

An LFER study of the protolytic equilibria of 4-aryl-2,4-dioxobutanoic acids in aqueous solutions

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Abstract: The protolytic equilibria of 13 4-aryl-2,4-dioxobutanoic acids (ADKs) were spectrophotometrically studied in aqueous solutions in the pH range 1–9 at 25±1 °C and an ionic strength of 0.1 mol l^{−1} (NaCl), with the exception of the 4-OH-derivative which was also potentiometrically studied in the pH range 7–10 at 25±1 °C and an ionic strength of 0.1 mol l^{−1} (NaCl). In solution, the compounds simultaneously exist in one diketo and two enolic forms; therefore, the determined acidity constants (pK_{a1} 1.87–2.29, pK_{a2} 6.63–8.13 and pK_{a3}(4-OH-) 9.52) represent system macro constants. The ¹H-NMR spectrum of the parent compound (4-phenyl-2,4-dioxobutanoic acid) (25 °C, pD 5.0) proved the existence of all tautomeric forms. Using the extended Hammett relation, the determined pK_a values were correlated with literature σ values. The predicted pK_a values were in fair accordance with the experimentally observed ones. Molecular, monoanionic and dianionic forms of the parent compound were optimized by the semi-empirical molecular orbital PM6 method using the implicit water solvation model (COSMO). The obtained geometries were used to explain the quality of the LFER models.

Keywords: acidity constants, 4-aryl-2,4-dioxobutanoic acids (ADK), linear free energy relationship (LFER).

INTRODUCTION

4-Aryl-2,4-dioxobutanoic acid (Ar-C(O)-CH₂-C(O)-COOH) (ADK) derivatives exert widespread biological activities.¹ The targeting of HIV-1 integrase (IN), the enzyme responsible for the integration of viral DNA in the host genome, is among the most important ones.^{2–4} Appropriate structural modifications on the phenyl ring were used to assess the different types of biological activity

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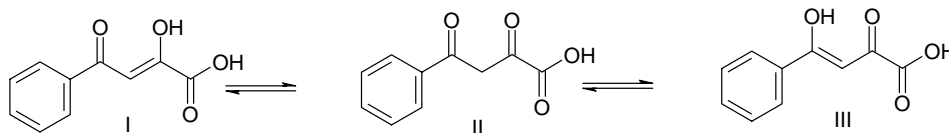
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(manuscript in preparation). Part of current studies on the physicochemical profiling of these compounds is presented in this manuscript, which describes the protolytic equilibria of 13 alkyl-, alkoxy-, hydroxy-, nitro- and halo-phenyl substituted derivatives (limited set) in aqueous solutions in the pH range 1–10 (Table I). The reported compounds (**1–13**) are inactive or exert low activity and are intended to be used to develop a model for a larger set incorporating active congeners. Acidity constants of the studied compounds in aqueous solutions have hitherto not been reported. The only reported pK_a values (pK_{a1} 3.68–4.14 and pK_{a2} 9.52–11.13, substances **1–3**, **7–10** and **13** (Table I)) were potentiometrically determined in an ethanol–water (3:1, v/v) mixture.⁵ As reported by others,^{6–8} these compounds simultaneously exist in two enolic forms (conformationally locked by the pseudo-ring) and one diketo form, having two rotatable bonds responsible for the conformational flexibility (Scheme 1).

TABLE I. Concentration equilibrium constants ($\overline{pK_a \pm \sigma}$) in aqueous solution of ADKs with the wavelengths used for the spectrophotometric determinations ($I = 0.1 \text{ mol l}^{-1}$ (NaCl); $t = 25 \pm 1 \text{ }^\circ\text{C}$)

Comp.	R–	pK_{a1}	λ / nm	pK_{a2}	λ / nm	pK_{a3}
1	H–	2.06±0.03	314.0	7.56±0.02	328.5	–
2	4-Me–	2.22±0.05	319.0	7.99±0.02	329.3	–
3	4-Et–	2.28±0.02	319.9	7.83±0.05	326.8	–
4	4- <i>i</i> -Pr–	2.29±0.05	319.9	7.85±0.02	329.3	–
5	4- <i>tert</i> -Bu–	2.21±0.03	321.6	7.77±0.06	331.9	–
6	3,4-di-Me–	2.09±0.04	321.6	7.92±0.04	326.8	–
7	4-F–	2.06±0.05	314.8	7.50±0.03	325.9	–
8	4-Cl–	2.09±0.04	316.5	7.30±0.03	334.4	–
9	4-Br–	2.06±0.03	318.2	7.53±0.03	333.6	–
10	4-NO ₂ –	1.87±0.06	328.5	6.63±0.02	324.2	–
11	3-OH–	2.18±0.04	314.0	7.45±0.04	332.7	–
12	4-OH–	2.29±0.05	331.9	7.73±0.01 ^a	–	9.54±0.07 ^a
13	4-MeO–	2.28±0.03	316.5	8.13±0.03	326.8	–

^aPotentiometrically determined values



Scheme 1. The diketo (II) and two enolic (I and III) forms of 4-phenyl-2,4-dioxobutanoic acid (**1**).

Aim of this work was to study the protolytic equilibria of a set of 13 ADKs. The determined acidity constants within the studied set are useful to develop a model in which more active and significantly less soluble congeners will be incorporated.

EXPERIMENTAL

Apparatus and reagents

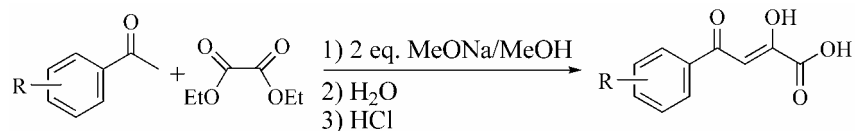
All used chemicals were of analytical reagent grade, purchased from Aldrich, Fluka, or Merck, and were used without further purification. The deuterated compounds had, at least, 99.5 % deuterium.

Melting points were determined in open capillary tubes on a Büchi apparatus and are uncorrected. The infrared spectra (IR) were recorded on an FT Perkin-Elmer 1725X spectrometer, (KBr disc). The ^1H - and ^{13}C -NMR spectra were recorded in $\text{DMSO}-d_6$ on a Varian Gemini 200 spectrometer at 200/50 MHz. Tetramethylsilane (TMS) was used as the internal standard for the ^1H -NMR spectra. The residual solvent signal of $\text{DMSO}-d_6$ was used as the internal standard at 39.70 ppm for ^{13}C -NMR calibration. The mass spectra (ESI-MS) were recorded on a ThermoQuest Navigator.

The ^1H - and ^{13}C -NMR spectra of compound **1** were recorded in deuterated acetate buffer ($c^{\text{tot}} = 0.1 \text{ mol l}^{-1}$, $\text{pD } 5.0$ ($\text{pD} = \text{pH}_{\text{measured}} + 0.4$)^{9,10}) on Bruker Avance spectrometer at 500/125 MHz. The ^1H -NMR spectrum was referenced to the HOD peak at 4.70 ppm as the internal standard; the TSP (3-(trimethylsilyl)-1-propanesulfonic acid- d_6 sodium salt) was not added to avoid interactions between the salt and the sample. The pH measurements in deuterated solutions were performed using a Corning pH-meter 120 with a Corning Ag/AgCl microelectrode (KCl solution). For determination of acidity constants, a GBC Cintra 6 spectrophotometer with 1 cm silica cells and a PHM240 pH-meter (Radiometer) with a combined GK2401B electrode (Radiometer) were used. The titrations were performed with a TTT-60 titrator with an ABU-12 autoburette (Radiometer).

