Supplementary Information

Quaternary Ammonium Arylspiroborate Esters as Organo-Soluble, Environmentally Benign Wood Protectants

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Anti-fungal activity

Cellulose paper bioassays were carried out as described previously,[1] with activity being measured at three concentrations, *i.e.* 5.3, 1.1 and 0.2 μmol cm^{-2} and fungal growth measured after seven, fourteen and twenty-one days.

Agar mini-block bioassays; preparation of specimens: *P. radiata* sapwood specimens measuring 10 x 10 x 5 mm were treated with acetonitrile solutions of the borate esters, and an aqueous solution of boric acid by use of the following method: The specimens were placed in a vacuum desiccator and a vacuum (-90 kPa) applied for 30 mins. The treatment solution was admitted to the desiccator under vacuum, after which the vacuum was released and the specimens left to adsorb solution at atmospheric pressure for 60 mins. Each specimen was weighed before and immediately after treatment to determine uptake and compound concentration. After treatment the specimens (except solvent controls) were wrapped in plastic bags and left for one week, then air-dried for two weeks. The specimens were then vacuum oven dried at -90 kPa and 40 ^{o}C for five days, after which they were reconditioned to a moisture content of approximately 9 %. The specimens were sterilized by gamma-irradiation at 25 Kgy prior to commencement of the fungal bioassay.

Fungal bioassay: Malt agar plates were inoculated at three equidistant points with pieces (9 x 9 mm) taken of the test fungi from seven day old cultures. The plates were then incubated at

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25 ^{o}C for five to seven days to allow the fungus to become established. Three sterile pieces of plastic mesh were then aseptically placed on each agar plate and one sterile test specimen was positioned on each piece of mesh. Twelve replicates were performed for each retention (three replicates per plate). Solvent-treated and untreated specimens were also included in the bioassay as controls. Replicates of treated and untreated specimens were also placed on sterile plates to monitor for mass change unassociated with fungal decay. The plates were then incubated at 25 ^{o}C and 63 % relative humidity for five weeks. At the completion of the bioassay the specimens were removed from the agar plates, cleaned of any fungal hyphae and weighed to estimate moisture content. The specimens were then reconditioned and weighed to determine mass loss.

Termiticidal activity

Cellulose paper bioassays: For each compound to be tested, a 0.10 mol dm^{-3} solution was prepared using the appropriate solvent and 0.01 and 0.001 mol dm^{-3} solutions prepared by serial dilution. Three cellulose papers (d = 4.7 cm) were treated with 0.35 cm^3 of each solution and air-dried. Controls were also prepared using the appropriate solvents and water. The treated papers were weighed prior to commencement of the bioassay. The cellulose papers were then placed in a 53 mm Petri dish and moistened with 0.4 cm^3 of deionised water. Twenty mature workers from a single colony of *Coptotermes acinaciformis* (Froggatt) were added to each Petri dish and the dishes placed on a bed of moistened cotton wool in sealed plastic containers. The containers were stored in an insectary maintained at 27 °C and 75% relative humidity for the duration of the bioassay (6 − 8 days). Periodically the termite mortality in each dish was assessed and any cadavers removed from the paper. At the completion of the bioassay the papers were washed with 70% ethanol and re-dried. The papers were then weighed to determine mass loss.

Wood specimen bioassays: Treatment of specimens: *P. radiata* sapwood specimens (20 x 20 x 10 mm) were treated in the same way that described for the mini-blocks used in the fungal bioassay. The termite bioassay involved placing a single test specimen on a 29 mm white plastic lid within a vented 250 mL glass jar containing finely ground *C. acinaciformis* mound material (100 % moisture holding capacity). Two grams of *C. acinaciformis*, taken from a single colony at Gunns Point in the Northern Territory, were added to the jar. Three replicates were used per retention. Solvent treated and untreated specimens were also included in the bioassay as controls. The jars were maintained in an insectary at 27 ^{o}C / 75% relative humidity. The test duration was eight weeks. Termite mortality within the test units was observed periodically and recorded. At the completion of the bioassay the test specimens were removed from the jars, cleaned and reconditioned. Wood consumption based on mass difference was determined.

Accelerated Leaching

Treatment of specimens: Specimens, 20 mm (radial) x 20 mm (tangential) x 10 mm (longitudinal), were treated via a full-cell schedule similar to that used for the fungal mini-block assays. This involved a 30 min. vacuum cycle (-85 kPa), after which the treatment solution was admitted to the treatment vessel while still under vacuum. The specimens were then left submerged in the treatment solution at atmospheric pressure for 1 hr.

