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The relationship between molecular structure and biological activity of alkali metal salts of vanillic acid: Spectroscopic, theoretical and microbiological studies

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ABSTRACT

In this paper we investigate the relationship between molecular structure of alkali metal vanillate molecules and their antimicrobial activity. To this end FT-IR, FT-Raman, UV absorption and ¹H, ¹³C NMR spectra for lithium, sodium, potassium, rubidium and caesium vanillates in solid state were registered, assigned and analyzed. Microbial activity of studied compounds was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis* and *Candida albicans*. In order to evaluate the dependence between chemical structure and biological activity of alkali metal vanillates the statistical analysis was performed for selected wavenumbers from FT-IR spectra and parameters describing microbial activity of vanillates. The geometrical structures of the compounds studied were optimized and the structural characteristics were determined by density functional theory (DFT) using at B3LYP method with 6-311++G^{**} as basis set. The obtained statistical equations show the existence of correlation between molecular structure of vanillates and their biological properties.

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1. Introduction

Biological activity of benzoic acid derivatives depends on their molecular and electronic structure. Such dependencies were reported in our previous works [1–3]. Knowledge of such correlations allows to replace trial and error method with a systematic approach in the search for new antimicrobial substances.

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a benzoic acid derivative used as a flavoring agent. It is an oxidized form of vanillin. It is also an intermediate in the production of vanillin from ferulic acid. The highest amount of vanillic acid in plants known so far is found in the root of *Angelica sinensis*, usually referred to as "dong quai", "dang gui" or "female ginseng", a herb indigenous to China, which is used in traditional Chinese medicine.

The antimicrobial effects of vanillin and vanillic acid were verified against several species and strains of *Listeria monocytogenes*, *Listeria innocua*, *Listeria grayi*, and *Listeria seeligeri* in a laboratory medium adjusted to pH values ranging from 5.0 to 8.0. Medium pH had little influence on the MIC of vanillin as determined by a broth dilution assay, and growth of all test strains was inhibited by concentrations ranging from 23 to 33 mM. Bactericidal effects increased with pH in media supplemented with vanillin. An inverse relationship was found for vanillic acid, and the lethality of the compound increased with declining pH. Mixtures of vanillin and vanillic

* Corresponding author. E-mail address: w-lewando@wp.pl (W. Lewandowski). acid exhibited additive inhibitory effects, particularly at lower pH. These natural antimicrobial compounds could prove useful either alone or in mixtures for the control of *Listeria* spp. in food products [4].

The aim of this paper is to study: (1) the molecular structure of alkali metal salt of vanillic acid (one of naturally occurring methoxy-derivatives of hydroxybenzoic acid); (2) the antimicrobial activities of the studied compounds; (3) dependencies between the molecular structure of investigated molecules and their biological activity. In this way it may become possible to find compounds with specific activity not by a process of trial and error, but on the basis of strictly described dependencies between the structure and biological activity of compounds [2]. In the present work many different analytical methods, which complement one another, were used: infrared (FT-IR), Raman (FT-Raman), electronic absorption spectroscopy, nuclear magnetic resonance (¹H and ¹³C NMR) and quantum mechanical calculations carried out with the GAUSSIAN 09 [5]. Optimized geometrical structures of studied compounds were calculated. The theoretical IR and NMR (¹H and ¹³C) spectra were obtained.

2. Experimental

Lithium, sodium, potassium, rubidium and caesium vanillates were prepared by dissolving the powder of vanillic acid in the water solution of the appropriate alkali metal hydroxide in a stoichiometric ratio. The mixed solution was slowly condensed at 70 °C. Then, the remaining solvent was removed by drying at 120 °C. The

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salts obtained were found to be anhydrous as indicated by the lack of bonds characteristic of water of crystallization in the IR spectra.

The IR spectra were recorded with an Equinox 55, BRUKER FT-IR spectrometer within the range of $400-4000 \text{ cm}^{-1}$. Samples in the solid state were measured in KBr matrix pellets which were obtained with hydraulic press under 739 MPa pressure. Raman spectra of solid samples in capillary tubes were recorded in the range of $100-4000 \text{ cm}^{-1}$ with a FT-Raman accessory of the Perkin-Elmer system 2000. The resolution of spectrometer was 1 cm^{-1} . The NMR spectra of DMSO saturated solution were recorded with the NMR AC 200 F, Bruker unit at room temperature. TMS was used as an internal reference. The UV spectra in water solution were recorded on a DR 5000 HACH-LANGE spectrophotometer between 190 and 400 nm. The compounds were studied in aqueous solutions with concentrations 10^{-4} mol/dm^3 .

To calculate optimized geometrical structures the density functional theory (DFT) hybrid method B3LYP with nonlocal correlation provided by Lee–Yang–Parr expression was used. The 6-311++G** basis set was used as the standard basis set available for all atoms in studied molecules. The method has been successfully applied to calculate geometrical parameters, IR spectra, atomic charges for systems similar to these studied in this paper All theoretical calculations were performed using the GAUSSIAN 09 (rev. 11.2) package of programs [5] running on a PC.

Five species of bacteria: Escherichia coli (PCM 2268), B. subtilis (PCM 2021), P. vulgaris (PCM 2269), P. aeruginosa (PCM 2270), S. aureus (PCM 2267) and one species of yeasts Candida albicans (PCM 2566) were used for antimicrobial tests. Bacterial cultures where purchased from Polish Collection of Microorganisms (PCM). Microbiological analysis was carried out according to the literature [6,7]. Solutions of tested compounds were prepared by dissolving 0.2 g of each of them in 9.8 ml of deionized water. The concentration of compounds in the culture broth was 0.1%. 4.75 ml of broth inoculated with bacteria was used and then 0.25 ml solution of tested compound was added. The studied bacteria were inoculated on enriched broth medium and yeast on Sabouraud broth medium and stored in 35°C (for yeast in 25 °C) for 24 h. The growth of tested cells was standardized turbidimetrically by measuring optical density at 600 nm with Hach Lange UV/vis spectrophotometer DR 5000. The microbiological tests for studied compounds were carried out in deionized water medium. The samples were incubated in 35 °C for bacteria and 25 °C for yeast. The number of colonies was directly proportional to optical density, which was estimated similarly after 24 and 48 h incubation. Statistical calculations were performed using Statistica 9.1 program [8].

3. Results and discussion

3.1. Vibrational spectra

The vibrational spectra of vanillic acid and the synthesized lithium, sodium, potassium, rubidium and caesium vanillates were recorded and assigned. The observed Raman and IR bands together with their relative intensities and band assignments of studied metal salts are presented in Table 1 (vanillic acid) and 2 (vanillates), respectively. The symbol " ν " denotes stretching vibrations, " β ": in-plane bending modes, " γ ": out-of-plane bending modes; " φ (CCC)": the aromatic ring out-of-plane bending modes, " α (CCC)": the aromatic ring in-plane bending modes, " δ (CH₃)": the methyl group bending vibrations, " ρ (CH₃)": the methyl group rocking deformations. The bands are numbered along with the notation used by Varsányi [9]. Theoretical calculations by B3LYP method at 6-311++G** level were used to obtain values of wavenumbers and intensities of IR and Raman spectra (Tables 1 and 2). Good

correlation between experimental and theoretical IR spectra was noted. The correlation coefficients for IR spectra are: 0.9975 (acid), 0.9973 (Li), 0.9973 (Na) and 0.9979 (K). Raman and IR experimental and calculated spectra of vanillic acid are presented in Fig. 1.

