Kinetics and Mechanism of Decarboxylation of Hydrogen Carbonate Coordinated to a Macrocyclic Chromium(III) Complex. A Comparison with the Carbonic Anhydrase Enzymes

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Decarboxylation of the cis-$[Cr(cycb)F(OCOOH)]^+$ complex, cycb = rac-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane, has been investigated at a range of temperatures and hydrogen ion concentrations in 1 M (Na,H)(Br,OH). The relative robustness of hydrogen carbonate complexes towards decarboxylation has been correlated with the acid dissociation constants of the coordinated hydrogen carbonate ligand. It is suggested that the rate-determining step for the decarboxylation reaction is proton transfer from an uncoordinated oxygen atom to the coordinated oxygen atom of the carbonate ligand. A unified mechanism for the decarboxylation path of both the carbonic anhydrase enzymes and simple carbonate complexes is presented.

Many biological processes which are effectively catalysed by metalloenzymes are also affected by less sophisticated metal complexes. One such important example is the hydration of carbon dioxide and the reverse process which are catalysed by the zinc-containing carbonic anhydrase enzymes (Scheme 1). This process has attracted con-

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$
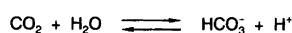
Scheme 1.

siderable interest and has been elucidated in significant depth.[1] A key step in the proposed catalytic sequence is the decarboxylation of a hydrogen carbonate ligand coordinated to a zinc(II) centre. This process has been estimated to have rate constants of about 3×10^6 and 4×10^4 s^{-1} for the human isoenzymes II and I, respectively.[1]

These rate constants are at least four orders of magnitude larger than those for decarboxylation of hydrogen carbonate coordinated to simpler metal complexes of cobalt(III), rhodium(III) and iridium(III), which all conform to the stoichiometry of Scheme 2. The decarboxylation rate constants, k_d, for these complexes all lie in a rather narrow range between 0.3 and 3 s^{-1}.[2]

$$MOCOOH \xrightarrow{k_d} MOH + CO_2$$
$$\Updownarrow K_A$$
$$MOCO_2 + H^+$$
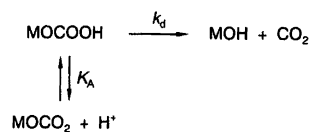
Scheme 2.

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Recently the first monomeric cationic carbonate complex of chromium(III) was characterized,[3,4] and kinetic data were analysed in some detail.[5] This work showed that this chromium(III) complex was significantly less reactive with regard to decarboxylation than the previously investigated complexes. The complexity of the investigated system, particularly the tendency to form the chelate carbonate complex, prevented an accurate characterization of the reactivity of the cis-$[Cr(cycb)(OH)(OCOOH)]^+$ complex [cycb = rac-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane]. The present work describes a kinetic investigation of the decarboxylation reaction of the cis-$[Cr(cycb)F(OCOOH)]^+$ complex in which chelation to form the chelate carbonato complex is prevented by the fluoride ligand.

Results and discussion

Stoichiometry. Cis-$[Cr(cycb)F(OH_2)]^{2+}$ is readily precipitated as the perchlorate salt from concentrated perchlorate solutions, and dissolution of cis-$[Cr(cycb)F_2]ClO_4$ in concentrated perchloric acid at room temperature is the basis for an efficient synthesis of the perchlorate salt of the former cation.

