# $^{11}$B NMR Investigations of Borate Esters in Aqueous Solutions and Wine

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$^{11}$B NMR studies have been performed in dilute solutions of boric acid resp. borate and some typical hydroxy-carboxylic acids as a function of $p$H. Typical chemical shifts have been found for the esters occurring at the different ranges of $p$H. The origin of the two $^{11}$B NMR signals observable in wine could be determined.

## Introduction

The element boron plays a role in medical and biological fluids [1] which is not yet well investigated. Further it is questionable if boron is needed as a trace element in the human food. But due to its toxicity, boron containing drugs are largely forbidden in this country [2]. With the daily food, boron is incorporated in amounts which are estimated to 1 through 40 mg per day [3], where the consumption of wine plays an important role, since wine contains boron in amounts of some mg/liter [4]. This results from the fact that the vine involves boron in its metabolic processes [4].

Boron has two isotopes, $^{11}$B and $^{10}$B, which are accessible to nuclear magnetic resonance (NMR) investigations, and $^{11}$B NMR studies in aqueous solutions with millimolal concentrations are possible due to the favourable NMR properties of this isotope as has been shown recently in a detailed investigation of aqueous solutions of borates and boric acid [5].

The $^{11}$B NMR signals of red wine [6] (see also Fig. 3), confirmed the cited content of boron but also indicated the existence of at least two boron containing complex species as is not unlikely, since wine is a complex fluid with many ingredients [7], for instance different carboxylic acids.

Many $^{11}$B NMR investigations on organic compounds have been published as can be verified from some recent reviews [8–11] but rather few data are available for aqueous solutions (see [5] and references therein).

Borate esters of diols in water have been identified by $^{11}$B NMR at high $p$H's [12], and just recently the $p$H dependence of the stability of esters of boric acid and borate with glycol, and glycolic, oxalic, and glyceric acid has been investigated using $^{11}$B NMR by van Duin et al. [13]. These NMR investigations have been extended to borate esters of further polyhydroxycarboxylates and polyols in alkaline aqueous solutions [14] with results on their stability and structure. An additional study involving e.g. (D-)-fructose and (D-)-glucose at $p$H 6–12 has been published recently [15].

For a clarification of the origin of the $^{11}$B NMR lines in wine, investigations of the behaviour of $^{11}$B signals in aqueous solutions of boric acid and different ingredients of wine as lactic acid, tartaric acid, and malic acid as a function of $p$H in the acidic and alkaline range have been performed. Since wines show $p$H's between 2 and 4, further boric acid solutions containing butandiols, ethanol, and succinic acid have been studied.

## Experimental

For the $^{11}$B NMR investigations at 28.88 MHz a Fourier spectrometer has been used which consists of a Bruker SXP 4–100 pulse console, an externally $^{1}$H stabilized 2.11 T Bruker iron magnet system, and a B – NC 12 data unit. The measurements have been performed at a temperature of $(299 \pm 1)$ K in not rotating cylindrical glass tubes of 10 mm diameter. The $^{11}$B NMR signal of the glass decays with a short time constant, so the rather long free induction decays of the samples investigated are not strongly disturbed. The chemical shift is defined by

$$\delta = (v_{\text{sample}} - v_{\text{ref.}})/v_{\text{ref.}} ,$$

where $\nu_{ref.}$ is the Larmor frequency of a 0.1 molal solution of $B(OH)_3$ in $H_2O$. The linewidth of the reference is about 75 Hz and something affected by the magnetic field inhomogeneity.

## Results

In Fig. 1 the chemical shifts of the ^{11}B NMR signals in aqueous solutions which contain 10 mmol $B(OH)_3$ and 186 mmol of the different carboxylic acids are given as a function of pH. The pH was adjusted by adding NaOH, therefore the concentrations decrease something for increasing pH. At low pH two [11]B signals were observed for all three presented acids, lactic, tartaric, and malic acid, one shifted something to higher values than the reference signal of $B(OH)_3$, the other at about $-9.9$ ppm. For increasing pH two or three signals are observable.

