Comparison of the acid–base properties of 5- and 6-uracilmethylphosphonate ($5Umpa^{2-}$ and $6Umpa^{2-}$) and some related compounds. Evidence for intramolecular hydrogen-bond formation in aqueous solution between (N1)H and the phosphonate group of $6Umpa^{2-}$

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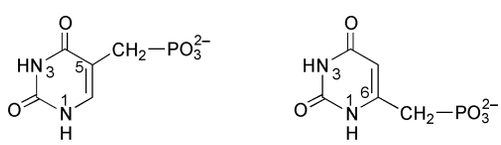
The acidity constants of 5-uracilmethylphosphonic acid, H_2(5Umpa), and 6-uracilmethylphosphonic acid, H_2(6Umpa),‡ were determined by potentiometric pH titrations in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$). Comparison of these constants with those of related uracil derivatives (partly taken from the literature) allows the conclusion that an intramolecular hydrogen bond is formed between (N1)H and the phosphonate group of $6Umpa^{2-}$; the formation degree of this hydrogen-bonded isomer is estimated to be 86 ± 7%. The X-ray crystal structure analysis of H_2(6Umpa) is reported but this solid state structure is dominated by intermolecular hydrogen bonds. In the context of the properties of $5Umpa^{2-}$ and $6Umpa^{2-}$ those of uracil are also discussed and from various comparisons of acidity constants it is concluded that deprotonation of uracil may occur at (N3)H as well as at (N1)H but that the (N3)-deprotonated species dominates with about 80% in aqueous solution at 25 °C and I = 0.1 M (Na^+). The search for other examples of uracil derivatives which allow hydrogen-bond formation in aqueous solution has led to orotic acid (= 6-uracilcarboxylic acid; [H(6Urca)]) and 5-uracilcarboxylic acid [H(5Urca)]; based on acidity constant comparisons it is concluded that in aqueous solution (25 °C; I = 0.1 M, KCl) H(5Urca) exists to about 92 ± 10% as a species with a hydrogen bond between (C5)COOH and (C4)O, and $6Urca^-$ to about 95 ± 5% as a species with a hydrogen bond between (C6)COO^- and (N1)H. The importance of intramolecular hydrogen-bond formation to the acid–base properties of compounds in solution is briefly emphasized.

1 Introduction

Cisplatin, *cis*-$(NH_3)_2PtCl_2$, one of the leading antitumor drugs,[1] is often employed also in combination therapy,[2] *e.g.* together with 5-fluorouracil.[3] Moreover, complexes prepared from the hydrolysis products of *Cisplatin* and uracil as well as related ligands ("Platinum Pyrimidine Blues") were considered at one point to be extremely promising antitumor agents.[4] This interest has also promoted the synthesis of phosphonate derivatives of uracil,[5] since phosphonates themselves are biologically very active compounds as, *e.g.*, the simple phosphonoformate[6] or the nucleotide analogue 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA).[7]

Indeed, the dimethyl esters of 5- and 6-uracilmethylphosphonic acids (Fig. 1) in combination with *Cisplatin* prolonged significantly the survival time of mice with lymphoid leukemia L-1210.[8] Further pharmacological tests will be facilitated by a detailed understanding of the solution properties of these compounds and therefore we have now studied the acid–base characteristics of 5-uracilmethylphosphonic acid, H_2(5Umpa), and 6-uracilmethylphosphonic acid, H_2(6Umpa), in aqueous solution. Comparison of these results with the corresponding properties of other uracil derivatives provides evidence for the formation of an intramolecular hydrogen bond between the phosphonate group and the (N1)H site in $6Umpa^{2-}$; its formation degree in aqueous solution is estimated. The solid state structure of H_2(6Umpa), however, is dominated by intermolecular hydrogen bonds. Further evidence for the occurrence of intramolecular hydrogen-bond formation in aqueous solution is given for 5-uracilcarboxylic acid as well as for 6-uracilcarboxylate, *i.e.* for the anion of orotic acid.



5Umpa^{2-} **6Umpa^{2-}**

Fig. 1 Chemical structures of 5-uracilmethylphosphonate ($5Umpa^{2-}$) and 6-uracilmethylphosphonate ($6Umpa^{2-}$).

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‡ The IUPAC names for 5-uracilmethylphosphonic and 6-uracilmethylphosphonic acids are uracil-5-ylmethylphosphonic and uracil-6-ylmethylphosphonic acids, respectively, and for 5-uracilcarboxylic and 6-uracilcarboxylic acids they are uracil-5-carboxylic and uracil-6-carboxylic acids, respectively.

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2 Experimental

2.1 Materials

5-Uracilmethylphosphonic acid and 6-uracilmethylphosphonic acid were synthesized as described.[5] Uridine (Sigma grade) was obtained from Sigma Chemical Co., St. Louis, MO, and uracil and thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] (*puriss.*) from Fluka AG, Buchs, Switzerland. Potassium hydrogen phthalate, HNO_3, NaOH (Titrisol) and $NaNO_3$ (all *pro analysi*) were from Merck AG, Darmstadt, Germany.

The titer of the NaOH used for the potentiometric pH titrations was determined with potassium hydrogen phthalate. The aqueous stock solutions of $5Umpa^{2-}$ and $6Umpa^{2-}$ were freshly prepared daily by dissolving the acids in deionized, ultrapure (MILLI-Q185 PLUS; from Millipore S.A., Molsheim, France) CO_2-free water and adding 2 equivalents of NaOH. Uridine, uracil and thymidine were simply dissolved in the above mentioned type of water.

