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NATURAL DEGRADATION WAYS OF HERBICIDES OF SULFONYLUREA GROUP

Currently in agriculture much attention is paid to development and use of low-cost and at the same time effective methods of fight against weeds. In this regard, widely purchased sulfonylurea herbicides. The paper presents the general characteristics, mechanism of action, degradation pathways (hydrolysis, photochemical transformation and microbial degradation) of metsulfuron-methyl and tribenuron-methyl. The key role of chromato-mass-spectrometry to establish the mechanisms of destruction these herbicides by soil microorganisms is shown.

Introduction. Derivatives of sulfonylurea is a group of chemical compounds, which comprise herbicides of new generation, showing a high biological activity upon application rates by 1–2 orders of magnitude lower compared to traditionally used preparations.

Typical representatives of the compounds of sulfonylureas are metsulfuron-methyl and tribenuron-methyl (Fig. 1).

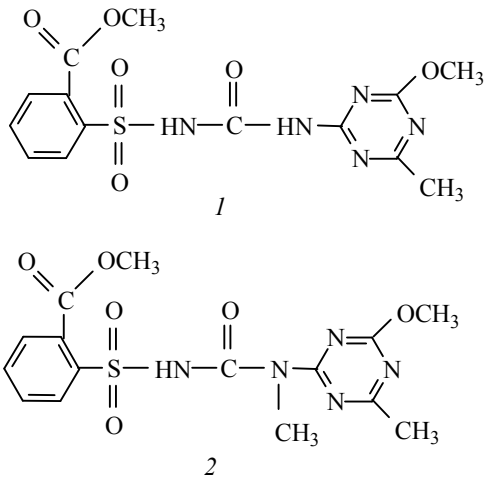

Fig. 1. The structural formulas compounds of sulfonylureas: *1* – metsulfuron-methyl; *2* – tribenuron-methyl

Herbicides are used for crops of cereals, flax, corn, cotton, peanuts, rice, soybeans and other crops to fight dicotyledonous weeds, including those resistant to 2,4-D [1].

Metsulfuron-methyl is the active ingredient of preparations such as “Akkurat”, “Laren Pro”, “Magnum”, “Meturon”, “Radgmetsol” (consumption rate of 8–10 g/ha), tribenuron-methyl – “Garmond”, “Grand”, “Granstar”, “Gyurza”, “Tameron”, “Tribune” (the rate of flow of 15–25 g/ha) [2].

Main part. The mechanism of action of herbicides of sulfonylureas group consists in inhibition of acetolactate synthase – key enzyme of biosynthesis of amino acid with alkyl side chains (Fig. 2).

Under the influence of these herbicides cells stop dividing and the plant dies. Among cultural plants, resistant to sulfonylureas there happens their metabolism.

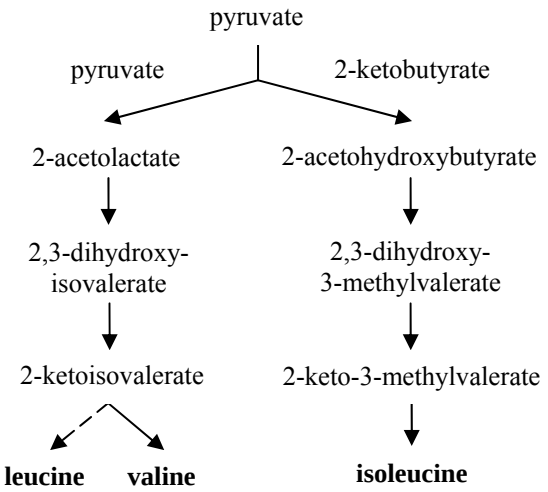

Fig. 2. The scheme of the biosynthesis of amino acids with alkyl side chains

Acetolactate synthase controls the synthesis of aliphatic amino acids with a branched carbon skeleton, the deficit of which disturbs the protein synthesis and slows down cell division. As a result the plant stops growing, and gradually dies. A significant fact is the absence of the acetolactate synthase of warm-blooded animals, in particular in humans, which explains the safety of these preparations for nonvegetables [3].

Herbicides decomposition in soil may occur under the action of chemical (hydrolysis), physical (photolysis) and biological (microbial degradation) factors.

Hydrolysis of sulfonylureas takes place due to moisture, adsorbed by soil particles. Hydrolysis speed depends on the chemical structure of herbicides, and on external conditions – temperature, humidity, level of acidity and soil type [1].