Synthesis of compounds 1–13

Examined compounds (**1–13**) were synthesized using a previously described procedure.¹¹ Compounds were obtained by addition of equimolar amounts of aryl ketones and diethyl oxalate (0.05 mol*) to a twofold molar quantity of sodium methoxide (Scheme 2), obtained by dissolution of sodium in dry methanol (2.3 g (0.1 mol) Na in 40 ml), mixed overnight and poured into ice-cold water.



Scheme 2. Synthesis of the examined compounds, R = **1**) H–; **2**) 4-Me–; **3**) 4-Et–; **4**) 4-*i*-Pr–; **5**) 4-*tert*-Bu–; **6**) 3,4-di-Me–; **7**) 4-F–; **8**) 4-Cl–; **9**) 4-Br–; **10**) 4-NO₂–; **11**) 3-OH–; **12**) 4-OH–; **13**) 4-MeO–.

After 3–4 hours of vigorous stirring at room temperature, the reaction mixture was filtered into water that had been acidified with conc. hydrochloric acid to pH 2–3. The obtained precipitate was collected by filtration and washed with ice-cold water. The filtrates were concentrated under reduced pressure to remove MeOH. Additional amounts of the compounds were harvested from the in this way obtained solutions. The crude products were crystallized from an appropriate solvent. Characterization of synthesized compounds **1–13** was performed by melting points, IR, ^1H - and ^{13}C -NMR spectroscopy, and ESI-MS (Electrospray Ionization Mass Spectrometry).

Characterization of compounds 1–13

The NMR integrals belonging to the diketo tautomers are not reported for the ^1H -NMR spectra. In the “aromatic” region, the overlaps of low intensity peaks of the diketo form with significantly stronger ones from the enol form were observed. The overlap of the enol aromatic peaks (mainly) was also observed. Thus, for some signals, full multiplicities are not reported and, as a conse-

*Compounds **5** and **7** were prepared starting with 0.025 mol of substituted acetophenones and equivalents of other reagents.

quence, some peak integrals (overlapped) have fractional values. The ESI-MS spectra were recorded in the negative mode.

4-Phenyl-2,4-dioxobutanoic acid (1): m.p. 143–144 °C*, decomposition; pale yellow powder (7.90 g, 80 %, AcOEt/PhH); $C_{10}H_8O_4$; M_w 192.17; MS: M^+ 191 (100 %); 119 (60 %). IR ν (cm^{-1}): 1720; 1625; 1276; 1244. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 10.91 (*b*); 8.08 (*d*, $J = 7.02$ Hz, 2H); 7.95 (*d*, $J = 9.54$ Hz); distinct maxima 7.76, 7.72, 7.69 (triplet-like overlapped peaks, $J_{1,2} = 7.20$ Hz, $J_{2,3} = 7.30$ Hz, “1.04 H”); distinct maxima 7.63, 7.59, 7.56 (triplet-like overlapped peaks, $J_{1,2} = 7.58$ Hz, $J_{2,3} = 7.02$ Hz, “2.19 H”); 7.48 (*d*, $J = 7.30$ Hz); 7.15 (*s*, 1H); 4.65 (*s*). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 195.71; 192.04; 190.85; 170.70; 163.58; 161.89; 134.99; 134.22; 129.44; 129.26; 128.85; 128.21; 98.22; 49.46.

4-(4-Methylphenyl)-2,4-dioxobutanoic acid (2): m.p. 141–142 °C, light yellow crystals (6.30 g, 61 %, AcOEt/PhMe); $C_{11}H_{10}O_4$; M_w 206.19; M^+ 205 (100 %) 133 (62 %). IR ν (cm^{-1}): 3520; 1603; 1290; 1248; 1142; 700. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 7.96 (*d*, $J = 8.08$ Hz, 2H); 7.91 (*d*, $J = 8.11$ Hz); 7.38 (*d*, $J = 8.07$ Hz, 2H); 4.57 (*s*); 2.56 (*s*); 2.41 (*s*, 3H). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.61; 170.00; 163.42; 145.03; 132.21; 129.94; 128.21; 97.87; 21.40.

4-(4-Ethylphenyl)-2,4-dioxobutanoic acid (3): m.p. 101–102 °C, light yellow crystals (7.30 g, 66 %, AcOEt/PhMe); $C_{12}H_{12}O_4$; M_w 220.22; M^+ 219 (100 %) 147 (82 %) IR ν (cm^{-1}): 3523; 1700; 1607; 1290; 1247. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 7.84 (*b*, overlapped with aromatic signals); 7.96 (*d*, $J = 8.42$ Hz, 2H); 7.89 (*d*, $J = 8.70$ Hz); 7.40 (*d*, $J = 8.14$ Hz, 2H); 7.25 (*d*, $J = 8.70$ Hz); 7.11 (*s*, 1H); 4.58 (*s*); 2.70 (*q*, $J_{1,3} = 15.16$ Hz, $J_{1,2} = 7.58$ Hz, 2H); 1.22 (*t*, $J = 7.58$ Hz, 3H). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.91; 170.22; 163.65; 151.15; 162.63; 129.08; 128.86; 128.62; 128.44; 98.05; 28.63; 15.28.

*4-(4-*i*-Propylphenyl)-2,4-dioxobutanoic acid (4)*: m.p. 96–97 °C, light yellow crystals (4.1 g, 35 %**, PhMe); $C_{13}H_{14}O_4$; M_w 234.25; M^+ 233 (100 %) 161 (71 %); IR ν (cm^{-1}): 3503; 1702; 1606; 1293; 1249. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.77 (*b*); 8.01 (*d*, 2H, $J = 8.43$ Hz); 7.93 (*d*, $J = 8.14$ Hz); 7.61 (*d*, $J = 7.30$ Hz); 7.44 (*d*, 2H, $J = 8.42$ Hz); 2.99 (*sp*, $J_{1,2} = 6.92$ Hz, $J_{1,3} = 13.84$ Hz, $J_{1,4} = 20.44$ Hz, 1H); 1.23 (*d*, $J = 6.73$ Hz, 6H). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.66; 170.29; 163.55; 155.50; 132.71; 129.03; 128.43; 127.37; 127.10; 97.97; 33.84; 23.66.

*4-(4-*tert*-Butylphenyl)-2,4-dioxobutanoic acid (5)*: m.p. 124–125 °C, pale yellow powder (4.60 g, 75 %, PhMe); $C_{14}H_{16}O_4$; M_w 248.27; M^+ 247 (100 %) 175 (46 %); IR ν (cm^{-1}): 3537.0; 1706.0; 1610.0; 1297.0; 1249.0. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.01 (*d*, $J = 8.71$ Hz, 2H); 7.92 (*d*, $J = 8.42$ Hz); 7.60 (*d*, $J = 8.43$ Hz, 2H); 7.09 (*s*, 1H); 4.55 (*s*); 1.32 (*s*, 9H). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.64; 170.27; 163.53; 157.71; 132.29; 128.75; 128.17; 128.28; 97.98; 35.22; 30.98.