The aquafluoro complex is acidic in aqueous solution, and the deprotonated complex reacts with carbon dioxide to give an uncharged carbonatofluoro complex. This complex can be isolated free from other chromium(III) species by ion-exchange chromatography of equilibrated solutions. Fig. 1 shows the spectral characteristics of the relevant complexes. The carbonato complex is reasonably stable in
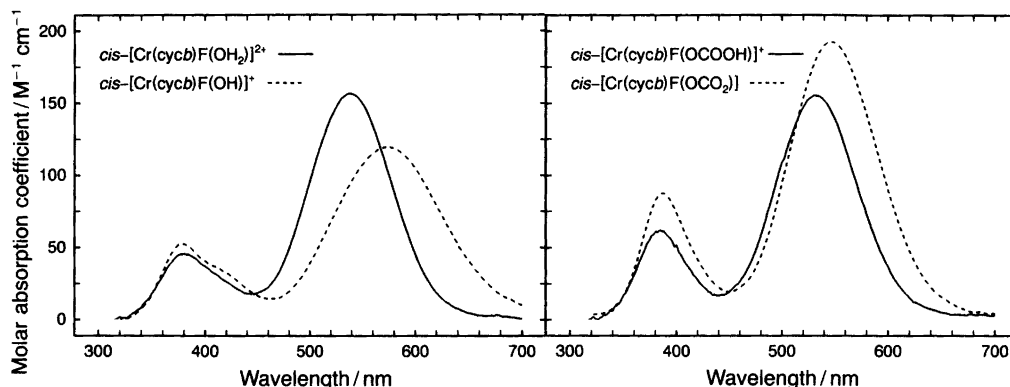
Fig. 1. Visible absorption spectra of cis-[Cr(cycb)F(OH₂)]²⁺, cis-[Cr(cycb)F(OH)]⁺, cis-[Cr(cycb)F(OCOOH)]⁺ and cis-[Cr(cycb)F(OCO₂)] in aqueous 1.00 M (Na,H)(Br,OH). The spectrum of the complex with coordinated water was measured in a solution with [H⁺] = 0.10 M, the spectra of complexes with coordinated hydroxide or carbonate ion were measured in solutions with [OH⁻] = 0.10 M, all at 25 °C. The spectrum of the complex with coordinated hydrogen carbonate was measured in a solution with [H⁺] = 0.08 M at 10 °C.

neutral and basic solutions but is rapidly decarboxylated in acidic solutions. In strongly basic solutions and at elevated temperatures fluoride ligand aquation occurs, leading to mixtures of the dihydroxy complex and the previously characterized complexes with monodentate and bidentate carbonate.[5]

Reactivity. The loss of carbon dioxide from the carbonate complex in reasonably acidic solution is a first-order process. At lower acidities, however, the reactivity corresponds to the formal Scheme 3. Interpretation of experi-
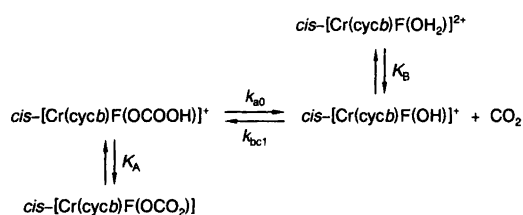
$$A \underset{k_{bc}}{\overset{k_a}{\rightleftharpoons}} B + C$$
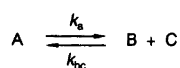
Scheme 3.

mental data within this scheme is conveniently performed using methods involving numerical integration as previously described.[6] Such a treatment shows k_a and k_{bc} to vary as function of the hydrogen ion concentration according to eqns. (1) and (2), corresponding to the detailed Scheme 4.

$$k_a = \frac{k_{a0}}{1 + K_A/[H^+]} \quad (1)$$

$$k_{bc} = \frac{k_{bc1}K_B/[H^+]}{1 + K_B/[H^+]} \quad (2)$$

cis-[Cr(cycb)F(OH₂)]²⁺

$\Bigg\updownarrow K_B$

cis-[Cr(cycb)F(OCOOH)]⁺ $\underset{k_{bc1}}{\overset{k_{a0}}{\rightleftharpoons}}$ cis-[Cr(cycb)F(OH)]⁺ + CO₂

$\Bigg\updownarrow K_A$

cis-[Cr(cycb)F(OCO₂)]

Scheme 4.

The parameters obtained are given in Table 1.