The main products of hydrolysis of metsulfuron-methyl and tribenuron-methyl, according to the literature [4, 5], are presented in Fig. 3 and Fig. 4 respectively.

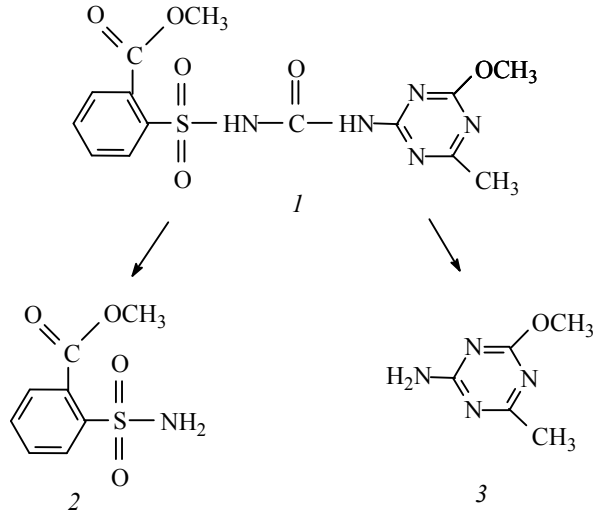

Fig. 3. The main products of hydrolysis of metsulfuron-methyl: *1* – metsulfuron-methyl; *2* – methyl-2-(aminosulfonyl)-benzoate; *3* – 4-methoxy-6-methyl-2-amino-1,3,5-triazine

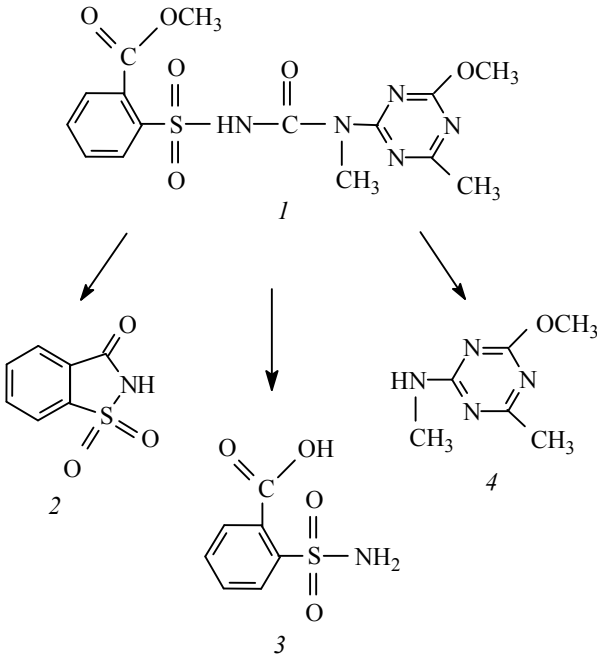

Fig. 4. The main products of hydrolysis of tribenuron-methyl: *1* – tribenuron-methyl; *2* – 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin); *3* – 2-(aminosulfonyl)-benzoic acid; *4* – 4-methoxy-6-methyl-2-aminomethyl-1,3,5-triazine

Photolysis is an ion-radical process of transformation of substances under action of quanta of absorbed electromagnetic radiation in the ultraviolet or visible regions of the spectrum.

Since derivatives of sulfonylureas have aromatic hydrocarbonic and heterocyclic chromophoric groups absorbing radiation in the ultraviolet region of the spectrum, then under its influence herbicides undergo transformations, leading, eventually, to degradation of these compounds.

Photolysis of metsulfuron-methyl is highly dependent on pH. Photodestruction occurs more intensively with unionized form of metsulfuron-methyl, and the products differ in different solvents, under the effect of radiation with different values for the wavelengths as well (Fig. 5). The mechanism of photochemical transformation of metsulfuron-methyl may occur in four main competitive ways: rupture of bonds C–N, N–CO, N–S and S–C in sulfonylurea fragment of the molecule [6].

Due to the fact that in the structure of tribenuron-methyl one of the atoms of nitrogen of urea-fragment has CH_3-group, pairing of benzene and triazine rings is broken, what makes difficult of photochemical excitation and destruction this compound under the influence of electromagnetic radiation of the visible and near ultraviolet regions of the spectrum.

The most important component of the herbicides decomposition in soil is degradation by soil microbiota due to the ability of microorganisms to adapt their enzyme systems to specific substrates and transform them. Some metabolites are more toxic than the original compounds. With the purpose of definition of such metabolites it is necessary to know the mechanisms of biodegradation of specific pesticides.

