Intermolecular Interaction of Glycyrrhizin with Cholesterol

L. A. Yakovishin* V. I. Grishkovets

a Sevastopol State University, 33 University Str., Sevastopol, 299053, Russia
b V. I. Vernadsky Crimean Federal University, 4 Vernadsky Ave., Simferopol, 295007, Russia
*email: chemsevntu@rambler.ru

Abstract. The 1:1 molecular complex of licorice triterpene glycoside glycyrrhizic acid (in the form of monoammonium salt) with cholesterol was obtained in 80% aqueous isopropyl alcohol for the first time. The complexation was studied by $^{13}$C NMR, UV, and ATR IR-Fourier spectroscopy. The hydrogen bonds and hydrophobic interactions are formed in the molecular complex.

Keywords: triterpene glycosides; licorice; glycyrrhizin; glycyrrhizic acid; cholesterol; molecular complex

Received: 27.10.2020. Accepted: 09.12.2020. Published:30.12.2020.

© Yakovishin L. A., Grishkovets V. I., 2020

Introduction

Glycyrrhizin (glycyrrhizic acid, 3-O-β-D-glucuronopyranosyl-(1→2)-O-β-D-glucuronopyranoside of 18β-glycyrrhetinic acid, GA; Fig. 1) is the dominant triterpene saponin from licorice roots Glycyrrhiza glabra L. and Glycyrrhiza uralensis Fisch. (Fabaceae) [1, 2]. GA has anti-inflammatory, antioxidative, antiviral, anticancer, hypcholesterolemic, and hepatoprotective properties [1–3]. The most important derivative of GA is its monoammonium salt (monoammonium glycyrrhizinate, glycyram, GC; Fig. 1). GC is used as an anti-inflammatory, hepatoprotective, antiallergic, mineralocorticoid, and antitussive drug [2–4].

Some biological properties of saponins explain their molecular complexation with sterols [1, 2, 5–7]. GA increases permeability and reduces the elastic modulus of cell membranes [8]. On the other hand, recent spectrophotometric titration did not confirm the complexation of GC with cholesterol (Chol; Fig. 1) and 1,2-dipalmitylophosphatidylcholine [9]. The authors of this paper have been suggested that the presence of 11-oxo group in the GC aglycone part prevents its complexation.

In order to consider the possibility of complexation of GC with Chol in various media, we studied their intermolecular interaction in aqueous isopropyl alcohol by NMR, IR, and UV spectroscopy.

Experimental

GC (purity ≥95% by HPLC) was purchased from Calbiochem. Chol and other chemicals in the highest grade of purity were obtained from Sigma-Aldrich.
The complex of Chol with GC 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 by vacuuming.

UV spectra of Chol solutions (0.50 · 10⁻⁴ M = const) with different concentrations of GC (0, 0.125 · 10⁻⁴, 0.25 · 10⁻⁴, 0.50 · 10⁻⁴, 1.0 · 10⁻⁴ M) were recorded on a LEKI SS2110UV spectrophotometer using a quartz cuvette (l = 1 cm) at 25 °C.

The IR spectra were recorded on a Simex FT-801 IR-Fourier spectrometer in the 4000–550 cm⁻¹ region (spectral resolution 4 cm⁻¹; 50 scans) using the universal optical attenuated total reflection (ATR) accessory with diamond crystal plate.

**IR spectrum of GC (ν, cm⁻¹):** 3197 (ОН, NH), 2928 (СН), 2907 (СН), 2868 (СН), 1719 (С=О), 1708 (С=О), 1641 (С(11)=О, C=C), 1587 (СОО –), 1451 (СН), 1425 (NH₄⁺), 1416 (COO⁻), 1387 (CH), 1357 (CH), 1318 (CH), 1260 (CH), 1211 (CH), 1162 (C–O–C, C–OH), 1037 (C–O–C, C–OH), 980 (=CH), 946 (CH), 918 (monosaccharide ring), 880 (CH), 818 (CH), 793 (CH), 694 (CH), 687 (CH), 679 (=CH), 663 (OH).

**IR spectrum of the complex of GC with 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), 925 (=CH), 881 (CH), 839 (C–C–C), 799 (CH), 738 (CH), 720 (CH), 694 (CH), 687 (CH), 679 (=CH), 662 (OH).

