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Interphase transmetallation of copper(II) by spiroboronates

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Abstract

Copper(II) species, including highly insoluble copper(II) hydroxide, undergo transfer into a non-polar organic phase containing two molar equivalents of a sodium spiroboronate. Biological testing has established that the products of phase transfer are excellent wood preservatives. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Boron; Spiroboronate; Copper(II); Second-sphere coordination; Phase transfer; Wood

1. Introduction

Boron is an element with a number of important properties. In particular, it is a trace mineral essential to the growth of plants and other biota, and yet it is quite toxic to some species at low levels. Boron has significant activity in the control of certain target species such as fungi and insects that degrade susceptible substrates such as timber. An important advantage in using boron to prevent degradation is that it has quite a low toxicity to mammals, including humans. Therefore, biocides containing boron are more desirable compared with those based on arsenic, chromium or tin which are used commonly in wood preservation.

A serious practical deficiency with commonly available boron compounds, including those typically used in wood preservation, is that they are leached readily from substrates when exposed to moisture. Generally, the boron compounds used are either simple inorganic compounds such as boric acid or boric acid salts, or boron esters of simple organic alcohols. All of these compounds provide some degree of protection. The boron esters are soluble in organic solvents, which is beneficial for some applications. However, they are generally unstable in the presence of water, hydrolysing readily to liberate boric acid, which then may be leached from the substrate.

Boric acid, and especially the borate anion $[B(OH)_4]^-$, combines with polyhydroxy organic compounds to produce chelate esters. Coordination compounds derived from polyhydric alcohols and phenols have been well documented. For example, syntheses of simple boronato complexes of *ortho*-hydroxymethylphenols (Fig. 1) have been recorded in the literature for many years and have even been used in wood preservation [1].

While anionic boron coordination compounds such as these in which the counter-cation is univalent (either hydrogen, an alkali metal, or ammonium) are well documented, anionic boron–diol complexes having a counter-cation bearing a formal charge greater than one are less well known. Some, however, have been reported; for example, a copper complex of pentaerythritol borate [2].

Some years ago Maynard [3] used 2-chloro-6-hydroxymethyl-4-nonylphenol (**1**) to prepare the spiroboronate **2**. Treatment of a solution of **2** in petroleum spirits with an aqueous solution of copper(II) sulfate resulted in a colour change in the organic phase from pale yellow to red–brown. It was inferred that this colour change was

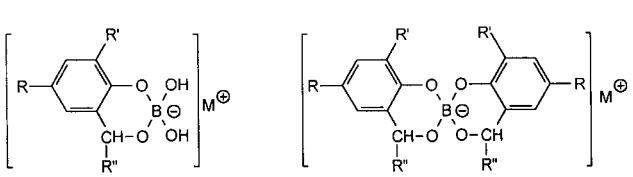

Fig. 1. Boron complexes of ortho-hydroxymethylphenols.

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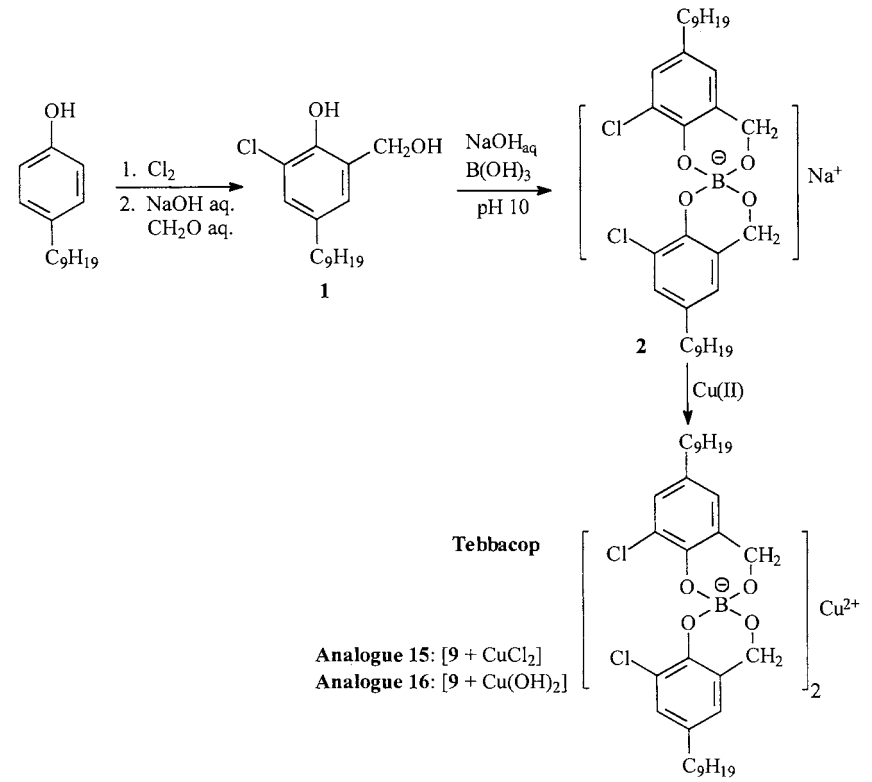


Scheme 1.

the result of interphase transmetallation of Cu(II); the resulting hydrocarbon-soluble material was called Tebbacop and was assumed to have the structure shown in Scheme 1.

Subsequently, it was found that this material was an exceptional wood preservative. In fact, the biological activity of Tebbacop was better than that of the spiroboronate alone and was superior to that of wood preservatives in common use. Commercially available 4-nonylphenol having a mixture of isomeric C_9 chains was originally chosen [3] as the starting material for the Tebbacop project because it is the least expensive of all alkylphenols. Another attractive feature associated with the use of this phenol in industrial processes is that it is a liquid at room temperature, which facilitates syntheses stemming from it.

The main objective of the present work was to prepare pure analogues of Tebbacop in order that a better understanding of the structure of the latter material might be obtained. Furthermore, it was anticipated that study of pure analogues would allow determination not only of the optimum combination of halo and alkyl substituents on the phenol ring, but also of the synthetic sequence which leads to the product most active biologically.

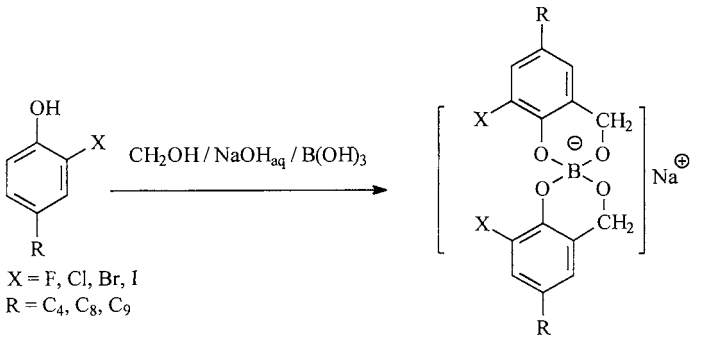
Herein we report on the synthesis of a number of spiroboronate analogues from various pure halogenated alkylphenols using a templated (one-pot) sequence, and the subsequent interphase transmetallation of one pure spiroboronate by copper(II).

