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IONISATION OF SOME FLAVANOLS AND DIHYDROFLAVONOLS

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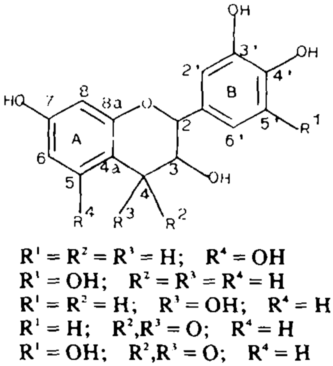
Abstract—Ionisation constants of catechin (**1**), robinetinidol (**2**), leuco-fisetinidin (**3**), fustin (**4**), dihydrorobinetin (**5**), dihydroquercetin (**6**) and a series of polyphenols are reported. The pK_a values of these flavonoids are shown to fit the linear relationships between pK_a and substituent σ constant for a series of related phenols.

Although use is made of the different acidities of flavonoids for the elucidation of hydroxylation patterns by UV analysis,[1] actual ionisation constants of only a few 3- and 5-hydroxy-flavones are known.[2-4] Herein we describe the determination of the ionisation constants of catechin (**1**), robinetinidol (**2**), leuco-fisetinidin (**3**), fustin (**4**) and dihydrorobinetin (**5**) present in wattle-bark extracts (*Acacia mearnsii*),[5] and the related dihydroflavonol, dihydroquercetin (**6**).



1: $R^1 = R^2 = R^3 = H$; $R^4 = OH$
2: $R^1 = OH$; $R^2 = R^3 = R^4 = H$
3: $R^1 = R^2 = H$; $R^3 = OH$; $R^4 = H$
4: $R^1 = H$; $R^2,R^3 = O$; $R^4 = H$
5: $R^1 = OH$; $R^2,R^3 = O$; $R^4 = H$
6: $R^1 = H$; $R^2,R^3 = O$; $R^4 = OH$

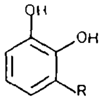
The potentiometrically determined ionisation constants of **1–6** (Table 1) are macroscopic constants and identifying these values with specific OH groups is not valid,[6] although an OH group of the pyran ring may be discounted as a possibility by virtue of the very weak acidity of aliphatic alcohols.[7] However, the A- and B-rings of **1–6** are not conjugated and ionisation of OH groups of one ring system should not appreciably affect ionisation of OH groups of the other. Hence, ionisations of OH groups of ring A are independent and distinguishable from those of ring B. The macroscopic ionisation constants (K'_a) are then related to the microscopic constants (K_a), which can be identified with specific OH groups, by[8]

$$K_a^{A\ \mathrm{or}\ B'} = K_a^A + K_a^B \qquad (1)$$

and

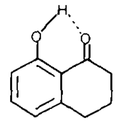
$$K_a^{B\ \mathrm{or}\ A'} = K_a^A K_a^B/(K_a^A + K_a^B) \qquad (2)$$

[the more acidic macroscopic constant is used in eqn (1)] where the constants with superscripts A and B refer to the ionisation constants of ring A and ring B, respectively. pK'_a values of **1–6** were assigned to ring A or ring B (see Table 1) as follows. Pyrocatechol (**7**) and pyrogallol (**8**) (as models for ring B) do not absorb light at $\lambda > 320$ nm up to the upper pH limit for $I = 0.1$. Hence, the ionisation constants of **1–6** determined spectrophotometrically at $\lambda > 320$ nm will be the microscopic constants of ring A. Only the dihydroflavonols (**4–6**) have suitable spectra and the following pK_a^A values were obtained, **4**: 7.07, **5**: 7.06 and **6**: 6.76 and 11.58. Thus, the $pK_a^{B'}$ values of **4–6** can be identified by elimination. By using these $pK_a^{B'}$ values as guides, the $pK_a^{B'}$ values (and hence $pK_a^{A'}$ values) of **1–3** can be identified since the B-rings of **1**, **3**, **4** and **6** are the same, as are those of **2** and **5**. Applying eqns (1) and (2) to the relevant pK'_a values gives the corresponding pK_a values (Table 2). pK_{a1}^A of **2** is the average of 9.68 from $pK_{a1}^{B'}$ and $pK_{a1}^{A'}$ and 9.75 from $pK_{a1}^{A'}$ and pK_{a2}^B.



