Polish J. Chem., **73**, 873–878 (1999) **COMMUNICATION**

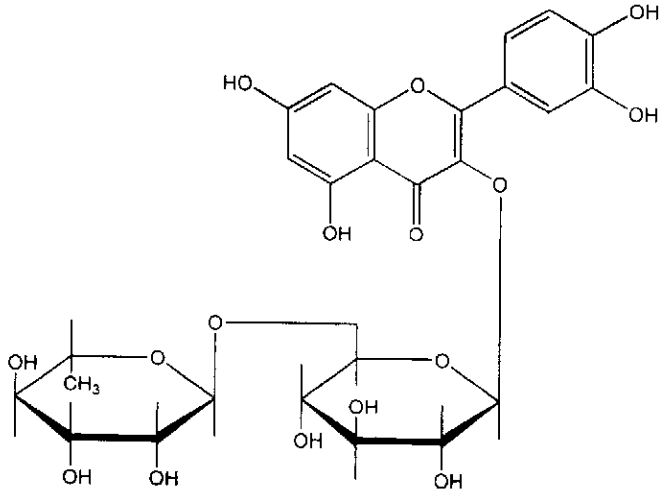
Cu(II) Complexes with Rutin

by M. Dyba[1], S. Solinas[2], N. Culeddu[3], M.-L. Ganadu[2] and H. Kozłowski[1]

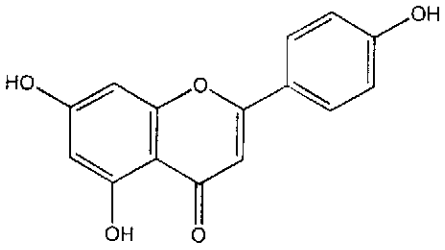
[1]*Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland*
[2]*Dipartimento di Chimica, Universita di Sassari, 07100 Sassari, Italy*
[3]*CNR IAT CAPA, via Vienna 2, 07100 Sassari, Italy*

(Received October 19th, 1998; revised manuscript January 8th, 1999)

Rutin, a natural pigment (Scheme 1), one of typical glucosides in the flavonoid series is widely distributed in natural plants. Its biological activity is well recognized and it is pharmacologically used to decrease the permeability and fragility of the blood vessels. Rutin ring system contains two very efficient binding sites for metal ions: {C(3′)–OH, C(4′)–OH} and {C(4)=O, C(5)–OH} donor pairs. The former, catechol-like site, is very efficient to coordinate various metal ions (see *e.g.* [1]).



Rutin



Apigenin

Scheme 1. Rutin and apigenin.

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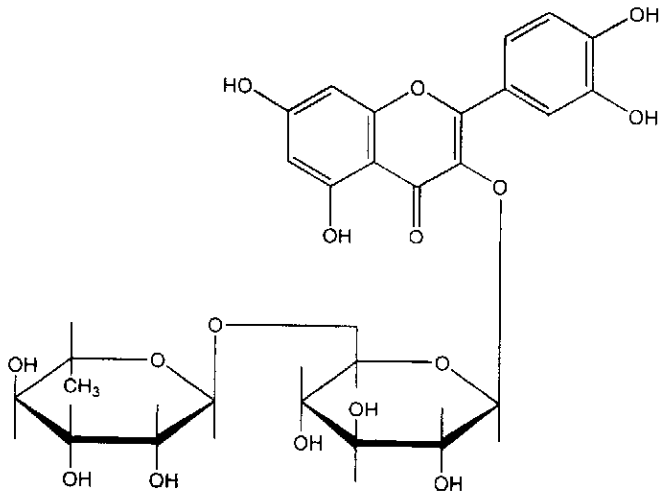
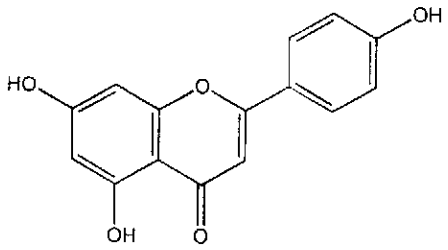


Rutin



Apigenin

Scheme 1. Rutin and apigenin.

Also the second pair of oxygen donors is effective in metal ion coordination, as it was found in anthracycline complexes [2,3].

The preliminary studies on binding ability of rutin and quercetin (rutin aglycon) have shown that indeed both ligands could be efficient in metal ion coordination [4]. In this work we collected the potentiometric, EPR and NMR data for Cu^{2+}-rutin and related systems, which allow to evaluate the coordination equilibria in aqueous solutions.