2. Results and discussion

2.1. Preparation of the spiroboronates

The spiroboronates **3**–**14** were prepared via a template reaction involving *ortho*-hydroxymethylation of the appropriate pure 2-halo-4-alkylphenol by stirring with aqueous formaldehyde at 50°C, followed by addition of aqueous boric acid and either sodium hydroxide or potassium hydroxide (Scheme 2). (For brevity of presentation, (1,1-dimethylethyl) is depicted as *t*-butyl, and (1,1,3,3-tetramethylbutyl) as *t*-octyl.) After 24 h the mixture was cooled slowly to room temperature, and the soft crystalline mass was filtered off, washed with water and hexanes and then dried.

The nature of the groups (halogen and alkyl) on the aromatic ring affects both the yield and the purity of the derived spiroboronates (Table 1), chlorinated analogues typically affording the cleanest products in good yield. In contrast, all attempts to prepare pure iodinated analogues failed, only mixtures of products being recovered. However, in each case where a mixture was formed, the presence of the desired spiroboronate was confirmed by both ^{1}H-NMR spectroscopy and FAB$^+$ mass spectrometry.



3: R = n-Bu, X = Br
4: R = t-Bu, X = CH_3
5: R = t-Bu, X = F
6: R = t-Bu, X = Cl
7: R = t-Bu, X = Br
8: R = t-Bu, X = I
9: R = t-octyl, X = Cl
10: R = t-octyl, X = Br
11: R = t-octyl, X = I
12: R = t-octyl, X= H
13: R = n-nonyl, X = Cl
14: R = n-nonyl, X = Br

Scheme 2.

Not unexpectedly, boronato species containing short-chain alkyl groups are insoluble in non-polar solvents (e.g. hexane, toluene). This is of particular relevance because solubility in these solvents is necessary for the subsequent interphase transmetallation reaction with copper(II) salts (see later).

The effect of pH on yield was established for the template reaction by preparing spiroboronates **9**, **10**, **11** under two sets of conditions (Table 2). In the first series of reactions the aqueous phase was pH 14, and in the second set of reactions the aqueous phase was adjusted to pH 10 by the addition of hydrochloric acid. This study established that the product yield was greatest when the aqueous phase was pH 14, rather than pH 10. That is, the presence of sodium borate (which requires the aqueous phase to be at pH 10) was not necessary for the formation of spiroboronates when a reaction via the template method was used. This is in contrast to the case when a hydroxymethylphenol was treated with a solution of boric acid in aqueous base alone, as this reaction requires the aqueous phase to be pH 10.

An investigation into the effect of product yield according to the base used was also undertaken. Treatment of either 2-bromo-4-(1,1-dimethylethyl)phenol or 2-bromo-4-(1,1,3,3-tetramethylbutyl)phenol with aqueous formaldehyde, boric acid, and aqueous potassium hydroxide rather than sodium hydroxide resulted in an improvement in the yield of the boron ester (Table 3). This improvement might reflect the different solubilities of spiroboronate products, since potassium salts are less soluble than sodium salts. In contrast, the yield of the chlorinated derivative did not improve when aqueous potassium hydroxide was used in place of sodium hydroxide.

Table 1
Spiroboronate yield and purity

Spiroboronate	R	X	Yield (%)
3	*n*-Butyl	Br	38
4	(1,1-Dimethylethyl)	CH_3	39
5	(1,1-Dimethylethyl)	F	30
6	(1,1-Dimethylethyl)	Cl	40
7	(1,1-Dimethylethyl)	Br	35
8	(1,1-Dimethylethyl)	I	Mixture
9	(1,1,1,3-Tetramethylbutyl)	Cl	72
10	(1,1,1,3-Tetramethylbutyl)	Br	76
11	(1,1,1,3-Tetramethylbutyl)	I	Mixture
12	(1,1,1,3-Tetramethylbutyl)	H	27
13	*n*-Nonyl	Cl	98
14	*n*-Nonyl	Br	79

2.2. Structure of the spiroboronates

Both the ^{1}H- and ^{13}C-NMR spectra of the spiroboronates exhibit characteristically different chemical shifts in the methylene resonances upon coordination of boron. Typically an AB quartet is observed in the coordination complexes due to the diastereotopic relationship between the methylene protons. In contrast, the methylene protons in the precursor 2-hydroxymethylphenol are observed as a singlet and at lower field. It is interesting to note that as the nature of the *ortho* halogen, and, to a lesser degree the *para* alkyl group, changes, the chemical shifts of the axial and equatorial methylene protons of the various spiroboronates also differ (Table 4).

Calculations (PC Spartan Plus, AM1) showed that not all of the methylene protons were equidistant from a halogen substituent, one being much closer compared with the methylene protons on the other arm of the spiro centre. Comparison of computed space-filling models of the fluoro, chloro, bromo and iodo ana-

Table 2
Effect of pH on yield of spiroboronate (R = (1,1,3,3-tetramethylbutyl))

Spiroboronate (X)	pH 14	pH 10
9 (Cl)	72	23
10 (Br)	76	47
11 (I)	Mixture	Mixture

Table 3
Effect of countercation on yield of spiroboronates **7**, **9**, and **10**

Spiroboronate (R)	X	Na^+ (%)	K^+ (%)
(1,1,-Dimethylethyl) (**7**)	Br	35	65
(1,1,3,3-Tetramethylbutyl) (**9**)	Br	76	90
(1,1,3,3-Tetramethylbutyl) (**10**)	Cl	72	63

logues showed that the methylene protons became closer to the halogen substituent as the van der Waals radius of the halogen increased. It is not surprising, therefore, that a variation in the chemical shift of the methylene protons is observed in the spiroboronate compounds as the halogen substituent becomes larger. Somewhat unexpected, however, is the effect of the alkyl substituent. Namely, subtle differences in the structure of the alkyl chain in the *para* position can lead to separation of the signals due to the methylene protons into an AB quartet.

^{11}B-NMR spectroscopy has been used to identify borate esters of organic hydroxy compounds since the early 1970s. The formation of anionic complexes of both 1:1 and 1:2 stoichiometry by the interaction of diols with borate anions has been demonstrated by the detection of discrete ^{11}B resonances for the three anions in the equilibrating system (Fig. 2) [4]. The exchange rate between $B(OH)_4^-$, its diester and tetraester is slow near room temperature on the ^{11}B-NMR time scale [5]. This generally enables the determination of the chemical shifts, line widths and the intensities of the signals due to discrete borate esters of polyols. 2-Hydroxymethylphenol has been shown [6] to form strong complexes, and the ^{11}B-NMR spectrum of 2-hydroxymethylphenol in borax solution at pH 12 has been reported as showing a single resonance at δ 2.2 (Fig. 2). This signal is observed even at lower pH values and at very low diol/borate ratios, indicating that 2-hydroxymethylphenol is a good chelating dihydroxy compound.

