

β -Thujaplicin: New Quantitative CZE Method and Adsorption to Goethite

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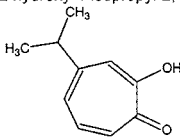
β -Thujaplicin (β -TH) is a toxic tropolone derivative present in the heartwood of western red cedar (*Thuja plicata*) and is used as a preservative and antimicrobial additive in a number of commercial goods. β -TH released from western red cedar timber used outdoor and from other products containing β -TH may transfer to soil and leach to groundwater and surface waters. The objective of this study was to quantify the adsorption of β -TH to goethite as a typical model for geosorbents. Adsorption was studied using pH-adjusted goethite suspensions with solid:solution ratios of 1:500, 0.01 M NaNO₃ electrolyte, and 20 °C. β -TH was determined using a new capillary zone electrophoresis (CZE) method providing a detection limit of 0.21 μ M. Near-sorption equilibrium was attained within 48 h. β -TH showed maximum adsorption at low pH (3.8) and a 70% drop in adsorption from pH 6.2 to 8.8. The Langmuir type adsorption isotherm at pH 5.5 approached a maximum adsorption of 220 μ mol/g (= 6.2 μ mol/m²), which is more than twice the amount of phosphate adsorbed under similar conditions. The affinity of β -TH for goethite is low as compared with organic ligands such as citrate, oxalate, and 2,4-dihydroxybenzoate. The adsorption data and FTIR analyses indicate that β -TH is most likely adsorbed as monodentate mononuclear surface complexes at the surface of goethite. Hydrophobic adsorption is thought to contribute to the adsorption, in particular at low pH. The strong adsorption of β -TH to goethite suggests low mobility in most soil environments, the risk of contamination increasing in soils with high pH (calcareous material), low contents of iron and aluminum oxides, phyllosilicates, and organic matter.

KEYWORDS: Adsorption envelope; adsorption isotherm; isopropyltropolone; hinokitiol; capillary zone electrophoresis; biotoxin; *Thuja plicata*

INTRODUCTION

β -Thujaplicin (β -TH) is a naturally occurring toxic compound belonging to the class of tropolones that contain an unsaturated seven member carbon ring (**Table 1**). Thujaplicins are isopropyl-substituted tropolones and exist in three structural isomers: α -, β -, and γ -thujaplicin (2). All isomers, in particular β -TH, are naturally occurring in several cupressaceous trees and shrubs such as western red cedar (*Thuja plicata* Donn), eastern white cedar (*Thuja occidentalis* L.), and hiba (*Thujopsis dolabrata* Sieb. et Zucc) (10). β -TH is the most intensively studied isomer and is well-known for its inhibitory effects on the growth of fungi, bacteria, insects, and plants (6, 7, 11). The content of thujaplicins is the primary reason of the well-known decay resistance of western red cedar, rendering heartwood of this species an invaluable material for outdoor woodwork (12, 13). β -TH also finds use as a food additive and in cosmetics due to

Table 1. Chemical Structure and Selected Properties of β -TH

Chemical name	β -thujaplicin
Synonyms	β -isopropyltropolone, hinokitiol
CAS no.	499-44-5
IUPAC name	2-hydroxy-4-isopropyl-2,4,6-cycloheptatriene-1-one
Chemical structure	
Molecular formula	C ₁₀ H ₁₂ O ₂
Molar mass	164.20 g·mol ⁻¹
pK _a	7.29 at 25 °C, I=0.0 (1)
Solubility in water	1.0 mM for the acid form (2) 7.3 mM at pH 9.5 at 25 °C (3)
log K _{ow}	1.89 (4) 1.83 (5)
log K _{oc}	1.0 (5)
MIC ^a (<i>Staphylococcus epidermis</i>)	0.2 mg·l ⁻¹ (6)
MIC ^a (<i>Daedalea dickinsii</i>)	0.2 mg·l ⁻¹ (7)
LD ₅₀ (mice)	191 mg·kg ⁻¹ (8) 256 mg·kg ⁻¹ (9)

^a MIC = minimum inhibitory concentration.

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its antibacterial properties (14). Recently, Matsumura et al. (8) have demonstrated β -TH to be toxic to cancer cells.

In western red cedar, β -TH is almost exclusively found in the heartwood (13) and thus may be expected to transfer to terrestrial environments from dead trees, twigs, and slash. The use of β -TH as a preservative in a number of commercial products and of western red cedar timber as fence posts, roofs, and wall coverings of houses represents other sources of β -TH. Different authorities now recommend the use of heartwood of western red cedar as an environmentally friendly alternative to pressure-impregnated wood. The natural occurrence and increasing commercial use of β -TH combined with its relatively high toxicity emphasize the need for evaluating the fate of β -TH in the terrestrial environment, including soils, surface water, and groundwater. Sorption of β -TH in soils and sediments is expected to be a major determinant of β -TH mobility in terrestrial ecosystems.

β -TH and tropolones in general form strong complexes with metal cations and in particular iron(III) (15). Hence, iron(III) (hydr)oxides (henceforth called iron oxides) are considered important adsorbents for β -TH in soil. This is based on the general correlation between stability constants of metal complexes with particular ligands in solution and constants for the binding of the same ligands to the corresponding metal oxides (16). Compounds adsorbed in this way, i.e., by ligand exchange, are said to form inner sphere surface complexes, which are typical for the binding of organic acids to goethite (17, 18). In the present work, goethite (α -FeOOH) was chosen as the model system since it is a common iron oxide in most soils with a large reactive surface and with a well-defined crystal structure (19).

