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The Use of Chromatographic Profiling Methods for Analyzing and Identifying Low Molecular Mass Organic Substances of Natural and Anthropogenic Origin

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Abstract

An approach for the determination of the individual and the group composition for low molecular mass organic substances of natural and anthropogenic origin basing on the analysis of chromatographic profiles ("fingerprints") and the spectral characteristics. Data are presented concerning the use of the approach for the identification of persistent organic pollutants in the environment of Siberia and Mongolia with the purpose of assessing the risk of the chemical compounds to affect human health and ecosystems, as well as of identifying the main sources of pollution. There are potentialities considered concerning the approach for the analysis of biologically active substances of plant or animal origin, the development of low-dose preparations for agriculture biomass based on Siberian coniferous plants as well as the studies of living systems. It is demonstrated that chromatographic profiles represent highly informative characteristics those could be used in order to recognize a "chemical image" of complex systems, to identify and predict properties inherent in these objects.

Key words: chromatographic profiles, spectral characteristics, gas chromatography-mass spectrometry, low molecular mass biologically active substances, persistent organic pollutants, environmental chemistry, chemistry of natural compounds, living systems

Contents

INTRODUCTION

One of the main objectives in various fields of science consists in pattern recognition, *i. e.*, either in the identification of the object under investigation, or in the determination of its properties from some its features. In various fields of science there are their own approaches to choosing diagnostic characteristics and pattern recognition methods. In the fields of natural sciences, such as phytochemistry, wood chemistry, medicinal chemistry, ecology, environmental chemistry, biology, biochemistry, biotechnology, microbiology, medicine, geochemistry, petroleum chemistry, archeology *etc.*, one of the major diagnostic and informative characteristics of the object under study is presented by its chromatographic profile (fingerprints). Currently, chromatographic profiling (metabolomics) is most efficiently used in molecular biology in the studies on the processes occurring in living systems, as well as in the development of methods for the diagnosis and treatment of various diseases.

Acquisition and analysis of data according to the individual and group composition of low

molecular mass organic substances of natural and anthropogenic origin is an integral part of modern fundamental and problem-oriented research work.

All the objects of living nature produce a huge amount of organic matter in the course of their life. The information concerning their individual and group composition can be used in order to study the molecular mechanisms of plant morphogenesis, for establishing the chemotaxonomic features of plants, searching for promising biologically active compounds, identifying biomarkers and signalling substances, in the creation of new medical, biorational and functional foods and drugs, for predicting their biological, physiological and consumer properties.

The determination of the composition and profile of organic compounds, especially persistent organic pollutants (POPs) in the environment is the basis ecogeochemical, environmental and hygienic studying the ecosystems of different levels. Data obtained could be used for identifying the major anthropogenic sources of pollution, for assessing their relative contribution to the overall level of pollution, for assessing the transboundary transport of pollutants, as well as in order to simulate and predict the behaviour of pollution levels and pollutants, in order to assess the effects of chemical hazards on human health and to develop efficient technologies for environmental protection.

To analyze complex mixtures, as well as to identify their components researchers widely use chromatographic system with sensitive, selective and specific detectors, such as a mass spectrometry detector, or a diode-array detector in the visible and ultraviolet regions of radiation.

One of the most informative approaches to the study of individual and group composition of substances of natural and anthropogenic origin is considered to be obtaining and analyzing their chromatographic profiles [1?4]. The chromatographic profile of any individual object serves as its individual characteristic, and it can therefore be used as an identification or diagnostic indicator for determining its authenticity, origin, in developing methods for determining the quality, for the identification and standardization of products and preparations on its basis, and for predicting their properties [4–8].

SPECTRAL ANALYTICAL METHODS IN ENVIRONMENTAL STUDIES

The analysis of environmental objects plays a key role in conducting environmental studies, examinations, assessing the condition of ecosystems, human population health and quality of life, levels of chemical hazard impact on humans and ecosystems, of environmental accidents and disaster investigations [9–11].

Any conclusion concerning the ecological condition of ecosystems should be based on reliable information about the content of priority persistent organic pollutants in the environment (polychlorinated dioxins and furans, biphenyls, pesticides, polycyclic aromatic hydrocarbons). Currently these pollutants are of a global environmental hazard due to the toxicity, persistence, ubiquity, the contamination of food and natural products.

The identification of the compounds of anthropogenic and natural origin in various environmental objects is based on the analysis of high-quality chromatographic profiles for complicated mixtures using chromatographic techniques in a total ion current mode (review analysis), selective detection of individual ions (targeted analysis), «ion extraction», with the use of complete mass spectral databases, data concerning the retention time values and abundance ratio for characteristic ions.

In 1994 under the leadership of V. A. Koptyug there was an automated information analytical centre developed which included an analytical testing centre accredited within the system of RosTechRegulirovaniye with respect to technical competence and independence, with modern analytical equipment, as well as an information centre that includes a multidisciplinary specialized library and database concerning the chemical aspects of environmental protection, ecology, the chemistry of naturally occurring compounds and renewable raw materials.

Basing on this complex, an information analytical technology was developed that allows: 1) performing comprehensive analyses to identify low molecular mass organic substances of human, synthetic and natural origin in various objects, 2) assessing the risk of chemical substances affecting ecosystems and human health taking into account their physicochemi-

cal, toxicological properties and biological activity, 3) predicting the properties of naturally occurring compounds, 4) studying the condition of living systems, and 5) developing the scientific basis for the creation of new substances and materials for medicine, agriculture and other industries [12].

Within the framework of the program "Evaluation of the Extent and Level of the Siberian Territory Contamination by Dioxins and Their Analogues" basing on an integrated approach developed for targeted and survey ecological analyzing the environmental objects and territories of different Siberian regions (Novosibirsk, Tomsk, Kemerovo Regions, Altai and Krasnoyarsk Territories, Republic of Buryatia, Lake Baikal, the oil- and gas-bearing provinces of the Khanty-Mansi Autonomous Area), Mongolia and Kazakhstan, the following parameters were determined [13–17]:

1) the levels of content for polychlorinated dioxins and furans (PCDD/PCDF) in various environmental samples (Table 1, Fig. 1);

2) the qualitative and quantitative characteristics of the levels of content for main organic pollutants;

3) specific pollutants those determine the condition of ecosystems and affect human health, which pollutants could be considered integral indicators;

4) characteristic ratios between individual components of organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dioxins and furans, polychlorinated biphenyls (PCBs), petroleum hydrocarbons (PH) and phenol (PhOH) those allow assessing natural and man-made contribution to the total level of pollution, ranking the regions with respect to anthropogenic load and revealing the sources of pollution;

5) the association types of individual hydrocarbons those characterize a particular area, type and capacity of human impact, which allows one to identify the source of contamination.

