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ARTICLE

Synthesis and Chemical Characterization of the Novel Agronomically Relevant Pentadentate Chelate 2-(2-((2-Hydroxybenzyl)amino)ethylamino)-2-(2-hydroxyphenyl)acetic Acid (DCHA)

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Iron chelates analogous to *o,o*-EDDHA/Fe^{3+} are the fertilizers chosen to treat iron chlorosis in plants growing on calcareous soil. The isomer *o,p*-EDDHA/Fe^{3+} presents less stability but faster assimilation by the plant than *o,o*-EDDHA/Fe^{3+}, because only five coordinating groups are able to complex Fe^{3+}. The new chelating agent 2-(2-((2-hydroxybenzyl)amino)ethylamino)-2-(2-hydroxyphenyl)acetic acid (DCHA) has been synthesized to obtain an iron fertilizer with intermediate stability between *o,o*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} and with fast assimilation. Its synthesis has been done starting from phenol, *N*-acetylethylendiamine, glyoxylic acid, and NaOH in a three-step sequence. The purity of the DCHA chelating agent, its protonation, and Ca^{2+}, Mg^{2+}, Fe^{3+}, and Cu^{2+} stability constants, together with its ability to maintain iron in solution in different agronomic conditions, have been determined. The results indicate that the chelate DCHA/Fe^{3+} has intermediate stability between those of *o,o*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} complexes and that it is capable of maintaining the Fe^{3+} in agronomic conditions. This new chelating agent may be effective in correcting iron chlorosis in plants.

KEYWORDS: Iron chelates; iron chlorosis; fertilizers; *o,o*-EDDHA/Fe^{3+}; *o,p*-EDDHA/Fe^{3+}; 2-(2-((2-hydroxybenzyl)amino)ethylamino)-2-(2-hydroxyphenyl)acetic acid (DCHA)

INTRODUCTION

Iron chlorosis is a major problem for crops cultivated in alkaline and calcareous soils (*1*), and the use of iron chelates derived from *o,o*-EDDHA/Fe^{3+} (**Figure 1**) in soil applications is the standard treatment to alleviate this plant disease (*2*, *3*). Although synthetic aminopolycarboxylate chelating agents are now under scrutiny due to their influence on metal availability and mobility, especially due to their persistence in the environment (*4*, *5*), nowadays they are the most efficient soil fertilizers to treat iron chlorosis in plants growing in calcareous soil. Commercial EDDHA/Fe^{3+} fertilizers are made by incorporating an iron salt to a mixture of reaction products obtained in the industrial synthesis production of *o,o*-EDDHA. This mixture contains *o,o*-EDDHA (**1**) as the major component, together with the regiosiomers *o,p*-EDDHA (**2**) and *p,p*-EDDHA in variable amounts, and is accompanied by polycondensation products and other byproducts recently revealed (*6*−*9*). Except for *p,p*-EDDHA, all of them are able to bind iron, but only *o,o*-EDDHA and *o,p*-EDDHA have been proved to be effective as iron suppliers to plants (*7*, *10*). The chelate *o,o*-EDDHA/Fe^{3+} is highly stable ($K \approx 10^{35}$) (*11*), whereas *o,p*-EDDHA/Fe^{3+} presents a significantly lower stability ($K \approx 10^{29}$) (*12*) than *o,o*-EDDHA/Fe^{3+} due to the pentacoordinated nature of the ligand that requires the incorporation of a water molecule to fill the octahedral environment around the metal. Despite this lower stability, *o,p*-EDDHA/Fe^{3+} is a better substrate for the iron chelate reductase of Fe-stressed cucumber plants (*10*) than *o,o*-EDDHA/Fe^{3+}. It was proposed that the open nature of the *o,p*-EDDHA/Fe^{3+} makes the iron more accessible to the iron chelate reductase before the reduction step. Hence, *o,p*-EDDHA/Fe^{3+} is a faster substrate for plant iron assimilation than *o,o*-EDDHA/Fe^{3+}. Recent studies found that Fe-stressed soybean plants show a faster response to *o,p*-EDDHA/Fe^{3+} than to *o,o*-EDDHA/Fe^{3+} in hydroponics (*10*). The counterpart to this higher activity is the lower stability of the complex that reduces the available amount of *o,p*-EDDHA/Fe^{3+} in the alkaline soil solutions compared to the more stable *o,o*-EDDHA/Fe^{3+} (*11*). When these chelates are

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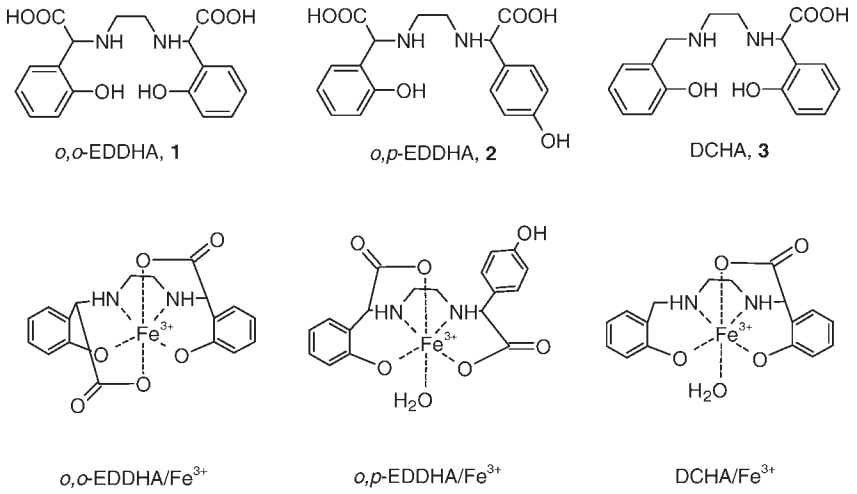


Figure 1. Structures of chelating agents *o,o*-EDDHA (**1**), *o,p*-EDDHA (**2**), and DCHA (**3**) and their Fe(III) chelates.

applied at once in calcareous soils, *o,o*-EDDHA/Fe^{3+} provides better iron nutrition to strategy I plants than *o,p*-EDDHA/Fe^{3+} (*13*, *14*).