In the present, it was necessary work to establish whether a complex of 1:1 or 2:1 stoichiometry is formed. The ^{11}B-NMR spectrum of spiroboronate **9** prepared by a template reaction as above exhibited a single peak at δ 2.2, in excellent agreement with the value obtained for boron tetraesters derived from 2-hydroxymethylphenol. Furthermore, samples of spiroboronate **9** prepared either from treatment of 2-chloro-4-(1,1,3,3-tetramethylbutyl)phenol with aqueous formaldehyde (i) at pH 10; (ii) using one molar equivalent of boric acid; or (iii) using five molar equivalents of boric acid: or from 2-chloro-6-hydroxymethyl-4-(1,1,3,3-tetramethylbutyl)phenol (i.e. not via a template reaction) exhibited this same chemical shift in the ^{11}B-NMR spectrum.

The IR data did not clearly establish the nature of the boron complex formed since compounds **3**–**14** also exhibit bands characteristic of free and/or hydrogen-bonded O–H stretching vibrations. In spite of the fact that the presence of OH bands would be expected if a boron:2-hydroxymethylphenol complex of 1:1 stoichiometry had formed (see Fig. 1), evidence in support of the exclusive formation of 1:2 complexes comes from the mass spectra. The FAB^+ and FAB^- spectra of the spiroboronates were acquired by ionisation of the analyte in a matrix of *meta*-nitrobenzyl alcohol. These

Table 4
Selected NMR data for spiroboronates **3**–**10** and **12**–**14**

Spiroboronate	R	X	CH_2OB		
			^{1}H (ppm)	^1J (Hz)	^{13}C (ppm)
3	*n*-Butyl	Br	4.48, 4.59	13.8	61.2
4	(1,1-Dimethylethyl)	CH_3	4.49	–	61.7
5	(1,1-Dimethylethyl)	F	4.57	–	61.4
6	(1,1-Dimethylethyl)	Cl	4.52, 4.58	13.9	61.4
7	(1,1-Dimethylethyl)	Br	4.49, 4.61	13.8	61.6
8 [a]	(1,1-Dimethylethyl)	I	4.43, 4.65	13.8	–
9	(1,1,3,3-Tetramethylbutyl)	Cl	4.55	–	61.3
10	(1,1,3,3-Tetramethylbutyl)	Br	4.51, 4.58	13.7	61.4
12	(1,1,3,3-Tetramethylbutyl)	H	4.48, 4.52	13.6	61.5
13	*n*-Nonyl	Cl	4.00, 4.55	14.0	61.1
14	*n*-Nonyl	Br	4.48, 4.57	13.9	61.2

[a] A mixture of spiroboronate products was formed in this reaction. Nevertheless, it was possible to extract the chemical shifts of the methylene protons from the ^{1}H-NMR spectrum.

experiments established that it is possible to detect the *ortho*-borate ester anion at low levels using FAB mass spectroscopy. In all cases, however, no molecular ions for borate esters with 1:1 stoichiometry were detected, whereas molecular ions indicative of 1:2 complexes were observed. Therefore, the presence of OH bands in the IR spectra must be attributable to water molecules associated with these spiroboronates. This is not unexpected given the polarity of these species; in fact, waters of crystallisation have been proposed previously for a boron–diol complex [7]. Further evidence in support of the presence of waters of crystallisation in a complex of 1:2 stoichiometry (as opposed to the formation of a 1:1 complex) comes from elemental microanalysis of the dried spiroboronate **9**, the calculated value requiring the inclusion of one molecule of water to be consistent with the observed data.

Interestingly, while a 1:1 spiroboronate complex is clearly not formed according to mass spectrometry, ions of higher molecular mass were observed in both the FAB^+ and the FAB^- spectra. Although these ions were consistent with macrocyclic species of various ring size (e.g. Fig. 3; FAB^+), clusters at high multiple mass are a common feature of this ionisation technique.

In an attempt to establish the structure of a spiroboronate unequivocally, a crystal of **9** was eventually grown from dimethylformamide solution. Although there were problems with refinement of the X-ray diffraction data due to twinned crystals, and some disorientation due to various conformational possibilities in the (1,1,3,3-tetramethylbutyl) side chains (final crystallographic R indices $[I > 2\sigma(I)]$ $R_1 = 0.2500$; full data not included), the gross structure was nevertheless revealed (Fig. 4). Spiroboronate **9** is indeed the 1:2 complex, as a DMF solvate. The spiroboronate anions pack into pairs with their hydrophobic ends adjacent and the rows of paired spiroboronates create hydrophilic channels containing DMF molecules and sodium cations.

2.3. Interphase transmetallation of the spiroboronates

With the structure of the spiroboronate anions secure, their ability to complex copper ions could be investigated. A series of experiments involving interphase transmetallation of boron salts with copper(II) reagents was undertaken in order to determine not only the optimum conditions for the transfer but also the structure of the ensuing product. Given that **9** is a -1 anion, it was assumed that the optimum transfer of copper(II) to an organic phase should require two moles of the borate anion for every mole of copper(II) chloride. This was confirmed by calculating the %w/w of copper(II) samples prepared using different ratios of borate anion:copper(II) chloride (Table 5).

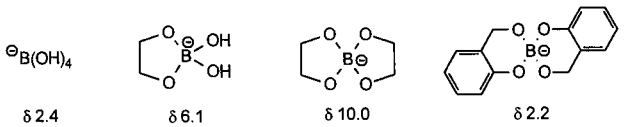


Fig. 2. ^{1}H-NMR data for boron complexes.

As expected, the maximum uptake of copper occurred when the molar ratio of borate anion **9**:copper(II) chloride was 2:1. The maximum value expected if no other ligands are coordinated to copper is 5.5 %w/w copper. The presence of additional ligands (as in, for example, an aqua complex) would increase the molecular mass of the solvated species and therefore a smaller %w/w copper value would result (the theoretical %w/w uptake of copper decreases by 0.1% for the inclusion of each additional mole of water solvate).

During these experiments it became apparent that the rate of addition of the aqueous copper(II) solution to a solution of the spiroboronate in toluene, together with the rate of stirring, both had an effect on the composition of the final product in the hydrocarbon phase. Slow addition of a dilute solution of copper(II) chloride to a solution of the spiroboronate with rapid turbulent stirring ensured optimum interphase transmetallation.

A series of reactions was worked up at various time intervals ranging from 5 min to several days in order to establish whether an immediate colour change translates to rapid and complete interphase transmetallation of the spiroboronate by copper(II) chloride. The results (Table 6) indicated not only that transfer of copper(II) from this salt is fast, but also that the resulting copper–boron complex is stable in the hydrocarbon medium for long periods.

In the experiments described so far spiroboronate **9** (sodium salt) was dissolved in toluene. Although this solvent dissolves **9** satisfactorily, many of the other spiroboronates prepared during this study were not soluble appreciably in either toluene or other non-polar solvents such as hexane or dichloromethane. To estab-

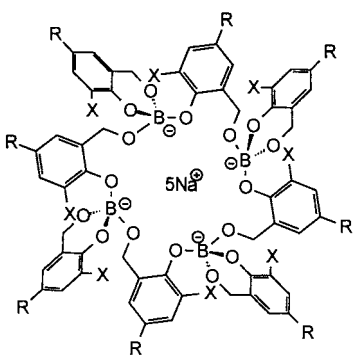


Fig. 3. Cationic cluster detected in FAB^+ mass spectrum.