With the use of the complex chromatographic methods, a detailed structure and profiles of persistent organic pollutants in different sites of the Lake Baikal ecosystem a detailed structure and profiles of persistent organic pollutants in different sites of the Lake Baikal ecosystem was for the first time revealed according to the results of long-term experimental research in cooperation with the Baikal Institute of Nature Management (BINM) of the SB RAS. The following parameters were determined: 1) specific pollutants for the southern, central and northern parts of the Lake Baikal (Fig. 2); 2) bioaccumulation coefficients for organochlorine and polyaromatic com-

TABLE 1

Content of polychlorinated dioxins and furans (PCDD/PCDF) in various environmental objects of the Siberian region

Environmental objects	PCDD/PCDF (DE) content
Drinking water	<0.1 pg/L
Natural surface water	1–10 pg/L
Wastewater	5–25 pg/L
Soils of industrial areas	0.2–90 ng/kg
Soils of urban areas	0.2–6.0 ng/kg
Soils of "background" agricultural areas	0–0.08 ng/kg
Soils of refuse tips	0.1–110 ng/kg
Soils of landfills for disposal and destruction of chemical wastes	0.03–4.0 μg/kg
Soils of snow blades	0.07–1.6 ng/kg
Snow cover of snow blades	3.4–6.6 pg/L
Snow cover under emission plumes	2–11 pg/L
Bottom sediments of rivers and lakes	0.003–4.0 ng/kg
Wastes (ash, sludge) of industrial enterprises	3–200 ng/kg
Atmospheric air and gas emissions of industrial enterprises	0.3–300 pg/m^3

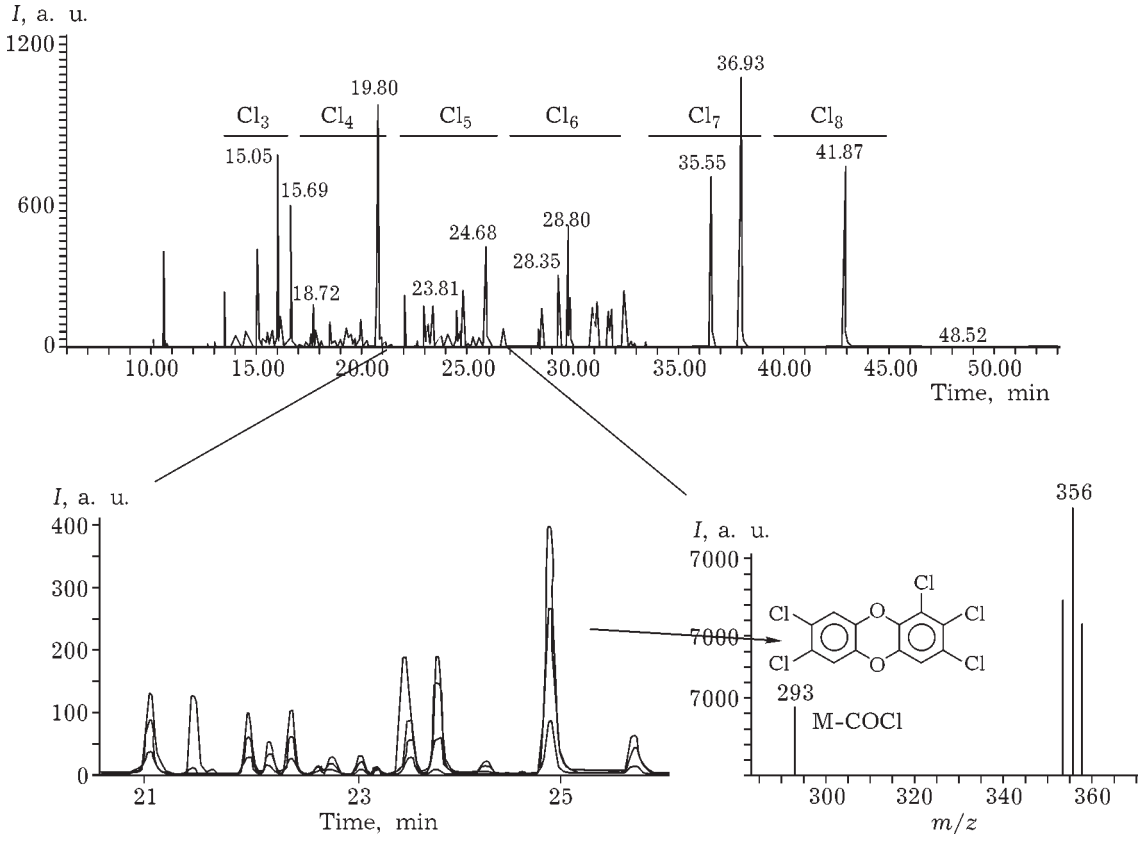


Fig. 1. Chromatography-mass spectrometry profile in the mode of selective detection of individual ions for polychlorinated dioxins (PCDDs), containing from 3 to 8 atoms of chlorine; the soil of waste incineration landfill in the Altai Territory (PCDD content in dioxin equivalents 3.8 μg/kg).

pounds; 3) the distribution of light and heavy PAHs; 4) the distribution of isomers of polychlorinated biphenyls in water, in bottom sediments and seal fat; 5) the characteristic profiles of aliphatic hydrocarbons (Fig. 3) determining the levels of man-caused and naturally occurring pollution [13]. The data obtained became a basis for the evaluation and prediction of the ecological condition of the Baikal natural territory and the development of methods for optimal monitoring large lake systems.

Together with the Institute of Computational Mathematics and Mathematical Geophysics of the SB RAS and the Institute of Inorganic Chemistry of the SB RAS we conducted a survey of the snow cover over the territories of the Priobskoye oil field, the Novosibirsk, Iskitim, Berdsk, Tomsk, Seversk, Kemerovo, Novokuznetsk, Barnaul, Zarinsk, Biysk and Beloyarsk cities and the neighbourhoods, for the content of man-caused organic substances. Data concerning the profiles and the content of polycyclic aromatic hydrocarbons and petroleum hydrocarbons obtained by means of chromatography-mass spectrometry (GC/MS) were used to develop and test the models of complex monitoring the aerosol contamination of the territory in the regions of associated petroleum gas flaring in the course of petroleum production, the emissions of power plants

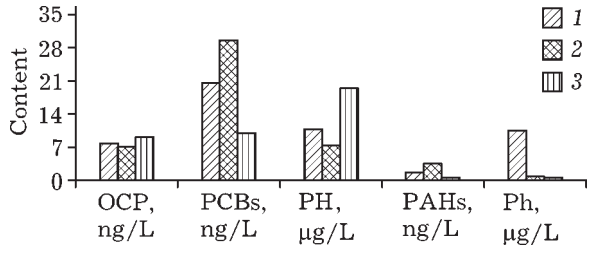


Fig. 2. Content of organic pollutants in the southern (*1*), central (*2*) and the northern (*3*) parts of the Lake Baikal. OCP – organochlorine pesticides, PCBs – polychlorinated biphenyls, PH – petroleum hydrocarbons, PAHs – polycyclic aromatic hydrocarbons, Ph – phenols.

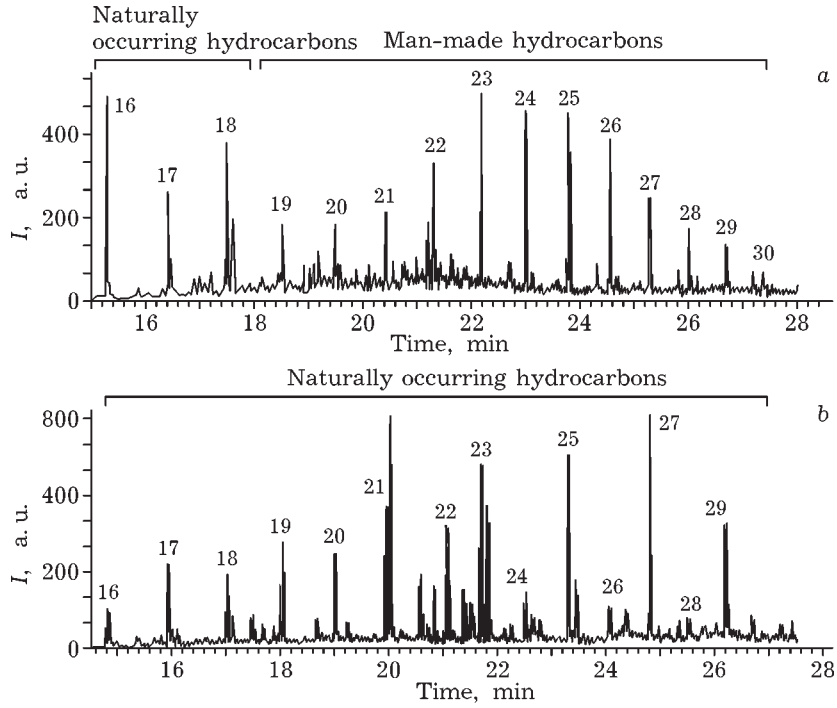


Fig. 3. Chromatography-mass spectrometry profiles of aliphatic hydrocarbons with respect to ion at m/z = 85 for two samples of bottom sediments from the Lake Baikal (the numbers above the peaks are corresponding to the number of carbon atoms in n-alkanes): a – hydrocarbons of natural and anthropogenic origin (19 mg/kg), b – hydrocarbons of natural origin (22 mg/kg).

and vehicles basing on the data of contact and remote observations, as well as to identify specific ratios between PAH, those allow adequately reveal pollution sources [18–20].