Quantitative determination of β -TH usually includes derivatization prior to analysis with high-performance liquid chromatography (HPLC) (20, 21) and gas chromatography (22, 23). To our knowledge, no capillary electrophoresis (CE) method for tropolone or its derivatives exists. Advantages of CE include that it is a cheap and robust method that only requires small sample volumes and a minimum of organic solvents. Hence, as part of the present study, we developed a new capillary zone electrophoresis (CZE) method to determine β -TH in aqueous solutions excluding the use of organic solvents or derivatization. The objectives of this work were to develop a CZE method for determination of β -TH directly in aqueous solutions and to apply this method to studies of adsorption of β -TH to goethite. The bonding of β -TH to goethite is discussed, and likely implications for β -TH mobility in soils are evaluated.

MATERIALS AND METHODS

Materials and Chemicals. Goethite (α -FeOOH) was synthesized by precipitation of Fe(III) from $\text{Fe}(\text{NO}_3)_3$ solutions using KOH followed by aging at 70 °C for 60 h (19). The repeatedly washed product was freeze-dried, ground in an agate mortar, and stored in the solid form. The purity of the goethite was verified by X-ray diffraction (Siemens X-ray Diffractometer D5000) and by IR transmission spectroscopy (Perkin-Elmer System 2000 FT-IR Spectrometer). After the sample was degassed for 19 h at room temperature, the specific surface area was determined to 35.6 m²/g with five point N²/BET determinations (Micromeritics Gemini III 2375).

β -TH (99%) was obtained from Sigma-Aldrich (Milwaukee, WI). Tetradecyltrimethylammonium bromide (TTAB) (99%) was obtained from Sigma (St. Louis, MO). Ethylenediaminetetraacetic acid tetrasodium salt (Na_4EDTA) (98%) was obtained from BDH (Poole, Dorset, U.K.). Boric acid (H_3BO_3) (99.8%) was obtained from Riedel-deHaën (Seelze, Germany). Sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) (99.0%) and sodium nitrate (NaNO_3) (99.5%) were obtained from Merck (Darmstadt, Germany). All chemicals were p.a. quality. Milli-Q water, i.e., triple deionized water, UV radiated, and filtered through 0.1 μm membrane filter (Milli-Q Element System Millipore) was used in all experiments.

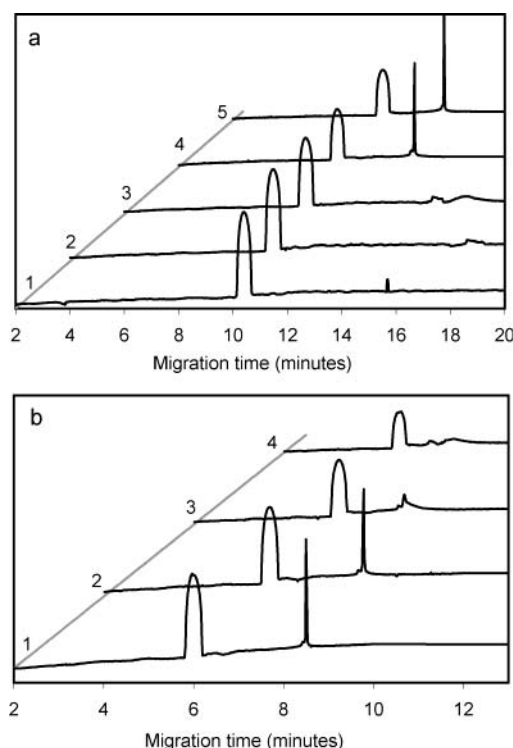


Figure 1. Electropherograms of 5 μM β -TH (the sharp peak) (a) 400 mM borate electrolyte at pH 1, 9.5; 2, 9.3; 3, 9.0; 4, 8.5; and 5, 8.3. (b) Borate electrolyte at pH 8.5 with a concentration of 1, 500 mM; 2, 400 mM; 3, 300 mM; and 4, 200 mM. The sharp peaks are signals for β -TH, and the broad peaks are caused by EOF (electro-osmotic flow).

β -TH has been referred to as slightly soluble in water by some authors (24, 25), and the water solubility of the basic and acid form of β -TH has been determined experimentally to approximately 7.3 (3) and 1.0 mM (2), respectively (Table 1). To ascertain that aqueous stock solutions were not supersaturated β -TH, stock solutions of 600 μM were prepared and found to be stable for periods of at least 3 months. All solutions of β -TH were prepared in amber glass or by wrapping laboratory equipment with aluminum foil, because β -TH is degraded by light (24). Borate electrolytes for CZE were prepared from boric acid adjusted to the appropriate pH with 1.0 M NaOH. The electrolytes were degassed by vacuum before use.

Instrumentation. β -TH was determined with a P/ACE 5510 CE instrument (Beckman, Fullerton, CA), equipped with a diode array detector. All experiments were performed in a 50/57 cm uncoated fused silica capillary with 75 μm i.d. (Composite Metal Services, Worcester, U.K.).

CZE Method Development. A quantitative method for determination of β -TH in aqueous solutions was developed based on sodium phosphate or sodium borate as the buffer component. All sodium phosphate electrolytes tested resulted in peak splitting in the electropherograms of pure β -TH under all conditions tested, i.e., 20–100 mM, pH 7.0–8.2, separation voltage 10–25 kV, and sample volumes from 30 to 90 nL. Furthermore, addition of the electrophoretic modifiers methanol, ethanol, 1-propanol, acetonitrile, or TTAB below the critical micelle concentration (CMC) to the electrolyte or samples were tested, but none of these modifications resulted in a single sharp peak for β -TH. Borate electrolytes with 90–150 mM and pH 8.5–9.0 produced one low broad peak for all combinations with applied voltage from 15 to 30 kV, temperatures from 15 to 35 °C, sample volumes from 30 to 90 nL, and addition of ethanol and acetonitrile to electrolytes and samples. However, borate electrolytes with 400–500 mM at pH 8.3–8.5 resulted in one sharp peak from β -TH in contrast to concentrations of 200–300 mM and at pH 9.0–9.5 (Figure 1). Further optimization of the applied voltage, sample volume, and temperature resulted in the final procedure for the CZE method used for the determination of β -TH in the adsorption experiments.

