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Molecular Complexation of Hederasaponin C with Cholesterol in Aqueous Isopropyl Alcohol

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Abstract. The 1:1 molecular complex of ivy triterpene glycoside hederasaponin C (HedC) with cholesterol (Chol) was obtained in aqueous isopropyl alcohol. The stability constant of $(3.3 \pm 0.7) \cdot 10^6$ $(mol/L)^{-1}$ was calculated for the complex. The complexation was studied by UV- and ATR IR-Fourier spectroscopy, and method of isomolar series. The hydrogen bonds and hydrophobic interactions are formed in the molecular complex.

Keywords: triterpene glycosides; hederasaponin C; cholesterol; molecular complex

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Introduction

Triterpene glycoside hederasaponin C (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside, HedC; Fig. 1) was discovered in the most species of the ivy genus *Hedera* L. (Araliaceae Juss.) [1]. HedC is the dominant ivy saponin. HedC was also founded in plants of various species of *Kalopanax*, in *Aralia elata*, *Acanthopanax sieboldianus* and *Schefflera octophylla* [1].

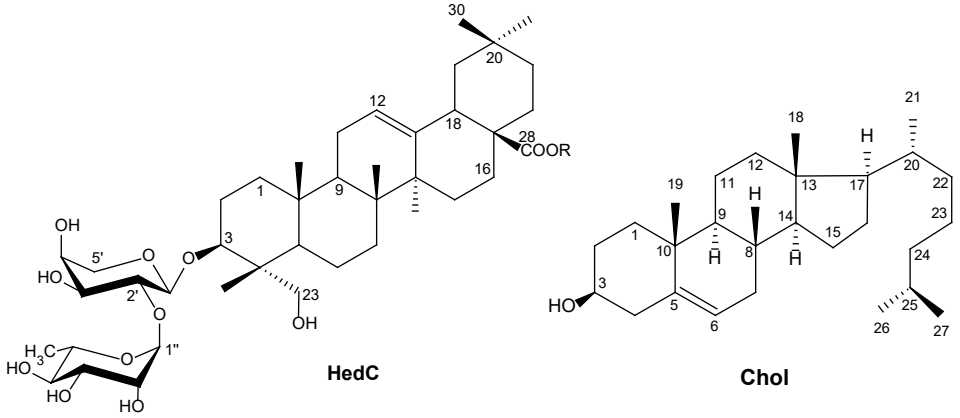

Fig. 1. Structures of HedC (R = ←βGlc$_p$-(6←1)-βGlc$_p$-(4←1)-αRha$_p$) and Chol

HedC is the component of antitussive drugs Prospan, Hedelix and other containing *Hedera helix* L. leaves [1]. A characteristic feature of triterpene glycosides is their ability to form molecular complexes with sterols [1–4]. The complexation of saponins with sterols is responsible for hemolytic, antitumor, ichthyotoxic, molluscicidal, antifungal, hypocholesterolaemic, and embryotoxic activity of triterpene glycosides [1, 2]. On the other hand, it was reported that some triterpene glycosides do not form a molecular complex with cholesterol (Chol; Fig. 1) [3].

The interaction of HedC with Chol has been studied by spectrophotometric titration in aqueous ethanol [3] and isomolar series [4]. A preparation of molecular complex of Chol with HedC and bisdesmoside ivy triterpene glycoside hederacoside B mixture in aqueous ethanol and its analysis by planar chromatography [2] was previously reported.

To study the complexation of HedC with Chol in various media we examined their interaction in 80% aqueous isopropyl alcohol.

Experimental

HedC was preparatively isolated from leaves of *Hedera canariensis* Willd. (Araliaceae Juss.) by column chromatography on SiO_2 and its structure was confirmed using chemical and physical methods [5].

The isomolar series were prepared by mixing 10^{-4} mol/L solutions of HedC and Chol in 80% aqueous isopropyl alcohol (v/v) at 25 °C for 40 min with continuous stirring. Spectroscopic analysis of isomolar series was performed on a LEKI SS2110UV spectrophotometer using a quartz cuvette (l = 1 cm) at 25 °C. Stability constant of the complex was calculated according to the A. K. Babko method based on isomolar curves [4, 6].

The complex of Chol with HedC was preparatively obtained by liquid-phase method. For this purpose, 1 mmol of the substances was mixed with 50 mL of 80% aqueous isopropyl alcohol (v/v). The obtained mixture was incubated at 50 °C for 1.5 h with continuous stirring. The organic solvent was removed under reduced pressure. Synthesized complex was analyzed by IR spectroscopy.

The IR spectra were recorded on the Simex FT-801 IR-Fourier spectrometer in the 4000–550 cm^{-1} region (spectral resolution 4 cm^{-1}; 50 scans) using ATR accessory with diamante crystal plate.

IR spectrum of HedC (ν, cm^{-1}): 3333 (OH), 2930 (CH), 2907 (CH), 2878 (CH), 1734 (C=O), 1624 (C=C), 1451 (CH), 1433 (CH), 1417 (CH), 1387 (CH), 1357 (CH), 1342 (CH), 1319 (CH), 1260 (CH), 1230 (CH), 1201 (CH), 1050 (C–O–C, C–OH), 1024 (C–O–C, C–OH), 979 (=CH).