By means of posing the inverse problems concerning the transport of pollutants in the atmosphere and basing on the results of experimental studies we reconstructed the fields of aerosol deposition to determine the total PAH emissions from anthropogenic sources. Using the models of PAH in the reconstruction as tracers (markers) allows increasing the reliability of determining the restorable characteristics of the fields of snow cover pollution with heavy metals and other components.

The patterns revealed allow one to estimate the degree of man-caused impact both in the cities and in the surrounding areas, to compare the intensity of supplying the pollutants from urban areas, to perform integral estimations of population morbidity in these areas taking into account the level of man-caused impact, and to assess additional hazards with respect to the health of inhabitants of the settlements entering the zones of a significant impact of city-caused emissions.

Together with the research workers of the Institute of Chemistry and Chemical Technology (ICCT) of the SB RAS (Krasnoyarsk), we performed a complex of studies concerning the qualitative and quantitative composition of carcinogenic PAHs released in various processes of aluminium production by means of the Soderberg technology. Based on a detailed analysis of chromatographic profiles of PAHs and their derivatives, released at different stages of the aluminium production process, we established main sources of the emission of carcinogenic PAHs inherent in the environmentally hazardous technology to propose the ways to reduce cancer risks at the Krasnoyarsk Aluminium Plant. With the use of GC/MS method, we studied the composition of polycyclic aromatic hydrocarbons contained in coal pitch species and in carbon materials obtained from them, in order to establish the mechanisms of thermochemical processes [21–24].

Within the framework of the Russian-Korean-Mongolian expedition in conjunction with the staff the BIP of the SB RAS, the Institute of Environmental Geoscience of the Mongolian Academy of Sciences of the MPR, the

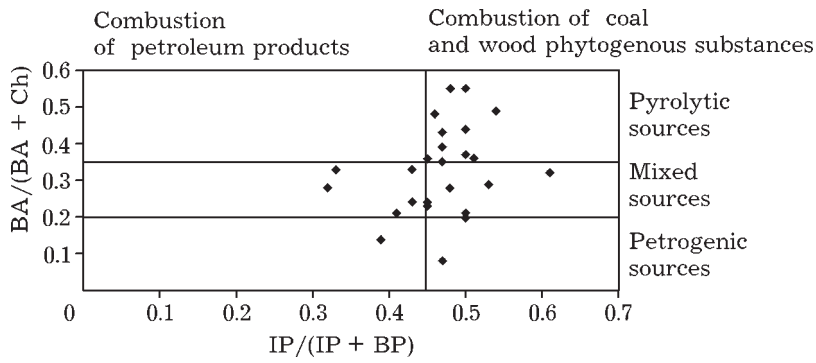


Fig. 4. Dependence of the ratio of diagnostic PAHs in bottom sediments of the river basin. Selenga River in Mongolia and Buryatia to identify the sources of pollution. BA – benz(a)anthracene, Ch – chrysene, IP – indeno(1,2,3-cd)pyrene, BP – benzo(ghi)perylene.

Miongji University, the Institute of Water Resources and Environmental Protection of the Korean Water Corporation and the Korean Environmental Institute according to the project "Integrated Model of Water Management in the Basin of the Selenga River" the UNEP program, we for the first time carried out a detailed examination of the surface water and the bottom sediment in the basin of the Selenga River over the Mongolia and Buryatia territory [25, 26]. The aim of the study consisted in determining the levels and sources and areas of the industrial pollution of the catchment basin of the Lake Baikal by persistent organic pollutants.

The analysis of the chromatographic profiles of specific pollutants allowed us to determine that the main pollution sources of the Selenga River basin are presented by coal combustion and motor transport (Fig. 4). It was suggested that the mouth of the Selenga River serves as a geochemical barrier filter that prevents the penetration of the Lake Baikal from the penetration of organic pollutants.

The results demonstrated a high efficiency of using the data concerning the qualitative and quantitative composition of OCPs, PAHs, aliphatic hydrocarbons and phenols in environmental objects, for regional ecogeochemical hygiene and environmental assessments of the condition of the environment as well as for efficient identifying the areas of strengthening and weakening the man-caused impact on the environment.

USING CHROMATOGRAPHIC METHODS FOR THE IDENTIFICATION AND INDIVIDUAL GROUP ANALYSIS OF BIOLOGICALLY ACTIVE COMPOUNDS

The complexes of biologically active compounds those exhibit a variety of biological activities (antioxidant, immunomodulatory, antibacterial, antiviral, anticoagulant, antifungal, antiallergic *etc.*) are widely used in medicine, cosmetics, food industry and agriculture [27?33]. Natural compositions usually represent multicomponential mixtures of biologically active compounds, so obtaining the data concerning their qualitative and quantitative composition is an important task in basic and applied research fields. The priority areas of basic research include: the development of methodology for spectral-chromatographic analyzing the complex mixtures of natural organic compounds, the phytochemical studying the objects of phytogenous raw materials, the studies on their metabolic profiles, revealing the chemotaxonomic and diagnostic features, the development of approaches to forecasting the physiological properties of natural mixtures basing on the knowledge of the composition, the development of scientific bases for the creation of novel biologically active compounds from renewable raw materials for medicine, agriculture, *etc.*

In the field of applied problems, of relevance is the identification of promising sources of biologically active compounds, the standardization of natural resources, the identification of indicator components of biologically active products and preparations for quality control,

the determination of authenticity and falsification of medicines and products based on natural raw materials.

Currently, there is skyrocketing the amount of bioactive natural compounds belonging to the group of flavonoids, sterols, carotenoids, alkaloids, vitamins, fatty acids *etc*., those are used to create new tools, products and preparations. In connection with the fact that even in the everyday life there are terms such as phytochemical substances, nutrients, bioactive supplements, functionalized and environmentally friendly products increasingly used, an especially important significance is attached today to naturally occurring biologically active substances those are used for their design and creation.

The use of the gas (GC) and high performance liquid chromatography (HPLC) with different kinds of detection (diode array, MS selective detectors) in combination with the corresponding bases of mass spectrometric and spectroscopic data chromatographic collections allows one in most cases to obtain information concerning the individual group composition of multi-component naturally occurring mixtures without isolating the individual compounds and without using a large-scale set of standard substances. The methodology of the individual-group analysis of naturally occurring objects consists in the creating the digital collections of standardized chromatographic profiles for the samples of natural origin and spectral analytical characteristics of the important groups of biologically active compounds obtained by modern methods of gas and liquid chromatography.

In order to determine the individual and the group composition of naturally occurring low molecular mass organic substances, an approach was proposed based on the analysis of chromatographic profiles obtained under standardized conditions, and of the spectral characteristics of main components (retention times, mass spectrum, UV spectrum, UVt spectral relationships) [34].

One of the most widespread and numerous classes of secondary plant metabolites is presented by phenolic compounds. A large group of natural phenolic compounds are presented by phenylpropanoids **I–V**, containing one or more $C_6–C_3$ fragments in the structure (hydroxycinnamic acids, anthocyanins, flavonoids, lignans and their derivatives, flavolignans). These compounds have recently become the subject of rapt attention in the course of searching for promising bioactive compounds and creating biologically active compounds on their basis [29].

