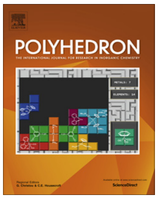
Polyhedron 156 (2018) 89–97

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly



The formation of Ca(II) enolato complexes with α- and β-ketoglutarate in strongly alkaline solutions

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ARTICLE INFO

Article history:
Received 26 July 2018
Accepted 4 September 2018
Available online 12 September 2018

Keywords:
Hyperalkaline solution
Oxocarboxylates
Enolato complexes
Calcium
Stability constants

ABSTRACT

In the present study, the proton dissociation and calcium complex formation reactions of α- and β-ketoglutarate (2- and 3-oxopentane-dioate, α-Ket^{2-} and β-Ket^{2-}, respectively) in neutral and alkaline aqueous solutions were explored. From potentiometric measurements, the equilibrium constants of the [Ca(α-Ket)]^0 and [Ca(β-Ket)]^0 complexes formed in neutral medium were determined to be $\log K^{\alpha}_{1,1} = 1.15 \pm 0.02$ and $\log K^{\beta}_{1,1} = 0.78 \pm 0.02$, respectively. In alkaline medium, it was found for both ligands that the methylene group underwent deprotonation yielding the carbanion-enolate anion. The deprotonation constants, $\mathrm{p}K_a{}^{\alpha}$ and $\mathrm{p}K_a{}^{\beta}$, were found to be 15.16 ± 0.01 and 14.41 ± 0.01, respectively. The calcium complexing behaviour of the two ligands was also studied in strongly alkaline medium. Under these conditions, the Ca^{2+} ion was capable of promoting the deprotonation of the ligands and forming various enolato-type complexes. The formation of both mono- and binuclear species were deduced, their formulae are suggested to be $[\mathrm{Ca}(\alpha\text{-Ket})\mathrm{H}_{-1}]^-$, $[\mathrm{Ca}_2(\alpha\text{-Ket})\mathrm{H}_{-3}]^-$, $[\mathrm{Ca}(\beta\text{-Ket})\mathrm{H}_{-1}]^-$, $[\mathrm{Ca}_2(\beta\text{-Ket})\mathrm{H}_{-1}]^+$ with the formation constants of $\log\ \beta^{\alpha}_{11-1} = -11.91 \pm 0.02$, $\log\ \beta^{\alpha}_{21-3} = -36.10 \pm 0.06$, $\log\ \beta^{\beta}_{11-1} = -11.58 \pm 0.01$, $\log\ \beta^{\beta}_{21-1} = -10.80 \pm 0.03$, respectively. Beside the formation of the rare Ca^{2+}-enolato complexes, the deprotonation of the hydrating water molecules around the Ca^{2+} ion was also invoked in some cases.

1. Introduction

The complex formation between calcium and hydroxycarboxylate ligands in highly alkaline aqueous solutions has profound practical relevance. It has been reported that various mono- (D-gluconate [1], D-heptagluconate [2] and L-gulonate [3]) as well as bi- or tricarboxylates (*e.g.*, citrate [4] and tartrate [5]) formed unexpectedly stable calcium(II) complexes in hyperalkaline (pH > 12) medium. This increased stability in caustic medium stems from the ability of calcium to induce the deprotonation of a rather weak acid, such as an aliphatic alcohol. Concerning oxocarboxylates (*i.e.*, α- and β-ketoglutarate), which are the oxidized forms of hydroxycarboxylates, analogous deprotonation is expected to occur on the methylene group adjacent to the oxo group resulting in the formation of an enolate ion. This may give rise to the formation of complexes with high stability, similarly to those observed for sugar-type ligands.

α-Ketoglutaric acid (2-oxopentanedioic acid, H$_2$(α-Ket), Scheme 1) is an important metabolite and substrate of several enzymes [6–8]. The two carboxylic groups of H$_2$(α-Ket) act as weak acids in water. From potentiometric measurements, the pK_a of the first (pK_1) and second (pK_2) deprotonation steps were determined to be 2.8 and 4.8 (I = 0.1 M) [9] as well as 2.0 and 4.6 (I was not reported) [10]. According to ^{1}H NMR measurements, α-ketoglutaric acid exists as an equilibrium mixture of the cyclic hemiketal (cyclo, 16%), keto (31%) and geminal diol (53%) forms at pH 0.5 (Scheme 1) [11,12]. ^{13}C NMR spectroscopic and UV spectrophotometric measurements yielded somewhat different distribution among the various species (30% cyclo, 35% geminal diol and 25% keto forms) [6]. However, it was shown that in neutral (or close-to-neutral) solutions, α-Ket^{2-} existed predominantly as the keto form with about 7% geminal diol [6,11].

The behaviour of α-Ket^{2-} in alkaline solutions was examined by Kozlowski and Zuman [13]. It was found that upon increasing the pH of a solution containing α-Ket^{2-}, the methylene group adjacent

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https://doi.org/10.1016/j.poly.2018.09.005
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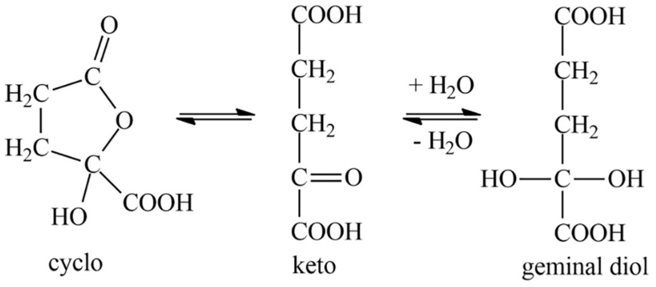
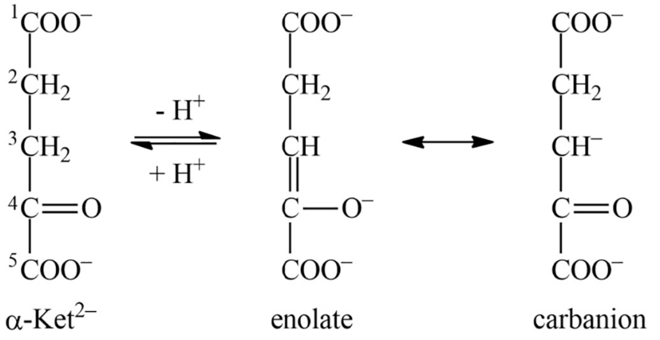


Scheme 1. The possible forms of α-ketoglutarate in acidic aqueous solutions.



Scheme 2. The structural formula of α-Ket^{2-} and the resonance structures of $(\alpha\text{-Ket})\text{H}_{-1}^{3-}$.

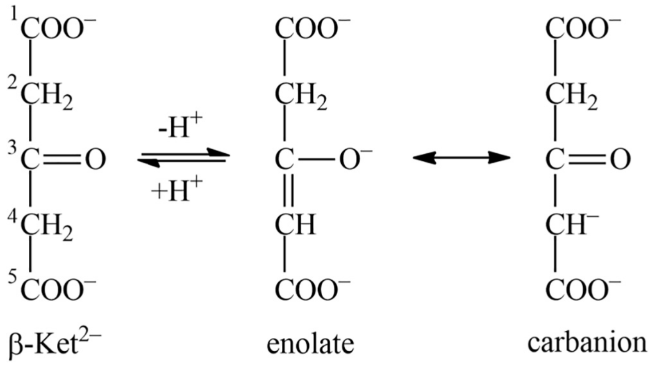
to the C=O group underwent deprotonation resulting in the formation of the carbanion and enolate resonance forms (Scheme 2). It was also demonstrated that a new peak appeared on the UV absorption spectrum corresponding to the absorption of the carbanion-enolate ion at 263 nm. Evaluating the spectral changes, the deprotonation constant of α-Ket^{2-} was found to be pK = 15.8 [13] (the ionic strength was not held constant).

To the best of our knowledge, only Schubert and Lindenbaum reported about the complex formation between calcium and α-Ket^{2-} in neutral medium [14]. In the presence of α-Ket^{2-}, the neutral $[\text{Ca}(\alpha\text{-Ket})]^0$ species was found to be formed. Its formation constant, log K_{11}, was determined to be 1.29 (I = 0.16 M) *via* ion-exchange measurements.

