Complexation of boric acid with vitamin C

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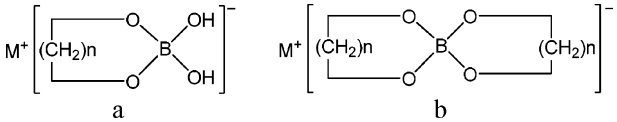
Mono-chelate (1 : 1) and bis-chelate (1 : 2) anionic complexes of boric acid with vitamin C (L-ascorbic acid, H_2A) were isolated from aqueous solutions in salt form with Li^+, Na^+ and Ca^{2+} ions. The complexes were characterized by FTIR, ^{13}C and ^{11}B MAS (magic angle spinning) NMR techniques. The spectral data agreed with the calculated structures that H_2A complexes with boric acid through its *cis*-enediol groups by forming five-membered chelate rings. Side-chain OH groups do not participate in complexation as corroborated by the results of the test experiments conducted with iso-propylideneascorbic acid (i-H_2A) where the side chain is blocked. Empirical relations, that can be used as diagnostics for the 1 : 1 and 1 : 2 ascorbatoborate complexes, were derived from the observed chemical shift values. Bis-chelate complexes were found to have higher thermal and hydrolytic stabilities than their mono-chelate homologs. The effect of the cation on the stabilization of the complexes was also investigated. The observed relative stability of the 1 : 1 Na–ascorbatoborate complex with respect to the analogous Ca complex, correlated with the recent reports about the role of alkali metal ions in the stabilization of ribose in the prebiotic world.

Introduction

The exact biological function of boron in animals and humans still remains to be clarified. Currently, it is accepted that boron stabilizes biological molecules by linking them through ester bridges.[1,2] At intracellular pH, nearly all boron exists as boric acid, which behaves as a Lewis acid and forms molecular addition compounds with amino- and hydroxy acids, carbohydrates, nucleotides and vitamins through electron donor–acceptor interactions.[3–8] Complexation of boric acid with organic molecules containing adjacent hydroxyl groups proceeds through esterification reactions. Partial esterification creates either monoesters (1 : 1 complexes) that retain the planar configuration with no charge or a tetrahedral configuration with negative charge. Complete esterification leads to the formation of bicyclic diester (1 : 2) structures with negatively charged tetrahedral borate anions,[3–8] as shown in Scheme 1. Monoesters of boron are quite labile and rapidly hydrolyze to their original components in aqueous solution, while diesters are expected to be thermodynamically stable and almost undissociable in water.[9,10] Neutralization with a metal cation helps stabilization of the ester structures.[11]

Complexation with sugars is particularly important in understanding the role of boron as a carrier for nucleotides and carbohydrates. An exciting article suggests that borate minerals could have played a crucial role in the early world by stabilizing the cyclic ribose during RNA synthesis.[12] Very recently, da Silva and co-workers have elegantly demonstrated possible clues on the roles of borate ions, alkaline and alkaline earth ions in the synthesis and stabilization of ribose during the pre-RNA period.[13] H_2A is a sugar acid with a *cis*-enediol group on the sugar ring and adjacent alcoholic hydroxy groups on the side chain (Fig. 1a), available for complex formation with boric acid. The possible link between boron and ascorbate metabolism was investigated based on the hypothesis that boron stimulates AFR (ascorbate free radical) reduction to ascorbate. Evidence that boron affects ascorbate metabolism includes the finding that ascorbate supplementation restores growth of the squash meristem retarded by boron deprivation.[14,15] To our knowledge, only two studies have been reported in the literature concerning the interaction of H_2A with H_3BO_3. The scarcity of the related literature possibly arises from the unstable nature of the H_2A molecule. Militzer first reported in 1945 that not the oxidation of H_2A but that of DHA (dehydroascorbic acid) was prevented by borate, suggesting that a complex is formed between borate and the primary oxidation product of H_2A.[16] Half a century later, Obi *et al.* published a speculative letter on the formation of borate esters with H_2A, i-H_2A and isopropylidene–H_2A (Fig. 1b) in 1998.[17] The authors claimed that the enediol groups of H_2A favorably participate in borate ester formation to yield a six-membered ring, though their ^{11}B NMR chemical shifts agreed with those of the borate esters of α,β-diols which produce five-membered rings.

In the present study, the complexation of boric acid with H_2A was reinvestigated. Negatively charged mono- (1 : 1) or bis-ascorbato (1 : 2) borate complex anions were precipitated in salt form with Li^+, Na^+ and Ca^{2+} ions in order to examine the effects of the borate and also the counter metal ions on the



Scheme 1 Boric acid esters (a) monoester, (b) bicyclic diester.