The UV spectra of phenolic compounds (Fig. 5), as a rule, exhibit several absorption bands, so the detection in the course of HPLC analysis is performed basing on the most characteristic wavelength values inherent in the mentioned compounds (280 nm for catechins, flavanonols, lignans, flavolignans; 320 nm for hydroxycinnamic acids, coumarins; 360 nm for flavanonols; 520 nm for anthocyanins).

For all the types of the main structural types of phenylpropanoids, we prepared and investigated the chromatographic profiles under standardized conditions, typical UV and mass spectral characteristics [34].

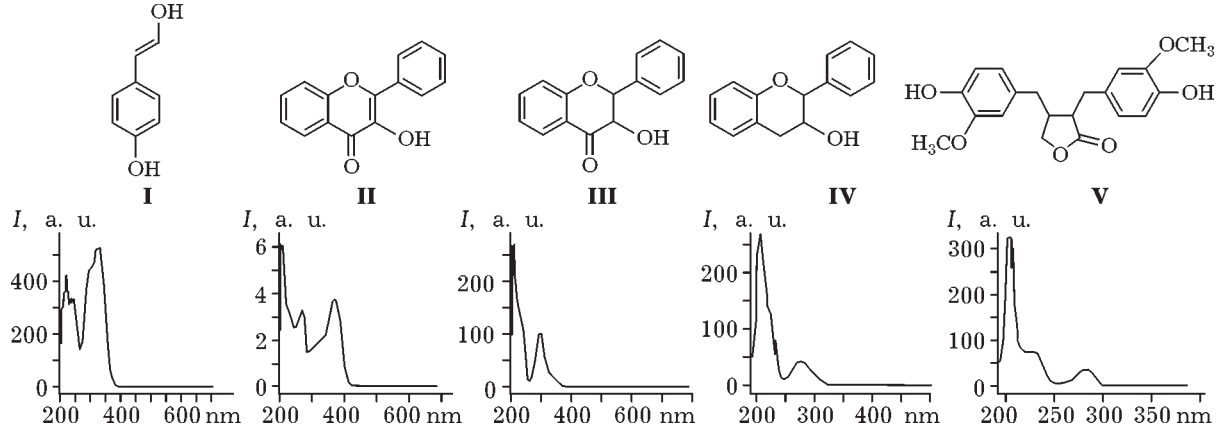


Fig. 5. Main types of naturally occurring phenylpropanoids **I–V** and their UV spectra.

The simplest group of phenylpropanoids such as hydroxycinnamic acids are contained in almost all higher plants in various combinations, in the free form or in the form of glycosides. The chromatographic profile and spectral characteristics of hydroxycinnamic acids we obtained in the course of analyzing the extracts isolated from larch *Larix Sibirica*.

The main type **II** flavonoids isolated from a number of plants comprise such well-known compounds as quercetin, kaempferol, isorhamnetin, myricetin, apigenin. Using the HPLC technique, we obtained the chromatographic profile of type **II** flavonoids inherent in medicinal plant Orthilia secunda. In order to obtain the chromatographic profile and UV spectral characteristics of type **III** flavonoids we analyzed an acetate extract from larch wood. The main peaks in the chromatogram correspond to dihydroquercetin and dihydrokaempferol.

The chromatographic profile of catechins those belong to the group of type **IV** flavonoids was obtained by using a sample of green tea extract, *Camellia sinensis*. The peaks in the chromatogram exhibit UV spectra inherent in catechins. The main components of the extract are presented by epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate. For the green tea catechins, we evaluated the range of the values of characteristic spectral ratios.

An important group of naturally occurring phenolic compounds is composed of lignans, the compounds whose structure includes two phenylpropane fragments of the general formula $(C_6–C_3)_2$, in most cases interconnected with β,β′-carbon atoms of the side chains. Lignans are widely distributed in the plant kingdom being available both in the free form and in the form of glycosides. They exhibit a broad spectrum of biological activities, being inherent in particular plant groups, and they could be used as a chemotaxonomic mark. In order to obtain the chromatographic profiles of lignans $(C_6–C_3)_2$ with a guayacyl type of aryl fragment substitution we analyzed different polar extracts of coniferous trees to identify their main components.

The main phenolic compounds of the seeds of magnolia vine (*Schisandra chinensis*) represent the lignans of other structural type; they belong to dibenzo-[a,c]-cyclooctadiene derivatives. With the use of GC/MS and HPLC we obtained the profiles of the magnolia-vine lignans, and identified their main UV spectral characteristics.

Flavolignans represent flavonoids those have in the structure an additional phenylpropanoid fragment. As an example of the totality of natural flavolignans we used the seed extract of Saint Mary's thistle (*Silybum marianum*). In the course of studying of the St. Mary's thistle extract by means of HPLC with diode array and mass selective detectors, we obtained its chromatographic profile and UV spectral characteristics inherent in the components of the extract. We identified isosilichristin, silichristin, silidianin, silybin A, silybin B, isosilybin (cf. [35]), for those we have calculated the ranges of characteristic spectral ratio values.

A high biological activity is demonstrated by anthocyanins, the group of natural phenolic compounds, whose extensive study is performed all over the world. The characteristic absorption band is observed for anthocyanins to be centred at $\lambda = (520 \pm 10)$ nm; there is no considerable absorption observed in this region for the majority of naturally occurring compounds, whereby this wavelength value is used as an analytical one in the course of the HPLC analysis of anthocyanins.

We obtained the chromatographic profiles and UV spectral characteristics of anthocyanins for blue honeysuckle *Lonicera caerulea*, black currant *Ribus nigrum*, bilberry *Vassinium myrtellus*, chokeberry *Aronia melanocarpa*, arrowwood *Viburnum*, bog whortleberry *Vaccinium uliginosum*, and European dewberry *Rubus caesius* under the conditions of isocratic elution. It was demonstrated that the UV spectra at the major peak are almost the same and correspond to anthocyanins (the characteristic peak at ~520 nm), whereas the chromatographic profiles differ to a considerable extent from each other being typical for each plant (Fig. 6).

The resulting spectral and chromatographic characteristics of the major phenylpropanoids were used as diagnostic and identification parameters in the course of phytochemical studies [34].

The potentialities of using the approach developed concerning the studies on the individual and group composition of different objects are demonstrated by the example of our own investigations and works performed in cooper-

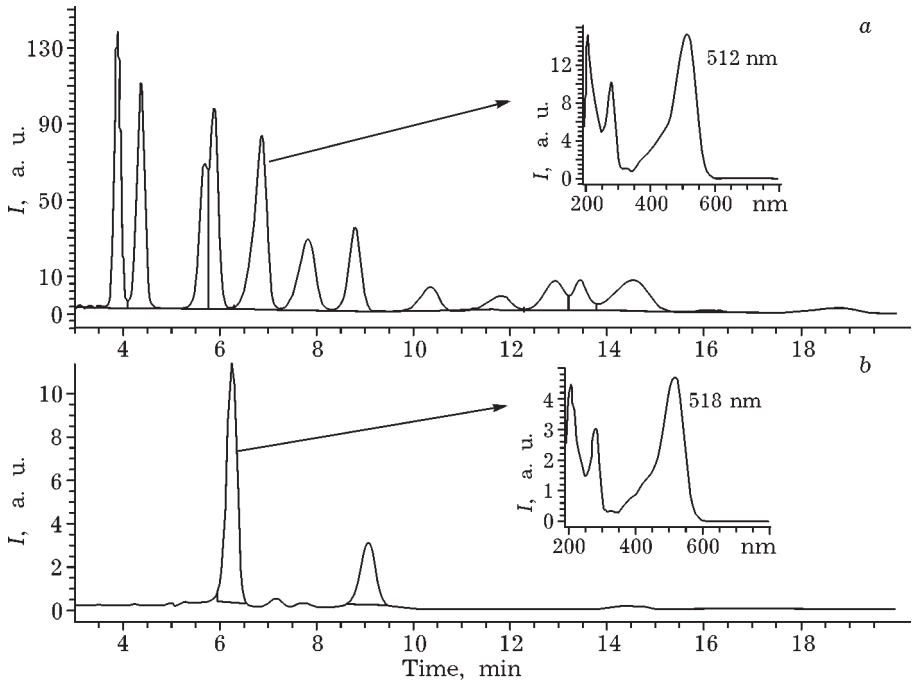


Fig. 6. Chromatographic profiles of anthocyanins from *Vaccinium myrtillus* (*a*) and *Aronia Melanocarpa* (*b*).