The buffer solutions (pH 4.00, 7.00, 9.00 based on the NBS scale; now NIST) for calibration (Section 2.2) were from Metrohm AG, Herisau, Switzerland.

2.2 Potentiometric pH titrations

The pH titrations for the determination of the acidity constants were recorded with a Metrohm E536 potentiograph connected to a Metrohm E665 dosimat and a Metrohm 6.0222.100 combined macro glass electrode. The instrument was calibrated with the buffers mentioned above.

The direct pH meter readings were used in the calculations; *i.e.* these acidity constants (25 °C; $I = 0.1$ M, $NaNO_3$) are so-called practical, mixed or Brønsted constants.[9] They may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[9] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[9,10] However, this conversion term and the ionic product of water (K_w) do not enter into our calculations because we evaluate the differences in NaOH consumption between a pair of solutions, *i.e.* with and without ligand (see below in Section 2.3).

All acidity constants were calculated by curve fitting procedures in the way and with the equipment described recently.[11]

2.3 Determination of acidity constants

$H(5Umpa)^-$ and $H(6Umpa)^-$. The acidity constants $K^H_{H(U)}$ and K^H_U [eqns. (2) and (3)] were determined by titrating under N_2 50 mL of aqueous 5.4×10^{-4} M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 3×10^{-4} M $5Umpa^{2-}$ or $6Umpa^{2-}$ (see Section 2.1) with 1.5 mL of 0.03 M NaOH. The differences in NaOH consumption between such a pair of titrations (with and without ligand) were used (every 0.1 pH) for the calculations in the pH range 5 to 10 and 4.5 to 9.5 for 5Umpa and 6Umpa, respectively. The final values (Table 1) are the average of 19 pairs of independent titrations for $H(5Umpa)^-$ and 32 for $H(6Umpa)^-$.

Uridine and thymidine. K^H_U [eqn. (4)] of Urd and Thd was obtained by titrating 50 mL of aqueous 10^{-4} M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 9×10^{-4} M nucleoside under N_2 with 2 mL of 0.1 M NaOH. The differences in NaOH consumption between such a pair of titrations were used (every 0.1 pH) for the calculations in the pH range 7.5 to 11; four experiments were carried out. In a second set, 20 mL of 0.015 M HNO_3 in the presence and absence of 4.5×10^{-3} M nucleoside (25 °C; $I = 0.1$ M, $NaNO_3$) were titrated with 2.5 mL 0.2 M NaOH. Again the differences in NaOH consumption were evaluated in the pH range (2 to 7.5 and) 7.5 to 11.4; two experiments were performed. The final result (Table 1) is for both nucleosides the average of six (4 plus 2) titration pairs. These experiments also allowed determination of the upper limits for $pK^H_{H(U)}$ (protonation of the nucleosides) and the lower limits for $pK^H_{(U-H)}$ (formation of dianions) which are 1 and 12, respectively.

Uracil. The acidity constant K^H_U [eqn. (4)] of uracil was measured by titrating under N_2 50 mL of aqueous 1.2×10^{-4} M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 3×10^{-4} M uracil with 1.5 mL of 0.03 M NaOH. The difference in NaOH consumption between such a pair of titrations was used (every 0.1 pH) for the calculations in the pH range 6 to 10.4. The final value in Table 1 is the average of 5 independent pairs of titrations.

2.4 NMR measurements

^1H and ^{13}C NMR spectra were recorded using a Varian Inova-600 spectrometer in D_2O [internal reference, sodium 3-(trimethylsilyl)propanesulfonate] and DMSO-d_6. pH* refers to the uncorrected pH meter reading. NaOD was applied to adjust the pH*.

2.5 Crystal structure determination of $H_2(6Umpa)$ §

Crystal data. $C_5H_7N_2O_5P$, $M = 206.10$, monoclinic, space group $P2_1/n$, $a = 5.387(1)$, $b = 7.948(2)$, $c = 18.060(4)$ Å, $\beta = 93.36(3)°$, $U = 771.9(3)$ Å^3, $T = 293(2)$ K, $Z = 4$, μ(Mo-Kα) = 0.349 mm^{-1}, 2598 reflections total, 1467 unique ($R_{int} = 0.034$), which were used in all calculations, and 974 observed ($F \geqslant 4\sigma(F)$). The final $wR2$ was 0.0801, $R1 = 0.0360$ (observed data).

X-Ray analysis. Diffraction data were collected on an Enraf–Nonius KappaCCD diffractometer to a resolution of $2\theta_{max} = 51.4°$. Data procession was performed using DENZO and SCALEPACK.[12] The structure was solved by direct methods[13] and refined by full-matrix least-squares based on F^2 using the SHELXTL PLUS[14] and SHELXL-93 programs.[15] All non-hydrogen atoms were refined anisotropically. All protons were localized with difference Fourier syntheses and refined with a common isotropic displacement factor. After convergence of the refinement hydrogen coordinates were fixed for the final cycles in order to save parameters.

3 Results and discussion

3.1 Definition of the acidity constants and results

$5Umpa^{2-}$ and $6Umpa^{2-}$ can accept two protons at their phosphonate groups (Fig. 1); it is also well known that uracil residues can be deprotonated in the upper pH range at their (N3)H site.[16] Hence, the deprotonation reactions (1)–(3) need to be considered, where $H_2(U)$ represents $H_2(5Umpa)$ or $H_2(6Umpa)$; in eqn. (4) U represents any other uncharged compound with a uracil residue.

$$H_2(U) \rightleftharpoons H(U)^- + H^+ \quad (1a)$$

$$K^H_{H_2(U)} = [H(U)^-][H^+]/[H_2(U)] \quad (1b)$$

$$H(U)^- \rightleftharpoons U^{2-} + H^+ \quad (2a)$$

$$K^H_{H(U)} = [U^{2-}][H^+]/[H(U)^-] \quad (2b)$$

§ CCDC reference number 167669. See http://www.rsc.org/suppdata/p2/b1/b101078f/ for crystallographic files in .cif or other electronic format.