4-(3,4-Dimethylphenyl)-2,4-dioxobutanoic acid (6): m.p. 175–177 °C, decomposition; white powder (6.30 g, 57 %, PhMe); M_w 220.22, $C_{12}H_{12}O_4$; M^+ 219 (100 %); 147 (64.3 %); IR ν (cm^{-1}): 1705; 1612; 1261. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 7.77 (*s*, 1H); 7.71 (*d*, $J = 7.86$ Hz, 1H); 7.28 (*d*, $J = 7.86$ Hz, 1H); 6.64 (*b*); 4.40 (*b*). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 164.42; 142.81; 137.34; 133.28; 130.30; 128.70; 97.93; 19.84; 19.55.

4-(4-Fluorophenyl)-2,4-dioxobutanoic acid (7): m.p. 145–147 °C, decomposition; white needles (crystalline) (3.60 g, 69 %, AcOEt/PhMe); M_w 210.16, $C_{10}H_7FO_4$; M^+ 209 (100 %), 137 (33.1 %); IR ν (cm^{-1}): 3522; 1631; 1601; 1240. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.17 (*dd*, $J = 8.71$ Hz, 2H); 7.41 (*t*, $J = 8.70$ Hz, 2H); 7.11 (*s*, 1H); 4.58 (*s*). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 189.93; 169.69; 168.38; 163.44; 131.72; 131.41; 131.21; 116.74; 116.30; 98.20; 49.33.

* At 143–144 °C the compound turns to a dark red color. This product melts at 155–156 °C. In the literature the latter temperature is reported as the melting point (Ref. 11 from the main text and *Tetrahedron* **60** (2004) 6479).

** Hydrolysis of the Et-ester described in main text is insufficiently efficient for compound **4**.

4-(4-Chlorophenyl)-2,4-dioxobutanoic acid (8): m.p. 164–165 °C, decomposition; white crystals (8.30 g, 73 %, AcOEt/PhMe); M_w 226.61, $C_{10}H_7ClO_4$; M^+ 225 (100 %), 153 (51.7 %); IR ν (cm^{-1}): 3504; 1630; 1591; 1323; 1286; 1241; 1142. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.08 (d , J = 8.70 Hz, 2H); 8.01 (d , J = 8.70 Hz); 7.63 (d , J = 8.69 Hz, 2H); 7.57 (d , J = 8.90 Hz); 7.10 (s , 1H); 4.59 (s). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 189.50; 170.44; 163.61; 163.63; 139.22; 133.68; 131.43; 130.66; 130.02; 129.50; 129.50; 129.28; 129.00; 98.20; 49.37.

4-(4-Bromophenyl)-2,4-dioxobutanoic acid (9): m.p. 163–164 °C, decomposition; white powder (3.95 g, 58 %, PhMe); M_w 271.06, $C_{10}H_7BrO_4$; M^+ 271 (100 %), 269 (82 %), 199 (8.8 %), 197 (8.7 %); IR ν (cm^{-1}): 3534; 1622; 1588; 1291; 1241. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 7.94 (d , J = 8.70 Hz, 2H); 7.72 (d , J = 8.70 Hz, 2H); 7.01 (s); 4.53 (s). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 189.13; 171.05; 163.44; 134.11; 132.43; 130.07; 128.34; 98.18.

4-(4-Nitrophenyl)-2,4-dioxobutanoic acid (10): m.p. 158–160 °C, light orange-red powder (9.50 g, 80 %, AcOH/PhMe); $C_{10}H_7NO_6$; M_w 237.17; M^+ 236 (100 %), 164 (60 %); IR ν (cm^{-1}): 3492; 1707; 1603; 1528; 1349; 1288; 1239. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.40–8.12 (m , “5.7 H”), within distinct strong doublet 8.32 (J = 6.70 Hz). Other observable peaks at: 8.38, 8.37, 8.35, 8.27, 8.26, 8.21, 8.20, 8.19, 8.18, 8.15; 7.125 (s , 1H); 4.67 (s). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 186.86; 172.31; 166.17; 163.26; 152.00; 150.37; 140.10; 130.99; 130.19; 129.48; 124.31; 124.021; 98.88.

4-(3-Hydroxyphenyl)-2,4-dioxobutanoic acid (11): m.p. 175 °C, light dull-orange powder (5.60 g, 54 %, AcOEt); $C_{10}H_8O_5$; M_w 208.17; M^+ 207 (100 %), 135 (24.6 %); IR ν (cm^{-1}): 3390; 1628; 1580; 1283; 1199. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.97 (b); 7.47 (d , J = 7.65 Hz, 1H); 7.39 (s , b , “1.38 H”); 7.33 (doublet-like, J = 7.88 Hz, “0.9 H”); 7.07 (d (peak broadening), J = 8.11 Hz, 1H); 6.99 (s , 1H); 4.48 (s). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 195.93; 191.98; 190.81; 170.27; 163.53; 158.25; 137.54; 136.32; 130.59; 130.33; 121.56; 119.88; 119.14; 114.71; 114.15; 98.22; 49.46.

4-(4-Hydroxyphenyl)-2,4-dioxobutanoic acid (12): m.p. 202–204 °C, dark yellow-orange powder (4.6 g, 44 %, water); $C_{10}H_8O_5$; M_w 208.17; M^+ 207 (100 %), 135 (10.4 %); IR ν (cm^{-1}): 3233; 1723; 1604; 1270; 1226. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 14.10 (b , 1H); 10.74 (b , 1H); 7.99 (d , J = 8.71 Hz, 2H); 7.86 (d , J = 8.71 Hz); 7.05 (s , 1H); 6.93 (d , J = 8.71 Hz, 2H) broadening in peak basis; 4.47 (s). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.79; 168.34; 163.70; 163.62; 131.085; 126.06; 116.19; 97.75.

4-(4-Methoxyphenyl)-2,4-dioxobutanoic acid (13): m.p. 158–159 °C, decomposition; yellow powder (7.80 g, 70 %, AcOEt/PhH); M_w 222.19, $C_{11}H_{10}O_5$; M^+ 221 (100 %), 149 (70.9 %); IR ν (cm^{-1}): 3468; 1688; 1602; 1295; 1261; 1180. 1H -NMR (200 MHz, 29 °C, DMSO- d_6) δ 8.06 (d , J = 8.70 Hz, 2H); 7.11 and 7.07 doublet overlapped with singlet, “3.3 H”; 4.54 (s); 3.89 (s , 3H). ^{13}C -NMR (50 MHz, 29 °C, DMSO- d_6) δ 190.66; 168.94; 164.46; 163.73; 131.25; 130.76; 127.57; 114.79; 114.42; 97.91; 55.96; 49.15.