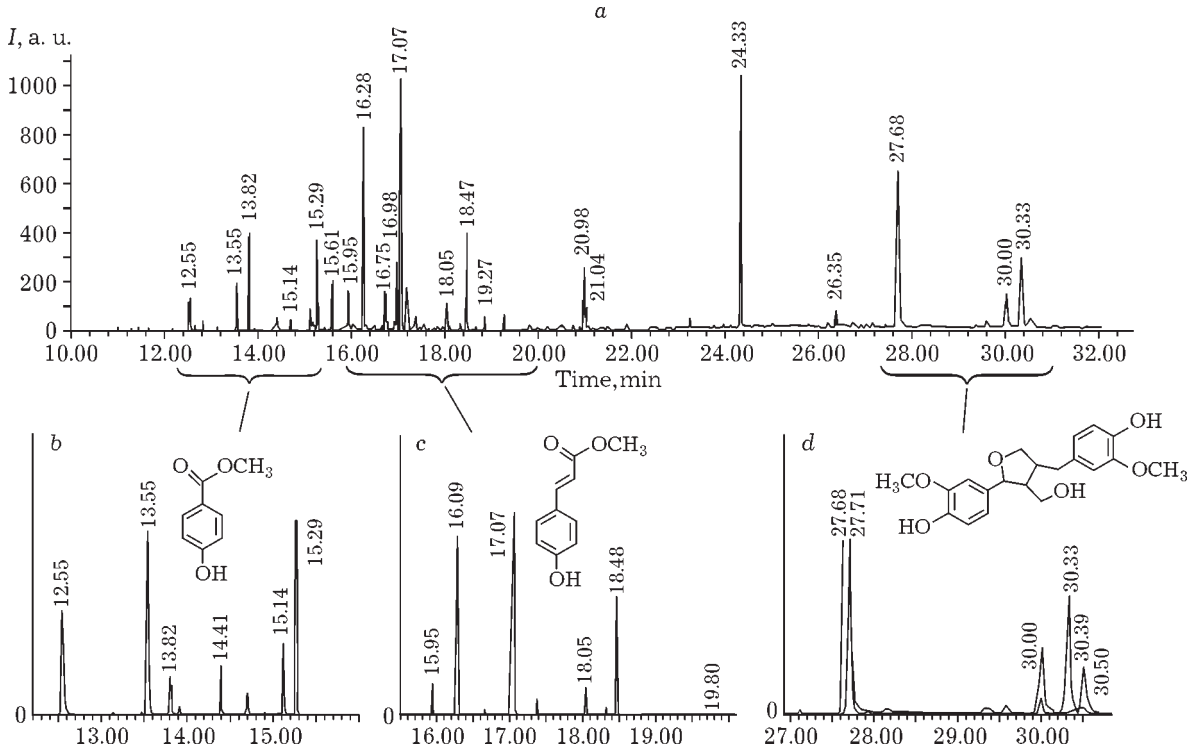


Fig. 7. Chromatography-mass spectrometry profiles of phenolic complex (*a*), hydroxybenzoic acids (*b*), hydroxycinnamic acids (*c*), lignans (*d*) from the Siberian abies.

ation with the laboratories of the Institute, the scientific research and educational institutes of the Siberian region [13].

With the help of the technique of «ion extraction» basing on molecular and characteristic fragmentary ions and on data concerning the diagnostic UV spectral ratios we obtained chromatographic profiles for the major groups of phenolic compounds from the wood green of Siberian fir (*Abies Sibirica*): hydroxybenzoic and hydroxycinnamic acids, flavonoids, lignans. We identified quercetin, kaempferol and isorhamnetin (flavonoids); 3,4-divanilyltetrahydrofuran, lariciresinol, isolariciresinol, matairesinol, pinoresinol, hydroxymatairesinol (lignans), 4-hydroxy-, 4-methoxy-, 4-hydroxy-3-methoxy-, 3,4-dimethoxy-, 3,4,5-trimetoxycinnamic acids, 4-hydroxy-, 4-methoxy-, 4-hydroxy-3-methoxy and 3,4,5-trimethoxybenzoic acids (Fig. 7).

As the result of investigations carried out in conjunction with the Central Siberian Botanical Garden (CSBG) of the SB RAS (Novosibirsk), for the first time the individual and group composition was established for the phenylpropanoid complex inherent in blue honeysuckle fruits of various ecogeographical origin introduced in the CSBG. It was demonstrated that its main components represent anthocyanins, flavonols and flavones, hydroxycinnamic acids (Fig. 8). In the group of anthocyanins, we identified the following compounds: cyanidin-3,5-diglucoside, cyanide-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, peonidin-3-glucoside; in the group of flavanonols and flavons we identified rutin and quercetin-3-glucoside, luteolin-3-glucoside and luteolin-3-rutinoside, diosmin and various quercetin based glycosides, as well as chlorogenic, neochlorogenic and dicaffeoylquinic acid in the group of hydroxycinnamic acids.

The analysis of the variability in the total content of flavones and flavonols, anthocyanins and hydroxycinnamic acids in the groups and individual components, depending on the genetic origin of the representatives of *Lonicera caerulea* L. demonstrated that the subspecies of *L. caerulea* studied differ in the quantitative content of phenolic compounds, whereas the componential composition remains constant. We established that in the case of cross breeding the honeysuckle samples geographically remote concerning the place of origin, in the hybrids of the first generation there is a significant increase observed in the content of bioflavonoid complex in fruits [36].

Together with the research workers of the Wood Chemical Engineering and Biotechnology Chair of the Krasnoyarsk SSTU, using the HPLC technique we investigated the individual and group composition of phenolic compounds from the alcoholic extract of the buds of balsam poplar *Populus balsamifera* (Fig. 9).

