### Title

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### Journal

The Journal of Organic Chemistry, 78(2)

### ISSN

0022-3263



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### Publication Date

2013-01-18

### DOI

10.1021/jo301135m

# Urushiol Detection using a Profluorescent Nitroxide

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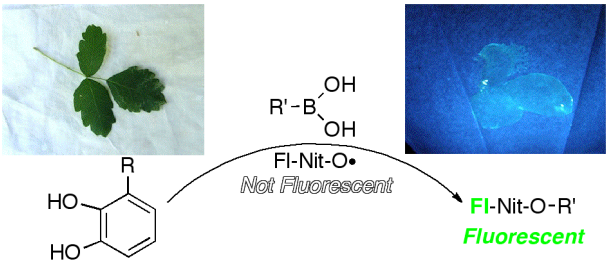



**ABSTRACT:** A method to visually detect minute amounts of urushiol, the toxic catechol from poison oak, poison ivy and poison sumac, has been developed utilizing the reaction of a profluorescent nitroxide with the *B*-*n*-butylcatecholboronate ester formed in situ from urushiol and *B*-*n*-butylboronic acid. The resulting *N*-alkoxyamine is strongly fluorescent upon illumination with a fluorescent lamp, allowing the location of the toxic urushiol contamination to be visualized. This methodology constitutes the groundwork for the future development of a spray to detect urushiol to avoid contact dermatitis, as well as to detect catecholamines for biomedical applications.

## INTRODUCTION

Urushiol is the causative agent of allergic contact dermatitis from exposure to *Toxicodendron spp*. (poison oak, poison ivy, and poison sumac). For susceptible individuals, exposure to tiny amounts of this invisible oil elicits contact dermatitis, which is contracted by either direct exposure to the plant source, or indirectly by exposure to contaminated tools, clothing, and pets. The deleterious effects of *Toxicodendron* dermatitis are a large personal and economic burden to the United States, causing significant workers' compensation claims and lost time for those engaged in a myriad of outdoor jobs,[1,2] accounting for greater than 10% of the total U.S. Forest Services lost-time injuries.[3] In 1988, the *National Institute for Occupational Safety and Health* (NIOSH) estimated that 1.07-1.65 million occupational skin injuries occurred yearly, with an estimated annual rate of 1.4 to 2.2 cases per 100 workers.[4] Approximately 50-70% of the adult American population is clinically allergic to urushiol.[1] Outside of North America, significant occupational hazards arise from exposure to the sap (kiurushi) of Asian lacquer trees (ex. *Toxicodendron verniciflua* in Japan,[5] *Melanorrhoea usitata* in Thailand[6]) used as a varnish in lacquerware. Symptoms of allergic contact dermatitis from urushiol exposure vary from mild annoyance to weeks of irritation and pain. Occasionally, exposure can lead to nephropathy[7] and even to fatal systemic anaphylaxis.[8]

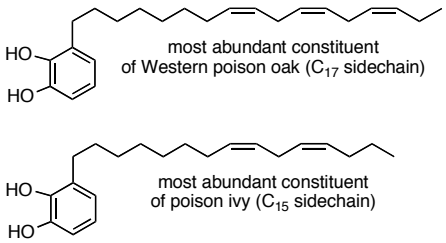


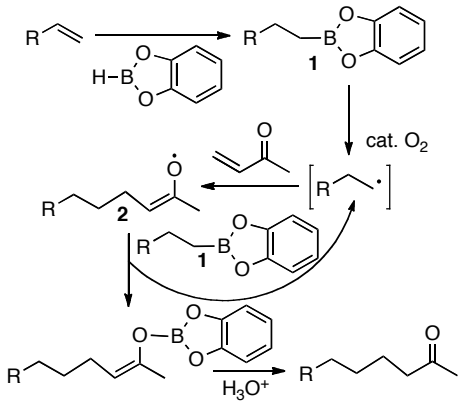

**Figure 1.** Structure of the most abundant components of urushiol from *Toxicodendron species*.

Chemically, urushiol (Figure 1) is the name given to a collection of related compounds consisting of 3-substutitued catechols, in which the long hydrophobic chain is a linear $C_{15}$ or $C_{17}$ alkyl group containing 0-4 degrees of predominately *cis* unsaturation.[9,10] Each of the different members of the *Toxicodendron species* contain mixtures of urushiol bearing $C_{15}$ or $C_{17}$ alkyl chains, with various degrees of unsaturation. They all share the catechol functionality, and a long, greasy alkyl chain, which facilitates migration into the skin. The majority of allergic contact dermatitis cases from urushiol result from unexpected exposure. Since as little as 2 mg or less of urushiol can lead to contact dermatitis,[1] a very sensitive method for detecting urushiol is warranted. The ability to visualize the distribution of this oil would allow sensitive individuals to avoid or wash away urushiol[11] to prevent or minimize contact dermatitis. Early work includes the development of a “black spot test” to help identify these plants in the field,[12] but this is not practical for detecting the distribution of contamination. Thus a fluorescent detection method is attractive, given the strong signal generated from very small amounts of fluorophore. A dilute solution of non-toxic spray, which reacts selectively to form a highly fluorescent species in the presence of urushiol, would enable the location of urushiol contamination to be determined quickly, without the need for specialized instrumentation beyond a hand-held fluorescent lamp. Reactions based on oxidation of the catechol to the orthoquinone, followed by condensation with ethylenediamine[13] or benzylamine[14] to give fluorescent heteroaromatic derivatives have been used traditionally to quantitate catechols and catecholamines. However, both ethylenediamine and benzylamine are considered very dangerous upon exposure to the skin.