The complexing properties of α-Ket^{2-} in the presence of several other metal ions were studied. Sr^{2+} ions were found to form the $\text{Sr}(\alpha\text{-Ket})^0$ species with log K_{11} being 1.14 [14]. In the presence of Cu^{2+} or Fe^{3+} ions, the formation of the $[\text{Cu}(\alpha\text{-Ket})]^0$ and $[\text{Cu}(\alpha\text{-Ket})_2]^{2-}$, as well as the $[\text{Fe}(\alpha\text{-Ket})]^+$ and the $[\text{Fe}(\alpha\text{-Ket})_2]^-$ species were detected [9]. Similarly to Cu(II) and Fe(III), Zn(II) also forms $[\text{Zn}(\alpha\text{-Ket})]^0$ and $[\text{Zn}(\alpha\text{-Ket})_2]^{2-}$ complexes [10]. The interactions of α-Ket^{2-} with Al(III) were examined in the acidic pH range and diverse complexation processes were observed *via* pH potentiometry, cyclic voltammetry and spectrophotometry [15]. Besides the mononuclear $[\text{AlH}(\alpha\text{-Ket})]^{2+}$, $[\text{Al}(\alpha\text{-Ket})]^+$, $[\text{Al}(\alpha\text{-Ket})\text{H}_{-1}]^0$, $[\text{Al}(\alpha\text{-Ket})_2]^-$ and $[\text{Al}(\alpha\text{-Ket})_2\text{H}_{-2}]^{3-}$ species, the binuclear $[\text{Al}_2(\alpha\text{-Ket})]^{4+}$ complex was also detected.

β-Ketoglutaric acid (3-oxopentanedioic acid, $\text{H}_2(\beta\text{-Ket})$) or most importantly its derivatives are important starting materials in different organic reactions [16,17]. $\text{H}_2(\beta\text{-Ket})$ can also be used in polymerization reactions as water soluble photoinitiator [18]. β-Ket^{2-} (Scheme 3) was recognized as one of the abiotic oxidation products of citrate released into manganese-rich soils by plants, bacteria and fungi [19]. The acid-base as well as the metal ion complexing properties of β-Ket^{2-} are rather poorly described.

The photoproduct of aerobactin, which contains a β-ketoglutaryl moiety, was shown to coordinate Fe^{3+} ions efficiently [20].



Scheme 3. The structural formula of β-Ket^{2-} and the resonance structures of $(\beta\text{-Ket})\text{H}_{-1}^{3-}$.

To the best of our knowledge, no further data are available about the metal complexes of β-Ket^{2-}.

In the present study, the deprotonation and Ca(II) complexation equilibria of α- and β-Ket^{2-} were studied by potentiometry, ^{1}H NMR spectroscopy and UV spectrophotometry. Our aim was to explore whether calcium was able to promote the deprotonation of an oxocarboxylic acid and to form enolato complexes in strongly alkaline aqueous solutions.

2. Experimental

2.1. Reagents and solutions

Sodium α-ketoglutarate and sodium β-ketoglutarate stock solutions were prepared by neutralizing α-ketoglutaric acid (Sigma–Aldrich, ≥99% purity) and β-ketoglutaric acid (Acros Organics, ≥97% purity) with NaOH (Analar Normapur, a. r. grade) solution adjusting the pH to ~8. CaCl_2 stock solutions were prepared by dissolving $\text{CaCl}_2{\cdot}2\text{H}_2\text{O}$ (Sigma–Aldrich, ≥99% purity) in water, and the exact calcium concentration was determined by complexometric titration using EDTA. The ionic strength was adjusted to 4.0 M with NaCl (Molar Chemicals, a. r. grade) in each sample. All reagents were dissolved in Milli-Q Millipore water.

2.2. Potentiometric titrations

Potentiometric titrations were conducted on a Metrohm 794 Titrando instrument using a combined calcium ion-selective electrode (Metrohm, type 6.0510.100). The measurements were performed at constant ionic strength (4.0 M) and temperature ((25.0 ± 0.1) °C), applying continuous stirring. For the calibrations, the response of the electrode at 4.0 M ionic strength was found to be nonlinear in the $-4.0 < \log([\text{Ca}^{2+}]/\text{M}) < -1.0$ range, thus, a nonlinear calibration procedure was applied by fitting splines onto the calibration curve using the Spline Calculus program [21]. During calculations, the $\log([\text{Ca}^{2+}]/\text{M})$ values were fitted as a function of added volume of the titrant with the aid of the PSEQUAD software [22]. Hereafter, for species X, $[\text{X}]_{\text{T},0}$ or $[\text{X}]_\text{T}$ denote its total initial concentration (titrations) or its analytical concentration (NMR and photometric measurements), while [X] represents its equilibrium concentration, respectively.

The titrations were performed as follows: for α-Ket^{2-}, 70 mL of a solution containing $[\text{CaCl}_2]_{\text{T},0} = 10^{-4}$ M was titrated with $[\text{CaCl}_2]_{\text{T},0}$ = 0.2015 M CaCl_2 up to 120 mL, where $[\alpha\text{-Ket}^{2-}]_{\text{T},0}$ was set to 0 M (calibration) as well as to 0.1071, 0.1429 and 0.1786 M. For β-Ket^{2-}, 75 mL of a solution containing $[\text{CaCl}_2]_{\text{T},0} = 10^{-4}$ M as initial concentration was titrated with $[\text{CaCl}_2]_{\text{T},0}$ = 0.1002 M CaCl_2 up to 120 mL, where $[\beta\text{-Ket}^{2-}]_{\text{T},0}$ was set to 0 M (calibration) as well as to 0.0500, 0.1186 and 0.1584 M. The experimental conditions were the same for the calibrations and measurements alike.

2.3. ^{1}H NMR spectroscopic measurements

The ^{1}H NMR spectra were recorded on a Bruker Avance DRX 500 MHz spectrometer equipped with a 5 mm inverse broadband probe head furnished with *z*-oriented magnetic field gradient capability. The magnetic field was stabilized by locking it to the ^{2}D signal of the solvent prior to the measurements. The sample temperature was set to (25 ± 1) °C during all measurements. For the individual samples, 64 scans were acquired to record the ^{1}H NMR spectra.

The NMR experiments were conducted under the same experimental conditions for both ligands. During the determination of the proton dissociation constant, [α/β-Ket^{2-}]$_T$ was kept constant (0.20 M), while [OH$^-$]$_T$ was systematically increased from 0.0 M to 2.7 M. During the calcium(II) complexation experiments, [α/β-Ket^{2-}]$_T$ = 0.01 M and [OH$^-$]$_T$ = 0.1 M was set in each solution, while [Ca^{2+}]$_T$ was varied between 0.0 M and 0.01 M. The ionic strength was set to 4.0 M with NaCl in all samples.

2.4. UV spectrophotometric measurements

UV absorption spectra were recorded on a Shimadzu UV-1650 PC double beam spectrophotometer in the 200–400 nm wavelength range at 4.0 M ionic strength and at (25 ± 2) °C. The measurements were carried out in a quartz cuvette having an optical path length of 1 cm.

During the deprotonation experiments, the ligand concentration was held constant while the concentration of NaOH was systematically increased. For α-Ket^{2-}, [α-Ket^{2-}]$_T$ was set to 0.001 M while [OH$^-$]$_T$ was varied between 0.05 M and 3.50 M. For β-Ket^{2-}, [β-Ket^{2-}]$_T$ was set to 0.0001 M or 0.0002 M and [OH$^-$]$_T$ was increased from 0.11 M to 2.00 M or to 0.90 M, respectively.

For studying the calcium(II) complexation in alkaline medium, [Ca^{2+}]$_T$ was varied at constant [α/β-Ket^{2-}]$_T$ and [OH$^-$]$_T$, or [α-Ket^{2-}]$_T$ was varied at constant [OH$^-$]$_T$ and [Ca^{2+}]$_T$. In total, seven α-Ket^{2-}-containing and six β-Ket^{2-}-containing solution sets were prepared. The applied concentrations of [α/β-Ket^{2-}]$_T$, [OH$^-$]$_T$ and [Ca^{2+}]$_T$ are listed in Table 1.

During data evaluation, the absorbance values were fitted as a function of [OH$^-$]$_T$ or [Ca^{2+}]$_T$ with the aid of the PSEQUAD software [22].

