Synthesis of 2,4-diketoacids and their aqueous solution structures

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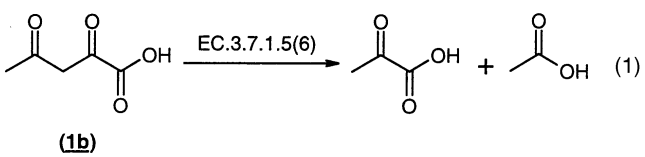
The synthesis of several 2,4-diketo carboxylic acids by standard methods was undertaken to study the substrate specificity of the carbon-carbon bond hydrolases. It was shown by ^{1}H- and ^{13}C-NMR experiments that compounds with 4-alkyl, 4-alkenyl and 4-alicyclic substituents exist in three main forms: 2,4-diketo, 2-enol-4-keto and 2-hydrate-4-keto. The equilibrium ratios of these aqueous solution structures were similar, but were markedly affected by the pH values (1.5–10.5). At pH 7.5 the ratio of these structures was approximately 4 : 5 : 1, but at low pH values the 2-hydrate predominated (≈50%) and at high pH values the 2-enolate carboxylate was dominant (≈80%) while the 2-hydrate was not detected. 4-Aryl substituents gave one pH-independent isomer formulated with C-2, C-3 and C-4 electrons delocalized in conjugation with the arene at C-4. This interpretation of a very rapid equilibrium between 2- and 4-enolate isomers to give a π-delocalized six-membered ring in conjugation with arene substituents is supported by the effect of divalent metal ions on the structural forms. Rate and equilibrium constants for several of these solution interconversions are influenced by pH. Mg^{2+} coordinates to the 2-enolate dianion of alkyl analogues, whereas Cu^{2+} forms a six-membered π-delocalized ring with the 2- and 4-oxo atoms in conjugation with the arenes. Exchange of ^{2}H from $^{2}H_2O$-enriched solvent occurs with the protons at C-3. The dimers of the 4-alkyl analogues *in aqua* were characterized as a product of self-aldol condensations. These data have facilitated enzyme mechanism studies of C—C bond hydrolysases (β-ketolases).

1,3-Diketones are substrates for carbon–carbon bond hydrolysis by β-ketolases. Some of us have recently reviewed the properties, physiological and metabolic functions of the ten enzymes (EC 3.7.1.1–10)[1] that belong to this group, and some others not yet listed.[2] Only two of them, namely fumarylacetoacetate hydrolase from beef liver (EC 3.7.1.2)[3] and acetopyruvate hydrolase from *Pseudomonas putida* (EC 3.7.1.6)[4] have been purified to electrophoretic purity. The two acetopyruvate hydrolases [EC 3.7.1.5(6)] cleave 2,4-diketopentanoic acid (**1b**) into pyruvate and acetate [eqn. (1)]. At present, **1b** is the only substrate known that is accepted by the enzyme from *P. putida*,[4] although many analogues have been found to be substrates for the other acetopyruvate hydrolase from rat liver.[5] The substrate specificity that is reported for this enzyme cannot be explained by the steric properties of these compounds only. So there must be other reasons based on the aqueous solution structures of the substrates, which are responsible for their suitability as substrates for the β-ketolases.



For a detailed investigation of the substrate specificity it is necessary to have more information about the aqueous solution structures of the 2,4-diketo acids, whether substrates or not, and possibly inhibitors for the enzyme activity. Most previous work on this topic was carried out on **1b**. In aqueous solution this compound partly forms an enol isomer, as was shown independently by Dieckmann[6] and by Hieber.[7] But they observed different amounts of the enol isomer by the use of different analytical methods. Later, Guthrie[8] found that in water and in buffered solutions three main structures exist in equilibrium. Beside the diketo isomer, an enol isomer and a hydrate exist (Fig. 1). It was suggested that this isomerization and addition/elimination takes place at the keto group in the 2-position. This was consistent with results from Michael and Smith,[9] who also described an enol formed from the keto group in the 2-position for ethyl 2,4-diketopentanoate (**1a**) dissolved in toluene, and from Emerson *et al.*[10] who later formulated such tautomers for ethyl 2,4-diketo-5,5-dimethylhexanoate (**2a**) and 2,4-diketo-5,5-dimethylhexanoic acid (**2b**). Guthrie also reported the pH-dependent ratio of the different

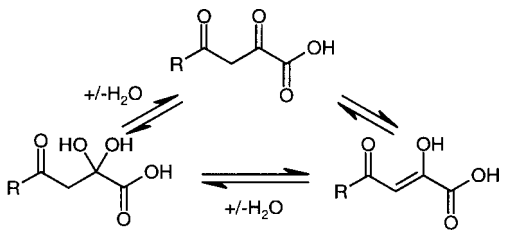


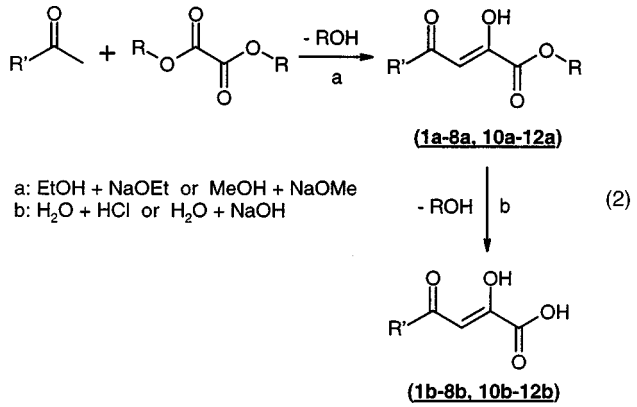
Fig. 1 Aqueous solution structures of the 2,4-diketo acids with an aliphatic side chain.

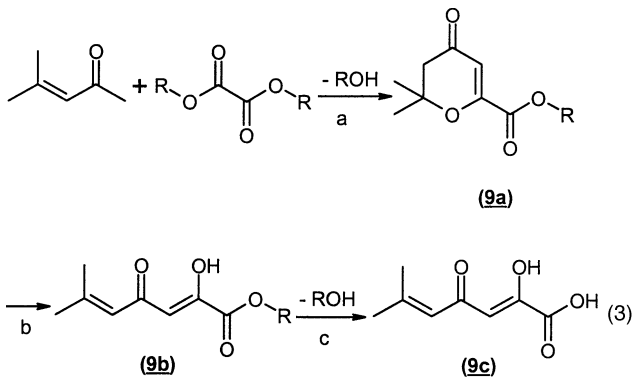
forms of **1b** at pH values lower than 7.0 and the pK values of the carboxylic and the enol protons.[8]

Results

Synthesis of 2,4-diketo acids

We have synthesized several compounds that possess the 2,4-diketobutanoic acid structure with different 4-alkyl and aryl groups by well-described strategies. Oxalic acid diester was condensed with a methyl ketone in a Claisen reaction to give the esters of 2,4-diketo acids. The esters were then hydrolysed in aqueous acid or base. The easy two-step reaction is shown in eqn. (2) and the synthesized compounds are listed in Table 1. Only 2,4-diketo-6-methylhept-5-enoic acid (**9c**) was made by a different procedure, because of its tendency to undergo an intramolecular addition to form 2-carboxy-6,6-dimethyl-4-oxodihydropyran [eqn. (3)].[17,18]





a: EtOH + NaOEt or MeOH + NaOMe
b: Na + $Cu(Ac)_2$
c: H_2O + HCl or H_2O + NaOH

Solution structures of the 2,4-diketo acids

All synthesized analogues form more than 98% enol isomer in deuterated chloroform. This was determined by ^{1}H- and ^{13}C-NMR spectroscopy. In an aqueous solution of 20 mM substrate in a 100 mM phosphate buffer at pH 7.5 the compounds show different solution structures from those in $CDCl_3$. All substrates without an aromatic system in the molecule show three structures, similar to those reported by Guthrie[8] (Fig. 1) for **1b** at lower pH values (pH 1.5–6.5). This was determined by ^{1}H-NMR spectroscopy with the use of a presaturation method for water suppression.[22] Table 2 shows the ratio of the three forms for substances with distinct ^{1}H-NMR signals for the protons of the side chain. The ratios have been calculated from the relative integral values. The compounds with an aromatic system in the side chain form a delocalized π-system (in an equilibrium between the two possible enol isomers) that is distinguished by a broad ^{1}H-NMR signal of a methine proton in the 3-position at about 6 ppm. Data on the synthetic products and the shifts of the protons in the 3-position of all substances for the different aqueous solution structures are listed in Table 1.