Leaching: Half of the specimens were retained as 'unleached' for chemical analysis and the other half were leached. The procedure involved saturating treated specimens with de-ionised water and placing them in a jar that contained at least three times the volume of water as of samples. The jars were placed in a shaking water bath maintained at 35 ^{o}C for five days with

daily changes of water. At the completion of the leaching cycle the specimens were dried in readiness for chemical analysis.

Boron analysis: Leached and unleached blocks for analysis were manually chipped, passed through a Bauer refiner and ground in a Wiley mill to pass a 2 mm screen. Moisture contents were determined by weight loss upon drying of the ground samples for 18 hours in an oven at 108 $^{\circ}$C. An appropriate amount of each of the ground samples (approximately 1 g) was weighed accurately into a crucible and 10 cm^3 of a saturated solution of Ba(OH)$_2$ added. After thorough mixing the crucibles were heated in an oven at 108 $^{\circ}$C for 48 hours, charred with a Bunsen burner and then ashed in a muffle furnace at 650 $^{\circ}$C for 2 hours. The ash was digested in 20 cm^3 of 10 % HCl (CAUTION: vigorous reaction), filtered under gravity through glass fibre filter paper and diluted with deionised water; to 100 cm^3 in the case of the leached samples, and 200 cm^3 in the case of the unleached samples. The solutions were then analysed for boron by inductively coupled plasma optical emission spectrometry (ICP-OES) using a GBC Integra XM spectrometer, calibrated with matrix-matched standards.

Raw Data for Mortality as a function of Time (see Fig. 3).

Compound	Conc. (mol dm^{-3})	Mortality (%) as a function of time (h)[a]						
		24	48	72	96	120	168	192
NBu$_4$[B(o-hmp)$_2$]	0.1	2 (0.6)	42 (4.5)	63 (1.2)	70 (1.0)	80 (2.7)	85 (2.0)	88 (2.3)
NBu$_4$[B(sal)$_2$]	0.1	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
B(OH)$_3$	0.1	0 (0.0)	7 (1.5)	25 (2.7)	38 (5.5)	60 (3.6)	90 (2.0)	95 (1.0)
NBu$_4$[B(o-hmp)$_2$]	0.01	8 (1.5)	27 (4.7)	42 (5.5)	45 (4.4)	52 (4.6)	73 (1.2)	75 (1.0)
NBu$_4$[B(sal)$_2$]	0.01	50 (8.7)	70 (5.2)	78 (3.8)	85 (2.7)	88 (2.1)	97 (1.2)	97 (1.2)
B(OH)$_3$	0.01	0 (0.0)	0 (0.0)	5 (0.0)	7 (0.6)	32 (4.7)	80 (1.0)	85 (2.0)
NBu$_4$[B(o-hmp)$_2$]	0.001	7 (0.6)	12 (0.6)	12 (0.6)	13 (0.6)	23 (2.5)	27 (2.9)	28 (3.2)
NBu$_4$[B(sal)$_2$]	0.001	0 (0.0)	5 (0.0)	17 (2.1)	22 (1.2)	28 (3.1)	40 (2.7)	43 (3.5)
B(OH)$_3$	0.001	0 (0.0)	0 (0.0)	3 (0.6)	5 (0.0)	7 (0.6)	7 (0.6)	7 (0.6)
Water	-	2 (0.6)	2 (0.6)	2 (0.6)	3 (0.6)	3 (0.6)	3 (0.6)	5 (1.0)
Acetonitrile	-	2 (0.6)	2 (0.6)	2 (0.6)	3 (0.6)	5 (1.0)	7 (0.6)	7 (0.6)

[a]Average of three replicates. Standard deviation in parentheses.

Images of filter papers after Termite Bioassay

L→R, papers treated with 0.001, 0.01 & 0.1 mol dm^{-3} solutions of compounds under investigation.

NBu$_4$[B(sal)$_2$] (4)

NBu$_4$[B(*o*-hmp)$_2$] (2)