Study of the mechanisms of herbicides biodegradation is based on the identification of their metabolites using modern physico-chemical methods of the structural-functional analysis.

In particular, Fig. 6 presents a chromatogram metsulfuron-methyl and intermediates of its microbial degradation.

The analysis showed that the chromatographic peak with retention time of 6.98 min corresponds to metsulfuron-methyl as $[M+H]^+$ with m/z 382.66; peak with retention time of 2.87 min – methyl-2-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)carbamo- yl]sulfamoyl]-4-hydroxybenzoate as $[M+Na]^+$ with m/z 420.36; peak with a retention time of 3.80 min – methyl-2-(aminosulfonyl)-benzoate as $[M+H]^+$ with m/z 216.24; peak with retention time of 5.62 min – 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin) as $[M+Na]^+$ with m/z 206.43; peak with retention time of 10.79 min – product with an open triazine cycle in the form of $[M+H]^+$ with m/z 398.24.

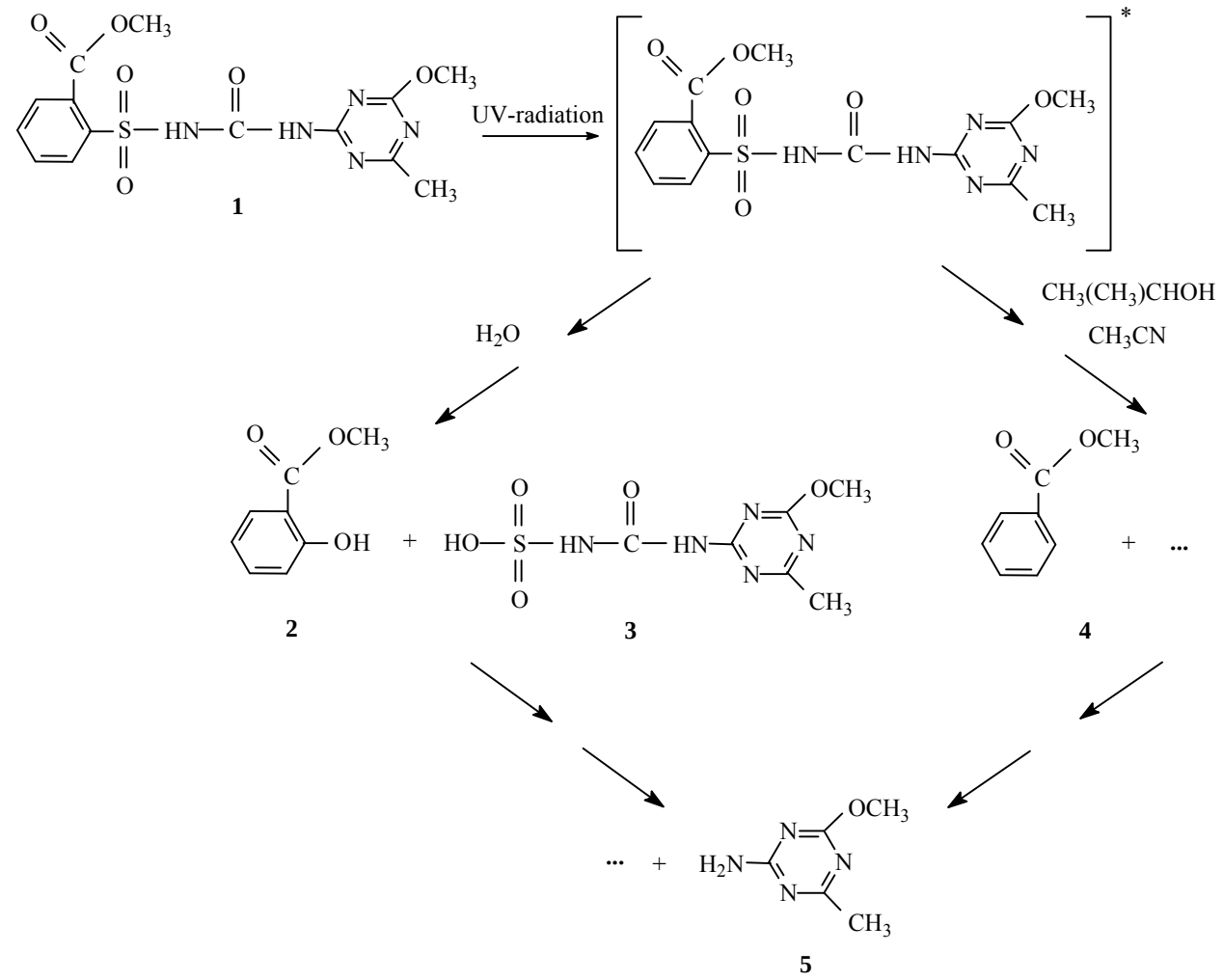

Fig. 5. Scheme photolysis metsulfuron-methyl:
