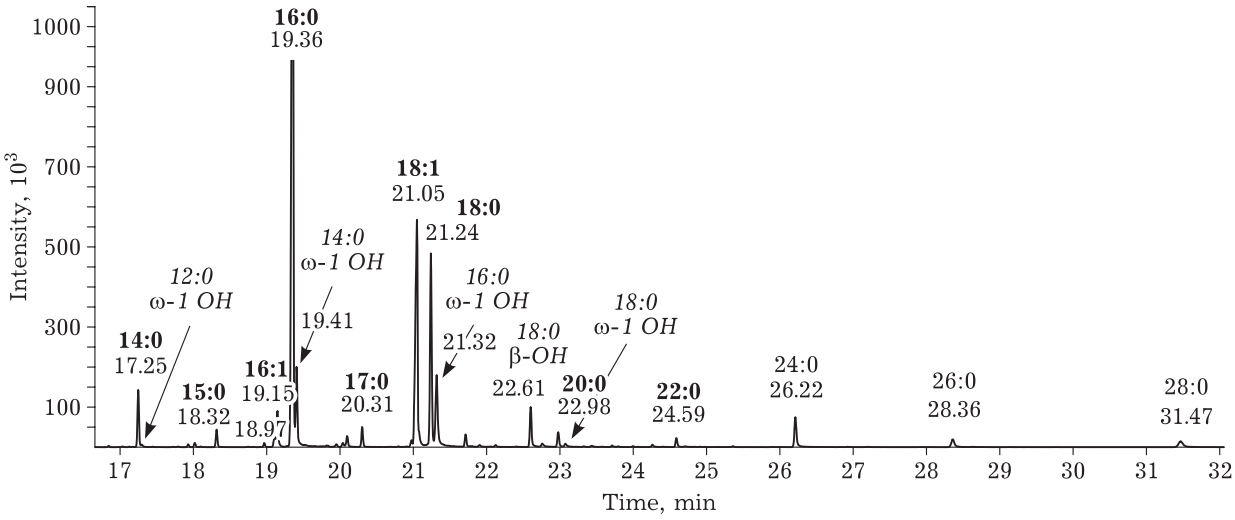

Fig. 15. Chromatographic profile of MEFA of wax moth over characteristic ion with m/z 74.

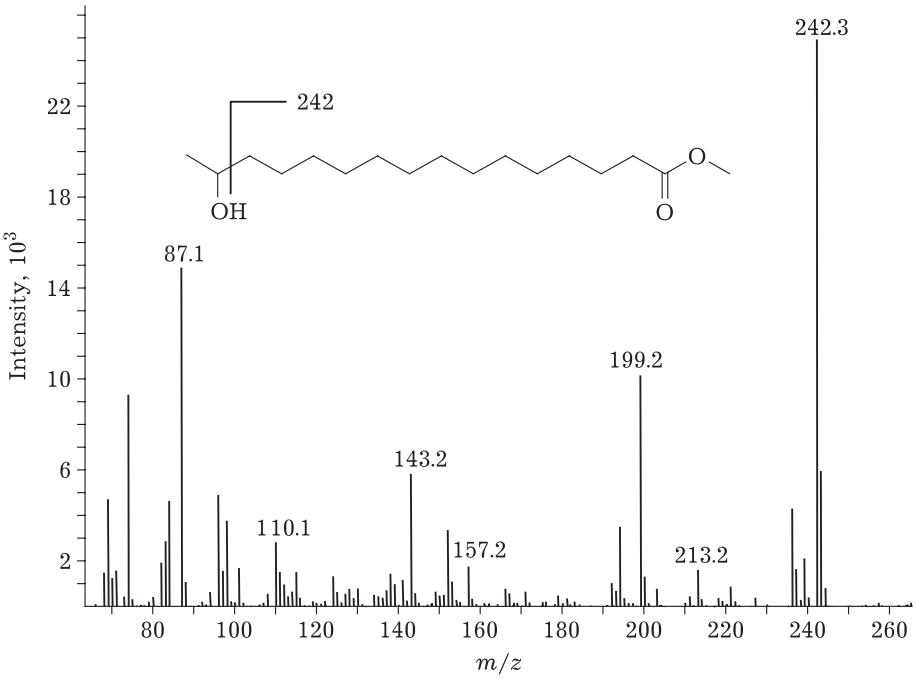

Fig. 16. Mass spectrum of methyl ester of (ω-1)-hydroxy-substituted hexadecanoic acid.

for terminally branched hydrocarbons, the number of carbon atoms can be either even or odd, which depends on the initial compound (valine – odd, leucine – even) [16, 18].

