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Molecular complexes of the triterpene glycosides with *L*-tyrosine and their biological activity

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Aim. *To investigate the complexation of L-tyrosine (Tyr) with hedera- genin 3-O-α-L-rhamnopyranosyl-(1 → 2)-O-α-L- arabinopyranoside (α-hederin) and its 28-O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl ester (hederasaponin C); to study the effect of glycosides, Tyr, and their mixtures on Avena sativa L. seed germination.* ***Methods***. *Electrospray ionization mass spectrometry.* ***Results***. *Mass spectra of mixes of glycosides with Tyr have been received and interpreted. The comparative analysis of biological activity of indivi- dual glycosides and their complexes with Tyr and others aromatic amino acids has been performed.* ***Conclusions***. *For the first time a composition of the complexes is established by mass spectrometry. The complex of α- hederin with Tyr has appeared most toxic.*

Keywords: α-hederin, hederasaponin C, L-tyrosine, complexa- tion, mass spectrometry, Avena sativa L.

Introduction. At present more and more attention is paid to the methods of obtaining molecular complexes of different biologically active substances with plant saponins and the elaboration of novel medical preparations on their basis [1–9]. In particular, the complexes with aromatic amino acids were synthesized. They include the most investigated complexation of amino acids with steroid saponins [10–13] which was confirmed by the time-of-flight plasma-desorption mass spectrometry with ^{252}Cf fission-fragment ionization as well as by NMR and UV-spectroscopy. There is a veterinary preparation "Clatiram" which is the complex of cloprostenol prostaglandin, *L*-tyrosine (Tyr) and glycyrrhizic acid, the main triterpene glycoside of Glycyrrhiza [14]. It is used for the purposes of regulating the reproductive function of animals.

There has been started the study on the complexation of triterpene glycosides of α-hederin (hederagenin3-O-α-L-rham-nopyranosyl-(1→2)-O-α-L-arabinopyranoside, glycoside 1, Fig. 1) and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glycopyranol-(1→6)-O-β-D-glycopyranosyl ester (hederasaponin C, glycoside **2**, Fig. 1). The complexes of glycosides **1** and **2** with amino acids, cholesterol, sildenafil, laevomycetin (chloramphenicol), caffeine and streptocid were obtained [15]. Glycosides **1** and **2** were discovered in representatives of the majority of *Hedera* L. species where they are prevailing saponins [16–21]. Glycosides **1** and **2** are a part of anticough drugs, containing *Hedera helix* L. leaves extract [16, 22].

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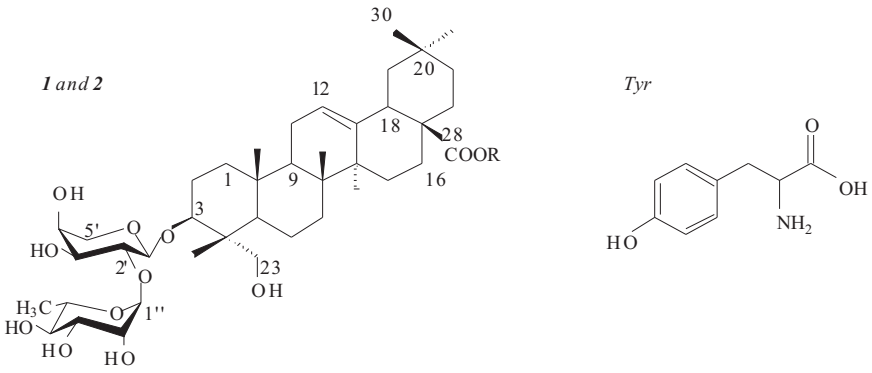

Fig. 1 The composition of molecular complex components (glycoside **1**: R = H; glycoside **2**: R = ←βGlc$_p$- (6←1)-βGlc$_p$-(4←1)-αRha$_p$

The electrospray ionization mass spectrometry was used to study the complexation of glycosides **1** and **2** with *L*-phenylalanine (Phe) [23], *L*-tryptophan (Trp) [15] and *L*-histidine (His) [24]. Besides, we have established the effect of these complexes on the germinating ability of seeds and further development of *Avena sativa* L. sprouts [24–26]. However, Tyr complexes were previously analyzed only by UV-spectrometry [27]. Therefore, the current article presents the results of mass-spectrometry analysis of the interaction of glycosides **1** and **2** with Tyr to systematize the data on the complexation of triterpene saponins with aromatic amino acids. Also we reviewed the effect of complexes on the germinating ability of seeds.

