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Quantum-Chemical Approach to the Justification of the Mechanisms of Sorption of the Triterpenoid Saponin by Highly Basic Anion Exchanger AB-17-2P in the Chloride Form

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Abstract—Computer simulation of interaction of the structural fragments of triterpenoid saponins with the anion exchanger AV-17-2P was carried out using the Gaussian 03 software. The results showed that a decrease in the energy of the system that includes the sum of the energies of electrostatic interactions and hydrogen bonding at the absorption by the anion exchanger of the polar part of the saponin molecule equals 18.9 kJ mol^{-1} and at the absorption of oleanolic acid, 31.9 kJ mol^{-1}, that is, the sorption capacity of the aglycone was higher than that of glucuronic acid.

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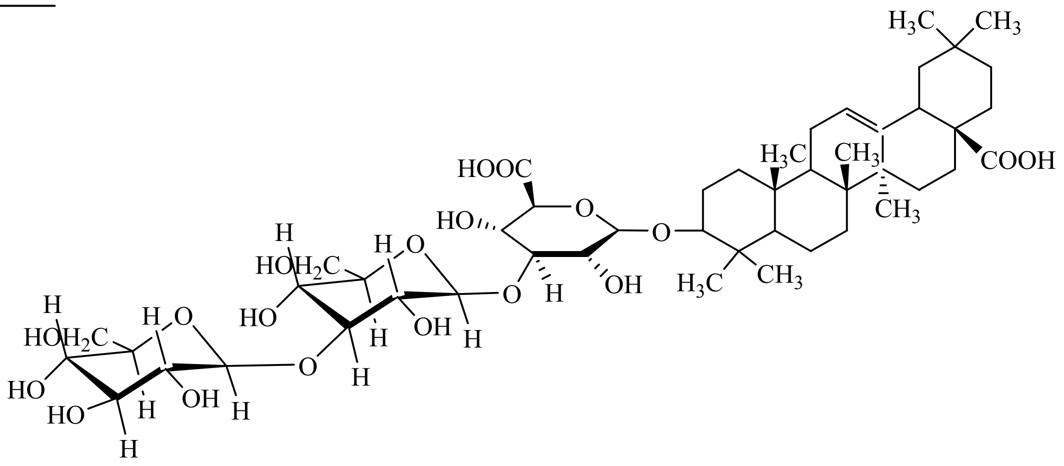
A wide range of therapeutic effects and minimal side effects of drugs and biologically active additives (BAA) based on substances of natural origin made them particularly popular in the recent years. The overwhelming majority of these means are based on the triterpene saponins, the compounds of glycoside structure. A large part of saponin derivatives is associated with the oleanolic acid derivatives with 2 to 4 sugar residues (Fig. 1).

Since the sources of the considered compounds are natural objects, the products are complex mixture of compounds containing, besides the main substance dozens or sometimes hundreds of related components. Therefore it is urgent to intensify the treatment of the extracts in order to isolate the main substance. The development of synthetic polymeric sorbents led to the widespread use of the latter for the separation and fine purification of complex mixtures. Studies of these processes using modern physicochemical methods of analysis allowed the assumption of the possible mechanism of interaction in the sorbate–sorbent system, but a detailed study of the structures formed and the contribution of forces of electrostatic interaction is only possible using computer simulation techniques.



The correct interpretation of the mechanism of absorption of compounds by sorbents at the molecular level will allow, in turn, choosing a theoretically reasonable sorbent, as well as a prediction of a number of specific interactions with substances of glycoside structures.

The performed studies [1] have shown the feasibility of using ionic macroporous sorbents, in particular, the polymer AB-17-2P, for purification of the isolated saponins fractions from lipophilic compounds. Analysis of the output curves of the sorption under equilibrium and kinetic conditions revealed that the ion-exchange absorption involved only one carboxy group which might belong to the saponin aglycone, the oleanolic or glucuronic acid that was a part of the carbohydrate component of the molecule. It can be assumed that the hydration of the carbohydrate moiety of saponin prevents the ion exchange with the involvement of the carboxy group of glucuronic acid, while the hydrophobic component , the aglycone, is absorbed through the ion-exchange and interacts with the benzene ring of the anion exchanger through specific interactions stabilizing the appearing saponin–sorbent structure (stacking effect).

