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A QUANTUM-CHEMICAL STUDY ON THE ANTIOXIDANT PROPERTIES OF NATURAL PHENOLIC COMPOUNDS

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*The influence of the structure of flavonolignans (silybin, silychristin), flavonoids (quercetin and taxifolin) on the antioxidant properties of silymarin - a standardized herbal extract obtained from the milk thistle seeds (lat. *Silybum marianum*), which contains a mixture of these compounds has been studied. Molecular structure was optimized at HF/6-31G(d,p) level of theory by means of the GAMESS program package. The O-H bond dissociation enthalpies for all OH groups and ionization potentials of molecules were calculated using density functional theory approach with B3P86 functional and 6-31G(d,p) basis set to analyze the redox properties. The solvation model IEF PCM was used to account for the solvent effects. It has been shown that the OH groups of the side phenolic ring give the maximum contribution to the hydrogen atom transfer mechanism (HAT). CH₂OH groups and OH groups of flavonoids moieties of silybin and silychristin weakly participate in the HAT (high dissociation enthalpies). The presence of 2,3-double bond in the flavonoid moiety affects the redistribution of charge and increases the contribution of the 3-OH group to a HAT mechanism for the quercetin molecule compared to taxifolin. The contribution of the electron transfer mechanism to the antioxidant activity is reduced in a series of quercetin > silychristin > taxifolin > silybin.*

Keywords: flavonolignans, flavonoids, antioxidant activity

INTRODUCTION

The formation of free radicals is one of the most versatile processes which occur in the cells and in the intercellular space of all living organisms. In small amounts, free radicals play a useful role in maintaining health, taking part in the millions of chemical reactions. They help digest food, fight pathogenic bacteria, fungi and viruses. But the influence of some factors, such as high temperature, electromagnetic radiation leads to failure of natural control mechanisms. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis and neurodegeneration in motor neuron diseases [1–3]. Oxidative damage in DNA can cause cancer [4]. And then antioxidants can come to the assistance by intercepting free radicals which attack vital targets when a natural neutralizing mechanism can't cope with this task.

There is a growing interest in the antioxidant properties of silymarin [5–7] – a standardized herbal extract obtained from the milk thistle seeds (lat. *Silybum marianum*) containing a mixture of flavonolignans (silybin, isosilybin, silychristin, silydianin), flavonoids (quercetin and taxifolin) and other polyphenolic compounds. Silymarin was

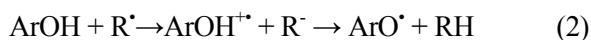
used as a hepatoprotective drug for the prevention and treatment of liver diseases of different etiologies [8]. Silica nanocomposites with silymarin may be more effective for the treatment of, for example, toxic liver disease. However, the creation of nanocomposites requires a detailed study of the properties of its components. The aim of this work was to study the influence of the structure of main components of silymarin (silybin, silychristin, quercetin and taxifolin) on its antioxidant properties.

THEORETICAL BACKGROUND

Molecular structure (Fig. 1) has been optimized by using Hartree-Fock theory with the 6-31G(d,p) basis set by means of the GAMESS [9]. The enthalpies of dissociation of O–H bonds for each OH-groups and ionization potentials of molecules were calculated using DFT (B3P86/6-31G(d,p)) (GAMESS) with functional B3P86, which gives the best agreement with experiment for ionization potentials and bond-dissociation enthalpies for phenolic compounds [10]. The improved solvation model IEF PCM (Integral Equation Formalism version of the Polarizable Continuum Model) [11] was used to account for the solvent effects.

Compounds with phenolic structure can realize the antioxidant properties by two mechanisms [10]:

by transferring the hydrogen atom (1) or electron transport (2).



R^{\bullet} is any radical involved in oxidative stress (including $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, ROO^{\bullet}) and, for example, the model-radical DPPH.

Reaction (1) corresponds to the homolytic dissociation of an O–H bond (when the total electron pair shared between radicals ArO^{\bullet} and R^{\bullet} which are formed) and can occur on each OH group of the phenolic compound depending on the bond dissociation enthalpy of the OH group. With a decrease in the bond dissociation energy, the probability increases of participation of this OH group in an antioxidant effect. Reaction (2) proceeds to form an intermediate – cation, therefore it is dependent on the ionization potential of the molecule, which by Koopmans theorem (within the method of molecular orbitals) is determined by the energy of the HOMO with the opposite sign [12].