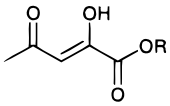
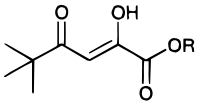
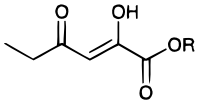
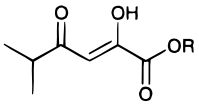
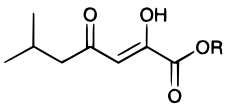
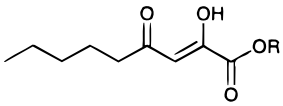
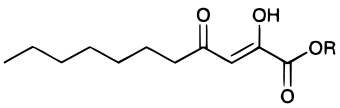
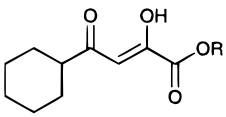
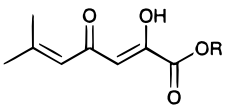
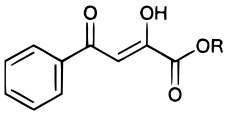
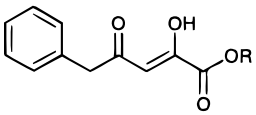
With 20 mM solutions of **1b**, 2,4-diketohexanoic acid (**3b**) and 2,4-diketo-5-phenylbutanoic acid (**10b**), the ^{1}H-NMR spectra were examined at different pH values. **10b** was just sufficiently soluble in buffer above pH 5.5. Under these conditions no significant change of the signals of this compound was found. In contrast, **1b** and **3b** show different ratios of the three structures. They have been measured for a pH range from 1.5 to 10.5 (**1b**) and from 1.5 to 7.5 (**3b**) and are in accordance with the data reported by Guthrie for **1b**.[8] At higher pH values the line-broadening leads to an overlap of the signals. The relative ratios of the three forms at different pH values are given in Fig. 2. They are based on the measured relative integral values for the ^{1}H-NMR signals of the methyl and ethyl groups for **1b** and **3b**. Additionally, the absorption maxima and the extinction coefficients of these solutions have been determined for the different pH values in a 0.05 mM solution in 0.25 mM phosphate buffer. These UV data are also given in Fig. 2.

To assign the integrated alkyl signals to the protons of the three forms it was necessary to determine the correct structure of the three forms of these compounds. This was done for the solutions of **1b**, 2,4-diketo-5,5-dimethylhexanoic acid (**2b**), **3b** and **10b**. More concentrated aqueous solutions (0.5 M) were used for ^{1}H- and ^{13}C-NMR measurements and heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra of each compound. A lower pH value was chosen because the substrates undergo a fast dimerization at higher pH values for **1b** and **3b**. The pH values used were 2.5 for **1b**, **2b** and **3b** and 6.5 for **10b**. We assign the singlets at about 6.0, 3.7 and 3.2 ppm to the enol, diketo and hydrate forms respectively. All these signals are generated from the protons bound to the carbon atom in the 3-position. Starting from those proton signals the $^1J_{C\text{-}H}$ and $^{2,3}J_{C\text{-}H}$ coupling constants for all three forms were determined by HMQC and HMBC (optimized for a coupling constant of 8 Hz). The results are shown in Fig. 3–5 and are listed in Table 3 for **3b**. They show that the enol and the hydrate must be formed on the keto group in the 2-position. The C atom in this 2-keto group only leads to a $^2J_{C\text{-}H}$ coupling to the proton in the 3-position in the diketo isomer. The carbon atom of the 4-keto group shows a $^2J_{C\text{-}H}$ coupling to the same proton and also $^3J_{C\text{-}H}$ couplings to all protons of the ethyl group. In the hydrate and enol forms only the 4-keto group is left and this gives the same couplings as the 4-keto carbon in the diketo structure. The carbon atom of the keto group in the hydrate may have a long relaxation time because the corresponding ^{13}C signal is very small. The quaternary carbon atom of the enol and the geminal diol only give a $^2J_{C\text{-}H}$ coupling to the protons in the 3-position. It is consistent that the 2-keto group is used to form the enol and the hydrate.

The aqueous solution structure of **10b** was determined by similar measurements. The results show broad signals for the proton in the C-3 position (about 30 Hz line half-width) as well as for the carbon atoms in the 2-, 3- and 4-positions. The shifts and the line shapes of these signals are not compatible with stable keto or enol forms but with a delocalized π-electron system on carbon atoms 2, 3 and 4, which can be explained by a fast interconversion between the two possible enol structures (Fig. 6). This can be seen from the shift values in Table 4.

The influence of metal ions on the structures was studied because Mg^{2+} ions activate and Cu^{2+} ions inhibit the β-ketolase activity with **1b** as substrate.[4] Solutions of 20 mM **1b**

Table 1 Results of the synthesis of 2,4-diketo acids

Compound		Yield/%	mp or bp/°C[a]	3-Proton shift in $CDCl_3$/ ppm	3-Proton shifts in H_2O/ppm[b]	Ref.
	R = H (**1b**)	49	98 (mp)	6.47	2.98/3.85/6.20	11
	R = C_2H_5 (**1a**)	62	96 (bp) 13 mbar	6.31		8
	R = H (**2b**)	65	63 (mp)	6.61	3.10/3.99/6.34	12
	R = C_2H_5 (**2a**)	70	121 (bp) 13 mbar	6.49		12
	R = H (**3b**)	68	81 (mp)	6.48	2.96/3.77/6.10	13
	R = C_2H_5 (**3a**)	71	106 (bp) 13 mbar	6.32		14
	R = H (**4b**)	45	Oil	6.49	3.03/3.92/6.15	15
	R = C_2H_5 (**4a**)	65	109 (bp) 13 mbar	6.35		15
	R = H (**5b**)	32	41 (mp)	6.43	3.01/4.09/6.15	5
	R = C_2H_5 (**5a**)	49	123 (bp) 13 mbar	6.30		16
	R = H (**6b**)	36	51 (mp)	6.45	3.02/3.92/6.09	5
	R = C_2H_5 (**6a**)	61	115 (bp) 0.66 mbar	6.32		14
	R = H (**7b**)	43	47 (mp)	6.46	3.03/3.99/6.03	14
	R = C_2H_5 (**7a**)	60	131 (bp) 0.66 mbar	6.35		14
	R = H (**8b**)	55	69 (mp)	6.49	3.03/3.91/6.12	—
	R = CH_3 (**8a**)	62	120 (bp) 13 mbar	6.40		—
	R = H (**9c**)	52	90 (mp)	6.38	2.97/3.86/5.95	17, 18
	R = C_2H_5 (**9b**)	31[c]	nd	6.26		17, 18
	R = H (**10b**)	50	152 (mp)	7.15	6.20 (br)[d]	19, 20
	R = C_2H_5 (**10a**)	79	139 (bp) 0.66 mbar	7.02		19, 20
	R = H (**11b**)	37	58 (mp)	6.44	5.75 (br)[d]	21
	R = C_2H_5 (**11a**)	52	145 (bp) 0.66 mbar	6.35		21

[a] mp: melting point; bp: boiling point at the reported pressure. [b] Proton shifts are in the order hydrate/diketone/enol at pH = 7.5. [c] Over two steps. [d] Broad signal at the equilibrium between the two enol isomers.