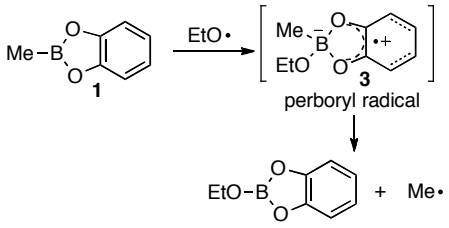
## RESULTS AND DISCUSSION

In order to develop a system to selectively detect catechols in the presence of other alcohols and diols (such as sugars), a reaction that takes place with catechols but not with other alcohols is required. *B*-Alkylcatecholboronate esters have been used to selectively generate alkyl radicals upon reaction with oxygen radicals.[15,16] Early work by Brown and Negishi[17,18] entailed enol oxygen radical **2** addition to *B*-alkylcatecholboronate ester **1** to give an alkyl radical, which then adds to an unsaturated ketone in a chain reaction (Scheme 1). The efficacy of oxygen radical addition specifically to *B*-alkylcatecholboronate esters **1** is due to stabilization of the intermediate perboryl radical **3**, by delocalization of the unpaired electron into the aromatic ring (Scheme 2). Direct ESR evidence for a delocalized perboryl radical **3** was observed below 270 K by Roberts.[19] A number of very useful synthetic methodologies have been developed from this chemistry.[20]

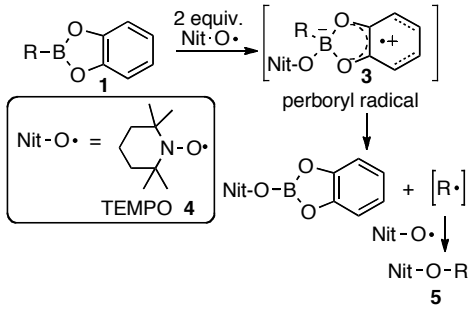
Scheme 1. Brown and Negishi's 1969 sequence for generating carbon radicals from *B*-alkylcatecholboronate ester **1**




Scheme 2. Roberts demonstrated that the addition of an oxygen radical to an *B*-alkylcatecholboronate ester **1** formed a perboryl radical **3**, visible by EPR

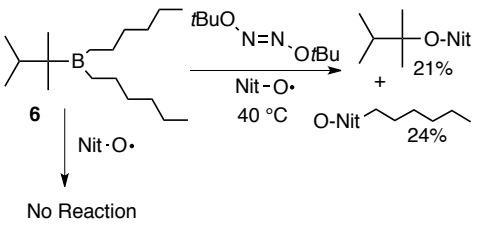



Scheme 3. Renaud's method of radical generation by addition of nitroxide to a *B*-alkylcatecholboronate ester **1**




Key to this urushiol detection is the seminal work by Renaud,[21-23] in which addition of two equivalents of the oxygen radical TEMPO **4**, a commercially available persistent nitroxide, results in formation of the carbon radical trapping product, *N*-alkoxyamine **5** (Scheme 3). We have found that trialkylboranes do not form a resonance-stabilized perboryl radical, and thus do not react with TEMPO. For example, trialkylborane **6** gave no reaction upon exposure to TEMPO, but did react with the "hotter" *t*-butoxy radical generated from stoichiometric di-*t*-butyl hyponitrite (Scheme 4).[24] Thus nitroxides react selectively with *B*-alkylcatecholboronate esters **1**, to generate *N*-alkoxyamines **5**.

Scheme 4. TEMPO did not add to trialkylborane **6**, however *t*BuO• generated from di-*t*-butyl hyponitrite did add to the trialkylborane **6**

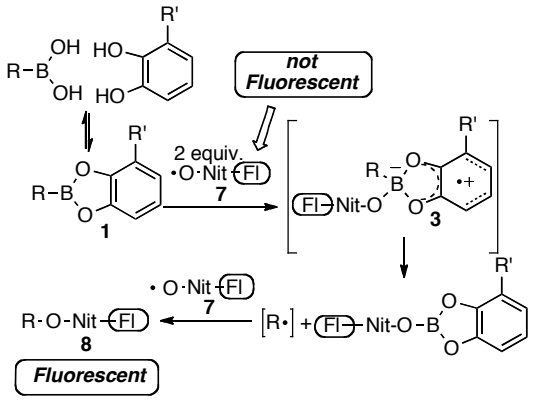



Profluorescent nitroxides[25-28] **7** (sometimes referred to as "pre-fluorescent nitroxides"[29]) are nitroxides bearing a short covalent tether to a fluorophore. The free nitroxide quenches the fluorescence (Figure 2). Upon reaction of the nitroxide with a carbon radical or reducing agent, to form an *N*-alkoxyamine **8** or an *N*-hydroxylamine, the fluorescence is restored. Reaction of a


**Figure 2.** Profluorescent Nitroxides **7**: the free nitroxide quenches fluorescence of a closely tethered fluorophore; fluorescence is restored upon reaction to form the *N*-alkoxyamine **8**.

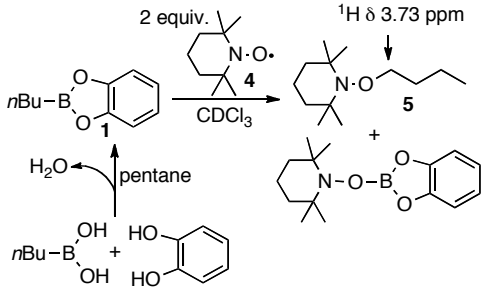
catechol with a *B*-alkyl boronic acid derivative to form a *B*-alkyl boronate ester **1** in situ, in the presence of a profluorescent nitroxide **7**, is expected to form a highly fluorescent *N*-alkoxyamine **8** (Scheme 5). Generation of a fluorescent signal would be a positive indicator for the presence of the catechol functionality.

