BIOLOGICALLY IMPORTANT PHYSICOCHEMICAL PROPERTIES OF KOJIC ACID DERIVATIVES

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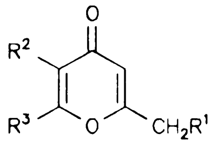
Physicochemical properties (hydrophobicity, acidity, metal complexing ability), which are expected to play a role in biological activity of kojic acid derivatives, were determined for 35 compounds. The structure–property relationships were analysed with the aim to predict the properties of new derivatives and to obtain the data for formulation of quantitative structure–time–activity relationships. Linear correlations were found between hydrophobicity expressed as decadic logarithm of the 1-octanol/buffer partition coefficient and the sum of fragment constants and correction factors according to Leo and Hansch as well as between acidity and a set of Hammett constants of the varying substituents.

Kojic acid is a metabolic product of numerous species of the genera *Aspergillus* and *Penicillium* with a wide spectrum of biological activity. It itself or its derivatives exhibit antibacterial[1 – 3], antiprotozoal, and insecticide[4,5] activity. Metal chelates and some derivatives were recommended as preservatives against several fungal diseases of plants[1]. Biological activity of these compounds is referred to their chelating ability[6].

Microbial origin of kojic acid implies its nonproblematic biodegradation. This fact makes it an attractive skeleton for development of biologically active compounds via derivatization. The task can be rationalized by the use of quantitative structure–time–activity relationships (QSTAR), for which the knowledge of certain physicochemical properties is needed. The most interesting properties in this context are: *1*) hydrophobicity, as characterized by 1-octanol/water partition coefficient, *2*) acidity, and *3*) metal ion chelating ability. The former two parameters determine the distribution of the compounds in biological systems, the latter two may have some relation to the mechanism of action. The determination of these properties and the analysis of their relation to structure in the series of 35 kojic acid derivatives are the main goals of this study.



EXPERIMENTAL

Chemicals

The derivatives (see Table I) were prepared by previously reported methods[7–19] from kojic acid (compound *I*) isolated from fermentation medium of *Aspergillus tamarii VIII* (ref.[20]). Other used chemicals (1-octanol, dimethyl sulfoxide – DMSO, $Cu(NO_3)_2$, NaOH, buffer constituents) were of analytical grade (Lachema, Brno, The Czech Republic).

Monitoring the Interphase Distribution

The distribution behaviour of the kojic acid derivates in the two-phase system 1-octanol/buffer (according to McIlvain[21], pH 4, 0.06 M citric acid and 0.082 M Na_2HPO_4) was characterized by the partition coefficients *P*, which were determined as the ratio of the transport rate parameters in the direction buffer–1-octanol (l_1) and backwards (l_2). The measurements were made in a glass vessel[22] temperated to 25 °C. The stirring frequency (1.3 s^{-1}) was adjusted so as no concentration gradients could appear in either phase and the motion of the phase interface was minimal. The measuring vessel was filled with redistilled deionized 1-octanol-satured water (175 ml), temperated to 25 °C and surfaced with the solution of the respective compound in freshly distilled 1-octanol saturated with water or buffer (20 ml). Samples (2 ml) for spectrophotometric determination of the compound concentration were withdrawn from water phase with a syringe at proper time intervals. The UV spectra were recorded in the complete spectral range; this measurement also served for monitoring the stability of the compounds in the given medium. The samples were replaced to the vessel immediately after each measurement.

Providing that at the beginning of partition the compound is present in 1-octanol only and its concentration is c_0, the time course of the concentration in aqueous phase (c_1) can be expressed by[22]

$$c_1 = c_1 l_2 V_2 \{1 - \exp[-S (l_1/V_1 + l_2/V_2)t]\}/(l_1 V_1 + l_2 V_2) . \qquad (1)$$

Here l_1 and l_2 are the transport rate parameters, V_1 and V_2 stand for the volume of aqueous and 1-octanol phases, *S* for the interface surface and *t* for time. The time course of the absorbance in the aqueous phase is expressed by Eq. (*1*) multiplied by εd (ε is the molar absorption coefficient, *d* is the length of the light path). Rearrangement of Eq. (*1*) as expressed by absorbance using the substitution of the exponential by the first two terms of the Taylor series yields[22]

$$A = \varepsilon\, d\, c_0\, l_2 S\, t/V_1 ; \quad t \to 0 \qquad (2)$$

$$\ln (A_e - A) = \text{const.} - S (l_1/V_1 + l_2/V_2)\, t . \qquad (3)$$

where A_e is the value for absorbance at the end of distribution. The values of transport rate parameters l_1 and l_2 were determined by a nonlinear regression analysis[23] of the time course of the absorbance of the compound in the aqueous phase according to Eq. (*1*) expressed through absorbance; Eqs (*2*) and (*3*) served for initial assesments of l_2 and l_1, respectively.