Comparisons with data for other systems. The overall stoichiometric reaction scheme derived for carbonate ligand decarboxylation in the present chromium(III) system is in agreement with the general scheme for this process established on the basis of data for carbonate complexes of predominantly cobalt(III) and rhodium(III). The decarboxylation rate constant, k_{a0} in Scheme 4, however, is more than one order of magnitude smaller than the constants for the cobalt(III) and rhodium(III) complexes referred to above, all being in the range 0.3–3 s⁻¹ at 25 °C.[2] These values are to be compared with the value of 0.0303(4) s⁻¹ for the present system and values of 0.0170(4) and 0.0015(12) s⁻¹ for cis-[Cr(cycb)(OH₂)(OCOOH)]²⁺ and cis-[Cr(cycb)(OH)(OCOOH)]⁺, respectively.[5]

Intimate mechanism of decarboxylation. It appears that the reduced reactivity of the present chromium(III) complexes is correlated with a reduced acidity of the coordinated hydrogen carbonate ligand. This is demonstrated by an approximate proportionality between the rate constant for decarboxylation, k_d, and the acid dissociation constant, K_A,

Table 1. Kinetic and thermodynamic parameters for the transformations shown in Scheme 4. Values relate to a 1 M (Na,H)(Br,OH) medium. Standard deviations are in parenthesis, cf. the Experimental section.

Parameter	Value (25 °C)	ΔH^a/kJ mol⁻¹	ΔS^a/J K⁻¹ mol⁻¹
k_{a0}/s⁻¹	0.0303(4)	73.5(10)	−28(3)
k_{bc1}/M⁻¹ s⁻¹	12.3(9)	63(4)	−14(13)
−log (K_A/M)	7.365(11)	25.2(14)	−56(4)
−log (K_B/M)	6.499(14)	42.8(15)	19(5)

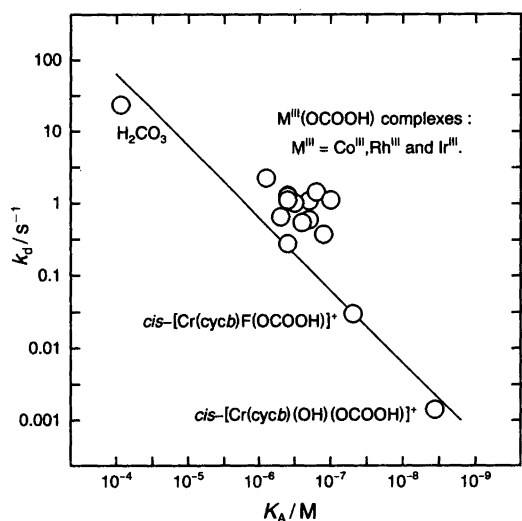$^a \Delta H^\circ$ or ΔH^*, and analogously for ΔS, depending upon the parameter.

Fig. 2. Correlation between rate of decarboxylation, k_d, and acid dissociation constant, K_A, at 25 °C, cf. Scheme 2. The straight line is drawn with a slope of 1. The data for the Co(III), Rh(III) and Ir(III) complexes from Table XXXIII of Ref. 2 are only for those complexes for which the acid dissociation constant reflects the acidity of the hydrogen carbonate ligand. Other data points are from Ref. 5 and Tables 1 and 2.

which including data for decarboxylation of carbonic acid, as shown in Fig. 2, extends over more than four orders of magnitude.

A positive correlation between k_d and K_A has previously been reported for a number of cobalt(III), rhodium(III) and iridium(III) complexes.[2] Inclusion of data for some aqua hydrogencarbonate complexes for which the first acid dissociation constant is obviously dominated by the acidity of the coordinated water ligand resulted, however, in a slope of about 0.2, and the correlation extended over only one order of magnitude.

It is well documented that differences in acid dissociation constants reside primarily in differences in the rate constants for proton dissociation. The rate constants for proton uptake are close to the diffusion-controlled limit,[7] and are consequently quite similar. The data in Fig. 2 are therefore evidence that the rate of the decarboxylation reaction may be correlated with the rate of dissociation of a proton. Since the rate expression for the decarboxylation path is not indicative of proton dissociation to the solvent, it is suggested here that the decarboxylation process for the simple hydrogencarbonate complexes involve a rate-determining intramolecular proton transfer followed by a fast release of carbon dioxide. This first process can reasonably be thought of as taking place by proton transfer from an uncoordinated oxygen atom of the monodentate carbonate ligand to the coordinated oxygen atom as shown in Scheme 5.