IR spectrum of Chol (ν, cm^{-1}): 3403 (OH), 3337 (OH), 2929 (CH), 2899 (CH), 2865 (CH), 2848 (CH), 1672 (C=C), 1460 (CH), 1434 (CH), 1377 (CH), 1364 (CH), 1341 (CH), 1333 (CH), 1318 (CH), 1275 (CH), 1268 (CH), 1253 (CH), 1234 (CH), 1220 (CH), 1190 (CH), 1169 (C–OH), 1132 (C–OH), 1106 (C–OH), 1052 (C–OH), 1022 (C–OH), 986 (=CH), 953 (CH).

IR spectrum of the complex of HedC with Chol (ν, cm^{-1}): 3316 (OH), 2930 (CH), 2900 (CH), 2865 (CH), 1732 (C=O), 1670 (C=C), 1625 (C=C), 1458 (CH), 1434 (CH), 1378 (CH), 1363 (CH), 1339

(CH), 1316 (CH), 1261 (CH), 1230 (CH), 1199 (CH), 1128 (C–O–C, C–OH), 1050 (C–O–C, C–OH), 1024 (C–O–C, C–OH), 983 (=CH), 958 (CH).

Results and discussion

The composition of the complex of HedC with Chol was determined by the isomolar series method. This method gave a molar ratio ≈1.0, which corresponded to a 1:1 complex (Fig. 2).

Such ratio was obtained for complex of HedC with Chol in 90% and 70% aqueous ethanol [3, 4], and for complexes of HedC with several drugs [7]. Stability constant of complex (3.3 ± 0.7) · $\cdot 10^6$ $(mol/L)^{-1}$ was calculated based on isomolar curves (A. K. Babko method) [4, 6]. The stability constants of complex in aqueous ethanol were of the order 10^{-4} [3, 4]. Thus, the stability constant of complex formed in 80% aqueous isopropyl alcohol was greater than in aqueous ethanol.

As the HedC concentration increases (at constant Chol concentration), the optical density of their solutions increases (hyperchromic effect) (Fig. 3). The absorption maximum of the solutions decreases insignificantly (hypsochromic shift) from 237 to 230 nm. Similar spectral changes were previously observed for molecular complexation of HedC with caffeine [7].

The complexation of HedC with Chol was studied by ATR FT-IR spectroscopy. The potential centers of intermolecular interactions in the molecules of HedC and Chol are OH groups. Indeed, upon the formation of complex in the IR spectra for the absorption bands of stretching vibrations of O–H bonds in Chol are observed shifts from 3403 and 3337 cm^{-1} to 3316 cm^{-1}, and in HedC — from 3333 to 3316 cm^{-1}. This may indicate to formation of hydrogen bonds between HedC and Chol.

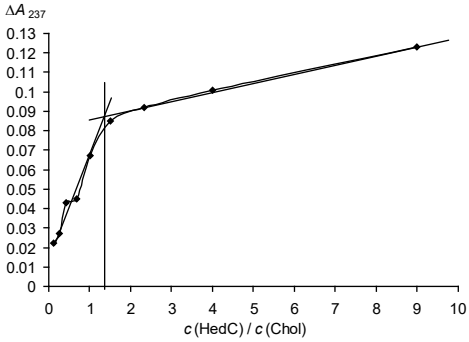

Fig. 2. Optical density change D*A* as a function of component ratio of isomolar series at 237 nm

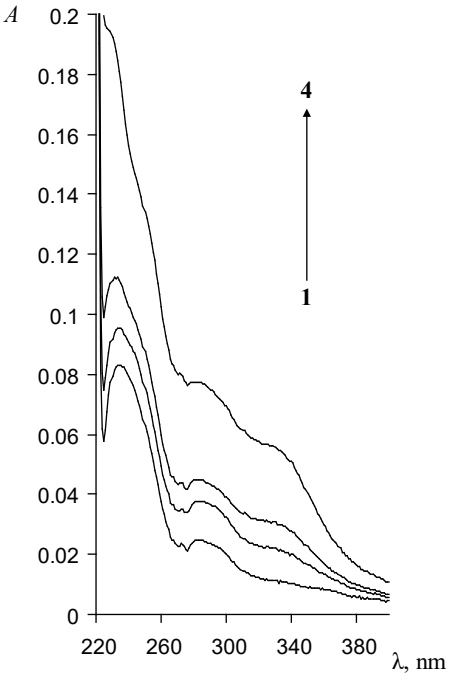

Fig. 3. UV spectra of Chol solutions ($0.50 \cdot 10^{-4}$ M = const) with different concentrations of HedC: 0 M (curve **1**), $0.125 \cdot 10^{-4}$ (curve **2**), $0.25 \cdot 10^{-4}$ (curve **3**), $0.50 \cdot 10^{-4}$ (curve **4**)

The complexation also causes changes in certain frequencies of absorption of CH bonds. These facts may indicate the presence of hydrophobic contacts between Chol and HedC molecules in the molecular complex. The presence of hydrophobic interactions explains the high stability of triterpene glycosides molecular complexes [4].

Conclusions

The 1:1 molecular complex of HedC with Chol has been prepared for the first time in aqueous isopropyl alcohol. The presence of molecular complexation of Chol with HedC has been proved by UV- and ATR IR-Fourier spectroscopy. Intermolecular interaction in the complex is carried out by hydrogen bonds formation and hydrophobic contacts. The results can be used to explain the mechanisms of the biological activity of triterpene glycosides.

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