Determination of Acidity

The acid dissociation constants K_a were determined by potentiometric titration method[24]. The aqueous solution of the compound (25 cm^3, 0.004 mol dm^{-3}, 0.1 mol dm^{-3} KNO_3 as the inert electrolyte, 1% (v/v) DMSO) in a thermostatted stirred vessel (25 °C) was titrated with 0.01 mol dm^{-3} NaOH. After each addition of NaOH the pH of the mixture was measured (OP 209/1 Radelkis, Hungary). The pK_a values were determined from Eq. (*4*), derived from the definition of K_a, the mass balance of the compound, and the charge balance of the system:

$$pK_a = -\log[H^+] + \log\{(c_{HA} - [Na^+] - [H^+] + [OH^-])/([Na^+] + [H^+] - [OH^-])\}, \tag{4}$$

where c_{HA} is the total concentration of the compound. Throughout the whole manuscript the square brackets denote concentration.

Determination of Copper Chelating Ability

Also in this case a potentiometric method was used, since the copper ions are chelated by anions of kojic acid derivatives and the protons are liberated into the medium. The experimental conditions were the same as before, the concentration of Cu^{2+} was determined by a complexometric method[25]. The solution (25 cm^3) containing 10^{-5} mol H^+ (as HNO_3) and 10^{-5} mol Cu^{2+} (as $Cu(NO_3)_2$) was titrated with 0.004 mol dm^{-3} solution of sodium salt of the respective derivative (prepared by neutralization with NaOH). Simultaneously, the pH value of the medium was monitored.

The consecutive equilibria of metal ions with ligands can be characterized by the corresponding association constants K_i (for the complex with i ligands bound) and by the global association constants $\beta_i = K_1K_2 \ldots K_i$. If maximum value of i is 2, the ligand number, n (the average number of bound ligands per one metal ion)

$$n = ([MA] + 2[MA_2])/([MA] + [MA_2] + [M]) = (c_{HA} - [HA] - [A])/c_M \tag{5}$$

can be expressed as

$$n = (\beta_1[A] + 2\beta_2[A]^2)/(1 + \beta_1[A] + \beta_2[A]^2). \tag{6}$$

Here A stands for anions of kojic acid derivatives and M for metal ions (for simplicity the charges are not given), c_M is the total concentration of the metal ion. The concentration of non-ionized derivatives can be calculated from the balance for protons as

$$[HA] = c_{HNO_3} + [OH^-] - [H^+]. \tag{7}$$

Then, from the second equality of Eq. (5), one gets using the definition of acid dissociation constant for expression of [A]

$$n = \{c_{HA} - (1 + K_a/[H^+])(c_{HNO_3} + [OH^-] - [H^+])\}/c_M. \tag{8}$$

Equation (*6*) with n expressed through Eq. (8) was used to determine the values of β_1 and β_2 by nonlinear regression analysis[23].

Estimation of Hydrophobicity

For this purpose the approach of Leo and Hansch[26,27] was used. The substituents R^1 were treated as being bound to benzyl carbon, i.e. their fragment values were calculated from the values for substituents on aliphatic carbon[27] by addition of 0.2. For the substituents R^2 and R^3 the aromatic fragment values for the fragments bound to the basic skeleton of kojic acid were used. The fragment value for OC(O)C(O)O (derivative *XIX*, Table I) is not reported, therefore the sum of fragment values for aromatic and aliphatic OC(O) was used. In some cases (*XIV*, *XV*, *XVI*, *XX*, *XXIII*) the fragment values of the substituents were calculated from the measured partition coefficients of suitable compounds. The details are given in remarks to Table I. Further corrections were made according to the published recommendations of the authors[26,27] using the factors F for: *1*) flexibility, $F_b = -0.12\ (n - 1)$ where n is the number of bonds (not counting those to hydrogen) in each chain outside the fragment; *2*) chain branching, $F_{cbr} = -0.13$; *3*) group branching, $F_{gbr} = -0.22$; *4*) geminal and vicinal proximity of two polar fragments, $F = \mathrm{const}\,(f_1 + f_2)$, where the values of const are given in Table II of ref.[27]; *5*) interaction of Cl, Br, and I through two carbon atoms with polar fragments, $F = 0.35$; *6*) ortho-effect, F is given in Table III of ref.[27]; *7*) hydrogen bond, $F = 0.63$; *8*) interaction of polar fragments on the aromatic ring, F is calculated from the data in Table IV of ref.[27].