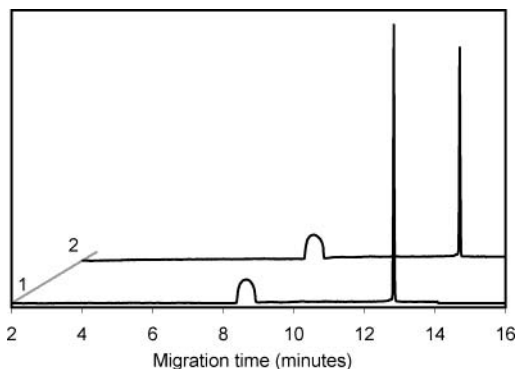


Figure 2. Electropherograms of (1) a sample containing $22 \mu\text{M}$ $\beta\text{-TH}$ (0.01 M NaNO_3 and $2 \text{ mM Na}_4\text{EDTA}$) and (2) a standard containing $16 \mu\text{M}$ $\beta\text{-TH}$ (0.01 M NaNO_3 and $2 \text{ mM Na}_4\text{EDTA}$).

Electrophoretic Conditions of the CZE Method. Samples were introduced hydrodynamically at 0.5 psi (3.5 kPa) for 15 s (90 nL) followed by a 3 s introduction of running electrolyte from a separate vial to minimize peak tailing and contamination of the running electrolyte. During sample introduction, the outlet end of the capillary was placed in a separate vial with electrolyte to avoid siphoning and to increase sample volume reproducibility (26). The running electrolyte consisted of $500 \text{ mM H}_3\text{BO}_3$ adjusted to $\text{pH } 8.5$ with NaOH . The capillary was held at a constant temperature of $23 \text{ }^\circ\text{C}$. The applied voltage was 15 kV (current approximately $110 \mu\text{A}$), and the UV detection was carried out at 250 nm .

The capillary was preconditioned daily before use by rinsing for 20 min with 0.1 M NaOH followed by 4 min with water. Between each run, the capillary was rinsed for 15 s with water, 2 min with 0.1 M NaOH , 1 min with water followed by preconditioning for 5 min with the electrolyte (i.e., 30 capillary volumes). At the end of each day, the capillary was rinsed for 4 min with 0.1 M NaOH , 4 min with water, and blown empty for 4 min with air (20 psi or 138 kPa) to avoid precipitations in the capillary during storage.

The limit of detection (LOD) for $\beta\text{-TH}$ was $0.21 \mu\text{M}$ determined as three times the standard deviation of 15 repeated measures of one standard solution containing $2.1 \mu\text{M}$ $\beta\text{-TH}$ ($\text{SD} = 0.074 \mu\text{M}$, $\text{CV } 3.5\%$), 10 mM NaNO_3 , and $2 \text{ mM Na}_4\text{EDTA}$. The standard curve for $\beta\text{-TH}$ is linear from $0.25 \mu\text{M}$ to the upper limit of quantification at about $50 \mu\text{M}$ (R^2 is $0.997\text{--}0.999$ for different days) since the peaks for higher concentrations of $\beta\text{-TH}$ show fronting and splitting. Electropherograms of a standard and a sample are shown in **Figure 2**. The method was found to be robust within $\text{pH } 8.3\text{--}8.5$, borate concentration $400\text{--}500 \text{ mM}$, sample injection volume $40\text{--}90 \text{ nL}$, and capillary temperature $20\text{--}30 \text{ }^\circ\text{C}$.

Adsorption Experiments. The adsorption of $\beta\text{-TH}$ to goethite was investigated as a function of time, pH , and total concentration of $\beta\text{-TH}$ in 0.010 M NaNO_3 at room temperature. Preliminary experiments with standards in tetra replicate and statistically compared at the 95% significance level showed that $\beta\text{-TH}$ does not adsorb to Pyrex glass and Teflon but adsorbs strongly to polypropylene and polyethylene tubing during periods of 48 h . Hence, centrifuge tubes made of Pyrex glass were used throughout the study.

In all adsorption experiments, a fixed amount of goethite (0.0500 g) was weighed out to 50 mL Pyrex glass centrifuge tubes with Teflon-lined screw caps. Aqueous NaNO_3 and $\beta\text{-TH}$ solutions were added to obtain $480 \mu\text{M}$ $\beta\text{-TH}$ for adsorption kinetics and $24\text{--}580 \mu\text{M}$ $\beta\text{-TH}$ for adsorption isotherm, and the pH was adjusted with NaOH or HNO_3 solutions making up a final volume of 25 mL . The centrifuge tubes were capped and placed in an end-over-end rotator (5.5 rpm) for 48 h . After the equilibration period, the pH was determined in aliquots of suspensions before centrifugation. The tubes were centrifuged, and 10 mL of the supernatant was withdrawn by a polypropylene syringe and filtered through a $0.45 \mu\text{m}$ regenerated cellulose membrane filter (Sartorius, Goettingen). The first 2 mL of filtrate was discarded based on results of initial experiments. The filtrate was added to $2 \text{ mM Na}_4\text{-EDTA}$ to mask metal cations, primarily iron(III), before analysis with

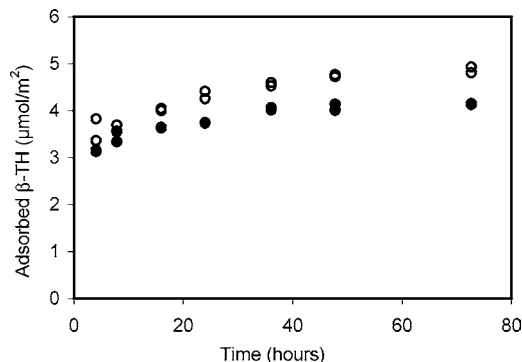


Figure 3. Effect of time on adsorption of $\beta\text{-TH}$ to goethite at $\text{pH } 4$ (\circ) and $\text{pH } 7$ (\bullet) (initial $\beta\text{-TH}$ concentrations $480 \mu\text{M}$, 0.01 M NaNO_3 electrolyte, goethite concentration 2.0 g/L , $20 \text{ }^\circ\text{C}$).