Rutin and its analogues were obtained from Aldrich with purity > 95%. All other reagents were of analytical grade. The purity of rutin was checked and the exact concentration of the solution was determined by the Gran method [5]. The stability constants were determined by pH-metric titrations of 2–2.5 cm^3 samples at 25°C. The rutin concentrations in each sample was 1.5×10^{-3} mol dm^{-3} and the metal ion to ligand molar ratios were 1:1, 1:2, 1:4 and 1:6. Due to the precipitation in pure water, the ligand and ligand with copper solutions were prepared in 1:1 v/v ethanol–water homogeneous mixture. The ionic strength (I) was adjusted to 0.1 mol dm^{-3} with KNO_3. The proton concentration was adjusted to 1.5×10^{-3} mol dm^{-3} with HNO_3. Argon was bubbled through solutions to remove oxygen. The solutions were prepared before determination and stored in the dark under argon atmosphere at low pH = 2.8 to avoid oxidation of the ligand. The titrations were performed with carbonate-free 0.1 mol dm^{-3} NaOH standard solution under argon atmosphere over pH range 2.8–11.8 for ligand and metal:ligand systems. Potentiometric measurements were made with a MOLSPIN 1000 titration system and micro-combined glass-silver/silver chloride electrode (Rusell CMAW711) calibrated in hydrogen ion concentration using 1:1 ethanol–HNO_3 solution [6]. The concentration stability constants $\beta_{prq} = [M_pH_rL_q]/[M]^p[H]^r[L]^q$ were calculated with the SUPERQUAD 5.20 computer program [7]. Absorption spectra were recorded with a BECKMAN DU-650 spectrophotometer in 1:1 v/v ethanol–water solution. The metal ion to ligand molar ratio was 1:6 and the metal concentration was 1×10^{-3} mol dm^{-3}. The EPR spectra were recorded on BRUKER ESP 300E spectrometer at X-band (9.3 GHz) at liquid nitrogen in 1:1:1 ethanodiol–water–ethanol solutions. The metal ion to ligand concentration ratios were 1:6 and the metal concentration was 2×10^{-3} mol dm^{-3}. NMR measurements were carried out on Varian VXR300-s by using standard procedures to obtain monodimensional ^1H and ^{13}C or homonuclear and heteronuclear (HETCOR) shift correlated 2D spectra. Typical parameters were ^1H frequency = 299.997 MHz, acquisition time = 3.7 sec., pw = 7 microsec. (pw30); ^{13}C frequency = 75.427 MHz, acquisition time = 1.8 sec., pw = 7 microsec. (pw45). Varian standard Presat and binom sequences were used to obtain solvent suppression.

According to potentiometric data, rutin possesses three protonation constants with pK's 7.49, 8.90 and 11.85 (Table 1). The pH dependence of the proton chemical shifts of the ring protons (Fig. 1) indicates that the first deprotonation with pK 7.49 occurs at C(7)–OH group. The chemical shifts of C(6) and C(8) protons occur at pH range 6–8 (Fig. 1). The second deprotonation with pK 8.90 occurs at catecholic C(4′)–OH site, what is strongly suggested by the distinct chemical shifts at C(5′) proton and much smaller at C(2′) and C(6′) protons in the pH range 8–10. The proton dissociation observed above pH 10.5 (pK = 11.85) does not change distinctly any

proton chemical shift, although the most likely is the deprotonation of the C(5)–OH phenolic group [3]. These pK values differ distinctly from those obtained earlier [4]. The main reason is the use of different solvents in the experiment. The spectroscopic results reported in [4] suggested two pK values ~10.0, which seems to be a doubtful result as the second pK value of catecholic site is well above 12.0 [1]. It should be mentioned here that no assignment of pK values to particular functions was given in [4]. The calculations of the potentiometric data, obtained for Cu^{2+}-rutin solutions, give two almost identical chemical models with very close and very good statistics (Table 1). The species distribution curves, shown in Fig. 2, clearly indicate that both chemical models differ distinctly in high pH region. In the model **A** the predominant complex is the $CuHL_2$ species, while the model **B** indicates at higher pH the equilibrium between the equimolar $CuH_{-1}L$ species and CuL_2 complex. The most sensitive method to check, whether there is one or two different Cu^{2+} species in solution, was EPR method. EPR measurements made for the Cu^{2+}-rutin solutions above pH 8 show two spectra overlapped with each other (Fig. 3). This indicates clearly two different Cu^{2+} complexes in equilibrium. Thus, according to these data the **B** model is more probable than the **A** one. The formation of a stable equimolar species suggests the involvement of the {C(5)–O^-, C(4)=O} donor set in the metal ion binding in this complex. The distinct steric hindrance, derived from the sugar moiety, may protect the formation of the bis-complexes, thus the CuL_2 complex could