Materials and Methods. Glycosides **1** and **2** were extracted from the leaves of *Hedera taurica* Carr. and *Hedera canariensis* Willd. The composition of glycosides was confirmed by the methods, presented in [17, 18].

The complexes of glycoside **1** were obtained by mixing the solutions containing 1 mmol glycoside and 1 mmol Tyr while for preparing the complexes of glycoside **2** the solutions were mixed in a molar ratio of 1:2. As a solvent, the mixture of 70 % aqueous solution of C_2H_5OH and $CHCl_3$ in a volume ratio of 3:1 was used. The solutions were kept at 50°C for 1.5 h at constant stirring. The organic solvents were removed in the vacuum; the obtained mixtures were used for mass-spectra filming.

The measurements were conducted using Bruker Daltonic micrOTOF-Q mass-spectrometer with direct-inlet probe, electrospray ionization, detection of negative and positive ions in the range of 50–3,000 Da. The voltage at the restrictor tube was ±4200 V, the gas drier parameters (nitrogen ACS, 5 l/min, 180 °C) and the energy of ions at the quadrupole (5.0 eV) were optimized to detect the peaks of pseudomolecular and associative ions. The solutions of substances in acetonitrile (Merck, HPLC/MS) in the concentration up to 1 mg/ml were used for direct inlet, the inlet rate did not exceed 0.05 μl/s. The *m/z* ratios and the values of relative intensities of the ion peaks (I_{rel}, %) are presented in Table 1.

The germinating ability of *A. sativa* L. seeds (*Poaceae (Gramineae)* family) was checked in the laboratory conditions at room temperature (23–25 °C) as described in [24]. The results are presented in Table 2.

Results and Discussion. Mass-spectrometry of mixtures of glycosides 1 and 2 with Tyr. The ion peak $[M^1 + M^{Tyr} – H]^-$ with *m/z* 930.5, corresponding to the formation of the complex of 1:1 composition, was registered in the mode of negative ions for the mixture of glycoside **1** and Tyr. The peaks of ions of three molecular complexes $[M^1 + M^{Tyr} + H]^+$, $[2M^1 + M^{Tyr} + H]^+$ and $[3M^1 + M^{Tyr} + H]^+$ were observed in the mass-spectrum of positive ions besides the peaks of protonated Tyr and glycoside 1, their self-associates as well as the adducts with Na^+ and K^+ ions (Fig. 2, Table 1). The ratio of peak intensities of these ions is 632:28:1. Therefore, the complex with Tyr, the composition of which is 1:1, is more typical for glycoside **1**. The formation of the analogous complex out of Tyr and glycoside 1 has recently been proven by the isomolar series method [27].

The complexes of glycoside **2** with Tyr are not registered in the negative ion mode. There are only the ions of deprotonated glycoside **2** and Tyr. However, the peaks $[M^2 + M^{Tyr} + H]^+$ and $[3M^2 + 2M^{Tyr} + H]^+$, corresponding to the complexes of 1:1 and 3:2 ratios, respectively, were registered in the positive ion mode