CZE (27). Adsorbed concentrations of $\beta\text{-TH}$ were calculated by the difference between total and equilibrium concentrations.

Contamination of the samples with metal cations and organic compounds was minimized by washing the laboratory equipment in a dishwasher that included rinsing in weak acid followed by deionized water. Utensils made of polyethylene were acid washed in 10% HCl for 30 min to remove metal contaminations and rinsed six times with deionized water. HCl -washed polyethylene and glass utensils were acid washed in 6.5% p.a. HNO_3 for 1 h and then rinsed six times with deionized water followed by one time with triple deionized water. Adsorption isotherms were fitted with the Langmuir equation using the software TableCurve 2D (28).

IR Analysis. Transmission FT-IR spectra of powdered $\beta\text{-TH}$, freeze-dried goethite suspension, and freeze-dried $\beta\text{-TH}$ -goethite were obtained using a Perkin-Elmer System 2000 FT-IR instrument. Spectra were scanned from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and averaged over 25 single scans. The $\beta\text{-TH}$ -goethite was prepared by the same procedure as used for the adsorption experiments, and after separation from the supernatant by centrifugation, the $\beta\text{-TH}$ goethite samples were freeze-dried, ground in an agate mortar, and mixed with powdered KBr to a sample content of 1 mg/cm^2 .

RESULTS AND DISCUSSION

Adsorption of $\beta\text{-TH}$ to Goethite. Adsorption Kinetics. Preliminary studies of the time dependence of the adsorption of $\beta\text{-TH}$ to goethite at $\text{pH } 4$ and 7 showed that about 75% of maximum adsorption was reached within 4 h and that the system was near equilibrium after 48 h (**Figure 3**). The fast initial adsorption indicates adsorption to readily accessible surface sites followed by a much slower adsorption to less accessible sites perhaps caused by steric hindrance. At $\text{pH } 4$, approximately 16% more $\beta\text{-TH}$ was adsorbed than at $\text{pH } 7$.

pH Dependence. The adsorption envelope for $\beta\text{-TH}$ to goethite, i.e., a plot of the maximum adsorbed amount vs pH (29), showed high and almost constant adsorption of $4.3 \mu\text{mol/m}^2$ from $\text{pH } 3.8$ to 6.2 , corresponding to 64% of total $\beta\text{-TH}$ added (**Figure 4**). Above $\text{pH } 6.2$, adsorption decreased to $1.8 \mu\text{mol/m}^2$ at $\text{pH } 8.8$, corresponding to 27% of total $\beta\text{-TH}$ added. This decreasing adsorption along with increasing pH was also observed in the preliminary kinetic studies. The adsorption envelope of monoprotic inorganic acids (30) and monoprotic organic acids (17, 18) bound to goethite by inner sphere surface complexation typically shows maximum adsorption at a pH near the pK_a . As is seen in **Figure 4**, this is not the case for adsorption of TH to goethite. The unexpected high adsorption at low pH indicates that hydrophobic interactions contribute to adsorption of $\beta\text{-TH}$ to goethite at low pH .

Adsorption Isotherm. The adsorption of $\beta\text{-TH}$ to goethite displayed a hyperbolically shaped isotherm at $\text{pH } 5.5$ (**Figure**

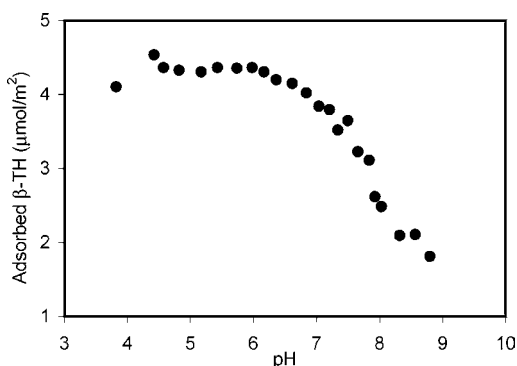


Figure 4. Effect of pH on β -TH adsorption to goethite (initial β -TH concentrations 480 μ M, 0.01 M NaNO₃ electrolyte, goethite concentration 2.0 g/L, 20 °C).

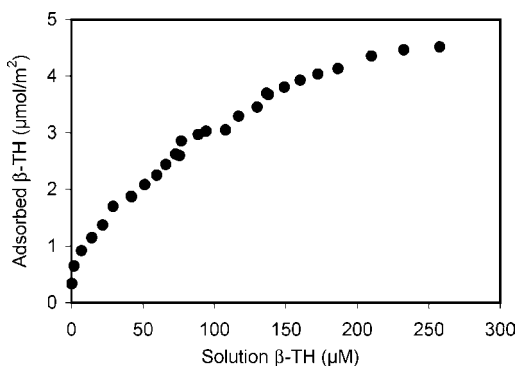


Figure 5. Adsorption isotherm for β -TH binding to goethite at pH 5.5 (0.01 M NaNO₃ electrolyte, goethite concentration 2.0 g/L, 20 °C).