7: $R = H$
8: $R = OH$

The acid-strengthening 4-keto group of **4–6** has no effect on the OH groups of ring B as shown by the similarity of the pK_a^B values of **1** and **3** with those of **4** and **6** and of **2** with those of **5**. This confirms that ring A and ring B of these flavonoids are unconjugated. Only the OH groups of ring A are affected by the 4-keto group as shown by the lower pK_{a1}^A values of the 7-OH group of **4** and **5** relative to that of **2**. pK_{a1}^A of **6** may also be identified with the 7-OH group since the UV spectra of the related 7-hydroxyflavanones and not those of 5-hydroxyflavanones are affected by addition of sodium acetate,[1] indicating that the 7-OH group is the more acidic. Hence, pK_{a2}^A of **6** corresponds to the 5-OH group. The acidity of this group is less than that of the second ionisation of ring A of **1** and may be explained by the acid-weakening effect of an intramolecular H-bond between the 5-OH and 4-keto group of **6**. A similar effect has been found with H-bonded 2-hydroxyacetophenone and 8 - hydroxy - 1(2H,3H,4H) - naphthalenone (**9**).[9] The existence of an intramolecular H-bond in the related 5-hydroxyflavanones has been established from IR data.[10,11]



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pK_{a1}^B and pK_{a2}^B values of the 3′, 4′, 5′-trihydroxyflavonoids (**2** and **5**) correspond to the 3′,5′-dihydroxyl group, if it is assumed that they follow the same ionisation order as **8**.[12] The acidity of the 4′-OH

Table 1. Macroscopic ionisation constants of flavanoids (**1**–**6**) at 20° and I = 0.1

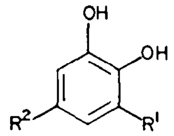
Flavonoid	pK'_{a1}	pK'_{a2}	pK'_{a3}	pK'_{a4}
1	8.79(B)[a]	9.44(A)	11.18(A)	13.25(B)[b]
2	8.71(B)	9.73(A)	11.09(B)	>13(B)[b]
3	8.89(B)	9.68(A)	13.20(B)[b]	
4	7.05(A)	9.01(B)	13.25(B)[b]	
5	7.06(A)	8.78(B)	11.12(B)	>13(B)[b]
6	6.74(A)	9.02(B)	11.55(A)	-

[a] A and B identify the values with ring A and B, respectively (see text). [b] Spectrophotometrically determined values; the other values determined by potentiometric titration.

Table 2. Microscopic pK_a of flavonoids (**1**–**6**)

	A-ring		B-ring		
Flavonoid	pK^A_{a1}	pK^A_{a2}	pK^B_{a1}	pK^B_{a2}	pK^B_{a3}
1	9.26	11.18	8.97	13.25	
2	9.72		8.76	11.07	>13
3	9.58		8.99	13.20	
4	7.05		9.01	13.25	
5	7.07		8.77	11.12	>13
6	6.74	11.55	9.02	-	

Table 3. pK_a of substituted pyrocatechols at 20° and I = 0.1



Phenol	R^1	R^2	pK_{a1}	pK_{a2}	σ_m[a]	σ_p[a]
7	H	H	9.37[b]	13.7[b]	0	0
8	OH	H	9.05[b]	11.2[b]	0.10	0.12[c]
11	H	Me	9.56	14.0	-0.08	-0.15
12	H	t-Bu	9.53	14.0	-0.07	-0.14
13	OMe	H	9.32	13.6	0.12	0.00[d]
14	H	COO^-	8.82	13.2	-0.02	0.24
15	H	Cl	8.62[e]	12.6[e]	0.37	0.23
16	H	SO_3^-	8.50[f]	12.8[f]	0.28	0.40
17	H	$COCH_2Cl$	7.40	12.0	0.45[g]	0.95[g]
18	H	CHO	7.21	11.8	0.48	1.04
19	H	NO_2	6.84[h]	11.1[h]	0.69	1.24
20	H	OH	9.12	11.6	0.10	-0.37
21	OH	COO^-	8.68	11.4	-0.02	0.24
22	OH	COOPr	7,89	10.9	0.37[i]	0.64[i]

[a] σ constants (refs. 20 and 21) of R' (except for 8 and 13). [b] Ref. 13. [c] σ_oOH (see text). [d] σ_oOMe [e] P. Pichet and R.L. Benoit, *Inorg. Chem.* 6, 1505 (1967). [f] M. Bartušek and O. Staňková, *Coll. Czech. Chem. Comm.* 30, 3415 (1965). [g] Calculated from pK_{a1} in 40% dioxane (ref. 13 and eqns therein).
[h] M. Bartušek, *Coll. Czech. Chem. Comm.* 32, 757 (1967).
[i] σ constants of the related COOEt substituent.

