Introduction. One possible method for reducing therapeutic doses of drugs, increasing their solubility, and expanding the spectrum of biological activity is to form clathrates with plant saponins [1]. This approach has already been examined for glycyrrhizic acid, the main triterpene saponin of licorice roots [1, 2].

We examined some of the most common triterpene glycosides of plants from various species of the genus Hedera L. (ivy) as promising complexants [3]. One of the dominant glycosides from ivy is α-hederin (hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside, Hed, Fig. 1) [4]. Hed was isolated from H. helix L. [5, 6], H. canariensis Willd. [7], H. taurica Carr. [8], H. nepalensis C. Koch. [9], H. rhombea Bean. [10], H. caucasisena Pojark. [11], H. scotica A. Cheval. [12], and H. colchica C. Koch. [13].

Hed is included in the well-known drugs for treating coughs, Hedelix and Prospan, composed of H. helix leaves [4, 14]. Hed inhibits the inactivation of β2 receptors in the lungs and bronchus [15]. Hederasaponin C (precursor of Hed) is the dominant glycoside of ivy leaves [4]. Complexes of Hed with amino acids [3, 16-18], cholesterol [19], caffeine [20], chloramphenicol [3], sulfanilamide [3], paracetamol [21], and sildenafil (in the basic form) [3] were prepared. However, complexation of Hed with sildenafil citrate (SC, Fig. 2) has not been previously studied by FT-IR and UV spectroscopy. SC is an inhibitor of phosphodiesterase-5 (PDE-5). Medicinal preparations based on SC (Viagra, etc.) are widely used for the treatment of erectile dysfunction [22].

Materials and methods. We used Hed sample...
obtained from leaves of *H. taurica* Carr. and *H. canariensis* Willd. (Araliaceae Juss.). The isolation and analytical methods have been published [7, 8]. SC was purchased from Shilpa Medicare Limited (India).

UV spectra (Fig. 3) were obtained at room temperature (20–22 °C) on a Unico UV-Vis 4802 spectrophotometer (USA) in quartz cuvettes (l=1 cm). The isomolar series were prepared by mixing aqueous solutions (10^-4 M) of Hed and SC at room temperature (20–22 °C) for 40–50 min with constant stirring. Fig. 4 shows the isomolar curve. The IR spectra (suspension in vaseline oil) were recorded on a InfraLUM FT-02 IR Fourier spectrometer (Russia) in the 4000–400 cm⁻¹ region with spectral resolution 1 cm⁻¹ (Fig. 5).

The complexes were prepared by mixing Hed (0.1 mmol) and SC (0.1 mmol) in 25 mL of solvent (aqueous ethanol (70 %) and chloroform, 3:1, v/v). The resulting mixture was held at 40–50 °C for 1.5 h with constant stirring. Organic solvents were removed by vacuum distillation. Dry residue was dried to constant weight.

IR spectrum of SC (vaseline oil, ν, cm⁻¹): 3607 (ОН), 3450 (NH), 3295 (NH), 1700 (С=О, CONH), 1602 (Ar), 1579 (СОО м), 1540 (Ar), 1356 (SO), 1340 (CN), 1279 (CN, NH), 1250 (C-O-C), 1215 (CH), 1172 (SO), 1156 (SO), 1078 (C-O-C, C-OH, CN), 1026 (C-O-C, C-OH), 939 (SN).

IR spectrum of Hed (vaseline oil, ν, cm⁻¹): ∼3360 (ОН), 1695 (C=O), 1647 (C=C), 1341 (CH), 1304 (CH), 1268 (CH), 1234 (CH), 1207 (CH), 1141 (C-O-C, C-OH), 1075 (C-O-C, C-OH), 1050 (C-O-C, C-OH), 1029 (C-O-C, C-OH), 981 (=CH).

IR spectrum of Hed and SC complex (vaseline oil, ν, cm⁻¹): ∼3400 (OH, NH), 1697 (C=O, CONH), 1648 (C=C), 1602 (Ar), 1579 (COO⁻), 1539 (Ar), 1340 (CH, CN), 1306 (CH), 1276 (CH, CN, NH), 1207 (CH), 1168 (SO), 1156 (SO), 1140 (C-O-C, C-OH), 1077 (C-O-C, C-OH, CN), 1047 (C-O-C, C-OH), 1029 (C-O-C, C-OH), 981 (=CH), 939 (SN).

Ichthyotoxicity was tested on *Poecilia reticulata* (Poeciliidae) using solutions of the glycoside and SC in distilled water. The action of each separate compound concentration was studied using 20 fish that were placed into solutions of the glycoside, SC, and their complex. The incubation time at which 100 % lethality occurred was determined. The confidence range was calculated at reliability level α=0.95.

**Result and discussion. FT-IR and UV spectroscopy.** UV spectroscopy confirmed that molecular interactions exist between Hed and SC. As the Hed concentration increases at constant SC concentration (10^-4 M), the optical density of their solutions increases as well (hyperchromic effect) (Fig. 3). The absorption maximum of the solutions decreases (hypsochromic shift) from 291 (Fig. 3, curve 1) to 280 nm (Fig. 3, curve 6). A hyperchromic effect has also been recently reported to occur upon formation of the complexes of Hed and hederasaponin C with caffeine [20, 23].