Scheme 5. Fluorescence generation by condensation of a catechol with a *B*-alkyl boronic acid, followed by reaction with a profluorescent nitroxide **7**, to form a stable fluorescent *N*-alkoxyamine **8**



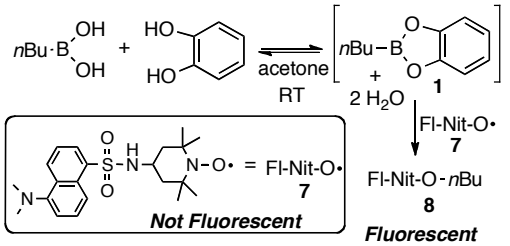

To explore the viability of this approach, a preformed *B*-alkylcatecholboronate ester **1** was prepared from *B*-*n*-butylboronic acid using Dean-Stark conditions in toluene. Although this was successful, a much easier procedure developed by Brown[30] provides the same *B*-*n*-butylcatecholboronate ester in minutes at room temperature: the reaction is run in pentane. Water generated under the condensation conditions phase separates, conveniently forming the catechol boronate **1** in good yield. Addition of two equivalents of orange-colored TEMPO **4** to an NMR tube containing one equivalent of the preformed *B*-*n*-butylcatecholboronate ester **1** resulted in the dissipation of the orange color, and formation of the *N*-*n*-butoxyamine of TEMPO **5** (Scheme 6), identified by the distinct *O*-methylene triplet at  3.73 in $CDCl_3$. Authentic *N*-*n*-butyloxyamine **5** was prepared from TEMPO **4** by the method of Whitesides[31] employing *n*-butyllithium, allowing confidence in the spectral assignment.

Scheme 6. Generation of the *N*-*n*-butoxyamine **5** from TEMPO **4** using preformed *B*-*n*-butylcatecholboronate ester **1**



Mixing two equivalents of profluorescent nitroxide dansyl-TEMPO[32] **7** with one equivalent of the preformed *B*-*n*-butylcatecholboronate ester **1** formed the highly fluorescent *N*-*n*-butoxyamine **8**; fluorescence was detected almost immediately. Even more gratifying was the generation of fluorescence from free catechol, via the reversible formation of the *B*-*n*-butylcatecholboronate ester **1** formed in situ. In this key experiment, two equivalents of dansyl-TEMPO **7** were mixed with one equivalent of *B*-*n*-butylboronic acid in acetone; addition of one equivalent of catechol under nitrogen immediately resulted in strong fluorescence upon illumination with a UV lamp (Scheme 7). The fluorescent *N*-alkoxyamine product **8** was isolated in 64% yield. It should be noted that alkyl radicals react with molecular oxygen at a rate of 5 x $10^9$ $M^{-1}$ $s^{-1}$ in hydrocarbon solvent,[33] whereas the rate of trapping of primary alkyl radicals by TEMPO is 1.2 x $10^9$ $M^{-1}$ $s^{-1}$,[34] approximately four times slower. Thus for the detection of urushiol in air, a significant portion of *n*-butyl radicals will trap $O_2$, forming peroxides, which will most likely undergo autooxidation. Thus the nitroxide trapping method will underestimate the amount of urushiol present, but enough fluorescent alkoxyamine will be formed to indicate the spatial distribution of urushiol contamination.

Scheme 7. Fluorescent *N*-alkoxyamine **8** formed by reaction with catechol using profluorescent nitroxide dansyl-TEMPO **7**




Spot tests on a paper towel give a visual display of the efficacy of this procedure in detecting the presence of catechol. In Figure 3a, the profluorescent dansyl-TEMPO **7** was spotted

against the fluorescent *N*-alkoxyamine **8**. As a control, a mixture of *B*-*n*-butylboronic acid and dansyl-TEMPO **7** was also spotted; illumination with a hand-held TLC fluorescent lamp (365 nm) only showed the *N*-alkoxyamine **8** to be fluorescent as observed by the human eye. A small sample of crystalline catechol likewise generated a spectacular fluorescent display upon addition of a drop of dilute *B*-*n*-butylboronic acid and dansyl-TEMPO **7** in acetone (Figure 3b).

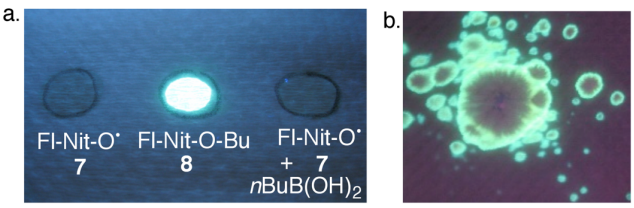



**Figure 3.** a. Spot tests (circled in pencil) on a paper towel of acetone solutions of dansyl-TEMPO **7**, the corresponding *N*-alkoxyamine **8**, and a control mixture of dansyl-TEMPO **7** and boronic acid; b. crystalline catechol on a paper towel upon addition of a dilute solution of *n*BuB(OH)$_2$ + Fl-NitO• **7** in acetone. Both are visualized by illumination under a TLC lamp at 365 nm.

Quantification of the fluorescent response was determined by fluorimetry. The fluorescence excitation and emission spectra of the profluorescent nitroxide dansyl-TEMPO **7**, and the corresponding *N*-alkoxyamine **8** are shown in Figure 4. The profluorescent nitroxide **7** does display a residual amount of fluorescent activity, but it is significantly suppressed compared to the *N*-alkoxyamine **8**.

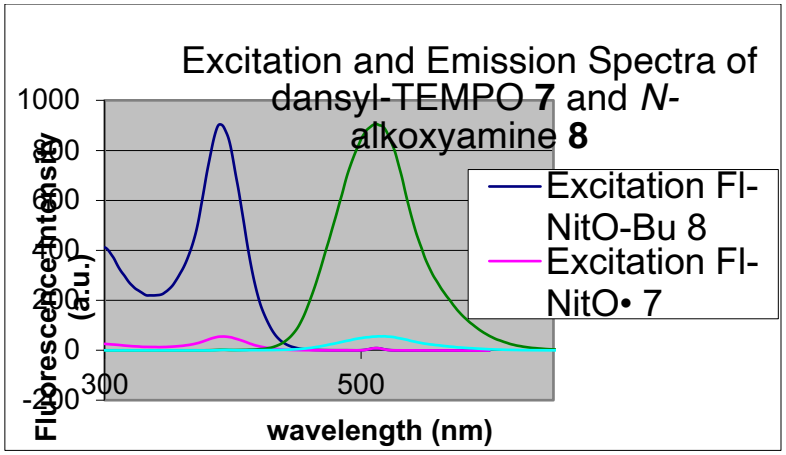



**Figure 4**. Fluorescence trace of dansyl-TEMPO **7** (excitation in red, emission in purple) and *N*-alkoxyamine **8** (excitation in blue, emission in green), $\lambda_{ex}$ = 350 nm, $\lambda_{em}$ = 512 nm.