Table 1 Negative logarithms of the acidity constants in aqueous solution for $H(5Umpa)^-$ and $H(6Umpa)^-$ as well as for some related species at 25 °C and $I = 0.1$ M ($NaNO_3$) as determined by potentiometric pH titrations[a,b]

No.	Protonated species	$pK^H_{R-P(O)_2(OH)}$ [eqn. (2)]	pK^H_U [eqns. (3), (4)]	Ref.
1	$CH_3P(O)_2(OH)^-$	7.53 ± 0.01		18
2	$CH_3CH_2P(O)_2(OH)^-$	7.77 ± 0.01		18
3	$CH_3CH_2OCH_2P(O)_2(OH)^-$	7.02 ± 0.01		18
4	$H(5Umpa)^-$	7.15 ± 0.01	10.25 ± 0.02	—
5	$H(6Umpa)^-$	6.17 ± 0.02	10.12 ± 0.02	—
6	$CH_3OP(O)_2(OH)^-$	6.36 ± 0.01		19
7[c]	5-Methyl-Ur = thymine (Th/5MUr)		9.82 ± 0.05	20
8[c]	6-Methyluracil (6MUr)		9.55 ± 0.05	20
9[c]	Uracil (Ur)		9.33 ± 0.05[d]	20
10[c]	1-Methyluracil (1MUr)		9.63 ± 0.05	20
11[c]	3-Methyluracil (3MUr)		9.90 ± 0.05	20
12[e]	Uridine (Urd)		9.19 ± 0.02	—
13[e]	Thymidine (Thd)		9.69 ± 0.03	—
14	Guanine (Gu)		9.36 ± 0.01	21
15	9-Methylguanine (9MGu)		9.56 ± 0.02	21
16	Guanosine (Guo)		9.22 ± 0.01	22

[a] The error limits given, if nothing else is mentioned, are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] So-called practical, mixed or Brønsted constants are listed (see also Section 2.2). [c] These values from ref. 20 were determined by potentiometric pH titration at 25 °C and I close to 0.1 M (Na^+); listed above are the so-called pG' values of ref. 20 (and not those given for $I = 0$). The given error limits are estimates based on the information provided in tables III–VII of ref. 20. [d] The value $pK^H_{Ur} = 9.33 \pm 0.03$ as determined now (see Section 2.3) is identical with the value given above, thus confirming the reliability of the constants taken from ref. 20. [e] In addition, we determined $pK^H_{H(U)} < 1$ [deprotonation of $H(Urd)^+$ or $H(Thd)^+$] and $pK_{(U-H)} > 12$ [formation of $(Urd - 2H)^{2-}$ or $(Thd - 2H)^{2-}$].

$$U^{2-} \rightleftharpoons (U - H)^{3-} + H^+ \quad (3a)$$

$$K^H_U = [(U - H)^{3-}][H^+]/[U^{2-}] \quad (3b)$$

$$U \rightleftharpoons (U - H)^- + H^+ \quad (4a)$$

$$K^H_U = [(U - H)^-][H^+]/[U] \quad (4b)$$

The release of the first proton from a $-P(O)(OH)_2$ group occurs with $pK_a < 2.5$, *e.g.* for $CH_3P(O)(OH)_2$ $pK_{a/1} = 2.10 \pm 0.03$;[17] consequently, above pH 4 only equilibria (2) and (3) are of relevance. These acidity constants were measured in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) by potentiometric pH titrations. For comparison purposes, we also studied equilibrium (4) for uridine (Urd), thymidine (Thd) and uracil (Ur). The results are given in Table 1 together with related data from the literature[18–22] which are used in the evaluations below.

The acidity constants of $H(5Umpa)^-$ and $H(6Umpa)^-$ have not been determined before and those of uridine, thymidine and uracil are in accord with expectations.[16] The self-consistency of the values listed in Table 1, despite the different sources and the wide time-span in which they were determined, is evident, *e.g.*, from the following comparisons: (i) The values $pK^H_{Urd} = 9.19 \pm 0.02$ (Table 1, entry 12) and $pK^H_{Ur} = 9.33 \pm 0.05$ (entry 9) are identical within the error limits with the selected constants 9.18 and 9.34, respectively, compiled for uridine (Urd) and uracil (Ur) in ref. 23; in addition, the value measured now for Ur is identical with the previous one (Table 1, footnote *d*). (ii) In another compilation[24] six values are listed for the deprotonation of thymine (Th) at 25 °C and $I = 0.1$ M; one of these values drops out and the average of the remaining five gives $pK^H_{Th} = 9.80 \pm 0.05$ (2σ) in excellent agreement with 9.82 ± 0.05 listed under entry 7 in Table 1. (iii) Substitution of the hydrogen atom at N1 of uracil and thymine by the sugar residue giving uridine and thymidine (Thd), respectively, has the same effect; *i.e.*
$pK^H_{Ur} - pK^H_{Urd} = (9.33 \pm 0.05) - (9.19 \pm 0.02) = 0.14 \pm 0.05$ and
$pK^H_{Th} - pK^H_{Thd} = (9.82 \pm 0.05) - (9.69 \pm 0.03) = 0.13 \pm 0.06$
(Table 1; entries 9, 12 and 7, 13). For comparisons to be made below in Section 3.3 it is important to note that the same difference is observed for guanine (Gu) and guanosine (Guo) (entries 14, 16), namely
$pK^H_{Gu} - pK^H_{Guo} = (9.36 \pm 0.01) - (9.22 \pm 0.01) = 0.14 \pm 0.01$; *i.e.* the properties of the (N1)H site in the guanine residue correspond in a relative sense to those of the (N3)H site in uracil and thymine moieties.