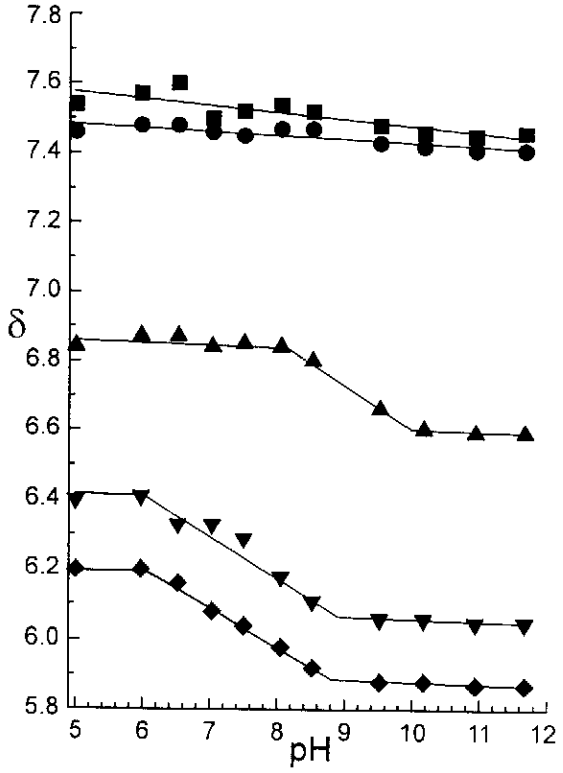


Figure 1. The pH dependence of the proton chemical shift of the ring protons in rutin: –■– C(2′), –●– C(6′), –▲– C(5′), –▼– C(6), –◆– C(8).

involve the catecholic site of rutin. The EPR spectrum of the Cu^{2+}-quercitin (rutin aglycon) basic solution is very similar to that of Cu^{2+}-rutin one. This excludes the involvement of the sugar donor system in the metal ion coordination.

Table 1. Stability constants of the proton (log K) and Cu(II) complexes (log β) of rutin at 25°C and I = 0.1 mol dm^{-3} (KNO_3) in 1:1 water–ethanol solution.

Species		
log K (HL)	11.85(1)	
log K (H_2L)	8.90(1)	
log K (H_3L)	7.49(1)	
	Model	
	A	**B**
log β $[CuH_2L]^+$	27.67(4)	27.93(5)
log β [CuHL]	23.00(6)	23.43(6)
log β $[CuL]^-$	17.13(7)	17.65(6)
log β $[CuH_{-1}L]^{2-}$		11.66(8)
log β $[CuH_2L_2]^{2-}$	42.06(10)	
log β $[CuHL_2]^{3-}$	33.92(12)	
log β $[CuL_2]^{4-}$		26.20(12)

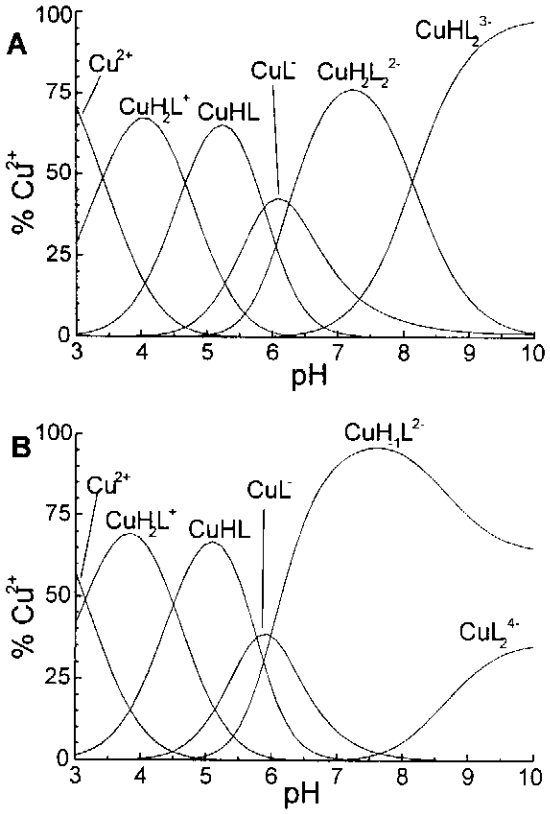


Figure 2. Species distribution curves for Cu^{2+}-rutin system for **A** and **B** models. Metal to ligand molar ratio 1:5, ligand concentration 1.5×10^{-3} mol dm^{-3}.