Table 2. Langmuir Equation Parameters for Ligand Adsorption to Goethite^a

ligand	K_{ads} (L/ μ mol)	Γ_{max} (μ mol/m ²)	R^2
phosphate (40, 41)	62	2.2	0.736
citrate (32)	0.47	1.4	0.837
2,4-dihydroxybenzoate (31)	0.24	0.27	0.965
oxalate (42)	0.17	2.0	0.982
β -TH	0.010	6.2	0.971
fluoride (39)	0.0051	4.6	0.975
lactate (32)	0.0014	1.2	0.997

^a Partly based on data from literature.

5) as often seen for adsorption of organic acids to goethite (31, 32). The isotherm was described well by the Langmuir equation with an adsorption maximum (Γ_{max}) of 6.2 μ mol/m² (= 220 μ mol/g) and an affinity constant (K_{ads}) of 0.010 L/ μ mol (Table 2).

In this study, the distribution coefficient (K_d) for β -TH between goethite and solution lies between 600 and 40 000 L/kg, and hydrophobic adsorption of β -TH directly to the goethite surface does not seem likely since this is presumed to yield an almost linear isotherm (33). The decreasing fraction of adsorbed β -TH with increasing solution concentration indicates saturation of adsorption sites at the goethite surface and thus inner sphere surface complexation (17, 18).

Adsorption Mechanism. Transmission FT-IR spectra of freeze-dried β -TH-goethite suspensions showed absorbance bands at 3130, 1790, 1659, 1384, 888, 794, and 646 cm^{-1} like IR spectra of freeze-dried goethite suspensions (data not shown). The band at 1384 cm^{-1} is due to NO₃⁻ (19) used as electrolyte. In addition, the IR spectra of β -TH-goethite displayed absorbance bands at 1589, 1574, 1506, 1422, 1364, and 1238 cm^{-1} , whereas

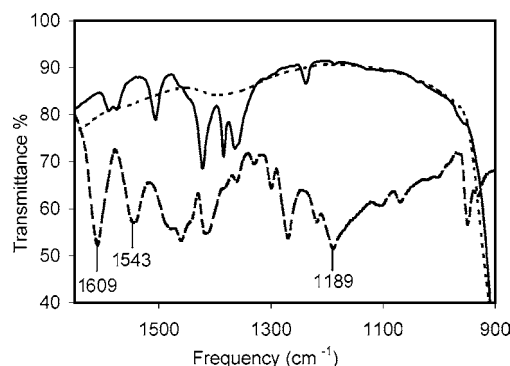


Figure 6. Transmission FT-IR spectra (1650–900 cm^{-1}) of freeze-dried goethite solution (---), powdered β -TH (---), and freeze-dried β -TH-goethite solution (—).

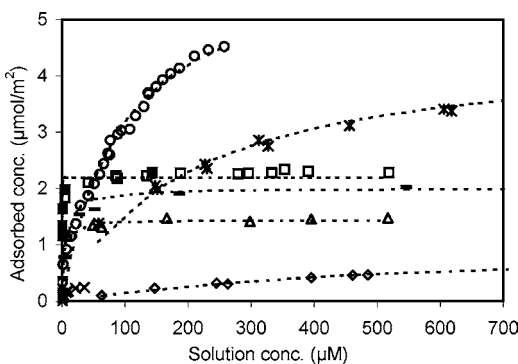


Figure 7. Comparison of adsorption isotherms fitted with the Langmuir equation of β -TH at pH 5.5 (○), fluoride at pH 4.0 (*) (39), phosphate at pH 4.0 (■) (40), phosphate at pH 4.0 (□) (41), oxalate at pH 4.0 (△) (42), citrate at pH 4.0 (▲) (32), 2,4-dihydroxybenzoate at pH 5.5 (×) (37), and lactate at pH 4.0 (◇) (32).

the IR spectra of powdered β -TH in this region exhibit absorbance bands at 1609, 1543, 1476, 1459, 1417, 1269, 1189, and 950 cm^{-1} (Figure 6). Thus, the absorbance bands in the β -TH spectrum ascribed to C=O stretching in the tropolone carbon ring (1609 and 1543 cm^{-1}) and the C–O–H group (1189 cm^{-1}) (34) display a significant shift in frequencies in comparison with β -TH-goethite spectra. This indicates that β -TH forms inner sphere surface complexes at the goethite surface, in accordance with the interpretation of IR spectra of organic ligands and the inner sphere surface complexes between these ligands and goethite for both dry and wet samples (35–38). On the basis of the IR spectra, it is not possible though to determine whether monodentate or bidentate complexes are formed.

The IR spectra are similar for freeze-dried β -TH-goethite suspensions at pH 4.0, 5.2, and 8.2 (data not shown) implying that inner sphere surface complexation contributes to β -TH adsorption over the full pH range tested. The suggestion of formation of inner sphere surface complexes is supported by the decreasing fraction of β -TH adsorbed with increasing equilibrium concentration of β -TH and the Langmuir adsorption isotherm indicating saturation of the adsorption sites at the goethite surface (18).