3. Results and discussion

3.1. Calcium complexation of α- and β-Ket^{2-} in neutral medium

Fig. 1 shows the Ca-ISE titration curves for α- and β-Ket^{2-} performed at *I* = 4.0 M and *T* = 25 °C. By increasing [α/β-Ket^{2-}]$_{T,0}$, the cell potential systematically decreases indicating complex formation. The three data sets were fitted simultaneously by minimizing the *F* fitting parameter. For a certain data set, F stands for the average difference between experimental and calculated values [22]. In the case of α-Ket^{2-}, the initial *F* = 0.45 log ([Ca^{2+}]/M) unit could be decreased to 0.024 assuming the formation of the [Ca(α-Ket)^0 complex with log β_{110} = 1.15. In general, β_{pq-r} can be defined as

$$\beta_{pq\text{-}r} = \frac{[\mathrm{Ca}_p\mathrm{L}_q\mathrm{H}_{\text{-}r}{}^{(2p\text{-}2q\text{-}r)+}][\mathrm{H}^+]^r}{[\mathrm{Ca}^{2+}]^p[\mathrm{L}^{2-}]^q c^{\mathrm{o}\,1+r\text{-}p\text{-}q}} \tag{1}$$

where L^{2-} = α/β-Ket^{2-} and $c^{\ominus}$ = 1 mol dm^{-3}. Accordingly, β_{110} is defined as

$$\beta_{110} = \frac{[\mathrm{CaKet}^0]c^{\mathrm{o}}}{[\mathrm{Ca}^{2+}][\mathrm{Ket}^{2\text{-}}]} \tag{2}$$

For β-Ket^{2-}, the initial *F* = 0.22 could be decreased to 0.016 assuming the [Ca(β-Ket)]^0 complex with log β_{110} = 0.78.

The difference in the stability of [Ca(α-Ket)]^0 and [Ca(β-Ket)]^0 is most probably due to the difference in the structure of the complexes. Previously, it was pointed out in the literature that complexes with five-membered chelate rings are usually more stable than those having six-membered rings [23–27]. Concerning the Ca(II) ketoglutarate species, the possible binding sites of the ligands are one carboxylate group and the oxo group. In [Ca(α-Ket)]^0 a five-membered chelate is formed, while in Ca[(β-Ket)]^0 a putatively less stable six-membered chelate is present (Scheme 4).

The value obtained for α-Ket^{2-} is not very much different from the one reported previously (log β_{110} = 1.29, *I* = 0.16 M) [14], the difference is most probably due to the differences in the ionic strength.

Fig. 2 demonstrates that these stability constants lead to significant formation of the [Ca(α-Ket)]^0 and [Ca(β-Ket)]^0 complexes (up to 58% and 38%, respectively).

3.2. Deprotonation of α- and β-Ket^{2-} in strongly alkaline medium

The ^{1}H NMR spectrum of α-Ket^{2-} consists of two well-separated triplets corresponding to the two methylene groups of the anion. Upon addition of NaOH to a solution containing [α-Ket^{2-}]$_T$ = 0.2 M, the peak at around 2.8 ppm (C3) broadens and shifts upfield (Fig. 3). This variation is the consequence of the deprotonation of this methylene group yielding the carbanion-enolate resonance forms ((α-Ket)H$_{-1}^{3-}$, Scheme 2).

Furthermore, the significant broadening of the signal indicates that the interconversion between α-Ket^{2-} and (α-Ket)H$_{-1}^{3-}$ is slow on the NMR timescale. That is, the deprotonation of the CH$_2$ group (and the parallel formation of the carbanion-enolate) is a relatively slow reaction compared to other deprotonation processes. For instance, the deprotonation rate constant of the pyruvate ion in alkaline medium can be estimated as 5.7·10^{-1} M^{-1} s^{-1} from the data given in Ref. [28].

Table 1
The concentrations of α/β-Ket^{2-}, OH$^-$ and Ca^{2+} applied for the UV spectrophotometric experiments performed in alkaline medium.

α-Ket^{2-}			β-Ket^{2-}		
[α-Ket^{2-}]$_T$/M	[OH$^-$]$_T$/M	[Ca^{2+}]$_T$/M	[β-Ket^{2-}]$_T$/M	[OH$^-$]$_T$/M	[Ca^{2+}]$_T$/M
0.001	0.010	0–0.179	0.00013	0.010	0–0.180
0.002	0.010	0–0.179	0.00040	0.010	0–0.180
0.001	0.015	0–0.103	0.00020	0.020	0–0.064
0.002	0.015	0–0.103	0.00015	0.040	0–0.029
0.001	0.070	0–0.0198	0.00025	0.040	0–0.029
0.0004–0.002	0.015	0.080	0.00025	0.080	0–0.0071
0.0004–0.002	0.040	0.008			

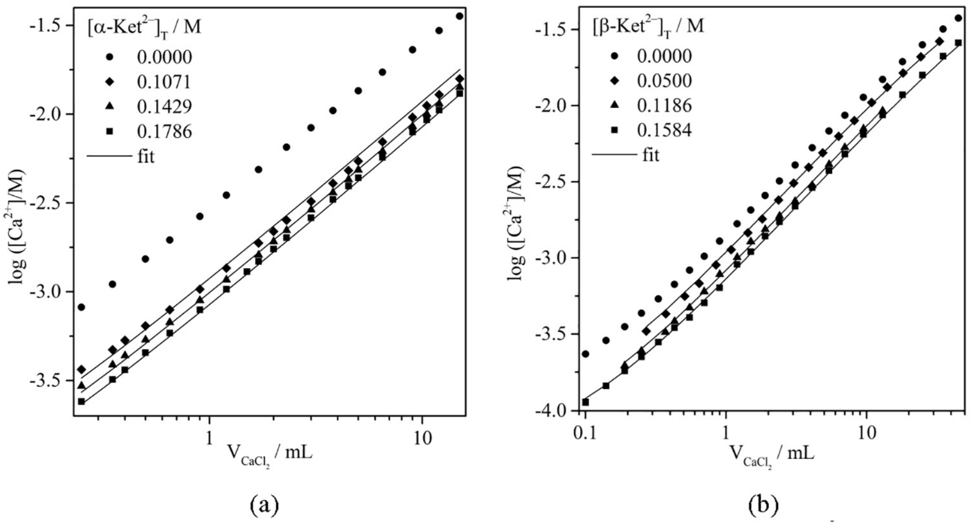
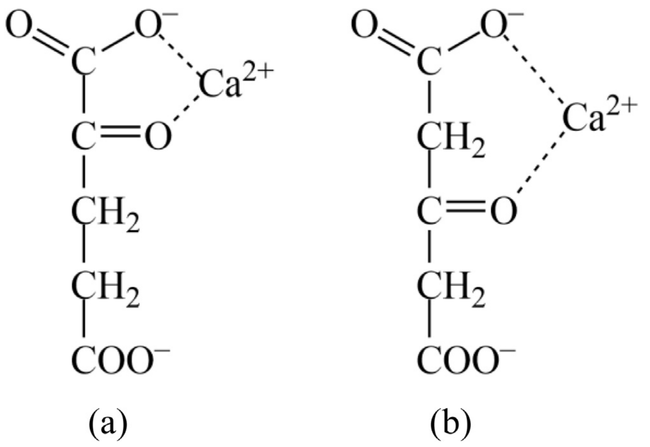


Fig. 1. Ca-ISE potentiometric titration curves of solutions containing α-Ket^{2-} (a) or β-Ket^{2-} (b) and Ca^{2+} in neutral medium as a function of the added titrant volume (log scale). Experimental conditions: I = 4.0 M (NaCl), T = (25.0 ± 0.1) °C, V_0 = 70 mL (a) or 75 mL (b); $[Ca^{2+}]_{T,0} = 10^{-4}$ M, titrant: $[CaCl_2]_T$ = 0.2015 M (a) or 0.1002 M (b). The initial concentrations of α/β-Ket^{-} are shown in the figures. Symbols represent the experimental, while solid lines denote the calculated data.



Scheme 4. The possible structure of the $[Ca(\alpha\text{-Ket})]^0$ (a) and $[Ca(\beta\text{-Ket})]^0$ (b) complexes.

To determine the acid dissociation constant of α-Ket^{2-}, UV spectrophotometric measurements were carried out for a solution of $[\alpha\text{-Ket}^{2-}]_T$ = 0.001 M (see Fig. 4). Upon increasing $[NaOH]_T$, a new peak next to the absorption band of Ket^{2-} emerges with a maximum at 263 nm, which is characteristic for the carbanion-enolate form [13]. Hence, these spectra were suitable for the determination of the deprotonation constant, pK_a, of α-Ket^{2-}. For these computations, the ionic product of water, pK_w, was taken as 14.26 [29].

