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DFT study on the radical scavenging capacity of apocynin with different free radicals

D. Y. Yancheva[1]*, S. S. Stoyanov[1], E. A. Velcheva[1], B. A. Stamboliyska[1], A. Smelcerovic[2]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., build. 9, 1113 Sofia, Bulgaria*

[2] *Department of Chemistry, Faculty of Medicine, University of Nis, Blv. Dr Zorana Djindjica 81, 18000 Nis, Serbia*

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Dedicated to Acad. Bogdan Kurtev on the occasion of his 100th birth anniversary

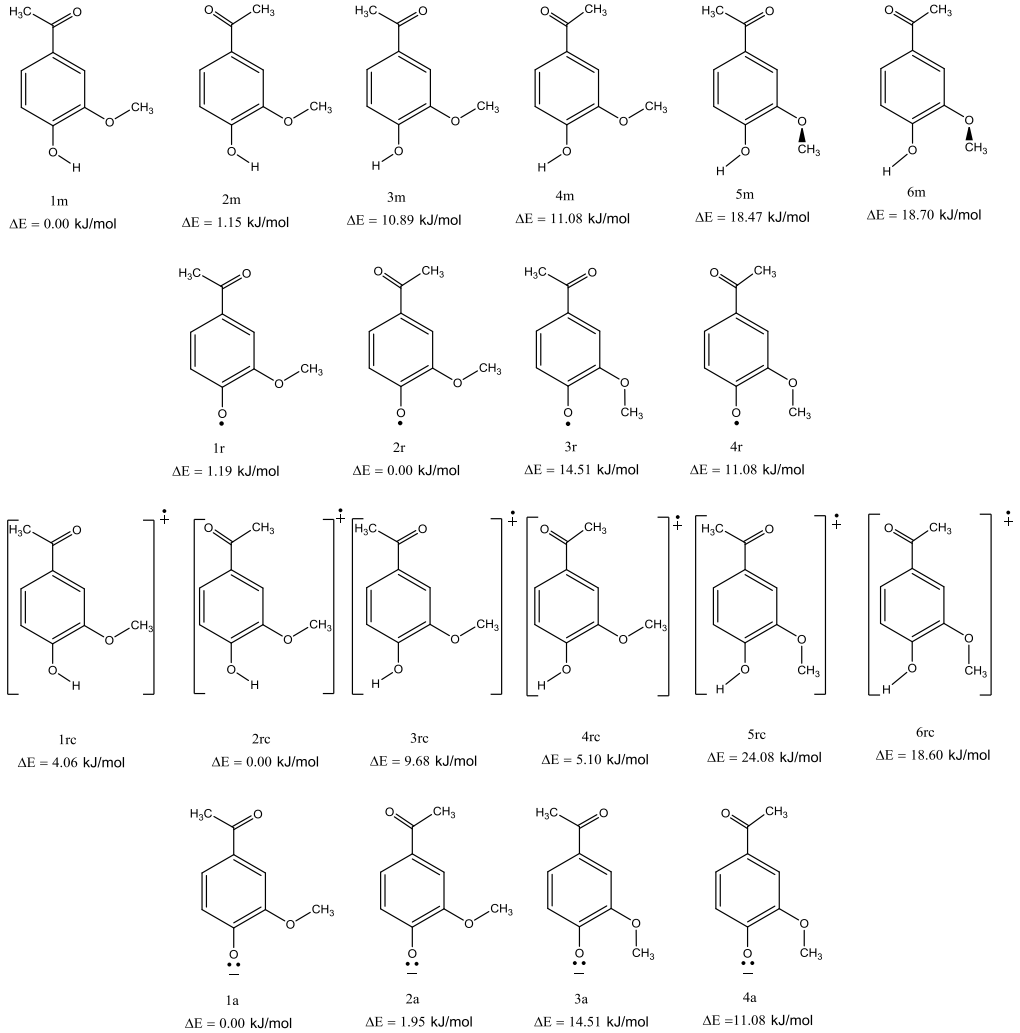
Apocynin is known as a nicotinamide adenine dinucleotide phosphate oxidase inhibitor and has been used as one of the most promising drugs in experimental models in vascular, inflammatory and neurodegenerative pathologies where the regulation of reactive oxygen species (ROS) plays a crucial role. Different possible mechanisms such as hydrogen atom transfer (HAT), single-electron transfer (SET-PT), sequential proton loss electron transfer (SPLET) were studied by DFT computations of the respective reaction enthalpies in polar and nonpolar solvents. The reactivity against various free radicals was accounted by analysing the thermodynamic data of apocynin reactions with hydroxyl, hydroxyperoxyl, alkoxyl and alkoxyperoxyl radicals. According to the calculations, the SPLET mechanism is preferred in ionization supporting solvents such as water. In this relation, the formation of oxyanion of apocynin was carried out in DMSO, and the structural and spectral changes arising from the conversion were followed by IR methods and DFT computations.