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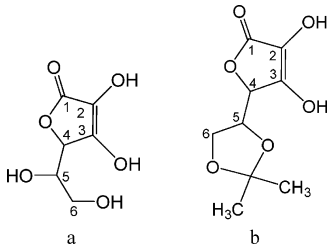
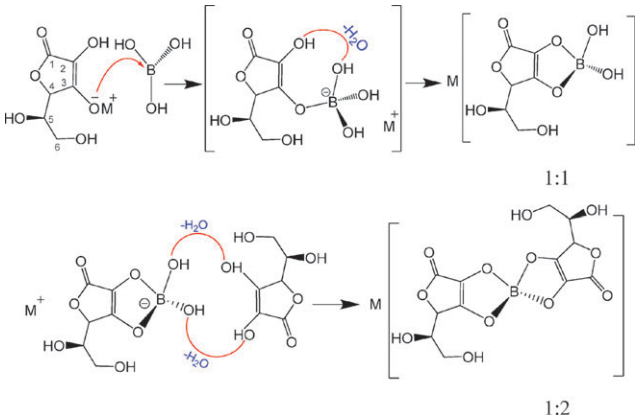


Fig. 1 (a) Ascorbic acid, (b) isopropylideneascorbic acid.

stabilization of ascorbate. Structural characterization of the isolated compounds and their relative stabilities in the aqueous phase are discussed.

Results and discussion

H_2A is a weak dibasic acid (pK_{a1} = 4.25 and pK_{a2} = 11.79). The monoanion (HA) forms at pH 4–5 with deprotonation of (C3)OH and the dianion (A) forms at pH 11–12 with deprotonation of the (C2)OH.[18] The monoanionic form is more stable due to the delocalization of the negative charge between the oxygens at the C1 and C3 positions.[19] Although it has several donor atoms capable of metal complex formation, the interaction of HA with metals mainly occurs through the O_{C3} atom monodentately or *via* (C3)O and (C2)O by chelation (see ref. 20 and the references therein). The reaction between HA and boric acid is simply based on Lewis acid–base interaction. Following the attack of O_{C3}^- of HA on the trigonal boron atom, the condensation reaction between O_{C2}–H and O–H of $B(OH)_3$ releases one water molecule and leads to a monoester structure (1 : 1) which may be precipitated in salt form where the negatively charged tetrahedral boron is neutralized by the metal ion. Further condensation with another H_2A molecule releases two water molecules and results in diester (1 : 2) formation (Scheme 1). Since boron is claimed to have an essential role in affecting calcium and magnesium metabolisms and lithium is an element with well-established pharmacological properties,[1,21] the complexes were intended to be prepared in salt form with these elements for either nutritional or pharmacological purposes. The compounds were precipitated as white crystalline solids. The crystals were air-stable but of too poor quality for X-ray analyses. The reluctance of covalently bound pure ascorbate complexes towards crystallization is a well-known handicap in their structure identification, which therefore requires the use of other characterization techniques such as NMR.[20] The compositions and melting points of the products are summarized in Table 1. All efforts towards isolation of the magnesium salt



Scheme 2 Proposed mechanism for the complexation of boric acid with ascorbic acid.

failed. A yellow coloration was observed during the concentration of the solution; an indication of ascorbate decomposition. Magnesium ion has been previously shown to behave differently than the other alkaline earth metals in the speciation of ribose–borate-bound species[13] and also in binding with H_2A,[22] probably due to its smaller cation size. In the present case, magnesium ions possibly compete with ascorbate for borate, and free ascorbate undergoes degradation according to the mechanism proceeding in aqueous acidic solutions under non-oxidative conditions, Scheme 2.[23]

FTIR spectra

A summary of the FTIR spectral data for the compounds is given in Table 2 with their assignments.[24–26] All the compounds showed a broad asymmetric band at 3600–3000 cm^{-1} associated with the hydrogen-bonded O–H stretching vibrations of ascorbic acid, water molecules and borate anions. The carbonyl stretching vibrations of the free H_2A shifted by a few wavenumbers to lower frequencies in the complexes. The combination band due to (C═O) + (C═C), and also the bending vibrations of hydrogen bonded –OH groups appeared as broad and strong bands at 1650 cm^{-1} and 1590 cm^{-1}. Another broad band observed at *ca.* 1420 cm^{-1}, together with the next strongest band at *ca.* 1380 cm^{-1} are assigned to the C–H deformation vibrations. The strong peak at 1320 cm^{-1} and the weak peak at *ca.*1250 cm^{-1} corresponding to the (O–H) enediol for the free acid disappeared in the spectra of the complexes showing the participation of the O_{C3} and O_{C2} atoms in chelate-type coordination.[27] The spectral pattern in the 1200–800 cm^{-1} region is consistent with the data

Table 1 Chemical compositions[a] and melting points of vitamin C–borate complexes

Compound	m.p./°C	C (%)	H (%)	H_2O (%)
NaHA	220	36.36	3.53	—
$Na[B(A)(OH)_2]\cdot H_2O$	88	27.78 (27.69)	3.85 (3.86)	6.9 (6.9)
$Li[B(A)(OH)_2]\cdot 1/2H_2O$	105	31.20 (30.76)	3.80 (3.42)	3.9 (3.8)
$Na[B(i\text{-}A)(OH)_2]\cdot 2.5H_2O$	98	33.12 (32.83)	5.98 (5.77)	13.6 (13.7)
$Ca[B(A)(OH)_2]_2\cdot 3H_2O$	93	27.07 (27.06)	3.99 (3.76)	9.90 (10.2)
$Ca[B(A)_2]_2\cdot 10H_2O$	85	30.67 (30.70)	4.38 (4.69)	18.7 (19.2)