Table 2 Relative ratios[a] of the three forms in water (buffer) at pH 7.5

Compound	Diketone/%	Enol/%	Hydrate/%
1b	41	44	15
3b	36	59	5
4b	42	48	10
2b	45	51	4
9c	37	48	15
10b		No separate isomer (see Fig. 6)	

[a] Ratios are calculated from the integrated signals of the protons of the side chain.

and 10 mM of the metal salts in 100 mM phosphate buffer at pH 7.5 were directly measured by ^{1}H-NMR spectroscopy. Sixteen metal ions (Table 5) were examined in this way. They have varying influence on the structures. Some ions increase the amount of the enol form and others that of the diketo form while several do not influence the ratios significantly. Copper and cobalt favour one isomer of **1b** that is similar to the π-delocalized form described for **10b**. This was seen by the loss of all signals of the three forms and by the appearance of new broad signals with chemical shifts similar to those of **10b**. In some cases there are small shift differences from the signals observed for the compound without metal ions in solution. This is due to overlapping of the signals from the hydrate and

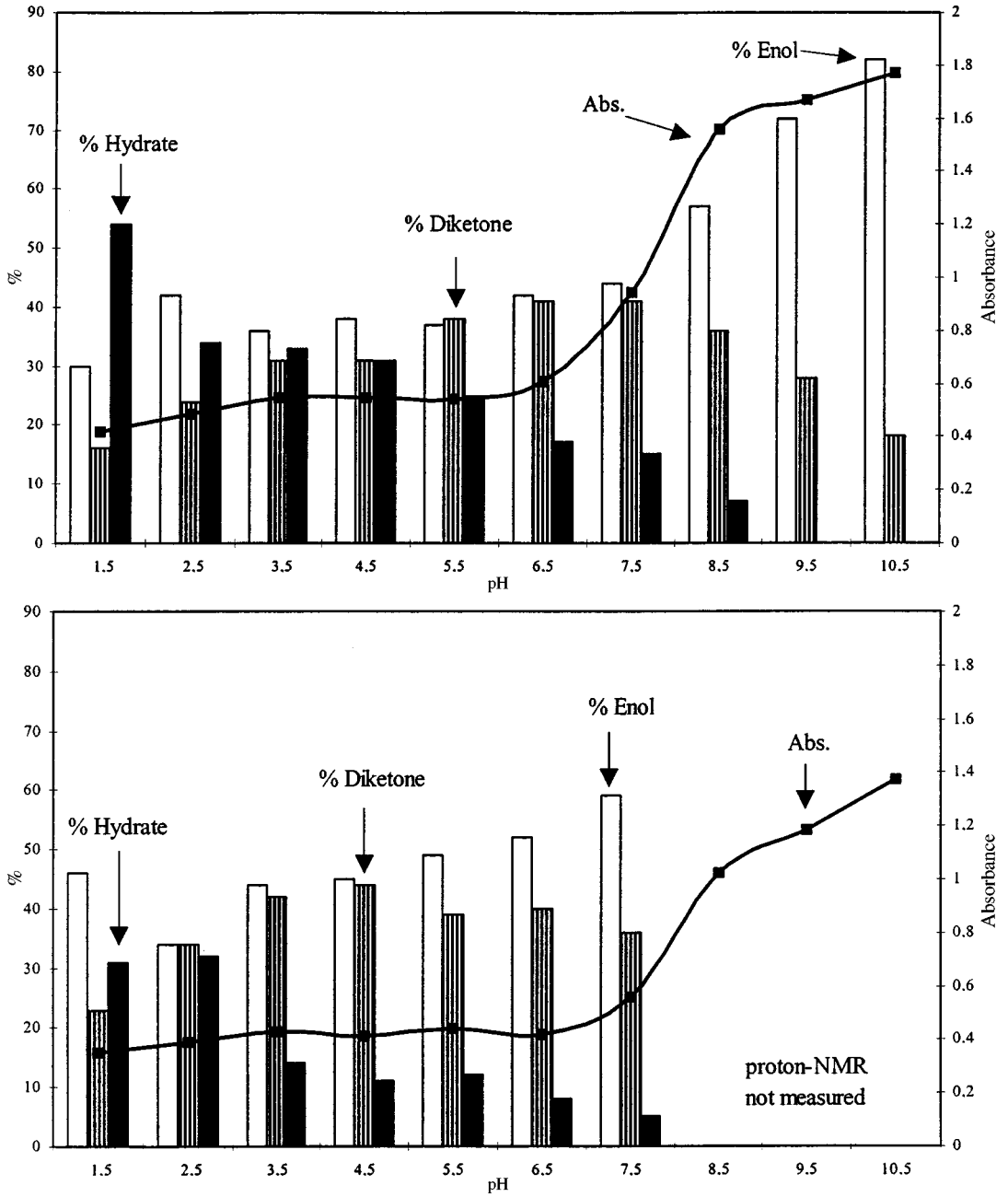


Fig. 2 Relative ratios of the different forms of (top) **1b** and (bottom) **3b**. % form is the amount of the different forms in solution determined by ^{1}H-NMR spectrometry. Abs. is the absorption at the maximum. Proton NMR of **3b** not measured because of overlapping ^{1}H-NMR signals.

the enol forms. Paramagnetic metal ions like Mn^{2+} and Fe^{2+} also lead to broader lines due to overlapping of the signals (Table 5). The isomeric delocalized form of **10b** is not influenced by the Mg^{2+} ions; no difference in the ^{1}H-NMR spectrum of this compound was found after the addition of Mg^{2+} ions.

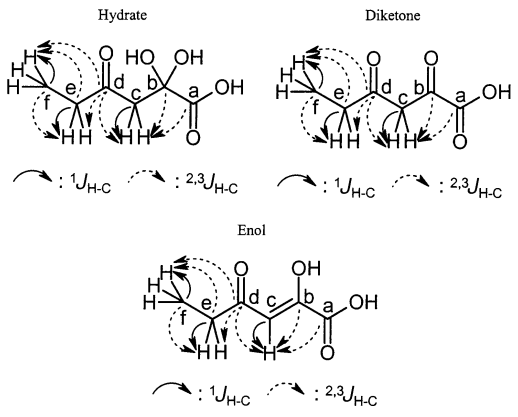


Fig. 3 C–H couplings in **1b** detected in HMQC and HMBC spectra.

Dynamic procedures in aqueous solution

For the rate of enolization a value of $k \approx 0.06$–0.18 s^{-1} was reported in acetic acid buffer at pH 4.97 with 0.174 mM **1b**.[8] Because we have used a phosphate buffer for all the other investigations we checked the enolization rate in this buffer at various pH values. The iodine reaction used for determination of the enolization rate in the acetic acid buffer was used again. We found a similar value ($k \approx 0.06$–0.08 s^{-1}) at all pH values from 3.5 to 8.5. Below pH 3.5 the enolization rates were slower and above pH 8.5 much faster (Table 6). The values measured by this method are relatively inaccurate (error of about 20%) because of the experimental errors described earlier.[8] However, we can show that the rate of enolization in the phosphate buffer system is similar to that in an acetic acid buffer at pH 4.97. We further showed that addition of metal ions (Mg^{2+}, Cu^{2+}) does not influence the rate constant significantly at pH 7.5 for **1b**.