3.2 Properties of the phosphonate group in $5Umpa^{2-}$ and $6Umpa^{2-}$

The addition of an electron-withdrawing group like an ethoxy unit to methylphosphonate giving an oxygen-ether bridge renders the phosphonate group less basic, *i.e.* the acidity increases by $\Delta pK_a = (7.53 \pm 0.01) - (7.02 \pm 0.01) = 0.51 \pm 0.01$ (Table 1, entries 1, 3). A uracil residue at the same position is expected to be less effective than an O atom and hence, less acidifying. Indeed, this is observed for $5Umpa^{2-}$: $\Delta pK_a = (7.53 \pm 0.01) - (7.15 \pm 0.01) = 0.38 \pm 0.01$.

However, the properties of $6Umpa^{2-}$ (Table 1, entry 5) evidently do not fit into this picture: $H(6Umpa)^-$ is by about 1 pK_a unit more acidic than $H(5Umpa)^-$. It is even more acidic than monoprotonated methyl phosphate (entry 6) which lacks a phosphorus–carbon bond. Why? Since, $H(5Umpa)^-$ behaves as expected, an explanation cannot be based on the assumption that the release of H^+ from its $-P(O)_2(OH)^-$ group is somehow inhibited, *e.g.* by the formation of a hydrogen bond to the carbonyl oxygen at C4. In other words, the explanation of the experimental fact regarding $H(6Umpa)^-$ must be based on a facilitated release of the proton in $H(6Umpa)^-$ itself.

The only chemically feasible explanation for such a facilitated release is to postulate the formation of a hydrogen bond between the $-PO_3^{2-}$ group and the (N1)H site in $6Umpa^{2-}$ (Fig. 1); clearly, deprotonation of the $-P(O)_2(OH)^-$ residue will favor such a bond. However, before its formation can be considered in more detail, it is first necessary to discuss the properties of the (N1)H site.

3.3 Comparison of the acid–base properties of the (N1)H and (N3)H sites in uracil and related residues

Which site in uracil, (N1)H or (N3)H, releases its proton first? Clearly, with $pK_{a/2} \simeq 14.2$ the dianion $(Ur - 2H)^{2-}$ can be formed,[25] but this is not of relevance here. The question may be answered superficially by comparing the pK_a value of uridine ($pK^H_{Urd} = 9.19 \pm 0.02$), where the proton must originate from (N3)H, with that of uracil ($pK^H_{Ur} = 9.33 \pm 0.05$) (Table 1); since the difference is small ($\Delta pK_a = 0.14 \pm 0.05$), one may conclude that at 25 °C and $I = 0.1$ M (Na^+) with uracil also the proton is mainly released from the (N3)H site. This tentative answer

contains some truth, as will be seen below, but a more detailed analysis is necessary.

There is evidence[26–28] that in alkaline solution the uracil monoanion exists in an approximately 1 : 1 ratio of (N1)- and (N3)-deprotonated forms which we designate as $(UrN1 - H)^-$ and $(UrN3 - H)^-$, respectively. With this in mind we may rewrite eqn. (4b) as eqns. (5a) and (5b). If the tautomer ratio is 1 : 1 the micro acidity constants for uracil are as given in eqn. (6). Of course, they are identical under the given assumption

$$K^{H}_{Ur} = \frac{[(Ur - H)^-][H^+]}{[Ur]} = \frac{([(UrN1 - H)^-] + [(UrN3 - H)^-])[H^+]}{[Ur]} \quad (5a)$$

$$= k^{H}_{(UrN1H)} + k^{H}_{(UrN3H)} \quad (5b)$$

$$K^{H}_{Ur} = k^{H}_{(UrN1H)} + k^{H}_{(UrN3H)} = 10^{-9.63} + 10^{-9.63} = 10^{-9.33} \quad (6)$$

and by 0.3 log unit different from the macro acidity constant (Table 1, entry 9), accounting for the fact that there are two ways to form $(Ur - H)^-$ but only one each for $(UrN1 - H)^-$ and $(UrN3 - H)^-$.

There is evidence that a decreasing solvent polarity, *i.e.* a decreasing relative permittivity (dielectric constant), favors the (N1)-deprotonated tautomer,[26–28] since this is the less polar one, whereas increasing salt concentrations (NaCl, $NaClO_4$, KCl, *etc.*) favor deprotonation of (N3)H.[28] This latter observation is expected if one takes into account that carbonyl oxygens are known to favor complex formation, be it innersphere or outersphere, at a neighboring N atom[22,29] due to hydrogen-bond formation with metal ion-coordinated water molecules and here the N3 site with two such groups is favored.