A monodentate carbonate ligand protonated at the co-ordinated oxygen atom would be expected to be a reasonably strong acid. In an attempt to estimate the acid dissociation constant of this species, protonation of coordi-
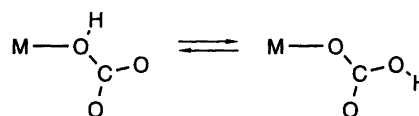
Scheme 5.

nated carbonate may be compared with protonation of coordinated hydroxide as shown in Scheme 6. The difference in protonation constants between these two processes
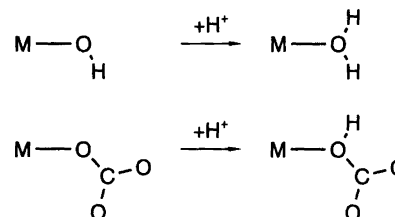
Scheme 6.

may be estimated by comparison with the protonation constants of the free ligands. Assuming a linear free-energy relation with a slope of 1 between these two types of processes, i.e. the protonation of coordinated and free ligands, and by applying appropriately corrected equilibrium constants, cf. Tables 1 and 2, an equilibrium constant of about $1 \ M^{-1}$ for the protonation of the co-ordinated oxygen atom of the carbonate ligand of the cis-$[Cr(cycb)F(OCO_2)]$ complex is obtained. This value corresponds to an equilibrium constant of about $10^{7.3}$ for the transfer of a proton from the coordinated oxygen atom to the uncoordinated oxygen atom of the carbonate ligand as shown in Scheme 5. This equilibrium constant is equivalent to a free energy change of about $-42 \ kJ \ mol^{-1}$.

Comparison with the carbonic anhydrase enzymes. This interpretation leads to the conclusion that there should not be significant mechanistic differences between the decarboxylation reactions of the metal complexes and of carbonic acid, cf. the correlation in Fig. 2. The mechanism presented here is also a simple but reasonable approximation to the geometrical changes of the carbonic acid decarboxylation which, from a detailed theoretical analysis,[8] can in simple terms be described as a concerted proton transfer and carbon–oxygen bond stretching.

This mechanism also provides a rationale for the effectiveness of the carbonic anhydrase enzymes as compared to the simpler metal complexes. Crystallographic studies[9] have shown that the enzyme may function as an effective polytopic frame for the zinc(II)-coordinated substrate. In view of the suggested mechanism for the reactions of the simple complexes one essential function of the enzyme seems therefore to be its ability to maintain the proton of the coordinated hydrogen carbonate ligand on the oxygen atom coordinated to the metal centre. Structural studies of the enzyme have indicated an environment suitable for the

stabilization of such a configuration, as demonstrated by the suggested substrate–enzyme geometry in Ref. 1. Theoretical evidence for solvent water participation in the proton transfer reaction of the uncatalysed system[10] may also be of relevance in this context when compared with the enzymatic possibilities of stereospecific substrate solvation and desolvation.

The possibility of bidentate coordination of the hydrogen carbonate substrate has been demonstrated in an NMR study of the manganese(II)-substituted human carbonic anhydrase I enzyme.[11] Whether or not this mode of substrate binding is relevant for the function of the native enzyme is open to question, as it should be recalled that the manganese(II) ion is generally considered to be about 0.07 Å larger than the zinc(II) ion[12] and to have an increased tendency towards a higher coordination number. This additional coordination is not easily fulfilled with anything other than the substrate at the congested active site of the enzyme. The catalytic efficiency of the manganese(II) enzyme is decreased as compared to the native zinc(II) enzyme. This fact may also support the view that the catalytically active species in this step of the catalytic cycle is a monodentate complex. In this context it is also relevant to mention that substitution of bidentate carbonate is usually a two-step process initiated by a metal–oxygen bond-breaking reaction forming the monodentate complex.[2]

Another aspect of the suggested coordination mode, relevant for the catalytic efficiency of the enzyme, is that a monodentate hydrogen carbonate ligand with the proton bound to the coordinated oxygen atom will be an excellent leaving group and will therefore be readily substituted by water. This is particularly the case for a labile metal centre such as zinc(II), for which the rate of water exchange in the aqua-ion is about 10^8 s^{-1}. The monodentate coordination is thus in agreement with both the fast decarboxylation and the fast release of coordinated hydrogen carbonate, both being necessary for the catalytic efficiency of the enzyme.