In order to detect urushiol contamination, the generation of a fluorescent species should be rapid. Time studies (Figure 5) were conducted utilizing a 2 mL solution of dansyl-TEMPO **7** ($1.5 \times 10^{-3}$ M) and *B*-*n*-butylboronic acid ($7.5 \times 10^{-4}$ M) in acetone (fluorescence of this solution is shown at time zero). To this was added two mL of catechol ($1.5 \times 10^{-3}$ M) in

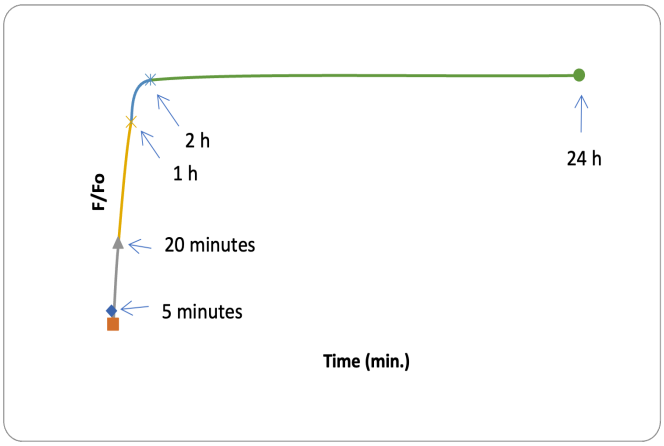



**Figure 5.** Time study of fluorescence upon addition of 3.0 µmol of catechol to a dilute acetone solution of 1.5 µmol of *B*-*n*-butylboronic acid and 3.0 µmol of dansyl-TEMPO **7** ($\lambda_{ex}$ = 350 nm, $\lambda_{em}$ = 512 nm).

acetone to give a 3.75 x $10^{-4}$ M solution containing a 2:1:2 molar ratio of dansyl-TEMPO:*n*Bu$(OH)_2$:catechol. The ratio of fluorescence intensity, $F/F_o$ (F = fluorescence and $F_o$ = initial fluorescence), was plotted as a function of time. The fluorescent signal increased over the first hour; by the second hour the fluorescence had reached a stable maximum that remained unchanged after 24 hours.

Optimization of the System

Acetone was the initial solvent examined, as precedent for safe human exposure is provided by the common use of acetone as fingernail polish remover. A few additional solvents were examined. The time study depicted in Figure 5 was repeated with THF, acetonitrile, and isopropanol as solvent; the fluorescent response as a function of time was compared to that using acetone (Figure 6). Acetone gave a rapid response, followed by THF and then acetonitrile. Isopropanol gave the slowest increase in the fluorescent signal, presumably as the nucleophilic solvent may compete with the catechol for reaction with the *B*-*n*-alkyl boronic acid. The *B*-*n*-butyl boronic acid is insoluble in dichloromethane and chloroform. The fluorescence reached the greatest intensity most quickly with acetone compared to the other solvents. Thus acetone continued to be the solvent of choice.

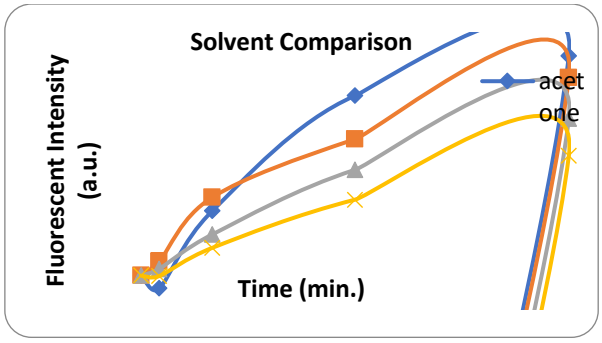


**Figure 6**. The effect of solvent on fluorescence intensity and reaction time in the reaction of catechol with profluorescent dansyl-TEMPO **7** and *B*-*n*-butyl boronic acid ($\lambda_{ex}$ = 350 nm, $\lambda_{em}$ = 512 nm).

Alkylboronic acids are renown for being marginally stable, dehydrating reversibly to the boroxine anhydrides,[35] which are particularly sensitive to degradation by oxygen.[36] Arylboronic acids, which also reactive in this manner, are more robust:[37] thus the possibility of replacing the *n*-butyl with a phenyl group was examined. In Figure 7, it is clear that the *B*-*n*-butylboronic acid is far more effective in generating a fluorescent signal than *B*-phenylboronic acid. This is not surprising, as the reaction mechanism for the formation of fluorescent *N*-alkoxyamine **8** entails generation of a carbon radical, following nitroxide addition to the catecholboronate ester **1**. The dissociation energy for a primary alkyl radical (for example, Et-H to Et•) has been measured at 101.1 ± 0.4 kcal/mole, whereas for a phenyl radical (Ph-H to Ph•) at 111.2 ±0.8 kcal/mole,[38] making the phenyl radical approximately 10 kcal/mole less stable than the butyl radical. Although anhydrous *B*-*n*-butylboronic acid tends to decompose upon storage, we found that a dilute acetone solution of *B*-*n*-butylboronic acid in the presence of water[39] was stable for at least several months. Thus the formulation of *B*-*n*-butylboronic acid as a component of a shelf-stable spray to detect urushiol should be viable. Although skin toxicity for $10^{-6}$ M *B*-*n*-butylboronic acid has not been determined, the use of the alkylboronic acid drug Velcade® (Bortezomib)[40] in chemotherapy for multiple myeloma, the use of the arylboronic acid 4-dihydroxy-borylphenylalanine (BPA) in boron neutron capture therapy,[41] and the topical use of the closely related benzoxaboroles as a broad spectrum antifungal,[42,43] make it likely that dilute solutions of an alkylboronic acid can be tolerated on the skin.