1 – metsulfuron-methyl; *2* – 2-hydroxymethylbenzoate; *3* – [[(4-methoxy-6-methyl-1,3,5-triazine-2-yl) amino]carbonyl]-sulfamylic acid; *4* – methylbenzoate; *5* – 4-methoxy-6-methyl-2-amino-1,3,5-triazine

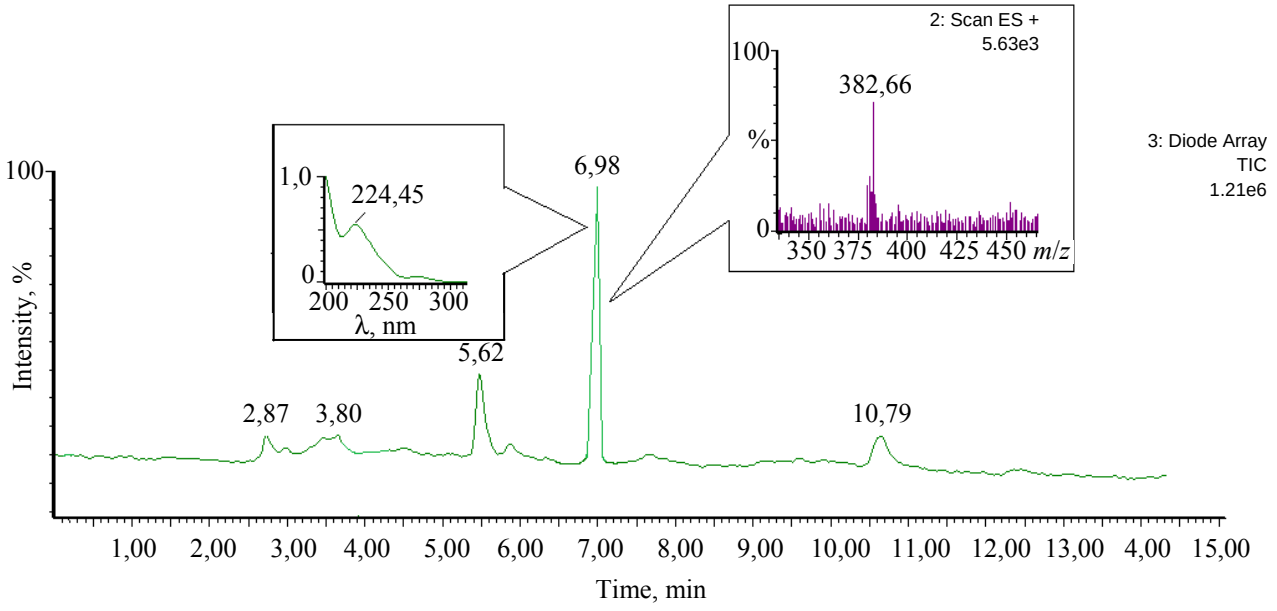

Fig. 6. Chromatogram of metsulfuron-methyl and intermediates of its microbial degradation.
In inserts – electronic and mass spectra of metsulfuron-methyl

Mechanism of metsulfuron-methyl degradation by soil microorganisms was proposed (Fig. 7) on the basis of the findings and analysis of the literature [7].

Under aerobic conditions metsulfuron-methyl (methyl-2-[[[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl) amino]carbonyl]amino]sulfonyl]-benzoate) (1) undergoes changes in the structure in the following ways:

– hydroxylation of the benzene ring leads to the formation of methyl-2-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-4-hydroxybenzoate (6);

– splitting of sulfonylurea fragment results in the formation of methyl-2-(aminosulfonyl)-benzoate (2) and 4-methoxy-6-methyl-2-amino-1,3,5-triazine (5), and the compound (2) after demethylation into 2-(aminosulfonyl)-benzoic acid (3) transforms into 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin) (4).