At present, quantum-chemical calculations along with the physicochemical methods provide a way to obtain the data necessary to elucidate the mechanisms of complex organic reactions [2]. Therefore it was possible to confirm or refute our assumptions by means of computer simulation of the interaction of each of the fragments of the saponin structure with the anion exchanger AV-17-2P. Quantum chemical calculations were carried out with the Gaussian 03 software recommended for organic compounds [2, 3], in the Hartree–Fock approximation with the 6-31G**(d,p) basis. This basis involves in the calculation the *d*-orbitals of the *p*-elements and *p*-orbitals on hydrogen atoms (valence-split basis), which significantly improves the accuracy of the calculation.

Accounting for the complexity of glycoside structure, the simulation of the sorption mechanism of saponin by the anion exchanger was performed by considering selected representative fragments of the glycoside, that is, the parts of the molecules whose atoms may be directly involved in the act of ion exchange. The carboxy groups belong to oleanolic acid, which is the saponin aglycone, and to glucuronic acid, therefore, we selected two fragments belonging to nonpolar and polar parts of the molecule: aglycone and the chain of sugars (glucuronic acid, D-glucose, D-xylose). The validity of this choice was assessed while comparing the lengths of the O–H bonds of the carboxy groups in the whole saponin molecule and in its fragments. The values are given below.

Molecule	Oleanolic acid, O–H bond length, nm	Glucuronic acid, O–H bond length, nm
Saponin	0.0988	0.0990
Fragment	0.0955	0.0955

As seen, in the molecule of saponin the O–H bond of the carboxy group of glucuronic acid is weaker than in the carboxy group of oleanolic acid. However, in the relevant fragments the bond lengths are equal due to redistribution of electron density. Nearly the same change in the bond lengths of the O–H bonds of carboxy groups of aglycone and glucuronic acid allowed regarding the selection of chosen fragments as appropriate.

The next step was a computer simulation of the interaction of selected fragments of the saponin molecule with the anion exchanger AB-17-2P in chloroform. As a parameter characterizing the stability of the formed structures and therefore the probability of their formation was chosen the difference between the Gibbs energies of the products of ion exchange reaction and the initial compounds. Such approach has already been discussed for the structures of the phenothiazine series [3]. To calculate the Gibbs energy, the structures of the starting compounds were optimized: anion exchanger, oleanolic acid, and carbohydrate moiety of saponin including the glucuronic acid, as well as the structures of anion exchanger–aglycone and anion exchanger–saponin carbohydrate moiety. In the framework of the method of supermolecular approximation [2], water molecules were gradually added to the investigated systems.

Table 1 shows the values of difference in the Gibbs energy of the reaction products and starting materials. The data in Table 1 show that in the nonhydrated state the structure of anion excahger–carbohydrate component is more stable. The reason for the sharp decrease in the Gibbs energy at the interaction of the carbohydrate moiety of saponin with the anion exchanger is the low value of the Gibbs energy of this representative fragment due to the intramolecular hydrogen bonds formed in the rings. The effect of intramolecular H-bonds on the value of the Gibbs energy has been demonstrated in a number of works [4, 5], but its mechanism has not yet been determined. At the hydration of these systems with 3 water

Table 1. Gibbs energy change in the interaction of the saponin fragments with the anion, a.u.

Number of water molecules in the system	Anion exchanger–oleanolic acid	Anion exchanger–carbohydrate part
0	–342.996	–460.054
3	–460.067	–459.970
5	–459.952	–422.947

molecules, the Gibbs energy of the two structures differs only slightly.

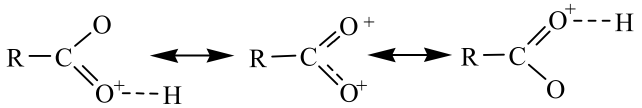
The oxygen atoms of the carboxy groups of both potential participants in the reaction of ion exchange interact with the atoms of the anion exchanger not only by electrostatic forces, but also through the formation of hydrogen bonds. The oxygen atoms O^1 and O^2 of the aglycone carboxy group form hydrogen bonds with oxygen atoms of water molecules O^3 and O^4. The water molecules in turn form hydrogen bonds with the carbon atoms of the methyl groups of the sorbent C^6 and C^7 (Table 2, Fig. 1).

Although such unusual hydrogen bonding C–H···Y for a long time has been considered as secondary molecular interactions contributing very little in the physicochemical properties of compounds, currently the number of such molecular events is constantly growing and this type of interaction is common in complex biological systems [6], including those considered in this paper.