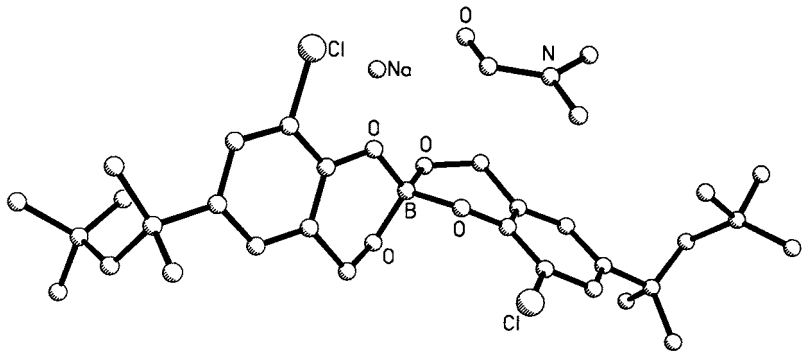


Fig. 4. The atomic arrangement in **9**.

lish whether a non-polar solvent is in fact necessary in this interphase transmetallation process, experiments were undertaken in which the polar solvent nitromethane either replaced the toluene used previously, or was added to the organic phase. The results of these experiments (Table 7) show that the addition of a polar solvent to the organic phase is in fact detrimental to the interphase transmetallation.

The decrease in the phase transfer parallels the volume of nitromethane added to the toluene. Unfortunately, the addition of trace amounts of nitromethane to toluene did not enhance dissolution of those spiroboronate analogues carrying a relatively short alkyl chain (i.e. those having either a butyl or (1,1-dimethylethyl) substituent in the *para* position), and therefore the potential of analogues **3**–**8** to undergo phase transmetallation remains untested. The capacity of spiroboronates **9** and **10**, recrystallised from nitromethane, to participate in interphase transmetallation was also tested. This work established that not only is it detrimental to have a polar solvent as such present in the organic phase, but also that a polar solvent of recrystallisation (i.e. a polar solvate) is similarly detrimental.

In contrast to copper(II) chloride, copper(II) hydroxide is practically insoluble in water. It is not surprising, therefore, that a slower reaction rate and a lower transfer of copper was observed when this salt is used. It was necessary to establish whether the pH of the aqueous copper(II) salt employed affected the biological activity of the resulting material. Therefore, Tebbacop analogues **15** and **16**, prepared by interphase transmetallation of spiroboronate **9** with either acidic copper(II) chloride or basic copper(II) hydroxide respectively, were tested using a rapid screening filter paper technique.

Table 5
Varying stoichiometry of either $CuCl_2{\cdot}2H_2O_{aq}$ or spiroboronate **9**

Molar ratio; **9**:$CuCl_2{\cdot}2H_2O$	Cu (%w/w)
4:1	2.1
2:1	5.1
1:1	1.4
1:2	1.4

Table 6
Effect of varying reaction time on interphase transmetallation of **9**

Reaction time	Cu (%w/w)
5 min	4.9
1 h	5.1
5 days	4.8

2.4. Biological testing

Coniophora puteana, a brown rot fungus, was selected as the sole screening organism because it gives consistent results and is a common test organism, being tolerant of many common fungicides. The results, expressed as the minimum inhibitory concentrations (MICs), indicate that while a sample of **15** showed good resistance to the test fungus (MIC, 0.01% Cu), sample **16** prepared from copper(II) hydroxide gave the best performance (MIC, 0.001% Cu). The exceptional performance of sample **16** can be explained in terms of the known fungicidal activity of copper(II) hydroxide [8], commercial applications of which are severely limited by its poor solubility in aqueous media. Utilising the interphase transmetallation methodology, however, relatively insoluble copper(II) hydroxide is apparently

Table 7
Effect of a polar organic solvent on interphase transmetallation of **9**

Solvent	Cu (%w/w)
CH_3NO_2	1.5
CH_3NO_2/toluene (1:1)	3.8
Toluene	5.1

'dissolved' in a non-polar hydrocarbon solvent by the spiroboronate complexant **9**. This has a great deal of potential in terms of applications for the resulting formulation, especially as a replacement for powdered copper(II) hydroxide. The fact that the formulation is soluble in organic solvents not only meets an important criterion for wood preservatives but also makes this material highly suitable for addition to oil-based paint products.

2.5. Second-sphere coordination

The main objective of this research was a thorough analysis of the interphase transmetallation reaction so that a more accurate notion of the structure of Tebbacop might be elucidated. It was hoped that a pure analogue might be crystallised and then studied by X-ray crystallography; despite intensive effort, however, a suitable crystal of any of the analogues could not be grown. Nonetheless, in the absence of conclusive proof from an X-ray crystal structure, reports of similar interphase transmetallation complexes and the spectroscopic data recorded herein for Tebbacop analogues have been helpful in formalising the structure of this complex.

It has been appreciated for many years [9] that the coordinating influence of many transition metal complexes extends beyond their covalently bonded first-sphere ligands to non-covalently bonded chemical species in the so-called second sphere [10]. Since the original proposal of second-sphere coordination by Werner in 1912, improvements in spectroscopic and crystallographic techniques have revealed that a very wide range of phenomena can be accounted for on the basis of second-sphere coordination.

It is proposed that a gamut of non-covalent interactions including hydrogen bonds, charge-transfer, van der Waals' and dipolar interactions are responsible for the complexation of copper(II) by the spiroboronate anions discussed in the present work. Electronic and vibrational spectroscopic techniques have been reported [11,12] to be useful and straightforward methods to detect second-sphere coordination and the results of analysis of the Tebbacop analogues using UV and IR are consistent with this phenomenon.

In the IR spectrum of spiroboronate **9** the OH absorption is present at 3393 cm^{-1}, whereas in the interphase transmetallation products **15** (from **9** + $CuCl_2$) and **16** (from **9** + $Cu(OH)_2$) only a single broad OH band was observed and at lower wavenumbers (3371 and 3360 cm^{-1}, respectively). In the 'fingerprint' region subtle differences are observed in the C=C aromatic stretching and the C–O stretching frequencies. These bands shift to lower wavenumber in **15** and **16**, consistent with donation of electrons from the ligand to the complexed ion. Interaction of the copper with the chelate oxygens (as indicated by C–O bands) appears to be weaker in the case of the spiroboronate complex **16** derived from $Cu(OH)_2$. These results suggest that weak interactions, such as hydrogen bonding interactions, are present between solvate water and the complex. Such interactions might involve either the chelate oxygens (evident from changes in C–O stretching frequency) or the chelate ring itself (evident in changes in C=C stretching frequency).