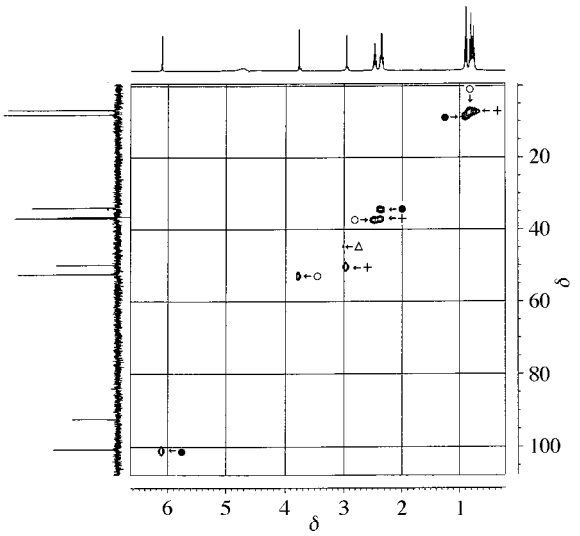


Fig. 4 HMQC spectrum of an aqueous solution of **3b**: (●) cross peaks belonging to the enol isomer, (○) cross peaks belonging to the diketo isomer, (+) cross peaks belonging to the hydrate form, (△) cross peaks that are artefacts or that belong to the dimer, a small amount of which is formed during the measurement.

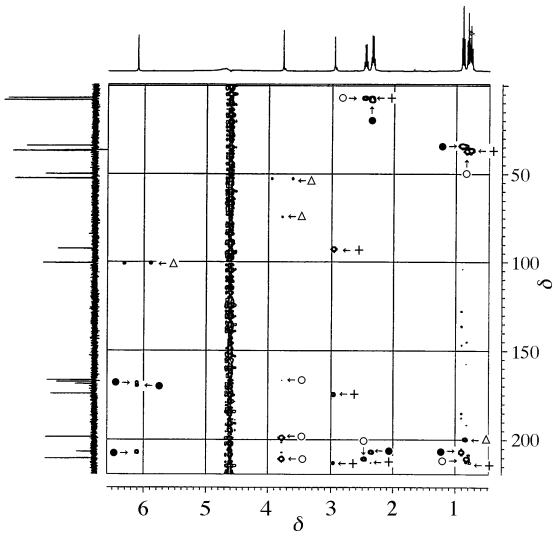


Fig. 5 HMBC spectrum of an aqueous solution of **3b**: (●) cross peaks belonging to the enol isomer, (○) cross peaks belonging to the diketo isomer, (+) cross peaks belonging to the hydrate form, (△) cross peaks that are artefacts or that belong to the dimer, a small amount of which is formed during the measurement.

The enolization rates suggest that there is an exchange of the protons at the 3-position in an aqueous solution. NMR spectra of a 0.1 M **1b** solution in a 0.5 M phosphate buffer at pH 7.5 with 50% D_2O were taken. Proton-deuterium exchange leads to several partially deuteriated compounds. The ^{1}H-NMR and ^{13}C-NMR spectra show signals of compounds deuteriated in the 3-position, in addition to the three structures described before (Table 7). The ^{13}C signals of the completely deuteriated hydrate and diketo forms were not detected because of the relatively low concentration of these compounds and the low sensitivities of these carbon atoms.

We also showed that slow dimerizations are perturbing side reactions. The dimerization reaction rate increases with pH and with the concentration of the 2,4-diketo acids. It further depends on the structure of the alkyl substituent. **1b**, **3b** and 5-methyl-2,4-diketohexanoic acid (**4b**) show a relatively fast dimerization, whereas with the more bulky and longer chain substituents it was slower. We prepared the dimer from a 0.5 M solution of **1b** in 1 M buffer at ambient temperature over 48 h, and examined NMR spectra from the resulting dimer directly. The product was 3-acetyl-4-carboxy-2,4-dihydroxy-6-oxohept-2-enoic acid (**12**). The structure was determined by heteronuclear correlation (HETCOR) and correlation spectroscopy *via* long range coupling (COLOC) spectra. The connectivities are shown in Fig. 7 and the ^{1}H- and ^{13}C-NMR data are given in Table 8. The *E*/*Z* configuration of the double bond was not considered. We did not find a lactone ring form under these conditions, as reported for the dimers of ethyl 2,4-diketopentanoate (**1a**), which was analysed after isolation.[23]

Discussion

2,4-Diketoacids form predominantly (98%) an enol structure at the 2-keto group when dissolved in an aprotic organic solvent ($CDCl_3$). The corresponding diketo isomer and the enol isomer at the 4-keto group are not detectable in this solvent. The conjugated enol must be energetically favourable. This enol isomer is also the predominant form (≈50%) in an aqueous solution at pH 7.5. The minor structures that occur

Table 3 NMR data for **3b** in water at pH 7.5

Position[a]	^{13}C shift/ppm (mult.[b])	^{1}H shift/ppm (mult.[c])	^{1}H integral	$^3J_{H\text{-}H}$/Hz
Diketo form				
a	166.8 (q)			
b	198.5 (q)			
c	52.5 (s)	3.77 (s)	2H	
d	210.5 (q)			
e	36.9 (s)	2.47 (q)	2H	7.2
f	6.9 (p)	0.79 (t)	3H	7.2
Hydrate form				
a	174.4 (q)			
b	92.4 (q)			
c	49.9 (s)	2.96 (s)	2H	
d	212.0 (q)			
e	36.6 (s)	2.33 (q)	2H	7.2
f	7.0 (p)	0.76 (t)	3H	7.2
Enol form				
a	168.9 (q)[d]			
b	167.7 (q)[d]			
c	100.6 (s)	6.10 (s)	1H	
d	206.7 (q)			
e	34.0 (s)	2.33 (q)	2H	7.2
f	8.0 (p)	0.91 (t)	3H	7.2

[a] Positions are marked on Fig. 3. [b] q: quaternary; s: secondary; p: primary. [c] s: singlet; t: triplet; q: quartet. [d] Interchangeable.

Table 4 NMR data for **10b** in water at pH 7.5

Position[a]	^{13}C shift/ppm (mult.[b])	^{1}H shift/ppm (mult.[c])	^{1}H integral
a	173.0 (q, br)		
b	183.0 (q, br)		
c	94.7 (t, br)	6.2 (s, br)	1H
d	199.9 (q, br)		
e	141.6 (s)		
f	135.8 (t)	7.72 (m_c)	2H
g	134.0 (t)	7.38 (m_c)	2H
h	128.6 (t)	7.32 (m_c)	1H
OH		5.6 (s, br)	1H

[a] Positions are marked on Fig. 6. [b] q: quaternary; t: tertiary; s: secondary; br: broad. [c] s: singlet; m_c : centred multiplet; br: broad.

Table 5 Influence of metal ions on the relative ratios of the structures of **1b** in aqueous solution

		% Structures				
Metal[a]	Salt[b]	Diketone[c]	Enol[c]	Hydrate[c]	Cyclic[c]	CH proton shift in enol[d,e]
Manganese	$MnSO_4$	← 87	→	13	0	2.24 (br)
Mercury	$HgSO_4$	23	72	5	0	2.20
Magnesium	$MgSO_4$	30	58	12	0	2.09
Zinc	$ZnCl_2$	29	58	13	0	2.13
Cadmium	$CdCO_3$	32	← 68	→	0	2.15
Calcium	$CaCl_2$	34	58	8	0	2.21 (br)
No metal ions		34	53	13	0	2.16
Lead	$PbCl_2$	34	← 66	→	0	2.16
Thallium	$Tl(NO_3)_2$	37	← 63	→	0	2.16
Aluminium[f]	$AlOH_3$	38	← 62	→	0	2.16
Barium	$BaCl_2$	38	← 62	→	0	2.16
Tin	$SnCl_2$	39	46	15	0	2.20
Cerium	$Ce_2(SO_4)_3$	40	← 60	→	0	2.16
Nickel	$NiSO_4$	41	← 59	→	0	2.16
Iron	$FeCl_2$	43	← 57	→	0	2.16 (br)
Cobalt	$CoSO_4$	30	20	0	50	2.16
Copper	$CuSO_4$	10	5	0	85	2.15

[a] Concentration of **1b** is 20 mM. [b] Concentration of the metal salt is 10 mM. [c] ^{1}H-NMR signal of the CH_3 group in **1b**. [d] br: broad. [e] Shift is relative to the H_2O signal (4.7 ppm) in the same solution. [f] Not dissolved.