Insect epicuticle is a complex multicomponent mixture of hydrocarbon isomers close to each other in structure and physicochemical properties. In some cases, this causes insufficient chromatographic separation of the peaks of individual compounds. For the purpose of reliable identification of methyl-branched hydrocarbons, an algorithm was developed to reveal the characteristic ions in the mass spectra of compounds to be identified. This algorithm is based on the statistical processing of mass spectra of linear and methyl-branched alkanes [27].

The mass spectrum of linear alkane $C_{33}H_{68}$ was chosen as the basis to develop the algorithm. In m/z region from 113 to 407, which includes the characteristic ions of the compounds under investigation, a linear dependence was plotted between the relative intensities of ion peaks and m/z, then the $\pm 3\sigma$-interval was plotted with respect to this linear dependence. In the case if the intensities of

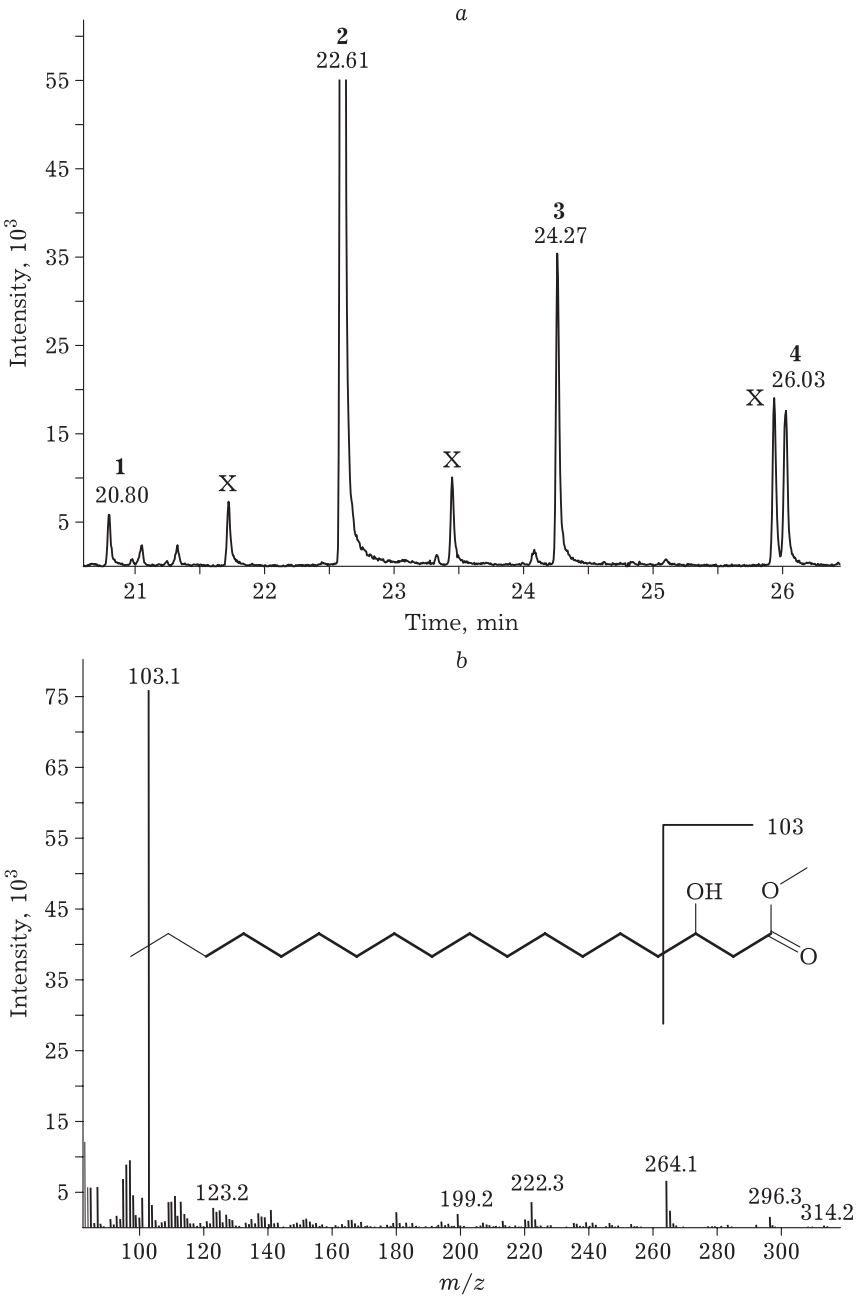

Fig. 17. Chromatographic profile of methyl esters of β-hydroxy-substituted fatty acids of wax moth over characteristic ion with *m/z* 103: 1 – C16:0 β-OH, 2 – C18:0 β-OH, 3 – C20:0 β-OH, 4 – C22:0 β-OH (*a*); mass spectrum of the methyl ester of β-hydroxyoctadecanoic acid C18:0 β-OH (*b*).

the peaks of ions related to methyl-branched alkanes exceeded the upper limit of 3σ-interval, these ions were considered to be characteristic for the certain compound, otherwise they were not.

The results obtained by applying this algorithm to identify methyl alkanes in the complex peak with retention time (r.t.) 130.10 min of the chromatographic profile of cuticular lipids of Italian locust are shown as an example in Fig. 19, *a*. It has been determined through the analysis of the mass chromatogram obtained by reconstruction over the assumed characteristic ions that this peak is due to two compounds with the gross formula $C_{39}H_{80}$.

For the compound with r.t. 130.08 min, the calculated linear retention index is 3757, and for the compound with r.t. 13.11 min – 3760 (see Fig. 19, *b*).

By applying the algorithm aimed at determination of characteristic ions, it has been established that only for ions with *m/z* 168/169,