[a] Calculated values in parentheses. A = $C_6H_6O_6$, i-A = $C_9H_{10}O_6$

Table 2 A summary of the FT-IR spectral data of vitamin C–borate complexes

Compound	ν(O–H)	ν(C=O)	ν(C=O) + ν_a(C=C)	C–H def. and ν(C–O$^-$)	ν_a(B–O)/BO_4 and ring (H_2A)	ν_s(BO)/BO_4
H_2A[24]	600–3000	1753vs	1750 + 1650	1495m, 1385m	1200–900	—
NaHA[24]	400–3200	1702vs	1628sh + 1550vs	1482m, 1359m	1200–900	—
Borax[25]	~3300	—	—	—	1220	(834, 815)d
$Na[B(A)(OH)_2]\cdot H_2O$	~3400	1744s	1650vs + 1590s	1421s, 1380sh	1200–900	823, 763
$Li[B(A)(OH)_2]\cdot 1/2H_2O$	33 600–3200	1705s	1650sh + 1590vs	1473m, 1386s	1200–900 shp	830, 753
$Ca[B(A)(OH)_2]_2\cdot 3H_2O$	~3400	1744vs	1650vs + 1590sh	1421vs, 1384vs	1200–900	824, 763
$Ca[B(A)_2]_2\cdot 10H_2O$	~3400	1750vs	1664vs + 1609sh	1480m, 1420m, 1352m	1200–900	—, 761

d = doublet, s = strong, vs = very strong, m = medium, sh = shoulder, shp = sharp.

reported by Davis and Mott for several carbohydrate borate complexes.[26] In this region, the individual assignment of bands is difficult due to the overlap of numerous (O_{C5}–H), (O_{C6}–H), (C–O) ring, (C–C) ring vibrations of ascorbate and in plane B–O–H bending vibrations of tetrahedral borate. The symmetric (B–O) doublet at 834 cm^{-1} and 815 cm^{-1}, which is a diagnostic for the tetrahedral borate anion,[25] was not observed. Instead, the compounds typically showed two signals at 822 cm^{-1} and 762 cm^{-1}, the latter being more intense. Several other peaks also appeared in this region down to 500 cm^{-1} due to the lattice vibrations of crystal water.

FT-IR spectra displayed similar features for the 1 : 1 complexes with minor changes in the peak positions and intensities due to the presence of different metal ions in the structure and different amount of water molecules that are involved in hydrogen-bonding interactions. The spectrum of the lithium compound displayed sharp peaks that are almost all positioned at lower wavenumbers than those assigned for the other 1 : 1 complexes. On comparing the FT-IR spectra of $Ca[B(A)(OH)_2]_2\cdot 3H_2O$ and $Ca[B(A)_2]_2\cdot 10H_2O$, the red-shift in the asymmetric (B–O) band in the spectrum of $Ca[B(A)_2]_2\cdot 10H_2O$ is noteworthy and can be assigned as the key point in distinguishing the IR spectra of the mono-chelate and bis-chelate esters of boric acid. In addition, the ν_s(B–O) around 820 cm^{-1} nearly disappeared in the spectrum of $Ca[B(A)_2]_2\cdot 10H_2O$.

NMR spectra

Solution ^{13}C NMR spectra of the 1 : 1 complexes yielded signals identical to those present in the spectrum of NaHA. It appears that complete hydrolysis in D_2O yielded HA and boric acid species during the recording of the spectrum. For $Ca[B(A)_2]_2$, several signals were observed for the C2 and C3 chemical shifts indicating that various species involving monodentate or bidentate ascorbate moieties are present in the solution due to the partial hydrolysis of the diester.

Since the compounds are inclined to hydrolysis in the solution phase, ^{13}C MAS NMR spectroscopy was applied to determine whether the *ene*-diol groups or side-chain alcoholic groups of HA are used in chelation with boron. Fig. 2 shows the ^{13}C MAS NMR spectra of $Ca(HA)_2\cdot 2H_2O$, **1** and **2**, for comparison. The splitting of the Ca–ascorbate signals is consistent with the previously reported crystal structure of $Ca(HA)_2\cdot 2H_2O$ in which the asymmetric unit contains two independent molecules differing in the orientation of the side chain and thus giving rise to a number of possible