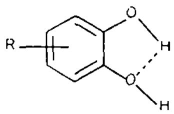
group of **2** and **5** would be expected to be very weak, due to the acid-weakening effect of the 3′,5′-di-O^- group and the influence of a strong H-bond between the central OH group and either of the two O^- groups. The UV spectra of **2** and **5**, at the upper pH limit for I = 0.1, gave no evidence for the ionisation of the 4′-OH group indicating that $pK^B_{a3} > 13$.

A suitable analytical wavelength was not available for determining pK^B_{a2} of **6** since the A-ring chromophore dominates the UV spectrum over the whole pH range. However, since the B-ring of **6** is the same as those of **1**, **3** and **4**, pK^B_{a2} of **6** should be similar to the pK^B_{a2} values of these flavonoids.

Linear relationships between pK_a and substituent σ constant have been found for series of phenols.[13-19] Equation (3) has been used to assign apparent σ constants for a variety of substituents from the thermodynamic constants (${}^TK_a$) of phenols in water at 20°–25°.[20,21] In order to correlate the pK_a

$$p^TK_a = -2.23\Sigma\sigma + 9.92 \qquad (3)$$

values of the flavonoids (**1**–**6**), values of σ_oOH and σ_mOH (as well as σ constants for the other substituents of ring A and ring B) are required. Although $\sigma_o\mathrm{OH} = 0.04$ and $\sigma_m\mathrm{OH} = 0.34$ have been proposed,[17] no statistical corrections were applied to the pK_{a1} values of **7** (9.85)[22] and resorcinol (9.15)[23] from which these σ constants were calculated. Using Jaffé's original value, $\sigma_m\mathrm{OH} = 0.10$, still gives close agreement between found and calculated pK_a values of resorcinol derivatives,[17] if statistical corrections are applied where necessary. In addition, a recently determined p^TK_{a1} value for **7** (9.36)[25] is different from the above value. Applying a statistical correction (+0.30) to 9.36, gives $\sigma_o\mathrm{OH} = 0.12$ from eqn (3). However, this constant is only valid if interaction between the OH groups of the *o*-dihydroxyl group remains constant for a series of substituted pyrocatechols. Hence, the slope of a plot of $\mathrm{p}K_{a1}$ vs σ constant for pyrocatechols should be similar to that of eqn (3) if the interaction remains constant.[16] The presence of two bands (OH stretching vibrations) in the IR spectrum of **7**[26-28] and the greater acidity of **7** relative to hydroquinone[23] have been interpreted as being due to intramolecular H-bonding (**10**), which could lead to a different slope.[16] However, the slope



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of the linear relationship [eqn (4)] between $\mathrm{p}K_{a1}$ (statistically corrected where necessary) and σ constant for **10** (Table 3) is similar to that of eqn (3) indicating that the effect of the H-bond remains constant as R is varied. It has been assumed that the

$$\mathrm{p}K_{a1} = 9.45 - 2.17\sigma \quad (r = 0.992, \quad s = 0.12) \qquad (4)$$