In the structure of carbohydrate part of saponin–3 molecules of water–anion (Fig. 2) the oxygen atoms O^1 and O^2 of the carboxy group of glucuronic acid form hydrogen bonds with the oxygen atoms O^4 and O^5 of two water molecules. The oxygen atoms of water molecules O^5 and O^7 form weak hydrogen bonds with the carbon atoms of the sorbent methyl groups C^6 and C^8 (Table 3).

Thus, the introduction of water molecules in the structure of the anion exchanger–saponin carbohydrate moiety leads to an increase in Gibbs energy, that is, the stability of the structure decreases, and by its energy becomes close to the Gibbs energy of the same structure with oleanolic acid.

In the system of carbohydrate part–anion–five water molecules the oxygen atoms of water molecules form H-bonds with the hydroxy groups of the carbohydrate ring, hindering sterically the electrostatic interaction of the oxygen atoms of the carboxy group of glucuronic acid with the sorbent: the sorbate–sorbent distance increases. The influence of the hydrogen bond results in the shift of the uniformly distributed electron density on the oxygen atoms of the carboxy group to one of the atoms, as reflect the corresponding resonance structures [7]:



Thus, the electrostatic interaction with one oxygen atom becomes stronger, but with another weaker, and the total Gibbs energy change is less than in the case of addition of 0–3 water molecules. Adding five water molecules in the structure of sorbent–aglycone practically does not change the value of the Gibbs energy: the number of embedded water molecules between the structures does not change, and the absence of intramolecular hydrogen bonds as well as the hydrophobicity of oleanolic acid do not cause the formation of resonance structures, and the electrostatic interaction occurs with two oxygen atoms of the carboxy group. Therefore, the structure with oleanolic acid becomes the more stable.

Thus, the hydrogen bonds formed in the absence of water molecules between the oxygen atoms of the

Table 2. The distances between the atoms and ions in the structure of anionexchanger–oleanolic acid–3 molecules of water, Å

Atom	O^1	O^2	O^3	O^4	O^5	C^6	C^7	C^8
O^1	0	2.232	2.644	2.745	5.006	3.906	3.577	1.296
O^2	2.232	0	4.767	2.745	5.408	5.338	5.300	1.246
O^3	2.644	4.767	0	4.526	5.924	3.286	3.338	3.772
O^4	2.745	2.745	4.526	0	2.647	3.375	3.479	3.126
O^5	5.006	5.408	5.924	2.647	0	3.397	3.472	5.679
C^6	3.906	5.338	3.286	3.375	3.397	0	2.460	5.014
C^7	3.577	5.300	3.338	3.479	3.472	2.460	0	2.460
C^8	1.296	1.246	3.772	3.126	5.679	5.014	2.460	0