Key words: apocynin; DFT; radical scavenging capacity; vibrational spectra; anion

INTRODUCTION

Imbalance between reactive oxygen species (ROS) synthesis and normal production of oxidants in the body causes many inflammatory and neurodegenerative pathologies. Atherosclerosis, osteoarthritis or rheumatoid arthritis, ischemia-reperfusion lung injury, brain injury *etc.* are all associated to excessive production of ROS. Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase, NOX) is the enzyme that triggers the ROS production, and inhibition of this enzyme represents a successful strategy in the treatment of many diseases. Apocynin (Scheme 1, other trivial names: 4-hydroxy-3-methoxy-acetophenone, acetovanillone), a plant phenol – the biologically active substance in the roots of *Picrorhiza kurroa* growing in alpine Hymalaya, has been established as effective and nontoxic inhibitor of NADPH oxidase [1, 2]. Plant extract from *Picrorhiza kurroa* have been used in India and Sri Lanka for the preparation of ethnical medicines since long ago [3, 4]. Extracts of this plant are commercialized in the USA for treatment of liver disease and other conditions related to severe oxidative damage. Due to its inhibitory activity to NADPH oxidase, apocynin exhibit potent anti-inflammatory effect. The high effectiveness and low toxicity of apocynin make it very promising lead compound and its clinical effects were documented in various models of neuro-degenerative diseases, including Alzheimer and Parkinson's disease [4] and cardiovascular diseases [5]. On the other hand, there are reports suggesting that apocynin is not actually inhibitor of vascular NADPH oxidase, but an antioxidant [6]. It was suggested that apocynin only inhibits NADPH oxidase in leukocytes, whereas in endothelial and vascular smooth muscle cells, it predominantly acts as an antioxidant. The authors demonstrated that apocynin selectively deactivates hydroxyl and hydroxyperoxyl radicals [6]. Oxidative side effects of apocynin were also reported due to pro-oxidant activity of its radical [7]. Despite the variety of experimental data supporting the radical-scavenging efficiency of apocynin [8, 9], there are no reports on the anticipated mechanism of its action. Therefore, the purpose of the present study is to investigate the possible mechanisms of antioxidant action taking into account the influence of the medium polarity. Different possible mechanisms such as hydrogen atom transfer (HAT), single-electron transfer (SET-PT), and sequential proton loss electron transfer (SPLET) will be studied by DFT computations of the respective reaction enthalpies in polar and nonpolar solvents. In order to estimate the reactivity against

* To whom all correspondence should be sent:
E-mail: *deni@orgchm.bas.bg*



Scheme 1. Conformers of neutral apocynin (**1m-6m**), its radical (**1r-4r**), radical cation (**1rc-6rc**) and oxyanion (**1a-4a**), the relative energies ΔE are given in kJ.mol^{-1} with respect to the most stable conformers.

various free radicals the enthalpies of the reactions between apocynin and hydroxyl, hydroxyperoxyl, alkoxyl and alkoxyperoxyl radicals will be analysed.

EXPERIMENTAL

Apocynin (98% purity) was purchased from Sigma-Aldrich Co and applied without further purification. CD_3OD (99% at. enrichment) was purchased from Merck and used to obtain CD_3ONa by reacting it with Na. Spectral quality $CDCl_3$ and DMSO-d_6 were purchased from Sigma-Aldrich Co.