Characteristic vibration bands of the carboxylic group are present in the spectra of vanillic acid, and are not observed in the spectra of salt. There are very intense, broad stretching bands: ν (C=O): 1682 cm ⁻¹ (IR), 1780 cm⁻¹ (IR_{calc.}), 1642 cm⁻¹ (R); ν (OH) in the range 2957–2571 cm ⁻¹ (IR) and ν C–(OH): 1298 cm⁻¹ (IR), 1357 cm⁻¹ (IR_{calc.}), 1301 cm⁻¹ (R). Deformation in plane vibration bands β (C=O): 766 cm⁻¹ (IR), 766 cm⁻¹ (R) and deformation out of plane vibration bands γ (C=O): 637 cm⁻¹ (IR), 777 cm^{-1} (IR_{calc}), 638 cm^{-1} (R). Moreover, in the spectra of acid the bands of lower intensity are present, originating from deformation vibrations β (OH) located at 1205 cm⁻¹ (IR), 1259 cm⁻¹ $(IR_{calc.})$, 1206 cm⁻¹ (R) and γ (OH) 918 and 588 cm⁻¹ (IR), 580 cm⁻¹ (IR_{calc}) , 919 and 585 cm⁻¹ (R) have also been observed. In the spectrum of the acid are also present bands from the aromatic ring and functional groups of the ring (hydroxyl -OHar and methoxy $-OCH_3$ groups), which are also present in the spectra of salt. Replacement of the carboxylic group hydrogen with a metal ion brought about characteristic changes in the IR and Raman spectra of the metal vanillates in comparison with the spectra of vanillic acid. One can observe appearance of bands of the symmetric and asymmetric vibrations of the carboxylate anion: $v_{as}(COO^{-})$ in the range: $1558-1549 \text{ cm}^{-1}$ (IR), $1554-1535 \text{ cm}^{-1}$ (IR_{calc}), 1566–1546 cm⁻¹ (R), ν_s (COO⁻) in the range: 1395–1385 cm⁻¹ (IR), 1426–1394 cm⁻¹ (IR_{calc.}), 1396–1371 cm⁻¹ (R); $\beta_s(COO^-)$ in the range: 961–957 cm⁻¹ (IR), 835–810 cm⁻¹ (IR_{calc.}), 968–953 cm⁻¹ (R), $\beta_{as}(COO^{-})$ in the range: 488–461 cm⁻¹ (IR), 490–461 cm⁻¹ (R) and $\gamma_{\rm s}(\rm COO^{-})$ in the range: 781–777 cm⁻¹ (IR), in the range $802-786 \text{ cm}^{-1}$ (IR_{calc.}), in the range $785-778 \text{ cm}^{-1}$ (R).

In the spectra of acid and salts there are present bands o from the hydroxyl group attached to the aromatic ring: ν (OH)_{ar} 3485 cm⁻¹ (IR), 3764 cm⁻¹ (IR_{calc}) in the spectrum of vanillic acid and 3455–3443 cm⁻¹ (IR), 3768–3749 cm⁻¹ (IR_{calc}), ν C–(OH)_{ar} 1225–1219 cm⁻¹ (IR), 1301–1270 cm⁻¹ (IR_{calc}), 1226–1217 cm⁻¹ (R); β (OH)_{ar} 1253–1236 cm⁻¹ (IR_{calc}); γ (OH)_{ar} 928 cm⁻¹ (IR), 932–929 and 645–640 cm⁻¹ (R) in the spectra of salts.

Stretching vibrations ν_{as} (CH₃) give the bands in the ranges: 2948–2938 cm⁻¹ (IR), 3147–3133 and 3088–3072 cm⁻¹ (IR_{calc.}), 2944–2939 cm⁻¹ (R) and ν_s (CH₃) in the ranges: 2841–2835 cm⁻¹ (IR), 3023–3012 cm⁻¹ (IR_{calc.}), 2841–2836 cm⁻¹ (R).

The $\delta_{as}(CH_3)$ (in plane bending) bands that occur in the ranges: 1474–1464, 1454–1447 and 1030–1026 cm⁻¹ (IR), 1506–1494, 1492–1481 cm⁻¹ (IR_{calc.}), 1474–1466, 1454–1448 and 1033–1026 cm⁻¹ (R) and $\delta_s(CH_3)$ in the range: 1379–1354 cm⁻¹ (IR), 1484–1478 cm⁻¹ (IR_{calc.}), 1381–1351 cm⁻¹ (R), as well as rocking vibration bands $\rho(CH_3)$ in the range: 1222–1216 and 1171–1168 cm⁻¹ (R_{calc.}). The bands of the ν O–(CH₃) vibrations are located in the range 1186–1182 cm⁻¹ (IR), 1060–1043 cm⁻¹ (IR_{calc.}), 1184–1178 and 1074–1064 cm⁻¹ (R).

3.2. NMR spectra

Theoretical and experimental ¹H and ¹³C NMR spectra of lithium, sodium and potassium vanillates were obtained (Table 3). Geometrical structures of studied compounds were optimized by B3LYP/6-311++G** method. The atom positions were numbered as in Fig. 2. The signals from protons no. 3 and 4 are shifted downfield in comparison to the appropriate signals in the spectrum of ligand. In the ¹H NMR spectra of vanillates no regularity can be observed in the chemical shifts of hydrogen along the series of alkali metal vanillates. Chemical shift values of signals traced for *m*-anisic acid

Wavenumbers [cm⁻¹], intensities and assignments of bands occurring in the experimental FT-IR, FT-Raman and theoretical FT-IR spectra of vanillic acid. The theoretical wavenumbers were calculated in B3LYP/6-311++G** level.

Vanillic acid						Assignment	No. ^a
IR	Calc.				Raman		
Exp.	Theoret. ^b	Int. _{IR}	Theoet. ^c	Int. _{IR}	Exp.		
	3775	98.71	3776	160.94		ν(OH)	
3485 vs ^a	3764	137.80	3760	253.82		$\nu(OH)_{ar}$	
3098 w	3228	2.21	3230	3.01	3083 w	ν(CH)	2
3016 sh	3209	1.50	3208	3.78	3027 vw	ν(CH)	20b
2988 w 2957-2571	3193	2.06	3192	5.02	2991 vw	$\nu(CH)$	20a
2337-2371	31/12	16.08	31/3	22.04		$\nu(OH)$	
	3079	28 78	3080	42.96		$v_{as}(CH_3)$	
	3016	43 58	3016	62 37		$\nu_{as}(CH_{a})$	
1682 vs	1780	443.02	1758	846.98	1642 m	$\nu(C=0)$	
1609 sh	1643	30.91	1638	22.65	1602 vs	v(CC)	8h
1599 vs	1633	140 73	1628	282.97	1002 10	v(CC)	8a
1524 vs	1544	176 54	1520	306.96	1518 w	$\nu(CC)$	19b
1474 m	1505	48 84	1497	74.08	1474 w	$\delta_{22}(CH_2)$	100
1454 vs	1492	10.73	1485	15.75	1454 w	$\delta_{as}(CH_2)$	
	1484	1.44	1486	0.93		$\delta_{c}(CH_{2})$	
1435 vs	1457	13.94	1454	18.99	1425 m	$\nu(CC)$	19a
1379 m					1381 m	$\delta_{\rm s}({\rm CH}_3)$	
1298 vs	1357	208.25	1350	296.77	1301 sh	$\nu C - (OH)$	
1283 vs	1415	37.02	1411	63.80	1286 s	v(CC)	14
1238 s					1238 w	$\nu(CH)$	7a
	1319	31.33	1319	41.36		β (CH)	3
	1301	211.39	1289	429.10		$\nu C - (OH)_{ar}$	
1205 s	1259	93.81	1246	133.94	1206 m	β (OH)	
	1227	89.37	1223	117.92		β (OH) _{ar}	
	1200	128.35	1196	179.92		$\rho(CH_3)$	
	1181	331.12	1167	519.85			
	1170	0.53	1175	0.90		$\rho(CH_3)$	
1186 m					1182 m	$\nu O - (CH_3)$	
1169 sh					1170 m	ν (CH)	13
	1139	81.94	1133	192.75		β (CH)	18a
1113 s	1087	132.13	1069	419.81	1123 vw	β (CH)	180
1020 -	1055	28.96	1051	18.02	1021	$\nu O = (CH_3)$	
1030 s					1031W	δ_{as} (CH ₃)	
918 m	022	F F2	010	15.24	919 m	$\gamma(OH)$	76
820 m	922	J.J.J 10.10	919	13.54	004 V W	$\nu(CH)$	11
820 III 806 m	840	9.54	844	14.55	909 m	γ(CH)	11
766 s	045	0.54	044	14.37	766 w	$\beta(C=0)$	
758 sh	810	26.09	809	57 31	700 W	$\mu(CCC)$	12
700 SH	010	20.05	803	57.51	742 3 725 w	$\alpha(CCC)$	12
637 m	777	63 37	771	93.88	638 m	$\nu(C=0)$	1
588 m	588	23 34	578	10 59	585 w	$\gamma(OH)$	
565 w	730	7.46	735	15.70	573 w	$\varphi(CC)$	16a
540 m	719	17.44	717	29.79	542 w	$\alpha(CCC)$	6a
507 m	-	-			498 w	$\varphi(CC)$	16b
451 w	570	34.21	568	67.49	450 w	$\alpha(CCC)$	6b
417 vw					417 vw	β (CH)	9b
	481	82.23	426	180.98		$\gamma(OH)_{ar}$	

^a Ref. [9].