Adsorption of β -TH as Compared to Other Ligands. In Figure 7, the adsorption of β -TH is compared with the adsorption of phosphate, citrate, oxalate, 2,4-dihydroxybenzoate, lactate, and fluoride ligands, which are all known to bind to goethite through inner sphere surface complexation (31, 32, 35, 39). The adsorption data used in the comparison are chosen at pH values resulting in maximum adsorption of the individual

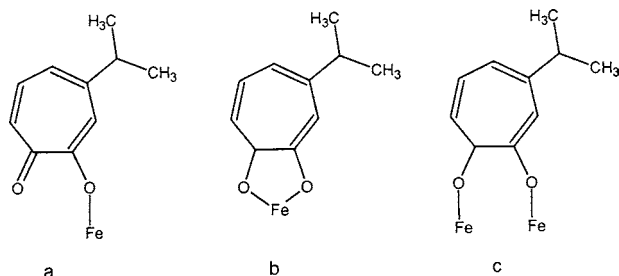


Figure 8. Structure of possible bonding of β -TH to the goethite surface: (a) monodentate mononuclear, (b) bidentate mononuclear, or (c) bidentate binuclear surface complexation.

compounds. All adsorption data have been fitted by the Langmuir equation.

It appears that β -TH exhibits a very high adsorption maximum (Γ_{\max}) as compared to phosphate, citrate, oxalate, 2,4-dihydroxybenzoate, and lactate. Fluoride shows an adsorption maximum closer to the one for β -TH. It is furthermore evident that β -TH displays a relatively small affinity for goethite, i.e., only lactate and fluoride are bound less strongly than β -TH (Table 2). Lactate and fluoride bind monodentate to goethite whereas the other compounds bind bidentate to goethite (31, 32, 35, 39).

Sorption Mechanism. Tropolones are known to form fairly stable metal complexes in aqueous solution (15). Hence, adsorption of β -TH to the goethite surface is expected to involve inner sphere surface complexation. This binding type is supported by the substantial changes observed for vibration frequencies of the HO-C-C=O moiety of β -TH during adsorption (Figure 6). Three types of surface hydroxyl groups exist at surfaces of hydrous oxides. These are singly, doubly, and triply coordinated groups, i.e., hydroxyl groups coordinated to one, two, or three iron atoms. The singly coordinated hydroxyl groups are generally considered the most reactive sites for inner sphere surface complexation (43). Therefore, the most likely types of inner sphere complexes at the goethite surface include monodentate mononuclear, bidentate mononuclear, and bidentate binuclear binding (17) (Figure 8).

The goethite used in this study consists of crystals that are needle-shaped and bound by (110) faces along the needle and (021) faces at the ends (41, 43); approximately 90% of the surface area consists of (110) faces and 10% of (021) faces (41). There are three and eight singly coordinated hydroxyl groups per nm^2 at the (110) and (021) faces, respectively (44–46). Hence, the average number of singly coordinated hydroxyl groups at the surface of goethite may be calculated to 3.5 sites/ nm^2 corresponding to 5.8 $\mu\text{mol}/\text{m}^2$. Adsorption of β -TH through bidentate binuclear surface complexation would result in an adsorption maximum of $5.8/2 = 2.9 \mu\text{mol}/\text{m}^2$, which is only half of the estimated Γ_{\max} of 6.2 $\mu\text{mol}/\text{m}^2$ (Table 2). Thus, monodentate binding of β -TH provides a more satisfactory agreement between the site density and the maximum adsorption capacity. This bonding mode is also able to explain the lower affinity of TH for goethite as compared with the other ligands shown in Figure 7, which all, except for lactate and fluoride, are thought to bind bidentate to goethite (31, 32, 35, 39) resulting in higher binding constants than for monodentate surface complexes (47). β -TH shows an adsorption maximum and adsorption affinity close to fluoride (Table 2), indicating that β -TH binds to goethite through monodentate mononuclear complexation like fluoride (39). A small portion of bidentate mononuclear surface complexes may form at the (021) faces,

but their contribution to total adsorption is thought to be insignificant (41).

The β -TH molecule consisting of a polar keto-hydroxy part opposite to a nonpolar isopropyl group has surfactant-like properties. Thus, hydrophobic interactions among the nonpolar moieties of β -TH molecules could contribute to adsorption at higher β -TH solution concentrations and may help explain the high adsorption capacity observed (48). The adsorption isotherm for β -TH (Figure 5) does not confer with the isotherm shapes typically observed for surfactants reflecting pronounced changes in adsorption energies depending on surface coverage (49). However, as indicated by the adsorption envelope, hydrophobic adsorption seems to take place and may completely dominate the adsorption process at low pH. At higher pH, hydrophobic adsorption and inner sphere surface complexation may take place concurrently producing adsorption isotherms, which are the sum of linear (hydrophobic) and Langmuir type (complexation) isotherms.

Implications for β -TH Sorption in Soil. The adsorption experiments demonstrate that β -TH is rather strongly bound to goethite. Other iron oxides such as ferrihydrite and lepidocrocite are considered to have similar sorption capacities per unit surface area. In soils, other metal oxides such as aluminum and manganese oxides and edges of clay phyllosilicates may contribute to β -TH bonding. In addition, β -TH may sorb to soil organic matter with a $\log K_{oc}$ value of 1.0 estimated from the $\log K_{ow}$ value for β -TH (Table 1). There are several unknown variables to be considered before sorption to soil materials can be correctly quantified. However, on the basis of our adsorption data for goethite and an estimate for K_{oc} , a minimum K_d can be calculated for β -TH sorption to soil. Assuming a soil material containing 0.5% (by weight) of goethite and 2% of carbon in soil organic matter, a K_d of 110 L/kg calculated at a β -TH solution concentration of 1 μM . Hence, β -TH has very limited mobility in soil and the risk of leaching to surface water and groundwater should be rather small unless the β -TH is transported with colloids.