The curves in Fig. 1 show the position of the ^{11}B NMR line which results from the weighted average of $B(OH)_3$ at 0 ppm and $B(OH)_4^-$ at $-17.6$ ppm as a function of pH, using a $pK_a = 9.2$ for the equilibrium $B(OH)_3 + H_2O \rightleftharpoons B(OH)_4^- + H^+$. This behaviour has been investigated in detail in [5] for 10 mmol aqueous boric acid solutions as a function of pH. The position of the measured signals marked with a full circle are well situated on these curves for pH > 8. Further the linewidths follow the behaviour found in [5]: at high pH the signals are narrow as expected for the highly symmetric $B(OH)_4^-$ species (the observed width is due to the inhomogeneity of the magnetic field), and for medium pH an exchange broadening is observed. At pH < 8 the signal positions deviate from the exchange equilibrium curve, that means from the chemical shift of $B(OH)_3$. This fact has also been found by van Duin et al. [13] for the esterification of boric acid with glycolic and glyceric acid. These signals between 0 ppm and $+0.6$ ppm can be assigned to an equilibrium between $B(OH)_3$ and the boric acid esters for which a schematical structure is given in Fig. 2, which is shortened according to the papers of van Duin et al. [13–15] by B°L, where B° denotes boric acid and L the polyhydroxy compound. The broad lines given in Fig. 1 e.g. for lactic acid support such an exchange.

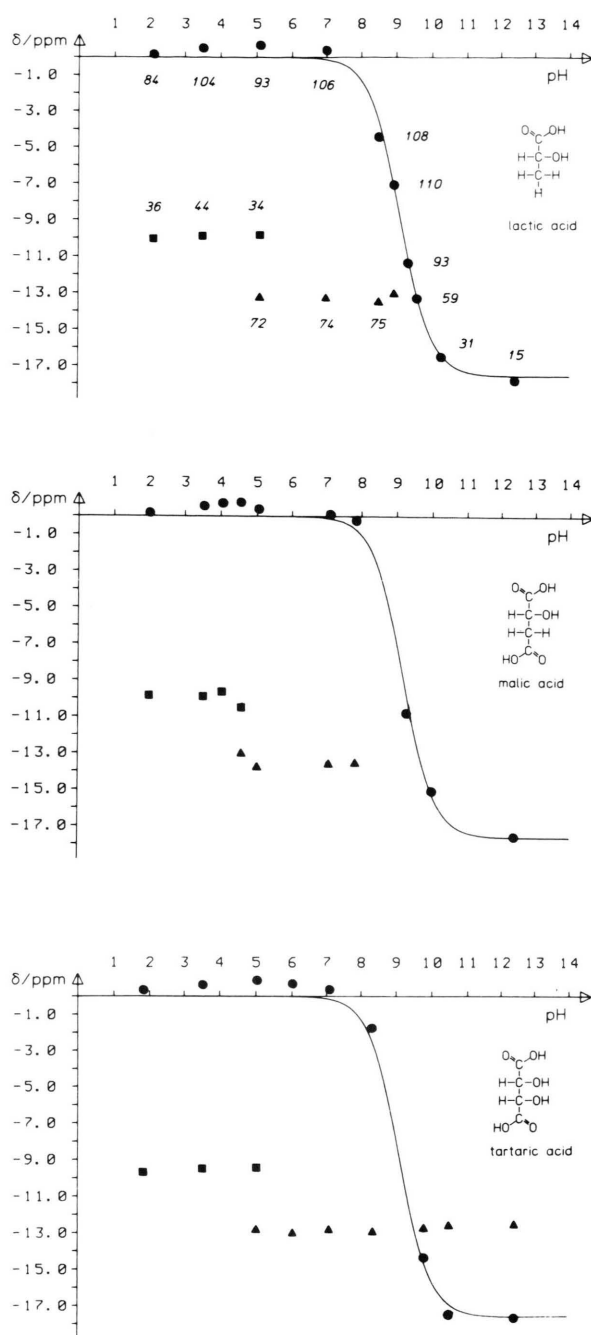The second signals in the spectra for pH $\leq$ 5 which appear for lactic acid between $-9.9$ and