Table 1
*Mass-spectra of the mixtures of Tyr with glycosides **1** and **2***

Ion	*m/z* (I_{rel}, %)	Ion	*m/z* (I_{rel}, %)
Mixture of Tyr and glycoside **1**		Mixture of Tyr and glycoside **1**	
$[M^{Tyr} - H]^-$	180,1 (26,68)	$[2M^1 + M^{Tyr} + H]^+$	1682,7 (0,83)
$[2M^{Tyr} - H]^-$	361,1 (1,94)	$[3M^1 + M^{Tyr} + H]^+$	2433,2 (0,03)
$[M^1 - H]^-$	749,4 (70)	Mixture of Tyr and glycoside **2**	
$[M^1\ldots(M^1 - H)\ldots M^1\ldots (M^1 - H)]^{2-}$ or $[M^1\ldots(M^1 - 2H) \ldots 2M^1]^{2-}$	1499,8 (3,02)	$[M^{Tyr} - H]^-$	180,2 (10,42)
$[(M^1 - H)\ldots M^1\ldots(M^1 - H)]^{2-}$ or $[M^1\ldots(M^1 - 2H)\ldots M^1]^{2-}$	1124,6 (3,71)	$[M^2 - H]^-$	1220,0 (16,96)
$[M^1\ldots(M^1 - H)\ldots M^1\ldots(M^1 - H)\ldots M^1]^{2-}$	1875,5 (0,17)	$[M^2 - 2H]^{2-}$	609,5 (23,55)
$[M^1 + M^{Tyr} - H]^-$	930,5 (9,42)	$[M^{Tyr} + H]^+$	182,0 (100)
$[M^{Tyr} + H]^+$	182,0 (100)	$[M^{Tyr} + Na]^+$	205,0 (2,06)
$[M^{Tyr} + Na]^+$	205,0 (0,85)	$[2M^{Tyr} + H]^+$	363,0 (1,23)
$[M^{Tyr} + K]^+$	221,0 (2,51)	$[2M^{Tyr} + Na]^+$	385,1 (0,32)
$[2M^{Tyr} + H]^+$	363,0 (12,23)	$[M^2 + M^{Tyr} + H]^+$	1402,5 (1,91)
$[2M^{Tyr} + Na]^+$	385,0 (4,97)	$[M^{Tyr} + K]^+$	220,0 (1,53)
$[M^1 + H]^+$	751,3 (38,05)	$[2M^{Tyr} + K]^+$	401,1 (0,57)
$[M^1 + Na]^+$	773,4 (4,78)	$[3M^{Tyr} + Na]^+$	566,3 (0,14)
$[M^1 + K]^+$	789,4 (0,74)	$[3M^{Tyr} + K]^+$	582,3 (0,20)
$[4M^1 + 2Na]^{2+}$	1523,6 (0,25)	$[4M^{Tyr} + H]^+$	725,3 (0,14)
$[2M^1 + H]^+$	1501,7 (2,23)	$[4M^{Tyr} + K]^+$	764,4 (1,02)
$[3M^1 + H]^+$	2252,1 (0,20)	$[3M^2 + 2M^{Tyr} + H]^+$	2013,2 (0,08)
$[M^1 + M^{Tyr} + H]^+$	932,3 (18,96)	–	–

(Fig. 3, Table 1). The peak intensities of these ions are in the ratio of 24:1 which indicated the prevalence of the complex of equimolar composition.

Previously, using the electrospray ionization mass-spectrometry we showed that for the glycosides **1** and **2** and amino acids (Trp, Phe and His) the complexes of 1:1 composition are the most typical [15, 23, 24]. The amino acid Tyr differs from Phe structurally only by the presence of a hydroxyl group. It should be noted that in the same experimental conditions the peak intensity $[M^1 + M^{Tyr} + H]^+$ is twice as high as $[M^1 + M^{Phe} + H]^+$. Phe forms with glycoside **1** more varied complexes than with glycoside **2**. The same regularity was observed for Tyr.

Biological activity. The toxic effect of triterpene glycosides is known to be the inhibition of the plant development. As a rule, this activity is inherent in monodesmosidic glycosides [28]. The effect of glycosides, Tyr and their complexes on the germinating ability and development of *A. sativa* L. sprouts was observed for 24–72 h (Table 2).

After 72 h it was determined that only the seeds treated with Tyr solution preserved 100 % germinating ability while for the seeds, preliminarily kept in the mixtures of glycosides **1** and **2** with Tyr, it was 44 and 80%, respectively which is 2.09 and 1.15 times less than for the seeds, kept in water. In addition, the mixture of glycoside **1** with Tyr inhibits the germinating

Table 2
The germination of Avena sativa seeds in laboratory conditions (concentrations of individual substances and substances in mixtures 10^{-4} M)