In conclusion, if this comparison between simple hydrogen carbonate complexes and the carbonic anhydrase enzymes is valid when considering the reactivity of the metal centres, there is evidence that the reactions may all conform to the same overall reaction scheme. The significant reactivity difference between these two types of reactions can be rationalized in terms of stabilisation of the proton on the uncoordinated oxygen atom in the simple metal complexes or on the coordinated oxygen atom in the zinc(II) enzymes.

Experimental

Caution! The perchlorate salts described here are potentially explosive and should be handled accordingly.

Chemicals. Cis-[Cr(cyc*b*)F$_2$]ClO$_4$ was prepared according to the literature.[4] Other chemicals were of best available commercial grades and were purified when necessary:

NaBr · 2H$_2$O was recrystallized from water. HBr (47 %, aq) was distilled from metallic tin. The amine and pyridine buffer substances were either distilled from solid KOH and kept protected from atmospheric carbon dioxide or were converted into the hydrobromide salts and recrystallized.

*Cis-[Cr(cyc*b*)F(OH$_2$)](ClO$_4$)$_2$.* 2.3 g *cis*-[Cr(cyc*b*)F$_2$]ClO$_4$ (4.5 mmol) were dissolved in 10 ml of 70 % perchloric acid and left in a Teflon flask at room temperature for a week. The resulting mixture was diluted with 10 ml of water and then cooled in ice-water. The red–violet crystals obtained were filtered off and washed three times with diethyl ether and then left to dry in the air. This crude product was dissolved in 25 ml of water at 100 °C, and traces of undissolved material were removed by filtration. 3 ml of a saturated aqueous solution of sodium perchlorate were added to the hot filtrate, which was then left to crystallize at 0 °C. The crystals were isolated as described above. Yield about 2.1 g (76 %) of the dihydrate salt.

The complex prepared in this way may contain some *cis*-[Cr(cyc*b*)(OH)(OH$_2$)](ClO$_4$)$_2$ · xH$_2$O, which is best removed by recrystallization from an acidic solution. Under these conditions aquation of the fluoride ligand is noticeable and prolonged heating should be avoided: 2.1 g *cis*-[Cr(cyc*b*)F(OH$_2$)](ClO$_4$)$_2$ · 2H$_2$O were suspended in 5 ml 1 M aqueous sodium hydroxide solution. The resulting mixture was placed on a glass filter and extracted with small portions of water at 60 °C until complete dissolution. This required about 60 ml. The resulting solution was rapidly acidified with 10 ml of 70 % perchloric acid and then cooled in ice-water. The red–violet crystals were filtered off, washed once with an ice-cold acetone–diethyl ether mixture (1:3 v/v) and then twice with diethyl ether. Yield 1.4 g (51 %) of the dihydrate salt. On standing, this salt apparently looses water of crystallization and is transformed into the anhydrous compound. Analyses, CrCl$_2$FO$_9$N$_4$C$_{16}$H$_{38}$: Cr, Cl, N, C and H.

The compound used for the kinetic investigations was recrystallized twice by the method described above.

*Cis-[Cr(cyc*b*)F(OH)]ClO$_4$·2H$_2$O.* 2.3 g purified *cis*-[Cr(cyc*b*)F(OH$_2$)](ClO$_4$)$_2$·2H$_2$O (3.8 mmol) were dissolved in 25 ml boiling water. This solution was made basic by the addition of a solution of 0.5 g sodium hydroxide in 3 ml of water. 10 ml of a saturated aqueous sodium perchlorate solution were then slowly added and the resulting mixture was left to crystallize at 0 °C. The blue–violet crystals were filtered off and washed three times with an acetone–diethyl ether (1:1 v/v) mixture. Yield 1.2 g (62 %). Analyses, CrClFO$_7$N$_4$C$_{16}$H$_{41}$: Cr, Cl, N, C and H.