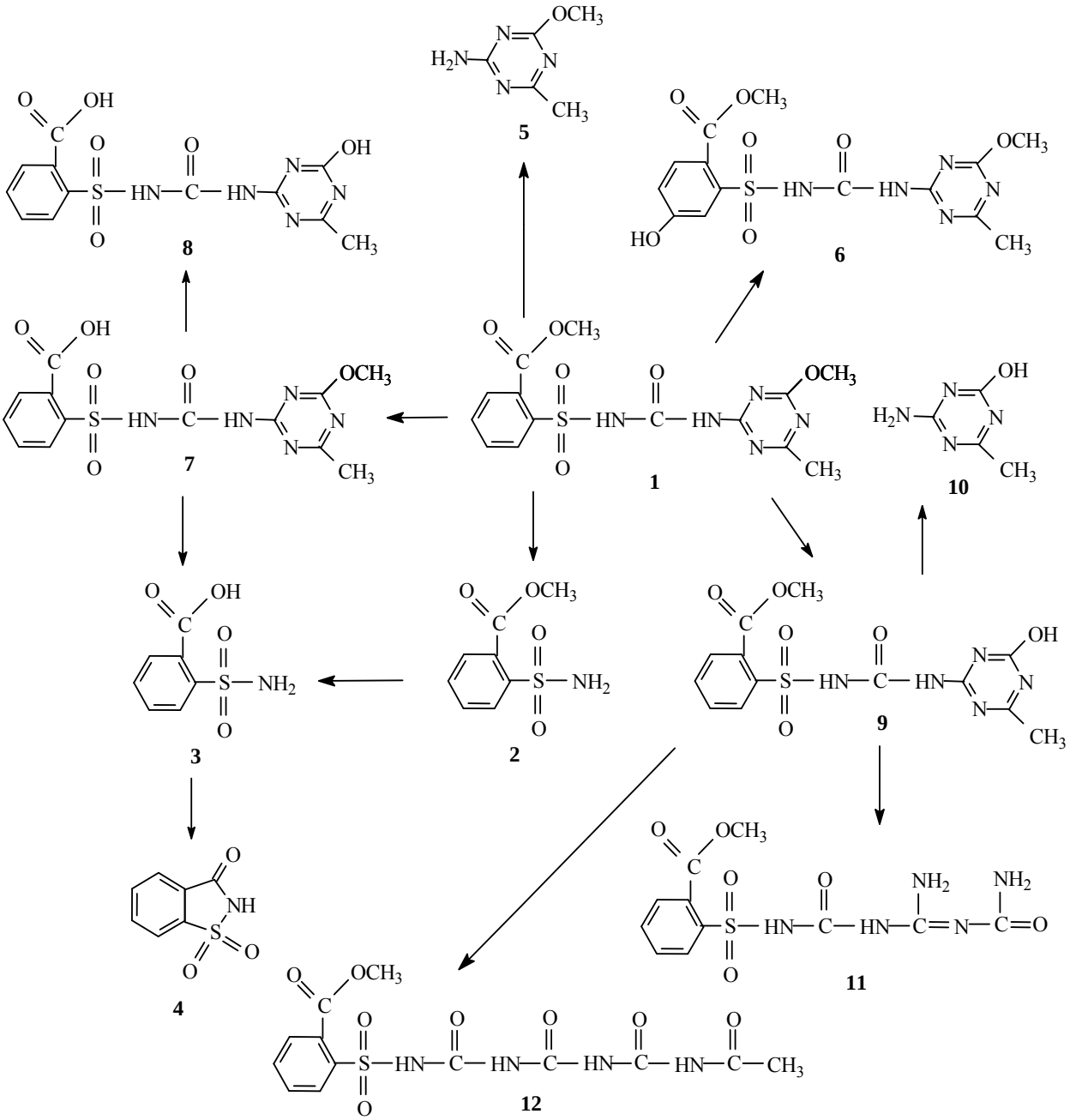

Fig. 7. The mechanism of metsulfuron-methyl degradation by soil microorganisms:
1 – metsulfuron-methyl; *2* – methyl-2-(aminosulfonyl)-benzoate; *3* – 2-(aminosulfonyl)-benzoic acid;
4 – 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin); *5* – 4-methoxy-6-methyl-2-amino-1,3,5-triazine;
6 – methyl-2-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-4-hydroxybenzoate;
7 – 2-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-benzoic acid;
8 – 2-[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-benzoic acid;
9 – methyl-2-[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-benzoate;
10 – 4-hydroxy-6-methyl-2-amino-1,3,5-triazine; *11, 12* – products with an open triazine cycle

Under anaerobic conditions there occurs demethylation of the methoxy group of triazine ring with the formation of methyl-2-[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-benzoate (9), which then turns into a 4-hydroxy-6-methyl-2-amino-1,3,5-triazine (10) and products with an open triazine cycle (11, 12).