That (N3)H deprotonation is facilitated at 25 °C and $I = 0.1$ M (Na^+) also follows from the following comparisons: replacement of the hydrogen by a methyl group at N1 gives 1-methyluracil with a $pK^{H}_{(1MUr)} = 9.63 \pm 0.05$ (Table 1, entry 10), whereas the same substitution at N3 leads to 3-methyluracil with $pK^{H}_{(3MUr)} = 9.90 \pm 0.05$ (entry 11); in other words, the (N3)H site is the more acidic one. For a more quantitative evaluation the micro acidity constants of eqn. (5b) need to be known. If we correct the acidity constant of 1-methyluracil for the (weak) electron-donating effect of the methyl group, we obtain the micro acidity constant for the (N3)H site. For this correction it is necessary to recall the similarity of the (N3)H site of a uracil residue with the (N1)H site of a guanine moiety [see part (iii) in the last paragraph of Section 3.1]. This allows us to estimate the effect of the methyl group by calculating the difference between the acidity constants (Table 1; entries 14, 15) of 9-methylguanine and guanine (deprotonation occurs at N1):[16] $\Delta pK_a = pK^{H}_{(9MGu)} - pK^{H}_{Gu} = (9.56 \pm 0.02) - (9.36 \pm 0.01) = 0.20 \pm 0.02$. This difference, which reflects the effect of the methyl group, needs to be subtracted from the acidity constant of 1-methyluracil to obtain the micro acidity constant of the (N3)H site for uracil: $pk^{H}_{(UrN3H)} = pK^{H}_{(1MUr)} - \Delta pK_a = (9.63 \pm 0.05) - (0.20 \pm 0.02) = 9.43 \pm 0.05$. Hence, for uracil, according to eqn. (5b), eqns. (7) hold. Application of these micro acidity constants now allows estimation of the ratio R of the two isomers [eqns. (8)]. From eqn. (8c) it follows that both tautomers occur in equilibrium but that the (N3)-deprotonated one most likely dominates.[30] The first ratio given in parentheses in eqn. (8c) represents the lower limit of $(UrN3 - H)^-$ following from $0.59 - 0.31 = 0.28$ [eqn. (8b)] and the second ratio the upper limit which follows from $0.59 + 0.31 = 0.90$. The conclusion that the (N3)H site is the more acidic one is also in accord with a recent first principles quantum mechanics calculation.[31]

$$k^{H}_{(UrN1H)} = K^{H}_{Ur} - k^{H}_{(UrN3H)} \quad (7a)$$

$$= 10^{-(9.33 \pm 0.05)} - 10^{-(9.43 \pm 0.05)} \quad (7b)$$

$$= 10^{-(10.02 \pm 0.31)} \quad (7c)$$

$$R = \frac{[(UrN3 - H)^-]}{[(UrN1 - H)^-]} = \frac{k^{H}_{(UrN3H)}}{k^{H}_{(UrN1H)}} \quad (8a)$$

$$= \frac{10^{-(9.43 \pm 0.05)}}{10^{-(10.02 \pm 0.31)}} = 10^{(0.59 \pm 0.31)} \quad (8b)$$

$$= \frac{3.89 \pm 2.78}{1} - \frac{80}{20}\left(\frac{66}{34}; \frac{89}{11}\right) \quad (8c)$$

A further question of interest in the present context is the effect of substituents at C5 and C6. Substitution of a hydrogen by a methyl group should give rise to a weak inductive effect,[32] meaning that a methyl group at C5 should lead to a slight negative polarization at N1 and N3, whereas the same group at C6 should have the opposite effect. Indeed, the pK_a value of 5-methyluracil is somewhat higher, indicating a slightly enhanced basicity, than that of 6-methyluracil (Table 1, entries 7, 8). Since in both instances the effect on N1 and N3 is the same, it is not expected that the tautomer ratio as discussed above changes significantly for these two uracil derivatives. Furthermore, approximately the same relative effects are observed for $5Umpa^{2-}$ and $6Umpa^{2-}$, the uracil residue of the former being slightly more basic than expected (Table 1, entries 4, 5). Hence, we assume that at 25 °C and $I = 0.1$ M ($NaNO_3$) also predominantly the (N3)H sites are deprotonated and that in any case, as indicated above, metal ion-complex formation with the uracil residues occurs largely at N3. That the pK^{H}_{Umpa} values are by about 0.5 pK units higher than the corresponding pK^{H}_{MUr} values (Table 1) of the two methyluracils (entries 7, 8) is the result of the charge effect of the $-PO_3^{2-}$ group.

3.4 Search for further evidence of hydrogen-bond formation between (N1)H and the phosphonate group in $6Umpa^{2-}$

After the discussion in Section 3.3 it is evident that (N1)H in $6Umpa^{2-}$ is available for hydrogen-bond formation with the $-PO_3^{2-}$ group and therefore, the increased acidity of the $-P(O)_2(OH)^-$ residue of $6Umpa^{2-}$, as indicated in the final paragraph of Section 3.2, may indeed be explained by such an interaction.

Therefore, we have attempted to find additional evidence for the formation of such a hydrogen bond in solution by recording the ^1H NMR spectra of $5Umpa^{2-}$ (pH* 8.9) and $6Umpa^{2-}$ (pH* 8.3) as well as of $H(5Umpa)^-$ and $H(6Umpa)^-$ (pH* 4.7) in D_2O. The measurements reveal that the CH_2 protons of the side chain are magnetically equivalent in all instances, *e.g.* $5Umpa^{2-}$: $\delta = 2.36$ ppm, d, ^2J (^1H ^{31}P) 18.6 Hz; $6Umpa^{2-}$: $\delta = 2.91$ ppm, d, ^2J (^1H ^{31}P) 21.0 Hz. There are no signs of a non-equivalence of the two methylene protons which, if present, should have led to two AB doublets, further split by ^{31}P coupling, and differential NOEs with the H(6) and H(5) proton of the uracil ring, respectively. This observation suggests that the two methylene protons are either in fixed positions, *i.e.* symmetrically displaced from the uracil plane, or rapidly oscillating about this plane. As a consequence, the P atom must be coplanar with the uracil ring (or oscillating about the uracil plane), with the possibility of one or two oxygen atoms pointing toward the (N1)H in the case of $6Umpa^{2-}$. Indeed, from model building it is evident that the (N1)H···O distance becomes shortest if both the P and one of the O atoms are in the plane of the uracil ring. This model suggests further that the resulting N–O distance (<3 Å) is considerably shorter than in the solid state of $H_2(6Umpa)$ [N(1)···O(13) = 3.157(3) Å; see Fig. 2 and the next paragraph below]. The coupling constants between ^{31}P and CH_2, C(6), and C(5) have been determined both for $5Umpa^{2-}$ (127.5, 5.8, 5.9 Hz, respectively) and for