Acidification of the mother liquor regenerated some of the starting material. This could be isolated as described above. It is essential to start with a carefully purified compound, as the common impurities, particularly *cis*-[Cr(cyc*b*)(OH)$_2$]ClO$_4$ · 2H$_2$O and the perchlorate salt of the difluoro complex, are not readily removed by recrystallization from basic solutions.

Cis-*[Cr(cycb)F(OCO₂)]* solutions. 25 mg cis-[Cr(cycb)-F(OH₂)](ClO₄)₂(·2H₂O), 50 mg sodium hydrogen carbonate and 10 mg sodium carbonate were dissolved in 5 ml of water at room temperature and left for 2.5 h. An excess of carbonate was removed by the addition of 250 mg barium hydroxide. Barium carbonate was removed by filtration and the filtrate was charged onto a 10×2 cm SP Sephadex C-25 filled column which had previously been treated with 0.01 M NaOH(aq). Elution with 0.01 M NaOH(aq) cleanly separated an apparently uncharged violet component prior to the blue cis-[Cr(cycb)F(OH)]⁺ ion of the starting material. The ionic strength of the eluate was adjusted to 1.00 M by means of sodium bromide. The visible absorption spectrum of a solution thus prepared is shown in Fig. 1.

Solid compounds containing the carbonato complex could be isolated by precipitation of more concentrated chromium(III) solutions with saturated sodium perchlorate solutions. However, an efficient purification method for solids thus prepared was not found. The solutions for the kinetic measurements were therefore prepared by the ion-exchange separation procedure as described above.

Cis-*[Cr(cycb)F(OCOOH)]⁺* spectrum. A solution of the hydrogen carbonate complex was made by rapidly acidifying a solution of the carbonate complex. The spectrum of the acidified solution was measured at 10 °C as a function of time on a Perkin-Elmer diode array spectrophotometer. The spectrum of the hydrogen carbonate complex was obtained by extrapolation to the time of acidification.

Kinetic measurements. The reactions were followed spectrophotometrically using a Cary 118C or a Perkin-Elmer Lambda-17 spectrophotometer controlled by an RC-Partner computer, in each case equipped with a Perkin-Elmer Digital temperature controller. The kinetic transformations were followed at a single wavelength, usually 540, 580 or 630 nm (Fig. 1). One such experiment is shown in Fig. 3. Carbon dioxide, hydrogen carbonate and carbonate were used as buffer components to maintain a suitable constant hydrogen ion concentration at lower acidities at the carbon dioxide uptake experiments. A range of pyridine buffers, including pyridine, 2-methylpyridine and 2,6-dimethylpyridine, were used to maintain a constant hydrogen ion concentration at the lower acidities at the decarboxylation experiments.[5] The kinetic experiments included the temperature range 10–65 °C, and the concentration ranges 1–3 mM for Cr(III), 0.1–5×10⁻¹⁰ M for H⁺ and 0–0.12 M for total carbonate.

Hydrogen ion concentration measurements. Hydrogen ion concentrations were measured with a Radiometer PHM 52 pH-meter equipped with standard glass and reference electrodes. The electrodes were calibrated by titrations of strong acid with strong base in 1.00 M NaBr solution. All measurements on solutions from the kinetic runs were performed at 25 °C relative to a 1.000 mM HBr and 0.999 M
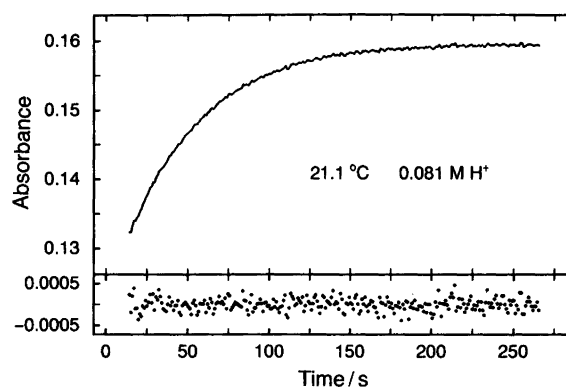

Fig. 3. Upper part: absorbance vs. time profile at 580 nm for aquation of cis-[Cr(cycb)F(OCOOH)]⁺ in 1.00 M (H,Na)Br. Lower part: differences between observed and calculated absorbance vs. time using a first-order rate constant of 0.0211 s⁻¹.