Determination of acidity constants

The acidity constants were spectrophotometrically determined (except for pK_{a2} and pK_{a3} of compound **12**, which were potentiometrically determined) at $t = 25 \pm 1$ °C and constant ionic strength 0.1 mol l $^{-1}$ (NaCl). Stock solutions of compounds **1–13** were prepared in ethanol ($c = 1.0 \times 10^{-2}$ mol l $^{-1}$, except for **5** where $c = 0.5 \times 10^{-2}$ mol l $^{-1}$ due to its lower solubility). Working solutions ($c = 1.0 \times 10^{-4}$ mol l $^{-1}$ for all but **5**, $c_5 = 0.5 \times 10^{-4}$ mol l $^{-1}$) were prepared in deionized water (the ethanol concentration was up to 1 %, vol) in the pH ranges 1.1–3.5 for pK_{a1} and 5.9–9.1 for pK_{a2} determination. HCl solutions were used for pH 1.1–3.5, phosphate buffers for pH 5.9–8.0 ($c^{tot} = 0.01$ mol l $^{-1}$), carbonate buffers for pH 8.1–9.1 ($c^{tot} = 0.01$ mol l $^{-1}$). The UV–Vis spectra of compounds **1–13** in their monoanionic form (HA $^-$) were recorded in acetate buffer ($c^{tot} = 0.01$ mol l $^{-1}$, pH 5.5*). The

* For 4-NO $_2$ - derivative (**10**) the acetate buffer pH 4.5 was used.

measured pH values were converted to $p\text{c}_\text{H}$ according to the relation:¹² $p\text{c}_\text{H} = -\log [\text{H}_3\text{O}^+] = \text{pH} - A$, where A is the correction factor ($A = 0.08$) determined by potentiometric titration of a standard HCl solution with a standard NaOH solution at 25 ± 1 °C and ionic strength 0.1 mol l^{-1} (NaCl). For solutions with $\text{pH} < 2$, the $p\text{c}_\text{H}$ values were calculated according to the concentration of the standard HCl solution.

The spectra were recorded over the 220–500 nm range at a scanning speed of 500 nm min^{-1} against the corresponding blank. For pK_a determination, the absorbances were measured at the wavelength of the absorption maximum or at the wavelength of the maximal differences in absorbances. Three sets of experiments were performed.

For the potentiometric determination of $pK_{\text{a}2}$ and $pK_{\text{a}3}$ of the compound **12**, 20.00 ml aliquots of the stock solution of **12** ($c_{\text{12}} = 9.9198 \times 10^{-4} \text{ mol l}^{-1}$) in 0.1 mol l^{-1} NaCl were titrated with 0.020 ml increments of the standard NaOH solution ($c = 0.1298 \text{ mol l}^{-1}$) until the pH 10 was attained. The values of $pK_{\text{a}2}$ and $pK_{\text{a}3}$ were found according to the formation function \bar{n} (the mean number of protons bound to the base (**12**)), with the data from three times repeated experiments. The formation function was calculated according to equation:¹³

$$\bar{n} = \frac{3c_{\text{12}} - [\text{H}_3\text{O}^+] - [\text{OH}^-]}{c_{\text{12}}} \quad (1)$$

where c_{12} is the concentration of **12**, $[\text{H}_3\text{O}^+]$ is calculated from $p\text{c}_\text{H}$, and $[\text{OH}^-]$ is the concentration of NaOH in the solution.

LFER calculations

Linear regressions were obtained using the BILIN program.¹⁴ The coefficients following the terms in Eqs. (5–10) are twofold standard deviations. The statistical parameters are reported as follows: n – number of observations; r – correlation coefficient; F – Fischer test, Q^2 – leave one out cross-validation; s_{PRESS} – standard deviation of Q .

Geometry optimization

The reported conformations of the molecular, monoanionic and dianionic forms of **1** (Figs. 5a–5c, respectively) were obtained by the semi-empirical MO PM6 method^{15a} with implicit water solvation (COSMO) (Keywords: EF, PM6, GNORM = 0.01, PRECISE, EPS = 78.4, RSOLV = 1.000, DISEX = 3.000, NSPA = 92) using the MOPAC 2007 package.^{15b} The VegaZZ 2.1.0 was used as the graphical user interface (GUI).¹⁶

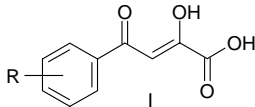
RESULTS AND DISCUSSION

The examined set (compounds **1–13**), comprised mainly of 4-substituted derivatives (**1–5**, **7–10**, **12**, **13**) and alkyl-, halo-, hydroxy-, alkoxy- and nitro-phenyl substituents, can be considered as sufficient for the derivation of a linear free energy relationship. Compounds **6** (3,4-di-Me-) and **11** (3-OH-) were included because the majority of the so far reported biologically active ADK derivatives have alkyl- or oxygen-containing substituents in position 3 of the phenyl ring. In this way, the model could be tested for further expansion. Using the extended Hammett correlation, the determined pK_a values were correlated with literature substituent constants¹⁷ (σ_p and σ_m ; σ_R and σ_I ; Table II).

ADKs in aqueous solution, in the studied pH range, act as diprotic acids, with the exception of compound **12**, which showed dissociation of carboxyl, α -hydroxyl, and 4-OH-phenyl groups. As mentioned before, ADKs in solution under-

go keto-enol tautomerization. The NMR spectra (Fig. 1), recorded in aqueous solution (pD 5.0), proved the existence of the diketo and both enolic forms, thus the acidity constants represent system macro constants.

TABLE II. Structures, experimentally determined pK_a values of the studied compounds (**1–13**) and the used substituent constants

						
Comp.	R–	pK_{a1}	σ_p	σ_m	σ_R	σ_I
1	H–	2.06	0	0	0	0
2	4-Me–	2.22	–0.14	0	–0.10 ⁵⁾	–0.03 ⁶⁾
3	4-Et–	2.28	–0.32* ¹⁾	0	–0.10 ⁵⁾	–0.05 ⁷⁾
4	4- <i>i</i> -Pr–	2.29	–0.28* ²⁾	0	–0.12 ⁵⁾	–0.06 ⁷⁾
5	4- <i>tert</i> -Bu–	2.21	–0.20	0	–0.13	–0.07 ⁸⁾
6	3,4-di-Me–	2.09	–0.14	–0.07	–	–
7	4-F–	2.06	0.21 ³⁾	0	–0.45 ⁹⁾	0.62
8	4-Cl–	2.09	0.23	0	–0.23	0.52
9	4-Br–	2.06	0.23	0	–0.25 ¹⁰⁾	0.50
10	4-NO ₂ –	1.87	0.78	0	0.27	0.64 ¹¹⁾
11	3-OH–	2.18	0	0.02 ⁴⁾	–0.06	0.18
12	4-OH–	2.29	–0.37	0	–0.43 ¹²⁾	0.23 ⁶⁾
13	4-MeO–	2.28	–0.27	0	–0.61	0.27
Comp.	R–	pK_{a2}	σ_p	σ_m	σ_R	σ_I
1	H–	7.56	0	0	0	0
2	4-Me–	7.99	–0.14	0	–0.14	–0.04
3	4-Et–	7.83	–0.13 ⁴⁾	0	–0.10 ⁵⁾	–0.05 ⁷⁾
4	4- <i>i</i> -Pr–	7.85	–0.13 ⁴⁾	0	–0.12 ⁵⁾	–0.06 ⁷⁾
5	4- <i>tert</i> -Bu–	7.72	–0.15 ⁴⁾	0	–0.15 ⁹⁾	–0.07 ⁸⁾
6	3,4-di-Me–	7.92	–0.14	–0.06 ⁴⁾	–	–
7	4-F–	7.50	0.21 ³⁾	0	–0.45 ⁹⁾	0.62
8	4-Cl–	7.30	0.28 ³⁾	0	–0.23	0.52
9	4-Br–	7.53	0.15* ²⁾	0	–0.30 [#]	0.44
10	4-NO ₂ –	6.63	0.78	0	0.15	0.64 ¹⁰⁾
11	3-OH–	7.45	0	0.12	–0.06	0.18
12	4-OH–	7.73	–	–	–	–
13	4-MeO–	8.13	–0.27	0	–0.61	0.27