^b calculations for isolated molecules.

^c calculations for the molecules in the solvent (water).

^d s, strong; m, medium; w, weak; v, very; sh, shoulder.

(4-metoxybenzoic acid) [10]. Lower values of σ protons in vanillic acid relative to the corresponding anisic acid protons were found.

The signals from C3, C4, C5, C6 and C8 carbon atoms in salts are shifted upfield in comparison to the appropriate signals in the spectrum of ligand, whereas the signals from carbons no. 2 and 7 are shifted downfield comparing to free ligand. This indicates an increase in the electron density around C2 and C7 carbons and a decrease in the electron density around the other carbon atoms. The differences between chemical shifts of carbon atoms along metal series are small and irregular. Only the values of chemical shifts of C2 atoms regularly increase in the series: acid \rightarrow Li \rightarrow Na \rightarrow K \rightarrow Rb \rightarrow Cs. The same relationship describes the calculated atomic charges mentioned before.

The linear correlation between calculated and experimental data of 13 C NMR is noted. Correlation coefficients of 13 C NMR for vanillic acid, lithium, sodium and potassium salts amount to 0.9816, 0.9767, 0.9779 and 0.9534, respectively. Corresponding values of correlation coefficient of 1 H NMR are lower.

3.3. UV spectra

The UV spectra of vanillic acid and vanillates were recorded. The UV spectrum of vanillic acid was recorded in water solution (the maxima of $\pi \rightarrow \pi^*$ bands are at 204, 256 and 289 nm) and ethanol (the maxima are at 205, 216, 259 and 289 nm). Replacement of the carboxylic group hydrogen with an alkali metal ion did not cause

Wavenumbers [cm⁻¹], intensities and assignments of bands occurring in the IR and Raman spectra of lithium, sodium, potassium, rubidium and caesium vanillates. The theoretical wavenumbers were calculated in B3LYP/6-311++G^{**} level.

Li vanillate						Na vanilla	ite				
IR					Raman	IR					Raman
Exp.	Theoret. ^a	Int. _{IR}	Theoret. ^b	Int. _{IR}	Exp.	Exp.	Theoret.	Int. _{IR}	Theoret. ^b	Int. _{IR}	Exp.
3445 s	3768	123.23	3745	218.92		3455 m	3773	117.07	3749	212.10	
3088 w	3217	4.19	3218	4.69	3080 w	3072 w	3216	5.09	3217	5.39	3094 w
3017 vw	3209	2.00	3205	5.91		3026 w	3208	2.96	3205	6.45	3028 w
2974 w	3187	4.54	3182	10.81		2976 w	3184	5.99	3180	12.11	2977 w
2948 w	3136	20.87	3149	23.63	2944 w	2938 m	3133	23.40	3148	24.07	2939 w
2011	3075	31.12	3090	44.57	22.44	2027	3072	33.05	3090	45.38	2026
2841 W	3014	47.75	3024	65.15	2841 W	2837 W	3012	50.73	3024	66.59	2836 W
1620 SII	1645	101 23	1628	2.30	1646 W	1500 m	1637	0.56 84 78	1628	2.95	1602 vs
1557 s	1535	101.23	1538	071.83	1566 w	1558 vs	1554	4.78	1547	1007.03	1563 w
1518 s	1535	49 54	1531	129 59	1510 w	1520 m	1538	84 20	1529	158 54	1505 W
1464 m	1505	32 15	1494	47 37	1515 11	1468 w	1506	41.60	1495	61 65	1467 sh
1454 m	1490	9.88	1482	14.55	1453 s	1447 m	1488	9.40	1481	14.65	1448 m
	1484	17.61	1480	7.02			1482	4.38	1479	1.75	
1421 sh	1449	130.91	1440	104.97	1417 w	1433 sh	1449	59.38	1441	75.76	1413 w
1393 vs	1426	536.10	1388	619.18	1396 s	1395 vs	1411	309.68	1375	852.86	1376 vs
1360 sh					1369 m	1389 sh					1360 sh
1281 s	1402	207.50	1401	495.67	1277 m	1283 s	1394	395.87	1396	175.65	1278 s
1250 m					1250 w	1258 m					1254 w
	1310	60.34	1300	80.56			1308	51.83	1300	67.44	
1219 m	1288	161.54	1272	339.50	1217 w	1225 s	1285	166.58	1273	342.77	1226 w
	1253	159.41	1238	306.25			1249	169.41	1236	325.81	
	1222	83.91	1216	107.99			1222	82.20	1217	111.27	
1100	1171	0.50	1169	0.91	1170	1100	1171	0.51	1169	0.97	1107
1186 m					11/8 m 1150 m	1186 m					1187 W
1153 511	1125	25.27	1170	6.26	1150 111	1120 311	1122	25 52	1107	4.60	1126 VW
1112 c	1155	23.57	1120	126.69	1117	1136 W	1133	24.22	1127	4.02	1154 W
11155	1057	44.45	1041	93.63	1117 W	11155	1060	46.21	1042	98.54	1064 vw
1030 s	1057	40.55	1041	55.05	1033 w	1026 s	1000	40.21	1042	50.54	1027 m
961 vw	835	12.10	819	1.10	967 vw	957 w	825	1.25	814	0.23	958 vw
928 w					930 w	928 w					932 m
883 m	938	4.34	929	6.11	881 vw	878 m	933	5.18	925	6.48	881 w
827 sh	911	16.61	904	31.41	832 vw	826 vw	914	16.27	907	30.46	822 vw
793 sh	854	4.12	848	5.59		814 vw	855	3.24	848	3.76	807 vw
779 s	798	54.78	790	113.20	782 m	777 s	802	52.65	792	96.75	778 m
746 vw	798	88.98	786	143.50	742 vw	746 w	793	80.39	784	163.77	743 w
727 w					722 vw	723 w					725 w
					644 m						645 m
565 vw	734	4.00	734	6.64	566 w	567 w	733	4.12	733	8.33	569 w
548 w	672	122.33	651	44.83	546 vw	540 w	658	22.48	649	36.37	542 w
498 W	583	0.78	5//	9.38	501 W	494 W	583	0.85	5/5	1.18	498 VW
486 VW	545	16.27	544	2.40	490 W	488 W	576	10.25	574	20.52	489 W
437 VW	545	10.27	544	2.49	401 W	433 W	570	19.25	574	50.52	417 vw
422 000	472	68 18	462	0.03	414 000	415 VW	469	28 55	463	0.01	417 000
	472	00.10	402	0.05			405	20.55	405	0.01	
K vanillate						Rb van	illate	Cs vanill	ate	Assignment	No.
IR					Raman	IR	Raman	IR	Raman		
Exp.	Theoret.	Int. _{IR}	Theoret. ^b	Int. _{IR}	Exd.	Exd.	Exd.	Exd.	Exp.		
2447 -	2740	210.59	2740	210.50	1	2447 -		2442 a	1	(011)	
3447S	3749	210.58	3749	210.56	2070	34475	2074	3443 \$	2075	$\nu(OH)_{ar}$	2
3001 w	3203	7.02	3210	7.20	3032 w	3022 VW	3019 VIV	3001 w	3004 w	$\nu(CH)$	206
2970 w	3179	12 51	3179	12 51	2974 w	2972 vw	2974 w	2963 w	2971 w	$\nu(CH)$	200 20a
2940 w	3147	24 71	3147	24 73	2939 w	2940 w	2940 w	2940 vw	2943 w	$v_{\rm eff}$	200
201011	3088	46.01	3089	46.02	2000 11	201011	201011	201010	201011	$v_{as}(CH_2)$	
2837 w	3023	67.32	3023	67.32	2836 w	2837 w	2835 w	2837 w	2836 w	$v_{s}(CH_{3})$	
1645 m	1641	4.24	1641	4.25	1646 w	1651 m		1645 m	1642 w	$\nu(CC)$	8b
1597 m	1628	107.18	1628	107.17	1602 vs	1599 m	1603 vw	1599 m	1601 vw	$\nu(CC)$	8a
1551 vs	1554	983.39	1554	983.44	1558 w	1549 s	1549 w	1551 vs	1546 w	$v_{as}(COO)$	
1520 m	1527	175.95	1527	175.93	1525 w	1516 s	1526 w	1518 m	1522 w	ν(CC)	19b
1464 w	1494	63.44	1494	63.43	1466 w	1466 w	1470 w	1465 m	1469 w	$\delta_{as}(CH_3)$	
1449 m	1481	14.50	1481	14.50	1448 m	1450 m	1450 m	1449 w	1448 m	$\delta_{as}(CH_3)$	
	1478	1.00	1478	1.00						$\delta_{\rm s}({\rm CH}_3)$	
1421 sh	1440	65.54	1440	65.54	1418 w	1418 sh	1422 vw	1418 sh		$\nu(CC)$	19a
1385 vs	1367	894.42	1367	894.35	1378 sh	1385 vs	1375 sh	1387 vs	1371 sh	$\nu_{\rm s}({\rm COO})$	
1364 sh					1366 sh	1354 sh	1355 m	1356 sh	1351 m	$\delta_{\rm s}({\rm CH}_3)$	
1283 s	1394	133.68	1394	133.67	1276 s	1284 s	1278 m	1283 s	1275 m	$\nu(CC)$	14
1256 m					1255 vw	1254 m	1250 vw	1254 m	1255 vw	$\nu(CH)$	7a