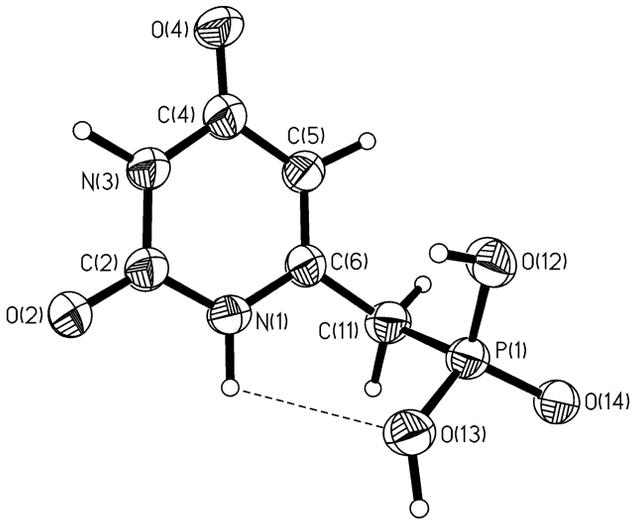

Fig. 2 Molecular structure and atom numbering of 6-uracilmethylphosphonic acid, H_2(6Umpa). The distance of 2.83(2) Å between (N1)H and the phosphonic acid oxygen O(13) (broken line) appears to be too long for an intramolecular hydrogen bond.

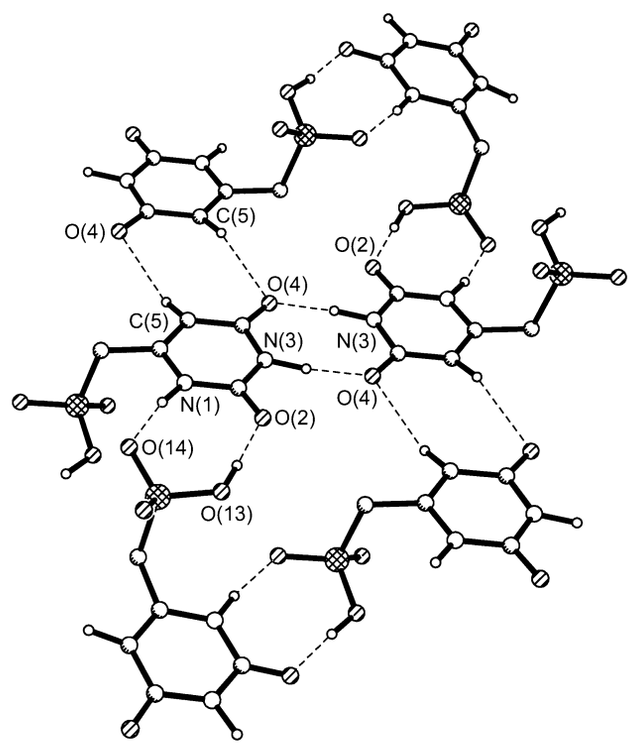

Fig. 3 Section of hydrogen-bonded network in H_2(6Umpa).

$6Umpa^{2-}$ (112.6, 7.4, 6.4 Hz, respectively). Although Karplus-type relationships for ^3J (PCCC) constants have been established for rigid systems,[33] the structural differences with the Umpa ligands do not permit a direct comparison. Hence, we have to conclude that our NMR results do not prove intramolecular H-bond formation in $6Umpa^{2-}$, but they are certainly also not inconsistent with its existence.

In the solid state structure of H_2(6Umpa) the twofold protonated phosphonate group is oriented in such a way that one of the OH groups [O(13)] is pointing toward (N1)H (Fig. 2). However, the intramolecular distance of 2.83(2) Å between O and the proton at N(1) is very long for a H bond. Moreover, a look at the packing pattern (Fig. 3) reveals that (N1)H is involved in intermolecular H bonding with the phosphonic acid residue [N(1)···O(14), 2.844(3) Å; O(2)···O(13), 2.568(3) Å]. The crystal structure is dominated by a network of additional intermolecular H bonds. Two molecules form dimers over a pair of H bonds [N(3)···O(4), 2.854(3) Å] about a center of inversion. These dimers are connected with each other by an additional pair of H bonds, again around an inversion center, involving also an aromatic proton [C(5)···O(4), 3.416(3) Å]. As a result, infinite layers along the crystallographic *y* axis are formed. These layers are interconnected by the already mentioned H bonds involving phosphonic acid oxygen atoms. The geometry of the uracil entity is normal.[34] Thus, although the X-ray crystal structure analysis of the neutral acid does not display intramolecular H bonding involving (N1)H, the overall orientation of the methylphosphonate entity is, in principle, favorable for such an interaction. Evidently, if both the P atom and the O atom, which is involved in intramolecular H bonding, move into the uracil plane [which is not the case in the solid state structure of H_2(6Umpa)], the (N1)H···O(13) distance becomes considerably shorter, as discussed above, with one of the lone electron pairs at the O atom pointing then directly toward the proton at N1. Of course, if deprotonated, as in our solution case discussed below, the $-PO_3^{2-}$ group is strongly basic and hence, a much better acceptor than the OH group in the solid state structure seen in Fig. 2.