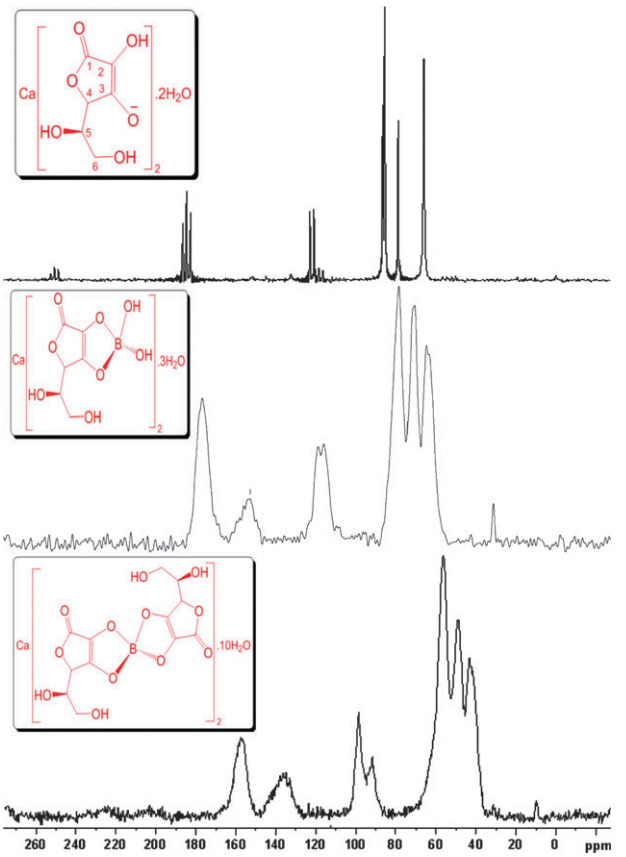


Fig. 2 ^{13}C MAS NMR spectra of $Ca(HA)_2\cdot 2H_2O$, $Ca[B(A)(OH)_2]_2\cdot 3H_2O$ and $Ca[B(A)_2]_2\cdot 10H_2O$.

hydrogen-bonding interactions.[28] On chelation to the NMR-active boron atom, all the signals were shifted and broadened due to the quadrupole relaxation of the boron nucleus, resulting in poorly resolved multiplets. Although the broadening has made detailed analysis difficult, particularly the calculation of the coupling constants, the general appearance of the spectra allowed qualitative conclusions to be drawn.

The assignments of solution ^{13}C NMR chemical shifts of H_2A and its metal ion salts have been reported in the literature (ref. 20 and the references therein). Upon acid ionization, drastic changes are observed for the chemical shifts of C3 (~20 ppm to higher frequency), C2 (to higher or lower frequency), C1 (to higher frequency) and C4 (to higher frequency). The positions of C5 and C6 are not significantly affected. The observed change for C3 is due to the related

Table 3 ^{13}C MAS NMR chemical shifts of vitamin C–borate complexes (ppm)

Compound	C1	C2	C3	C4	C5	C6
H_2A[30]	175	118	155, 152	77, 75	68	60, 59.4, 58.8
NaHA[30]	178	111	176	81	68	66
$Na[B(A)(OH)_2]\cdot H_2O$	169	110, 103	147	70	62	57
$Ca(HA)_2\cdot 2H_2O$	188, 185	122, 124	182	86, 84	78	64
$Ca[B(A)(OH)_2]_2\cdot 3H_2O$	177	118, 115	154	77	70	64
$Ca[B(A)_2]_2\cdot 10H_2O$	168	111, 104	146	70	62	57
$Na[B(i\text{-}A)(OH)_2]2.5H_2O$	168	110, 103	146	71	67	58

variations in bond lengths and delocalization of the electron distribution throughout the ene-diol and carbonyl groups as a result of ionization of the acid at this position. Consequently, the C2 carbon shows a considerable upfield shift if the binding to the metal is through the O3 atom only, as for the alkaline ascorbate salts. On the other hand, a downfield shift of C2 is indicative of chelation through O_{C2}–H and O_{C2}–H.[29]

Table 3 summarizes the ^{13}C MAS NMR chemical shifts and their assignments. All carbon resonances, including those of C5 and C6, shifted upfield in the solid state NMR spectra of the boron ascorbate complexes. The most significant shift is for the C3 atom for which $\Delta\delta = -28$ ppm upon 1 : 1 complex formation and $\Delta\delta = -36$ ppm upon 1 : 2 complex formation. This observation suggests the participation of the ene-diol oxygens in complexation with boron. It is particularly noteworthy that the changes in the chemical shifts of the C1, C4, C5 and C6 atoms show a double $\Delta\delta$ on moving from the corresponding calcium mono-chelate to bis-chelate borate ester structures. Table 4 shows the variation in the chemical shifts ($\Delta\delta$) due to complexation.

From the observed $\Delta\delta$ values, the following empirical equations can be derived for distinguishing the 1 : 1 and 1 : 2 ascorbatoborate complexes (MA is the original metal ascorbate salt):

$$\delta(C3)_{1:1} = \delta(C3)_{MA} - 28$$

$$\delta(C3)_{1:2} = \delta(C3)_{MA} - 36$$

$$\delta(C2)_{1:2} = \delta(C3)_{MA} - 16$$

$$\delta(C1,C4,C5)_{1:1} = \delta(C1,C4,C5)_{MA} - 8$$

$$\Delta\delta(C1,C4,C5,C6)_{1:2} = 2 \times \Delta\delta(C1,C4,C5,C6)_{1:1}$$

Additional evidence for the chelation sites was provided by the NMR spectrum of the boron complex prepared with isopropylideneascorbic acid (i-H_2A), a derivative of H_2A where the side chain is blocked by the isopropylidene group

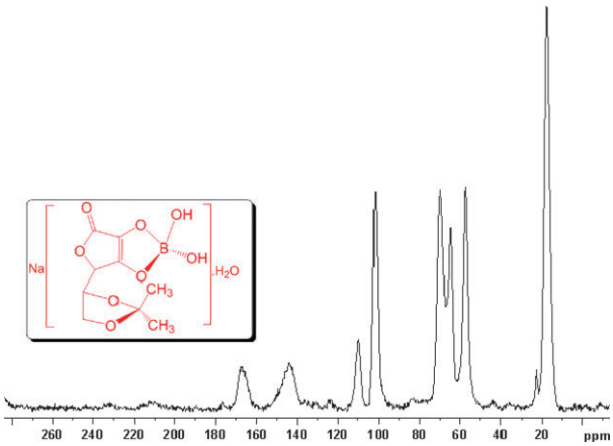


Fig. 3 ^{13}C MAS NMR spectrum of $Na[B(i\text{-}A)(OH)_2]_2\cdot 5H_2O$.