Demethylation of methyl ether at benzene ring leads to the formation of 2-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl]sulfamoyl]-benzoic acid (7), which is further converted in the 2-[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)carbamoyl] sulfamoyl]-benzoic acid (8) and compound (3).

Thus, chromato-mass-spectrometric analysis allowed us to identify four main metabolites of metsulfuron-methyl degradation.

Fig. 8 presents the chromatograms of tribenuron-methyl and products of its microbial degradation obtained in one experiment using two different detectors.

The analysis showed that the chromatographic peak with retention time of 12.21 min corresponds to tribenuron-methyl as $[M+H]^+$ with m/z 396.83; peak with retention time of 3.89 min – methyl-2-(aminosulfonyl)-benzoate as $[M+H]^+$ with m/z 216.12; peak with retention time of 5.81 min – 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin) as $[M+Na]^+$ with m/z 206.27; peak with retention time of 9.51 min – 4-methoxy-6-methyl-2-amino-methyl-1,3,5-triazine as $[M+H]^+$ with m/z 155.63; peak with retention time 9.95 min – 2-hydroxy-4-methyl-6-dimethylamino-1,3,5-triazine as $[M+H]^+$ with m/z 155.21.

Mechanism of degradation of tribenuron-methyl by soil microorganisms (Fig. 9) was proposed on the basis of the findings and analysis of the literature [8].

Under aerobic conditions tribenuron-methyl (methyl-2-[[[[(6-methyl-4-methyl-1,3,5-triazine-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate) (1) undergoes changes in the structure in the following ways:

– splitting of sulfonylurea fragment with emitting CO_2 leads to the formation of methyl-2-(amino-sulfonyl)-benzoate (2) and 4-methoxy-6-methyl-2-aminomethyl-1,3,5-triazine (7);

– compound (2) after demethylating into 2-(aminosulfonyl)-benzoic acid (3) transforms into 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin) (4);

– compound (7) is subjected to methylation of amine group and to O-demethylation with the formation of 2-hydroxy-4-methyl-6-dimethylamino-1,3,5-triazine (8);

– compound (7) is subjected to N-demethylation with the formation of 4-methoxy-6-methyl-2-amino-1,3,5-triazine (9);

– compound (1) is subjected to hydroxylation on CH_3-group of triazine ring with the formation of methyl-2-[[[[(6-oxymethyl-4-methoxy-1,3,5-triazine-2-yl)metilamino]carbonyl]amino]sulfonyl]-benzoate (5);

– compound (5) is subjected to N-demethylation with subsequent splitting sulfonylamine fragment with the formation of N-[4-methoxy-6-(hydroxymethyl)-1,3,5-triazine-2-yl]-urea (6).