RESULTS AND DISCUSSION

Structures of the studied derivatives and their 1-octanol/buffer (pH 4) partition coefficients are summarized in Table I. As the pK_a values of the studied derivatives are higher than six (see below), at pH 4 they should be practically completely non-ionized. The partition coefficients can be used for testing of computational approaches to their estimation, since the partitioning of ion pairs[26] is supressed under the conditions. As we intend to use the data for QSTAR analysis, it is advantageous to know how well the partition coefficients of non-synthesized compounds can be predicted. The sum of fragment values and the correction factors of the derivatives, for substructures of which the data have been tabulated[26,27] (all except *V*, *VI*, *XII*, and *XXVII*) are also given in Table I. The experimental partition coefficients correlate with the sum of calculated contributions of the varying substituents as

$$\log P = 0.723\ \Sigma(f + F) - 0.090 \qquad (9)$$
$$n = 31,\ s = 0.391,\ r = 0.883,\ F = 102.7\ .$$

The slope of the dependence is somewhat lower than unity and the fit is far from being perfectly linear (22% of the variance unexplained). This can be due to the heterocyclic nature of the kojic acid skeleton, which may exhibit multiple interactions with the varying substituents. Nevertheless, Eq. (*9*) does allow for a rough assessment of hydrophobicity from the structure of kojic acid derivatives.

Acidicity, metal complexing equilibrium constants, ΔpK_a (see below) and the Hammett constants[28] for some of the derivatives with the hydroxyl group in the position 5 (R^2 = OH) are summarized in Table II. Electronic behaviour of the substituents was characterized by the Hammet constants for benzene derivatives, as the γ-pyrone skeleton of kojic acid could exhibit aromatic character. The influence of the substituent

TABLE I
Structure and hydrophobicity of the studied derivatives of kojic acid. P 1-octanol/buffer (pH 4) partition coefficient, f fragment constants, F correction factors[26,27], both for the varying substituents R^1, R^2, and R^3

No.	R^1	R^2	R^3	$\log P$	f_1	F_1	f_2	F_2	f_3	F_3	$\sum(f+F)$
I	OH	OH	H	-0.617	-1.44^{g}	0.23^{h}	-0.44^{i}	$0.38^{j,k}$	0.23	0^{l}	-1.04
II	Cl	OH	H	0.635	0.26^{g}	0.35^{m}	-0.44	0.38	0.23	0	0.78
III	Br	OH	H	0.615	0.40^{g}	0.35	-0.44	0.38	0.23	0	0.92
IV	I	OH	H	0.672	0.79^{g}	0.35	-0.44	0.38	0.23	0	1.31
V	N_3	OH	H	-0.086	$-^{n}$	–	-0.44	0.38	0.23	0	–
VI	NCS	OH	H	0.061	–	–	-0.44	0.38	0.23	0	–
VII	$S(CH_2)_2CH_3$	OH	H	1.320	1.62^{g}	$-0.26^{h,o}$	-0.44	0.38	0.23	0	1.53
VIII	$S(CH_2)_4CH_3$	OH	H	1.430	2.94^{g}	$0.40^{h,o}$	-0.44	0.38	0.23	0	2.61
IX	$S(CH_2)_2OH$	OH	H	-0.693	-0.91^{g}	$0.32^{2h,o}$	-0.44	0.38	0.23	0	-0.42
X	$SCH(CH_3)_2$	OH	H	1.168	1.62^{g}	$-0.39^{h,o,p}$	-0.44	0.38	0.23	0	1.76
XI	$SCH_2COOC_2H_5$	OH	H	0.418	0.13^{g}	$0.16^{2h,o}$	-0.44	0.38	0.23	0	0.46
XII	$SC(S)N(CH_3)_2$	OH	H	0.915	–	–	-0.44	0.38	0.23	0	–
XIII	$SC_6H_{11}{}^{a}$	OH	H	1.748	$2.62^{g,q}$	$-0.02^{h,o}$	-0.44	0.38	0.23	0	2.77
XIV	$SC_5H_4N^{b}$	OH	H	1.142	$0.39^{i,r}$	$0.15^{h,k,o}$	-0.44	0.38	0.23	0	0.61
XV	$SC_7H_5N_2{}^{c}$	OH	H	1.505	$1.20^{i,s}$	0.15	-0.44	0.38	0.23	0	1.52
XVI	$SC_7H_4NS^{d}$	OH	H	2.082	$1.75^{i,t}$	0.15	-0.44	0.38	0.23	0	2.07
XVII	OH	OCH_3	H	-0.257	-1.44^{g}	0.23^{h}	0.28^{i}	$0.35^{j,k,o}$	0.23	0	-0.35
XVIII	OH	$OC(O)C(CH_3)_3$	H	0.705	-1.44	0.23	2.31^{i}	$0.33^{j,o,2u}$	0.23	0	0.78
XIX	OH	$OC(O)C(O)OC_2H_5$	H	-1.646	-1.44	0.23	$-0.50^{i,v}$	$0.01^{j,o}$	0.23	0	-1.47
XX	OH	$OC(O)CH(CH_3)OC_6H_4Cl^{e}$	H	1.602	-1.44	0.23	$2.88^{i,w}$	$0.26^{2j,o}$	0.23	0	2.16