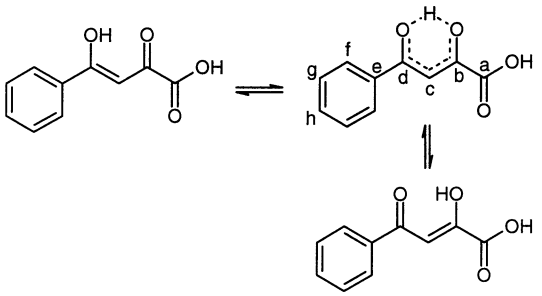


Fig. 6 Aqueous solution structures of **10b** with two possible extreme enol structures.

Table 6 Rate constants for the enolization of **1b**

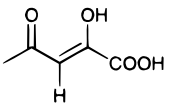
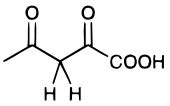
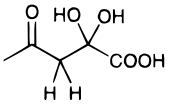
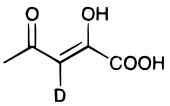
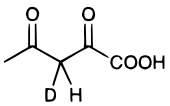
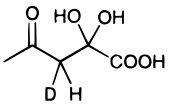
Solution pH	Rate constant/s^{-1}
1.5	0.015
2.5	0.026
3.5	0.064
4.5	0.066
5.5	0.061
6.5	0.085
7.5	0.079
8.5	0.065
9.5	>0.5
10.5	>0.5

depend on the substituent at the 4-keto group. An alkyl or alkenyl group gives two other forms, a diketo isomer and a hydrate of the 2-keto group. Aryl substituents take part in conjugated systems and a pseudodienolate is formed (Fig. 6), possibly due to a interconversion between the two formal enolate structures.

Alkyl and alkenyl substituents

The diketo and enol isomers and the H_2O adducts have at pH 7.5 an approximate ratio of 4 : 5 : 1 that is not significantly influenced by the nature of the side chain. The energy barrier between the forms must be relatively high because the rates of isomerization and of addition/elimination are slower than 1.0 s^{-1}. This can been concluded from the sharp ^{1}H-NMR signals for all three forms. Guthrie[8] has calculated the pK_a values and the equilibrium constants for the **1b** system in acetic acid. They show that the enol is preferred at higher pH values and the hydrate is the dominant form at lower pH values. The diketone can exist in large amounts only in the neutral range. Our measured ratios of **1b** in phosphate-buffered systems are consistent with this. The change of the enolization rate constant of **1b** conforms with the change of the pH value. Between the pK_a values of the 2-enolate (7.6) and the carboxylates (1.30, 2.79 and 3.21) the enolization rate is about 0.07 s^{-1}. At lower pH values the enolization rate is much lower, and at higher pH values much faster, so that the activation energy of the isomerization is influenced by the protonation of the carboxyl and enol groups. This is consistent with the line

Table 7 ^{1}H- and ^{13}C-NMR data for various forms of **1b** in deuteriated water[a]. Signals of the proton and carbon in the 3-position

Compound	^{1}H shift (ppm)	^{13}C shift (ppm) $^{1}J_{C\text{-}D}$/Hz
	6.11	102.4
	3.80	53.4
	2.98	51.1
	———	102.1 24.5
	3.76 Rel. int. value 1 : 1[b]	53.1 19.5
	2.95 Rel. int. value 1 : 1[b]	50.8 19.5

[a] Starting concentration of the non-deuterated substance is 0.5 M. [b] Relative integral when compared to that of the same fully protonated form in the same solution.

resolution of the ^{1}H-NMR spectra observed at these pH values, the enolization rate being much faster than the time scale for NMR measurements. (Fig. 8).

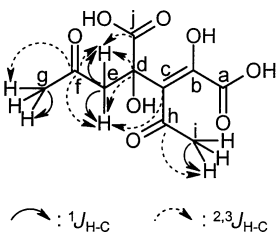


Fig. 7 C–H couplings in **12** detected in HETCOR and COLOC spectra.

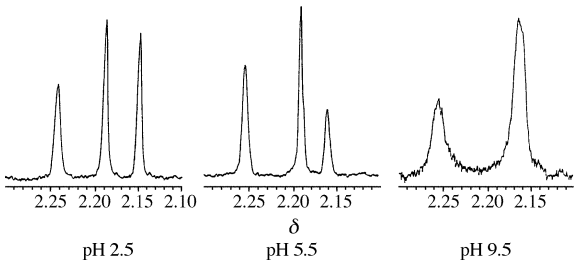


Fig. 8 pH-dependent line broadening of ^{1}H-NMR signals of **1b**. The signals of the methyl group in the three forms are shown at different pH values.

Some divalent metal ions influence the isomeric ratio of the 2,4-diketo acids in solution. They can be divided into two different groups that lead to different coordination structures. The best representative of the first group is Cu^{2+}. This ion forms a coordination bond to the oxygens of the two keto groups, as shown for compound **1b** in Fig. 9. The ratio of the isomers completely changes and an equilibrium between the two possible enol isomers appears. The enolization rate between these two must be much faster than 1.0 s^{-1}, since only one broad ^{1}H-NMR signal of the proton in the 3-position can be detected. Similar coordination structures were reported by Krebs and Johnson[24] and by Kawai *et al.*[25] An analogous system is well-known for the acetoacetone–Cu^{2+} coordination system.[26] The second group of metal ions, for which Mg^{2+} is the best representative, shows a different behaviour. They shift the ratio from the diketo to the enol isomer, but the enolization rate must still be lower than 1.0 s^{-1}, because the NMR signals are well-resolved. This behaviour can be explained by coordination of the 2-enolate and carboxylate oxygens to the metal ion, as was formulated by Kawai *et al.*[25] for a platinum complex of **1b**. This complex leads to a stabilization of the enol isomer (Fig. 10).

The dimerization was assumed to be an aldol-type reaction[8] and seems to be similar to the dimerization reported by Berner and Kolsaker.[23] The dimer we found in solution is the product of an aldol addition between a carbanion formed at

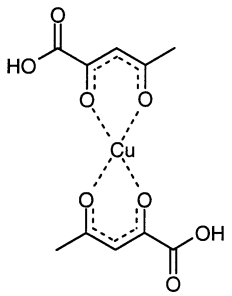


Fig. 9 Possible aqueous solution structure of the **1b**–copper complex.

Table 8 NMR data for **12** in water at pH 7.5

Position[a]	^{13}C shift/ppm (mult.[b])	^{1}H shift/ppm (mult.[c])	^{1}H integral	$^{2}J_{H\text{-}H}$/Hz
a	174.6 (q)			
b	162.7 (q)			
c	118.2 (s)			
d	85.4 (q)			
e	46.5 (s)	3.48 (d)	1H	16.7
		3.06 (d)	1H	16.7
f	212.5 (p)			
g	30.8 (q)	2.03 (s)	3H	
h	195.6 (q)			
i	28.2 (s)	2.27 (s)	3H	
j	174.9 (q)			

[a] Positions are marked on Fig. 7. [b] q: quaternary; s: secondary; p: primary. [c] s: singlet; d: doublet.