* σ_p^+ ; [#] σ_R^+ ; 1) *J. Org. Chem.* **23** (1958) 1215; 2) *J. Am. Chem. Soc.* **80** (1958) 4979; 3) *Summ. Sci Techn., Ser. Gen. Ques. Org. Chem.* p. 163 (1979); 4) *Chem. Scripta* **9** (1976) 200; 5) *J. Am. Chem. Soc.* **90** (1968) 1757; 6) *Can. J. Chem.* **46** (1968) 2929; 7) *J. Am. Chem. Soc.* **100** (1978) 7765; 8) *J. Org. Chem.* **29** (1964) 1222; 9) *Can. J. Chem.* **61** (1983) 2376; 10) *Prog. Phys. Org. Chem.* **13** (1981) 119; 11) *Prog. Phys. Org. Chem.* **10** (1973) 81; 12) *J. Am. Chem. Soc.* **85** (1963) 3146; The other σ_p and σ_m values are taken from *J. Org. Chem.* **23** (1958) 420; The other σ_R and σ_I values are taken from *J. Am. Chem. Soc.* **94** (1972) 9113

As the differences in the acidities of the carboxyl and α -hydroxyl groups are sufficiently high (Table I), the pK_{a1} and pK_{a2} of the diprotic acids could be separately determined. The values of pK_{a2} and pK_{a3} of compound **12** are too close to

be determined spectrophotometrically, thus they were potentiometrically determined. Briefly, as this will be reported separately, the 4-OH-phenyl group dissociates at a lower pH than the α -hydroxyl group. The formed phenolate ion is tautomericly equilibrated *via* a quiynoid structure and transfers the negative charge to the former aroyl oxygen. The negative charge on the “aroyl” oxygen, in turn, stabilizes the α -hydroxyl oxygen; this results in the higher pK_a value of the α -hydroxyl.

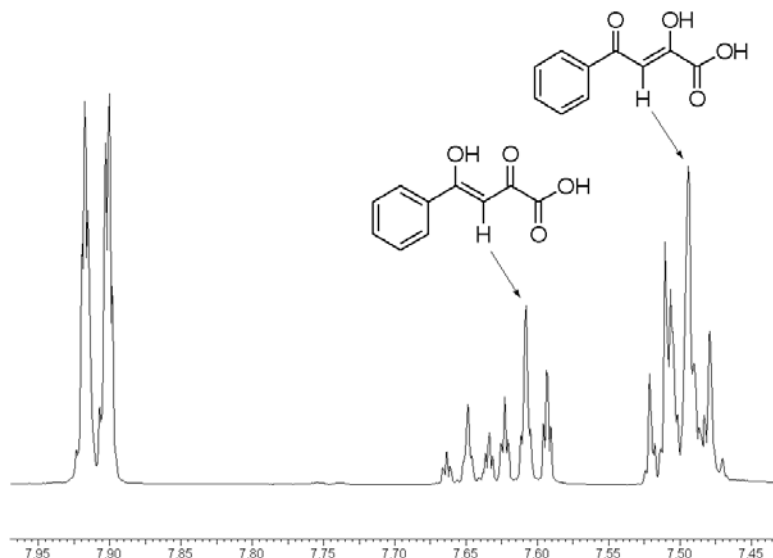


Fig. 1. 1D ^1H -NMR spectrum of compound **1** (region 7.4–8.0 ppm)* at pD 5.0 and $t = 25^\circ\text{C}$ in D_2O .

For the spectrophotometric determination of the acidity constants, two transformed forms of the classical spectrophotometric equation¹⁸ were applied:

$$A = A_{\text{H}_2\text{A}} - K_{\text{a1}} \frac{A - A_{\text{HA}^-}}{[\text{H}_3\text{O}^+]} \quad (2)$$

$$A = A_{\text{A}^{2-}} - \frac{1}{K_{\text{a2}}} [\text{H}_3\text{O}^+] (A_{\text{HA}^-} - A) \quad (3)$$

where $A_{\text{H}_2\text{A}}$, A_{HA^-} , $A_{\text{A}^{2-}}$, and A represent the absorbances of the molecular (H_2A), monoanionic (HA^-) and dianionic (A^{2-}) forms of the ADKs and their mixture at specified wavelengths, respectively. Eqs. 2 and 3 gave linear dependences when the spectrum of only one “pure” form (HA^-) was required for the determination of K_{a1} and K_{a2} . The absorption spectra used for the determination of the acidity constants of compound **1**, as a representative, are shown in Fig. 2a and 2c. The values of K_{a1} and K_{a2} were calculated by linear regression analysis from the slope of the corresponding lines (Fig. 2b and 2d).

* As the singlet of $-\text{CH}_2-$ protons from the diketo form, present at 4.50 ppm, is significantly weaker, just the region with the signals from the two enolic forms is shown.

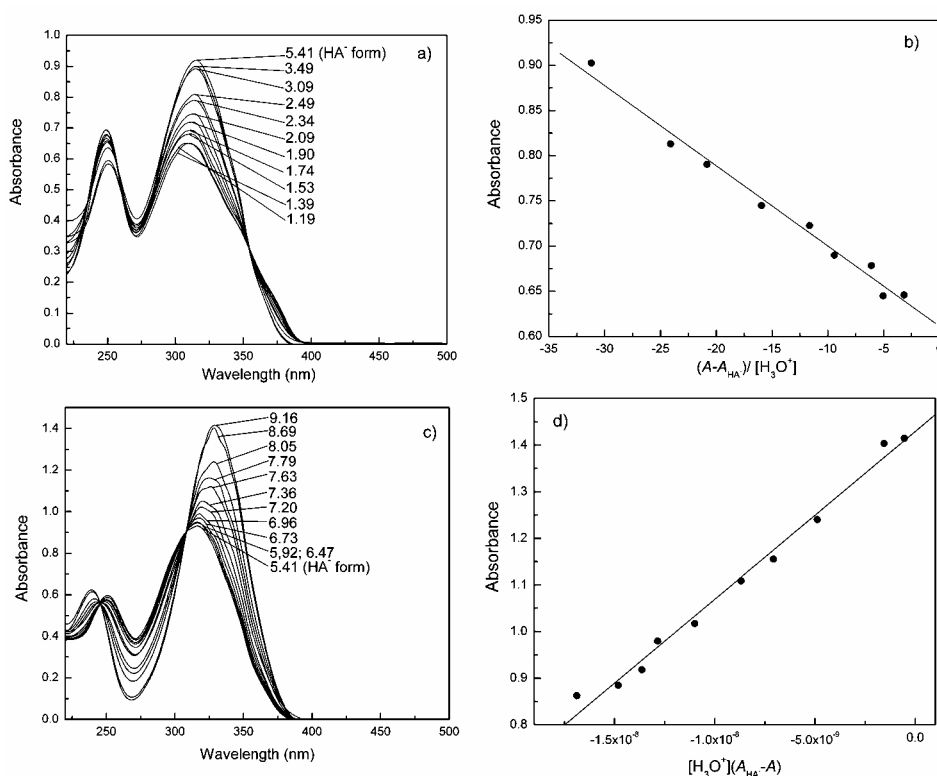


Fig. 2. a) Absorption spectra of compound **1**, used for the determination of K_{a1} in solutions of different acidity, the pH values are indicated on the figure; b) spectrophotometric determination of K_{a1} according to Eq. 2; $c_1 = 1.0176 \times 10^{-4} \text{ mol l}^{-1}$; $\lambda = 314.0 \text{ nm}$; c) absorption spectra of compound **1** used for the determination of K_{a2} in solutions of different acidity, the pH values are indicated on the figure; d) spectrophotometric determination of K_{a2} according to Eq. 3; $c_1 = 1.0021 \times 10^{-4} \text{ mol l}^{-1}$; $\lambda = 328.5 \text{ nm}$.