TABLE I
(*Continued*)

No.	R^1	R^2	R^3	log *P*	f_1	F_1	f_2	F_2	f_3	F_3	$\sum(f+F)$
XXI	OH	$OCH_2C_6H_5$[e]	H	0.255	-1.44	0.23	1.98[i]	0.14[j,o]	0.23	0	1.14
XXII	OH	$OC(O)C_6H_2Cl_3$[e]	H	1.921	-1.44	0.23	3.50[i]	-0.18[j,k,o,2v]	0.23	0	2.34
XXIII	OH	$OC(O)CH_2C_4H_3S$[f]	H	0.681	-1.44	0.23	1.54[i,y]	0.25[j,o]	0.23	0	0.81
XXIV	$OC(O)CH_3$	$OC(O)CH_3$	H	0.250	-0.40[g]	0.09[h,o]	0.33[i]	0.25[j,o]	0.23	0	0.50
XXV	Cl	OCH_3	H	0.161	0.26[g]	0.35[m]	0.28[i]	0.35[j,k,o]	0.23	0	1.47
XXVI	Br	$OC(O)CH_3$	H	0.505	0.40[g]	0.35	0.33[i]	0.25[j,o]	0.23	0	1.56
XXVII	$SC(S)N(CH_3)_2$	OCH_3	H	1.139	-	-	0.28[i]	0.35[j,k,o]	0.23	0	-
XXVIII	OH	OH	Br	0.272	-1.44[g]	0.23[h]	-0.44[i]	0.38[j,k]	1.09[i]	0.09[2k,x]	-0.09
XXIX	OH	OH	I	0.546	-1.44	0.23	-0.44	0.38	1.35[i]	0.09	0.17
XXX	OH	OH	$C(O)CH_3$	-0.377	-1.44	0.23	-0.44	0.38	-0.20[i]	1.18[2k,o,z]	-0.29
XXXI	Cl	OH	Cl	0.950	0.26[g]	0.35[m]	-0.44	0.38	0.94[i]	0.09[2k,x]	1.58
XXXII	Cl	OH	Br	1.085	0[illegible]	0.35	-0.44	0.38	1.09[i]	0.09[2k,x]	1.73
XXXIII	Br	OH	Br	1.274	0.40[g]	0.35	-0.44	0.38	1.09	0.09	1.87
XXXIV	$OC(O)CH_3$	OH	$C(O)CH_3$	-0.509	-0.40[g]	0.09[h,o]	-0.44	0.38	-0.20[i]	1.18[2k,o,z]	0.61
XXXV	$OC(O)CH_3$	$OC(O)CH_3$	NO_2	0.380	-0.40	0.09	0.33[i]	0.25[j,o]	-0.03[i]	0.79[2k]	1.03