The corresponding anion was obtained by adding 0.08 mol.l^{-1} DMSO-d_6 solution of the parent compound to excess of dry CD_3ONa. The reaction mixture was filtered to remove the remains of solid CD_3ONa and put immediately into a spectroscopic cell to record the IR spectra. The conversion was practically complete (no bands of the parent compound were seen in the spectrum after metalation). The IR spectra in DMSO-d_6 solution were recorded on Bruker Tensor 27 FT spectrometer in a 0.129 mm CaF_2 sample cell at a resolution of 2 cm^{-1} and 64 scans.

The quantum chemical calculations were performed using the Gaussian 09 package [10] of programs. Geometry of the species studied was performed by analytical gradient technique without any symmetry constraint. The results were obtained using the density functional theory (DFT), employing the M05-2X [11] in conjunction with 6-311++G(3df,3dp) basis set. To establish the stability order for the neutral, radical and ionic species in solvent we used the Integral Equation Formalism Polarizable Continuum Model (IEF-PCM) [12] on the same level of theory.

The preferred geometry of the molecule of apocynin, its anion, radical and radical cation was found by constructing and optimizing the most probable conformers with planar and nonplanar geometry and different mutual orientations of the carbonyl, methoxyl and hydroxy group at M05-2X/6-311++G(3df,3dp) level of theory. For every structure, the stationary points found on the molecular potential energy hypersurfaces were characterized using standard analytical harmonic vibrational analysis. The absence of imaginary frequencies, as well as of negative eigenvalues of the second-derivative matrix, confirmed that the stationary points correspond to minima on the potential energy hypersurface.

Dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) of the most stable conformers were calculated according [13].

$$BDE = H(ArO^{\cdot}) + H(H) - H(ArOH)$$
$$IP = H(ArOH^{+}) + H(e^{-}) - H(ArOH)$$
$$PDE = H(ArO^{\cdot}) + H(H^{+}) - H(ArOH^{+})$$
$$PA = H(ArO^{-}) + H(H^{+}) - H(ArOH)$$
$$ETE = H(ArO^{\cdot}) + H(e^{-}) - H(ArO^{-})$$

All reaction enthalpies were calculated for 298 K. The enthalpies of hydrogen atom, *H(*H*)*, proton, *H(*H^{+}*)*, and electron, *H*(e^{-}), for each solvent were obtained by the same method and basis set. Solvation enthalpy of electron was determined as:

$H(e^{-})_{solv} = H(DMSO^{\cdot-})_{solv} - H(DMSO)_{solv} - H(e^{-})_{gas}$, where $H(e^{-})_{gas}$ is 3.145 kJ.mol^{-1} following the procedure already applied in similar studies [14, 15].

Theoretical IR spectra were calculated for the most stable conformers of the neutral and anion species at B3LYP/6-311++G(2df,p) theory level [16, 17].

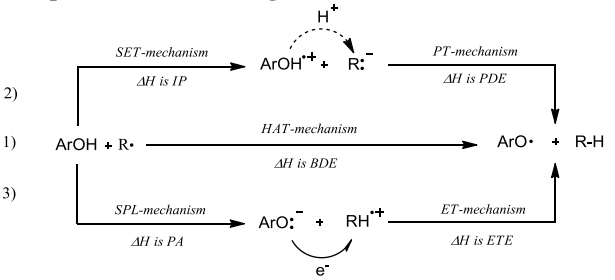
RESULTS AND DISCUSSION

Conformational isomers

Structures of the most probable conformational isomers of apocynin, its anion, radical and radical cation were optimized using density functional theory M05-2X /6-311++G(3df,3dp) in gas phase and different media. Earlier optimization of apocynin in gas phase yielded *s-cis* (with carbonyl group oriented towards the methoxyl group – conformer **1m**) as the most stable form, followed by *s-trans* (with carbonyl group oriented opposite to the methoxyl group – conformer **2m**) [18]. Our optimization in benzene, water and DMSO provided that the same conformers are the most favourable in these solvents as well. The optimized geometries of the conformers are shown in Scheme 1. The most stable form of the neutral molecule is stabilized by the formation of an intramolecular hydrogen bond with the participation of H-atom from the hydroxyl group and the O-atom from the methoxyl group. The most unfavourable conformations are those with methoxyl groups out of the plane of phenyl ring. All conformations of the radical, radical cation and oxyanion of apocynin show planar structure with *s-cis* form being more favourable for the oxyanion, while *s-trans* form – for the radical and radical cation (Scheme 1).