(Fig. 1b). Similar shifts were observed for C2 and C3 of $Na[B(i\text{-}A)(OH)_2]\cdot 2.5H_2O$, verifying that the *cis*-enediol groups are involved in the formation of borate esters with vitamin C (Fig. 3, Table 3).

$Ca[B(A)(OH)_2]_2\cdot 3H_2O$ was also investigated with ^{11}B MAS NMR and the spectrum was interpreted in comparison to that of H_3BO_3 (Fig. 4a). The spectrum of H_3BO_3 showed a typical quadrupole doublet of boron in a trigonal environment[31] while the spectrum of the complex displayed four peaks at *ca.* 4, 7, 11 and 16 ppm (Fig. 4b). The characteristic sharp signal at 4 ppm is consistent with the calculated value of the tetrahedral ^{11}B chemical shift for 5-ring diol esters,[32] thus confirming the presence of the tetrahedral boron atom in a five-membered chelate ring. The observed unsymmetrical split pattern of the spectrum might be due to orientational disorder in the rapidly precipitated complex. The packing of differently oriented ascorbatoborate moieties in the solid state results in a variety of intermolecular distances between chemically equivalent nuclei which contribute to the observed spectra.

The observed multinuclear NMR data correlated well with the minimum energy structures calculated for the 1 : 1 and 1 : 2 complexes, as shown in Fig. 5.

Table 4 Changes in ^{13}C MAS NMR chemical shifts of vitamin C–borate complexes

Compound	$\Delta\delta$(C1)	$\Delta\delta$(C2)	$\Delta\delta$(C3)	$\Delta\delta$(C4)	$\Delta\delta$(C5)	$\Delta\delta$(C6)
$Na[B(A)(OH)_2]\cdot H_2O$[a]	−9	−8	−29	−11	−6	−9
$Ca[B(A)(OH)_2]_2\cdot 3H_2O$[b]	−8	−5,−8	−28	−7	−8	[c]
$Ca[B(A)_2]_2\cdot 10H_2O$[b]	−17	−10,−16	−36	−14	−17	−7

[a] Values in ppm with respect to NaHA. [b] Values in ppm with respect to $Ca(HA)_2\cdot 2H_2O$. [c] Not significant.

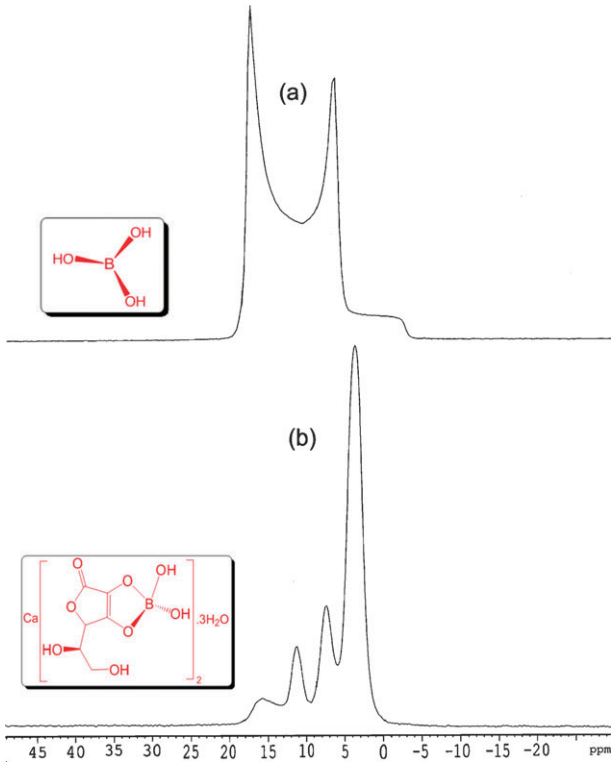


Fig. 4 ^{11}B MAS NMR spectra of a) H_3BO_3 and b) $Ca[B(A)(OH)_2]_2 \cdot 3H_2O$.

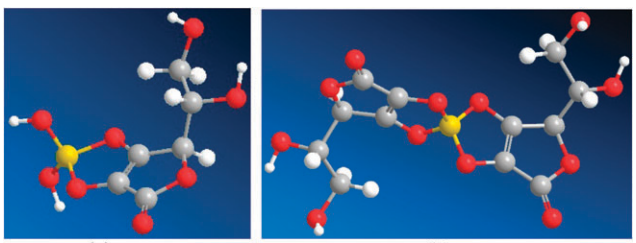


Fig. 5 Proposed structures for (a) $[B(A)(OH)_2]^-$ and (b) $[B(A)_2]^-$ moieties.