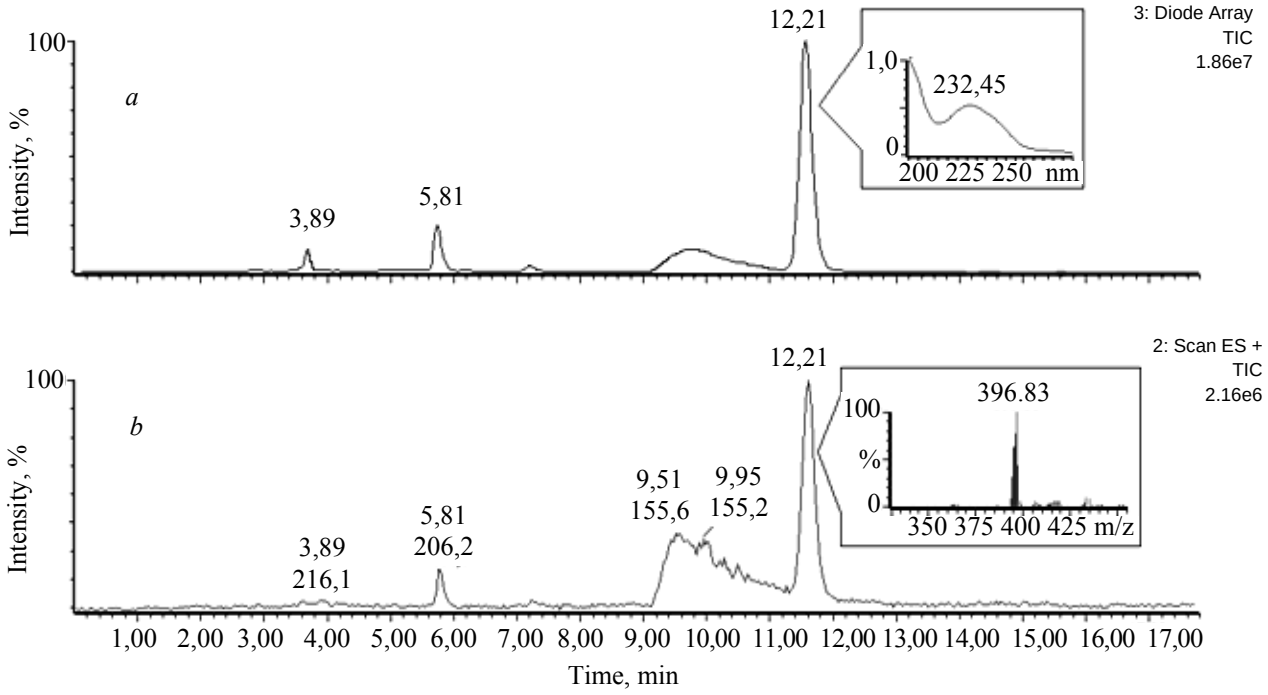

Fig. 8. Chromatograms of tribenuron-methyl and intermediates of its microbial degradation, obtained with the help of diode-matrix detector (*a*) and mass detector (*b*).
In inserts – electronic and mass-spectra of tribenuron-methyl

Under anaerobic conditions tribenuron-methyl is subjected to microbiological transformation with the formation of methyl-2-[[[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)methylamino]carbonyl]-amino]sulfonyl]-benzoate (10).

Thus, as a result of chromato-mass-spectrometric analysis we have identified four main metabolites of degradation of tribenuron-methyl, two of which – methyl-2-(aminosulfonyl)-benzoate and 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin) – are common to both representatives of sulfonylureas group.

One of the key stages in the study of the mechanisms of the herbicides biodegradation in soil is sample preparation, which depends essentially on the method and completeness of extraction of residual quantities of these compounds and their metabolites.