The UV spectrum obtained for the interphase transmetallation product **15** is consistent with the complexed copper(II) being either tetrahedral or at the limits of tetragonal distortion, since these geometries account for the observed red–brown colour of these complexes. It is fortuitous that a red–brown colour is highly desirable for wood preservation since the treated wood then retains a more natural appearance. The presence of an absorption at ca. 346 nm is characteristic of the spiroboronates **3**–**14**. The disappearance of this maximum as it shifts under the charge transfer band at 280 nm, together with the appearance of a d–d transition at 414 nm, is characteristic of the copper(II) complexes **15** and **16** formed from spiroboronate **9**. Smithson and Williams [11] have suggested that it is possible to distinguish between complex formation and second-sphere interactions by studying d–d transitions in the UV spectra. Separation of the d states is a function of the polarisation of the cation by the ligand, and therefore depends upon the distance between the ligand and the metal. In contrast to complexes of transition metals formed in the first sphere, weak interactions between transition metals and ligands in the second-sphere would be expected to have little effect on this d–d splitting because of the large separation between the metal and the ligand (i.e. the spiroboronate). The presence of a d–d transition at ca. 414 nm for Tebbacop analogues **15** and **16** is therefore in agreement with an indirect mode of complexation, namely, in the second-sphere. In contrast, when dilute aqueous ammonia is added to the aqueous phase a shift in the d–d transition is observed (406 nm), consistent with the exchange of some aqua ligands in the first sphere with ammine ligands.

Attempts at complexing other transition metal cations (cobalt(II) chloride or nickel(II) chloride) with a spiroboronate to enable comparative studies were unsuccessful; apparently these complexants are selective for copper(II).

Broadening of the ^{1}H- and ^{13}C-NMR signals of the interphase transmetallation complex implies that copper is in the +2 oxidation state. In the ^{11}B-NMR spectra the same chemical shift (2.2 ppm) was observed for both the spiroboronate **9** and its copper complex **15**, again supporting the proposal that covalent bonding of copper(II) does not occur in **15**. In contrast to the mass spectra of the precursor spiroboronates, no ion defining the molecular structure was detected in either the

FAB^+, FAB^- or electrospray mass spectra of the interphase transmetallation products. It can be argued that this lack of a molecular ion also suggests that principally a non-covalent interaction is present in these products.

Atomic absorption spectroscopy (AAS) has proved to be a useful tool in the study of Tebbacop analogues. These studies have shown that the optimum transfer of copper(II) occurs when approximately one molar equivalent of copper(II) is treated with two molar equivalents of the complexant (i.e. approximately stoichiometric transfer). The presence of waters of hydration around both copper and in the bulk of the sample can account for a lower than stoichiometric value. Furthermore, this value might vary between samples because of different degrees of hydration of the bulk material. The effect of polar solvents (e.g. nitromethane) present in the organic hydrocarbon layer during interphase transfer was also studied by AAS and was shown to be detrimental because such solvents (even in trace amounts in the crystal lattice) compete with copper ions for the sites of potential second-sphere interaction.

Tebbacop analogues are viscous oils, irrespective of the purity of the spiroboronate from which they are derived, and can be studied by X-ray photoelectron spectroscopy (XPS). Although no shake-up lines were observed in the Cu–2p region for the Tebbacop analogue **15**, this was ascribed to reduction of copper(II) to copper(I) in the instrument, rather than to copper(I) itself being present in the analyte (copper(II) chloride showed the same result; see earlier also). In contrast to a spectrum of the spiroboronate **9**, which displayed an obvious band at 1072.1 eV assigned to the binding energy of the Na 1s electron [13], such absorption was absent from the spectrum of the Tebbacop analogue. Thus, the term transmetallation is clearly appropriate for this process.

The Tebbacop analogue **16** prepared by interphase transmetallation of spiroboronate **9** by copper(II) hydroxide was also analysed by XPS. In contrast to samples **15** derived from copper(II) chloride, shake-up lines in the Cu 2p region were observed in the spectra of all samples **16**. Although line-width analysis indicated that more than one copper species was present, the shake-up lines were very similar to those of copper(II) hydroxide alone. However, the Cu 2p region is complicated and extracting information is difficult. It is tentatively suggested that copper(II) hydroxide is retained in samples **16** as such, whereas in the case of copper(II) chloride the metal ion is transferred as a hydrate. The formulation **16** prepared from spiroboronate **9** and copper(II) hydroxide is more active biologically than **15** prepared from spiroboronate **9** and copper(II) chloride. Clearly, the active copper agent in these samples is not the same. If the active agent is in fact copper(II) hydroxide (hydrated and solvated by the spiroboronate through second-sphere interactions), copper(II) hydroxide as opposed to a hydrated copper(II) species would be released into tested substrates. Tebbacop analogue **16** would therefore be expected to be more active since copper(II) hydroxide is known to be an effective, albeit poorly soluble, fungicide in its own right.

Notwithstanding that they are suggestive rather than definitive in terms of structure, all of the spectroscopic results described above can together be interpreted reasonably in terms of second-sphere coordination. The spatial disposition of the copper cation in relation to the second-sphere ligand can be inferred from the crystal structure of the precursor sodium spiroboronate **9**. The arrangement in a unit cell of **9** suggests that opportunities for interaction are limited to above and below the aromatic rings, since there are only small vacancies in the vertical plane between the stacked spiroboronates.

3. Experimental

Melting points were determined on a Reichert–Kofler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1000 or a Perkin–Elmer 1600 FTIR spectrometer. The spectra for solids were recorded as Nujol mulls or as a film on sodium chloride. NMR spectra were obtained using a Bruker AM400 spectrometer operating at 400.13 MHz for ^1H, 100.62 MHz for ^{13}C and 128.4 MHz for ^{11}B nuclei. Unless otherwise stated spectra were recorded in deuterochloroform using a 5 mm probe. ^{11}B spectra were externally referenced to $Et_2O{\cdot}BF_3$. Low-resolution mass spectra were recorded on a VG-7070 mass spectrometer operating at a nominal accelerating voltage of 70 eV. High-resolution mass spectra were recorded at a nominal resolution of 5000 or 10 000 as appropriate; all spectra were obtained as electron impact spectra using perfluorokerosene as the internal standard unless otherwise stated. XPS were measured on a Perkin–Elmer XSAM 800 X-ray photoelectron spectrometer using Mg–K_α (1253.6 eV) X-radiation as the excitation source. The binding energies were corrected by using the value of 285.0 eV for the C(1s) level resulting from contaminated carbon. Liquid samples were left to evaporate as thick films on aluminium foil or in a sample stub that has a top like a shallow dish. Powdered samples were pressed to form pellets then cut and attached to a sample stub with a sticky carbon disk. Samples were degassed overnight at about 10^{-7} Torr before the measurements were run at room temperature (r.t.) under a vacuum of about 10^{-10} Torr.

Flash column chromatography was performed using Kieselgel 60 (230–400 mesh) silica gel and a silica:sample ratio (w/w) of ca. 30:1.