**IR spectrum of Chol (ν, cm⁻¹):** 3216 (ОН, NH), 2928 (CH), 2903 (CH), 2868 (CH), 2848 (CH), 1717 (C=O), 1698 (C=O), 1669 (C=C₈), 1648 (C(11)=O, C=C₉), 1586 (COO⁻), 1459 (CH), 1450 (CH), 1433 (CH), 1424 (NH₄⁺), 1418 (COO⁻), 1386 (CH), 1379 (CH), 1362 (CH), 1339 (CH), 1316 (CH), 1277 (CH), 1261 (CH), 1211 (CH), 1163 (C–O–C, C–OH), 1038 (C–O–C, C–OH), 1030 (C–O–C, C–OH), 978 (=CH), 947 (CH), 919 (monosaccharide ring, =CH), 880 (CH), 818 (CH), 795 (CH), 719 (CH), 692 (CH), 685 (CH), 679 (=CH), 662 (OH).

¹³C NMR spectra were recorded on a Bruker WM-250 spectrometer (62.9 MHz for ¹³C) in C₂D₅N at 30 °C. NMR spectra are reported in Tables 1 and 2.

---

**Fig. 1.** Chemical structures of GA, GC, Chol and schematic representation of the possible orientation of GC and Chol molecules during their intermolecular interaction.
Results and discussion

The intermolecular interaction of GC with Chol was studied by UV spectroscopy. As the GC concentration increases (at constant Chol concentration), the optical density of their solutions increases (hyperchromic effect) (Fig. 2). The absorption maximum of the solutions increases (bathochromic shift) from 237 to 250 nm. Similar spectral changes were previously noted for molecular complexes of some triterpene glycosides [10] and cyclodextrins [11].

The complex of Chol with GC was preparatively obtained by liquid-phase method in 80% aqueous isopropyl alcohol. The potential centers of intermolecular interactions in the molecules are COOH groups of GC and OH group of Chol. The lipophilic nature of the aglycone part of GC and sterane system with the hydrocarbon “tails” in Chol may contribute to hydrophobic contacts between them.

The nature of intermolecular interactions in the complex was confirmed by ATR FT-IR spectroscopy. Upon the formation of complex in the IR spectra for the absorption bands of stretching vibrations of Chol O–H bonds are observed shifts from 3403 and 3337 cm⁻¹ to 3216 cm⁻¹, and for GC — from 3197 to 3216 cm⁻¹. We also found a low-frequency shift of the absorption band of C=O bond in one of GC carboxyl groups at 1708 cm⁻¹ by 10 cm⁻¹. Similar shifts of the absorption bands of C=O bonds in IR spectra were previously observed during the interaction of ivy triterpene glycosides with Chol [12], as well as during the formation of GA and GC complexes [11]. In addition, the band of stretching vibrations of C–O bonds in C–OH for GC at 1037 cm⁻¹ shifts by –7 cm⁻¹ and for Chol at 1169, 1052, and 1022 cm⁻¹ — by –6, –14, and +8 cm⁻¹, respectively. IR spectroscopic data indicate about the formation of a hydrogen bond between Chol OH group and C=O group in one of GC carboxyl groups:

\[ \text{C=O}_{\text{GC}} \cdots \text{H–O}_{\text{Chol}} \]

Fig. 2. UV spectra of Chol solutions (0.50 · 10⁻⁴ M = const) with different concentrations of GC: 0 M (curve 1), 0.125 · 10⁻⁴ (curve 2), 0.25 · 10⁻⁴ (curve 3), 0.50 · 10⁻⁴ (curve 4), 1.0 · 10⁻⁴ (curve 5) (a) and ATR FT-IR spectra of GC, Chol, and GC–Chol molecular complex (b)
The complexation also causes changes in certain frequencies of absorption of CH bonds: $2868 \rightarrow 2863$ cm$^{-1}$ and $2907 \rightarrow 2903$ cm$^{-1}$ (for GC), and also $2899 \rightarrow 2903$ cm$^{-1}$, $953 \rightarrow 947$ cm$^{-1}$, and $799 \rightarrow 795$ cm$^{-1}$ (for Chol). These facts may indicate the presence of hydrophobic contacts between Chol and GC molecules.