The initial value of the F parameter (0.50 absorbance unit) dropped to 0.006 by assuming the formation of $(\alpha\text{-Ket})H_{-1}^{3-}$ with pK_a = 15.17. The molar absorbance of the deprotonated species at 263 nm was found to be $\varepsilon = 3800\ M^{-1}\ cm^{-1}$. These values are in reasonable agreement with literature data reported by Kozlowski and Zuman (pK_a = 15.8 and $\varepsilon = 4800\ M^{-1}\ cm^{-1}$) [13]. The extent of deprotonation reaches 31% at most (at 3.5 M NaOH).

The carbanion-enolate form, however, can undergo protonation on both the enolate (O$^-$) and the carbanion (CH$^-$) moieties which can be deprotonated *vice versa*. Hence, the value for pK_a presented here is composed of the deprotonation constants for the oxo (p$K_{a,oxo}$) and the enol (p$K_{a,enol}$) forms of ketoglutarate.

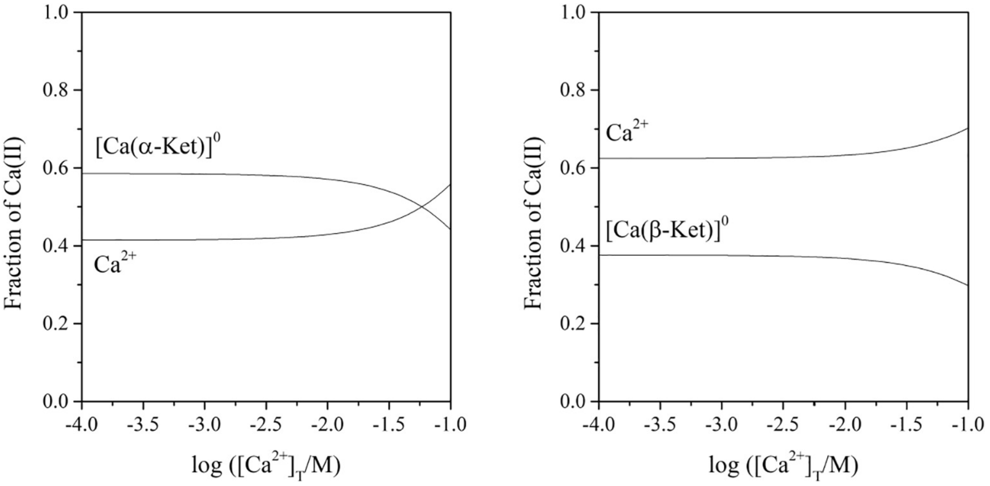


Fig. 2. Distribution of Ca(II) among the various aqueous species in a solution containing $[\alpha/\beta\text{-Ket}^{2-}]_T$ = 0.1 M as a function of log ($[Ca^{2+}]_T$/M). The simulations were based on stability constants determined at I = 4 M (NaCl) and T = 25 °C.

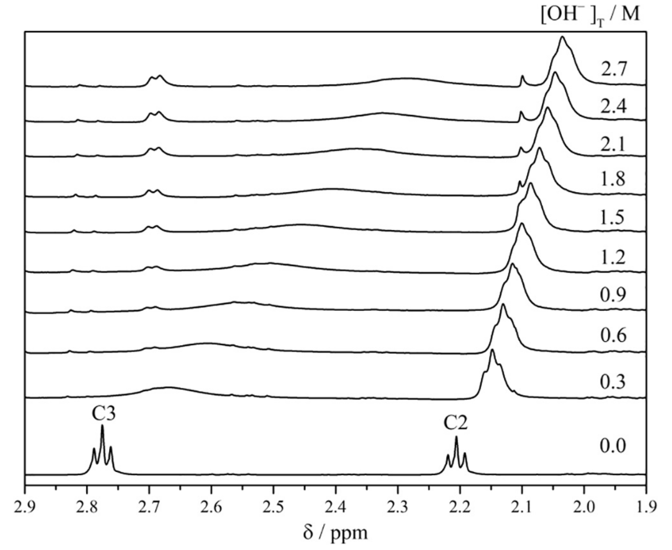


Fig. 3. ^1H NMR spectra of solutions containing $[\alpha\text{-Ket}^{2-}]_T = 0.20$ M and $[OH^-]_T = 0$–2.7 M; $I = 4.0$ M (NaCl), $T = (25 \pm 1)$ °C. C2 and C4 represent the signals of protons residing on the C2 and C3 methylene groups.

$$\frac{1}{K_a}=\frac{[\mathrm{Ket}^{2-}]c^o}{[\mathrm{KetH}_{-1}{}^{3-}][\mathrm{H}^+]}=\frac{[\mathrm{oxo}^{2-}]c^o}{[\mathrm{KetH}_{-1}{}^{3-}][\mathrm{H}^+]}+\frac{[\mathrm{enol}^{2-}]c^o}{[\mathrm{KetH}_{-1}{}^{3-}][\mathrm{H}^+]}=\frac{1}{K_{a,\mathrm{oxo}}}+\frac{1}{K_{a,\mathrm{enol}}} \qquad (3)$$

For oxaloacetic acid, the $pK_{a,\mathrm{enol}}$ and $pK_{a,\mathrm{oxo}}$ were found to be 12.4 and 13.2 [13], while for pyruvic acid the difference is much larger ($pK_{a,\mathrm{enol}} = 11.54$ and $pK_{a,\mathrm{oxo}} = 16.58$) [28]. Consequently, $pK_a \approx pK_{a,\mathrm{oxo}}$, which can also be assumed for ketoglutarate since its structure is similar to that of oxaloacetic acid.

The ^1H NMR spectrum of the solution containing $[\beta\text{-Ket}^{2-}] = 0.2$ M consists of one singlet corresponding to the two chemically identical methylene groups (C2 and C4). On the increase of $[\mathrm{NaOH}]_T$, this peak broadens and shifts upfield (Fig. 5). In parallel, two new peaks appear corresponding to C2 at around 3.7 ppm and the deprotonated C4 at around 3 ppm (see Scheme 3). The marked increase in the intensity of these peaks refers to the increasing conversion of the deprotonation, which is considerably higher than that of $\alpha\text{-Ket}^{2-}$. Similarly to $\alpha\text{-Ket}^{2-}$, the remarkable widening of the NMR signals of $\beta\text{-Ket}^{2-}$ corroborates that the rate of the carbanion-enolate formation is slow on the ^1H NMR timescale.

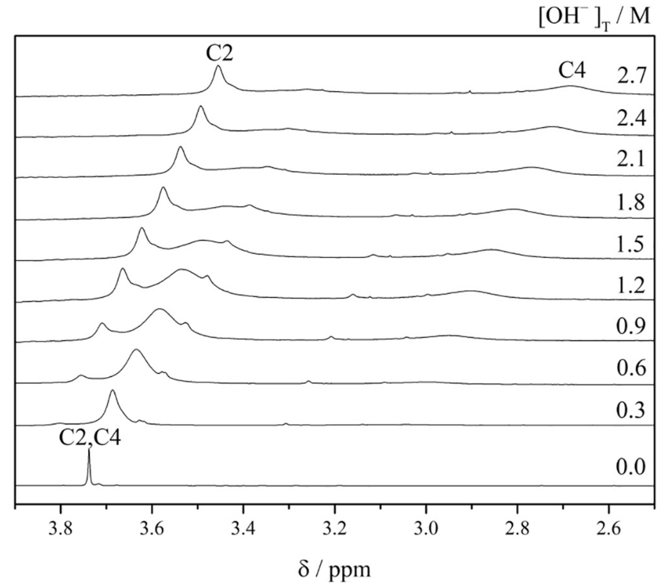


Fig. 5. ^1H NMR spectra of solutions containing $[\beta\text{-Ket}^{2-}]_T = 0.20$ M and $[OH^-]_T = 0$–2.7 M; $I = 4.0$ M (NaCl), $T = (25 \pm 1)$ °C. C2 and C4 represent the signals of protons residing on the C2 and C4 methylene groups.