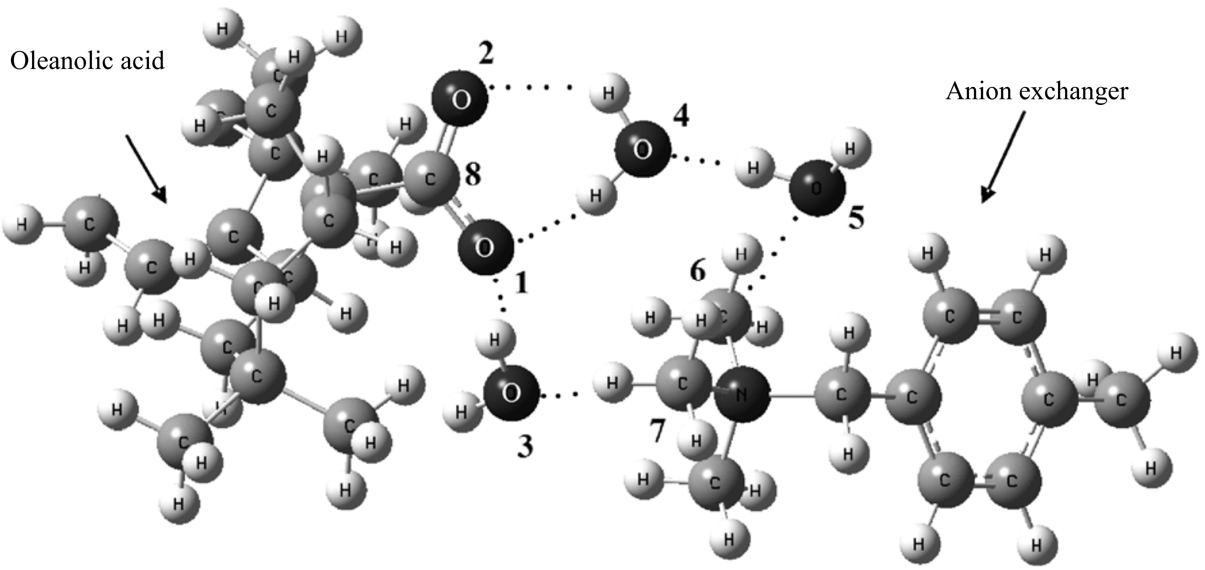


Fig. 1. Optimized structure of the anionite–oleanolic acid–3 water molecules. The dotted line shows the hydrogen bond.

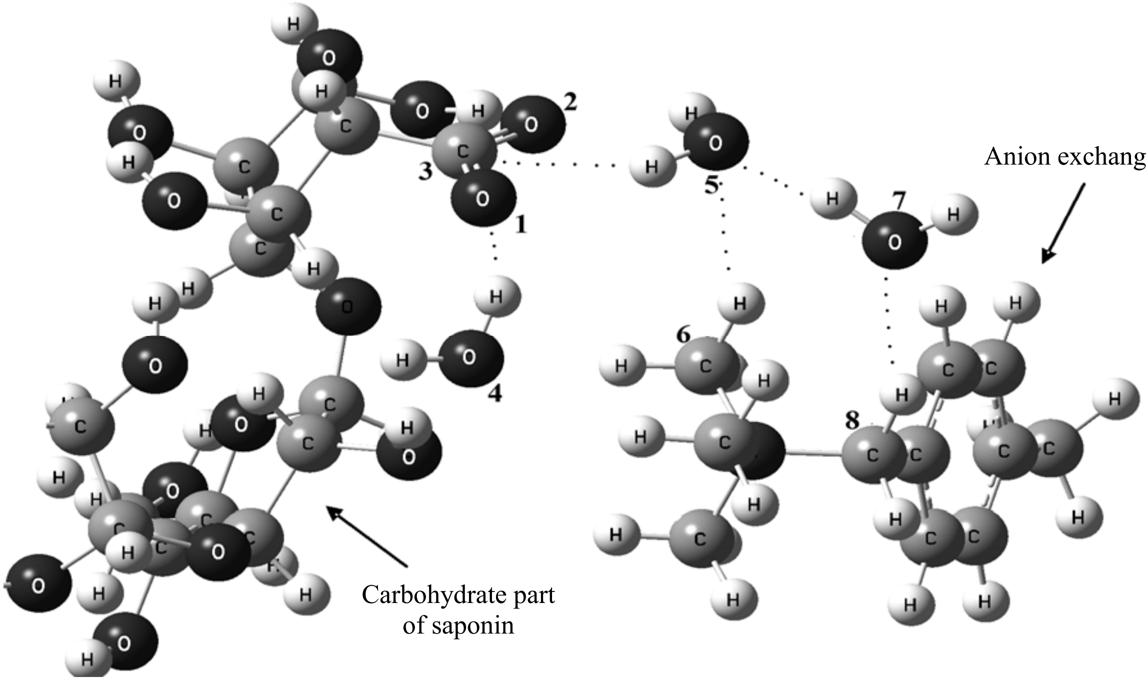


Fig. 2. Optimized structure of the anion–carbohydrate moiety of saponin –3 water molecules. The dotted line shows the hydrogen bond.

carboxy group of aglycone (glucuronic acid) and the hydrogen atoms of methyl groups of the anion exchanger become much weaker, and the interaction with the sorbent occurs through water molecules, the total energy of the interaction being the sum of energies of ionic and hydrogen bonds.

The influence of hydration on the interaction of selected fragments with the matrix of the sorbent was evaluated quantitatively by calculating the energy of the electrostatic interaction between the counterions and the matrix of the sorbent by the formula:

$$E_\mathrm{electr} = z_i z_f e^2/4\pi\varepsilon\varepsilon_0 r_s = k(z_i z_f/r_s), \qquad (1)$$

where z_i and $\underline{z}_\mathrm{f}$ are the charges of the fixed ion and the counterion; e is electron charge, equal to 1.6×10^{-19} C; ε is the electric permittivity of the medium (calculated

Table 3. The distances between the atoms and ions in the structure of anion exchanger–carbohydrate part of saponin–3 molecules of water, Å