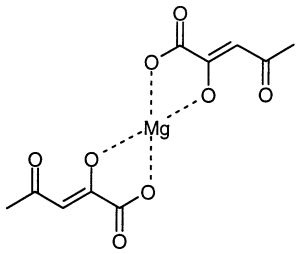
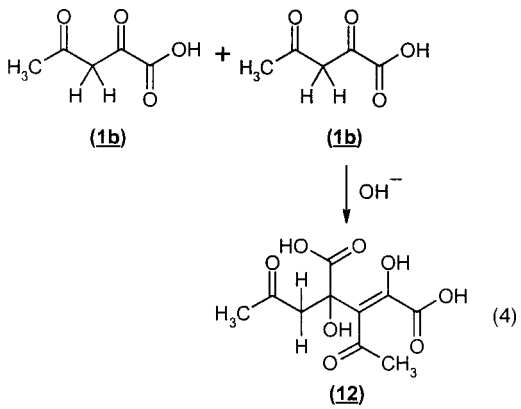


Fig. 10 Possible aqueous solution structure of the **1b**–magnesium complex.

the 3-position and the 2-keto group of a second monomer [eqn. (4)]. An elimination step does not take place in aqueous solution but one keto group enolizes. This shows that the diketo isomer is necessary for this reaction and explains that the dimerization only takes place in aqueous solutions with alkyl-type side chains, since in aqueous solution the diketone only exists in the case of these compounds. The increase of the dimerization rate with pH can be explained by the deprotonated form generated at alkaline pH.



(4)

Conjugated aryl substituents

Compounds with a phenyl substituent at the 4-keto group show a broad ^{1}H-NMR signal at 6.2 ppm for the proton in the 3-position and broad ^{13}C-NMR signals for the carbon atoms in the 2-, 3- and 4-positions. This is a typical sign of a fast interconversion between isomeric forms where a CH group is in the 3-position. A delocalized π-electron system conjugated with the aromatic ring, as shown in Fig. 6, explains such spectral data. The 2- and 4-enolates can be derived as the extreme structures of this conjugated system. The energy level of this delocalized system has to be much lower than either of the other 2- or 4-enolate isomers, because of its stability. In addition, this structure does not show any tendency to dimerize and addition of Mg^{2+} ions does not lead to a stable enol isomer. The pH value does not influence this structure. It does not undergo any reaction similar to that of analogues that have separate isomers.

The benzyl substituent at the 4-keto group also shows a similar broad ^{1}H-NMR signal for the proton at the 3-position and the protons at the benzylic positions show a broad signal with a lowfield shift. This can be explained by a similar delocalization where both keto groups take part in a delocalized system. Fig. 11 shows two extreme structures of such a delocalized system.

Significance of the solution structures

The 2,4-diketo acids examined for hydrolysis by acetopyruvate hydrolase from rat liver (EC 3.7.1.5) all have aliphatic side chains.[5] Cleavage of analogues such as 2,4-diketo-5-phenylbutanoic acid (**10b**) was not reported. So one of the

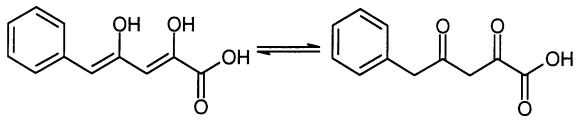


Fig. 11 Possible extreme structures of **11b** in aqueous solution.

three forms of the compounds with an alkyl side chain that are formed in water may be accepted as the substrate by the enzyme. This shows that other factors, in addition to steric properties, are responsible for the acceptance of 2,4-diketo-acids by acetopyruvate hydrolases [EC 3.7.1.5(6)]. Details of the enzymic experiments with acetopyruvate hydrolase (EC 3.7.1.6) from *P. putida* are presented elsewhere.

Experimental

Materials and methods

All commercial starting materials were used without purification. Organic solvents for the reactions have been purified by common methods.[27] All the water- and air-sensitive reactions were performed under an N_2 atmosphere. Silica gel 60 F_{254} TLC plates (Merck) were used and the spots observed under UV light. Melting points were determined with a Büchi 530 apparatus and are uncorrected. The boiling points for distillation refer to uncorrected temperatures.

For NMR spectra in $CDCl_3$ (250 mM), the $CHCl_3$ signal was taken as a reference (7.24 ppm). In water the compounds (20 mM) were dissolved in 100 mM phosphate buffer. The pH value was adjusted to 7.5 except for the pH dependence measurements. A D_2O vortex capillary was added for a lock signal. ^{1}H–^{2}D exchange experiments used 50% D_2O without the capillary and with 0.1 mM of **1b** in 0.5 mM buffer. HMQC and HMBC spectra were obtained in 1 M phosphate buffer, at substance concentrations of 0.5 M. As reference the H_2O signal was taken in all cases (4.70 ppm) and for suppression of the water signal the presaturation method was used.[22] The dimer 3-acetyl-4-carboxy-2,4-dihydroxy-6-oxohept-2-enoic acid (**12**) was prepared from **1b** (0.5 M) in 1 M buffer. The spectra were taken directly on the resulting solution. All one-dimensional HETCOR and COLOC spectra were measured on a Varian Gemini 200 system. The HMQC and HMBC spectra were recorded on Bruker DRX-400 and Varian Unity-600 systems with the gradient technique.

The UV spectra of the pure compounds have been taken on 0.05 mM solutions in a 0.25 mM buffer. Enolization rate measurements were made with a solution of 0.02 mM iodine and 0.15 mM KI in a 100 mM phosphate buffer (0.9 ml). **3b** (0.1 mM, 100 μl) was added and the decrease in the extinction of the iodine absorption maximum (292 nm) was measured. The spectra of the substances were obtained with a Spectronic Genesis 2PC and the kinetic experiments with a Shimadzu UV-240 Graphicord.

General synthetic procedures

Procedure A. Sodium (2.38 g, 103 mmol) was added to absolute ethanol (133 ml). The mixture was cooled to 0 °C and a mixture of diethyl oxalate (14.03 g, 96 mmol) and the ketone (96 mmol) was added slowly over a period of 20 min. A precipitate formed and stirring was continued for 4 h at room temperature. The precipitate was filtered, washed with absolute ethanol (20 ml) and dissolved in 2 N sulphuric acid (150 ml) and ether-extracted (3 × 150 ml), dried over Na_2SO_4 and ether removed. The residue was distilled under reduced pressure.

Procedure B. Sodium (2.38 g, 103 mmol) was added to absolute ethanol (133 ml) and cooled to 0 °C. A mixture of diethyl oxalate (14.03 g, 96 mmol) and the ketone (96 mmol)

was added slowly over a period of 20 min. Stirring was continued for 4 h at room temperature. Water (10 ml) was added with stirring and the ethanol (80–100 ml) was removed under reduced pressure. The residue was poured into 2 N sulphuric acid (150 ml), extracted with ether (3 × 150 ml, dried over Na_2SO_4 and the ether was evaporated. The residue was then distilled under reduced pressure.

Procedure C. The pure esters (7.7 mmol) from procedures A or B were dissolved in 5 N NaOH (10 ml) and acetone (1 ml) at 0 °C. The mixture was stirred and the hydrolysis was monitored by TLC. After complete hydrolysis 5 N sulphuric acid (10 ml) was added slowly. If the product precipitated directly it was filtered, otherwise the solution was extracted with ether, dried over Na_2SO_4 and then evaporated. The residue was recrystallized in the solvent, specified below with the melting points.

Procedure D. The pure esters (7.7 mmol) from procedure A or B were dissolved in 5 N sulphuric acid (10 ml), stirred and the process of the reaction monitored by TLC. The acids were isolated as for (C).

Syntheses

Ethyl 2,4-diketopentanoate (**1a**) was made by procedure A with acetone (5.57 g). A yellow liquid (9.49 g, 62% yield) resulted with bp 96 °C/13 mbar (lit.: 117–119 °C/38 mbar[11]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.30 (t, 3H, $^3J_{H\text{-}H}$ = 7.2 Hz), 2.19 (s, 3H), 4.27 (q, 2H, $^3J_{H\text{-}H}$ = 7.2 Hz), 6.31 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 27.6, 62.5, 102.1, 162.0, 167.0, 200.0.