To determine the deprotonation constant of $\beta\text{-Ket}^{2-}$, UV spectrophotometric measurements were carried out at constant ionic strength (4.0 M) Upon addition of NaOH to a solution containing $[\beta\text{-Ket}^{2-}]_T = 10^{-4}$ M (Fig. 6) or $2{\cdot}10^{-4}$ M (Fig. S1), CH deprotonation results in the appearance of a new absorption band at 266 nm corresponding to the carbanion-enolate ion ($(\beta\text{-Ket})\mathrm{H}_{-1}^{3-}$). By the data evaluation, the pK_a of $\beta\text{-Ket}^{2-}$ was determined to be 14.41 with $F = 0.006$ absorbance unit. The molar absorbance of the deprotonated species was found to be $\varepsilon = 18600\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$. The pK_a determined in this work is composed of $pK_{a,\mathrm{oxo}}$ and the $pK_{a,\mathrm{enol}}$ constants as in the case of the α isomer.

The difference in the deprotonation constants of $\alpha\text{-Ket}^{2-}$ and $\beta\text{-Ket}^{2-}$ is most probably due to the fact that the $(\beta\text{-Ket})\mathrm{H}_{-1}^{3-}$ has more extended conjugation (through five bonds) providing higher stability over $(\alpha\text{-Ket})\mathrm{H}_{-1}^{3-}$, in which such conjugation is not possible. Additionally, the extended conjugation in $(\beta\text{-Ket})\mathrm{H}_{-1}^{3-}$ is a plausible explanation for its higher molar absorbance.

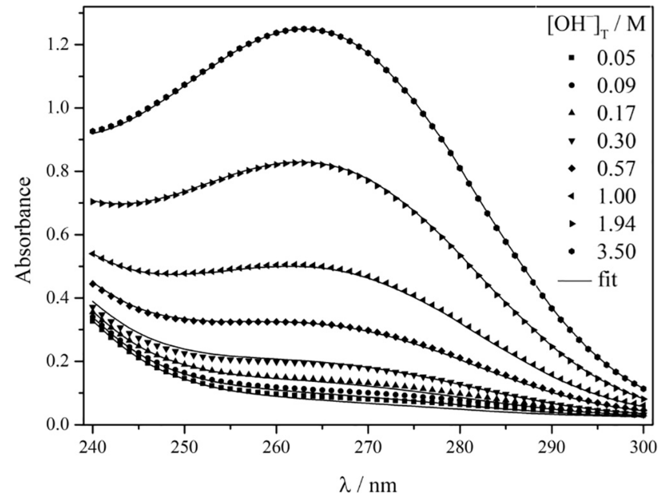


Fig. 4. UV absorption spectra of solutions containing $[\alpha\text{-Ket}^{2-}]_T = 0.001$ M and $[OH^-]_T = 0$–3.5 M; $I = 4.0$ M (NaCl), $T = (25 \pm 2)$ °C. Symbols represent the experimental, while solid lines denote the calculated data.

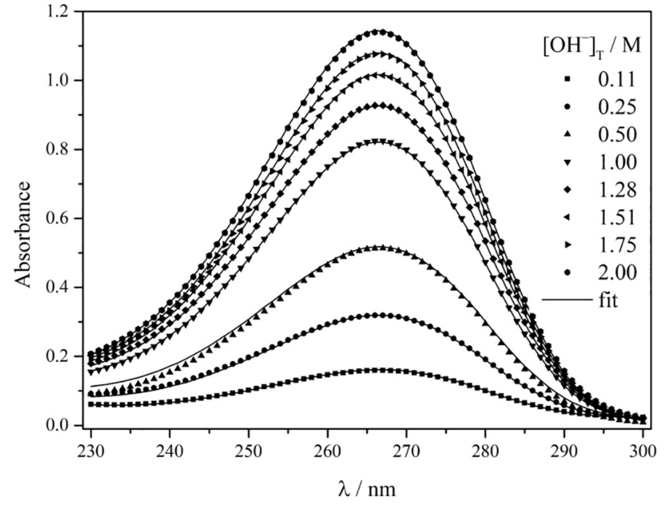


Fig. 6. UV absorption spectra of solutions containing $[\beta\text{-Ket}^{2-}]_T = 0.0001$ M and $[OH^-]_T = 0$–2.0 M; $I = 4.0$ M (NaCl), $T = (25 \pm 2)$ °C. Symbols represent the experimental, while solid lines denote the calculated data.

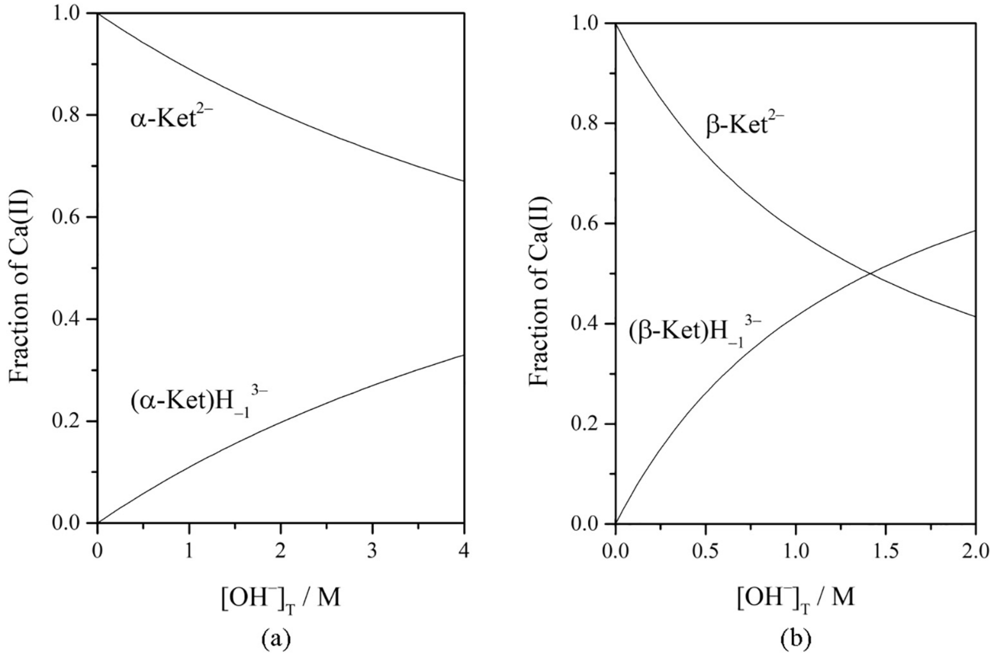


Fig. 7. Distribution among the various aqueous species in a solution containing $[\alpha\text{-Ket}^{2-}]_T$ = 0.001 M (a) or $[\beta\text{-Ket}^{2-}]_T$ = 0.0001 M (b) as a function of $[OH^-]_T$. The simulations were based on stability constants determined at *I* = 4 M (NaCl) and T = 25 °C.

Fig. 7 shows that the extent of deprotonation is 31% for $\alpha\text{-Ket}^{2-}$ and 59% for $\beta\text{-Ket}^{2-}$ under the experimental conditions applied during the UV spectrophotometric measurements. The higher value for $\beta\text{-Ket}^{2-}$ is in good agreement with the qualitative findings deduced from the NMR spectra.

3.3. Calcium complexation of α- and β-Ket^{2-} in highly alkaline medium

Upon addition of Ca^{2+} to a solution containing $[\alpha\text{-Ket}^{2-}]_T$ = 0.01 M and $[OH^-]_T$ = 0.1 M, the peak of C3 broadens on the ^{1}H NMR spectra (Fig. 8). This pattern is similar to that observed for deprotonation (Fig. 3) suggesting the formation of the carbanion-enolate form. Contrary to the spectra in Fig. 3, the upfield shift of this broad signal is not seen implying lower electron density on the ^{1}H nuclei. This finding proves that in parallel to deprotonation, the coordination of Ca^{2+} ions takes place, too.

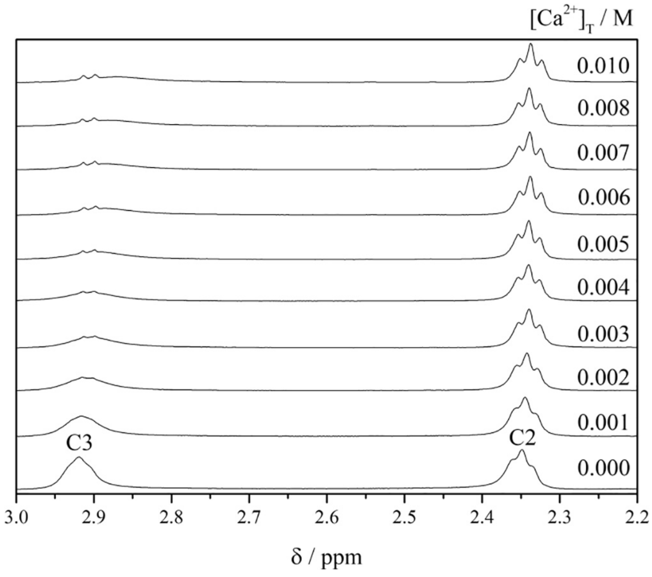


Fig. 8. ^{1}H NMR spectra of solutions containing $[\alpha\text{-Ket}^{2-}]_T$ = 0.01 M, $[OH^-]_T$ = 0.1 M and $[Ca^{2+}]_T$ = 0–0.01 M; *I* = 4.0 M (NaCl), *T* = (25 ± 1) °C.