Relative stabilities of 1 : 1 and 1 : 2 complexes

Thermal stabilities of the complexes were examined by recording their TGA (thermogravimetric analysis) and DTA (differential thermal analysis) curves (Fig. 6 and Fig. 7). Decomposition of the complexes generally proceeded in the following order: at low temperatures, the crystal water molecules were removed first. This was a single-step process for 1 : 1 complexes and multi-step process for 1 : 2 complexes. Then, water elimination from the ascorbate moieties took place. Finally, the organic group degraded *via* an exothermic process, where the TGA curve correlated with the exothermic peak in the DTA curve. Thermal decomposition left behind some greyish black pyrolytic carbon residue in the crucibles deposited on the expected metal borate end product (M_2O–uB_2O_3 or $MO \cdot B_2O_3$). The observed total mass losses were therefore found to be greater than the calculated values.

As a confirmation of the stabilization of ascorbate by borate,[33] the exothermic DTA peak temperatures of the complexes were noted to be higher than those of the starting metal ascorbate salts. Among the 1 : 1 complexes, the ligand decomposition temperatures were observed to depend also on the type of counter metal ion, showing an increase from the Li (186 °C) to Na (304 °C) and Ca (315 °C) complexes. Considering that the lithium ion has the smallest radius and thus the strongest resulting polarization of the organic moiety, $Li[B(A)(OH)_2] \cdot 1/2H_2O$ reasonably appears to be the least stable complex. Upon comparing the thermal stabilities of the 1 : 1 and 1 : 2 complexes, $Ca[B(A)_2]_2 \cdot 10H_2O$ was found to be stable up to *ca.* 15 °C higher than $Ca[B(A)(OH)_2]_2 \cdot 3H_2O$. This observation is consistent with those in the literature that bis-chelates of boron are more stable.[10]

Hydrolysis of the borate complexes in aqueous solutions releases ascorbate anions, which then undergo hydrolytic degradation as well. A decrease in the absorbance of the characteristic $\pi \rightarrow \pi^*$ transition of the ascorbate group over time would consequently be an indication of the hydrolytic stabilities of the complexes. This transition occurs between 243 and 265 nm depending on the pH of the solution.[34] Data presented here were recorded at 30 min intervals over 9 h, at the natural pH values of the 0.01 M complex solutions, without making any pH adjustment. Fig. 8 shows the solution UV spectra of NaHA and the corresponding 1 : 1 borate complex, $Na[B(A)(OH)_2]$. The absorbance of NaHA descended to nearly zero in 9 h due to the complete hydrolysis of ascorbate groups while non-zero absorbance was recorded for the 1 : 1 borate complex in the same time interval. Complexation with boron obviously increases the hydrolytic stability of ascorbate.

Fig. 9 shows the UV spectra of calcium ascorbate and its 1 : 1 and 1 : 2 borate complexes. The differences noted in the initial absorbance values and at further points are probably due to differences in the molar absorption coefficients (ε) of the complexes. Following the changes in the blue and green curves, the degree of hydrolysis after 9 h, $\{(A_0 - A_t/A_0) \times 100\%\}$, was found to be *ca.* 50% for the 1 : 2 complex and *ca.* 100% for the 1 : 1 complex. This result confirms again that bis-chelate complexes are more stable than the mono-chelate complexes.

A comparison of hydrolytic behavior of the 1 : 1 Na– and Ca–ascorbatoborate complexes gave a fascinating result that emphasizes the effect of the inorganic ions on the stabilization of the borate complexes. Following the blue curve in Fig. 8 and the green curve in Fig. 9, one can see that in the same time interval the hydrolytic stability of the 1 : 1 Na complex is greater than that of the Ca complex. The observed relative stability of the Na complex with respect to the Ca complex, correlates well with the very recent report from da Silva *et al.* about the role of inorganic ions in the emergence of life.[13] They stated that borate–ribose (furanose) complexes particularly with alkali cations are stable towards pH and temperature effects even under adverse environmental conditions. It appears that Ca ions interact with the borate moieties in the complex structure and destabilize the structure by removing boron.

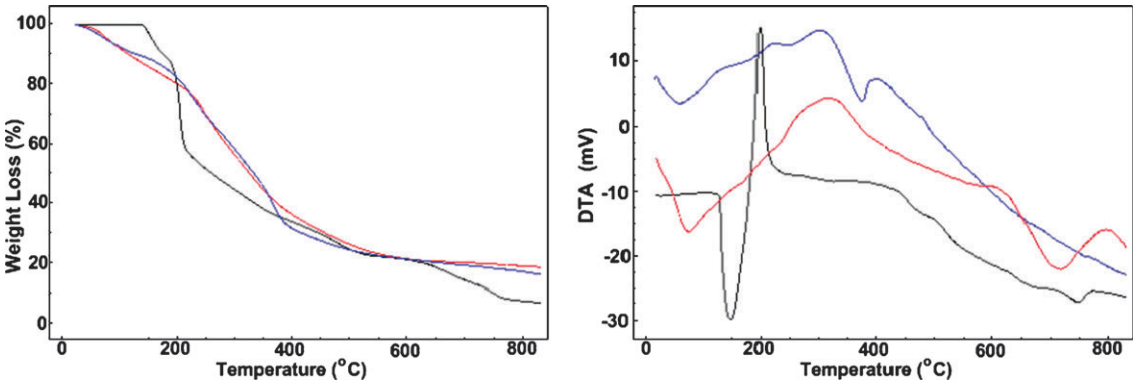


Fig. 6 (a) TG and (b) DTA curves of NaHA (black line), $Li[B(A)(OH)_2]\cdot 1/2H_2O$ (blue line) and $Na[B(A)(OH)_2]\cdot H_2O$ (red line).