3.5 Extent of hydrogen bond formation in $6Umpa^{2-}$ in aqueous solution

The formation degree of the hydrogen bond between (N1)H and the phosphonate group may be estimated in the following way. As a first approximation one may assume that the properties of the free $-P(O)_2(OH)^-$ group in $H(6Umpa)^-$ are reflected by the pK_a value of $H(5Umpa)^-$. However, since the 5,6-substituent effect is not identical (see Section 3.3), we assume that it is reciprocal, an assumption justified by other observations,[21] and deduct it from the $pK^H_{H(5Umpa)}$ value of $H(5Umpa)^-$. This substituent effect amounts to $(10.25 \pm 0.02) - (10.12 \pm 0.02) = 0.13 \pm 0.03$ (Table 1, entries 4, 5 in column 4); to be on the safe side we increase the corresponding error to ±0.20 log unit. Hence, the acidification as expressed by log Δ and attributable to hydrogen-bond formation of the $-P(O)_2(OH)^-$ group of $H(6Umpa)^-$ is as given in eqn. (9). If we define the 'open' isomer, *i.e.* the $6Umpa^{2-}$ species without a hydrogen bond, as $(6Umpa)^{2-}_{op}$ and the closed species with the intramolecular hydrogen bond (see also Fig. 2) as $(6Umpa)^{2-}_{NHO}$ we may consider the position of the intramolecular equilibrium (10a). The dimensionless equilibrium constant K_I [eqn. (10b)] can be calculated by following known routes.[22,35–37] The interrelation between log Δ of eqn. (9) and K_I is given by eqn. (11), and the percentage of the isomer closed by the hydrogen bond follows from eqn. (12).

$$\log \Delta = pK^H_{H(5Umpa)} - (0.13 \pm 0.20) - pK^H_{H(6Umpa)} \quad (9a)$$

$$= (7.15 \pm 0.01) - (0.13 \pm 0.20) - (6.17 \pm 0.02) \quad (9b)$$
$$= 0.85 \pm 0.20$$

$$(6Umpa)^{2-}_{op} \rightleftharpoons (6Umpa)^{2-}_{NHO} \quad (10a)$$

$$K_I = \frac{[(6Umpa)^{2-}_{NHO}]}{[(6Umpa)^{2-}_{op}]} \quad (10b)$$

$$K_I = 10^{\log \Delta} - 1 \quad (11)$$

$$\%(6Umpa)^{2-}_{NHO} = 100K_I/(1 + K_I) \quad (12)$$

Hence, one obtains $K_I = 10^{(0.85 \pm 0.20)} - 1 = 6.08 \pm 3.26$ and a formation degree of 86 ± 7% for $(6Umpa)^{2-}_{NHO}$.

Understandably, the formation degree of this six-membered "chelate" is much higher than that observed for hydrogen-bonded macrochelates which is on the order of 40%.[21,36–38]

Table 2 Negative logarithms of the acidity constants in aqueous solution for the 5- and 6-uracilcarboxylic acids as well as for some related species at 25 °C and $I = 0.1$ M ($NaNO_3$/KCl) as determined by potentiometric pH titrations[a]

No.	Protonated species	$pK^H_{R\text{-}COOH}$	pK^H_U	Ref.
1	Uridine (Urd)		9.19 ± 0.02	—
2	Orotidine (Ord) = 6-Urd-carboxylic acid	0.5 ± 0.3	9.12 ± 0.02	40
3	Uracil (Ur)		9.33 ± 0.05	—
4[b]	6-Ur-carboxylic acid [H(6Urca)] = orotic acid	2.07 ± 0.10	9.45 ± 0.10	39
5[b]	5-Ur-carboxylic acid [H(5Urca)]	4.16 ± 0.10	8.89 ± 0.10	39
6	CH_3COOH	4.57 ± 0.01		44
7	HCOOH	3.58 ± 0.01		44

[a] See footnote *a* of Table 1. The above entries 1 and 3 are from entries 12 and 9 of Table 1, respectively. [b] Measured at 25 °C and $I = 0.1$ M (KCl);[39] the error limits are estimates added now.

3.6 Intramolecular hydrogen-bond formation in 5-uracilcarboxylic acid (isoorotic acid) and 6-uracilcarboxylate (orotate)

The results described in Section 3.5 prompted a literature search to see if other uracil derivatives exist for which intramolecular hydrogen-bond interactions may be of relevance. This has led us to orotic acid,[39] which plays, with its derivatives orotidine[40] and orotidine 5′-monophosphate,[41,42] an important role in the metabolism of pyrimidine nucleotides.[43] The acidity constants of orotic acid, also known as 6-uracilcarboxylic acid, H(6Urca), are listed in Table 2 together with the corresponding values for 5-uracilcarboxylic acid, H(5Urca), and some other related compounds.[39,40,44]

In Section 3.3 we have seen that the substituent effects in 5-methyluracil and 6-methyluracil as well as in $5Umpa^{2-}$ and $6Umpa^{2-}$ are as expected for substituents with a weak inductive effect (*ortho*, *para* directing),[32] the uracil residue with the 5-substituent being the more basic one. The corresponding expectation also holds for 5-uracilcarboxylate and 6-uracilcarboxylate, but the carboxylate group, being a deactivating *meta* directing substituent,[32] now makes the 5-substituted derivative more acidic (*cf.* Table 2, entries 4, 5 in column 4), leaving the 6-uracilcarboxylate almost unaffected (see entries 3, 4 in column 4). Furthermore, comparison of the first four entries in column 4 shows that substitution of the hydrogen at N1 by the ribose residue has little effect, as has the presence of the carboxylate group in position 6.