The complex composition was determined by the method of isomolar series [24]. This method gave a molar ratio ~1.0 (Fig. 4), which corresponded to a 1:1 Hed-SC complex. Such a ratio was obtained for Hed complexes with aromatic proteinogenous amino acids in aqueous solutions [3].

Strong vibration bands of vaseline oil CH bonds are present in the IR spectra of all samples at 2900, 1460 and 1380 cm⁻¹ (Fig. 5). In the spectrum of Hed (Fig. 5) at the 3330–3360 cm⁻¹ region a broad intense band of associated OH groups' stretching vibrations of monosaccharide residues was revealed. Stretching vibrations of bonds
involving oxygen atoms (C=O, C-OH) appear at the 1200–1000 cm⁻¹ region. A strong band of stretching vibrations of C=O bond in the carboxyl group Hed was observed at 1695 cm⁻¹. The band at 1647 cm⁻¹ is attributed to the double bond C=C of the aglycone (hederagenin). At the 1400–1200 cm⁻¹ region there are the absorption bands of CH bonds’ deformation vibrations.

Stretching vibrations of citrate OH group and the NH bond in the CONH group of SC appear at the 3600–3300 cm⁻¹ (Fig. 5). The absorption band of symmetric stretching vibrations of SO bonds splits into two peaks with a higher (at 1172 cm⁻¹) and lower (at 1156 cm⁻¹) intensity in the IR spectrum. The absorption frequency of SO bonds’ asymmetric stretching vibrations is 1356 cm⁻¹. The CO groups’ stretching vibrations are observed at 1700 cm⁻¹ (COOH citrate and CONH sildenafil) and at 1579 cm⁻¹ (COO⁻ citrate). The IR spectrum of SC also contains characteristic absorption bands of C=O-C, CN and aromatic rings.

NH bonds’ absorption bands at 3450 and 3295 cm⁻¹ characterizing individual SC are absent in the IR spectrum of Hed−SC complex (Fig. 5). However, the broadened absorption band of the NH and OH bonds at 3400 cm⁻¹, the shape and frequency value of which indicate hydrogen bonds formation, can be observed [25].

The vibration band of the CO in the COO⁻ of citrate does not shift upon complexation. In the spectra of the complex and individual SC it is found at 1579 cm⁻¹. Obviously, this group is not involved in molecular interaction. In the complex IR spectrum, absorption band at 1697 cm⁻¹ correspond to stretching vibrations of the CO (COOH citrate and Hed, and CONH sildenafil). The slight shift is observed for the absorption bands of C=O-C and C-OH bonds.

**Fig. 4.** Optical density A as a function of component ratio of isomolar series at λ=280 nm: c(Hed) =10⁻⁴ M, c(SC)=10⁻⁴ M.

**Fig. 5.** FT-IR spectra of Hed, SC and complex of Hed with SC.

The absorption band of the SO (symmetric stretching vibrations) in the SC-Hed complex has been found at 1168 cm⁻¹, i.e. its shift is -4 cm⁻¹ compared with SC spectrum. While another absorption band at 1156 cm⁻¹ does not shift. The bands at 1168 and 1156 cm⁻¹ have almost the same intensity, although in the individual SC spectrum the absorption band at 1172 cm⁻¹ was more intense.

**Biological activity.** Triterpene glycosides are known to exhibit various medical and biological activities, in particular, pronounced toxicity for mollusks and fish [4]. We examined the effects of Hed, SC, and their complex on the fish P. reticulata. The incubation time during which 100 % fatality of the fish took place, was 25,1±0,6 min for Hed (0.50·10⁻³ M), 159,7±6,8 min for SC (0.50·10⁻³ M), and reached 13,4±1,8 min for complex Hed−SC (on 0.50·10⁻³ M of each of components).

**Conclusion.** Thus, the complex formation involves Hed hydroxyl and carboxyl groups, the CO and SO bonds of SC. Interaction occurs through hydrogen bonds’ formation. Hydrophobic interactions between nonpolar aglycone of Hed and SC aromatic rings cannot be excluded. The complex of Hed with SC has appeared most toxic.

**Acknowledgements.** The authors are grateful to Kashutsky S.N. (InterChem, Odessa, Ukraine) for providing the sample of SC.
Molecular complex of the triterpene glycoside α-hederin and sildenafil Citrate (Viagra)

1-Ch-Фур'є- та УФ-спектроскопічний аналіз і біологічна активність

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Резюме. Описано утворення молекулярного комплексу тритерпенового глікозиду α-хедерину (3-O-α-L-рамипіранозил-(1→2)-O-α-L-арабіпіранозил-α-D-лактозид) із цитратом сildenafілу (віагрою). Уперше комплексоутворення підтверджено методами 1-Ch-Фур'є- та УФ-спектроскопії. Глікозид утворює комплекс з цитратом сildenafілу складу 1:1. Міжмолекулярна взаємодія супроводжується пікромеморіальним ефектом. Вивчено іхтіотоксичність молекулярного комплексу проти Poecilia reticulata.

Ключові слова: тритерпенові глікозиди, α-хедерин, цитрат сildenafілу, молекулярний комплекс, УФ-спектроскопія, 1-Ch-Фур'є-спектроскопія, Poecilia reticulata, іхтіотоксичність.

References