Biological testing was carried out by Y. Xiao and D. Eden at the wood-processing division of the New Zealand Forest Research Institute Ltd. (FRI) in Rotorua. Sterile antibiotic assay (AA) discs dampened with mineral salt solution [14] were placed in contact with active *C. puteana* growing on malt agar plates for 2 days. The discs were supported on aerial growth so that no contamination with malt extract occurs. They were then transferred aseptically to the test substrate. The gas-sterilised filter papers were individually dipped in treatment solutions for 10 s while holding at the edge with sterile forceps. Each treatment concentration had three replicates. In addition, filter papers were dipped in dichloromethane, toluene and water to serve as treatment controls. The treated papers were moistened with mineral salt solution and inoculated in the centre with an AA disc infected with *C. puteana*. Controls were treated in the same way. The Petri dishes containing the papers were incubated for 2 weeks at 25°C in polythene bags to prevent drying. Extent of growth was calculated for three measured diameters at 120°C.

3.1. General procedure for preparation of the spiroboronates

The 2-halo-4-alkylphenol (2 mmol) and aqueous formaldehyde (37 %w/w) were stirred mechanically at 50°C. To this mixture was added an aqueous solution of boric acid (1.1 mmol) and sodium hydroxide (1.1 mmol) and this mixture was stirred for 24 h, during which time a soft crystalline mass formed. The mixture was cooled slowly to r.t. before being diluted with water and stirred rapidly to remove water-soluble components. The crystals were filtered off and washed thoroughly with water and hexanes before drying in a desiccator.

*3.1.1. Bis[3-bromo-5-butyl-2-hydroxybenzenemethanolato(2-)-O,O′]borate(1-) sodium salt (**3**)*

2-Bromo-4-butylphenol gave **3** as colourless crystals (38%), m.p. 178–80°C. (Found: $M^{+\bullet}$; 548.0181, $C_{22}H_{26}B^{79,81}Br_2NaO_4$. Calc.: 548.0168.) ν_{max} 3532, 1643, 1279, 1138, 1101, 1009, 964 cm^{-1}. δ_H (DMSO-d_6) 0.88 (t, *J* 7.3 Hz, 6H, $2CH_3$), 1.27 (sx, *J* 7.5 Hz, 4H, $2CH_2$), 1.47 (p, *J* 7.7 Hz, 4H, $2CH_2$), 2.41 (t, *J* 7.5 Hz, 4H, $2CH_2$), 4.48, 4.59 (2d, *J* 13.8 Hz, 4H, 2H-7), 6.64 (d, *J* 1.7 Hz, 2H, 2H-6), 7.02 (d, *J* 1.8 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 13.7 $2CH_3$, 21.6, 33.4, 33.6 $6CH_2$, 61.2 2(C-7), 110.0 2(C-3), 123.5 2(C-6), 128.0 2(C-4), 129.2 2(C-6a), 130.7 2(C-5), 151.6 2(C-2a). *m*/*z* (FAB^+) 571 (M + Na, 45%), 549 (M + H, 75), 548 (M^+, 100), 505 (M–C_3H_7, 18).

*3.1.2. Bis[2-hydroxy-3-methyl-5-(1,1-dimethylethyl)-benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (**4**)*

2-Methyl-4-(1,1-dimethylethyl)phenol gave **4** as colourless crystals (39%), m.p. > 300°C (dec.). (Found: $M^{+\bullet}$; 418.2295, $C_{24}H_{32}BNaO_4$. Calc.: 418.2291.) ν_{max} 3637, 3448, 1172, 1153, 971, 919, 898 cm^{-1}. δ_H (DMSO-d_6) 1.20 (s, 18H, $2C(CH_3)_3$), 2.01 (s, 6H, $2CH_3$), 4.49 (s, 4H, 2H-7), 6.61 (d, *J* 2.2 Hz, 2H, 2H-6), 6.80 (d, *J* 2.0 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 16.5 $2CH_3$, 31.7 $2C(CH_3)_3$, 33.3 $2C(CH_3)_3$, 61.7 2(C-7), 118.3 2(C-3), 123.0 2(C-6), 124.2 2(C-4), 124.7 2(C-6a), 136.8 2(C-5), 153.2 2(C-2a). *m*/*z* (FAB^+) 441 (M + Na, 33%), 418 (M^+, 56), 403 (M–CH_3, 16).

*3.1.3. Bis[3-fluoro-2-hydroxy-5-(1,1-dimethylethyl)-benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (**5**)*

2-Fluoro-4-(1,1-dimethylethyl)phenol gave **5** as colourless crystals (30%), m.p. > 300°C. (Found: $M^{+\bullet}$; 426.1794, $C_{22}H_{26}BF_2NaO_4$. Calc.: 426.1790.) ν_{max} 3633, 3351, 1515, 1010, 985, 931 cm^{-1}. δ_H 1.18 (s, 18H, $2C(CH_3)_3$) 4.57 (s, 4H, 2H-7), 6.63 (s, 2H, 2H-6), 6.82 (d, *J* 13.5 Hz, 2H, 2H-4). δ_C 31.4 $2C(CH_3)_3$, 33.6, $2C(CH_3)_3$, 61.4 2(C-7), 110.0 (d, *J* 19.0 Hz, 2C-3), 116.1 2(C-6), 128.2 2(C-4), 138.0 (d, *J* 5.0 Hz, 2C-6a), 142.6 (d, *J* 11.0 Hz, 2C-5), 151.0 (d, *J* 238 Hz, 2C-2a). *m*/*z* (FAB^+) 449 (M + Na, 10%), 427 (M + H, 34), 426 (M^+, 42), 411 (M–CH_3, 8).

*3.1.4. Bis[3-chloro-2-hydroxy-5-(1,1-dimethylethyl)-benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (**6**)*

2-Chloro-4-(1,1-dimethylethyl)phenol gave **6** as colourless crystals (40%), m.p. 229°C (dec.). (Found: $M^{+\bullet}$; 460.1199, $C_{22}H_{26}B^{35,37}Cl_2NaO_4$. Calc.: 460.1169.) ν_{max} 3435, 1627, 1289, 1122, 1097, 916 cm^{-1}. δ_H (DMSO-d_6) 1.24 (s, 18H, $2C(CH_3)_3$), 4.52, 4.58 (2d, *J* 13.9 Hz, 4H, 2H-7), 6.79 (s, 2H, 2H-6), 7.02 (d, *J* 2.3 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 31.3 $2C(CH_3)_3$, 33.5 $2C(CH_3)_3$, 61.4 2(C-7), 119.3 (C-3), 119.8 2(C-6), 123.3 2(C-4), 127.5 2(C-6a), 138.7 2(C-5), 150.5 2(C-2a). *m*/*z* (FAB^+) 483 (M + Na, 6%), 461 (M + H, 20), 460 (M^+, 26), 445 (M–CH_3, 5).