2,4-Diketopentanoic acid (**1b**) was made by procedure C from **1a** (1.22 g). A colourless crystalline solid (0.49 g, 49% yield) resulted with mp 98 °C (CCl_4) (lit.: 98–100 °C[8]). ^{1}H-NMR (200 MHz, $CDCl_3$): 2.28 (s, 3H), 6.47 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 26.9, 101.6, 164.4, 168.2, 198.8. UV (H_2O, c = 0.1 mM): λ_{max} = 294 nm, $\varepsilon/c = 0.946 \times 10^{-4}$.

Ethyl 2,4-diketo-5,5-dimethylhexanoate (**2a**) was made by procedure B with 3,3-dimethyl-2-butanone (9.60 g). A yellow liquid (13.66 g, 70% yield) resulted with bp 121 °C/13 mbar (lit.: 116–118 °C/13 mbar[12]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.16 (s, 9H), 1.33 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 4.30 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.49 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 26.7, 41.6, 62.4, 97.8, 162.3, 167.5, 209.2.

2,4-Diketo-5,5-dimethylhexanoic acid (**2b**) was made by procedure C from **2a** (1.62 g). A colourless crystalline solid (0.90 g, 65% yield) resulted with mp 63 °C (CCl_4) (lit.: 64 °C[28]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.20 (s, 9H), 6.61 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 26.7, 41.5, 97.8, 164.8, 167.8, 208.7.

Ethyl 2,4-diketohexanoate (**3a**) was made by procedure A with 2-butanone (6.91 g). A yellow liquid (12.11 g, 71% yield) resulted with bp 106 °C/13 mbar (lit.: 108–111 °C /11 mbar[13]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.12 (t, 3H, $^3J_{H\text{-}H}$ = 7.5 Hz), 1.31 (t, 3H, $^3J_{H\text{-}H}$ = 7.2 Hz), 2.49 (q, 2H, $^3J_{H\text{-}H}$ = 7.5 Hz), 4.29 (q, 2H, $^3J_{H\text{-}H}$ = 7.2 Hz), 6.32 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 8.5, 13.9, 34.2, 62.5, 101.3, 162.2, 166.0, 204.2.

2,4-Diketohexanoic acid (**3b**) was made by procedure C from **3a** (1.35 g). A colourless crystalline solid (0.75 g, 68% yield) resulted with mp 81 °C (CCl_4) (lit.: 83 °C[14]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.19 (t, 3H, $^3J_{H\text{-}H}$ = 7.5 Hz), 2.55 (q, 2H, $^3J_{H\text{-}H}$ = 7.5 Hz), 6.48 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 8.9, 33.5, 100.4, 164.1, 168.0, 202.5. UV (H_2O, c = 0.1 mM): λ_{max} = 291 nm, $\varepsilon/c = 0.559 \times 10^{-4}$.

Ethyl 2,4-diketo-5-methylhexanoate (**4a**) was made by procedure B with 3-methyl-2-butanone (8.25 g). A yellow liquid (12.51 g, 65% yield) resulted with bp 109 °C/13 mbar (lit.: 90–93 °C/8 mbar[29]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.13 (d, 6H, $^3J_{H\text{-}H}$ = 6.9 Hz), 1.32 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 2.61 (sep, 1H, $^3J_{H\text{-}H}$ = 6.9 Hz), 4.29 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.35 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 18.6, 39.0, 62.5, 100.0, 162.4, 167.1, 207.2.

2,4-Diketo-5-methylhexanoic acid (**4b**) was made by procedure C from **4a** (1.48 g). A slightly yellow oil (0.57 g, 45% yield) resulted that does not crystallize. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.20 (d, 6H, $^3J_{H\text{-}H}$ = 7.0 Hz), 2.81 (sep, 1H, $^3J_{H\text{-}H}$ = 7.0 Hz), 6.49 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.7, 38.6, 99.4, 164.2, 168.4, 206.0.

Ethyl 2,4-diketo-6-methylheptanoate (**5a**) was made by procedure B with 4-methyl-2-pentanone (9.96 g). A yellow liquid (9.76 g, 49% yield) resulted with bp 123 °C/13 mbar (lit.: 112 °C/3 mbar[30]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.92 (d, 6H, $^3J_{H\text{-}H}$ = 6.5 Hz), 1.33 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 2.26 (m, 1H), 2.31 (d, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 4.30 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.30 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 22.5, 26.0, 49.6, 62.5, 101.9, 162.3, 167.6, 202.2.

2,4-Diketo-6-methylheptanoic acid (**5b**) was made by procedure D from **5a** (1.62 g). A colourless crystalline solid (0.45 g, 32% yield) resulted with mp 41 °C (CCl_4) (lit.: 48 °C[16]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.96 (d, 6H, $^3J_{H\text{-}H}$ = 6.7 Hz), 2.14 (m, 1H), 2.61 (d, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.43 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 23.4, 25.5, 48.6, 101.3, 164.3, 167.7, 200.1.

Ethyl 2,4-diketononanoate (**6a**) was made by procedure B with 2-heptanone (10.94 g). A yellow liquid (12.60 g, 61% yield) resulted with bp 115 °C/0.66 mbar (lit.: 144–147 °C/8 mbar[14]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.85 (t, 3H, $^3J_{H\text{-}H}$ = 6.4 Hz), 1.30 (m, 7H), 1.61 (quint, 2H, $^3J_{H\text{-}H}$ = 7.7 Hz), 2.44 (t, 2H, $^3J_{H\text{-}H}$ = 7.7 Hz), 4.30 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.32 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.8, 14.0, 22.4, 24.6, 31.3, 40.8, 62.4, 101.6, 162.1, 166.7, 203.3.

2,4-Diketononanoic acid (**6b**) was made by procedure C from **6a** (1.84 g). A colourless amorphous solid (0.58 g, 36% yield) resulted with mp 51 °C (benzene, petroleum ether) (lit.: 52 °C[14]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.89 (t, 3H, $^3J_{H\text{-}H}$ = 6.4 Hz), 1.31 (m, 5H), 1.65 (quint, 2H, $^3J_{H\text{-}H}$ = 7.6 Hz), 2.48 (t, 2H, $^3J_{H\text{-}H}$ = 7.6 Hz), 6.45 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.8, 22.3, 24.8, 31.2, 40.2, 100.9, 164.2, 168.3, 202.1.

Ethyl 2,4-diketoundecanoate (**7a**) was made by procedure B with 2-nonanone (12.28 g). A yellow liquid (12.65 g, 60% yield) resulted with bp 131 °C/0.6 mbar (lit.: 165–170 °C/10 mbar[14]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.86 (t, 3H, $^3J_{H\text{-}H}$ = 6.4 Hz), 1.31 (m, 9H), 1.64 (quint, 2H, $^3J_{H\text{-}H}$ = 7.5 Hz), 2.46 (t, 2H, $^3J_{H\text{-}H}$ = 7.5 Hz), 4.33 (q, 2H, $^3J_{H\text{-}H}$ = 7.2 Hz), 6.35 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 14.1, 22.6, 24.9, 28.9, 29.1, 31.6, 40.9, 62.5, 101.6, 162.2, 166.8, 203.3.

2,4-Diketoundecanoic acid (**7b**) was made by procedure C from **7a** (2.00 g). A colourless amorphous solid (0.75 g, 43% yield) resulted with mp 47 °C (petroleum ether) (lit.: 51 °C[14]). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.87 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz), 1.29 (m, 7H), 1.66 (quint, 2H, $^3J_{H\text{-}H}$ = 7.3 Hz), 2.49 (t, 2H, $^3J_{H\text{-}H}$ = 7.3 Hz), 6.46 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.1, 22.6, 25.2, 28.9, 29,2, 31.6, 40.1, 100.7, 164.3, 168.4, 201.9.