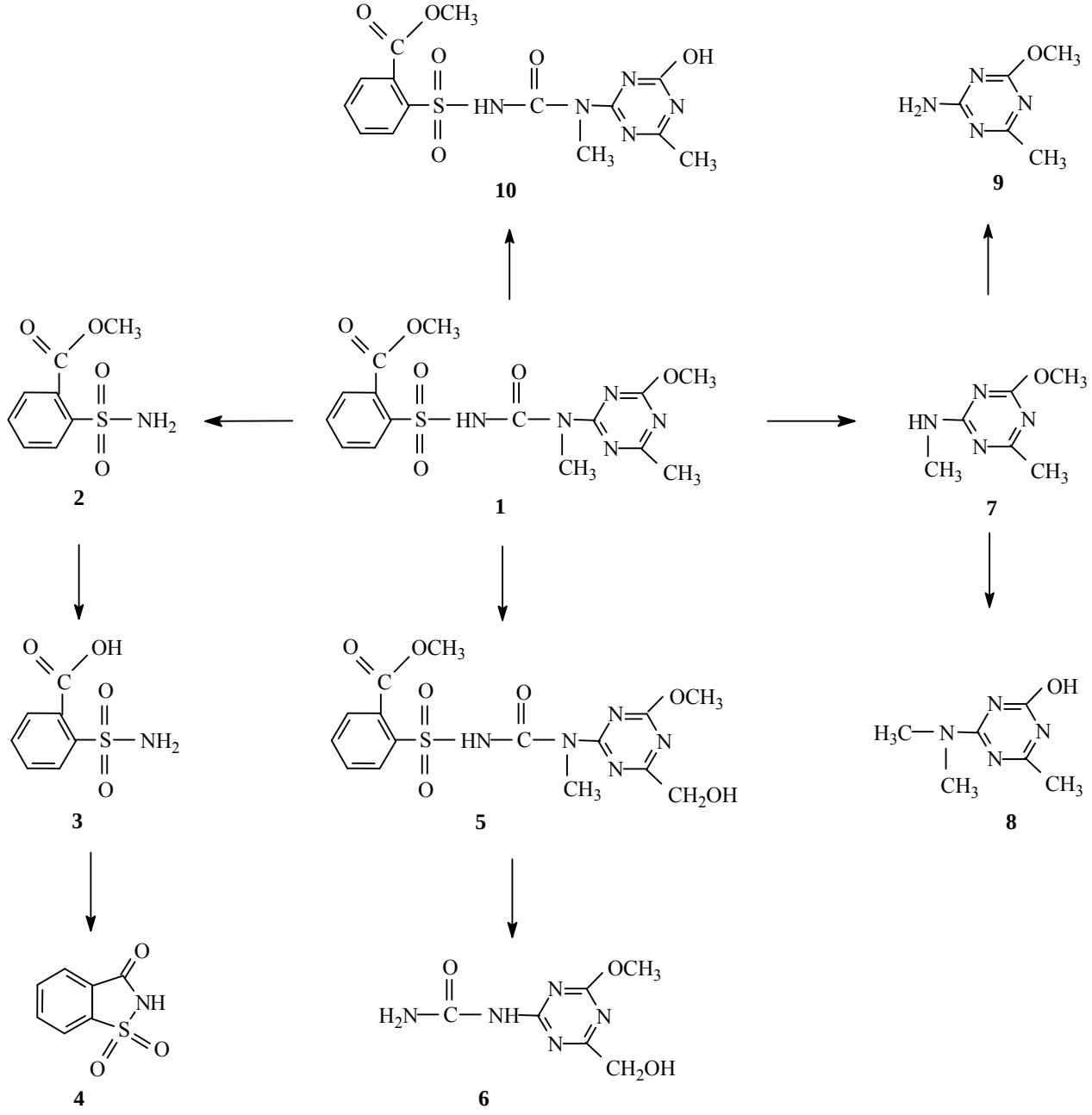

Fig. 9. The mechanism of degradation of tribenuron-methyl by soil microorganisms:
1 – tribenuron-methyl; *2* – methyl-2-(aminosulfonyl)-benzoate;
3 – 2-(aminosulfonyl)-benzoic acid; *4* – 1,2-benzizothiazol-3(2H)one-1,1-dioxide (saccharin);
5 – methyl-2-[[[[(6-oxymethyl-4-methyl-1,3,5-triazine-2-yl)methylamino]carbonyl]amino]sulfonyl]-benzoate;
6 – N-[4-methoxy-6-(hydroxymethyl)-1,3,5-triazine-2-yl]-urea; *7* – 4-methoxy-6-methyl-2-aminomethyl-1,3,5-triazine; *8* – 2-hydroxy-4-methyl-6-dimethylamino-1,3,5-triazine; *9* – 4-methoxy-6-methyl-2-amino-1,3,5-triazine;
10 – methyl-2-[[[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)methylamino]carbonyl]amino]sulfonyl]-benzoate

The problem is that the above-mentioned compounds are adsorbed by components of different soil types in different ways. In this regard, the completeness of extraction of residual quantities of herbicides and their metabolites will strongly depend on the method of extraction, the composition of the extracting agent and soil type.

There are the following methods of removing pollutants from soil [9]:

– thermal desorption;
– solvent (liquid) extraction;
– extraction in the microwave field;
– extraction by a subcritical water;
– supercritical fluid extraction;
– vapour phase analysis.

Each method has its advantages and disadvantages, whose consideration is beyond the scope of this article.

In this paper, we used the method of liquid extraction metsulfuron-methyl, tribenuron-methyl and metabolites of their degradation out of the model system consisting of a sterile agro-turf-podzol soil and culture of the designated microorganisms-destructors. And we have chosen as a solvent methylene chloride, providing the fullest extraction of metsulfuron-methyl, tribenuron-methyl and their metabolites.

Conclusion. The research was performed according to the task "Analysis of the ways biotransformation of pesticides of sulfonylurea group for development of technology for remediation of natural environment". The results of our research indicate that the method of chromatography-mass spectrometry is purely informative to establish mechanisms of biotransformation and biodegradation of pesticides. Elaborated approaches have formed the basis for the techniques of monitoring above mentioned herbicides and metabolites of their degradation in the environmental objects. They will be used to create biological products intended for remediation of natural environments, polluted by herbicides of sulfonylureas group.

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