Moreover, based on the UV-spectrophotometric results, at this particular solution composition and in the absence of Ca^{2+}, the extent of deprotonation would be only 2%. Thus, the extent of the spectral changes in the Ca^{2+}-containing system attests the formation of complexes in which the carbanion-enolate ion is formed.

The complex formation was also examined by UV spectrophotometry. The amount of Ca^{2+} is limited by its solubility in NaOH because of the precipitation of portlandite, $Ca(OH)_2(s)$. However, the applied NaOH-concentrations (0.01, 0.015 or 0.07 M over seven series) allowed to increase the Ca^{2+} concentration to 0.18, 0.1 or 0.02 M, respectively, which exceeded the concentration of the ligand (0.001–0.002 M).

The results of these measurements are shown in Figs. 9 and S2–S6. During data processing, the absorbance values were fitted as a function of $[Ca^{2+}]_T$. Additionally, the formation constants of the two hydroxido complexes of calcium(II), $CaOH^+$ and $Ca(OH)_2^0$, were taken from Ref. [30]. The initial value of *F* (0.079 absorbance unit) could be lowered to 0.016 assuming the formation of the $[Ca(\alpha\text{-Ket})H_{-1}]^-$ complex. The respective formation constant was calculated to be $\log \beta_{11-1} = -11.82$. The systematic difference between the experimentally observed and calculated absorbances were, however, still much larger than acceptable, which indicated the formation of further complex or complexes.

Several chemically meaningful compositions were tested, and the *F* fitting parameter could be further decreased to 0.0045 with the assumption of the $[Ca_2(\alpha\text{-Ket})H_{-3}]^-$ and the $[Ca(\alpha\text{-Ket})H_{-1}]^-$ species with the corresponding formation constants being $\log \beta_{11-1} = -11.91$ and $\log \beta_{21-3} = -36.10$. Since it is very unlikely that $(\alpha\text{-Ket})H_{-1}^{3-}$ undergoes a second deprotonation step, the $[Ca_2(\alpha\text{-Ket})H_{-3}]^-$ multinuclear complex should be a mixed hydroxido-enolato species, in which one OH^- ion is bound to each calcium ion.

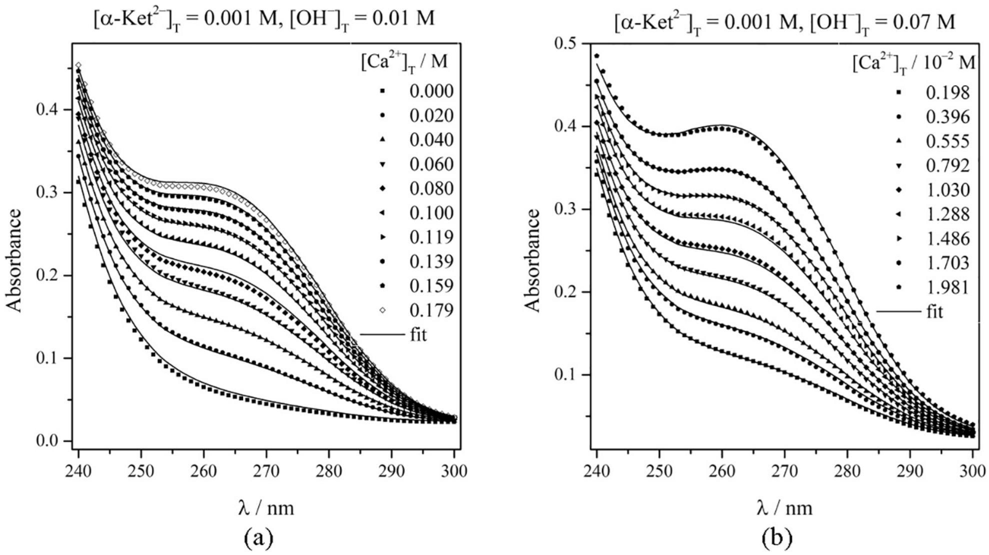


Fig. 9. UV absorption spectra of solutions containing $[\alpha\text{-Ket}^{2-}]_T = 0.001$ M and $[OH^-]_T = 0.01$ M (a) or 0.07 M (b) and increasing amount of Ca^{2+}; $I = 4$ M (NaCl), $T = (25 \pm 2)$ °C. Symbols represent the experimental, while solid lines denote the calculated data.

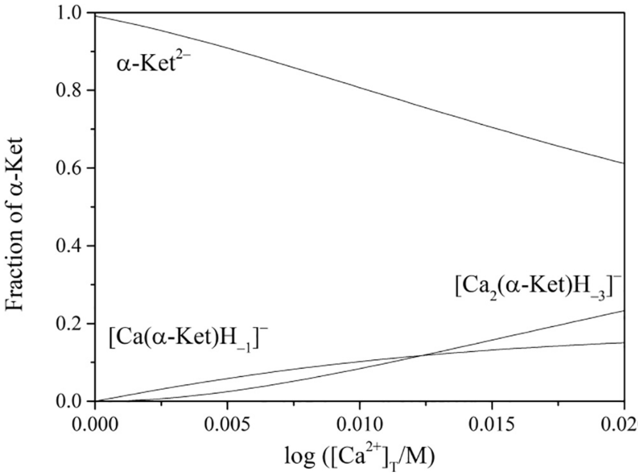


Fig. 10. Distribution among the various aqueous species in a solution containing $[\alpha\text{-Ket}^{2-}]_T = 0.001$ M and $[OH^-]_T = 0.07$ M as a function of log ($[Ca^{2+}]_T$/M). The simulations were based on stability constants determined at $I = 4$ M (NaCl) and T = 25 °C.

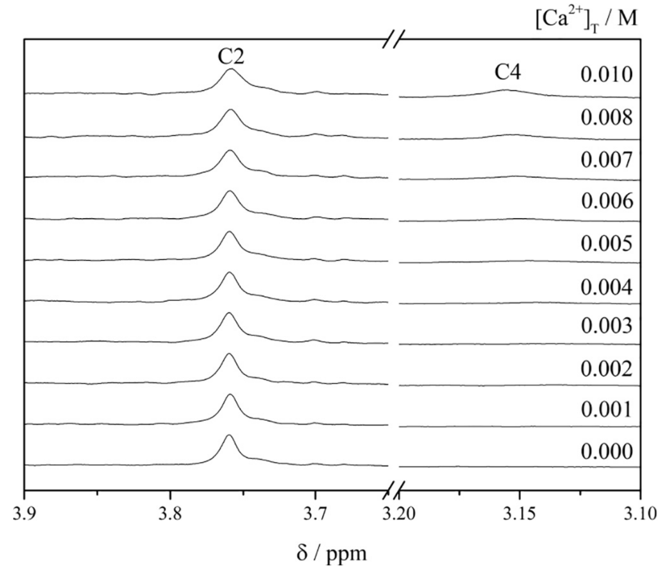


Fig. 11. ^1H NMR spectra of solutions containing $[\beta\text{-Ket}^{2-}]_T = 0.01$ M, $[OH^-]_T = 0.1$ M and $[Ca^{2+}]_T = 0$–0.01 M; $I = 4.0$ M (NaCl), $T = (25 \pm 1)$ °C.

The concentration distribution diagram is seen on Fig. 10, where the fraction of $\alpha\text{-Ket}^{2-}$ is shown as a function of log ($[Ca^{2+}]_T$/M).