Methyl-4-cyclohexyl-2,4-diketobutanoate (**8a**). Sodium (1.97 g, 85 mmol) was added to absolute methanol (44 ml). The mixture was cooled to 0 °C and then a mixture of dimethyloxalate (9.35 g, 79 mmol) and acetylcyclohexane (10.0 g, 79 mmol) was added slowly over a period of 20 min. Stirring was continued 4 h at room temperature. 2 N sulphuric acid (120 ml) was added under stirring and methanol was destilled out of the reaction mixture under reduced pressure (80–100 ml). This aqueous solution was extracted three times with ether (3 × 150 ml), the organic extract was dried over Na_2SO_4 and the ether was distilled on a steam bath. The residue was distilled under reduced pressure. A yellow liquid (10.4 g, 62% yield) resulted with bp 120 °C/13 mbar. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.30 (m, 5H), 1.79 (m, 5H), 2.42 (m, 1H), 3.88 (s, 3H), 6.40 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 25.5, 25.6, 28.9, 48.7, 53.1, 100.3, 162.7, 167.3, 206.0.

4-Cyclohexyl-2,4-diketobutanoic acid (**8b**) was made by procedure C from **8a** (1.40 g). A colourless amorphous solid (0.67 g, 55% yield) resulted with mp 69 °C (H_2O). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34 (m, 5H), 1.88 (m, 5H), 2.41 (m, 1H), 6.49 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 25.4, 25.6, 29.1, 47.8, 98.8, 163.6, 169.9, 204.0.

2-Carboxyethyl-6,6-dimethyl-4-oxo-dihydro-4*H*-pyran (**9a**). Sodium (8.00 g, 348 mmol) was added to absolute ethanol (250 ml). The mixture was cooled to 0 °C and a mixture of diethyl oxalate (50.20 g, 342 mmol) and mesityl oxide (33.97 g, 346 mmol) was added slowly over a period of 40 min. After adding about 30% of this mixture a turbid reaction mixture was obtained. A precipitate formed and stirring was continued for 2 h at room temperature. The mixture was diluted with ether (150 ml) and then poured into 2 N sulphuric acid (800 ml). This solution was extracted with ether (3 × 300 ml). The organic extract was dried over Na_2SO_4 and the ether was distilled. The residue was purified by distillation. A yellow liquid (42.3 g, 62% yield) resulted with bp 132 °C/13 mbar (lit.: 167 °C/5 mbar[17]), which crystallized after a few hours. It has a mp of 58 °C (lit.: 59–60 °C[17]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.33 (t, 3H, $^3J_{H\text{-}H}$ = 7.5 Hz), 1.48 (s, 6H), 2.53 (s, 2H) 4.31 (q, 2H, $^3J_{H\text{-}H}$ = 7.5 Hz), 6.18 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.0, 25.8, 47.7, 62.5, 82.6, 107.3, 157.3, 162.1, 193.7.

Ethyl 2,4-diketo-6-methylhept-5-enoate (**9b**). To a solution of sodium (1.15 g, 50 mmol) in absolute ethanol (30 ml) **9a** (10.0 g, 50 mmol) was added at room temperature. The mixture was stirred for 2 h and a yellow solid precipitated. Water (100 ml) was added at 0 °C to dissolve the precipitate. After acetic acid was added a yellow suspension appeared. A solution of copper acetate (4.0 g, 22 mmol) in water (30 ml) was added slowly, the suspension changed colour from yellow to green and the green precipitate was filtered. The green solid was dissolved in $CHCl_3$ (200 ml) and extracted with 2 N sulfuric acid until the green colour in the organic layer disappeared. The organic layer was dried over Na_2SO_4 and the solvent removed by distillation. A yellow oil (5.1 g, 51% yield) resulted, which was not further purified. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.35 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 1.96 (d, 3H, $^4J_{H\text{-}H}$ = 1.0 Hz), 2.32 (d, 1H, $^4J_{H\text{-}H}$ = 1.0 Hz), 4.32 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 5.90 (dd, 3H, $^4J_{H\text{-}H}$ = 1.0 Hz, $^4J_{H\text{-}H}$ = 1.0 Hz), 6.26 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.1, 21.5, 28.5, 62.3, 103.2, 122.4, 159.5, 162.4, 169.1, 190.4.

2,4-Diketo-6-methylhept-5-enoic acid (**9c**) was made by procedure C from **9b** (1.60 g). A colourless crystalline solid (0.72 g, 52% yield) resulted with mp 90 °C (ether, petroleum ether) (lit.: 92–93 °C[17]). ^{1}H-NMR (200 MHz, $CDCl_3$): 2.02 (d, 3H, $^4J_{H\text{-}H}$ = 1.1 Hz), 2.27 (d, 3H, $^4J_{H\text{-}H}$ = 1.1 Hz), 5.91 (dd, 1H, $^4J_{H\text{-}H}$ = 1.1 Hz, $^4J_{H\text{-}H}$ = 1.1 Hz), 6.38 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$): 21.8, 28.7, 102.1, 121.8, 160.9, 164.1, 170.8, 189.0.

Ethyl 2,4-diketo-4-phenylbutanoate (**10a**) was made by procedure A with acetophenone (11.52 g) that crystallized to a yellow solid with mp 42 °C (lit.: 42 °C[19]). A yellow liquid (16.8 g, 79% yield) resulted with bp 139 °C/0.66 mbar (lit.: 167 °C/5 mbar[31]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 4.34 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 7.02 (s, 1H), 7.50 (m, 3H), 7.95 (m, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.1, 62.5, 97.9, 127.8, 128.9, 133.8, 134.6, 162.2, 169.8, 190.7.

2,4-Diketo-4-phenylbutanoic acid (**10b**) was made by procedure D from **10a** (1.90 g). A yellow crystalline solid (0.86 g, 50% yield) resulted with mp 152 °C (ether, petrol ether) (lit.: 157 °C[5]). ^{1}H-NMR (200 MHz, $CDCl_3$): 7.15 (s, 1H), 7.60 (m, 3H), 8.10 (m, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$): 98.5, 128.8, 130.0, 134.9, 135.8, 163.4, 172.0, 191.5. UV (H_2O, c = 0.1 mM): λ_{max} = 324 nm, $\varepsilon/c = 0.644 \times 10^{-4}$.

Ethyl 2,4-diketo-5-phenylpentanoate (**11a**) was made by procedure A with 1-phenylpropan-2-one (12.86 g). An orange liquid (11.8 g, 52% yield) resulted with bp 145 °C/0.6 mbar (lit.: 146–150 °C/2 mbar[21]). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz), 3.77 (s, 2H), 4.32 (q, 2H, $^3J_{H\text{-}H}$ = 7.1 Hz), 6.35 (s, 1H), 7.30 (m, 5H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.1, 47.7, 62.6, 101.7, 127.6, 128.9, 129.1, 133.6, 161.9, 166.9, 200.6.

2,4-Diketo-5-phenylpentanoic acid (**11b**) was made by procedure C from **11a** (2.02 g). A yellow crystalline solid (0.68 g, 37% yield) resulted with mp 58 °C (toluene) (lit.: 62 °C[21]). ^{1}H-NMR (200 MHz, $CDCl_3$): 3.48 (s, 2H), 6.44 (s, 1H), 7.40 (m, 5H). ^{13}C-NMR (50 MHz, $CDCl_3$): 47.0, 101.1, 127.6, 129.0, 129.5, 133.4, 163.7, 168.9, 199.4.

3-Acetyl-4-carboxyl-2,4-dihydroxy-6-oxo-hept-2-enoic acid (**12**). **1b** (0.65 g) was added to a 1 M aqueous phosphate buffer solution (10 ml) and stirred for 48 h at room temperature. The product was not isolated and the yield was not determined, but samples were taken from the reaction mixture for NMR measurements. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.03 (s, 3H), 2.27 (s, 3H), 3.06 (d, 1H, $^3J_{H\text{-}H}$ = 16.7 Hz), 3.48 (d, 1H, $^3J_{H\text{-}H}$ = 16.7 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$): 28.2, 30.8, 46.5, 85.4, 118.2, 162.7, 174.6, 174.9, 195.6, 212.5.

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