RESULTS AND DISCUSSION

To analyze the contribution of the various mechanisms to antioxidant activity of silymarin,

quantum-chemical calculations on silybin, silychristin, quercetin and taxifolin have been carried out. Flavonolignans silybin and silychristin are the major components of silymarin. Quercetin is a reference phenolic antioxidant, and taxifolin is its dihydroflavonol counterpart (Fig. 1). The difference between those two compounds comes from the presence of the 2,3-double bond in quercetin. Since the intermolecular interactions between molecules in the human body take place in a liquid medium, calculations were made taking into account the solvation effects [13].

To estimate the contribution of hydrogen atom transfer, the bond dissociation enthalpies (Table 1) of the O–H bonds were calculated for all OH groups of the molecules studied (Fig. 1):

$$\Delta H_{\text{diss}} = \Delta H_{\text{f}}(\text{ArO}^{\bullet}) + \Delta H_{\text{f}}(\text{H}^{\bullet}) - \Delta H_{\text{f}}(\text{ArOH}),$$

where ArOH – phenolic compound, ArO^{\bullet} and H^{\bullet} – radicals formed by homolytic dissociation of O–H bond.

Geometry optimization of each ArO^{\bullet} radical was performed starting from the optimized structure of the parent molecule, after the H atom was removed from the 3, 5, 7, 16, or 20 position and additionally from the $\text{CH}_2\text{-OH}$ group.

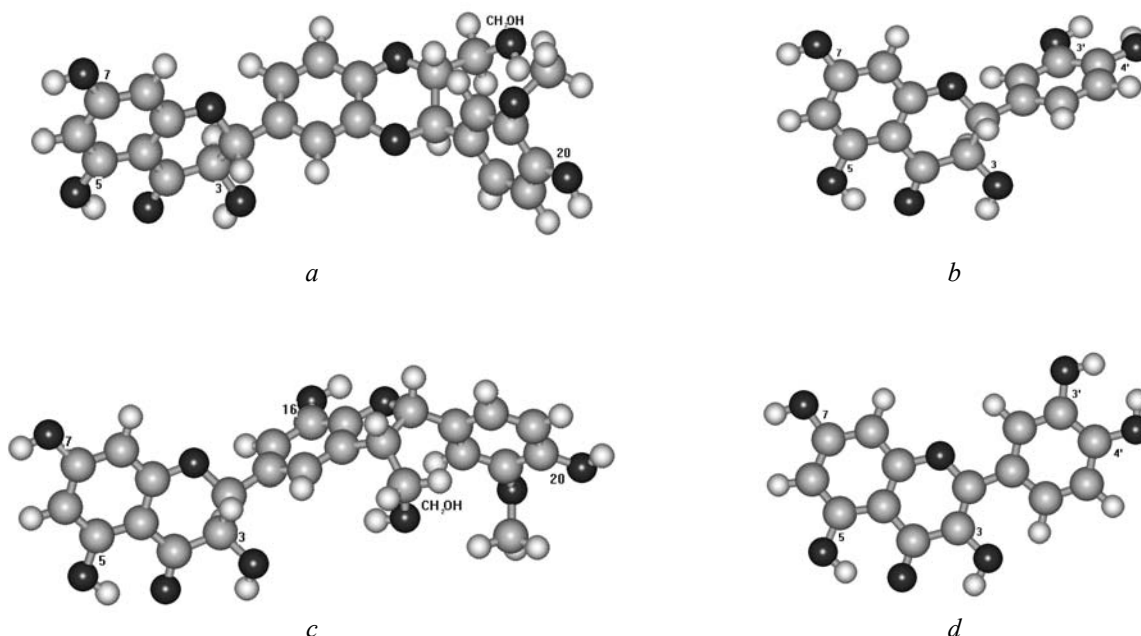


Fig. 1. The three-dimensional optimized structure of silybin (a), taxifolin (b), silychristin (c) and quercetin (d) molecules with the numbers of carbon atoms, which are attached to OH group

Table 1. The enthalpies of dissociation of O-H bonds for various OH groups (kcal/mol)