Table 2 (continued).

K vanillate	K vanillate			Rb vani	Rb vanillate Cs va		ate	Assignment	No.		
IR					Raman	IR	Raman	IR	Raman		
Exp.	Theoret.	Int. _{IR}	Theoret. ^b	Int. _{IR}	Exp.	Exp.	Exp.	Exp.	Exp.		
	1299	72.93	1299	72.91						β (CH)	3
1221 vs	1270	353.10	1270	353.03	1218 w	1219 s	1218 w	1221 s	1217 w	$\nu C - (OH)_{ar}$	
	1236	332.37	1236	332.40						$\beta(OH)_{ar}$	
	1216	102.84	1216	102.82						$\rho(CH_3)$	
	1190	42.44	1190	42.39						$\rho(CH_3)$	
1182 m					1183 w	1184 m	1183 w	1182 m	1184 w	$\nu O - (CH_3)$	
					1151 w		1150 vw		1150 vw	$\nu(CH)$	13
1130 m	1127	23.32	1127	23.31	1131 w	1130 sh	1129 w	1129 sh	1133 vw	B(CH)	18a
1113 s	1117	123.41	1117	123.43	1115 w	1115 s	1115 w	1113 vs	1113 w	β (CH)	18b
	1043	95.47	1043	95.40	1074 vw		1074 vw		1074 vw	$\nu O - (CH_3)$	
1026 s					1026 m	1026 s	1030 w	1026 vs	1026 w	$\delta_{2c}^{-}(CH_2)$	
	810	1.90	810	1.90	959 vw		953 w		968 w	$\beta_{\rm s}(\rm COO)$	
928 w					931 m	928 w	931 m	928 w	929 m	$\nu(OH)_{\rm ar}$	
881 m	922	7 19	922	7 20	886 w	880 m	882 w	881 m	882 vw	$\nu(CH)$	7b
836 vw	906	31.78	906	31.78		827 w	826 w	826 vw	825 vw	ν (CH)	11
810 sh	847	5 63	847	5.63	797 m	027 11	020 11	808 sh	796 m	$\gamma(CH)$	••
781 vs	786	92.69	786	92.67	783 s	777 s	785 s	781 s	782 s	$\gamma_{\rm c}(\rm COO)$	
746 sh	779	179 10	779	176 11	750 w	746 m	739 w	745 w	745 w	$\alpha(CCC)$	12
727 w	110	170110		170111	727 w	727 w	726 w	727 w	729 w	$\alpha(CCC)$	
					641 m		643 m		640 m	$\nu(OH)_{\rm ar}$	•
571 vw	731	7.44	731	7.44	574 w	567 w	574 w	569 w	566 w	$\varphi(CC)$	16a
536 w	647	40.22	647	40.22	538 w	538 w	542 w	538 w	538 w	$\alpha(CCC)$	6a
494 vw	575	1.41	575	11.41	490 w	492 w	498 w	494 w	490 w	$\varphi(CC)$	16b
461 w	574	30.36	574	30.37	465 w	462 w	461 w	461 w	462 w	$\beta_{\rm rec}(\rm COO)$	
438 vw	543	4.68	543	4.67	442 vw	457 w	458 w		456 vw	$\alpha(CCC)$	6b
420 w					417 w	422 w	417 vw	430 vw	428 vw	B(CH)	9b
	417	174.09	417	174.10						$\gamma(OH)_{ar}$	00

^a Calculations for isolated molecules.

 $^{\rm b}\,$ Calculations for the molecules in the solvent (water).

significant changes in the UV spectrum. For vanillates the maxima of $\pi \rightarrow \pi^*$ bands were found to be shifted hipsochromically and located within the range of 202–203, 251–254 and 284–285 nm.

3.4. Structural parameters

The geometrical parameters calculated at the $B3LYP/6-311++G^{**}$ level for vanillic acid and Li, Na and K vanillates

are gathered in Table 4. In acid molecule almost all bonds are of similar length, being longer than the conjugated C–C bond of the benzene ring (approx, 1.390 Å for benzene). The C2–C3 distance is the shortest one and the C3–C4 bond is the longest one in the ring. Alkali metal ions influence mostly the bond lengths and angles in the carboxylic group (which was expected) and they influence the methoxy and hydroxy group geometry.



Fig. 1. Calculated (a) and experimental FT-IR (b), Raman (c) spectra of vanillic acid.

36

he experimental and theoretical chemical shifts of	f protons in the ¹ H NMR and carbons in the ¹³ C NMI	R spectra of vanillic acid and alkali metal vanillates, δ [ppm]
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Atom position ^a	Vanillic a	acid		Vanillate	S									
				Li	Li		Na			К			Rb	Cs
	Exp.	Calc.		Exp.	Calc.		Exp.	Calc.		Exp.	Calc.		Exp.	Exp.
H1	12.47	5.39	6.05	-	-	-	-	-	-	-	-	-	-	-
H2	7.45	7.50	7.72	7.44	7.77	7.77	7.44	7.79	7.77	7.45	7.65	7.73	7.46	7.46
H3a,b	3.80	3.75	3.88	3.75	3.78	3.88	3.74	3.73	3.84	3.74	3.71	3.83	3.75	3.73
H3c	3.80	4.09	4.27	3.75	4.02	4.21	3.74	3.97	4.20	3.74	3.92	4.20	3.75	3.73
H4	9.82	5.76	6.21	9.15	5.55	5.87	9.01	5.37	5.77	-	5.27	5.75	-	-
H5	6.68	7.12	7.20	6.67	6.96	7.09	6.64	6.88	7.01	6.75	6.86	6.97	6.84	6.87
H6	7.33	8.04	7.95	7.33	8.01	7.81	7.31	8.01	7.80	7.32	7.94	7.79	7.36	7.36
C1	123.56	113.79	123.99	122.43	113.93	133.16	122.17	114.69	134.78	131.20	114.20	135.95	129.09	130.05
C2	112.80	124.23	115.41	113.16	130.31	114.30	113.24	132.50	114.57	113.31	135.91	114.35	113.31	113.40
C3	147.29	150.16	151.62	146.34	150.05	151.18	146.12	149.73	151.11	146.30	149.31	151.05	146.67	146.69
C4	151.16	157.57	157.96	148.14	155.87	154.50	147.66	154.58	153.86	148.03	153.69	153.71	149.33	149.28
C5	115.10	117.99	117.47	114.12	116.62	116.10	113.92	116.09	115.81	114.14	116.03	115.74	114.53	114.58
C6	121.69	132.70	131.02	120.86	130.15	127.84	120.86	130.30	127.78	122.28	130.07	127.31	122.60	122.53
C7	167.28	169.41	171.73	168.61	188.75	183.11	169.67	182.29	180.05	169.20	180.02	179.57	169.61	169.99
C8	55.60	55.60	56.26	55.35	55.37	56.57	55.32	55.05	56.35	55.32	55.39	56.17	55.40	55.37

^a Atom numbering in Fig. 2.