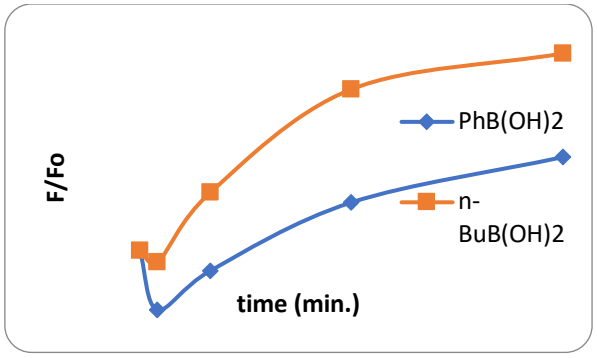



**Figure 7.** Comparison between the use of *B*-*n*-butylboronic acid and *B*-phenylboronic acid on fluorescence intensity and reaction time in the reaction of catechol with profluorescent nitroxide **7** and *B*-(alkyl or aryl)boronic acid ($\lambda_{ex}$ = 350 nm, $\lambda_{em}$ = 512 nm).

Possible Competing Reactions

Scaiano has shown that both catechols and phenols react slowly by transferring a phenoxy hydrogen to nitroxides,[44,45,44] and has demonstrated the use of profluorescent nitroxides to probe this antioxidant activity. In the urushiol detection system, reduction of the profluorescent nitroxide by phenols would form fluorescent *N*-hydroxyamines (second line, Scheme 8). In Figure 8, replacement of catechol by phenol resulted in only a negligible increase of fluorescence after 2 hours, indicating that phenols will not generate a significant fluorescent signal.

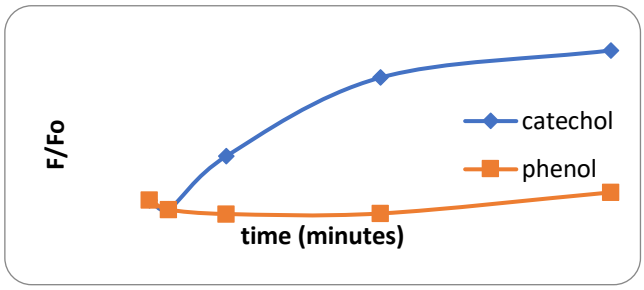
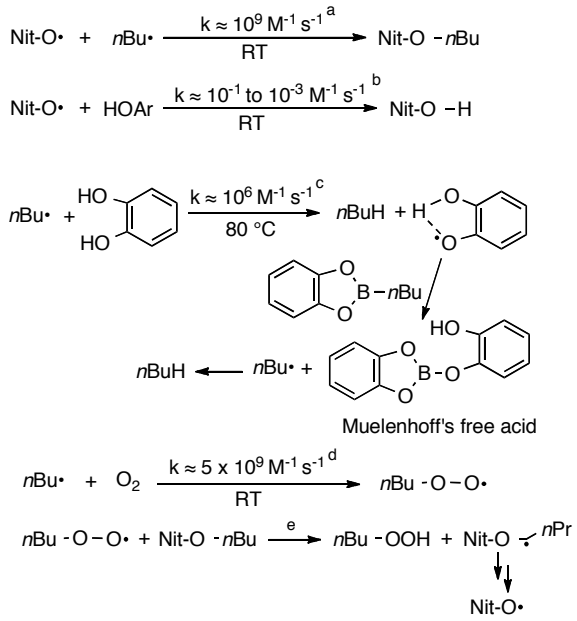



**Figure 8.** Time study of fluorescence upon addition of 3.0 □mol of phenol (red line) or 3.0 □mol of catechol (blue line) to a solution of 3.0 □mol dansyl-TEMPO and 1.5 μmol of *B*-*n*-butylboronic acid in acetone**.**

TEMPO **4** traps primary alkyl radicals at rate constants of approximately $10^9$ $M^{-1}$ $s^{-1}$ at room temperature[34] (this rate is slightly solvent dependant[46]). The rates of H-abstraction by TEMPO-type nitroxides from phenols such as Trolox and BHT in protic solvents have been measured to be $10^{-1}$ to $10^{-3}$ $M^{-1}$ $s^{-1}$ at room temperature.[44,47] Catechol has been shown to act as an effective hydrogen donor to carbon radicals.[48,49] A possible concern in the presence of high concentrations of urushiol might be the reduction of the *n*-butyl radical by abstraction of a hydrogen from the catechol moiety of urushiol (third line of Scheme 8). The resulting oxygen-centered catechol radical would then be expected to add to a *B*-*n*-butylcatecholboronate ester **1**, generating another *n*-butyl radical and Meulenhoff's free acid **9**.[48] The rate of hydrogen atom abstraction from catechols by alkyl radicals has been recently determined by Renaud to be $10^6$ $M^{-1}$ $s^{-1}$ at 80° C. Thus in the presence of large amounts of catechol (for example, 1 M), and very low concentrations of nitroxide (for example, $3.0 \times 10^{-3}$ M), these two reactions could become competitive, and would lead to an underestimation of the urushiol concentration. For the detection of trace amounts of urushiol, hydrogen abstraction from the catechol by alkyl radical is expected to be a very minor reaction. The trapping of molecular oxygen by the *n*-butyl radical is much more likely. The resulting peroxyl radical could participate in autooxidation, or abstract a $\beta$ hydrogen from alkoxyamine **8** to reform the nitroxide, a pathway recently unveiled by ab initio calculations.[50]

Scheme 8. Potential competing reactions




[a] reference 34; [b] reference 44,45; [c] reference 49; [d] reference 33, [e] reference 50.

