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Intermolecular Interaction of Glycyrrhizin with Cholesterol

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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

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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, hypocholesterolemic, 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-dipalmitoylphosphatidylcholine [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 ($0.50 \cdot 10^{-4}$ M = const) with different concentrations of GC (0, $0.125 \cdot 10^{-4}$, $0.25 \cdot 10^{-4}$, $0.50 \cdot 10^{-4}$, $1.0 \cdot 10^{-4}$ 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 (OH, NH), 2928 (CH), 2907 (CH), 2868 (CH), 1719 (C=O), 1708 (C=O), 1641 (C(11)=O, C=C), 1587 (COO^-), 1451 (CH), 1425 (NH_4^+), 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 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 the complex of GC with Chol (ν, cm^{-1}): 3216 (OH, NH), 2928 (CH), 2903 (CH), 2863 (CH), 1717 (C=O), 1698 (C=O), 1669 (C=C_{Chol}), 1648 (C(11)=O, C=C_{GC}), 1586 (COO^-), 1459 (CH), 1450 (CH), 1433 (CH), 1424 (NH_4^+), 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), 741 (CH), 719 (CH), 692 (CH), 685 (CH), 679 (=CH), 662 (OH).

^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer (62.9 MHz for ^{13}C) in C_5D_5N at 30 °C. NMR spectra are reported in Tables 1 and 2.

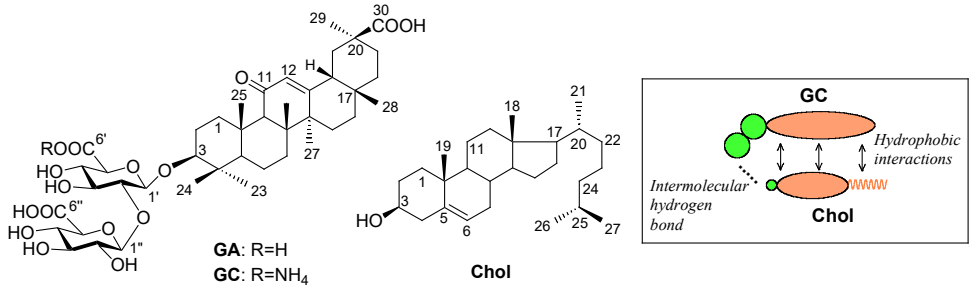


Fig. 1. Chemical structures of GA, GC, Chol and schematic representation of the possible orientation of GC and Chol molecules during their intermolecular interaction

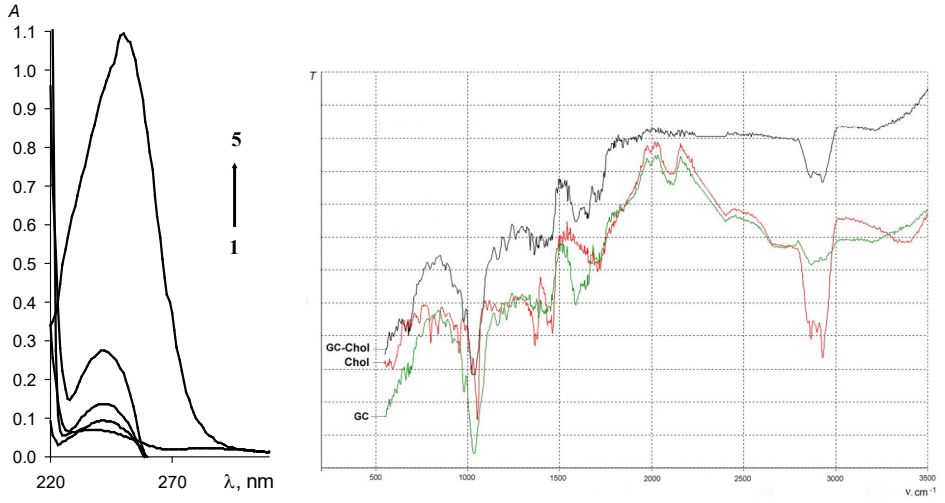
Results and discussion

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:

$$C{=}O_{GC}\cdots H{-}O_{Chol}.$$



a *b*

Fig. 2. UV spectra of Chol solutions ($0.50 \cdot 10^{-4}$ M = const) with different concentrations of GC: 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), $1.0 \cdot 10^{-4}$ (curve 5) (*a*) and ATR FT-IR spectra of GC, Chol, and GC–Chol molecular complex (*b*)

Table 1

^{13}C NMR spectral data for free GC and GC in the molecular complex with Chol (δ, ppm, 0 — TMS, C_5D_5N, 30 °C)

C-atom	GC	GC in complex with Chol	$\Delta\delta = \delta_{GC-Chol} - \delta_{GC}$	C-atom	GC	GC in complex with Chol	$\Delta\delta = \delta_{GC-Chol} - \delta_{GC}$
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′				GlcUA″			
1′	105.10	105.12	0.02	1″	106.63	106.61	–0.02
2′	82.82	82.82	0	2″	76.71	76.74	0.03
3′	77.15	77.16	0.01	3″	77.65	77.67	0.02
4′	73.45	73.47	0.02	4″	73.51	73.50	–0.01
5′	78.02	78.00	–0.02	5″	78.23	78.15	–0.08
6′	172.92	172.94	0.02	6″	174.19	174.03	–0.16

The complexation also causes changes in certain frequencies of absorption of CH bonds: 2868 → 2863 cm^{-1} and 2907 → 2903 cm^{-1} (for GC), and also 2899 → 2903 cm^{-1}, 953 → 947 cm^{-1}, and 799 → 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″

Table 2

^{13}C NMR spectral data for free Chol and Chol in the molecular complex with GC (δ, ppm, 0 — TMS, C_5D_5N, 30 °C)

C-atom	Chol	Chol in complex with GC	$\Delta\delta = \delta_{GC-Chol} - \delta_{Chol}$	C-atom	Chol	Chol in complex with GC	$\Delta\delta = \delta_{GC-Chol} - \delta_{Chol}$
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				

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 (C^3–O–H…O=$C^{6''}$) and hydrophobic contacts were confirmed by ^{13}C 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.

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