Atom	O^1	O^2	O^3	O^4	O^5	C^6	C^7	C^8
O^1	0	2.244	1.273	2.710	2.797	3.652	4.967	5.635
O^2	2.244	0	1.254	4.727	2.790	3.489	5.006	5.740
O^3	1.273	1.254	0	3.706	3.103	3.799	5.439	6.067
O^4	2.710	4.727	3.706	0	4.924	4.460	6.246	5.957
O^5	2.797	2.790	3.103	4.924	0	3.540	2.652	4.661
C^6	3.652	3.489	3.799	4.460	3.540	0	3.735	2.523
C^7	4.967	5.006	5.439	6.246	2.652	3.735	0	2.509
C^8	5.635	5.740	6.067	5.957	4.661	2.523	2.509	0

by the Debye equation [8]); r_s is the distance between the ions. The values obtained are listed in Table 4.

The energy of hydrogen bond, which is the second component of the total interactions, was calculated proceeding from the principle that the occurrence of the act of ion exchange determines the weakest H-bond between either a fragment of the sorbent (saponin) and a water molecule, or between two water molecules [9]. The strength of hydrogen bonds was assessed by the magnitude of the shift in the OH group vibration. To determine the frequency of vibration by computer simulation, we calculated the IR spectra of the structures of free water molecule, water molecule–glucuronic acid, and water molecule–aglycone of saponin.

In the structure of sorbent–glucuronic acid the weakest among the H-bonds is the bond between water molecules, therefore it was calculated using the following empirical relation [4, 9]:

$$E_H = 261.7\Delta\nu/\nu^0_{OH}, \tag{2}$$

where E_H (kJ mol^{-1}) is the energy of the hydrogen bond; $\Delta\nu$ (cm^{-1}) is the frequency shift in the stretching vibrations of free OH group at the formation of hydrogen bond, ν^0_{OH} is stretching vibration of free OH group. The value of the hydrogen bond energy was 16.5 kJ mol^{-1}.

Table 4. The change in the electrostatic energy and the energy of the hydrogen bond at the interaction of the saponin fragments with the anion, kJ mol^{-1}

	E_{electr}		E_H	
nH$_2$O	Sorbent–oleanolic acid	Sorbent–carbohydrate part	Sorbent–oleanolic acid	Sorbent–carbohydrate part
0	11.9	9.9	28.7	16.5
3	10.7	9.3		
5	10.7	7.9		

In the structure of sorbent–aglycone the weakest is the H-bond between water molecule and the oxygen atom of the carboxy group. The calculation of its value was carried out along the equation recommended for H-bond with carbonyl compounds [5]:

$$E_H = 1.38(\Delta\nu - 40)^{1/2}, \tag{3}$$

where E_H (kJ mol^{-1}) is the energy of the hydrogen bond; $\Delta\nu$ (cm^{-1}) is the frequency shift in the stretching vibrations of free OH group and OH group in the hydrogen bond. The value of the hydrogen bond energy was 28.7 kJ mol^{-1}.

Matching the values of the energy of the H-bonds with the published data [4, 5], as well as with the values of the energies of hydrogen bonds calculated from the experimentally obtained IR spectra of samples of the sorbent saturated with saponins (Table 5) confirm the validity of the calculations.

Thus, the hydrogen bonding is stronger than the electrostatic interaction, and it determines the course of the ion exchange. Such a mechanism of sorption is given in [4,10–13] analyzing the structure and functions of sulfonate cation exchangers.

Table 5. The energy of hydrogen bonds in the system of sorbent–saponin–water molecules

Bond	E_H, kJ mol^{-1}	
	calculation	experiment
$H_2O\cdots H_2O$	16.5	13.07
$H_2O\cdots {}^{-}OOC$	28.7	26.4

A decrease in the energy of the system at the absorption of polar part of the saponin molecule by the anion exchanger is the sum of the energies of electrostatic interaction and hydrogen bonding, and it equals 18.9 kJ mol^{-1}, at the absorption of oleanolic acid 31.9 kJ mol^{-1}, that is, the sorption capacity of the aglycone is higher compared with glucuronic acid. It can be assumed that at the quantum-chemical simulation of these structures with a large number of water molecules the energy will change, but the trend of the strengthening the bonding with oleanolic acid anion will continue, while the steric hindrance in the interaction of the sorbent with glucuronic acid will increase due to hydrophilicity, reducing the probability of its sorption.

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