[a] S-cyclohexane; [b] S-(2-pyridine); [c] S-(2-benzimidazole); [d] S-(2-benzothiazole). [e] $C_6H_{5-X}Cl_X$ = benzene and its 4-Cl and 2,4,6-tri-Cl derivatives; [f] C_4H_3S = 2-thiophene; [g] benzyl type = aliphatic type + 0.2; [h] vicinal interaction of two polar fragments; [i] aromatic type; [j] geminal interaction of two polar fragments; [k] interaction of two polar fragments on the aromatic ring; [l] no correction needed; [m] vicinal interaction halogen–polar fragment; [n] missing data; [o] flexibility; [p] chain branching; [q] log *P*(cyclohexane) = 3.44 used; [r] log *P*(pyridine) = 0.65 used; [s] log *P*(benzimidazole) = 1.46 used; [t] log *P*(benzothiazole) = 2.01 used; [u] group branching; [v] estimated as the sum of aliphatic type and aromatic type OCO fragments; [w] log *P*(4-Cl-anisole) = 2.78 used; [x] ortho-effect; [y] log *P*(2-CH_3-thiophene) = 2.33 used; [z] H-bond.

R^1 was expressed through the Hammett constant of the group CH_2R^1 for the para position and that of R^3 by ΔpK_a, which is the difference between pK_a values of 2-R^3-phenol and phenol. The latter choice was motivated by the fact, that the hydroxyl group in the position 5 could resemble the phenolic hydroxyl. Acidity of the derivatives (Table II) is correlated with their structure as

$$pK_a = -2.076\,(\sigma_p + \Delta pK_a) + 7.703 \qquad (10)$$
$$n = 11,\ s = 0.193,\ r = -0.960,\ F = 106.2\,.$$

As indicated by the values of the statistical parameters, the fit is quite satisfactory and can be used for the prediction of acidity of non-synthesized derivatives.

The substituent R^3= C(O)CH_3 in the compounds *XXX* and *XXXIV* can have quite specific influence on the ionization of the hydroxyl group in the position 5 due to the possible formation of the hydrogen bond, which is indicated also by a better agreement between calculated (Eq. (9)) and observed hydrophobicity (Table I), when the corresponding H-bond correction factors were used. The unknown ΔpK_a parameter for the C(O)CH_3 group can be estimated from Eq. (*10*) as being equal to 0.13 for the

TABLE II
Acidity, metal complexing ability (K_1 is the association constant for a 1 : 1 complex formation, K_2 is the association constant for the formation of the complex with two ligands and $\beta_2 = K_1K_2$ is the global association constant), ΔpK_a (the difference between pK_a values of 2-R^3-phenol and phenol)[28], and the Hammett constants for para positions[28] for some ionizable kojic acid derivatives (structures in Table I)

No.	pK_a	$\log K_1$	$\log K_2$	$\log \beta_2$	$\Delta pK_a(R^3)$	$\sigma_p(CH_2R^1)$
I	7.722	6.253	5.961	12.21	0.00	0.00
II	7.368	6.767	6.399	13.17	0.00	0.12
III	7.204	7.954	8.012	15.97	0.00	0.14
IV	7.464	7.154	6.112	13.27	0.00	0.11
V	7.567	6.767	5.557	12.32	0.00	0.15
VI	7.270	6.193	5.443	11.64	0.00	0.38
XXVIII	6.150	6.006	4.617	10.62	0.70	0.00
XXIX	6.057	6.218	4.677	10.89	0.63	0.00
XXX	7.437	6.805	5.701	12.51	–	0.00
XXXI	5.936	5.324	6.013	11.34	0.68	0.12
XXXII	6.248	5.483	6.535	12.02	0.70	0.12
XXXIII	6.013	6.213	5.884	12.10	0.70	0.14
XXXIV	7.172	6.519	5.732	12.25	–	0.31

compound *XXX* and to –0.05 for the compound *XXXIV*. These values or their average could be used to estimate roughly pK_a of non-synthesized derivatives.

The association constants for copper complex formation do not correlate well with the Hammett constants and ΔpK_a values presented in Table II (the values of the correlation coefficient are $r = -0.727$ for $\log K_1$, $r = -0.338$ for $\log K_2$ and $r = -0.584$ for $\log \beta_2$).

Tables I and II contain the physicochemical properties of the studied kojic acid derivatives, which can be used for construction of QSTAR. The dependences of these properties on structure of the studied derivatives will enable one to design the structures of compounds with the desired properties. The lack of collinearity between acidity and the metal chelating ability will promote the elucidation of the mechanism of action as both the properties might play a role in the biological effects of the kojic acid derivatives.

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