OH group of **10** in the position which the substituent is the more acid-strengthening will ionise first.[24] Hence, the algebraically greater σ constant is used in this correlation except for pyrogallols (**8** and **22**) for which the sum of $\sigma_m\mathrm{OH}$ and $\sigma_m\mathrm{R}^2$ is used. Although the pyrocatechols with ionic substituents (**14**, **16** and **21**) are not included in this correlation, since their σ constants vary with ionic strength,[29] inclusion of the points for these pyrocatechols gives a similar straight line. Using the Davies equation[30] to extrapolate the intercept of eqn (4) to zero ionic strength gives $\sigma_o\mathrm{OH} = 0.12$, the same value as obtained above. Using this value, a plot of $\mathrm{p}K_a$ (again statistically corrected where necessary) for **7**, **8**, **11**–**22** (except for $\mathrm{p}K_{a2}$ of **7**, **11**–**19** since $\sigma_o\mathrm{O}$ is not valid, see below) and for a series of monohydric phenols at $I = 0.1$[31,32] against $\Sigma\sigma$ gives the straight line,

$$\mathrm{p}K_a = 9.65 - 2.11\Sigma\sigma \quad (r = 0.991, \quad s = 0.15). \qquad (5)$$

Since $\mathrm{p}K_{a2}$ of **8** corresponds to the *m*-OH group,[12] $\sigma_m\mathrm{O}^- = 0.71$[21] is used for correlating the $\mathrm{p}K_{a2}$ values of **8**, **21** and **22**. The best fit for 1,2,4-trihydroxybenzene (**20**) is obtained with $\mathrm{p}K_{a1}$ and $\mathrm{p}K_{a2}$ corresponding to the 2-OH and 4-OH group, respectively.

The best correlation of the $\mathrm{p}K_{a2}$ values of **7**[†], **11**–**19** is obtained with the average of the substituent σ constants (σ_{av}):

$$\mathrm{p}K_{a2} = 13.66 - 2.57\sigma_{av} \quad (r = 0.991, \quad s = 0.13). \qquad (6)$$

A similar result has been obtained with the $\mathrm{p}K_{a1}$ values of 4-substituted pyrocatechols determined in 40% dioxane[‡] and was rationalised on the basis that the intramolecular H-bond (**10**) causes both OH groups to be equally affected by the substituent.[13] This argument is also applicable to the ionisation of the second OH group since a strong intramolecular H-bond is possible between the OH group and the *o*-O$^-$ group. The greater slope of eqn (6) relative to those of eqns (3)–(5) is expected since an electron-withdrawing substituent would weaken the H-bond and would also relieve the electrostatic repulsion between the resulting two O$^-$ groups yielding an even lower $\mathrm{p}K_{a2}$ value and vice versa. Hence, a $\sigma_o\mathrm{O}^-$ value, calculated from $\mathrm{p}K_{a2}$ of **7**, is not valid for the second ionisation of **10**.

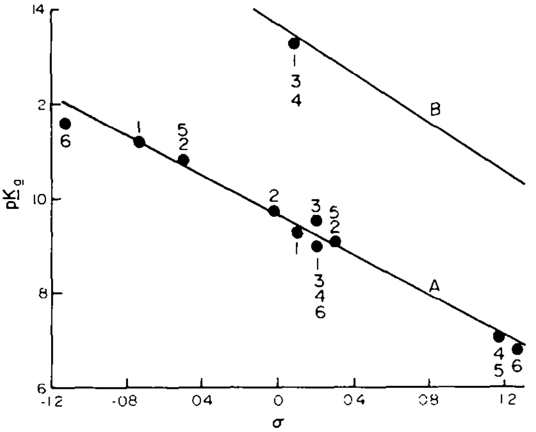

Fig. 1. Correlations of $\mathrm{p}K_a$ with σ: straight lines A and B defined by eqns (5) and (6), respectively.

In order to include the $\mathrm{p}K_a$ values of the flavonoids (**1**–**6**, Table 2) in these correlations, the following σ constants for the substituents of ring A and ring B were utilised: 4a-CH$_2$R, $\sigma_p = -0.15$[20] and $\sigma_o = -0.13$[21] as for -Me; 4a-CHROH, $\sigma_p = 0.08$[21] as for -CH$_2$OH; 4a-COR, $\sigma_p = 1.04$[20] as for -CHO and $\sigma_o = -0.54$ calculated from $\mathrm{p}^{\mathrm{T}}K_a$ of **9**[9] by eqn (3) and then substracting -0.08 for the *m*-methylene group; 8a-OCHR$_2$, $\sigma_m = 0.12$[20] as for -OMe; 1′(2-chromanyl), $\sigma_m = \sigma_p = 0.08$[21] as for -CH$_2$OH. Figure 1 shows that the points for **1**–**6** correlate well with the straight lines defined by eqns (5) and (6). A slightly better fit is obtained with $\mathrm{p}K_{a1}^{\mathrm{A}}$ and $\mathrm{p}K_{a2}^{\mathrm{A}}$ of **1** corresponding to the 5-OH and 7-OH group, respectively. However, for **6** by far the better fit is with the reverse of this ionisation order, confirming that the 7-OH group is the more acidic and that the 5-OH group is H-bonded as in **9**.