As mentioned previously, K_{a2} and K_{a3} for the 4-OH-derivative (**12**) were determined potentiometrically according to the formation function. In the pH interval where the H_2A^- , HA^{2-} and A^{3-} forms are present, the formation function gave the following linear dependence:¹³

$$\frac{[H_3O^+]^2(2 - \bar{n})}{\bar{n}} = K_{a2} \frac{[H_3O^+](\bar{n} - 1)}{\bar{n}} + K_{a2}K_{a3} \quad (4)$$

K_{a2} ($pK_{a2} 7.73 \pm 0.01$) and K_{a3} ($pK_{a3} 9.52 \pm 0.07$) were determined from the slope and the intercept of the corresponding line from three times repeated experiments (Fig. 3).

Considering the obtained pK_a values (Table I), the distribution of the species at some physiologically important pH values can be calculated. As the pK_{a1} of all the studied compounds lies within the interval 1.87–2.29, at pH 1.5, 30 to 70 %

of the ADKs (depending on the specific pK_{a1} value) are present in the H_2A form (H_3A for compound **12**). The pK_{a2} values of compounds **1–11** and **13** lie within the 6.63–8.13 range, thus at pH 7.4, 15 to 85 % of these compounds (depending on the specific pK_{a2} value) is present in the HA^- form, and at pH 9, most of ADKs **1–11** and **13** are completely deprotonated (A^{2-} form). The situation for compound **12** is slightly more complicated with H_2A^- and HA^{2-} being the dominant species in the pH range 6.7–8.6 and HA^{2-} and A^{3-} in the pH range 8.6–10.5. Representative distribution diagrams for compounds **1** and **12** are shown in Figs. 4a and 4b, respectively.

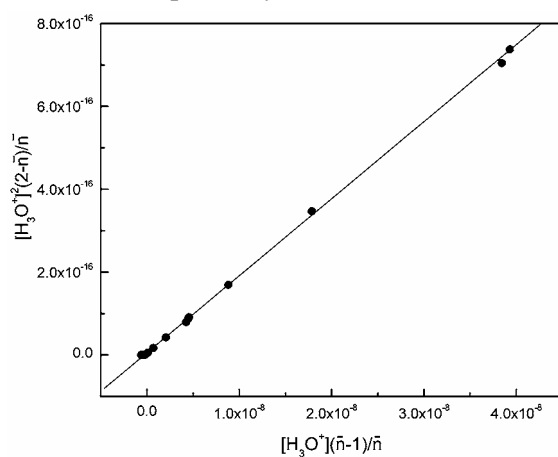


Fig. 3. Potentiometric determination of K_{a2} and K_{a3} for compound **12** according to Eq. (4), $c_{12} = 9.9198 \times 10^{-4} \text{ mol l}^{-1}$.

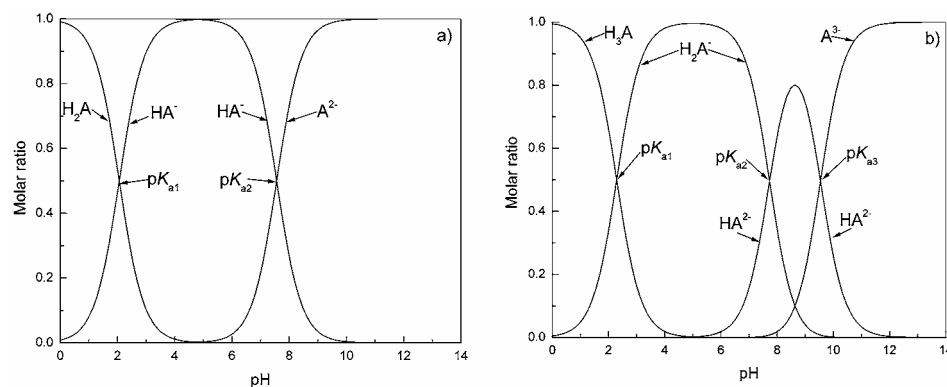


Fig. 4. Distribution diagrams: a) compound **1** and b) compound **12**.

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The determined acidity constants (pK_{a1} and pK_{a2}) were correlated with literature substituent constants in order to build a linear model of the Hammett type. Correlation of the determined pK_{a1} values with the σ_p and σ_m substituent constants has moderate statistics:

$$\text{p}K_{\text{a}1} = -0.382 (\pm 0.070) \sigma_{\text{p}} + 1.622 (\pm 1.080) \sigma_{\text{m}} + 2.151 (\pm 0.022) \quad (5)$$

($n = 13$; $r = 0.969$; $s = 0.034$; $F = 76.069$; $Q^2 = 0.916$; $s_{\text{PRESS}} = 0.040$)

The constant term in Eq. (5) is far for the experimentally obtained $\text{p}K_{\text{a}1}$ of the unsubstituted compound **1**. Exclusion of the parent compound (**1**) results in a statistically better correlation.

$$\text{p}K_{\text{a}1} = -0.381 (\pm 0.037) \sigma_{\text{p}} + 1.694 (\pm 0.570) \sigma_{\text{m}} + 2.159 (\pm 0.012) \quad (6)$$

($n = 12$; $r = 0.992$; $s = 0.018$; $F = 280.480$; $Q^2 = 0.960$; $s_{\text{PRESS}} = 0.029$)

Factorization of the influences of the substituent on resonance and inductive ones does not allow the inclusion of the disubstituted derivative (**6**):

$$\text{p}K_{\text{a}1} = -0.380 (\pm 0.180) \sigma_{\text{R}} - 0.392 (\pm 0.140) \sigma_{\text{I}} + 2.174 (\pm 0.055) \quad (7)$$

($n = 12$; $r = 0.925$; $s = 0.055$; $F = 26.574$; $Q^2 = 0.771$; $s_{\text{PRESS}} = 0.069$)

Again, exclusion of the parent compound (**1**) results in a statistically better correlation:

$$\text{p}K_{\text{a}1} = -0.333 (\pm 0.130) \sigma_{\text{R}} - 0.423 (\pm 0.980) \sigma_{\text{I}} + 2.202 (\pm 0.043) \quad (8)$$

($n = 11$; $r = 0.968$; $s = 0.037$; $F = 59.605$; $Q^2 = 0.891$; $s_{\text{PRESS}} = 0.049$)

Even the terms in Eqs. (7) and (8) are not standardized; the inductive term has a somewhat higher weight. In the pH range 1–5, the diketo form is present in a significantly lower amount compared to the enolic form I in the existing tautomeric mixture (Scheme 1) (this is clearly seen in the NMR spectra, exemplified by the ^1H -NMR spectrum of the parent compound (**1**) at $\text{pD} = 5.0$, Fig. 1). Therefore, resonance transmission of the substituent effects to the carboxylic group could be expected to be dominant. However, the optimized structures of the enolate molecular and monoanionic forms with the implicit water solvation model (COSMO) clearly show that the carboxyl group of the molecular form and the carboxylate anion of the monoanionic form of the compounds is torsionally distorted from the rest of the molecule, as exemplified on the parent compound (**1**) (Figs. 5a and 5b). Accordingly, the higher weight of the inductive effect reproduces the real situation in water, which was used as the medium in the present study of protolytic equilibria.