Radical scavenging properties of apocynin

Three possible mechanisms – hydrogen atom transfer (HAT), single-electron transfer (SET-PT), sequential proton loss electron transfer (SPLET) were studied by M05-2X/6-311++G(3df,3dp) computations according to Scheme 2.



Scheme 2. Possible mechanisms of apocynin reaction with free radicals.

The ability of apocynin to form radical, radical cation and oxyanion as well as the transformation of its radical cation and oxyanion into radical, were estimated in polar and nonpolar medium. The corresponding bond dissociation enthalpies (BDE), ionization potentials (IP), proton dissociation enthalpies (PDE), proton affinities (PA), and electron transfer enthalpies (ETE) of apocynin are collected in Table 1. These enthalpies were analysed in order to estimate whether the apocynin is prone to react via HAT, SET-PT or SPL-ET in polar and nonpolar medium.

In gas phase and benzene, *i.e.* the nonpolar (lipid) phase, the apocynin would preferably inactivate free radicals by directly transferring a hydrogen atom to free radicals and forming an apocynin radical. The energy needed to transfer an electron or a proton to free radicals is much higher and it can be concluded that HAT would be the

Table 1. DFT bond dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) values of apocynin and vanillin in kJ/mol.

compound	BDE	IP	PDE	PA	ETE	miLogP
			Gas Phase			
apocynin	376.9	789.3	901.6	1420.7	270.2	1.18
vanilin	378.9	806.3	886.7	1409.4	283.6	1.07
			Benzene (2.28)[a]			
apocynin	372.4	673.6	129.2	432.2	360.6	
vanilin	375.1	682.2	123.3	442.2	373.3	
			Water (80.10)[a]			
apocynin	365.8	508.9	71.7	218.3	362.4	
vanilin	369.0	517.1	66.8	211.3	372.6	

Table 2. DFT reaction enthalpies (in kJ/mol) of apocynin and vanillin with various free radicals according to Scheme 2.

Compound	Radical	ΔH_{BDE}	ΔH_{IP}	ΔH_{PDE}	ΔH_{PA}	ΔH_{ETE}
			Gas phase			
	OH	-116.3	-434.9	308.1	-148.3	107.0
	OCH_3	-58.2	-255.1	198.8	-183.0	126.6
	OOH	20.9	-240.6	261.5	-158.5	179.4
apocynin	$OOCH_3$	28.4	-156.0	184.4	-141.1	169.5
	L	36.0	-50.1	86.1	-196.7	232.7
	OL	-60.4	-59.0	-1.4	-141.0	80.6
	OOL	-3.9	-72.4	101.6	-115.3	144.4
	OH	-114.3	-417.9	293.1	-234.9	120.6
	OCH_3	-56.4	-238.1	183.8	-194.3	140.0
	OOH	23.0	-223.6	246.6	-169.8	192.8
vanilin	$OOCH_3$	30.5	-139.0	169.5	-152.4	182.9
	L	38.0	-33.1	71.1	-208.2	246.2
	OL	-58.3	-42.0	-16.3	-152.5	94.2
	OOL	-2.1	-55.5	86.6	-126.8	158.0
			Benzene			
	OH	-123.5	-352.8	229.3	-148.3	24.8
	OCH_3	-62.1	-202.4	140.3	-135.9	73.8
	OOH	14.7	-177.9	192.6	-99.5	114.2
apocynin	$OOCH_3$	16.6	-116.0	132.7	-259.3	275.9
	L	31.9	-35.4	67.3	-191.4	223.3
	OL	-64.6	-54.4	-10.2	-120.9	56.2
	OOL	23.6	-67.1	90.7	-93.8	117.4
	OH	-120.8	-344.2	223.4	-158.3	37.5
	OCH_3	-59.3	-193.8	134.5	-145.9	86.5
	OOH	17.4	-169.2	186.7	-109.5	126.9
vanilin	$OOCH_3$	19.4	-107.4	126.8	-269.3	288.7
	L	34.6	-26.8	61.4	-201.4	236.0
	OL	-61.9	-45.8	-16.1	-130.8	68.9
	OOL	26.3	-58.5	84.8	-103.8	130.1
			Water			
	OH	-133.5	-292.8	148.9	-102.0	-33.1
	OCH_3	-71.3	-165.0	139.9	-103.6	32.3
	OOH	5.8	-134.1	93.7	-61.7	67.5
apocynin	$OOCH_3$	13.9	-136.2	150.0	-60.6	74.4
	L	24.7	-23.7	46.7	-188.6	211.7
	OL	-72.9	-40.8	-32.0	-98.6	25.7
	OOL	17.3	-64.1	81.4	-68.8	86.1
	OH	-130.3	-284.7	144.0	-109.0	-22.9
	OCH_3	-68.1	-156.9	135.0	-110.5	42.5
	OOH	9.0	-126.0	88.8	-68.7	77.7
vanilin	$OOCH_3$	17.1	-128.0	145.1	-67.6	84.7
	L	27.9	-15.5	41.8	-195.6	222.0
	OL	-69.6	-32.7	-36.9	-105.5	35.9
	OOL	20.6	-56.0	76.5	-75.8	96.3