Analogous measurements were performed for $\beta\text{-Ket}^{2-}$ as well. Increasing the calcium concentration in a solution containing $[\beta\text{-Ket}^{2-}]_T = 0.01$ M and $[OH^-]_T = 0.1$ M, a new peak emerges on the ^1H NMR spectra at around 3.15 ppm (Fig. 11). This change is similar to that observed in the course of the deprotonation suggesting the formation of the carbanion-enolate ion. The positions of both peaks are significantly shifted to higher chemical shifts (*ca.* 3.75 and 3.15 ppm) compared to those seen in Fig. 5 (ca. 3.5 and 2.7 ppm, $[OH^-]_T = 2.7$ M). In conclusion, the electron density decreases in the Ca^{2+}-containing system due to complex formation.

Furthermore, under these experimental conditions, the extent of deprotonation without the presence of Ca^{2+} would be only 6%. The magnitude of these spectral variations indicates complex formation, just like in the case of $\alpha\text{-Ket}^{2-}$.

To determine the composition and stability product(s) of the forming complex(es), UV spectrophotometric measurements were performed. Six solution series were prepared where the concentration of Ca^{2+} was systematically increased while $[\beta\text{-Ket}^{2-}]_T$ and $[OH^-]_T$ were held constant (Figs. 12 and S7–S10). For the calculations, the formation constants of the two hydroxido complexes of calcium(II), $CaOH^+$ and $Ca(OH)_2^0$, were taken from Ref. [30]. During data evaluation the absorbance values were fitted with respect to $[Ca^{2+}]_T$. The initial *F* parameter (0.21 absorbance unit) decreased to 0.016 with the assumption of the plausible $[Ca(\beta\text{-Ket})H_{-1}]^-$ species with log $\beta_{11-1} = -11.80$, but the agreement between the experimental and calculated data was unsatisfactory.

The fitting could be significantly improved ($F = 0.006$) assuming the formation of both the $[Ca(\beta\text{-Ket})H_{-1}]^-$ and $[Ca_2(\beta\text{-Ket})H_{-1}]^+$ species with log $\beta_{11-1} = -11.58$ and log $\beta_{21-1} = -10.80$. These complexes most likely contain a deprotonated $\beta\text{-Ket}^{2-}$ unit ($(\beta\text{-Ket})H^{3-}_{-1}$).

The concentration distribution diagram (Fig. 13) shows that the extent of complex formation is significant; that is, the concentration of the free ligand drops to as much as 50% at the highest concentration of $CaCl_2$.

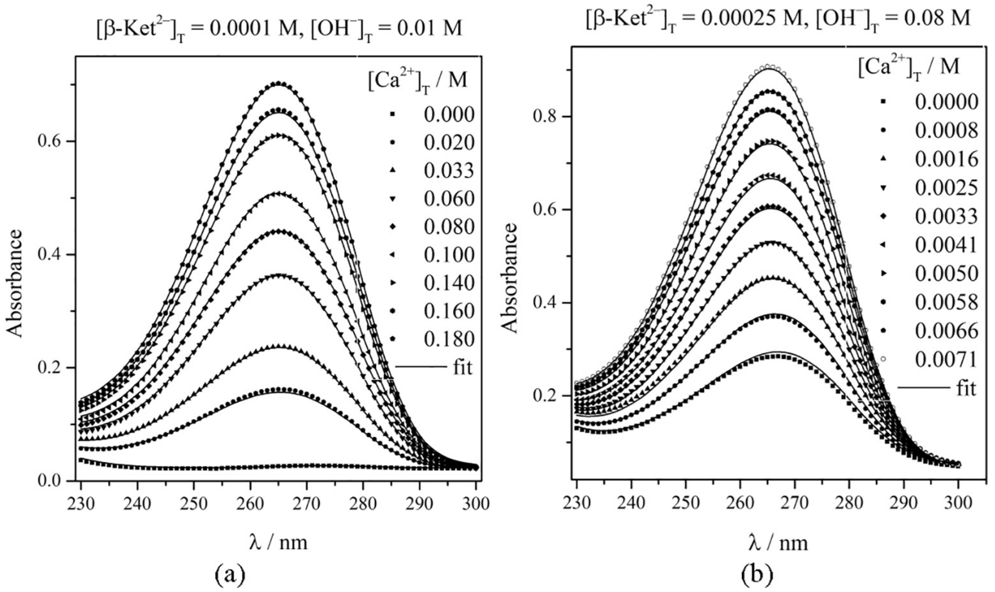


Fig. 12. UV absorption spectra of solutions containing $[\beta\text{-Ket}^{2-}]_T = 0.0001$ M and $[OH^-]_T = 0.01$ M (a) or $[\beta\text{-Ket}^{2-}]_T = 0.00025$ M and $[OH^-]_T = 0.08$ M (b) and increasing amount of Ca^{2+}; $I = 4$ M (NaCl), $T = (25 \pm 2)$ °C. Symbols represent the experimental, while solid lines denote the calculated data.

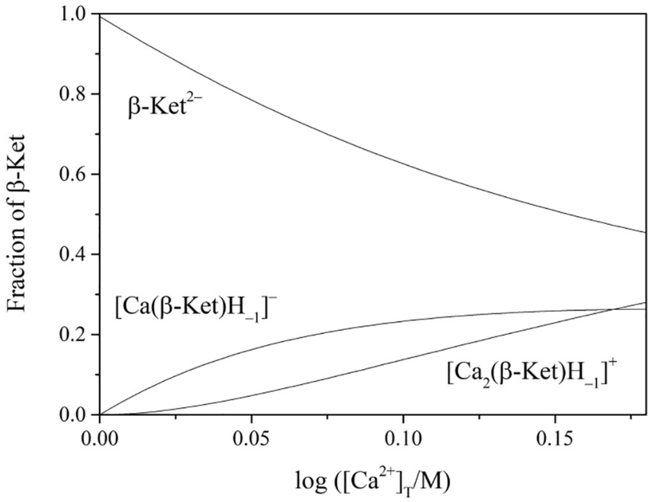


Fig. 13. Distribution among the various aqueous species in a solution containing $[\beta\text{-Ket}^{2-}]_T = 0.001$ M and $[OH^-]_T = 0.01$ M as a function of log ($[Ca^{2+}]_T$/M). The simulations were based on stability constants determined at $I = 4$ M (NaCl) and T = 25 °C.

Table 2
Stability products, log β_{pq-r}, for the $Ca_pL_qH_{-r}^{(2p-q-r)+}$ complexes in systems containing Ca^{2+}, L^- and OH^- ions, where $L^- = \alpha\text{-Ket}^{2-}$ or $\beta\text{-Ket}^{2-}$. The equilibrium constants are given with their ±3 *SE*[a] values and correspond to 25 °C and 4 M ionic strength.

Reaction	α-Ket^{2-} log β_{pq-r}	β-Ket^{2-} log β_{pq-r}
$Ca^{2+} + L^{2-} = [CaL]^0$	1.15 ± 0.02	0.78 ± 0.02
$L^{2-} = LH_{-1}^{3-} + H^+$	−15.17 ± 0.01	−14.41 ± 0.01
$Ca^{2+} + L^{2-} = [CaLH_{-1}]^- + H^+$	−11.91 ± 0.02	−11.58 ± 0.01
$Ca^{2+} + LH_{-1}^{3-} = [CaLH_{-1}]^-$	3.26[b]	2.83[b]
$2\ Ca^{2+} + L^{2-} = [Ca_2LH_{-1}]^+ + H^+$	–	−10.80 ± 0.03
$2\ Ca^{2+} + L^{2-} = [Ca_2LH_{-3}]^- + 3\ H^+$	−36.10 ± 0.06	–

[a] Standard error.

[b] Formation constants were corrected with the deprotonation constant of the ligands, expressed as log β_{11-1} − log β_{01-1}.

The formation constants for the two oxocarboxylates determined in the present work are summarized in Table 2.

To conclude, both ligands form an aqueous species with 1:1:–1 composition in highly alkaline medium. It is interesting to note that the higher stability of $[Ca(\beta\text{-Ket})H_{-1}]^-$ compared to that of $[Ca(\alpha\text{-Ket})H_{-1}]^-$ (−11.58 *vs.* −11.91) reflects the difference found for the pK_a values. Conversely, the formation constants corrected with respect to the deprotonation constant of the ligand (3.26 for $[Ca(\alpha\text{-Ket})H_{-1}]^-$ and 2.83 for $[Ca(\beta\text{-Ket})H_{-1}]^-$) supports the notion that $(\alpha\text{-Ket})H_{-1}^{3-}$ is a stronger base than $(\beta\text{-Ket})H_{-1}^{3-}$.