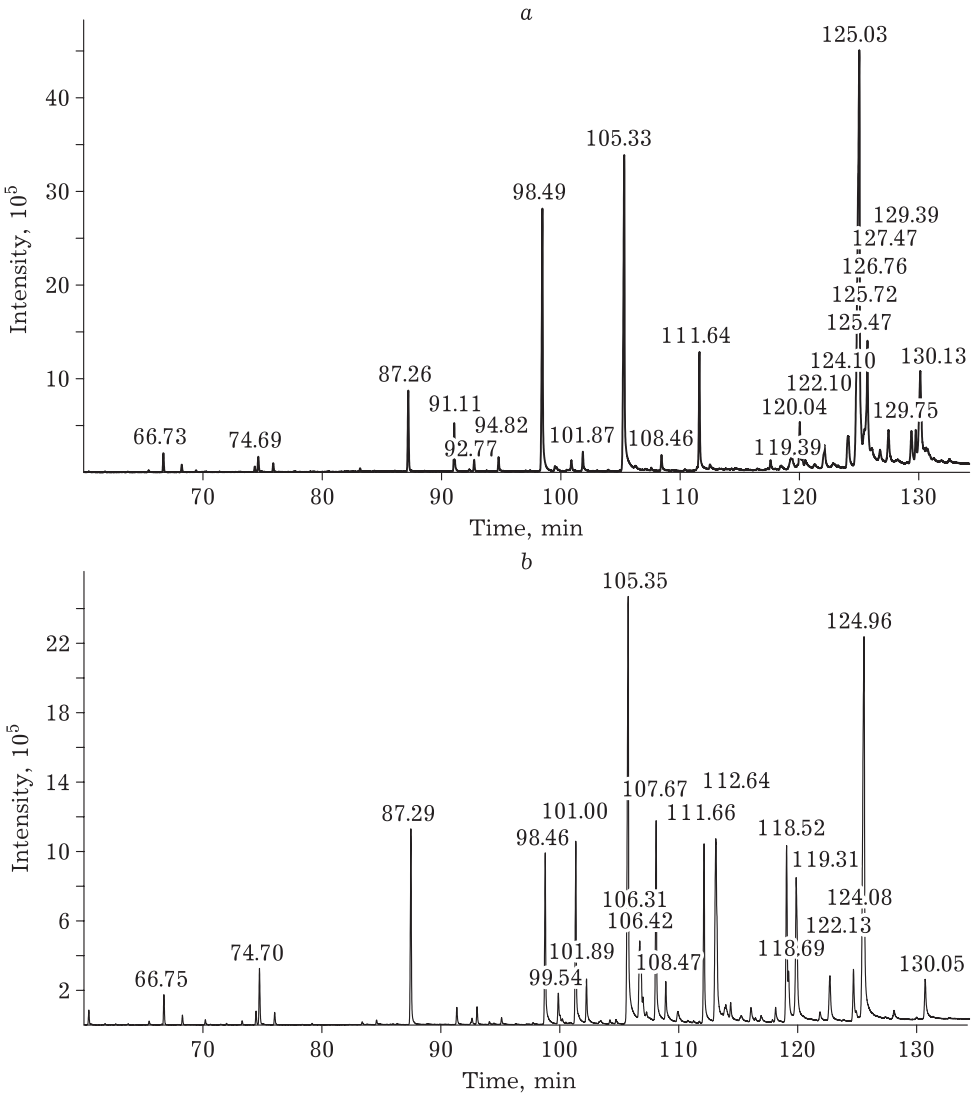

Fig. 18. Chromatographic profiles of epicuticular hydrocarbons of the nymphs of Italian locust (*a*) and migratory locust (*b*) over characteristic ion with m/z 85.

196/197, 252/253, 322/323, 378/379 and 406/407 the intensities exceed the upper limit of the 3σ-interval (Fig. 20).

Thus, it has been determined involving the principles of biochemical feasibility that the former compound is symmetric 13,25-dimethylheptatriacontane decomposing into two identical ion pairs with m/z 196/197 and 378/379, while the latter giving characteristic ions with m/z 168/169, 252/253, 322/323 and 406/407 is 11,21-dimethylheptatriacontane (Fig. 21). Experimental LRI of the identified compounds correspond to the literature data [26, 27, 50], NIST 14 MS.

In spite of substantial diversity of the identified epicuticular methyl-branched hydrocarbons of Colorado potato beetle imago, it has been shown that they form 8 isomeric-homologous series of mono-, di-, and trimethyl-branched alkanes with the main chain length C_{26}–C_{37}, synthesised via certain biochemical pathways [51].

The data obtained in the work were used to study the changes in the composition of insect cuticular lipids during ontogenesis, changes of habitats, and interaction with entomopathogenic fungi [26, 27].

CONCLUSION

It is demonstrated as a result of the studies that chemical fingerprinting methodology can be efficiently used to investigate the characteristic target metabolites of living systems. Using gas chromatography – mass spectrometry and the tools of chemical fingerprinting, the chromatographic pro-