LITERATURE CITED

- (1) Martell, A. E.; Smith, R. M. *NIST Standard Reference Database No. 46, v5.0—Critically Selected Stability Constants of Metal Complexes*; NIST Standard Reference Data: Gaithersburg, 1998.
- (2) Raa, J.; Goksoy, J. Studies on the effects of the heartwood toxin β -thujaplicin on the metabolism of yeast. *Physiol. Plant* **1965**, *18*, 159–176.
- (3) Yui, N.; Nozoe, T. The solubilities and the ionization constants of tropolone and its related compounds. (I). Hinokitiol and its bromo derivatives. *Sci. Rep. Tohoku Univ. Ser. 1* **1951**, *35*, 83–89.
- (4) Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR. Hydrophobic, Electronic, and Steric Constants*; ACS Professional Reference Book; American Chemical Society: Washington, DC, 1995.
- (5) USEPA. *EPI Suite v3.10 for Windows*; U.S. Environmental Protection Agency: Washington, DC, 2000.
- (6) Inamori, Y.; Shinohara, S.; Tsujibo, H.; Okabe, T.; Morita, Y.; Sakagami, Y.; Kumeda, Y.; Ishida, N. Antimicrobial activity and metalloprotease inhibition of hinokitiol-related compounds, the constituents of *Thujopsis dolabrata* S. and *Z. hondai* Mak. *Biol. Pharm. Bull.* **1999**, *22*, 990–993.
- (7) Inamori, Y.; Sakagami, Y.; Morita, Y.; Shibata, M.; Sugiura, M.; Kumeda, Y.; Okabe, T.; Tsujibo, H.; Ishida, N. Antifungal activity of hinokitiol-related compounds on wood-rotting fungi and their insecticidal activities. *Biol. Pharm. Bull.* **2000**, *23*, 995–997.