Boric acid

Water

MeCN

*Geometry optimisations used for logP$_{oct}$ calculations.*

The semi-empirical methods AM1, PM3, and MNDO, as well as the MM+ molecular mechanics forcefield, were tested for their ability to reproduce the X-ray structure of NBu$_4$[B(*o*-hmp)$_2$] (2).[1] All methods worked well for NBu$_4$, but only AM1 gave the tetrahedral spiro-borate structure observed experimentally, as shown below (X-ray structure shown in green, AM1 in red). Table S1 summarizes the performance of the various methods tested.
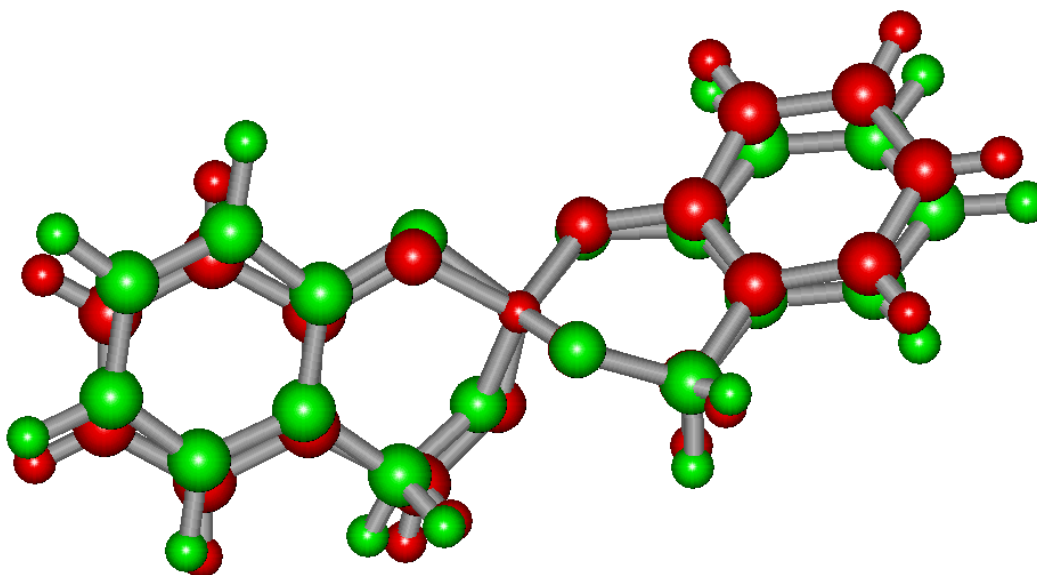
Comparison of geometrical details determined using various methods

Data source	B-O1	B-O2	B…N
Xtal	1.482	1.460	4.524
AM1	1.486	1.515	4.271
PM3	1.950	1.930	3.653
MNDO	1.448	1.474	5.180
MM+	1.416	1.550	4.627
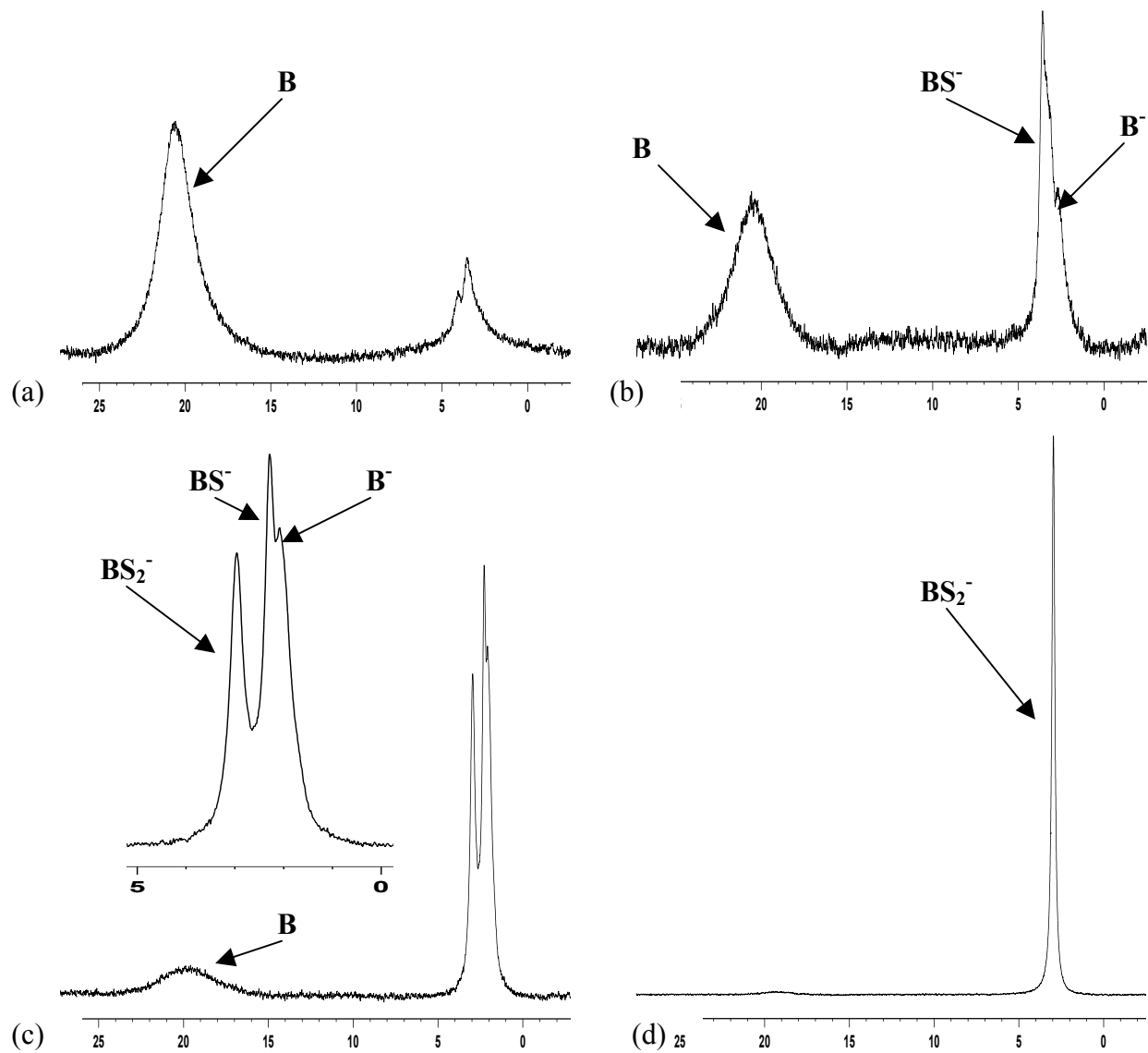
^{11}B NMR spectra of d_6-DMSO solutions containing 2-hydroxymethyl phenol:boric acid: TBAOH in H_2O, with ratios (a) 0.25:1:1, (b) 0.5:1:1, (c) 1:1:1 and (d) 2:1:1.