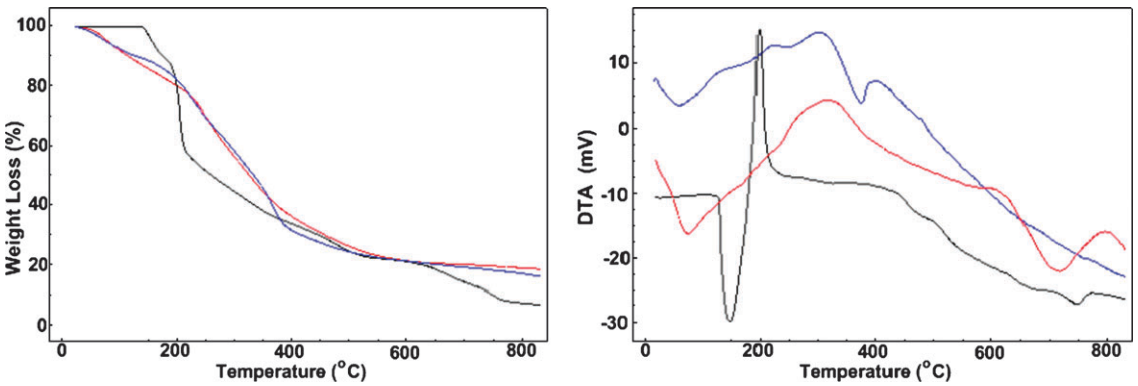


Fig. 7 (a) TG and (b) DTA curves of $Ca(HA)_2\cdot 2H_2O$ (black line), $Ca[B(A)(OH)_2]_2\cdot 3H_2O$ (blue line) and $Ca[B(A)_2]_2\cdot 10H_2O$ (red line).

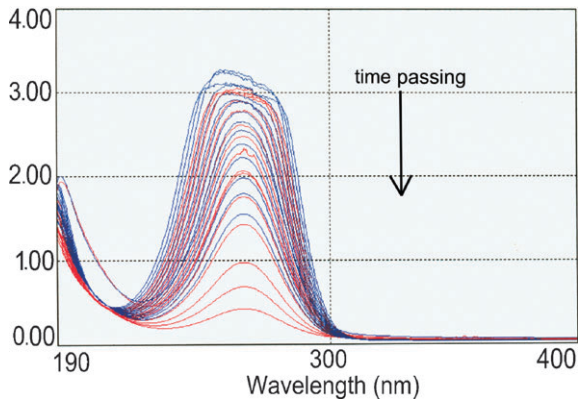


Fig. 8 UV spectral curves for NaHA (pH = 9.2) (red curves) and $Na[B(A)(OH)_2]\cdot H_2O$ (pH = 8.3) (blue curves) solutions.

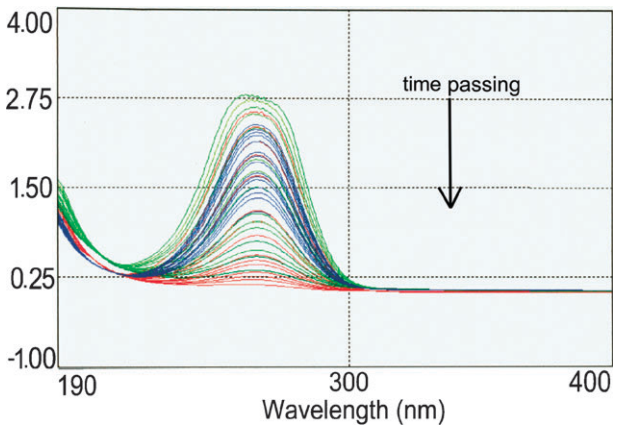


Fig. 9 UV spectral curves for $Ca(HA)_2\cdot 2H_2O$ (pH = 8.6) (red curves), $Ca[B(A)(OH)_2]_2\cdot 3H_2O$ (pH = 7.8) (green curves) and $Ca[B(A)_2]_2\cdot 10H_2O$ (pH = 7.6) (blue curves) solutions.

Summary and conclusions

The ascorbatoborate complexes were isolated in salt form with Li^+, Na^+ and Ca^{2+} ions by flash precipitation from aqueous solutions. Complexation reaction was simply based on the addition of the anionic ascorbate ligand to the Lewis acid boron center followed by water elimination between the hydroxy groups of the organic ligand and of boric acid through esterification. The complexes were prepared in the form of mono-chelate (1 : 1) and bis-chelate (1 : 2) borate esters, which are readily soluble in water but slowly undergo hydrolytic dissociation to boric acid and ascorbate in aqueous solutions as indicated by solution NMR studies. This slow hydrolysis behavior may allow them to be used in nutritional and/or pharmaceutical applications before being completely hydrolyzed and excreted from the body.