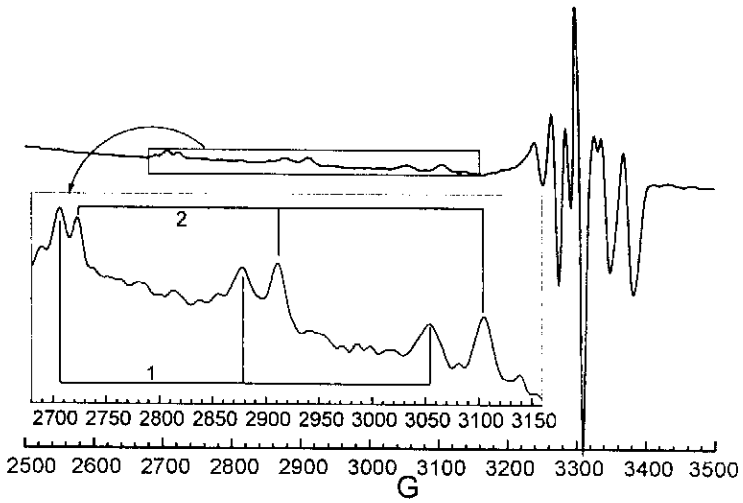


Figure 3. EPR spectrum at 120 K of Cu^{2+}-rutin system in 1:1:1 ethanodiol–water–ethanol solutions in pH = 10. Metal to ligand molar ratio 1:6, ligand concentration 1.2×10^{-2} mol dm^{-3}. (1) and (2) correspond to $CuH_{-1}L$ and CuL_2 species, respectively (see Table 2).

Apigenin is a rutin derivative (Scheme 1), having only one donor set able to form chelate with metal ion, which corresponds to the {C(4)=O, C(5)–O^-} rutin donor set. The EPR spectrum of the Cu^{2+}-apigenin solution is exactly the same as that assigned above for the equimolar complex (Table 2). This strongly supports the assumption made above, that in the $CuH_{-1}L$ complex Cu^{2+} ion binds rutin *via* {C(4)=O, C(5)–O^-} donor set. The parameters ($A_{\|}$ and $g_{\|}$) of the second EPR spectrum, assigned above to CuL_2 complex, are very close to the parameters observed for the bis-complexes of Cu^{2+} with catechol-like ligands [1]. This indicates, that in CuL_2 species metal ion is coordinated by two rutin ligands *via* {C(3′)–O^-, C(4′)–O^-} donor sets. The di-phenolic ring is not crowded by sugar rings and the formation of the bis-complex is sterically favoured. Thus, the Cu^{2+} ion starts the coordination at {C(4)=O, C(5)–O^-} site forming CuH_2L and CuHL species. Very basic catechol site begins the coordination at higher pH and that is why CuL_2 species is formed above pH 7. The resolution of the EPR spectra at pH below 6 did not allow to establish the binding mode in the CuL complex.

Table 2. Spectroscopic parameters for Cu^{2+} complexes formed by rutin for model **B**.

Species	EPR		UV-Vis
	$g_{\|}$	$A_{\|}$ [G]	
$[CuH_2L]^+$	2.415	121	727(47)
[CuHL]	unresolved		703(62)
$[CuH_{-1}L]^{2-}$	$^1g_{\|} = 2.278$	$^1A_{\|} = 177$	647(78)[a]
$[CuL_2]^{4-}$	$^2g_{\|} = 2.249$	$^2A_{\|} = 190$	
Cu-apigenin	$g_{\|} = 2.278$	$A_{\|} = 177$	

[a]shoulder.

Rutin is a very effective natural ligand for Cu^{2+} ions, serving two powerful donor sets, the catecholic oxygen pairs as well as the mixed phenolic-keto set of oxygen. Both donor sets compete for Cu^{2+} ion binding and strong steric effects allow the formation of only one bis-complex with two sets of the catecholic oxygens bound to the metal ion.

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