only mechanism of radical scavenging exerted in lipid phase. Regarding the relative activity for apocynin compared to vanillin, based on the similar BDE values the two compounds are predicted to show similar activity via HAT. The IP calculated in water is higher than the water BDE of apocynin, but significantly lower than the IP calculated in benzene due to the favourable solvation of the charged species involved in the reactions. The ability of apocynin to donate an electron is found to be greater than those of vanillin in agreement with the reported oxidation potentials determined by electrochemical measurements [9].

According to the calculations, in water the proton affinity of apocynin becomes considerably lower than its BDE and IP values which favours the deprotonation and transfer of the proton to free radical (first step of SPLET mechanism). The second step of the SPLET requires subsequent transfer of an electron from apocynin to the cation produced from the free radical. This process is described by ETE and the calculated value shows that this step too can be achieved more easily than HAT. Deprotonation of apocynin is predicted as slightly more difficult in comparison to vanillin.

The oxidation processes in biological systems involve various free radicals. Among the ROS, hydroxyl radicals, ·OH, are the most reactive and show little selectivity towards the possible sites of attack [20]. Hydroxyperoxyl radicals, ·OOH, are less reactive, but they can diffuse into remote cellular locations [21] and initiate the lipid peroxidation [22]. The lipid alkoxyl radicals, ·OR, are formed from the reduction of peroxides and are less reactive than ·OH, but significantly more reactive than the lipid peroxyl radicals ·OOR [23, 24]. Different reactivity of free radicals is connected with different scavenging capacity of the antioxidants. The reactivity of apocynin against different free radicals was evaluated by including the free radicals in the reactions (Scheme 1) and calculating the respective reaction enthalpies according to the following equations:

$$\Delta BDE = BDE(ArOH) - BDE(ROH)$$
$$\Delta IP = IP(ArOH) - IP(ROH)$$
$$\Delta PDE = PDE(ArOH) - PDE(ROH)$$
$$\Delta PA = PA(ArOH) - PA(ROH)$$
$$\Delta ETE = ETE(ArOH) - ETE(ROH)$$

The free radicals (R·) included in the study were as follows: ·OH; ·OCH_3; ·OOH; ·$OOCH_3$; ·R; ·OR; and ·OOR, where R = (*Z*)-hex-2-ene-4-yl. The calculation results (Table 2) show that the enthalpies required for each of these reactions are of a very different magnitude, which enable to judge on ability of apocynin to scavenge particular type of free radicals. In nonpolar phase apocynin and vanillin would react exothermically with ·OH and alkoxyl (·OCH_3 and ·OR) radicals via HAT mechanism with resulting negative ΔH_{BDE} value, but not peroxyl (·OOH, ·$OOCH_3$ and ·OOR) and alkyl radicals (·R). Taking into account the negative water ΔH_{PA} and ΔH_{ETE} – negative or positive, but smaller than ΔH_{PA}, it seems that apocynin would readily scavenge ·OH and alkoxyl (·OCH_3 and ·OL) radicals via SPLET mechanism in water. Scavenging of ·OOH by apocynin would only be possible via SET-PT mechanism in water according to the calculated ΔH_{IP}.