EXPERIMENTAL

(+)-Catechin (**1**) was isolated from cube cambier (obtained from the leaves of *Uncaria gambir*)[35] and (±)-fustin (**4**) was extracted from the heatrwood of *Rhus glabra*.[36,37]

(−)-Robinetinidol (**2**), (+)-leuco-fisetinidin (**3**) and (+)-dihydrorobinetin (**5**) were generously supplied by Dr H. M. Saayman of this lab. 4-Hydroxypyrocatechol (**20**) was prepared by hydrolysis of 1,2,4-triacetoxybenzene (Koch-Light).[38] The other phenols are commercially available and were purified by either vacuum distillation or recrystallisation. Aqueous (boiled out water, cooled under N$_2$) solns of the phenols were stored under N$_2$ and standardised by potentiometric titration after addition of an excess of gemanium dioxide.[39]

A Bjerrum–Calvin potentiometric titration technique[40] was used to determine the ionisation constants of the more acidic phenols ($\mathrm{p}K_a < 11.5$). A spectrophotometric extrapolation technique[41] was used for the less acidic phenols. UV measurements were made with a Beckman ACTA MVI and a Beckman DU spectrophotometer. In all cases, the medium was 0.1 M (KCl/HCl/KOH) at 20 ± 0.1°. A Beckman Centuary SS pH meter with a Metrohm EA 147U electrode and a Metrohm Model E426 potentiometer with a Metrohm EA 125U electrode were used. The electrode systems were calibrated as [H$^+$] probes as previously described.[42,43] The ionisation constants quoted are therefore

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[†] Published $\mathrm{p}K_{a2}$ values for **7** show considerable variation, ranging from 11.6 to 13.7.[33] However, $\mathrm{p}K_{a2}$ 13.1 has been suggested as the minimum value at 25° and $I = 0.1$.[34]

[‡] However, incorporating $\mathrm{p}K_{a1}$ values of 3-substituted pyrocatechols results in a slightly better linear relationship with the greater σ constant and not with σ_{av}.

concentration quotients. All measurements were made under a N_2 atmosphere and as an added precaution against oxidation of the phenols during spectrophotometric measurements, hydrazine hydrate (0.01 M) was used as an antioxidant.[44] A typical potentiometric titration was as follows. 0.1 N KOH was used to titrate 50 ml of an aqueous solution of 0.004 N HCl and 0.996 N KCl through which N_2 was bubbled. Thereafter, an identical solution plus 0.006 M phenol was titrated. At least three such titrations were performed for each phenol. pK_a values for overlapping ionisations were calculated by least-squares treatment using the equations for two[45] and three[46] overlapping ionisations. Standard deviations for pK_a values are of the order of ± 0.02 for $pK_a < 10$ and ± 0.05 for $pK_a > 10$.