In the series of vanillic acid \rightarrow Li \rightarrow Na \rightarrow K vanillates, the C1–C7, C4–O4, O1–M/H1, O2–M, and O3–C8 distances increase, whereas the C7–O2 and C7–O1 (Li \rightarrow K) bonds shorten. Moreover, the C2–C1–C6 and C1–C7–O1 angles decrease, and the C1–C2–C3, as well as C5–C6–C1 angles increase along the series: vanillic acid \rightarrow Li \rightarrow Na \rightarrow K vanillates. The existence of some regular changes in the distances and angles of bonds suggests that other parameters, such as atomic charges of molecules or wavenumbers in the IR or Raman spectra, should also change regularly in the same fashion for vanillates. Nevertheless one should bear in mind that the calculations were performed for isolated molecules, whereas in real systems the intermolecular interactions exist, which affect the electronic systems of compounds.

Correlation between theoretical and experimental [11] obtained bond lengths and angles of vanillic acid molecule was studied and good agreement was found, R=0.9398 for bonds, R=0.8782 for angles.

Geometric aromaticity indices (HOMA, Aj, BAC and I_6 —Bird's index) were calculated and presented in Table 5. Substitution of the carboxylic group hydrogen with the metal atom causes an increase in the aromaticity indices. Aromatics regularly increase in the series: acid \rightarrow Li \rightarrow K. Comparing the aromaticity indices for vanillic acids with those for *m*-anisic and benzoic acids [10] it can be seen that the aromatics of these compounds increase in the series: vanillic acid \rightarrow *m*-anisic acid \rightarrow benzoic acid.

Comparing the values of dipole moment for molecule of vanillic acid and its salts one can observe an increasing tendency: 4.198 D for free acid; 4.799 D for lithium salt; 7.441 D for sodium; for potassium salt 11,458 D. The energy (hartree) shows a decreasing tendency: -610.76; -617.76; -772.52; -1210.20



Fig. 2. Vanillic acid, numbering scheme used in the present work.

(1 hartree = 2625.5 kJ/mol). Obtained results show that the degree of ionic bonding increases from H to Na atom, because of an increase symmetrization of carboxylate ion and in aromaticity of molecule.

Atomic charges. The alkali metal cations affect the electronic density of ligand (Table 6), The substitution of metal ions in the place of the carboxylic group hydrogen causes a decrease in the electronic charge density on the C1 atom and an increase on C2. C3. C4. C5. C6. C8. O3. O4. H2. H4. H5 and H6 atoms compared with the appropriate atomic charges calculated for acid. Electron density around these atoms increases in the series: acid \rightarrow Li \rightarrow Na \rightarrow K. The atomic charges on other atoms change insignificantly, depending on the applied method. Among the three applied methods in atomic charge calculations the best statistical correlations were obtained for NBO and ATP analysis and the changes of carbon and proton signals found in experimental NMR spectra (exemplary correlation for atomic charges and values of chemical shifts from ¹H NMR spectra are presented in Fig. 3). This suggests that NBO and ATP methods describe the electronic charge distribution in vanillate molecules and similar systems in a better way than Mulliken method. Metal substitution in the carboxyl group causes an increase in a total charge of the carboxylic ion and a decrease on the remaining functional group.

Figs. 4–5.

3.5. Microbial activity

Vanillic acid and its salts preparations tested at a concentration of 0.1% showed a varied impact on the growth of tested



Fig. 3. The relation between the obtained Mulliken, ATP and NBO atomic charges and change of proton signals from experimental NMR spectra of vanillic acid.

The bond lengths and angles of the structures of vanillic acid and its alkali metal salts calculated in B3LYP/6-311++G** level (atom numbering in Fig. 2).