Comparable relative activity for apocynin and vanillin, found by the theoretical estimation of BDE values, is in good accordance with the experimental data from crocin bleaching inhibition [9]. Apocynin and vanillin were tested also in oxygen radical absorbance assay (ORAC) which utilizes AAPH-derived hydroperoxyl radical to mimics the lipid peroxyl redicals involved in the lipid peroxidation chain reaction *in vivo* [8]. In this test apocynin and vanillin exhibited comparable activity as well. In a cell-based antioxidant assay - oxidative haemolysis inhibition assay (OxHLIA), where oxidation of erythrocyte membranes is induced by AAPH-derived hydroperoxyl radical, apocynin showed superior activity compared to vanillin [8], but those result was attributed to superior lipophilicity of apocynin and higher resulting access to lipophilic biomembrane of erythrocytes in OxHLIA [8].

Since the SPLET mechanism, preferred in water, involves deprotonation product of apocynin, it was worthwhile to generate the corresponding oxyanion of apocynin and characterize it in more details. Therefore, the apocynin was converted into oxyanion and the spectral, structural, and electronic changes resulting from the conversion were followed by experimental IR methods and DFT computations.

IR spectra of apocynin and its anion

The IR spectra of apocynin in DMSO-d_6 and its oxyanion are shown in Fig. 1. Numerical values of experimental vibrational frequencies and band intensities in region 1800–1100 cm^{-1} are compared with the theoretical ones in Table 3. For a better corresponding between experimental and calculated

Table 3. Theoretical and experimental vibrational frequencies and IR integrated intensities of apocynin and its oxyanion.

No	Theoretical data			Experimental data[a]			
				DMSO-d_6		$CDCl_3$	
	$\nu_{theor.}$[b]	A[c]	Approximate description[d]	ν_{exp}	A[e]	ν_{exp}	A[e]
			Apocynin molecule				
1.	1638	378.1	ν(C=O)	1668	s	1674	s
2.	1581	32.6	ν_{Ph}(C=C)	1599	sh	1608	m
3.	1570	389.1	ν_{Ph}(C=C)	1590	s	1596	m
4.	1489	238.3	ν_{Ph}(C=C)	1520	m	1519	m
5.	1449	85.8	$\delta^{as}(CH_3)$	1466	w	1465	m
6.	1438	14.0	$\delta^{as}(CH_3)$	} 1455	vw	1455	w
7.	1438	7.9	$\delta^{s}(CH_3)$				
8.	1422	14.6	$\delta^{as}(CH_3)$	-[f]	-[f]	-[f]	-[f]
9.	1417	25.1	$\delta^{as}(CH_3)$	-[f]	-[f]	-[f]	-[f]
10.	1410	189.2	ν_{Ph}(C=C)	1424	m	1429	m
11.	1367	86.1	δ(C-OH)	1397	w	1389	w
12.	1341	56.3	$\delta^{s}(CH_3)$	1360	w	1358	w
13.	1277	2.3	δ_{Ph}(CH)	1291	sh	-[f]	-[f]
14.	1253	813.9	ν(C-CO), ν(C-OCH_3)	1281	vs	1283	vs
15.	1234	48.7	ν(C-OH), δ_{Ph}(CH)	1263	sh	1261	w
16.	1197	169.5	ν(C-OCH_3), δ_{Ph}(CH)	1228	m	1218	m
17.	1178	454.7	δ_{Ph}(CH), δ(C-OH)	1177	w	1172	w
18.	1157	29.2	δ_{Ph}(CH), δ(C-OH)	1138	w	1138	w
19.	1136	1.2	γ(CH_3)	-[f]	-[f]	-[f]	-[f]
20.	1114	78.8	δ_{Ph}(CH)	1128	w	1119	w
21.	1054	56.0	δ_{Ph}(CH)	-[f]	-[f]	1071	w
22.	1015	2.6	γ(CH_3)	-[f]	-[f]	1032	w
			Apocynin oxyanion				
23.	1584	57.3	ν(C=O)	1628	m		
11	1537	594.2	ν_{Ph}(C=C)	1573	s		
12.	1494	23.5	ν_{Ph}(C=C)	1528	sh		
13.	1479	1812.0	ν(C-O^-)	1517	s		
14.	1450	100.7	$\delta^{as}(CH_3)$	1466	w		
15.	1435	10.5	$\delta^{as}(CH_3)$	1455	w		
16.	1433	81.1	$\delta^{s}(CH_3)$	1455	w		
17.	1424	12.4	$\delta^{as}(CH_3)$	1434	w		
18.	1421	14.2	$\delta^{s}(CH_3)$	-[f]	-[f]		
19.	1415	145.5	$\delta^{as}(CH_3)$	-[f]	-[f]		
20.	1364	877.0	δ_{Ph}(CH)	1376	s		
21.	1334	41.1	$\delta^{s}(CH_3)$	-[f]	-[f]		
22	1284	3.2	δ_{Ph}(CH)	1310	w		
23.	1265	1069.8	ν(C-CO), δ_{Ph}(CH)	1288	m		
24.	1200	201.9	δ_{Ph}(CH), ν(C-CO)	1233	m		
25.	1186	430.0	ν(C-OCH_3), γ(CH_3)	1213	m		
26.	1152	145.4	γ(CH_3), ν(C-OCH_3)	1177	m		
27.	1134	1.6	γ(CH_3)	1130	w		
28.	1111	259.0	δ_{Ph}(CH)	1113	w		
29.	1048	138.2	γ(CH_3)	-[f]	-[f]		
30[g].	1011		ν(O-CH_3)	-[f]	-[f]		