Fig. 1. Chemical shift of ^{11}B NMR signals in aqueous solutions of $B(OH)_3$ and of the carboxylic acids L(+)-lactic acid, L(−)-malic acid, and D(−)-tartaric acid as function of pH. The most acidic solutions contain 10 mmol $B(OH)_3$ and 186 mmol carboxylic acid. The pH has been adjusted by adding NaOH, so the concentration of the samples decreases something as function of increasing pH. The size of the symbols gives about the experimental error. The italic numbers in the case of lactic acid stand for the linewidths in Hz.

$-10.0$ ppm, for tartaric acid between $-9.4$ and $-9.7$ ppm, and for malic acid near $-9.9$ ppm can be assigned to a diester $B^-L_2$, where $B^-$ stands for the borate (see Figure 2). This results from the fact that the $pK_a$'s of the acids are in the range from 3.0 to 3.8 and so the deprotonation $L \rightleftharpoons L^- + H^+$ takes place at low $p$H's. A maximum of the concentration $[B^-L_2]$ of the species $B^-L_2$ is reached when $p$H = $pK_a$ (carboxylic acid) since then $[L] = [L^-]$ and $B^-L_2$ is formed according to $B^\circ + L + L^- \rightleftharpoons B^-L_2 + 3 H_2O$. This picture is corroborated by a nearer inspection of the $pK_a$'s at 25 °C (lactic acid: 3.79, tartaric acid: 2.98 and 4.34, and malic acid: $pK_{a1}$ 3.40) and the intensities of the signals for $B^\circ L$ and $B^-L_2$ as a function of $p$H for the different acids. For instance for lactic acid at $p$H = 2.1 the intensity of the signal at $+0.2$ ppm is about 10 times that of the signal at $-9.9$ ppm, whereas for $p$H = 3.5 the intensities have reversed and are now about 3 to 4. The high symmetry of the $B^-L_2$ species is reflected in the rather narrow lines found (see Figure 1). It should be mentioned that as a function of increasing $p$H the $B^-L_2$ − signal vanishes first for malic acid, since its $pK_{a1}$ is smaller than that of lactic acid and also than the mean of $pK_{a1}$ and $pK_{a2}$ for tartaric acid. A more quantitative analysis is difficult to perform due to the linewidths and the low boron concentrations used in this study.
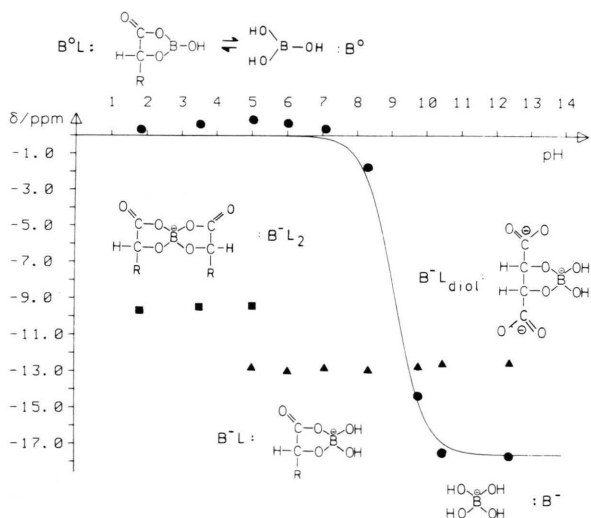


Fig. 2. Structures (schematical) of the esters of boric acid and borate with carboxylic acids present in the different ranges of $p$H and chemical shift, in case tartaric acid, as an example.