$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$		Vanillic acid		Vanillates			
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Calc.	Exp. [11]	Li Calc.	Na Calc.	K Calc.	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Pond longths [Å]						
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	C1-C2	1 407	1 290	1 404	1 402	1 405	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C1 C2	1.407	1,385	1.404	1.405	1.403	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	62-64	1,304	1.381	1.385	1.365	1.300	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C3-C4 C4-C5	1.410	1.397	1.409	1.400	1.400	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	65-66	1.390	1.373	1.369	1.369	1.300	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C5-C6	1.390	1.383	1.392	1.394	1.390	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C1C6 C1C7	1.397	1.391	1.395	1.395	1.390	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	CIC7	1.478	1.4/6	1.490	1.501	1.519	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C7=01	1.210	1.274	1.275	1.270	1.264	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	C7=02	1.364	1.266	1.277	1.272	1.265	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	01—Н1/М	0.968	1.137	1.850	2.203	2./15	
$\begin{array}{ccccccc} C2-H2 & 1.080 & 1.003 & 1.081 & 1.081 & 1.081 & 1.081 \\ C3-03 & 1.372 & 1.366 & 1.375 & 1.378 & 1.371 \\ 03-C8 & 1.425 & 1.426 & 1.423 & 1.422 & 1.430 \\ C8-H3a & 1.094 & 0.975 & 1.095 & 1.095 & 1.093 \\ C8-H3b & 1.094 & 1.012 & 1.095 & 1.095 & 1.093 \\ C8-H3c & 1.088 & 0.954 & 1.089 & 1.089 & 1.089 \\ C4-04 & 1.355 & 1.364 & 1.360 & 1.363 & 1.366 \\ 04-H4 & 0.968 & 0.838 & 0.967 & 0.967 & 0.969 \\ C5-H5 & 1.083 & 0.995 & 1.083 & 1.084 & 1.084 \\ C6-H6 & 1.082 & 1.027 & 1.082 & 1.082 & 1.082 \\ Angles [·] & & & & & & & & & & & & & & & & & & &$	02—M			1.853	2.207	2.717	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C2—H2	1.080	1.003	1.081	1.081	1.081	
03-C8 1.425 1.426 1.423 1.422 1.430 C8-H3a 1.094 1.012 1.095 1.095 1.093 C8-H3b 1.088 0.954 1.089 1.089 1.089 C4-O4 1.355 1.364 1.360 1.363 1.366 O4-H4 0.968 0.838 0.967 0.967 0.969 C5-H5 1.083 0.995 1.083 1.084 1.084 C6-H6 1.082 1.027 1.082 1.082 1.082 C1-C2-C3 119.58 119.23 119.75 119.92 120.34 C2-C3-C4 120.25 120.07 120.20 120.27 120.03 C3-C4-C5 119.95 120.61 119.90 119.76 119.84 C4-C5-C6 119.91 119.78 119.91 119.83 119.82 C1-C7-01 125.50 119.14 119.85 118.52 117.75 C1-C7-02 113.08 118.68 119.64 <td< td=""><td>C3-03</td><td>1.372</td><td>1.366</td><td>1.375</td><td>1.378</td><td>1.371</td></td<>	C3-03	1.372	1.366	1.375	1.378	1.371	
C8-H3a1.0940.9751.0951.0951.095C8-H3b1.0880.9541.0891.0891.089C4-041.3551.3641.3601.3631.366C4-041.3551.3641.3601.3631.086C4-041.3551.3641.0891.0891.089C5-H51.0830.9951.0831.0841.084C6-H61.0821.0271.0821.0821.082Angles [']11.925120.07120.27120.03C3-C4-C5119.95120.61119.90119.76119.84C4-C5-C6119.91119.78119.91119.93119.84C4-C5-C6119.91119.78119.91119.93119.84C4-C7-O2113.08118.66118.89119.59119.10C1-C7-O2121.30119.14119.85118.52117.75O1-C7-O2121.33122.18120.51123.06124.52C7-O1-H1/M106.36114.9383.1088.1593.47C7-O2-M82.8887.9093.34C1-C2-H2119.31119.84118.20117.96118.42C2-C3-C3126.10125.92126.08125.96126.14C3-O3126.10125.92126.08125.96126.14C3-O3126.10125.92126.08125.96126.14C3-O3118.52117.35118.24118.15118.55 <td>03—C8</td> <td>1.425</td> <td>1.426</td> <td>1.423</td> <td>1.422</td> <td>1.430</td>	03—C8	1.425	1.426	1.423	1.422	1.430	
C8+H3b 1.094 1.012 1.095 1.095 1.095 C8+H3c 1.088 0.954 1.089 1.089 1.089 C4-O4 1.355 1.364 1.360 1.363 1.366 O4+H4 0.968 0.838 0.967 0.967 0.967 C5-H5 1.082 1.027 1.082 1.082 1.082 Angles [*] 1.027 1.082 1.082 1.082 C2-C3-C4 120.25 120.07 120.20 120.27 120.34 C2-C3-C4 120.25 120.07 120.20 120.27 120.33 C3-C4-C5 119.95 120.61 119.90 119.76 119.88 C5-C6 119.91 119.33 119.83 118.52 117.75 C1-C7-O1 125.50 119.14 119.89 119.59 119.10 C1-C7-O2 113.08 118.68 119.64 118.42 117.75 C1-C7-O2 121.43 122.18	C8—H3a	1.094	0.975	1.095	1.095	1.093	
C8-H3c 1.088 0.954 1.089 1.089 1.089 1.089 C4-O4 1.355 1.364 1.360 1.363 1.366 O4-H4 0.968 0.838 0.967 0.967 0.969 C5-H5 1.083 0.995 1.082 1.082 1.082 Angles ['] 1 1.082 1.082 1.082 C1-C2-C3 119.58 119.23 119.75 119.92 120.34 C3-C4-C5 119.95 120.61 119.90 119.76 119.84 C4-C5-C6 119.91 119.78 119.91 119.83 119.84 C4-C5-C6 119.91 119.78 119.93 119.84 120.25 120.06 120.82<	C8—H3b	1.094	1.012	1.095	1.095	1.093	
C4-O4 1.355 1.364 1.360 1.363 1.366 O4-H4 0.968 0.838 0.967 0.967 0.969 C5-H5 1.082 1.027 1.082 1.082 1.082 Angles [*]	C8—H3c	1.088	0.954	1.089	1.089	1.088	
O4-H4 0.968 0.838 0.967 0.967 0.969 C5-H5 1.082 0.995 1.083 1.084 1.082 C6-H6 1.082 0.27 1.082 1.082 1.082 Angles [*] C1-C2-C3 119.58 119.23 119.75 119.92 120.33 C2-C3-C4 120.25 120.07 120.20 120.27 120.33 C3-C4-C5 119.95 120.61 119.90 119.76 119.84 C4-C5-C6 119.91 119.78 119.91 119.93 119.83 C2-C1-C6 119.96 120.16 119.89 119.59 119.10 C1-C7-01 125.50 119.14 119.85 118.52 117.75 O1-C7-02 113.08 118.68 119.64 118.42 117.73 O1-C7-02 121.43 122.18 120.51 123.06 124.52 C7-O1-H1/M 106.36 114.93 83.10 88.15	C4—04	1.355	1.364	1.360	1.363	1.366	
C5-H5 1.083 0.995 1.083 1.084 1.084 C6-H6 1.082 1.082 1.082 1.082 Angles [*]	04—H4	0.968	0.838	0.967	0.967	0.969	
$G-H6$ 1.0821.0271.0821.0821.082Angles [^]	C5—H5	1.083	0.995	1.083	1.084	1.084	
Angles [*]119.23119.75119.92120.3 $C1-C2-C3$ 120.25120.07120.20120.27120.03 $C3-C4-C5$ 119.95120.61119.90119.76119.84 $C4-C5-C6$ 119.91119.78119.91119.93119.88 $C5-C6-C1$ 120.36120.14120.43120.53120.82 $C2-C1-C6$ 119.96120.16119.89119.59119.10 $C1-C7-01$ 125.50119.14119.85118.52117.75 $C1-C7-02$ 113.08118.68119.64118.42117.73 $01-C7-02$ 121.43122.18120.51123.06124.52 $C7-01-H1/M$ 106.36114.9383.1088.1593.47 $C7-02-M$ 82.8887.9093.34 $C1-C2-H2$ 119.31119.84118.20117.96118.42 $C2-C3-O3$ 126.10125.92126.08125.96126.14 $C3-O3-C8$ 118.52117.35118.24118.15118.55 $O3-C8-H3a$ 110.98110.07111.04111.0910.87 $O3-C8-H3a$ 105.97105.10106.03106.06105.89 $O3-C4-O4$ 120.05119.79120.02120.05119.98 $C4-O4-H4$ 108.03108.23107.73107.53107.88 $C4-C5-H5$ 118.66118.24118.61118.60118.92 $C4-C6-H6$ 120.71119.95120.79120.80120.20 <td>C6—H6</td> <td>1.082</td> <td>1.027</td> <td>1.082</td> <td>1.082</td> <td>1.082</td>	C6—H6	1.082	1.027	1.082	1.082	1.082	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Angles [°]						
C2-C3-C4120.25120.07120.20120.27120.03 $C3-C4-C5$ 119.95120.61119.90119.76119.84 $C4-C5-C6$ 119.91119.78119.91119.93119.82 $C5-C6-C1$ 120.36120.14120.43120.53120.82 $C2-C1-C6$ 119.96120.16119.89119.59119.10 $C1-C7-01$ 125.50119.14119.85118.52117.75 $C1-C7-02$ 113.08118.68119.64118.42117.73 $O1-C7-02$ 121.43122.18120.51123.06124.52 $C7-01-H1/M$ 106.36114.9383.1088.1593.47 $C7-02-M$ 82.8887.9093.34 $C1-C2-H2$ 119.31119.84118.20117.96118.42 $C2-C3-O3$ 126.10125.92126.08125.96126.14 $C3-O3-C8$ 118.52117.35118.24118.15118.55 $O3-C8-H3a$ 110.98110.07111.04111.09110.87 $O3-C8-H3a$ 110.98110.07111.04111.09110.87 $O3-C8-H3a$ 105.97105.10106.06105.89105.89 $C3-C4-O4$ 120.05119.79120.02120.05119.98 $C4-O4-H4$ 108.03108.23107.73107.53107.88 $C4-O4-H4$ 108.03108.23107.73107.53107.88 $C4-O6-H5$ 120.71119.95120.79120.80120.2	C1-C2-C3	119.58	119.23	119.75	119.92	120.34	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C2-C3-C4	120.25	120.07	120.20	120.27	120.03	
C4-C5-C6119.91119.78119.91119.93119.88 $C5-C6-C1$ 120.36120.14120.43120.53120.82 $C2-C1-C6$ 119.96120.16119.89119.59119.10 $C1-C7-01$ 125.50119.14119.85118.52117.75 $C1-C7-02$ 113.08118.68119.64118.42117.73 $O1-C7-O2$ 121.43122.18120.51123.06124.52 $C7-O1-H1/M$ 106.36114.9383.1088.1593.47 $C7-O2-M$ 82.8887.9093.34 $C1-C2-H2$ 119.31119.84118.20117.96118.42 $C2-C3-O3$ 126.10125.92126.08125.96126.14 $C3-O3-C8-H3a$ 110.98113.07111.04111.09110.87 $O3-C8-H3a$ 110.98110.07111.04111.09110.87 $O3-C8-H3a$ 105.97105.10106.03106.06105.89 $C3-C4-O4$ 120.05119.79120.02120.05119.98 $C4-C5-H5$ 118.66118.24118.61118.60118.92 $C4-C6-H6$ 120.71119.95120.79120.80120.20	C3-C4-C5	119.95	120.61	119.90	119.76	119.84	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C4-C5-C6	119.91	119.78	119.91	119.93	119.88	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C5-C6-C1	120.36	120.14	120.43	120.53	120.82	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C2-C1-C6	119.96	120.16	119.89	119.59	119.10	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C1-C7-01	125.50	119.14	119.85	118.52	117.75	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C1-C7-02	113.08	118.68	119.64	118.42	117.73	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	01	121.43	122.18	120.51	123.06	124.52	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C7-01-H1/M	106.36	114.93	83.10	88.15	93.47	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	C7-02-M	_	_	82.88	87.90	93.34	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C1-C2-H2	119.31	119.84	118.20	117.96	118.42	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C2-C3-03	126.10	125.92	126.08	125.96	126.14	
03-C8-H3a110.98113.07111.04111.09110.8703-C8-H3b110.98110.07111.04111.09110.8703-C8-H3c105.97105.10106.03106.06105.89C3-C4-O4120.05119.79120.02120.05119.98C4-O4-H4108.03108.23107.73107.53107.88C4-C5-H5118.66118.24118.61118.60118.92C5-C6-H6120.71119.95120.79120.80120.20	C3-03-C8	118.52	117.35	118.24	118.15	118.55	
03-C8-H3b 110.98 110.07 111.04 111.09 110.87 03-C8-H3c 105.97 105.10 106.03 106.06 105.89 C3-C4-O4 120.05 119.79 120.02 120.05 119.98 C4-O4-H4 108.03 108.23 107.73 107.53 107.88 C4-C5-H5 118.66 118.24 118.61 118.60 118.92 C5-C6-H6 120.71 119.95 120.79 120.80 120.20	03—C8—H3a	110.98	113.07	111.04	111.09	110.87	
O3-C8-H3c 105.97 105.10 106.03 106.06 105.89 C3-C4-O4 120.05 119.79 120.02 120.05 119.98 C4-O4-H4 108.03 108.23 107.73 107.53 107.88 C4-C5-H5 118.66 118.24 118.61 118.60 118.92 C5-C6-H6 120.71 119.95 120.79 120.80 120.20	03-C8-H3b	110.98	110.07	111.04	111.09	110.87	
C3-C4-O4 120.05 19.79 120.02 120.05 119.98 C4-O4-H4 108.03 108.23 107.73 107.53 107.88 C4-C5-H5 118.66 118.24 118.61 118.60 118.92 C5-C6-H6 120.71 119.95 120.79 120.80 120.20	03-C8-H3c	105.97	105.10	106.03	106.06	105.89	
C4-O4-H4 108.03 108.23 107.73 107.53 107.88 C4-C5-H5 118.66 118.24 118.61 118.60 118.92 C5-C6-H6 120.71 119.95 120.79 120.80 120.20	C3-C4-04	120.05	119 79	120.02	120.05	119 98	
C4-C5-H5 18.66 18.24 18.61 18.60 18.92 C5-C6-H6 120.71 119.95 120.79 120.80 120.20	C4-04-H4	108.03	108.23	107 73	107 53	107.88	
C5-C6-H6 120.71 119.95 120.79 120.80 120.20	C4-C5-H5	118.66	118 24	118.61	118 60	118 92	
	C5-C6-H6	120.71	119.95	120.79	120.80	120.20	