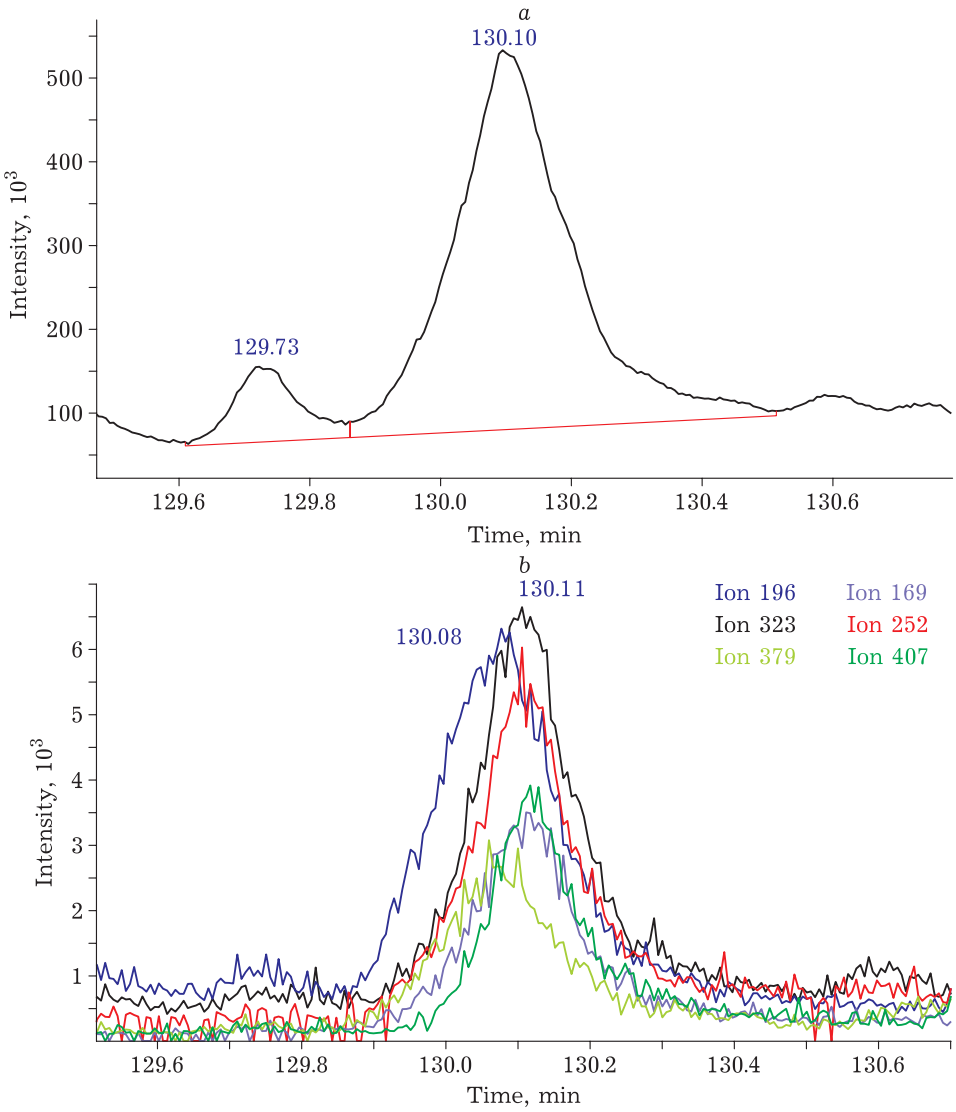

Fig. 19. Fragment of the chromatogram over full ion current (*a*) and fragment of the mass chromatogram obtained by reconstruction over assumed characteristic ions with m/z 169, 196, 252, 323, 378 and 407 from the peak with r.t. 130.10 min of the chromatographic profile of cuticular lipids of Italian locust (*b*).

files of characteristic target metabolites have been obtained, including saturated and unsaturated fatty acids, aliphatic hydrocarbons, alcohols, aldehydes, alkylresorcins, esters of fatty acids and fatty alcohols, in the studies of variability of the composition of cuticular lipids of barley leaves and stems as a result of directed gene editing.

The chromatographic profiles of fatty acids and their hydroxy-substituted derivatives, di- and triterpenoids of lach needles and birch leaves, which are the nutrition basis for gypsy moth – the most dangerous West Siberian forest pest in the Altai territory, Novosibirsk and Tomsk Regions. The data obtained can be used to detect the possibilities for expansion of the pest habitats to the north under the handing climate conditions.

The data obtained on the chromatographic profiles of phenol metabolites of larch wood are used to develop extraction-based methods to isolate biologically active complexes from the biomass of coniferous trees of Siberia for the purpose of elaborating now-generation low-dose agents to stimulate immunity, growth and development of plants with fungicidal and antistress properties.

The chromatographic profiles have been obtained for cuticular lipids (linear and methyl-branched hydrocarbons, free and bound fatty acids) of economically significant agricultural pests:

wax moth, Colorado potato beetle at different stages of development, and two locust species – Italian locust and migratory locust inhabiting different climatic zones.

An algorithms has been developed to determine characteristic ions in the mass spectra of isomeric methyl-branched alkanes of insect epicuticular lipids using chromatogram reconstruction over characteristic ions in complex, chromatographically hard-to-separate mixtures of the compounds close to each other in composition. The algorithm can be used for reliable identification of substances in complex mixtures of isomeric and structurally similar organic compounds of different classes.

Thus, chemical fingerprinting tools expand the possibilities of the analysis of characteristic target metabolites and allow one to determine isomeric homological series of the metabolites of different classes, which is important for studying and establishing the biochemical paths of their synthesis in living systems. This opens additional possibilities to investigate adaptation mechanisms of living systems under the action of abiotic and biotic factors and environmental pollution under the conditions of changing climate, and to reveal the changes in the composition of secondary metabolites during directed selection and gene editing of agricultural crops.