Correlation of the determined $\text{p}K_{\text{a}2}$ values with the σ_{p} and σ_{m} substituent constants has much better statistics than the corresponding equation describing $\text{p}K_{\text{a}1}$ (Eq. (5)):

$$\text{p}K_{\text{a}2} = -1.319 (\pm 0.220) \sigma_{\text{p}} - 1.706 (\pm 1.600) \sigma_{\text{m}} + 7.677 (\pm 0.062) \quad (9)$$

($n = 12$; $r = 0.977$; $s = 0.094$; $F = 93.574$; $Q^2 = 0.927$; $s_{\text{PRESS}} = 0.118$)

The 4-OH-substituted derivative could not be included in Eqs. (9) and (10); the explanation is offered above. Exclusion of the 4-*tert*-Bu- derivative (**5**) gives a superior correlation ($r = 0.985$, $F = 128.15$, $Q^2 = 0.931$). For this, no explana-

tion can be offered. Factorization of the influences of the substituent on resonance and inductive ones, with exclusion of the disubstituted derivative (**6**), gave a good correlation:

$$pK_{a2} = -1.416 (\pm 0.290) \sigma_R - 1.168 (\pm 0.220) \sigma_I + 7.591 (\pm 0.086) \quad (10)$$

$$(n = 11; r = 0.983; s = 0.083; F = 114.494; Q^2 = 0.949; s_{\text{PRESS}} = 0.102)$$

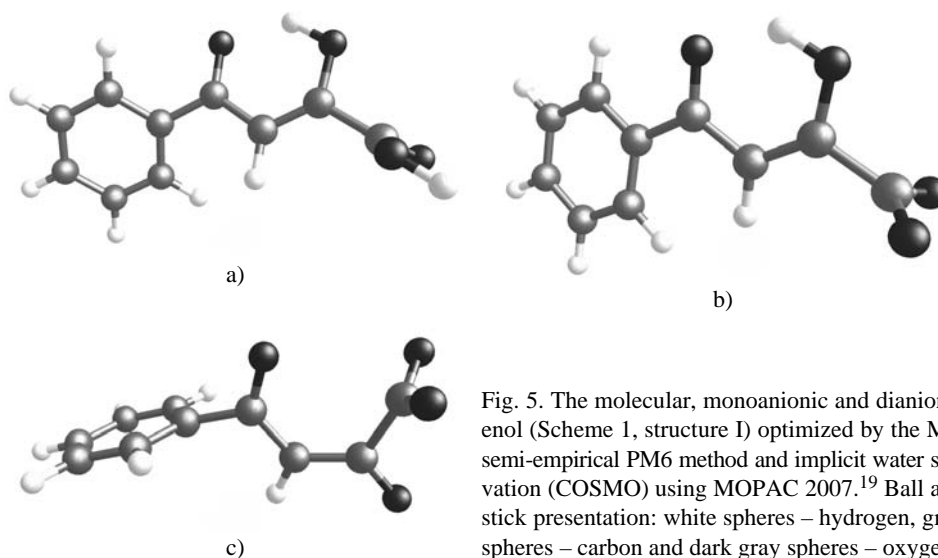


Fig. 5. The molecular, monoanionic and dianionic enol (Scheme 1, structure I) optimized by the MO semi-empirical PM6 method and implicit water solvation (COSMO) using MOPAC 2007.¹⁹ Ball and stick presentation: white spheres – hydrogen, gray spheres – carbon and dark gray spheres – oxygen.

Additional exclusion of the 4-*tert*-Bu- derivative (**5**) gave the best of the in this article reported correlations ($r = 0.993$, $F = 264.285$, $Q^2 = 0.977$).

At this stage, obvious question arises: Why the Hammett correlations of pK_{a2} (Eqs. (9) and (10)) are statistically significantly better than corresponding ones correlating pK_{a1} ? The optimized structures¹⁹ of the molecular, monoanionic and dianionic forms of the compounds (exemplified by the parent compound (**1**), Figs. 5a–5c) offer the probable explanation. The molecular form of enol I (Scheme 1) has an almost perfect coplanar $>C(O)-CH=C(OH)-$ (keto-enol) moiety, stabilized by an intramolecular H-bond. In the parent compound, the $>C=O \cdots H-O-$ distance is 1.71 Å, with a H-bond energy of -0.2997 kcal mol⁻¹. The aryl group is twisted from the plane of the keto-enol moiety by 37.61°, while the carboxyl group is twisted by 101.70°. In the monoanionic form, a similar distortion of the carboxylic group exists (91.25° for the parent compound (**1**)), *i.e.*, the carboxylic group is almost perpendicular to the plane of the keto-enol moiety. Transmission of the resonance effect is efficiently suppressed in this manner. Accordingly, the inferior statistics of Eqs. (5)–(8) in respect to Eqs. (9) and (10) could be reasonably explained. On the other hand, in the monoanionic form, the aryl group is twisted in respect to the keto-enol moiety by a minor amount (7.83° for the pa-

rent compound (**1**)), *i.e.*, the aryl ring is almost coplanar with the keto-enol moiety, therefore transmission of resonance and inductive effects is very efficient and could be described by the extended correlation of the Hammett type. As a note, in the dianionic form (exemplified by the parent compound (**1**), Fig. 5c) the keto-enol moiety is again coplanar, but the aroyl keto and ionized vinyl hydroxyl are in the *Z* configuration with respect to each other, because of the repulsion between the charge on the enolate anion and the lone pair of the aroyl keto group. Time scales of the rotation around the aroyl keto-vinyl =CH– and the deprotonation of the –OH group should be examined in the future.

The values of pK_a predicted from Eqs. (5)–(10) are given in Table III and shown in Fig. 6. The substituent constants were taken from different sources and the main criterion was to choose those derived from the ionization of carboxylic acids or solvolysis of the corresponding derivatives. To the best of our knowledge, a set of substituents constants derived from compounds having an enolizable interface between a phenyl ring and the reaction center (carboxylic group in the present case) has not been reported in the literature. For compounds **3** and **4**, σ_p^+ values were used in Eqs. (5) and (6). This could be an indication that hyperconjugation of 4-Et- (**3**) and 4-*i*-Pr- (**4**) substituents could influence the overall structures of these molecules, which is possible, particularly considering the tautomeric form III (Scheme 1). The σ_R of compounds **2**, **7**, **8**, and **13** (π -electron delocalization) are derived from the benzoic acid ionization model, while the others are π -electron delocalizations derived from ^{13}C -NMR shifts.