The location of GC carboxyl group involved in the interaction with Chol was determined by $^{13}$C NMR spectroscopy. The value of the chemical shift of the C-30 atom in the carboxyl group of the aglycone portion of GC remains practically unchanged (Table 1). However, there is a change in the chemical shift of the C-6$^\text{"}$

### Table 1

$^{13}$C NMR spectral data for free GC and GC in the molecular complex with Chol ($\delta$, ppm, 0 — TMS, C$_5$D$_5$N, 30 °C)

| C-atom | GC               | GC in complex with Chol | $\Delta \delta = \delta_{\text{GC-Chol}} - \delta_{\text{GC}}$ | C-atom | GC               | GC in complex with Chol | $\Delta \delta = \delta_{\text{GC-Chol}} - \delta_{\text{GC}}$ |
|--------|------------------|-------------------------|--------------------------------------------------|--------|------------------|-------------------------|--------------------------------------------------|
| Aglycone part                               |                      |                                                      |        |                  |                          |                                   |
| 1      | 40.03            | 40.04                   | 0.01                                              | 16     | 26.78            | 26.75                   | −0.03                                            |
| 2      | 26.96            | 26.96                   | 0                                                  | 17     | 32.23            | 32.23                   | 0                                                |
| 3      | 89.28            | 89.30                   | 0.02                                               | 18     | 48.81            | 48.82                   | 0.01                                             |
| 4      | 40.14            | 40.15                   | 0.01                                               | 19     | 41.84            | 41.89                   | 0.05                                             |
| 5      | 55.52            | 55.51                   | −0.01                                              | 20     | 44.19            | 44.19                   | 0                                                |
| 6      | 17.66            | 17.68                   | 0.02                                               | 21     | 31.72            | 31.71                   | −0.01                                            |
| 7      | 33.05            | 33.07                   | 0.02                                               | 22     | 38.51            | 38.51                   | 0                                                |
| 8      | 43.55            | 43.58                   | 0.03                                               | 23     | 28.17            | 28.16                   | −0.01                                            |
| 9      | 62.20            | 62.19                   | −0.01                                              | 24     | 16.91            | 16.92                   | 0.01                                             |
| 10     | 37.29            | 37.29                   | 0                                                  | 25     | 16.79            | 16.78                   | −0.01                                            |
| 11     | 199.62           | 199.63                  | 0.01                                               | 26     | 18.87            | 18.87                   | 0                                                |
| 12     | 128.75           | 128.75                  | 0                                                  | 27     | 23.66            | 23.65                   | −0.01                                            |
| 13     | 169.63           | 169.63                  | 0                                                  | 28     | 28.85            | 28.85                   | 0                                                |
| 14     | 45.63            | 45.61                   | −0.02                                              | 29     | 28.85            | 28.85                   | 0                                                |
| 15     | 26.78            | 26.75                   | −0.03                                              | 30     | 179.22           | 179.21                  | −0.01                                            |
| Carbohydrate part                           |                      |                                                      |        |                  |                          |                                   |
| GlcUA$^{1}$                                 | 105.10              | 105.12                                               | 0.02                                                | GlcUA$^{1}$ | 106.63           | 106.61                                               | −0.02 |
|     2$^{1}$                                 | 82.82               | 82.82                                                | 0                                                  |     2$^{2}$ | 76.71            | 76.74                                                  | 0.03 |
|     3$^{1}$                                 | 77.15               | 77.16                                               | 0.01                                               |     3$^{2}$ | 77.65            | 77.67                                                  | 0.02 |
|     4$^{1}$                                 | 73.45               | 73.47                                               | 0.02                                               |     4$^{2}$ | 73.51            | 73.50                                                  | −0.01 |
|     5$^{1}$                                 | 78.02               | 78.00                                               | −0.02                                              |     5$^{2}$ | 78.23            | 78.15                                                  | −0.08 |
|     6$^{1}$                                 | 172.92              | 172.94                                              | 0.02                                               |     6$^{2}$ | 174.19           | 174.03                                                  | −0.16 |
atom of the carboxyl group of the terminal residue of glucuronic acid (GlcUA") in the disaccharide fragment GC by −0.16 ppm compared to individual GC (Fig. 1). A smaller effect was also noted on the neighboring C-5" atom (Δδ = −0.08 ppm).

In addition, it is noted Δδ (up to 0.05 ppm) for a number of GC aglycone and Chol C-atoms (Tables 1 and 2). The greatest effects were found for some C-atoms in the B–E rings of GC, in the B–D rings, and side chain of Chol, as well as all methyl groups of Chol. These data may indicate about hydrophobic interactions between the aglycone part of GC and Chol (Fig. 1).

Conclusions

The results of this work confirm the molecular complexation between GC and Chol. The interaction is accompanied by bathochromic shift and a hyperchromic effect. The formation of an intermolecular hydrogen bond between OH group at C-3 of Chol and C=O group of terminal glucuronic acid residue in the carbohydrate part of GC (C3–O–H...O=C") and hydrophobic contacts were confirmed by 13C NMR and ATR FT-IR spectroscopy. The results of this work can be used to study of mechanisms of biological activity of GA, GC and other saponins.