[a]Measured after having decomposed the complex bands into components. [b]Scaled infrared frequencies [cm^{-1}]. [c]Predicted intensities [$km.mol^{-1}$] [d]Vibrational modes: ν, stretching; δ, in-plane bending; γ, out of plane banding; superscripts: s – symmetrical, as – asymmetrical, Ph - phenyl; [e]Relative intensities: vw, very weak; w, weak; m, moderate; s, strong; vs, very strong; sh, shoulder. [f]These bands were not detected in the IR spectrum.

value the native theoretical IR frequencies of the neutral molecule and anion were scaled according to the following equation:

$$\nu^{sc} = 1.0195 \cdot \nu^{native} - 51.4\ (cm^{-1})$$

obtained from the linear correlation between the experimental and calculated native frequencies of the neutral compound **1**. The correlation coefficient R was 0.9961, standard deviation S. D. = 34.2 cm^{-1}; number of data points n = 16. The mean absolute

deviation $MAD = n^{-1}\sum\left|(\rho\nu_i^{theor.(native)} + b) - \nu_i^{exp.}\right|$ was used as a measure for the deviation between the theoretical and experimental values. After scaling the native theoretical frequencies according to the above-mentioned equation. MAD of 10.9 cm^{-1} was obtained for neutral apocynin and 10.5 cm^{-1} – for the anion. These values are lower than the MADs reported by Velcheva *et al.* [25]. This better result should be explained with the higher theory levels and with solvation model which were used.

The highest frequency vibration in the spectrum of the molecule is the hydroxyl stretching. In solid state the position of the bands is 3303 cm^{-1}. In $CDCl_3$ it appears as moderate sharp band at 3553 cm^{-1}. This frequency is shifted down by 40 cm^{-1}, compared with that of 4-hydroxy-acetophenone in the same solution. These data indicated that the OH group is intramolecular hydrogen bonded *i.e.* conf. **1m** and **2m** are predominant in this solution. The IR spectra measured in DMSO and DMSO-d_6 solutions have different appearance. A multiplet broad band is present between 3400 and 2700 cm^{-1}, due to the formation of hydrogen bonds of the apocynin mainly with the solvent.

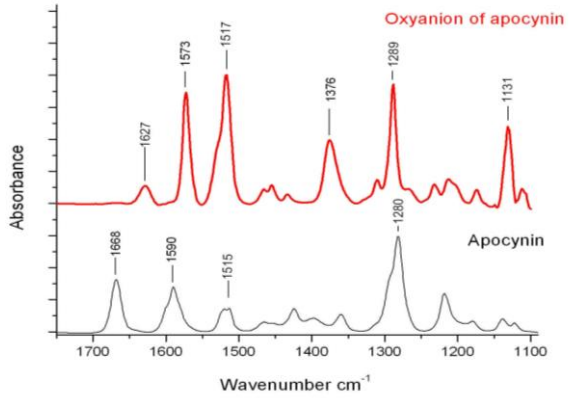


Fig. 1. Infrared spectra of apocynin and of its oxyanion (with counter ion Na^+) in DMSO-d_6.