As proof of concept for the detection of urushiol, a successful field test is demonstrated in Figure 9 with *Toxicodendron diversilobum* (western poison oak). A dilute acetone solution of dansyl-TEMPO **7** and *B*-*n*-butylboronic acid (2:1 molar ratio) was dripped onto a freshly harvested triad of poison oak leaves (figure 9a). A new paper towel was laid over the wet leaves, and pressure applied to make a "print." Immediate illumination with a long-wavelength UV TLC lamp resulted in the fluorescent image shown in Figure 9b. The particularly strong fluorescent spots are likely due to ruptures in the leaves while applying pressure, exposing the paper towel to localized areas containing high concentrations of urushiol.

a. 

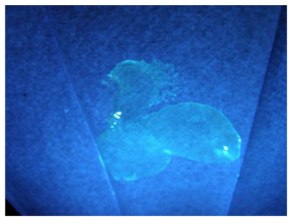
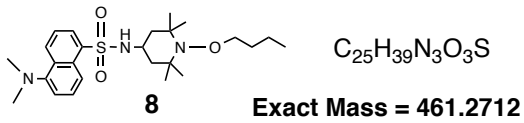
b. 

**Figure 9.** a. Fresh triad of poison oak leaves; b. print of the same leaves on a paper towel after treatment with a dilute acetone solution of dansyl-TEMPO **7** (3 □molar) and *B-n*-butylboronic acid (1.5 □molar), followed by illumination by a 365 nm TLC lamp.

Six months after the creating the fluorescent leaf image, a small piece of the still fluorescent print on the paper towel was analyzed by TOF-DART (Direct Analysis in Real Time) Mass Spectrometry:[51] the M+1 peak of the *N*-alkoxyamine product **8** was observed for both an authentic and paper towel-derived sample at 462.2808, confirming the formation and stability of the *N*-alkoxyamine **8** (Figure 10).



**8**

$C_{25}H_{39}N_3O_3S$

**Exact Mass = 461.2712**

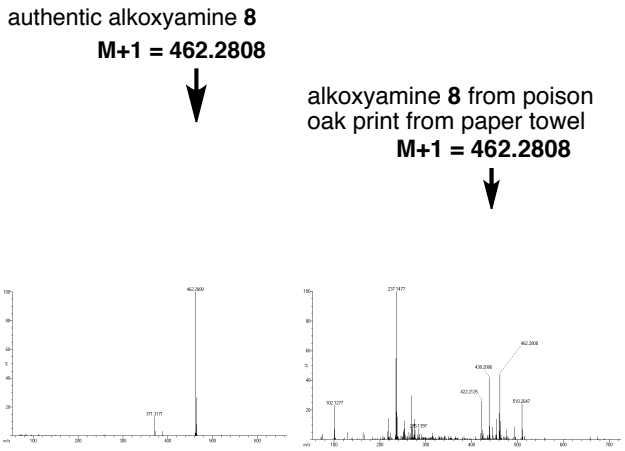



**Figure 10.** TOF-DART MS confirmation of the formation of *N*-alkoxyamine **8** from an authentic sample, and from the paper towel 6 months after the leaf print was prepared. (Note: the 371 peak is plasticizer on the glass rod used for sampling.)

Catecholamines, including dopamine, epinephrine and norepinephrine, are biologically important neurotransmitters. They are involved in the regulation of both the central and peripheral nervous systems, and are implicated in a number of diseases, including Alzheimer's[52]

and Parkinson's.[53] The ability to detect these species at very low concentration using this methodology based on *B*-alkylboronic acids and profluorescent nitroxides should lead to a number of biomedical applications. In preliminary experiments to detect L-DOPA in water, in analogy to urushiol in organic solvents, a fluorescent signal was observed. However, dansyl is a solvatochromic fluorophore;[54-55] dilute solutions of dansyl-TEMPO **7** with *B*-*n*-butylboronic acid in water gives a faint yet visible fluorescent signal *in the absence of catechol* (see Supporting Information). Use of the two-photon method[56] in a laboratory setting is an alternative strategy to avoid the fluorescence enhancement of dansyl fluorophore in water, but would not be applicable to a fast "in the field" test for urushiol. Thus a less solvatochromic fluorophore is needed. This sensitivity to water also impacts the urushiol detection system. Efforts are now being directed at examining alternative nonsolvatochromic fluorophores with non-hydrolyzable linkages to the nitroxide as the profluorescent nitroxide partner, in order to avoid generation of a weak signal in the presence of water.

## CONCLUSION

Fluorescent detection to illuminate the location of urushiol contamination from contact with *Toxicodendron* species has been developed, utilizing the reversible reaction of catechols with a *B*-*alkyl*boronic acids and a profluorescent nitroxide. The generation of the fluorescent signal is rapid: detection with a hand-held fluorescent lamp has been demonstrated, showing promise to identify the location of urushiol contamination in the home or field, without costly equipment, thus minimizing the severity of contact dermatitis for sensitive individuals. Investigation of the use of alternative fluorophores to dansyl is underway. The extension of this

methodology to detect catecholamines for biomedical applications is under active development in our laboratory.

## EXPERIMENTAL SECTION

**4-Azido-2,2,6,6-tetramethyl-1-piperidine-1-oxyl free radical (4-azido-TEMPO).** Following a modified procedure of Misharin *et al*,[57] a solution of 4-methylsulfonyloxy-TEMPO (4.045 g, 16.16 mmol) and sodium azide (2.100 g, 32.32 mmol) in DMSO (54 mL, 0.3 M) was heated to 110 °C under $N_2$ overnight. Upon cooling to rt, ether (450 mL) was added and the solution was washed with a saturated solution of sodium bicarbonate (200 mL) followed by water (4 x 100 mL). The organic layer was dried over sodium sulfate, filtered and then concentrated. The resulting solid was recrystallized from hexanes to yield orange crystals (2.283 g, 72%).

TLC $R_f$ = 0.83 (1:1 hexanes:ethyl acetate, UV, PAA).

IR ($CDCl_3$) 2099 $cm^{-1}$ (-$N_3$ stretching).