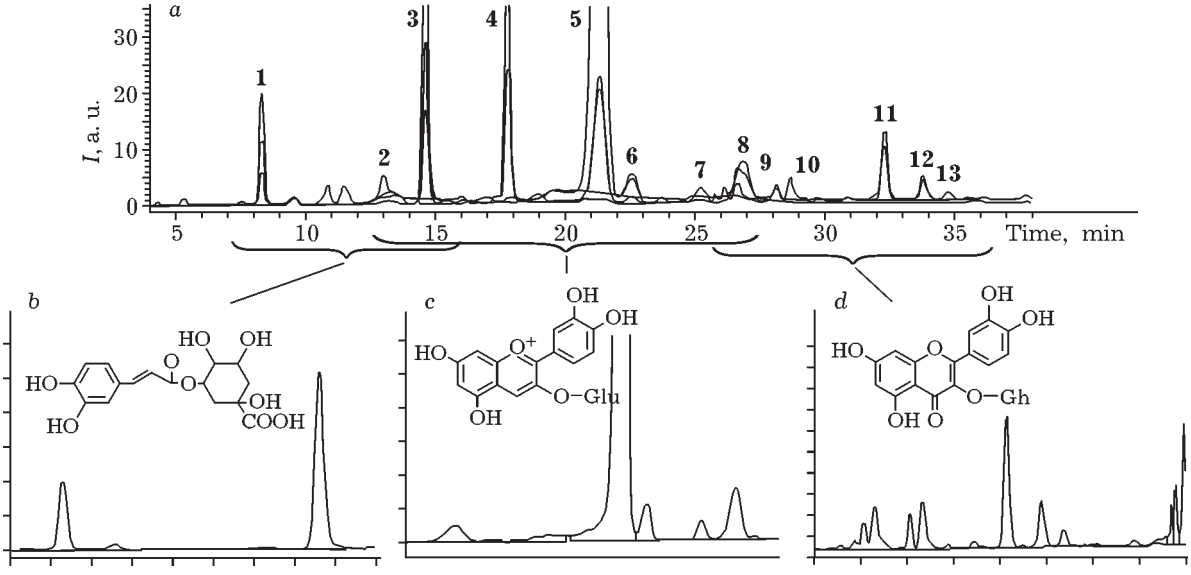


Fig. 8. Chromatographic profiles of phenylpropanoid complex (*a*), hydroxycinnamic acids (*b*, λ = 320 nm), anthocyanins (*c*, λ = 520 nm), flavonols and flavones (*d*, λ = 360 nm) from the extract of blue honeysuckle.

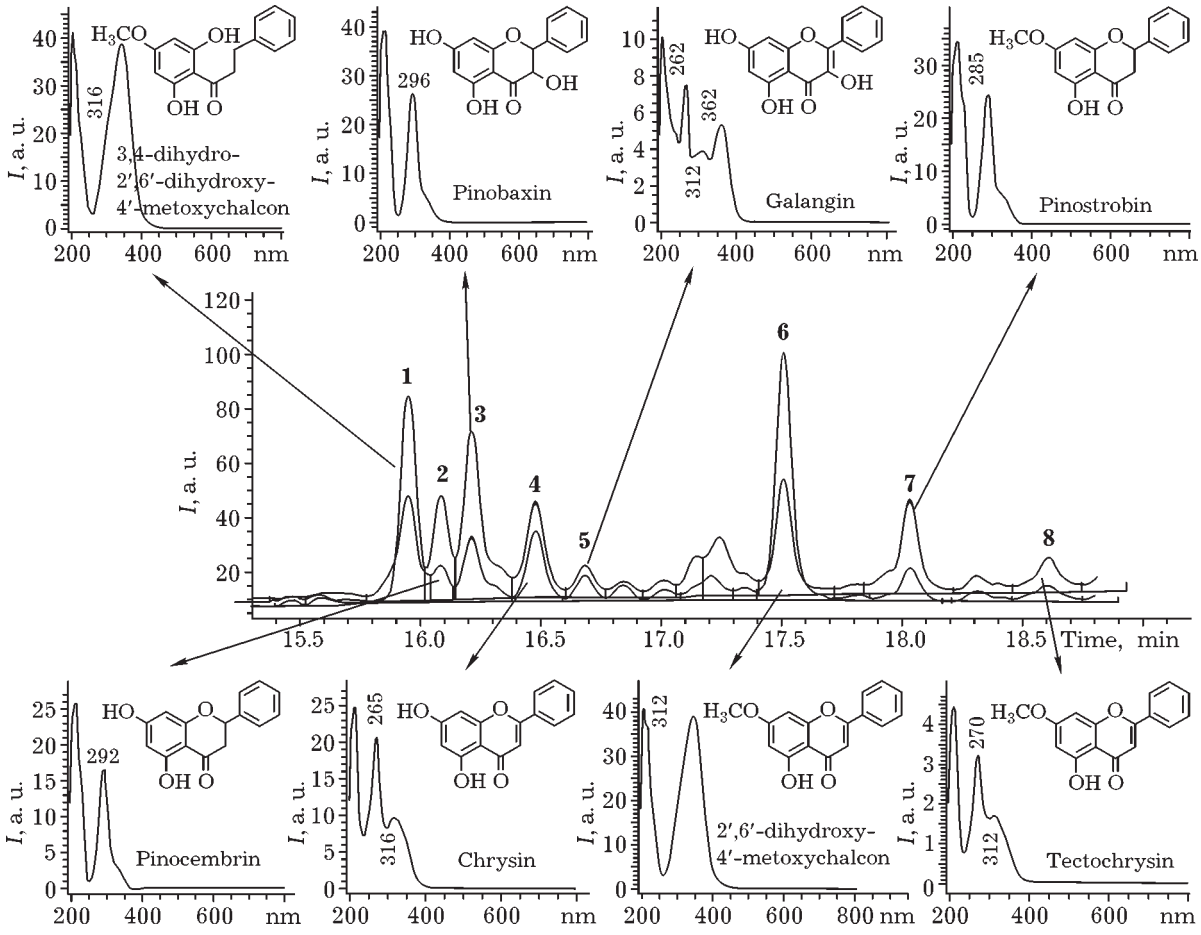


Fig. 9. Chromatographic profiles (HPLC) of flavonoids from the alcoholic extract of balsam poplar buds (λ = 290, 326 nm) and UV spectra of major peaks.

It was demonstrated that the main flavonoids of the extract are presented by pinobaxin, pinostrobin, pinocembrin, chrysin, galangin, 3,4-dihydro-2′,6′-dihydroxy-4′-metoxychalcon, 2′,6′-dihydroxy-4′-metoxychalcon, as well as hydroxycinnamic acids were identified [37].

With the use of HPLC and GC/MS methods we obtained chromatographic profiles of fatty and resin acids, sterols, tocopherols, di- and triterpenoids, lipid fractions of the products of phytogenous and animal origin. It should be noted that the use of modern chromatographic methods allows reliably identifying the minor components of vegetable oils and animal fats (tocopherols, carotenoids, sterols, squalene), whose qualitative and quantitative composition is individual for oil and fat products and determines their biological properties. The results of studying the profiles of fatty acids, carotenoids, phytosterols, tocopherols allow researchers to predict the main properties of oil and fat products, in particular biological properties, resistance with respect to oxidation, in order to develop criteria for their identification [13].

Thus, as the result of phytochemical studies we developed an approach to determining the group and individual composition of the multicomponential mixtures of biologically active compounds *via* analyzing the chromatographic profiles, retention time values and spectral characteristics (spectral ratios, UV and mass spectra).

For a large group of biologically active compounds belonging to the groups of flavonoids, anthocyanins, lignans, hydroxycinnamic acids, catechins, sterols, flavolignans, capsaicinoids, fatty acids, terpenoids, sugars, vitamins, using modern methods of GC/MS and HPLC

with diode-array and mass selective detection under standardized conditions, we obtained the following identification parameters: chromatographic profiles ("fingerprints"), retention time, UV and mass spectra, characteristic spectral ratios. It has been demonstrated that hydroxycinnamic acids can be characterized by the spectral ratio values A (320/280), A (320/220) and A (220/280), whereas A (254/220) A (280/254) A (280 / 220) A (360/254) and A (360/220) are inherent in flavonoids, the values of A (254/220) A (280/254) and A (280/220) are inherent in lignans and flavolignans, A (520/280) and A (280/254) are inherent in anthocyanins.

The approach developed could be efficiently used for the studies in the field of phytochemistry, for developing the methodology of spectral chromatographic analysis of multi-component compositions consisting of low molecular mass organic compounds of natural origin, for developing the scientific bases for making novel biologically active compounds from renewable raw materials for medicine, agriculture, food and cosmetic industries.

USING CHROMATOGRAPHIC PROFILING IN THE DEVELOPMENT OF BIOLOGICAL ACTIVE PREPARATIONS FOR AGRICULTURE FROM RENEWABLE RAW OF SIBERIA

The creation of modern physiologically active compounds for medicine and agriculture based on renewable plant raw materials is one of the priorities of the of natural compounds chemistry. The conifers of Siberia serve as agents producing biologically active substances of broad action range. The development of new crop protection chemicals based on modern biological active substances of natural origin, so-called "biorational pesticides", and the creation of biological crop protection technology on their basis belongs to one of the urgent tasks of fundamental and applied research [38].