Table 2

| C-atom | Chol | Chol in complex with GC | ∆δ = δGC–Chol – δChol | C-atom | Chol | Chol in complex with GC | ∆δ = δGC–Chol – δChol |
|--------|------|------------------------|------------------------|--------|------|------------------------|------------------------|
| 1      | 37.92| 37.94                  | 0.02                   | 15     | 24.59| 24.62                  | 0.03                   |
| 2      | 32.71| 32.71                  | 0                      | 16     | 28.58| 28.61                  | 0.03                   |
| 3      | 71.35| 71.38                  | 0.03                   | 17     | 56.49| 56.52                  | 0.03                   |
| 4      | 43.59| 43.58                  | −0.01                  | 18     | 12.09| 12.13                  | 0.04                   |
| 5      | 142.07| 142.06                 | −0.01                  | 19     | 19.68| 19.72                  | 0.04                   |
| 6      | 121.29| 121.31                 | 0.02                   | 20     | 36.11| 36.13                  | 0.02                   |
| 7      | 32.31| 32.34                  | 0.03                   | 21     | 19.02| 19.06                  | 0.04                   |
| 8      | 32.26| 32.29                  | 0.03                   | 22     | 36.57| 36.59                  | 0.02                   |
| 9      | 50.60| 50.62                  | 0.02                   | 23     | 24.22| 24.24                  | 0.02                   |
| 10     | 36.99| 37.02                  | 0.03                   | 24     | 39.80| 39.83                  | 0.03                   |
| 11     | 21.45| 21.48                  | 0.03                   | 25     | 28.29| 28.32                  | 0.03                   |
| 12     | 40.12| 40.15                  | 0.03                   | 26     | 22.74| 22.78                  | 0.04                   |
| 13     | 42.60| 42.63                  | 0.03                   | 27     | 22.99| 23.03                  | 0.04                   |
| 14     | 57.00| 57.04                  | 0.04                   |        |      |                        |                        |
Acknowledgements
This work was carried out in the frame of an internal grant of Sevastopol State University (identifier 30/06-31).

References
1. Hostettmann K, Marston A. Saponins. Cambrige: Cambridge University Press; 1995. 548 p.
2. Tolstikov GA, Baltina LA, Grankina VP, Kondratenko RM, Tolstikova TG. Solodka: Bioraznoobrazie, Khimiya, Primenenie v Medicine [Licorice: Biodiversity, Chemistry, and Application in Medicine]. Novosibirsk: Geo; 2007. 311 p. Russian.
3. Asl MN, Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res. 2008;22(6):709–24. doi:10.1002/ptr.2362
4. Pavlova SI, Uteshev BS, Sergeev AV. Licorice root: possible mechanisms of antitoxicant, anticarcinogen, and antitumor properties (a review). Pharm Chem J. 2003;37(6):314–7. doi:10.1023/A:1026005931751
5. Sidhu GS, Oakenfull DG. A mechanism for the hypocholesterolaemic activity of saponins. Brit J Nutrit. 1986;55(3):643–9. doi:10.3109/10717544.2015.919544
6. Lorent JH, Quetin-Leclercq J, Mingeot-Leclercq MP. The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org Biomol Chem. 2014;12(44):8803–22. doi:10.1039/c4ob01652a
7. Popov AM. Mechanisms of biological activity of ginsenosides: comparison with holothurian glycosides. Vestnik DVO RAN. 2006;6:92–104.
8. Selyutina OYu, Polyakov NE, Korneev DV, Zaitsev BN. Influence of glycyrrhizin on permeability and elasticity of cell membrane: perspectives for drugs delivery. Drug Deliv. 2016;23(3):858–65. doi:10.3109/10717544.2014.919544
9. Wojciechowski K, Orczyk M, Guthberlet T, Geue T. Complexation of phospholipids and cholesterol by triterpenic saponins in bulk and in monolayers. Biochim Biophys Acta Biomembr. 2016;1858(2):363–73. doi:10.1016/j.bbamem.2015.12.001
10. Yakovishin LA, Grishkovets V.I. Ivy and licorice triterpene glycosides: promising molecular containers for some drugs and biomolecules. Stud Nat Prod Chem. 2018;55:351–83. doi:10.1016/B978-0-444-64068-0.00011–5
11. Li S, Purdy WC. Cyclodextrins and their applications in analytical chemistry. Chem Rev. 1992;92(6):1457–70. doi:10.1021/cr00014a009
12. Yakovishin LA, Grishkovets VI. Molecular complexes of ivy triterpene glycosides with cholesterol. Khimiya Rastitel’nogo Syr’ya. 2018;4:133–40. doi:10.14258/jcprm.2018043607