*3.1.5. Bis[3-bromo-2-hydroxy-5-(1,1-dimethylethyl)-benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (**7**)*

3-Bromo-4-(1,1-dimethylethyl)phenol gave **7** as colourless crystals (35%), m.p. 200–1°C. (Found: $M^{+\bullet}$; 548.0197, $C_{22}H_{26}B^{79,81}Br_2NaO_4$. Calc.: 548.0168.) ν_{max} 3591, 3339, 1287, 1117, 1031, 1017, 970, 929 cm^{-1}. δ_H (DMSO-d_6) 1.21 (s, 18H, $2C(CH_3)_3$), 4.49, 4.61 (2d, *J* 13.8 Hz, 4H, 2H-7), 6.80 (s, 2H, 2H-6), 7.16 (d, *J* 1.9 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 31.4 $2C(CH_3)_3$, 33.6 $2C(CH_3)_3$, 61.6 2(C-7), 110.1 2(C-3), 120.7 2(C-6), 126.3 2(C-4), 127.6 2(C-6a), 139.5 2(C-5), 151.4 2(C-2a). *m*/*z* (FAB^+) 571 (M + Na, 13%), 547 (M + H, 28), 548 (M^+, 36), 533 (M–CH_3, 8).

3-Bromo-4-(1,1-dimethylethyl)phenol and potassium hydroxide in place of sodium hydroxide gave bis-[3-bromo-2-hydroxy-5-(1,1-dimethylethyl)benzenemethanolato(2-)-O,O′]borate(1-) potassium salt as colourless crystals (65%), m.p. > 300°C. (Found: $M^{+\bullet}$; 563.9918, $C_{22}H_{26}B^{79,81}Br_2KO_4$. Calc.: 563.9907.) *m*/*z* (FAB^+) 603 (M + K^+, 1%), 565 (M + H^+, 4), 564 (M^+, 5).

3.1.6. Bis[3-chloro-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzenemethanolato(2)-O,O′]borate(1-) sodium salt (9)

3-Chloro-4-(1,1,3,3-tetramethylbutyl)phenol gave **9** as colourless needles (72%) (pyridine–nitromethane), m.p. 224–5°C. (Found: C, 60.94; H, 7.74; Na, 3.84. $C_{30}H_{42}B^{35,37}Cl_2NaO_4 \cdot H_2O$. Calc.: C, 61.13; H, 7.53; Na, 3.90%.) ν_{max} 3393, 1655, 1648, 1169, 1117, 1091, 1021 cm^{-1}. δ_B (DMSO-d_6) 2.2. δ_H (DMSO-d_6) 0.72 (s, 18H, 2C(CH_3)$_3$), 1.26 (s, 12H, 2C(CH_3)$_2$), 1.64 (s, 4H, 2CH_2), 4.55 (s, 4H, 2H-7), 6.79 (s, 2H, 2H-6), 7.00 (s, 2H, 2H-4). δ_C (DMSO-d_6) 31.5 2C(CH_3)$_2$, 31.6 2C(CH_3)$_3$, 32.0 2C(CH_3)$_3$, 37.3 2C(CH_3)$_2$, 56.2 2CH_2, 61.3 2(C-7), 119.1 2(C-3), 120.6 2(C-6), 124.2 2(C-4), 127.2 2(C-6a), 137.3 2(C-5), 150.3 2(C-2a). m/z (FAB^+) 593 (M + Na, 48%), 571 (M + H, 64), 570 (M^+, 60), 499 (M–C_5H_{11}, 42). m/z (FAB^-) 547 (M–Na, 100%).

3-Chloro-4-(1,1,3,3-tetramethylbutyl)phenol and potassium hydroxide in place of sodium hydroxide gave bis[3-chloro-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzene-methanolato(2)-O,O′]borate(1-) potassium salt as colourless needles (63%) (pyridine/nitromethane), m.p. > 230°C. (Found: 586.2192 $C_{30}H_{42}B^{35,35}Cl_2KO_4$. Calc.: 586.2190.) m/z (FAB^+) 1213 (2M + K^+, 55%), 625 (M + K^+, 100), 586 (M^+, 20).

3.1.7. Bis[3-bromo-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (10)

3-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol gave **10** as colourless needles (76%) (nitromethane), m.p. 217–8°C. (Found: $M^{+\bullet}$; 660.1438, $C_{30}H_{42}B^{79,81}Br_2NaO_4$. Calc.: 660.1420.) ν_{max} 3634, 3479, 1630, 1153, 1101, 955 cm^{-1}. δ_H (DMSO-d_6) 0.72 (s, 18H, 2C(CH_3)$_3$), 1.26 (s, 12H, 2C(CH_3)$_2$), 1.63 (s, 4H, 2CH_2), 4.51, 4.58 (2d, J 13.7 Hz, 4H, 2H-7), 6.83 (s, 2H, 2H-6), 7.15 (s, 2H, 2H-4). δ_C (DMSO-d_6) 31.5 2C(CH_3)$_2$, 31.6 2C(CH_3)$_3$, 32.0 2C(CH_3)$_3$, 37.3 2C(CH_3)$_2$, 56.2 2CH_2, 61.4 2(C-7), 109.8 2(C-3), 121.3 2(C-6), 127.0 2(C-4), 127.3 2(C-6a), 138.0 2(C-5), 151.2 2(C-2a). m/z (FAB^+) 683 (M + Na, 60%), 660 (M^+, 66), 589 (M–C_5H_{11}, 64).

3-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol and potassium hydroxide in place of sodium hydroxide gave bis[3-bromo-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzene-ethanolato(2-)-O,O′]borate(1-) potassium salt as colourless needles (90%) (nitromethane), m.p. > 205°C (dec.). (Found: $M^{+\bullet}$; 676.1165, $C_{30}H_{42}B^{79,81}Br_2KO_4$. Calc.: 676.1159.) m/z (FAB^+) 715 (M + K^+, 100%), 677 (M + H^+, 71), 676 (M^+, 72), 605 (M–C_5H_{11}, 36).

3.1.8. Bis[2-hydroxy-5-(1,1,3,3-tetramethylbutyl)-benzenemethanolato(2-)-O,O′]borate(1-) sodium salt (12)

4-(1,1,3,3-Tetramethylbutyl)phenol gave **12** as colourless crystals (27%), m.p. > 262°C (dec.). (Found: $M^{+\bullet}$; 502.3240, $C_{30}H_{44}BNaO_4$. Calc.: 502.3230.) ν_{max} 3675, 3584, 3331, 3237, 1269, 1104 cm^{-1}. δ_H (DMSO-d_6) 0.70 (s, 18H, 2C(CH_3)$_3$), 1.25 (s, 12H, 2C(CH_3)$_2$), 1.63 (s, 4H, 2CH_2), 4.48, 4.52 (2d, J 13.6 Hz, 4H, 2H-7), 6.33 (d, J 8.4 Hz, 2H, 2H-6), 6.75 (d, J 1.8 Hz, 2H, 2H-3), 6.88 (dd, J 8.3, 2.1 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 31.6 2C(CH_3)$_2$, 31.7 2C(CH_3)$_3$, 32.0 2C(CH_3)$_3$, 37.2 2C(CH_3)$_2$, 56.4 2CH_2, 61.5 2(C-7), 115.4 2(C-3), 121.5 2(C-6), 123.8 2(C-4), 125.2 2(C-6a), 135.9 2(C-5), 154.9 2(C-2a). m/z (FAB^+) 525 (M + Na, 33%), 503 (M + H, 30), 502 (M^+, 33), 487 (M–CH_3, 5), 431 (M–C_5H_{11}, 42).