By means of gas chromatography and liquid chromatography, researchers obtained chromatographic profiles for lipid, carbohydrate and phenolic bioactive complexes isolated via sequential extraction using solvents with increasing polarity from the biomass of fir and larch wood green. Their individual group composition was studied and key components were identified.

The composition was investigated for a larch wood phenolic complex obtained using ultrasonic and microwave impact. By means of HPLC and GC/MS methods we demonstrated that the main groups of phenolic compounds are presented by flavonoids (dihydroquercetin, dihydrokaempferol, quercetin), lignans (lariciresinol, secoisolariciresinol, matairesinol, pinoresinol, conidendrin, 3,4-divanillyltetrahydrofuran), hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, 3,4-dimethoxycinnamic) and polymeric phenolic compounds.

The main components were determined for a phenolic complex isolated from abies wood waste green, waste of Novosil preparation: hydroxycinnamic glycosides and *p*-hydroxybenzoic acids, flavonoids and lignans. Basing on this complex a low-dose preparation "Abistim" was developed [39]. From the larch wood, with the help of CO_2 extraction we obtained biologically active lipid-phenol complex that served the base for making preparation "Biofungistim" [40] and the main components of the complex were identified. They are fatty and diterpene acids, terpene hydrocarbons, alcohols, aldehydes, squalene and sterols (campesterol, sitosterol, and stigmasterol), phenolic acids, acetophenones, phenols, lignans (matairesinol, conidendrin, secoisolariciresinol, pinoresinol, and laricinesinol).

For all the compositions isolated, researchers performed long-term field and laboratory testing for physiological activity in a wide range of cereals, legumes, vegetables and bulbs of different cultivars. Novel low-dose preparations for crop production, such as "Biofungistim" obtained from larch wood, "Abistim" and "Pihtoros" [41] obtained from Siberian abies wood green exhibit growth-promoting, and fungicidal and antistress properties (biological efficiency ranging within 40–100 %) allow one to treat fungal, bacterial and viral diseases, promotes increasing the productivity and quality of vegetable and grain crops.

STUDYING THE TRANSFORMATION AND METABOLISM OF CHEMICAL SUBSTANCES IN LIVING SYSTEMS

Kynurenine and its derivatives, being the metabolites of tryptophan enzymatic reactions form a human eye lens; they act as UV filters to protect the retina from irradiation. It is as-

sumed that thermal and photochemical reactions involving kynurenines could result in irreversible modifying the lens proteins, and subsequently, in the development of cataract. The mechanisms of these reactions are poorly understood, so the study of the chemical properties kynurenines is of great importance for photochemistry, biology and medicine. The studies concerning the metabolic transformations of the kynurenine UV filters were carried out jointly with ITC of the SB RAS, ICG of the SB RAS, the Novosibirsk State University and the Novosibirsk Regional Hospital with the use of HPLC methods and with involving of our own collection of spectral chromatographic characteristics.

Using the complex of spectral and chromatographic methods we studied the composition of the products of photochemical and thermochemical reactions of kynurenine, and obtained data concerning the kinetic parameters of the processes within the range of 50–90 °C [42, 43]. Basing on these results, a scheme was proposed for thermochemical kynurenine conversion, including the processes of deamination, decarboxylation, and cyclization. For the first time the formation of hydroxyquinoline was revealed.

A study was performed concerning 48 samples of cataract lens from human eyes and the metabolic profiles were obtained (Fig. 10) [44, 45].

Basing on the transformation patterns proposed for kynurenine UV filters in human eye lens (Scheme 1) we established qualitative and quantitative composition of kynurenine derivatives in the hydro-alcoholic extracts of cataractous lenses and the total content of the derivatives. It was established that the concentration of individual components could vary within the range of 50 times. So, for example, the concentration of 3-OH-kynurenine glycoside (3-OHKG) ranges from 6 to 320 nmol/g.

