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Molecular complex of the triterpene glycoside α-hederin and sildenafil citrate (viagra): FT-IR and UV spectroscopy analysis and biological activity

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Summary. Molecular complex of the triterpene glycoside α-hederin (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside) with sildenafil citrate (viagra) has been prepared. The complexation has been confirmed by FT-IR and UV spectroscopy for the first time. The glycoside forms complex with sildenafil citrate in a 1:1 molar ratio. Intermolecular interaction has been accompanied by a hyperchromic effect. The ichtyotoxic activity of the molecular complex was investigated on *Poecilia reticulata*.

Keywords: triterpene glycosides, α-hederin, sildenafil citrate, molecular complex, UV spectroscopy, FT-IR spectroscopy, *Poecilia reticulate*, ichtyotoxic activity.

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. caucasigena* 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 β_2 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 ingibitor 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

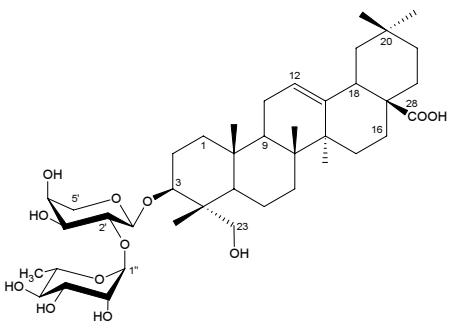

Fig. 1. Chemical structure of α-hederin (Hed).

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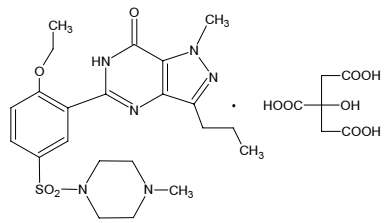

Fig. 2. Chemical structure of sildenafil citrate (SC).

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 (OH), 3450 (NH), 3295 (NH), 1700 (C=O, CONH), 1602 (Ar), 1579 (COO^-), 1540 (Ar), 1356 (SO), 1340 (CN), 1301 (CH), 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 (OH), 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).

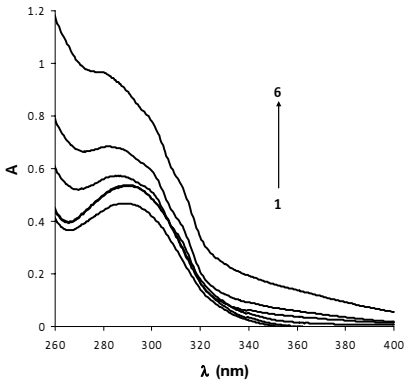

Fig. 3. UV spectra of SC aqueous solutions (10^{-4} M = const) with different concentrations of Hed: 0 M (1), $0.25\cdot10^{-4}$ M (2), $0.50\cdot10^{-4}$ M (3), $0.25\cdot10^{-3}$ M (4), $0.50\cdot10^{-3}$ M (5) and 10^{-3} M (6).

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 $t_{LD_{100}}$ during 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

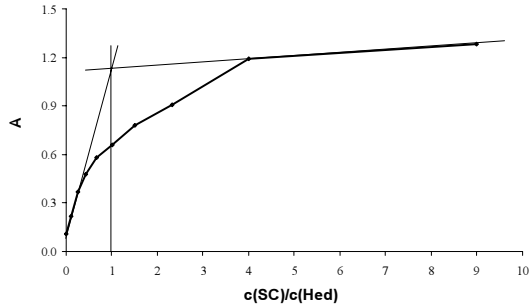

Fig. 4. Optical density A as a function of component ratio of isomolar series at λ=280 nm: c(Hed) =10⁻⁴ M, c(SC)=10⁻⁴ M.

involving oxygen atoms (C-O-C, 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.

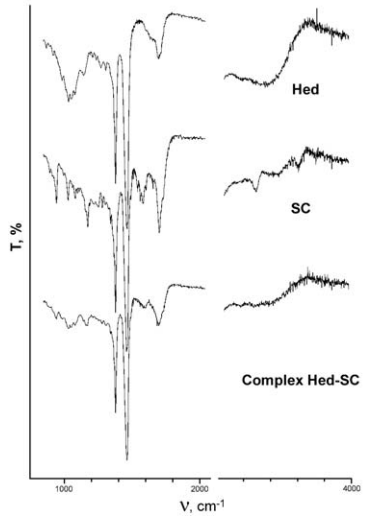

Fig. 5. FT-IR spectra of Hed, SC and complex of Hed with SC.

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 $t_{LD_{100}}$, during which 100 % fatality of the fish took place, was 25,1±0,6 min for Hed ($0.50 \cdot 10^{-3}$ M), 159,7±6,8 min for SC ($0.50 \cdot 10^{-3}$ M), and reached 13,4±1,8 min for complex Hed-SC (on $0.50 \cdot 10^{-3}$ 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.

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Молекулярний комплекс тритерпенового глікозиду α-хедерину і цитрату силденафілу (віагри): ІЧ-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*-рамнопіранозил-(1→2)-О-α-L-арабінопіранозиду хедерагеніну) із цитратом силденафілу (віагрою). Уперше комплексоутворення підтверджено методами ІЧ-Фур'є- та УФ-спектроскопії. Глікозид утворює комплекс з цитратом силденафілу складу 1:1. Міжмолекулярна взаємодія супроводжується гіперхромним ефектом. Вивчено іхтіотоксичну активність молекулярного комплексу проти *Poecilia reticulata.*

Ключові слова: тритерпенові глікозиди, α-хедерин, цитрат силденафілу, молекулярний комплекс, УФ-спектроскопія, ІЧ-Фур'є-спектроскопія, *Poecilia reticulate*, іхтіотоксичність.

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