$^1$H-NMR (600 MHz, $CDCl_3$ + phenylhydrazine): $\delta$ 3.60 (tt, J=12.4, 4.2 Hz, 1H), 1.87 (dd, J=12.4, 4.2 Hz, 2H) 1.57 (dd, J=12.4, 12.4 Hz, 2H), 1.23 (s, 6H), 1.16 (s, 6H) ppm.

$^{13}$C-NMR (150 MHz, $CDCl_3$ + phenylhydrazine): $\delta$ 59.6, 52.8, 44.1, 32.2, 20.2 ppm.

**4-Amino-2,2,6,6-tetramethyl-1-piperidine-1-oxyl free radical (4-amino-TEMPO).** Following a modified procedure of Misharin *et al*,[57] to a flame-dried round bottom flask was added 4-azido-TEMPO (1.815 g, 9.201 mmol) and anh. THF (20 mL). Triphenylphosphine (4.827 g, 18.40 mmol) was added in portions over a period of three hours under $N_2$. Effervescence was observed. Aqueous $NH_4OH$ (20 mL, 14.8 M) was added and the reaction mixture was stirred overnight. Most of the solvent was removed in vacuo using gentle heating. The concentrated

reaction mixture was taken up in $CHCl_3$ (100 mL) before adding a solution of 10% acetic acid (50 mL). The phases were separated and 50 mL of 10% acetic acid solution was added. The combined aqueous layers were neutralized with solid sodium bicarbonate, followed by a small amount of 2N NaOH at the end of the neutralization to make the solution slightly basic as determined by pH paper. This solution was then extracted with $CHCl_3$ (3 x 100 mL). The organic layer was dried over sodium sulfate, filtered, and then concentrated to yield a viscous orange oil (1.071 g, 70%).

TLC $R_f$ = 0.58 (1:1 ethyl acetate:methanol, UV, PAA).

$^1$H-NMR (600 MHz,$CDCl_3$ + phenylhydrazine): $\delta$ 3.05 (tt, J=15.2, 4.8 Hz, 1H), 1.74 (dd, J= 15.2, 4.8 Hz, 2H) 1.28 (dd, J=15.2, 15.2 Hz, 2H), 1.16 (s, 6H), 1.14 (s, 6H) ppm.

$^{13}$C-NMR (150 MHz, $CDCl_3$ + phenylhydrazine): $\delta$ 59.2, 49.7, 42.3, 32.6, 20.2 ppm.

***N*-dansyl-TEMPO (7).** Following a modified procedure,[58] dry $CH_2Cl_2$ (67.0 mL), 4-amino-TEMPO (1.439 g, 8.402 mmol), dansyl chloride (2.266 g, 28.402 mmol) and triethylamine (2.3 mL, 16.8 mmol, freshly distilled from calcium hydride) were added to a flame-dried round bottom flask and place under $N_2$. The reaction mixture was allowed to stir for 16.5 h. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (2:1 hexanes:ethyl acetate) to yield a fluffy orange solid (3.04 g, 89%).

TLC $R_f$ = 0.48 (1 :1 hexanes:ethyl acetate, UV, $I_2$, PAA).

$^1$H-NMR (600 MHz, $CDCl_3$ + phenylhydrazine): $\delta$ 8.54 (d, J=10.2 Hz, 1H), 8.28-8.26 (m, 2H), 7.54-7.50 (m, 2H), 7.17 (d, J=9 Hz, 1H), 3.51-3.45 (m, 1H), 2.88 (s, 6H), 1.52-1.40 (m, 4H), 1.10 (s, 6H), 1.00 (s, 6H) ppm.

$^{13}$C-NMR (150 MHz, $CDCl_3$ + phenylhydrazine): $\delta$ 151.3, 135.9, 130.5, 129.9, 129.7, 129.4, 128.8, 123.2, 118.8, 115.2, 59.2, 46.2, 45.8, 45.4, 32.1, 19.5 ppm.

***B*-*n*-butylcatecholboronate ester** (**1**). Method A (Dean Stark): Following a modified procedure from Green and Wagner,[59] to a flame-dried 25 mL round bottom flask was added toluene (8 mL), catechol (324 mg, 2.94 mmol) and *B*-*n*-butylboronic acid (300 mg, 2.94 mmol). A Dean Stark apparatus was attached and the solution was refluxed for 4 h. A separate water layer formed in the Dean Stark trap. The solution was concentrated in vacuo to yield 516 mg of the title compound as a yellow oil (99.6 % crude yield; $^{1}$H NMR shows contamination with traces of toluene).

Method B (in pentane): Following a modified procedure of Brown et. al,[60] to a flame-dried 10 mL round bottom flask was added catechol (216 mg, 1.96 mmol), *B*-*n*-butylboronic acid (200 mg, 1.96 mmol), and small scoop of sodium sulfate. The contents of the flask were dissolved in 1.6 mL of pentane and stirred under nitrogen at room temperature for 50 minutes. The solid was removed by filtration and the solvent removed in vacuo to give 287 mg (83.2 %) of the desired product as a yellow oil.

Note: it has been previously reported[61] that the carbon attached to the boron is not visible by $^{13}$C-NMR due to the quadrupole relaxation of $^{11}$B. In addition, $^{1}$H-$^{11}$B ($^{11}$B, I=3/2) coupling in the $^{1}$H-NMR spectrum of the butyl fragment is evident in the multiplet seen at 1.64-1.59 ppm.

$^{1}$H-NMR (600 MHz, $CDCl_3$): $\delta$ 7.21 (dd, J=7.2, 4.2 Hz, 2H), 7.07 (dd, J=7.2, 4.2 Hz, 2H), 1.64-1.59 (m, 2H), 1.45-1.39 (m, 2H), 1.32-1.29 (m, 2H), 0.96-0.93 (t, J=9.9 Hz, 3H) ppm.

$^{13}$C-NMR (150 MHz, $CDCl_3$): 148.3, 122.5, 112.2, 25.8, 25.2, 13.7 ppm.