Parameter	Compound					
	H_2O (control)	**1**	**2**	Tyr	**1**-Tyr mixture	**2**-Tyr mixture
24 h later						
Germination, %	0	0	0	0	0	0
Stem length, mm	–	–	–	–	–	–
Amount with the root, %	92	92	84	96	100	96
Root length, mm	2,6 ± 0,6	3,0 ± 1,4	3,3 ± 0,7	2,8 ± 0,6	1,6 ± 1,1	2,5 ± 1,1
48 h later						
Germination, %	56	28	40	64	8	56
Stem length, mm	1,0 ± 0,6	0,6 ± 0,9	0,5 ± 1,3	1,9 ± 0,6	1,5 ± 0,9	1,1 ± 0,6
Amount with the root, %	94	100	88	96	100	96
Длина корня, мм	4,6 ± 0,5	3,3 ± 0,8	3,5 ± 1,2	5,9 ± 1,1	3,5 ± 0,6	4,9 ± 0,6
72 h later						
Germination, %	92	60	86	100	44	80
Root length, mm	4,2 ± 0,6	3,5 ± 1,3	3,7 ± 0,6	4,6 ± 0,8	2,4 ± 0,7	4,6 ± 0,7
Amount with the root, %	96	100	96	100	100	100
Root length, mm	7,1 ± 0,6	5,5 ± 1,1	6,4 ± 0,8	7,6 ± 0,9	4,5 ± 0,8	3,3 ± 0,6

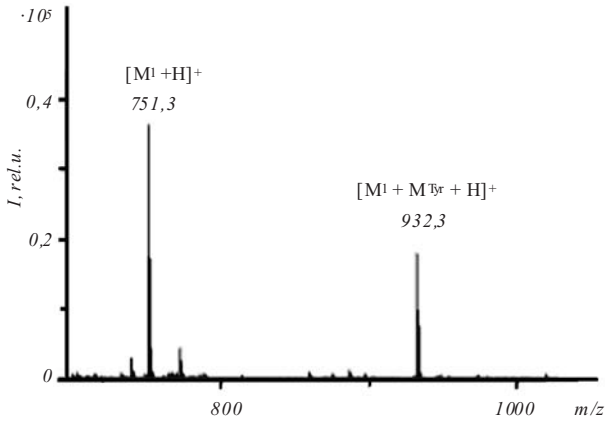

Fig. 2 The fragment of mass-spectra of positive ions of the mixture of glycoside **1** and Tyr

ability stronger than the solution of individual glycoside **1**. The seeds, treated with the complex of glycoside **2** with Tyr and the solution of individual glycoside **2**, have similar germinating ability. Therefore, the mixture of glycoside **1** with Tyr reduces the germinating ability of the seeds to the greatest extent.

The share of sprouts with roots when using all the substances and in the control group is 96–100 %. However, the length of the sprout root after the treatment with the complex of glycoside **2** with Tyr was approximately half the length of those treated with glycoside **2** and Tyr separately, as well as compared to the control. The complex of glycoside **1** and Tyr inhibits the root growth to a lesser degree.

The sprout length after the seed treatment with **1**-Tyr complex is approximately twice less than that of the Tyr-treated seeds and the control group. The effect of **2**-Tyr complex leads to the occurrence of the sprouts with approximately the same length as in control and Tyr-treated plants.

Previously we established that the complexes of glycosides **1** and **2** with Phe [25] and His [24] are less toxic than individual glycosides. *Vice versa*, the complexes with Trp proved to be more toxic [26]. The complexes of glycoside **1** with Tyr and Trp have equal inhibiting effect on the germinating ability 72 h after the seed treatment. The germinating ability of the seeds,