^a 1 Å = 10^{-10} m.



Fig. 4. The degree of growth inhibition exhibited by studied compounds in relation to *E. coli* (EC), *P. vulgaris* (PV), *P. aeruginosa* (PA), *B. subtilis* (BS), *S. aureus* (SA) and *C. albicans* (CA) evaluated by means of turbidimetry method by measuring optical density of water solutions at 600 nm after 24 h incubation.



Fig. 5. The degree of growth inhibition exhibited by studied compounds in relation to *E. coli* (EC), *P. vulgaris* (PV), *P. aeruginosa* (PA), *B. subtilis* (BS), *S. aureus* (SA) and *C. albicans* (CA) evaluated by means of turbidimetry method by measuring optical density of water solutions at 600 nm after 48 h incubation.

bacteria (E. coli, P. aeruginosa, B. subtilis, S. aureus and P. vulgaris) and the fungus C. albicans, depending on the incubation time 24 and 48 h. In E. coli the highest growth inhibition after 24 h of incubation was induced by lithium vanillate 24.8%, and the least by rubidium vanillate: 8.6%. However, after 48 h incubation, the observed growth inhibition was low, within 0.9-7.0%. In the case of P. vulgaris after 24 h incubation growth inhibition was found only in the case of vanillic acid, sodium and potassium vanillate. Vanillic acid and its salts, in the case of bacteria, P. aeruginosa showed a similar degree of inhibition, both after 24 h and 48 h and it ranged within the 12.1-23%. In case of the bacteria B. subtilis growth inhibition after 24 h of incubation was low-from 0.7% to 8.1%. After 48 h incubation, the strongest effect was obtained for lithium vanillate, up to 34.4%. In the case of *S. aureus* it has been demonstrated only after 48 h incubation, the strongest in the case of lithium vanillate 33.7% and the weakest in the case of rubidium vanillate-15.7%. After 24 h incubation, growth inhibition was not

Table 5

The calculated aromaticity indices for vanillic acid and vanillates lithium, sodium and potassium.

Aromaticity indices	Vanillic acid	Vanillates		
		Li	Na	К
НОМА	0.9592	0.9668	0.9691	0.9649
I_6	92.1141	93.0157	93.4541	93.6131
A_j	0.9897	0.9919	0.9929	0.9933
BAC	0.8551	0.8689	0.8751	0.8785
$\Delta(CC)^a$	0.026	0.024	0.023	0.020

^a Differences between the longest and the shortest bonds in the ring.

observed. For the fungus *C. albicans* growth inhibition after incubating for 24 h was the strongest in the case of sodium vanillate. After 48 h incubation, the stimulation of growth was found at the level of 31–37%.

Table 6

The atomic charge [ea] calculated by B3LYP/6-311++G** method for vanillic acid and lithium, sodium and potassium vanilliates.

	Vanillic acio	d		Vanillates											
Atom				Li			Na			K					
	Mulliken	ATP	NBO	Mulliken	ATP	NBO	Mulliken	ATP	NBO	Mulliken	ATP	NBO			
C1	0.629	-0.360	-0.182	1.958	-0.335	-0.166	2.133	-0.282	-0.159	0.831	-0.326	-0.154			
C2	-0.047	-0.007	-0.236	0.061	-0.033	-0.236	0.073	-0.054	-0.242	0.372	-0.091	-0.247			
C3	-0.541	0.374	0.256	-0.675	0.386	0.251	-0.694	0.395	0.249	-0.493	0.562	0.248			
C4	-0.189	0.611	0.313	-0.188	0.581	0.302	-0.279	0.560	0.295	-0.541	0.726	0.288			
C5	-0.174	-0.147	-0.246	-0.244	-0.135	-0.250	-0.171	-0.122	-0.252	0.007	-0.141	-0.254			
C6	-0.296	0.045	-0.151	-0.602	0.022	-0.161	-0.653	-0.003	-0.169	-0.585	-0.036	-0.176			
C7	0.215	1.461	0.787	-0.351	1.422	0.765	-0.447	1.369	0.765	-0.084	1.910	0.777			
C8	-0.304	0.508	-0.202	-0.327	0.509	-0.200	-0.324	0.511	-0.200	-0.313	0.636	-0.199			
01	-0.301	-0.873	-0.607	-0.346	-1.043	-0.821	-0.478	-1.026	-0.820	-0.539	-1.358	-0.821			
02	-0.207	-0.769	-0.700	-0.340	-1.029	-0.830	-0.468	-1.012	-0.828	-0.549	-1.353	-0.829			
03	-0.263	-0.878	-0.571	-0.264	-0.883	-0.575	-0.265	-0.887	-0.577	-0.277	-1.157	-0.578			
04	-0.242	-0.799	-0.661	-0.251	-0.798	-0.670	-0.257	-0.796	-0.674	-0.300	-1.032	-0.680			
H1/M	0.299	0.302	0.485	0.188	0.828	0.887	0.445	0.854	0.917	1.015	1.029	0.942			
H2	0.211	0.092	0.232	0.206	0.096	0.236	0.213	0.098	0.235	0.195	0.127	0.231			
H3a	0.166	-0.024	0.175	0.165	-0.026	0.174	0.163	-0.028	0.173	0.177	-0.034	0.173			
H3b	0.167	-0.024	0.175	0.165	-0.026	0.174	0.163	-0.028	0.173	0.177	-0.034	0.173			
H3c	0.190	0.008	0.193	0.170	0.002	0.189	0.168	-0.002	0.187	0.195	0.004	0.186			
H4	0.297	0.338	0.488	0.296	0.333	0.486	0.294	0.329	0.485	0.317	0.418	0.483			
H5	0.193	0.060	0.222	0.183	0.050	0.217	0.179	0.044	0.215	0.207	0.057	0.212			
H6	0.193	0.083	0.231	0.200	0.079	0.228	0.204	0.079	0.227	0.187	0.092	0.225			
COOH/M	0.006	0.121	-0.035	-0.849	0.178	0.001	-0.948	0.185	0.034	-0.157	0.228	0.069			
OCH ₃	-0.044	-0.410	-0.230	-0.091	-0.424	-0.238	-0.095	-0.434	-0.244	-0.041	-0.585	-0.245			
OH	0.055	-0.461	-0.173	0.045	-0.465	-0.184	0.037	-0.467	-0.189	0.017	-0.614	-0.197			