However, the situation changes dramatically if one considers the properties of the carboxylic acid groups (Table 2, column 3). The pK_a of 5-uracilcarboxylic acid is too high; it is about 0.6 p*K* units higher than that of formic acid (entry 7), whereas the pK_a of 6-uracilcarboxylic acid is clearly too low, being about 1.5 p*K* units below that for formic acid. Why?

Let us first consider 5-uracilcarboxylic acid by taking into account that the uracil residue leads to an acidification of monoprotonated methylphosphonate of $\Delta pK_a = (7.53 \pm 0.01) - (7.15 \pm 0.01) = 0.38 \pm 0.01$ (*cf.* Table 1, entries 1, 4); hence, the substitution of the same residue at formic acid should give rise to an even more pronounced effect because the substituent is now closer to the acidic site. We assume for the present case an acidification of $\Delta pK_a = 0.5$ with an error limit also of ±0.5 to be on the safe side; *i.e.* one obtains for the expected (calculated) acidity constant $pK^H_{H(5Urca)/calc} = pK^H_{HCOOH} - \Delta pK_a = (3.58 \pm 0.01) - (0.5 \pm 0.5) = 3.08 \pm 0.5$.[45] Since the negative logarithm of the measured acidity constant of 5-uracilcarboxylic acid, $pK^H_{H(5Urca)} = 4.16 \pm 0.10$ (Table 2, entry 5) is much higher, there must be an effect which inhibits the release of the proton and this can only be the formation of a hydrogen bond between (C4)O and (C5)COOH. Indeed, Li *et al.*[39] have already concluded that 5-uracilcarboxylic acid "is structurally favorable for intramolecular hydrogen bonding and resonance enhances (this) interaction between the carboxyl hydrogen and the adjacent oxygen . . . and probably accounts for the higher pK_1'' value, and we now designate this hydrogen-bonded species as $(5UrcaH)_{OHO}$. Application of equations analogous to eqns. (10)–(12) gives $\log \Delta = (4.16 \pm 0.10) - (3.08 \pm 0.5) = 1.08 \pm 0.51$ and $K_I = 10^{(1.08 \pm 0.51)} - 1 = 11.0 \pm 14.1$ and consequently, a formation degree of 92 ± 10% for this isomer. Since this value also applies to an aqueous solution at 25 °C and $I = 0.1$ M (KCl), it is not surprising that it is within its error limits identical with the results obtained for $(6Umpa)^{2-}_{NHO}$ (see Section 3.4), which also contains a six-membered "chelate".

The low acidity constant of 6-uracilcarboxylic acid ($pK^H_{H(6Urca)} = 2.07 \pm 0.10$; Table 2, entry 4) can be explained by a facilitated deprotonation of the carboxylic acid group due to hydrogen-bond formation between (N1)H and the carboxylate group; *i.e.* this situation is analogous to that discussed in Section 3.5 for $6Umpa^{2-}$ and we designate the hydrogen-bonded isomer of 6-uracilcarboxylate as $(6UrCOO)^-_{NHO}$. Since the exchange of the hydrogen at N1 by a ribose residue affects the acid–base properties of the uracil residue only a little, *i.e.* by $\Delta pK_a = 0.33 \pm 0.10$ [= (9.45 ± 0.10) − (9.12 ± 0.02); see Table 2], an even smaller effect is expected on the (C6)COOH group. Hence, we assume that this effect amounts to $\Delta pK_a = 0.3 \pm 0.2$ in the maximum; together with the acidity constant, $pK^H_{6Urd\text{-}COOH} = 0.5 \pm 0.3$, of orotidine (Table 2, entry 2), where N1 has no hydrogen, the acidic properties of 6-uracilcarboxylic acid should be well represented by $pK^H_{H(6Urca)/calc} = (0.5 \pm 0.3) + (0.3 \pm 0.2) = 0.8 \pm 0.36$ if no hydrogen bond is formed. The acidification, due to intramolecular hydrogen bonding in $6Urca^-$, is then given by $\log \Delta = (2.07 \pm 0.10) - (0.8 \pm 0.36) = 1.27 \pm 0.37$ (*cf.* ref. 46). Application of eqns. (11) and (12) provides $K_I = 17.6 \pm 15.9$ and $\%(6UrCOO)^-_{NHO} = 95 \pm 5$ for the formation degree of the hydrogen-bonded isomer. It is satisfying to note that this hydrogen bond is also found in the solid state. We have used the published[47] X-ray structural data of orotic acid, H(6Urca), to calculate the distance between the hydrogen atom of the (N1)H site and the carbonyl oxygen of the (C6)COOH group: it amounts to 2.337 Å, clearly indicating hydrogen bonding.

These results, as well as the evaluations in Section 3.5, clearly prove that intramolecular hydrogen bonding in aqueous solution may reach rather high formation degrees and that the acid–base properties of uracil derivatives may be significantly affected by such hydrogen bonds.

4 Conclusions

The present study proves that hydrogen-bond formation is not only common in the solid state where it is often observed, but it can also have a significant effect on the acid–base properties of a compound in aqueous solution. Furthermore, provided the various acidity constants are known, it is also possible, as shown here, to estimate the formation degrees of the hydrogen-bonded species in solution.

The presented results regarding the acid–base properties of $5Umpa^{2-}$ and $6Umpa^{2-}$ now also allow the metal ion-binding properties of these ligands to be studied.[48]

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