The concentration of borate species, $[BS^-]$, $[BS_2^-]$, $[B]$, $[B^-]$ were measured from the relative area of the corresponding peak of the ^{11}B NMR spectrum and the total boron concentration, 0.05 M. A relative error of 5% was estimated for these values. The absolute error for these values, estimated to be the sample standard error, s, was calculated as follows,

$$s_{[Borate]} = 0.05 \times [Borate]$$

eq S3. 1

The value for $[OH^-]_{free}$ was the same as $[B]$ and the error also 5%.

$[B_{free}]$ $[S]$ were calculated from:

$$[B_{free}] = [B] + [B^-]$$
eq S3. 2

$$[S] = [S]_0 - ([BS^-] + 2[BS_2^-])$$

eq S3. 3

The absolute error of the sum was obtained as the square root of the sum of the squares of the absolute errors of the components, *e.g.*

$$s_{[S]} = \sqrt{(s_{[S]_0})^2 + (s_{[BS^-]})^2 + 2 \times (s_{[BS_2^-]})^2}$$

eq S3. 4

The error in the concentration of substrate added was assumed to be zero, so S3. 4 becomes,

$$s_{[S]} = \sqrt{(s_{[BS^-]})^2 + 2 \times (s_{[BS_2^-]})^2}$$

eq S3. 5

The equilibrium expressions are:

$$\beta_1 = [BS^-]/[B_{free}]\bullet[S]\bullet[OH^-_{free}]$$
$$\beta_2 = [BS_2^-]/[B_{free}]\bullet[S]^2\bullet[OH^-_{free}]$$
$$\beta_3 = \beta_2/\beta_1 = [BS_2^-]/[S]\bullet[BS^-]$$

The relative error of the β values were calculated as the square root of the sum of the squares of the component concentration values, *e.g.*

$$\frac{s_\beta}{\beta_2} = \sqrt{\left(\frac{s_{[BS_s^-]}}{[BS_2^-]}\right)^2 + \left(\frac{s_{[B_{free}]}}{[B_{free}]}\right)^2 + 2\times\left(\frac{s_{[S]}}{[S]}\right)^2}$$

eq S3. 6

In general, only a single measurement was made for each borate species derived from each substrate, so equilibrium constants were calculated for a sample size of one. For a more rigorous determination of the equilibrium constants and estimation of their uncertainties, this sample size would need to be increased by taking measurements for a number of different substrate:boron ratios, such that true measurements of the sample standard deviation, s, could be obtained.

[1]   J. M. Carr, P. J. Duggan, D. G. Humphrey, E. M. Tyndall, *Aust. J. Chem.* **2005**, *58*, 21.