The developed approaches and the data obtained are of fundamental and applied significance for studies in the areas of biology, biochemistry, bioorganic chemistry, chemical ecology, genetics,

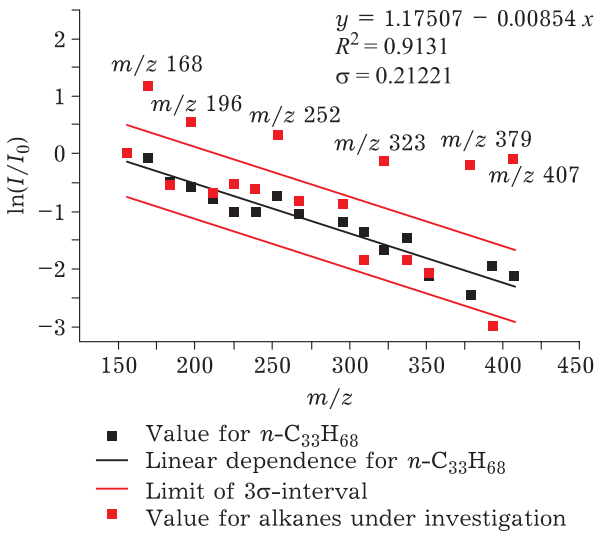

Fig. 20. Application of the algorithm to determine the characteristic ions of a complex peak with retention time 130.1 min in the chromatographic profile of cuticular lipids of Italian locust.

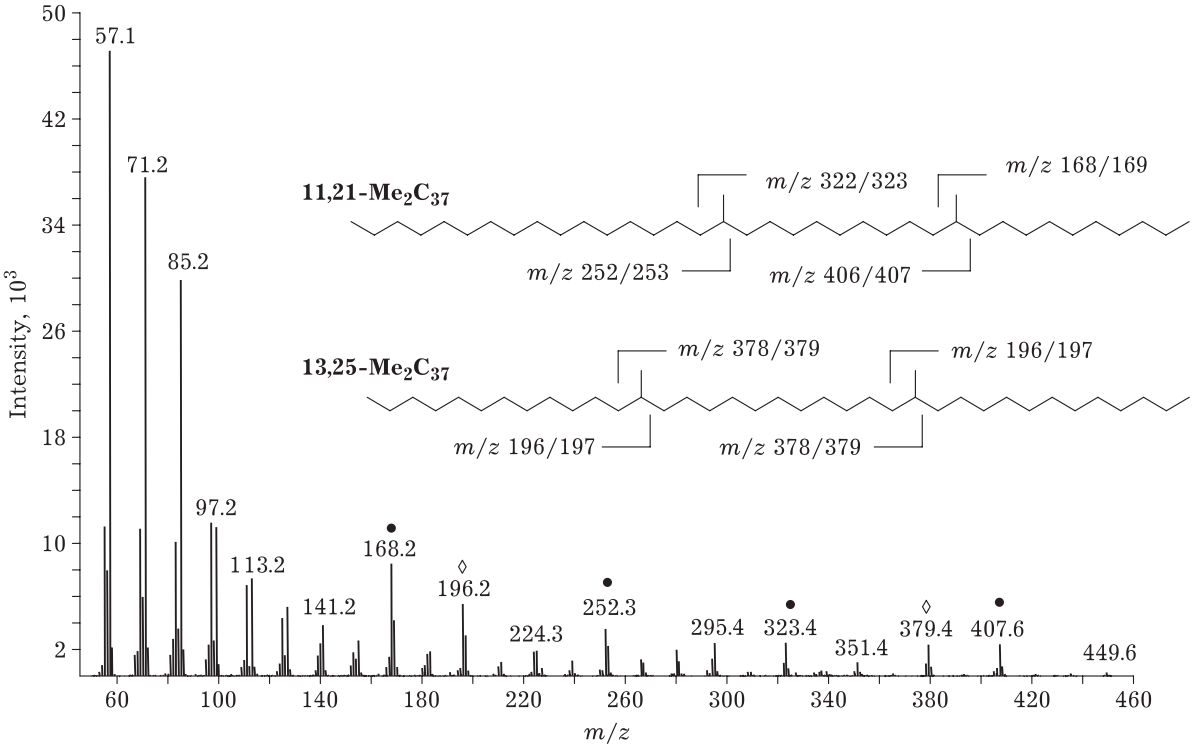

Fig. 21. Mass spectrum at the peak with retention time 130.10 min of the chromatographic profile of Italian locust. Designations: ◊ – characteristic ions formed in the decomposition of symmetric dimethyl-substituted alkane 13,25-Me_2C_{37}; • – characteristic ions formed in the decomposition of non-symmetric dimethyl-substituted alkane 11,21-Me_2C_{37}.

agriculture, phytochemistry and other research areas.

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