TABLE III. Experimentally obtained (pK_{a1} and pK_{a2}) and predicted acidity constant values

Comp.	R–	pK_{a1}	Predicted pK_{a1}				pK_{a2}	Predicted pK_{a2}	
			Eq. (5)	Eq. (6)	Eq. (7)	Eq. (8)		Eq. (9)	Eq. (10)
1	H–	2.06	2.15	* ^a	2.17	*	7.56	7.68	7.59
2	4-Me–	2.22	2.20	2.21	2.23	2.25	7.99	7.86	7.84
3	4-Et–	2.28	2.27	2.28	2.23	2.26	7.83	7.85	7.79
4	4- <i>i</i> -Pr–	2.29	2.26	2.27	2.24	2.27	7.85	7.85	7.83
5	4- <i>tert</i> -Bu–	2.21	2.23	2.24	2.25	2.28	7.72	7.87	7.88
6	3,4-di-Me–	2.09	2.09	2.09	*	*	7.92	7.96	a
7	4-F–	2.06	2.07	2.08	2.10	2.09	7.50	7.40	7.50
8	4-Cl–	2.09	2.06	2.07	2.06	2.06	7.30	7.31	7.31
9	4-Br–	2.06	2.06	2.07	2.07	2.07	7.53	7.48	7.50
10	4-NO ₂ –	1.87	1.85	1.86	1.87	1.88	6.63	6.65	6.63
11	3-OH–	2.18	2.18	2.19	2.13	2.15	7.45	7.47	7.47
12	4-OH–	2.29	2.29	2.30	2.25	2.25	7.73	*	*
13	4-MeO–	2.28	2.25	2.26	2.30	2.29	8.13	8.03	8.14

^aOmitted from equation derivation

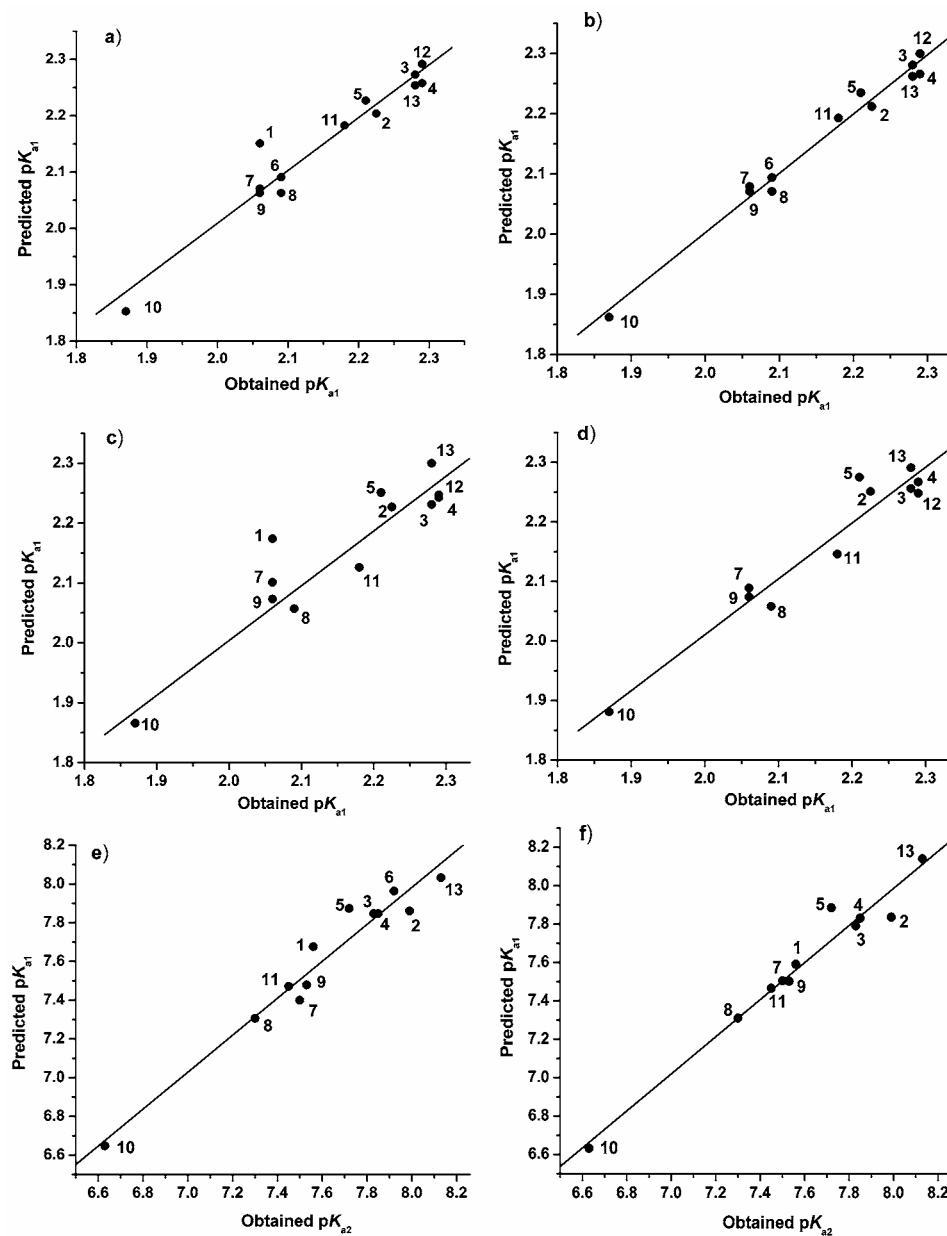


Fig. 6. Experimentally obtained vs. calculated pK_a values for compounds **1–13**. Graph a) corresponds to Eq. (5), graph b) to Eq. (6), graph c) to Eq. (7), graph d) to Eq. (8), graph e) to Eq. (9) and graph f) to Eq. (10).

The selection of the substituent constants could roughly indicate possible differences between the amounts of all three possible tautomeric forms in aqueous

solution for the molecular or ionized forms of every studied compound, as well as differences in the mode of their solvation. This account offers rationale for the magnitudes of the experimentally obtained pK_a values, which have been discussed in the presented correlations. These partially “empirically” derived models by the classical LFER approach could be extended to a larger set of congeners.

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ИЗВОД

ЛИНЕАРНЕ КОРЕЛАЦИЈЕ СЛОБОДНЕ ЕНЕРГИЈЕ (LFER) ПРОТОЛИТИЧКИХ РАВНОТЕЖА 4-АРИЛ-2,4-ДИОКСОБУТАНСКИХ КИСЕЛИНА У ВОДЕНИМ РАСТВОРИМА

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Протолитичке равнотеже 13 једињења из класе 4-арил-2,4-диоксобутанских киселина (АДК) спектрофотометријски су проучаване у воденим растворима у рН интервалу 1–9 при температури 25 ± 1 °C и јонској јачини раствора 0.1 mol l^{-1} (NaCl), са изузетком 4-ОН-деривата који је проучаван и потенциометријски у рН интервалу 7–10 при истим условима. Како АДК у воденом раствору подлежу кето-енолној таутомерији и истовремено постоје у дикето и два енолна облика, то одређене киселинске константе (pK_{a1} 1.87–2.29, pK_{a2} 6.63–8.13 и pK_{a3} (4-ОН-) 9.52) представљају макро константе за дати систем. ¹H-NMR спектар основне супстанце (4-фенил-2,4-диоксобутанска киселина) (25 °C, pD 5.0) потврђује присуство свих таутомерних облика. Употребом проширене Хаметове корелације, одређене pK_a вредности корелисане су са литературним σ вредностима. Предвиђене pK_a вредности добро се слажу са експериментално добијеним. Молекулски, моноанјонски и дианјонски облици основне супстанце су оптимизовани семиемпиријском молекулско–орбиталном PM6 методом са имплицитним моделом солватације у води (COSMO). Добијене геометрије су употребљене за објашњење квалитета LFER модела.

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