The strongest band in the IR spectrum of apocynin in DMSO/DMSO-d_6 at 1281 cm^{-1} corresponds to ν(C-CO). The band for the carbonyl stretch is found at 1668 cm^{-1} with strong intensity. The C-O vibrations (No 14-18) are greatly mixed with δ_{Ph}(CH), including the one associated with the C-OH bond at 1263 cm^{-1}. The bands characterizing the benzene ring appear at their typical positions.

The conversion of apocynin molecule into the oxyanion causes:

- a strong decrease in the *ν*(C=O) frequency: predicted 54 cm^{-1}, and observed 40 cm^{-1} (Table 3 and Fig. 1);
- a strong decrease in integral intensity $A_{C=O}$ of the ν(C=O) band. This result is not typical, as the intensity of carbonyl band usually increases by the conversion of carbonyl compounds into anions. The result is, however not surprising, as it was previously observed in other acetophenones containing electron-releasing substituents in the phenyl ring [26];
- essential intensities increases of the aromatic skeletal bands ν_{Ph}(C=C) similarly to [25, 26];
- a very strong frequency increase of the phenoxyl vibration: predicted 245 cm^{-1}, and observed 256 cm^{-1}.

CONCLUSION

DFT calculations in gas phase, benzene and water were used to study the reactivity of apocynin against different free radicals via hydrogen atom transfer, single-electron transfer, sequential proton loss electron transfer mechanisms. It was found that in nonpolar medium the preferred mechanism of the antioxidant action is direct hydrogen atom transfer, while in polar medium – SPLET related to the formation of an oxyanion. Therefore the oxyanion of apocynin was generated in DMSO-d_6 solution and characterized in more details. The spectral, structural, and electronic changes resulting from the conversion were followed by experimental IR methods and DFT computations.

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ТЕОРЕТИЧНО ИЗСЛЕДВАНЕ НА РАДИКАЛ-УЛАВЯЩАТА СПОСОБНОСТ НА АПОЦИНИН СПРЯМО РАЗЛИЧНИ СВОБОДНИ РАДИКАЛИ

Д. Я. Янчева[1]*, С. С. Стоянов[1], Е. А. Велчева[1], Б. А. Стамболийска[1], А. Шмелцерович[2]

[1] Институт по органична химия с Център по фитохимия, Българска академия на науките, ул. „Акад. Г. Бончев“, бл. 9, 1113 София, България

[2] Катедра по химия, Медицински факултет, Университет в Ниш, бул. „Д-р Зоран Джинджич“ 81, 18000 Ниш, Сърбия

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(Резюме)

Апоцининът е добре известен инхибитор на ензима никотинамид-аденин-динуклеотид-фосфат оксидаза и се използва като едно от най-обещаващите лекарствени средства в експериментални модели на сърдечно-съдови, възпалителни и невродегенеративни патологични състояния, където регулацията на реактивни кислородни частици играе решаваща роля. Различни вероятни механизми на радикал-улавящото действие на апоцинина като директен пренос на водород (HAT), пренос на единичен електрон (SET-PT) и пренос на протон последван от електронен пренос (SPLET) бяха изследвани чрез пресмятане на енталпиите на съответните реакции с използване на теория на плътностния функционал (DFT) в полярни и неполярни разтворители. Реактивоспособността спрямо различни свободни радикали беше оценена чрез анализ на термодинамичните данни за реакциите на апоцинина с хидроксилни, хидроксипероксидни, алкоксилни и алкоксипероксидни радикали. Въз основа на изчисленията, беше установено, че SPLET механизмът е предпочетен в полярни разтворители, благоприятстващи йонизацията, например вода. В тази връзка оксианионът на апоцинина беше получен в разтвор на диметилсулфоксид и спектралните и структурни промени, породени от превръщането, бяха проследени с ИЧ спектроскопия и DFT пресмятания.