Besides, both ligands form complexes in which the metal:ligand ratio is 2:1, but they are in different deprotonation states, α-Ket^{2-} was found to form $[Ca_2(\alpha\text{-Ket})H_{-3}]^-$, while β-Ket^{2-} was shown to form $[Ca_2(\beta\text{-Ket})H_{-1}]^+$.

Similarly to the deprotonated ligands, each Ca^{2+} complex exists as resonance structures. The stability of the 1:1:–1 complexes are, however, higher than that of the 1:1:0 species indicating the presence of stronger chelate rings. Additionally, the log β_{11-1} formation constants are very similar to that of the $[CaGlucH_{-1}]^0$ complex [1] in which an OH group is deprotonated. In the deprotonated ketoglutarate complexes, thus, Ca^{2+} ions interact with the negatively charged alcoholate group more likely than with the neutral oxo group. This assumption entails that in these complexes the enolate form is the preferred one.

4. Conclusions

In neutral medium both α-Ket^{2-} and β-Ket^{2-} was found to form complexes with 1:1 composition. The equilibrium constant of $[Ca(\alpha\text{-Ket})]^0$ is higher than that of $[Ca(\beta\text{-Ket})]^0$ which is most probably due to the difference in the size of the chelate ring forming during the coordination. The five-membered chelate in $[Ca(\alpha\text{-Ket})]^0$ is most stable than the six-membered chelate in $[Ca(\beta\text{-Ket})]^0$.

At strongly alkaline solutions both ligands undergo CH deprotonation yielding the carbanion-enolate form ($(\alpha/\beta\text{-Ket})H_{-1}^{3-}$). β-Ket^{2-} was found to be stronger acid than α-Ket^{2-}; the difference in their proton dissociation constant can be ascribed for the different structure of the deprotonated ligands. That is, $(\beta\text{-Ket})H_{-1}^{3-}$ contains a more extended conjugated system providing higher stability over $(\alpha\text{-Ket})H_{-1}^{3-}$.

In alkaline medium Ca^{2+} was shown to be able to induce the deprotonation of the methylene group of α/β-Ket^{2-} forming calcium enolato complexes. The formation of the 1:1:–1 complexes was observed for both ligands; these species are likely to contain a deprotonated ketoglutarate unit. The stability constant (corrected with the deprotonation constant of the ligands) of $[Ca(\alpha\text{-}Ket)H_{-1}]^-$ is somewhat larger than that of $[Ca(\beta\text{-}Ket)H_{-1}]^-$, which is in line with the observation that α-Ket^{2-} is a weaker acid than β-Ket^{2-}, consequently $(\alpha\text{-}Ket)H_{-1}^{3-}$ is stronger base and stronger complexant than $(\beta\text{-}Ket)H_{-1}^{3-}$.

In addition to the 1:1:–1 complexes, the formation of binuclear complexes with 2:1:–1 (for β-Ket^{2-}) and 2:1:–3 (for α-Ket^{2-}) was extracted from the UV spectrophotometric measurements. The structure of the $[Ca_2(\beta\text{-}Ket)H_{-1}]^+$ complex is most probably includes the binding of the second calcium ion to the second available carboxylate group of the ligand and results in the formation of a symmetrical binuclear complex. That is, it may be suggested that both Ca^{2+} ions are coordinated to the ligand in a bidentate manner, where the enolate oxygen is shared between the two calcium ions.

The formation of the 2:1:–3 complex for α-Ket^{2-} is somewhat unexpected. The presence of this species can be observed only at the highest pHs employed. Under these conditions the Ca^{2+} ion undergoes hydrolysis [30] and is partially present as $CaOH^+$. Since it is unlikely that $KetH_{-1}^{3-}$ undergoes a second deprotonation step, the formation of this multinuclear complex should be a calcium-hydroxido-enolate complex, in which one OH^- ion is bound to each calcium ion.

Acknowledgement

The authors are grateful for the technical assistance provided by Ilona Halasiné-Varga. This work was supported by the GINOP-2.3.2-15-2016-00013 and the NKFIH K 124 265 Grants. All these supports are highly appreciated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.poly.2018.09.005.

References

[1] A. Pallagi, É. Bajnóczi, S.E. Canton, T. Bolin, G. Peintler, B. Kutus, Z. Kele, I. Pálinkó, P. Sipos, Environ. Sci. Technol. 48 (2014) 6604.
[2] A. Pallagi, Z. Csendes, B. Kutus, E. Czeglédi, G. Peintler, P. Forgó, I. Pálinkó, P. Sipos, Dalton Trans. 42 (2013) 8460.
[3] B. Kutus, C. Dudás, G. Peintler, I. Pálinkó, P. Sipos, Carbohyd. Res. 460 (2018) 34.
[4] A. Gácsi, B. Kutus, Á. Buckó, Z. Csendes, G. Peintler, I. Pálinkó, P. Sipos, J. Mol. Struc. 1118 (2016) 110.
[5] A. Gácsi, B. Kutus, Z. Csendes, T. Faragó, G. Peintler, I. Pálinkó, P. Sipos, Dalton Trans. 45 (2016) 17296.
[6] T.S. Viswanathan, R.E. Johnson, H.F. Fischerm, Biochemistry 21 (1982) 339.
[7] I.G. Morgunov, S.V. Kamzolova, V.A. Samolienko, Appl. Microbiol. Biotechnol. 97 (2013) 8711.
[8] S.V. Kamzolova, M.N. Chiglintseva, J.N. Lunina, I.G. Morgunov, Appl. Microbiol. Biotechnol. 96 (2012) 783.
[9] J.T. Smith, V.N. Doctor, Inorg. Nucl. Chem. 37 (1975) 775.
[10] H. Scheidegger, W. Felty, D.L. Leussing, J. Am. Chem. Soc. 92 (1970) 808.
[11] A.J. Cooper, A.G. Redfield, J. Biol. Chem. 250 (1975) 527.
[12] A.J.L. Cooper, J.Z. Ginos, A. Meister, Chem. Rev. 83 (1983) 321.
[13] J. Kozlowski, P. Zuman, Bioelectroch. Bioener. 28 (1992) 43.
[14] J. Schubert, A. Lindenbaum, J. Am. Chem. Soc. 74 (1952) 3529.
[15] X. Yang, S. Bi, X. Wang, J. Liu, Z. Bai, Anal. Sci. 19 (2003) 273.
[16] J. Sporar, J. Bezensek, U. Ursic, A. Golobic, J. Svete, B. Stanovnik, Heterocycles 84 (2012) 449.
[17] J. Svetlik, L. Veizerova, V. Kettmann, Tertahedron Lett. 49 (2008) 3520.
[18] S.K. Ghosh, M. Nazimuddin, B.M. Mandal, Macromol. Chem. Rapid Commun. 13 (1992) 583.
[19] Y. Wang, A.T. Stone, Geochim. Cosmochim. Ac. 70 (2006) 4463.
[20] F.C. Kupper, C.J. Carrano, J.-U. Kuhn, A. Butler, Inorg. Chem. 45 (2006) 6028.
[21] G. Peintler, Spline Calculus, Version 2.12a, University of Szeged, Hungary, 2008.
[22] L. Zékány, I. Nagypál, G. Peintler, PSEQUAD for Chemical Equilibria, Update 5-5.10, Hungary, 2000-2008.
[23] L. Cattalini, A. Cassol, G. Marangoni, G. Rizzardi, E. Rotondo, Inorg. Chim. Acta 3 (1969) 681.
[24] G.J. McDougall, R.D. Hancock, J. Chem. Soc., Dalton Trans. (1978) 1438.
[25] J. Schaubroeck, A.M. Goeminne, Z. Anorg, Allg. Chem. 492 (1982) 188.
[26] V.A. Kogan, S.G. Kochin, A.S. Antsyshkina, G.G. Sadikov, A.D. Garnovskii, Mendeleev Commun. 7 (1997) 239.
[27] A. Pallagi, P. Sebők, P. Forgó, T. Jakusch, I. Pálinkó, P. Sipos, Carbohyd. Res. 345 (2010) 1856.
[28] Y. Chiang, A.J. Kresge, P. Pruszynski, J. Am. Chem. Soc. 114 (1992) 3103.
[29] Á. Buckó, B. Kutus, G. Peintler, I. Pálinkó, P. Sipos, unpublished results.
[30] B. Kutus, Á. Gácsi, A. Pallagi, I. Pálinkó, G. Peintler, P. Sipos, RSC Adv. 6 (2016) 45231.