Molecule	3-OH	5-OH	7-OH	16-OH	20-OH	CH ₂ -OH	3'-OH	4'-OH
silybin	114.8	118.0	119.7		95.5	112.8		
silychristin	114.7	117.7	119.7	91.6	93.5	111.0		
taxifolin	114.9	104.2	104.5				84.1	84.1
quercetin	86.9	106.2	103.5				85.2	82.6

The enthalpies of dissociation of the O-H bonds (Table 1) have been analyzed. The lowest values were for the OH groups of the side phenolic ring (20-OH group of silybin and silychristin and 3'-OH, 4'-OH of quercetin and taxifolin) and for the additional OH group in position 16 (16-OH) of silychristin. These groups provide a maximum contribution to the hydrogen transfer mechanism. The dissociation enthalpy values for these groups vary in the range of 82–95 kcal/mol. Involvement of OH groups of flavonoids moieties (3-OH, 5-OH and 7-OH) in the transfer of hydrogen atoms is small (ΔH_{diss} values for these groups are more than 19 kcal/mol above). The role of 3-OH group is more significant only for quercetin molecule (ΔH_{diss} value is comparable with the corresponding values for the side ring). The charges on the oxygen and hydrogen atoms of the 3-OH group and on the carbon atoms of 2,3-bonds of the quercetin and taxifolin are shown in Table 2. Formation of 2,3-double bond in the molecule of

quercetin leads to a redistribution of charges, reduction of the negative charge on the oxygen atom, and explains the decrease in enthalpy of homolytic dissociation of this O-H bond.

The hydrogen atom transfer from the CH₂-OH group of silybin and silychristin seems to be less effective (ΔH_{diss} are 112.8 and 111.0 kcal/mol, respectively), indicating that this group weakly participate in the H-transfer mechanism.

The ionization potentials (Table 3) allow us to estimate the contribution of the mechanism of electron transfer in the antioxidant properties of molecules. The ionization potential of the molecule of quercetin was the lowest (6.17 eV). Thus, the presence or absence of the 2,3-double bond are the most influences on ionization potential. For other molecules studied the contribution of electron transfer in antioxidant activity was lower than that for quercetin.

Table 2. The charges on the oxygen and hydrogen atoms of 3-OH group and on the carbon atoms of 2,3-bond of the molecules of quercetin and taxifolin (a.u.)

Atom	taxifolin	quercetin
C ₂	0.090	0.136
C ₃	0.049	0.005
O ₃	-0.463	-0.402
H ₃	0.364	0.377

Table 3. The ionization potential (eV)

Molecule	silybin	silychristin	taxifolin	quercetin
Ionization potential	6.52	6.32	6.41	6.17

CONCLUSION

It has been shown that the OH groups of the side phenolic ring (20-OH group for silybin and silychristin and 3'-OH, 4'-OH for quercetin and taxifolin) and additionally OH-group in position 16 for silychristin give the maximum contribution to the hydrogen atom transfer mechanism. It has been found that the presence of 2,3-double bond affects

the redistribution of charge and increases the contribution of the 3-OH group to the hydrogen atom transfer mechanism for the quercetin molecule compared to taxifolin one.

The contribution of the electron transfer mechanism to the antioxidant activity is reduced in a series of quercetin – silychristin – taxifolin – silybin.

Квантовохімічне дослідження антиоксидантних властивостей природних фенольних сполук

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Методами Хартрі-Фока (базис 6-31G(d,p)) і ТФГ (B3P86/6-31G(d,p)) з використанням сольватаційної моделі IEF PCM було вивчено вплив структури флаволігнанів (силібін, силікристин), флавоноїдів (кверцетин і таксіфолін) на антиоксидантні властивості силімарину - стандартизованого рослинного екстракту з насіння розторопші плямистої (лат. *Silybum marianum*), що містить суміш цих сполук. Було показано, що ОН-групи бічного фенольного кільця дають максимальний внесок в механізм перенесення атома водню. Було встановлено, що наявність 2,3-подвійного зв'язку впливає на перерозподіл заряду і збільшує внесок групи 3-ОН в механізм перенесення атома водню для молекули кверцетину. Внесок механізму з перенесенням електрона в антиоксидантну активність знижується в ряду кверцетин > силікристин > таксіфолін > силібін.

Ключові слова: флаволігнани, флавоноїди, антиоксидантна активність

Квантовохімічне дослідження антиоксидантних властивостей природних фенольних сполук

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Received 30.07.2016, accepted 03.11.2016