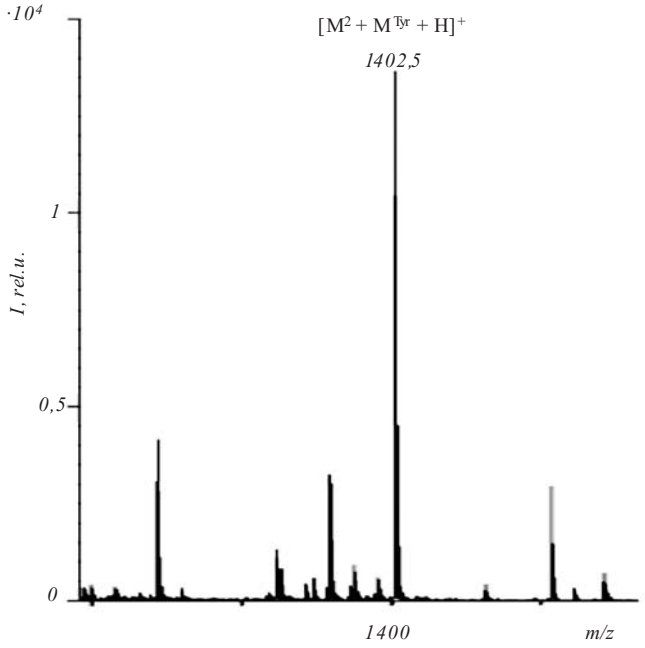

Fig. 3 The fragment of mass-spectra of positive ions of the mixture of glycoside **2** and Tyr

kept in their solutions, is only 44 % which is approximately twice less compared to the control group. These complexes appeared to be the most phytotoxic compared to glycosides **1** and **2** as well as to their complexes with all aromatic amino acids.

The least inhibiting effect on the germinating ability was demonstrated by the solutions, containing the mixtures of glycoside **1** with His and glycoside **2** with Phe, His, Tyr. Their germinating ability is 88 % (complexes **1**–His, **2**–Phe, **2**–His) and 80 % (complex **2**–Tyr).

Conclusions. Therefore, for Tyr and glycosides **1** and **2** the most common are the complexes with molar ratio of 1:1. Glycoside 1 forms the complexes with a higher composition variety. The mixture of Tyr with glycoside **1** has the highest inhibiting effect on the seed germinating ability and limits the sprout length.

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Молекулярные комплексы тритерпеновых гликозидов с L-тирозином и их биологическая активность

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Резюме

Цель. *Исследовать комплексообразование L-тирозина (Tyr) с 3-O-α-L-рамнопиранозил-(1 → 2)-O-α-L- арабинопиранозидом хедерагенина (α-хедерином) и его 28-O-α-L-рамнопиранозил-(1 → 4)-O-β-D-глюкопиранозил-(1 → 6)-O-β-D-глюкопиранозиловым эфиром (хедерасапонином C). Изучить влияние гликозидов, Tyr и их смесей на всхожесть семян Avena sativa L.* ***Методы***. *Масс-спектрометрия с ионизацией электрораспылением.* ***Результаты***. *Получены и интерпретированы масс-спектры смесей гликозидов с Tyr. Проведен сравнительный анализ биологической активности индивидуальных гликозидов и их комп- лексов с Tyr и другими ароматическими аминокислотами.* ***Выводы***. *Впервые масс-спектрометрически установлен состав комплексов. Наиболее токсичным оказался комплекс α-хедерина с Tyr.*

Ключевые слова: α-хедерин, хедерасапонин C, L-тирозин, комплексообразование, масс-спектрометрия, Avena sativa L.

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Молекулярні комплекси тритерпенових глікозидів з *L*-тирозином та їхня біологічна активність

Резюме

Мета. *Дослідити комплексоутворення L-тирозину (Tyr) з 3-O-α-L-рамнопіранозил-(1 → 2)-O-α-L-арабінопіранозидом хедерагенину (α-хедерином) та його 28-O-α-L-рамнопіранозил-(1 → 4)-O-β-D-глюкопіранозил-(1 → 6)-O-β-D-глюкопіранозиловим естером (хедерасапоніном C). Вивчити вплив глікозидів, Tyr та їхніх сумішей на проростання насіння Avena sativa L.* ***Методи***. *Мас-спектрометрія з іонізацією електророзпиленням.* ***Результати***. *Отримано та інтерпретовано мас-спектри сумішей глікозидів з Tyr. Проведено порівняльний аналіз біологічної активності індивідуальних глікозидів і їхніх комплексів з Tyr та іншими ароматичними амінокислотами.* ***Висновки***. *Вперше методом мас-спектрометрії встановлено склад комплексів. Найтоксичнішим виявився комплекс α-хедерину з Tyr.*

Ключові слова: α-хедерин, хедерасапонін С, L-тирозин, комплексоутворення, мас-спектрометрія, Avena sativa L.

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