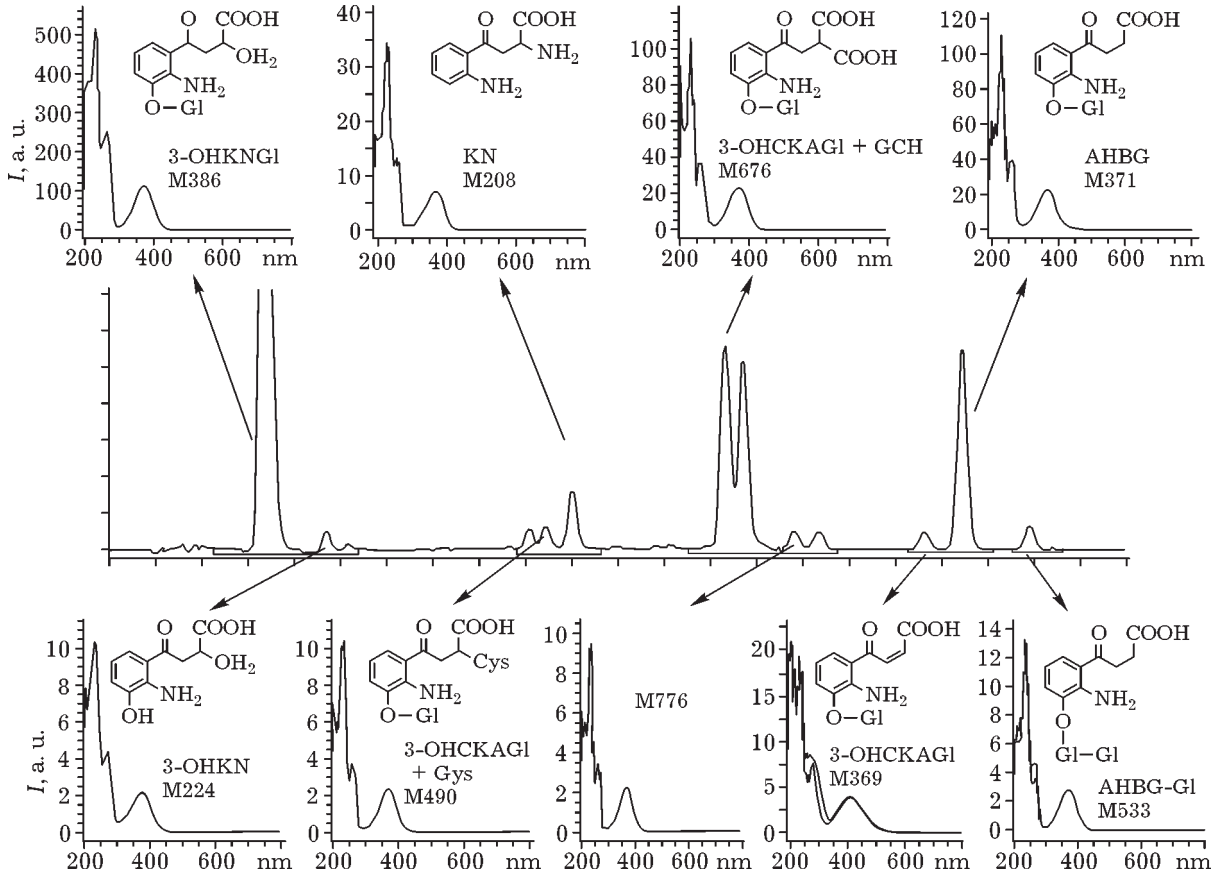


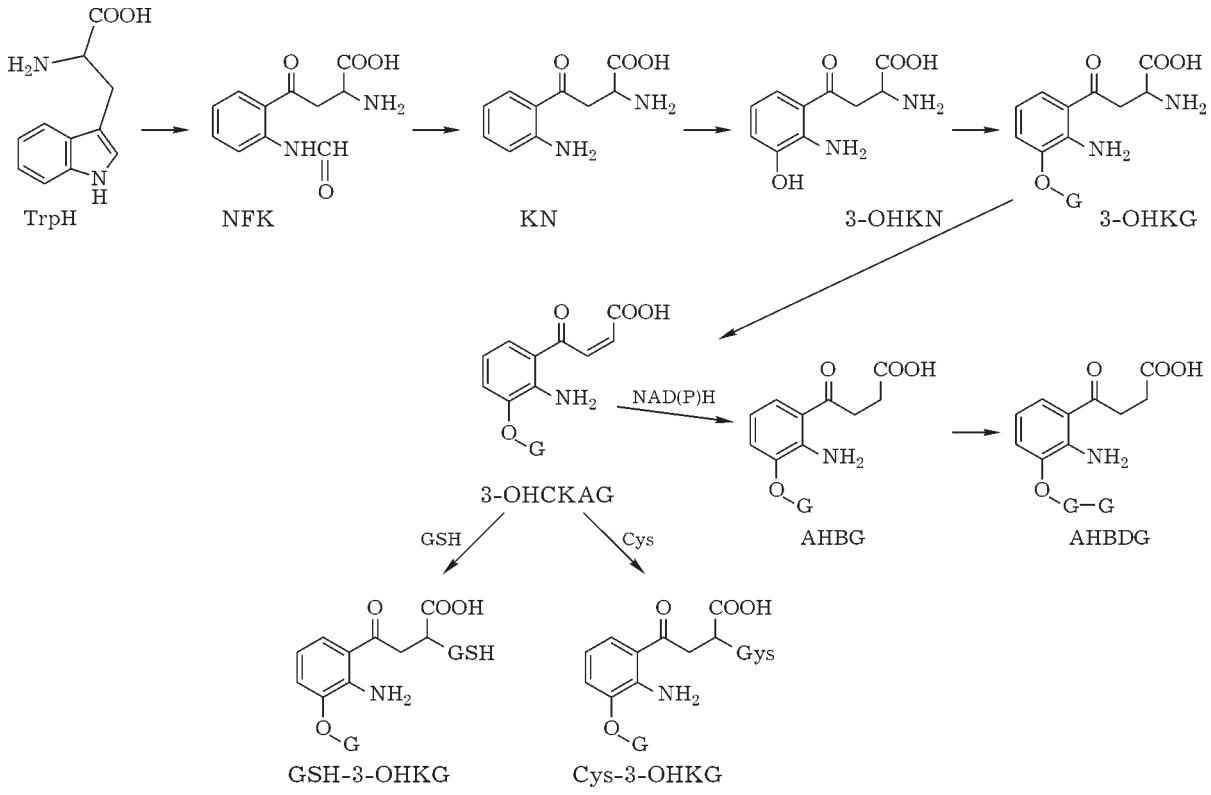
Fig. 10. Chromatographic profiles, UV spectra and molecular mass values for the major low molecular mass compounds of human eye cataract lens.

The identification was performed concerning the main components of the extracts of human eye cataractous lenses. It was for the first time found that the composition of the lens involves deaminated 3-OH-kynurenine glycoside (3-OHCKAG); an additional evidence of this consists in the fact that an adduct of 3-OHCKAG with glutathione is registered.

The chromatographic profiles of eye lens UV filters were obtained and investigated for the two lines of rats (Wistar and OXYS) depending on the age (from 1 day to 2 years). Wistar rats are considered the norm, whereas the OXYS rats represent premature aging rats being the first domestic model of senile cataract created at the Institute of Cytology and Genetics of the SB RAS (Novosibirsk). It was found that, contrary to a popular belief, the lenses of rats contain kynurenine. It was demonstrated that the maximum concentration of kynurenine corresponds to the age of about 0.5 months, whereas by 2 years the value demonstrates a decrease to almost zero level. Changing the concentration of tryptophan is symbatic with kynurenine concentration changing before one year old, whereas by two years the concentration of tryptophan exhibits a slight increase [46].

Thus, the investigations performed resulted in revealing the composition and structure of the kynurenine UV filters of human eye cataractous lenses to obtained their metabolic profiles. For the first time, we detected deaminated hydroxykynurenine glycoside in a human eye lens, a key intermediate, resulting in an irreversible transformation of human eye lens proteins. We have experimentally confirmed one of the schemes of the irreversible modification of lens proteins with the participation of kynurenine UV filters, leading to the development of the cataract. Potentialities are demonstrated with respect to using Wistar and OXYS rats as the models for studying the development of the cataract. The data obtained concerning the molecular processes of cataractogenesis open up possibilities for the development of medical diagnostics, the prophylaxis and treatment of cataracts.

Together with the IEVSFE of the SB RAAS, using HPLC technique we performed pharma-



Scheme 1.

cokinetic studies on the accumulation and excretion of drug Aversectin with potent antiparasitic activity, by the action of the natural mixture of eight 16-membered macrocyclic lactols obtained *via* microbiological synthesis with the use of the culture of *Streptomyces avermitilis*. Techniques were developed for determining the content of aversectin plasma, fat, and muscle and liver tissues of farm animals by means of HPLC with UV detection. Pharmacokinetic data were obtained for aversectin complex in the course of testing in farm animals of the Mountain Altai region (sheep, deer, cows), in the case of oral administering, which data were used for the development of new and efficient anti-parasitic drugs for agriculture and modern technologies for using them [47, 48].

Together with researchers from the ICEL of the SB RAMS, using GC and GC/MS we obtained metabolic profiles and studied the patterns of the accumulation and distribution of free fatty acids (as the markers of the pathogenic activation of lipid oxidation by peroxides), tocopherols (as the markers of antioxidant activity) in the peripheral blood and lymphatic systems of an organism under normal conditions in the course of modelling the ischemia and chronic hepatitis and correcting them via adding biologically active supplements to the diet of animals, in the case of low energy laser radiation influence upon an organism. The results obtained indicate an important role of the lymphatic system in the mechanism of the oxidative homeostasis in an organism [49–51].

CONCLUSIONS

One of the most important areas of modern chemical analysis consists in the development of the methodology of the spectral chromatographic study of complicated compositions containing low-molecular mass organic substances of natural and anthropogenic origin with identifying the components, determining their origin, with the ability of predicting the properties of biologically active substances and novel materials obtained from the mentioned substances.

The problem of analyzing the complex mixtures of organic substances do not have an universal solution, and one of the most promising approaches to solve this problem consists in obtaining chromatographic profiles («fingerprints») of the objects under investigation.

Studying the chromatographic profiles of man-made chemical substances, especially of persistent organic pollutants, allows one to identify the sources of pollution, to develop the scientific basis of ecosystem analysis, of assessing the hazards of the action of chemical substances on human health, as well as to develop efficient environmental protection technologies.

Obtaining and analyzing the chromatographic profiles (metabolic profiles) for living systems are of a great importance for chemosystematics, biochemistry, medicine, biology and agriculture. In addition, they offer great potentialities for the efficient use of renewable plant and animal raw materials for the production of biologically active substances of a wide range of actions for the development of novel diagnosis methods, for the prevention and treatment of a certain range of diseases. Chromatographic profiles also allow one to solve many applied problems connected with determining the source of raw materials, the authenticity and quality of natural products.

In order to obtain highly informative chromatographic profiles and to solve the problems of recognizing the «chemical patterns» of complicated objects and systems, it is most promising to use the methods of high performance gas liquid chromatography with mass-selective and diode-array detectors in combination with databases on physicochemical properties, biological activity, origin, behaviour in the environment, on the methods of analysing and identifying the organic substances of natural and anthropogenic origin.

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