From about $p$H = 5 a further signal is observable at $-13.3$ ppm in lactic, at $-12.8$ ppm in tartaric, and at $-13.8$ ppm in malic acid solutions. The intensity of these signals increase at the expense of the signals of the $B^-L_2$ species since [L] becomes smaller for $p$H $\gtrsim$ 5. The signals must be ascribed to the species $B^-L$ (see Figure 2). If the $p$H exceeds the $pK_a$ = 9.2 of boric acid, in lactic and malic acid solutions only the borate signal is observed, whereas for the tartaric acid solutions also in the very alkalic range signals near $-12.6$ ppm are observable. It is well known [12−15] that at high $p$H polyhydroxycarboxylic acids are esterified by the diol function, the stability of the diol type esters $B^-L_{diol}$ being higher than that of the $\alpha$ − hydroxycarboxylic type $B^-L$ (see e.g. [13]).

Due to our low field and the small boron concentrations the spectral resolution was not high enough to discriminate between the two $B^-L$ possibilities for tartaric acid in the very alkalic range.

The results given in Fig. 1 are for the D(−)-tartaric acid, no significant differences have been found for L(+)-tartaric acid.

In Fig. 2 the structures of all the esters are given schematically for the different ranges of $p$H and chemical shifts.

The relative abundance of the esters as a function of $p$H, that means the signal intensities strongly depend on the ratio of the concentrations of boron acid and the polyhydroxy compound. According to the investigations in wine we had to look primarily for low $p$H and small boron concentrations as well as for further compounds occurring in wine: At $p$H = 5.6, solutions containing 10 mmol $B(OH)_3$ and 186 mmol (1.3)-butandiol, 189 mmol (2.3)-butandiol, 1143 mmol ethanol, and 2412 mmol ethanol have been investigated. Only one $^{11}$B signal was observed at 0 ppm. The linewidths in the butandiol − solutions are those found for boric acid, in the ethanol solutions they are 20% larger.

For solutions containing 186 mmol succinic acid at $p$H = 2.46 also only a signal at 0 ppm was observable. So for these solutions no esterification is observed in the acidic range as expected.

In Fig. 3 $^{11}$B spectra from red wines are presented. The signals at 28.87 MHz taken with the SXP spectrometer show a small signal-to-noise ratio according to the low Larmor frequency and the small boron concentration. The two signals are found near 0 ppm and $-10$ ppm. This could be confirmed by a mea-
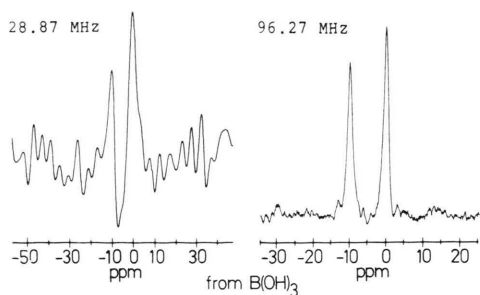
Fig. 3. ¹¹B NMR signals of samples of about 1 ml of red wine (i) at 28.87 MHz (2.11 T) obtained with the SXP spectrometer using 388 300 pulses within 5.5 h, without line broadening, (ii) at 96.27 MHz (7.05 T) measured with a Bruker CXP 300 spectrometer by H. Förster, Bruker, Rheinstetten, using 43 144 pulses within 1 h 12 min, line broadening 50 Hz.

surement at 96.27 MHz with a much better signal-to-noise ratio by H. Förster, Bruker Rheinstetten, using a CXP 300 spectrometer. It is obvious from the investigations above that the signal near 0 ppm can be ascribed to the exchange of $B(OH)_3$ with the boric acid esters $B°L$ of the different hydroxycarboxylic acids L abundant in wine. The signal near $-9.9$ ppm arises from the species $B^-L_2$ of the various hydroxycarboxylic acids (the $pH$ of wines is around 3).

A boron concentration of about 2 to 3 mg/liter was estimated from the signal-to-noise ratios in wine and diluted aqueous boric acid solutions using the SXP-spectrometer. The ratios of the signal intensities in the ¹¹B NMR spectra are different for wines of different origin. Further, ¹¹B signals have also been detected in grapes and juice of grapes. A more detailed ¹¹B NMR investigation of wines is in progress.

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