- (8) Matsumura, E.; Morita, Y.; Date, T.; Tsujibo, H.; Yasuda, M.; Okabe, T.; Ishida, N.; Inamori, Y. Cytotoxicity of the hinokitiol-related compounds, γ -thujaplicin and β -dolabrins. *Biol. Pharm. Bull.* **2001**, *24*, 299–302.
- (9) Morita, Y.; Matsumura, E.; Tsujibo, H.; Yasuda, M.; Sakagami, Y.; Okabe, T.; Ishida, N.; Inamori, Y. Biological activity of α -thujaplicin, the minor component of *Thujopsis dolabrata* Sieb. Et Zucc. Var. *Hondai* Makino. *Biol. Pharm. Bull.* **2001**, *24*, 607–611.
- (10) Haluk, J.-P.; Roussel, C. Caractérisation et origine des tropolones responsables de la durabilité naturelle des Cupressacées. Application potentielle en préservation du bois. *Ann. For. Sci.* **2000**, *57*, 819–829.
- (11) Inamori, Y.; Nishiguchi, K.; Matsuo, N.; Tsujibo, H.; Baba, K.; Ishida, N. Phytogrowth-inhibitory activities of tropolone and hinokitiol. *Chem. Pharm. Bull.* **1991**, *39*, 2378–2381.
- (12) DeBell, J. D.; Morrell, J. J.; Gartner, B. L. Tropolone content of increment cores as an indicator of decay resistance in western red cedar. *Wood Fiber Sci.* **1997**, *29*, 364–369.
- (13) Nault, J. Radial distribution of thujaplicins in old growth and second growth western red cedar (*Thuja plicata* Donn). *Wood Sci. Technol.* **1988**, *22*, 73–80.
- (14) Nkagawa, Y.; Tayama, K. Mechanism of mitochondrial dysfunction and cytotoxicity induced by tropolones in isolated rat hepatocytes. *Chem.-Biol. Interact.* **1998**, *116*, 45–60.
- (15) Dutt, Y.; Singh, R. P. Metal complexes with tropolones. *Talanta* **1969**, *16*, 1369–1382.
- (16) Kummert, R.; Stumm, W. The surface complexation of organic acids on hydrous γ -Al₂O₃. *J. Colloid Interface Sci.* **1980**, *75*, 373–385.
- (17) Evanko, C. R.; Dzombak, D. A. Influence of structural features on sorption of NOM-analogue organic acids to goethite. *Environ. Sci. Technol.* **1998**, *32*, 2846–2855.
- (18) Evanko, C. R.; Dzombak, D. A. Surface complexation modeling of organic acid sorption to goethite. *J. Colloid Interface Sci.* **1999**, *214*, 189–206.
- (19) Schwertmann, U.; Cornell, R. M. *Iron Oxides in the Laboratory—Preparation and Characterization*, 1st ed.; Dyllick-Brenzinger, C., Ed.; VCH Verlagsgesellschaft mbH: Weinheim, Germany, 1991.
- (20) Endo, M.; Mizutani, T.; Matsumura, M. High-performance liquid chromatographic determination of hinokitiol in cosmetics by the formation of difluoroborane compounds. *J. Chromatogr.* **1988**, *455*, 430–433.
- (21) Hanafusa, F.; Nakamura, K.; Togano, S.; Ohta, T. Determination of hinokitiol in cosmetic products by HPLC. *Bunseki Kagaku* **1989**, *38*, 124–128.
- (22) Johnson, E. L.; Cserjesi, A. J. Gas-liquid chromatography of some tropolone-TMS ethers. *J. Chromatogr.* **1975**, *107*, 388.
- (23) Nault, J. A capillary gas chromatographic method for thujaplicins in western red cedar extractives. *Wood Sci. Technol.* **1987**, *21*, 311–316.
- (24) Coombs, R. W.; Trust, T. J. The effect of light on the antibacterial activity of β -thujaplicin. *Can. J. Microbiol.* **1973**, *19*, 1177–1180.
- (25) Furuya, T.; Asada, Y.; Matsuura, Y.; Mizobata, S.; Hamada, H. Biotransformation of β -thujaplicin by cultured cells of eucalyptus perriniana. *Phytochemistry* **1997**, *46*, 1355–1358.
- (26) Mayer, B. X. How to increase precision in capillary electrophoresis. *J. Chromatogr. A* **2001**, *907*, 21–37.
- (27) Westergaard, B.; Hansen, H. C. B.; Borggaard, O. K. Determination of anions in soil solutions by capillary zone electrophoresis. *Analyst* **1998**, *123*, 721–724.
- (28) Jandel Scientific. *TableCurve2D for Windows v2.03*, 1989.
- (29) Hingston, F. J.; Atkinson, R. J.; Posner, A. M.; Quirk, J. P. Specific adsorption of anions on goethite. *Trans. 9th Int. Cong. Soil Sci.* **1968**, *1*, 669–678.
- (30) Hingston, F. J.; Posner, A. M.; Quirk, J. P. Anion adsorption by goethite and gibbsite. I. The role of the proton in determining adsorption envelopes. *J. Soil Sci.* **1972**, *23*, 177–192.
- (31) Tejedor-Tejedor, M. I.; Yost, E. C.; Anderson, M. A. Characterization of benzoic and phenolic complexes at the goethite/aqueous solution interface using cylindrical internal reflection Fourier transform infrared spectroscopy. 2. Bonding structures. *Langmuir* **1992**, *8*, 525–533.
- (32) Filius, J. D.; Hiemstra, T.; van Riemsdijk, W. H. Adsorption of small weak organic acids on goethite: Modeling of mechanisms. *J. Colloid Interface Sci.* **1997**, *195*, 368–380.
- (33) Chiou, C. T.; Porter, P. E.; Schmedding, D. W. Partitioning equilibria of nonionic organic compounds between soil organic matter and water. *Environ. Sci. Technol.* **1983**, *17*, 227–231.
- (34) Ikegami, Y. The infrared spectra of tropolone compounds. VI. The infrared and Raman spectra of tropolone, 3- and 4-isopropyltropolones. *Bull. Chem. Soc. Jpn.* **1963**, *36*, 1118–1125.
- (35) Parfitt, R. L.; Fraser, A. R.; Russell, J. D.; Farmer, V. C. Adsorption on hydrous oxides I. Oxalate, and benzoate on goethite. *J. Soil Sci.* **1977**, *28*, 29–39.
- (36) Persson, P.; Nordin, J.; Rosenqvist, J.; Lövgren, L.; Öhman, L.-O.; Sjöberg, S. Comparison of the adsorption of o-phthalate on boehmite (γ -AlOOH), aged γ -Al₂O₃, and goethite (α -FeOOH). *J. Colloid Interface Sci.* **1998**, *206*, 252–266.
- (37) Boily J.-F.; Persson, P.; Sjöberg, S. Benzenecarboxylate surface complexation at the goethite (α -FeOOH)/water interface: II. Linking IR spectroscopic observations to mechanistic surface complexation models for phthalate, trimellitate, and pyromellitate. *Geochim. Cosmochim. Acta* **2000**, *64*, 3453–3470.
- (38) Sheals, J.; Sjöberg, S.; Persson, P. Adsorption of glyphosate on goethite: molecular characterization of surface complexes. *Environ. Sci. Technol.* **2002**, *36*, 3090–3095.
- (39) Hiemstra, T.; van Riemsdijk, W. A. Fluoride adsorption on goethite in relation to different types of surface sites. *J. Colloid Interface Sci.* **2000**, *225*, 94–104.
- (40) Geelhoed, J. S.; Hiemstra, T.; van Riemsdijk, W. H. Phosphate and sulfate adsorption on goethite: single anion and competitive adsorption. *Geochim. Cosmochim. Acta* **1997**, *12*, 2389–2396.
- (41) Hiemstra, T.; van Riemsdijk, W. A. surface structural approach to ion adsorption: the charge distribution (CD) model. *J. Colloid Interface Sci.* **1996**, *179*, 488–508.
- (42) Mesuere, K.; Fish, W. Chromate and oxalate adsorption on goethite. 1. Calibration of surface complexation models. *Environ. Sci. Technol.* **1992**, *12*, 2357–2364.
- (43) Cornell, R. M.; Schwertmann, U. *The Iron Oxides. Structure, Properties, Reactions, Occurrence and Uses*; VCH Verlagsgesellschaft mbH: Weinheim, Germany, 1996.
- (44) Barron, V.; Torrent, J. Surface hydroxyl configuration of various crystal faces of hematite and goethite. *J. Colloid Interface Sci.* **1996**, *177*, 407–410.
- (45) Venema, P.; Hiemstra, T.; Weidler, P. G.; van Riemsdijk, W. H. Intrinsic proton affinity of reactive surface groups of metal (hydr)oxides: Application to iron (hydr)oxides. *J. Colloid Interface Sci.* **1998**, *198*, 282–295.
- (46) Boily, J.-F.; Lützenkirchen, J.; Balmès, O.; Beattie, J.; Sjöberg, S. Modeling proton binding at the goethite (α -FeOOH)-water interface. *Colloid Surf. A* **2001**, *179*, 11–27.
- (47) Parfitt, R. L.; Russell, J. D. Adsorption on hydrous oxides. IV. Mechanisms of adsorption of various ions on goethite. *J. Soil Sci.* **1977**, *28*, 297–305.
- (48) Somasundaran, P.; Fuerstenau, D. W. Mechanisms of alkyl sulfonate adsorption at the alumina-water interface. *J. Phys. Chem.* **1966**, *70*, 90–96.
- (49) Scamehorn, J. F.; Schechter, R. S.; Wade, W. H. Adsorption of surfactants on mineral oxide surfaces from aqueous solutions. *J. Colloid Interface Sci.* **1982**, *85*, 463–478.

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