**1-*n*-Butoxy-2,2,6,6-tetramethylpiperidine** (**5**). A. Preparation of authentic sample: Following the procedure of Whitesides,[62] a flamed-dried 25 mL round bottom flask was charged with

TEMPO 4.28 (500 mg, 3.20 mmol) and dry toluene (17.8 mL). The solution was stirred and cooled to -78 °C under nitrogen before adding *n*-butyllithium (0.90 mL, 2.24 mmol, 2.5 M in hexanes) dropwise. The reaction mixture was allowed to warm to room temperature, and then washed with 18 mL of water. The solution was dried over magnesium sulfate, filtered and concentrated. The product was purified by flash column chromatography (4:1, hexanes:ethyl acetate) to give the desired product as a yellow oil (358 mg, >100%, slightly contaminated with the *N*-hydroxylamine).

TLC $R_f$ = 0.69 (4:1, hexanes:ethyl acetate, UV, $I_2$, PAA).

$^1$H-NMR (500 MHz, $CDCl_3$): δ 3.73 (t, J=6 Hz, 2H), 1.51-1.31 (m, 10H), 1.15 (s, 6H), 1.09 (s, 6H), 0.92 (t, J=6 Hz, 3H) ppm.

$^{13}$C-NMR (125 MHz, $CDCl_3$): δ 70.4 33.5, 32.0, 27.0, 24.8, 14.0, 13.6, 11.1, 8.2 ppm.

B. Formation by reaction of TEMPO **4** with *B-n*-butylboronic ester **1** in an NMR tube: an NMR tube was charged with *B-n*-butylboronic ester **1** (10.5 mg, 0.0600 mmol), $CDCl_3$ (700 L) and TEMPO **4** (15.0 mg, 0.100 mmol). The tube was capped and $^1$H-NMR spectra were taken to monitor the reaction. Spectral data was consistent with that of the authentic sample prepared previously. In particular, the -$OCH_2$- peak in the $^1$H-NMR at 3.73 (t) was diagnostic of the formation of *N*-alkoxyamine **5**. (*Note:* the product **5** is volatile, and is easily lost on the vacuum pump.)

*O-n*-Butyl-dansyl-TEMPO (**8).** A. Preparation of authentic sample: following the literature procedure,[57] a solution of dansyl-TEMPO 4.16 (500 mg, 1.24 mmol) in dry toluene (17.8 mL, 0.18M) was cooled to -78 °C before adding *n*-butyllithium (2.5 M in hexanes, 0.35 mL, 0.87 mmol) dropwise. The reaction mixture was allowed to warm to room temperature, and then

was quenched with water (17 mL). The organic layer was separated and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (2:1 hexanes:ethyl acetate) to give the title compound as a viscous oil (239 mg, 83.9 %).

TLC $R_f$ = 0.27 (2:1 hexanes:ethyl acetate, UV, $I_2$, PAA).

IR ($CDCl_3$): 1574 (N-O) 1373 (N-O) 1318 (S-O) 1143 (S-O) $cm^{-1}$.

$^1$H-NMR (600 MHz, $CDCl_3$): $\delta$ 8.54 (d, J=8.4 Hz, 1H), 8.28-8.26 (m, 2H), 7.58-7.51 (m, 2H), 7.19 (d, J=7.2 Hz, 1H), 3.61 (t, J=6.6 Hz, 2H), 3.43-3.40 (m, 1H), 2.89 (s, 6H), 1.57 (s, NH), 2.05-1.20 (m, 8H), 1.00 (s, 6H), 0.94 (s, 6H), 0.89-0.85 (m, 3H) ppm.

$^{13}$C-NMR (150 MHz, $CDCl_3$): $\delta$ 152.1, 135.8, 130.4, 129.9, 129.7, 129.2, 128.4, 123.2, 118.7, 115.2, 60.5, 59.6, 46.3, 45.9, 45.4, 32.6, 30.7, 20.3, 19.5, 14.0 ppm.

HRMS (ESITOF) Calcd for $C_{25}H_{40}N_3O_3S$ (M + 1) 462.2785; Found 462.2785.

B. Formation of **8** by reaction of dansyl-TEMPO with *B-n*-butylboronic acid and catechol: catechol (136 mg, 1.24 mmol) was added to an acetone solution (24.7 mL) of dansyl-TEMPO (1.00 g, 2.47 mmol), $PbO_2$ (591 mg, 2.47 mmol), and *B-n*-butylboronic acid (126 mg, 1.24 mmol) under nitrogen and stirred overnight. The next day, the reaction mixture was filtered through a pad of Celite® and the solvent removed in vacuo. The resulting oil was dissolved in 25 mL of $CH_2Cl_2$ and washed with 2 M NaOH (2 x 25 mL) followed by 25 mL of deionized water. The solution was dried over $MgSO_4$, filtered and then concentrated in vacuo. The crude product was purified by flash column chromatography (2:1, hexanes:ethyl acetate) to give the title compound as a viscous oil (64%). Spectral data was consistent with that of the authentic sample prepared with *n*-butyl lithium. (Note: Lead oxide was initially used to ensure the nitroxide was not reduced by the catechol to the *N*-hydroxyamine, however it was later found to be unnecessary, and was not used in subsequent experiments.)

$R_f$ = 0.27 (hexanes:ethyl acetate, 2:1, UV, $I_2$, PAA).

## ACKNOWLEDGMENT

The University of California Santa Cruz Committee on Research is acknowledged for partial support of this research. We thank JEOL for obtaining the DART MS data. We thank a reviewer for pointing out the possible use of the two-photon method to circumvent the fluorescence enhancement of dansyl-TEMPO in water. We thank another reviewer for a discussion of side reactions in the presence of oxygen.

**Supporting Information**. General experimental methods, spot tests of dansyl-TEMPO **7** and *B-n*-butylboronic acid on a paper towel with water, and with catechol, $^1$H and $^{13}$C-NMR spectra of all intermediates and products, and the DEPT spectrum of *O-n*-butyl-dansyl-TEMPO **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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