^a $e = 1.6021892 \times 10^{-19} C.$

Results of statistical analysis performed for spectral (i.e. wavenumbers of selected bands) and biological data (percent growth inhibition) for vanillic acid and vanillinates alkali metals.

Tested bacteria	Time incubat.	Assignment (correlation coefficients)	MODEL (regression function)	P-Value	R-SQ ADJ. R-SQ	Sign. F
E. coli	24	8b (0.72)	0.260.8b - 0.306.18b	0.122	0.998	7.65×10^{-5}
	10	18b (0.72)		0.191	0.747	0.51 10.6
E. coli	48	18b(0.71)	$0.158 \cdot 18b - 0.174 \cdot \beta_{as}(COO)$	0.015	0.999	8.51×10^{-6}
		$\beta_{\rm as}({\rm COO})(-0.8)$		0.131	0.749	
P. vulgaris	24	19b (-0.77)	-11.832.19b+16.254.18b	0.034	0.964	4.41×10^{-3}
		18b (0.78)		0.033	0.705	
P. vulgaris	48	8b (0.81)	$0.699 \cdot 8b - 0.667 \cdot v_{as}(COO)$	0.046	0.991	$5.60 imes10^{-4}$
		$v_{as}(COO)(-0.81)$		0.061	0.739	
P. aeruginosa	24	8b (0.65)	0.111.8b-0.083.18b	0.198	0.999	1.02×10^{-5}
Ŭ.		18b (0.92)		0.474	0.749	
P. aeruginosa	48	$\delta_{as}(CH_3)(0.76)$	$0.209 \cdot \delta_{as}(CH_3) - 0.181 \cdot \nu C - (OH)_{ar}$	0.079	0.999	1.27×10^{-5}
		ν C —(OH) _{ar} (-0.60)		0.164	0.749	
B. subtilis	24	8a (-0.37)	-0.968 8a + 1.480 18b	0.289	0.999	1.03×10^{-5}
		18b (0.38)		0.264	0.749	
B. subtilis	48	19a (0.63)	0.933·19a – 0.975·14	0.251	0.985	1.24×10^{-3}
		14 (0.49)		0.275	0.731	
S. aureus	24	14 (0.87)	5.597·14 – 6.300·18b	0.723	0.980	1.83×10^{-3}
		18b (0.73)		0.729	0.725	
S. aureus	48	14 (0.80)	-0.194.14+0.290.18b	0.958	0.994	2.72×10^{-4}
		18b (0.87)		0.945	0.743	
C. albicans	24	8a (-0.83)	$0.113.8a - 0.180.\beta_{as}(COO)$	0.010	0.999	$5.21 imes10^{-6}$
		$\beta_{\rm as}({\rm COO})$ (0.76)	• • • •	0.094	0.749	
C. albicans	48	19b (0.95)	$0.126 \cdot 19b - 0.073 \cdot v_{as}(COO)$	0.001	0.999	2.30×10^{-7}
		$\gamma_{\rm s}({\rm COO})(-0.84)$		0.030	0.750	

In the multiple regression analysis the most important problem is the choice of a proper set of two or more predictor variables (highest intensity of the bands). The general rule is based on the need to consider variables which have statistically significant correlation with the predicted variable (percent of growth inhibition) and at the same time they are not statistically connected between themselves. This rule can be obeyed using analysis of correlation coefficients matrix and the elimination of those predictors which coefficients are:

- nonsignificant for connections with predicted variable;
- significant for connections between predictors.

In fact there exist a lot of econometric methods, but quite often the method of trials and computers simulations should be used. If to much predictors are included for a small number of samples it can be wrongly concluded that all predictors are significant In our case analysis of correlation coefficients between predictors for highest intensity of the bands and percent of growth inhibition was performed. In each case we choose two predictors that were the best in the sense of the maximum correlation but not strongly connected between themselves.

Table 7 displays models with two predictors for each for the tested bacteria at 24 and 48 h of incubation separately. Models were verified taking into account:

- 1) Values of coefficients of linear determination (R^2) for models: all are about 99% which means that variability of growth inhibition percent could be explained by the regression models in 99%.
- 2) Analysis of variance with test *F* which says if jointed effect of the two variables on predicted values is statistically significant. Here all the significance levels *F* are small enough that the action of two predictors is strong.
- 3) Verification of significance of regression coefficients with *p*-values. In our models the significance level was 0.1.

Concluding there are models that are positively verified with the above criteria (grey rows in Table 7).

It is important to mention that here only the simplest linear statistical model was presented. Better solutions could be obtained using more samples, additional analyses will be performed in the nearest future.

4. Conclusion

In this work we have studied the relationship between molecular structure and biological activity of vanillic acid and alkali metal vanillates.

The intensities and wavenumbers of the majority of aromatic system bands in IR and Raman spectra increased in the case of salts comparing to the free ligand, although for some of them the intensity and wavenumbers decreased. Calculations showed a good correlation between experimental and theoretical IR spectra. The correlation coefficients for IR spectra are: 0.9975 (acid), 0.9973 (Li), 0.9973 (Na) and 0.9979 (K).

Alkali metal ions influence on the bond lengths and angles in the carboxylic group and they influence the methoxy and hydroxy group geometry.

Good agreement was obtained between the experimental and calculated data of ¹³C NMR is noted, but corresponding values of correlation coefficient of ¹H NMR are lower.

The calculated aromaticity indices (HOMA, A_j , BAC and I_6 —Bird's index) for vanillic acid and vanillates lithium, sodium and potassium increase in series acid \rightarrow Li \rightarrow Na \rightarrow K. Obtained results of total energy of studied molecules decreases along the series: vanillic acid \rightarrow potassium vanillate. The values of dipole moment show increasing tendency in this series. The aromaticity of salts is higher for the acid molecule compared to salts: HOMA, A_j , BAC and I_6 aromaticity indices.

Alkali metal vanillinate showed strong activity against *S. aureus* and *B. subtilis* after 48 h of incubation and *E. coli* after 24 h of incubation. Caesium and rubidium vanillinates show relatively

higher antimicrobial properties than the other tested alkali metal salts.

Satisfactory correlations between molecular structure of vanillates and their biological activity have been revealed. The use of spectral data for structure describing is sufficient to create statistically important regression models that characterize and allow to predict antimicrobial properties of structure similar compounds. High determination coefficients obtained for regression functions show good dependency between parameters used in these equations.

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