3.1.9. Bis[3-chloro-2-hydroxy-5-nonylbenzene-methanolato(2-)-O,O′]borate(1-) sodium salt (13)

2-Chloro-4-nonylphenol gave **13** as glistening flaky crystals (98%), m.p. 211–2°C. (Found: $M^{+\bullet}$; 600.2769, $C_{32}H_{46}B^{35,37}Cl_2NaO_4$. Calc.: 600.2734.) ν_{max} 3542, 3354, 1210, 1156, 1017, 949, 901 cm^{-1}. δ_H (DMSO-d_6) 0.85 (t, J 7.0 Hz, 6H, 2CH_3), 1.24 (m, 10H, 20CH_2), 1.49 (t, br, J 6.8 Hz, 8H, 4CH_2), 2.40 (t, J 7.4 Hz, 4H, 2CH_2), 4.00, 4.55 (2d, J 14.0 Hz, 4H, 2H-7), 6.59 (d, J 1.7 Hz, 2H, 2H-6), 6.86 (d, J 1.7 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 13.9 2(CH_3), 22.0, 28.5, 28.6, 28.8, 30.6, 31.1, 31.2, 34.0 16(CH_2), 61.1 2(C-7), 119.4 2(C-3), 122.8 2(C-6), 126.3 2(C-4), 127.9 2(C-6a), 130.0 2(C-5), 150.7 2(C-2a). m/z (FAB^+) 623 (M + Na, 48%), 601 (M + H, 36), 600 (M^+, 48), 487 (M–C_8H_{17}, 8).

3.1.10. Bis[3-bromo-2-hydroxy-5-nonylbenzene-methanolato(2-)-O,O′]borate(1-) sodium salt (14)

2-Bromo-4-nonylphenol gave **14** as colourless crystals (79%), m.p. 218–9°C. (Found: $M^{+\bullet}$; 688.1722, $C_{32}H_{46}B^{79,81}Br_2NaO_4$. Calc.: 688.1733.) ν_{max} 3630, 3525, 1606, 1286, 1151, 1094, 1024, 969, 946 cm^{-1}. δ_H (DMSO-d_6) 0.86 (t, J 7.0 Hz, 6H, 2CH_3), 1.24 (m, 20H, 10CH_2), 1.48 (p, br, J 6.5 Hz, 8H, 4CH_2) 2.40 (t, J 7.4 Hz, 4H, 2CH_2), 4.48, 4.57 (2d, J 13.9 Hz, 4H, 2H-7), 6.63 (d, J 1.8 Hz, 2H, 2H-6), 7.02 (d, J 1.8 Hz, 2H, 2H-4). δ_C (DMSO-d_6) 13.9 2(CH_3), 22.0, 28.5, 28.62, 28.8, 28.9, 31.2, 33.9 16(CH_2), 61.2 2(C-7), 110.0 2(C-3), 123.5 2(C-6), 127.9 2(C-4), 129.1 2(C-6a), 130.7 2(C-5), 151.6 2(C-2a). m/z (FAB^+) 711 (M + Na, 8%), 688 (M^+, 24) 575 (M–C_8H_{17}, 6).

3.1.11. Bis[bis[3-chloro-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzenemethanolato(2-)-O,O′]borate(1-)] copper(II) salt (15)

A solution of copper(II) chloride (1 mmol) in water containing a trace amount of ammonia (to maintain the solution at ca. pH 5) was added dropwise (during several minutes) to a solution of spiroboronate **9** (2 mmol) in hexanes with rapid turbulent stirring. When almost all of the copper(II) solution had been added the mixture became deep red–brown in colour. After 15 min the mixture was transferred to a separating funnel and left to settle. The organic phase was separated,

washed once with water, dried ($MgSO_4$) and then filtered through a Gelman teflon filter (pore size 0.45 μm). The solvent was allowed to evaporate at r.t. and then removed under vacuum at r.t. leaving **15** as a red–brown oil. MIC 0.01% Cu. ν_{max} 3371, 1618, 1161, 1113, 1016 cm^{-1}. δ_B (DMSO-d_6) 2.2, br.

*3.1.12. Bis[bis[3-chloro-2-hydroxy-5-(1,1,3,3-tetramethylbutyl)benzenemethanolato (2-)-O,O′]borate(1-)] copper(II) salt (**16**)*

To a slurry of copper(II) hydroxide (5–10 mmol) in water containing a trace amount of ammonia was added a solution of spiroboronate **9** (1.0 mmol) in hexanes. This heterogeneous mixture was stirred for 3–5 days at r.t. After allowing the mixture to settle over several hours the organic phase was carefully separated by Pasteur pipette and filtered through a sintered glass crucible packed with Celite and anhydrous magnesium sulphate. Finally, the solution was filtered through a Gelman Teflon filter (0.45 μm pore size) and the solvent evaporated as above, leaving **16** as a red–brown oil. MIC 0.001% Cu. ν_{max} 3360, 1620, 1163, 1117, 1085, 1022 cm^{-1}. δ_B (DMSO-d_6) 2.2, br.

References

[1] D.R. Stern, F.J. Weck, US Patent Appl. 3 266 981, 16 August, 1966.
[2] I. Zviedre, V. Bel'skii, E. Schvarts, Latv. PSR Zinat. Akad. Vestis Kim. Ser. (1985) 672 [Chem. Abstr. 104 (1986) 79562g].
[3] N.P. Maynard, NZ Patent Appl. 226 (1988) 377.
[4] W.G. Henderson, M.J. How, G.R. Kennedy, E.F. Mooney, Carbohydr. Res. 28 (1973) 1.
[5] M. Van Duin, J.A. Peters, A.P.G. Kieboom, H. Van Bekkum, Tetrahedron 41 (1985) 3411.
[6] J. Böeseken, Adv. Carbohydr. Chem. 4 (1949) 189.
[7] V. Grundstein, E. Shvarts, I. Lange, Latv. PSR Zinat. Akad. Vestis. Kim. Ser (1989) 33 [Chem. Abstr. 110 (1989) 224431u].
[8] W.H. Furness, N.J. Haddonfield, US Patent Appl. 24 324, 28 May, 1957.
[9] (a) A. Werner, Z. Anorg. Chem. 3 (1893) 267. (b) A. Werner, Justus Liebigs Ann. Chem. 386 (1912) 1. (c) A. Werner, Chem. Ber. 45 (1912) 121.
[10] H.M. Colquohoun, J.F. Stoddart, D.J. Williams, Angew Chem. Int. Ed. Engl. 25 (1986) 487.
[11] J.M. Smithson, R.J.P. Williams, J. Chem. Soc. (1958) 457.
[12] J. Chatt, L.A. Duncanson, L.M. Venanzi, J. Chem. Soc. (1956) 2712.
[13] J. Chastain (Ed.), Handbook of X-Ray Photoelectron Spectroscopy, Perkin–Elmer, Minneapolis, MN, 1992.
[14] Mineral salt solution: a solution supplement with vitamins which has been found suitable for growth of *C. puteana* when 1.0 ml is added to a 9 cm filter paper.