All the complexes were shown to contain negatively charged tetrahedral borate anions counterbalanced by metal cations. Li, Na and Ca ions were purposely selected to aid in the metabolic processes, where boron is claimed to be active, for the cumulative treatment of the metal ion, boron and the bio-molecule deficiencies or to be used in nutritional supplements.

Due to their hydrolytic instabilities, structural analyses of the complexes were performed with solid state NMR studies. The observed MAS NMR data verified that vitamin C forms complexes with boric acid through its *cis*-enediol groups by forming a 5-membered chelate ring. Side-chain OH groups do not participate in complexation. Systematic changes were noted in the chemical shift values, and empirical relations were derived that can be used as diagnostics for the 1 : 1 and 1 : 2 ascorbate–borate complexes.

The remarkable stability of 1 : 2 borate ester structures was confirmed. Bis-chelate vitamin C–borate complexes were found to have higher thermal and hydrolytic stabilities than their mono-chelate analogs. The hydrolytic dissociation of the 1 : 2 Ca complex over 9 h at pH = 7.6 was about 50%. This time interval may well be adequate to allow calcium bis-ascorbatoborate to function as a pharmacophore, for particular use in strengthening the immune system and the skeletal system.

The effect of the cation on the stabilization of the complexes was also investigated. The observed relative stability of the 1 : 1 Na–ascorbatoborate complex with respect to the corresponding Ca complex, correlates with recent reports[13] about the role of alkali metal ions in the stabilization of ribose in the prebiotic world.

The results of this study may help in better understanding of (i) the occurrence of boron complexes in biological systems, (ii) the sugar binding properties of boric acid and thus the role of boron as a carrier for nucleotides and carbohydrates, and (iii) the effect of the inorganic ions on the stabilization of borate complexes.

Experimental

Preparations

The reactions were performed in aqueous solutions prepared with deionized water that was deoxygenated prior to use. The reagents H_2A (Merck) and H_3BO_3 (Sigma, 99.5%) were used as received. Isopropylideneascorbic acid (i-H_2A) was prepared as described by Jabs *et al.*[35] Na–, Mg–, Li– and Ca–ascorbate solutions were prepared by reacting the H_2A solutions with either $NaHCO_3$ (Merck), $Mg(OH)_2$ (Merck), LiOH (Merck) or $Ca(OH)_2$ (Merck) in appropriate ratios to produce monoascorbate anions by removing only the first acidic proton, (C3)O–H.

Monoesters, $M[B(A)(OH)_2]$ (M = Li, Na, A = $C_6H_6O_6$) and $M[B(A)(OH)_2]_2$ (M = Mg, Ca), were prepared by adding solid H_3BO_3 into the solution containing the metal ascorbate salt in 1 : 1 molar ratio. Because borate formation is reversible, H_3BO_3 was added in solid form to avoid confusion between the interacting trigonal and tetrahedral boron species. The resulting mixture was stirred for 1 h. The solution was then concentrated in vacuum and cold acetone was added into the concentrated solution. The precipitates were vacuum filtered and kept in a desiccator over solid $CaCl_2$.

The diester, $Ca[B(A)_2]_2$, was prepared by adding solid H_2A into the solution containing the Ca–monoester, in equimolar amount. The subsequent steps were performed as described for the monoesters.

Instrumentation

C, H contents were determined by an CHNS-932 LECO model analytical instrument. Crystal water determination and thermal analyses (TGA, DTA) were performed using a Shimadzu DTG-60H system, under a dynamic nitrogen atmosphere (100 mL min^{-1}), at a heating rate of 10 °C min^{-1}, in platinum sample vessels with reference to α-Al_2O_3. Melting points were determined using an Electrothermal 9100 model instrument. FTIR spectra were measured in the 450–4000 cm^{-1} range with a Perkin-Elmer Spectrum One instrument, using the KBr pellet technique. UV spectra were recorded using a PG Instruments T80 + UV/Vis spectrophotometer. Solution ^{13}C-NMR spectra were recorded using a Bruker AV 400 spectrometer over 200–0 ppm and 8–0 ppm ranges, respectively, at 295 K in D_2O. Solid state ^{13}C-NMR spectra were recorded over 280–0 ppm using a Bruker Avance Ultrashield TM 300 MHz WB instrument, by using a 4 mm MAS prob at a 5 KHz spin rate and contact time of 2 ms. The measurement temperature was 293 K. Solid state ^{11}B-NMR spectra were recorded using a Bruker Avance Ultrashield TM 400 MHz WB instrument by using a 4 mm MAS prob in 50/−30 ppm with selective 90° and 180° pulses at a 12 KHz spin rate, and the recycle delay was 60 s. All experiments were conducted on powder samples prepared from boric acid containing boron isotopes at their natural abundance. The spectrometer frequency was 128.38 MHz for ^{11}B, and thus 1 ppm amounts to 128.38 Hz.

Calculations

Calculation of the complex structures were performed at the DFT 6–311G level. All parameters were optimized to obtain the minimum energy structure and bond lengths.

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