NaBr solution and were corrected to the temperature of the kinetic experiment by the temperature dependence of the acid dissociation constants of the protonated buffer substances.[5]

Determination of acid dissociation constants. Acid dissociation constants were determined as previously described by titrations at 25 and 40 °C. Results for cis-[Cr(cycb)F(OH₂)]²⁺ are given in Table 1. Cis-[Cr(cycb)-F(OCOOH)]⁺ was too labile in acidic solution to be titrated, and the acid dissociation constant for this species was determined from the kinetic measurements, cf. the data in Table 1.

Methods of calculation. All parameter values were determined by minimizations within the framework of 'non-linear regression analysis. The method used to determine the acid dissociation constants has been described previously.[13] Rate constants for kinetic runs followed at a single wavelength were determined by minimization of eqn. (3), where $A(t)_{obs}$ and $\sigma[A(t)]$ are the absorbance and

$$\sum_t \frac{[A(t)_{obs} - A(t)_{calc}]^2}{\sigma^2[A(t)]} \tag{3}$$

standard deviation upon absorbance, respectively, at time t. $A(t)_{calc}$ was calculated from the relevant component concentrations as a function of time obtained by integrating the set of coupled differential equations [eqn. (4)] corresponding to Scheme 3.

$$-\frac{d[A]}{dt} = \frac{d[B]}{dt} = \frac{d[C]}{dt} = k_a[A] - k_{bc}[B][C] \tag{4}$$

Single experiments treated in this way tend to give highly correlated values for k_a and k_{bc}, but this numerical problem could be overcome by treating several experiments simultaneously. The final calculations were consequently carried

out by simultaneous minimization of all experiments as a function of the relevant parameters. The data in Table 1 were determined in this way.

A complicating feature of reaction kinetic investigations involving carbon dioxide is that the rate of equilibration in the carbon dioxide–water system is frequently of the same order of magnitude as the rates of the complex formation reactions. This calls for an expansion of the set of differential equations to describe the kinetic transformations. The possible influence of the kinetic transformations in the isolated carbon dioxide system for the kinetic results of the present system was investigated by comparing the results of a treatment as an equilibrium system with the results of a treatment including the kinetic transformations shown in Scheme 7. Selected relevant literature parameters are given
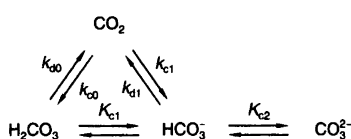
Scheme 7.

in Table 2. The data in Table 1 were those obtained by including the kinetics of the transformations in the carbon dioxide system. The results of this treatment of the carbon dioxide system as an equilibrium system were largely similar, but with somewhat smaller standard deviations on the different parameters, particularly those associated with the k_{bc1}-reaction (Scheme 4). This clearly demonstrates that the kinetic transformations in the present system are sufficiently slow not to be significantly influenced by the kinetics of the equilibrium reactions in the carbon dioxide water system.

Table 2. Selected literature parameters (Ref. 2) for the carbonic acid system, *cf.* Scheme 7. Standard deviations used for the calculation are given in parenthesis.

Parameter, P	Value (25 °C)	$-R\dfrac{\partial \ln P}{\partial T^{-1}}$ / kJ mol^{-1}
k_{co}/s^{-1}	0.061(3)	63(3)
k_{oo}/s^{-1}	23.7(8)	63(3)
$-\log (K_{c1}/M)$	3.45(2)	5(2)
$-\log (K_{c2}/M)$	9.57(3)	11(3)
$-\log (k_{c1}K_w/M\ s^{-1})$	9.83(8)	114(5)
$-\log (K_w/M^2)$	13.80(2)	57(1)

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