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REFERENCES

[1] T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids*, Part II. Springer-Verlag, Berlin (1970).
[2] G. M. Saxena and T. R. Seshadri, *Proc. Indian Acad. Sci.* **46A**, 218 (1957).
[3] H. Kido and W. C. Fernelius, Pennsylvania State Univ., Contract No. AT(30-1)-907 (1960).
[4] K. Yamamoto and K. Takamizawa, *Nippon Kagaku Zasshi* **88**, 345 (1967).
[5] S. E. Drewes and D. G. Roux, *Biochem. J.* **87**, 167 (1963).
[6] G. M. Fleck, *Equilibrium in Solution*, p. 92. Holt, Rinehart & Winston, New York (1966).
[7] P. Ballinger and F. A. Long, *J. Am. Chem. Soc.* **82**, 794 (1960).
[8] E. Q. Adams, *J. Chem. Soc.* **38**, 1503 (1916).
[9] L. B. Magnusson, C. Postmus and C. A. Craig, *J. Am. Chem. Soc.* **85**, 1711 (1963).
[10] H. L. Hergert and E. F. Kurth, *Ibid.* **75**, 1622 (1953).
[11] B. L. Shaw and T. W. Simpson, *J. Chem. Soc.* 655 (1955).
[12] M. Bartušek and J. Zelinka, *Coll. Czech. Chem. Comm.* **32**, 992 (1967).
[13] J. Corse and L. L. Ingraham, *J. Am. Chem. Soc.* **73**, 5706 (1951).
[14] A. I. Biggs and R. A. Robinson, *J. Chem. Soc.* 388 (1961).
[15] M. Papoport, C. K. Hancock and E. A. Meyers, *J. Am. Chem. Soc.* **83**, 3489 (1961).
[16] L. A. Cohen and W. M. Jones, *Ibid.* **85**, 3397 (1963).
[17] P. J. Pearce and R. J. J. Simkins, *Canad. J. Chem.* **46**, 241 (1968).
[18] J. Durmis, M. Karvaš and Z. Maňásek, *Coll. Czech. Chem. Comm.* **38**, 215 (1973).
[19] H. W. Gibson and F. C. Bailey, *J. Chem. Soc.* Perkin II 196 (1976).
[20] L. A. Cohen and W. M. Jones, *J. Am. Chem. Soc.* **85**, 3402 (1963).
[21] G. R. Barlin and D. D. Perrin, *Quart. Rev.* **2M**, 75 (1966).
[22] R. Kuhn and A. Wassermann, *Helv. Chim. Acta* **11**, 3 (1928).
[23] C. T. Abichandani and S. K. K. Jatkar, *J. Indian Inst. Sci.* **21A**, 417 (1938).
[24] H. H. Jaffé, *Chem. Rev.* **53**, 191 (1953).
[25] R. F. Jameson and M. F. Mason, *J. Chem. Soc.* Dalton 2610 (1972).
[26] L. Pauling, *J. Am. Chem. Soc.* **58**, 94 (1936).
[27] L. L. Ingraham, J. Corse, G. F. Bailey and F. Stitt, *Ibid.* **74**, 2297 (1952).
[28] E. A. Robinson, H. D. Schreiber and J. N. Spencer, *J. Phys. Chem.* **75**, 2219 (1971).
[29] H. Zollinger and W. Wittwer, *Helv. Chim. Acta* **39**, 347 (1956).
[30] C. W. Davies, *Ion Association*, p. 41. Butterworths, London (1962).
[31] C. Postmus, L. B. Magnusson and C. A. Craig, *Inorg. Chem.* **5**, 1154 (1966).
[32] K. E. Jabalpurwala and R. M. Milburn, *J. Am. Chem. Soc.* **88**, 3224 (1966).
[33] L. G. Sillén and A. E. Martell, *Stability Constants* p. 400. The Chemical Society, London (1971).
[34] C. A. Tyson and A. E. Martell, *J. Am. Chem. Soc.* **90**, 3379 (1968).
[35] A. G. Perkin and E. Yoshitake, *J. Chem. Soc.* **81**, 1160 (1902).
[36] D. G. Roux and K. Freudenberg, *Liebigs Ann.* **613**, 56 (1958).
[37] D. G. Roux and E. Paulus, *Biochem. J.* **77**, 315 (1960).
[38] M. Healey and R. Robinson, *J. Chem. Soc.* 1625 (1934).
[39] P. Pichet and R. L. Benoit, *Inorg. Chem.* **6**, 1505 (1967).
[40] H. M. Irving and H. S. Rossotti, *J. Chem. Soc.* 2904 (1954).
[41] A. Albert and E. P. Serjeant, *The Determination of Ionization Constants* p. 52. Chapman & Hall, London (1971).
[42] W. A. E. McBryde, *Analyst* **94**, 337 (1969).
[43] W. A. E. McBryde, *Ibid.* **96**, 739 (1971).
[44] L. Sommer, *Coll. Czech. Chem. Comm.* **28**, 2101 (1963).
[45] H. M. Irving and H. S. Rossotti, *J. Chem. Soc.* 3397 (1953).
[46] V. T. Athavale, L. H. Prabhu and D. G. Vartak, *J. Inorg. Nucl. Chem.* **28**, 1237 (1966).