These findings led us to design a novel pentacoordinate Fe chelate (DCHA/Fe^{3+}) having two phenolate groups and a carboxylate moiety, in addition to the two amino groups, coordinated to iron. This compound will be essentially different from *o,p*-EDDHA/Fe^{3+}, and it is predicted to combine a good stability in nutrient solutions and calcareous soils (due to the presence of the two phenolates as in *o,o*-EDDHA) and a fast action to solve iron chlorosis due to its open nature like that of *o,p*-EDDHA/Fe^{3+}. The new chelate DCHA/Fe^{3+} has been synthesized and then studied using a previously described methodology (*11*) to predict its capacity to solve iron chlorosis. This methodology includes the calculation of the protonation and stability constants of DCHA with Fe^{3+} and other cations existing in soils such as Ca^{2+}, Mg^{2+}, and Cu^{2+} and the modeling of the behavior of DCHA/Fe^{3+} under agronomic conditions.

EXPERIMENTAL PROCEDURES

General. All reagents used in this work were of analytical grade. All aqueous solutions were prepared with water according to type I grade (*15*). NMR spectra were recorded at 22 °C on a Bruker Avance DPX 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C) or on a Bruker Avance 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C). Chemical shifts are given in part per million relative to D_2O (^{1}H, 4.78 ppm), D_2O/Na_2CO_3 (^{13}C, 165.7 ppm), or DMSO-d_6 (^{1}H, 2.5 ppm; ^{13}C, 40.6 ppm). IR spectra were taken on a Perkin-Elmer 781 spectrometer. ESI-MS spectra were carried out in MeOH, using an ESQUIRE-LC (Bruker Daltonic, Bremen, Germany) ion trap spectrometer in negative mode of detection. The stainless steel capillary was held at a potential of 5.0 kV. Nitrogen was used as nebulizer gas at a flow rate of 3.98 L min^{-1} (nebulizer pressure = 11 psi) at 150 °C. All commercially available products were used without further purification. The details of the methods employed to determine the purity, the stability constants, the pM values, and the stability of the chelating agents have been described in an earlier paper (*11*).

Synthesis of DCHA. *Synthesis of the Amine Hydrochloride 5*. Monoacetyl ethylenediamine (1.08 g, 10.62 mmol) was added to a stirred flask containing 25.0 g (265.6 mmol) of melted phenol and 0.64 g (7.97 mmol) of NaOH (50% w/w) at 30−35 °C. Then, 1.57 g (10.62 mmol) of glyoxylic acid (50% in water) was added slowly dropwise to the solution, keeping the temperature below 40 °C. The mixture was kept at 70−75 °C for 2 h and then at room temperature for 20 min. After the addition of water (60 mL), the reaction was extracted with CH_2Cl_2 (3 × 20 mL), and the solvent was evaporated under reduced pressure to yield 1.9 g (96%) of amide **4** as a solid: ^{1}H NMR (D_2O) δ 7.17−7.04 (m, 2H, ArH), 6.82−6.66 (m, 2H, ArH), 4.07 (s, 1H, CH), 3.26−3.16 (m, 2H, CH_2), 2.84−2.74 (m, 1H, CH_2), 2.71−2.54 (m, 1H, CH_2), 1.83 (s, 3H, CH_3); ^{13}C NMR (D_2O) δ 176.8 (C=O), 174.9 (C=O), 156.6, 130,4, 130.3, 122.8, 120.1, 116.8 (ArC), 64.9 (CH), 45.9 (CH_2), 38.3 (CH_2), 22.2 (CH_3).; IR (KBr) ν 3423 (NH), 1656 (C=O, amide I), 1578, 1444 (NC=O, amide II), 1377, 1104, 742 cm^{-1}.

The hydrolysis of the acetamido group in **4** was carried out by reflux with HCl 15% v/v (60 mL, 2 h) to yield 2.83 g (90%) of amine hydrochloride **5** as a yellow solid after evaporation of the solvent: ^{1}H NMR (D_2O) δ 7.29−7.23 (m, 2H, ArH), 6.89−6.86 (m, 2H, ArH), 5.08 (s, 1H, CH), 3.28−3.20 (m, 4H, $2CH_2$); ^{13}C NMR (D_2O) δ 171.0 (C=O), 155.14, 132.8, 131.8, 121.2, 116.8, 116.3 (ArC), 61.2 (CH), 43.1 (CH_2), 35.7 (CH_2); IR (KBr) ν 3000−2400 (broad, NH_2^+), 1728 (C=O), 1230, 761 cm^{-1}.

Synthesis of Imine 6. Amine hydrochloride **5** (300 mg, 1.06 mmol) was dissolved in 3 mL of water and the pH of the solution adjusted to 7.2 with 10% $NaHCO_3$. Then, a solution of salicylaldehyde (130 mg, 1.06 mmol) in 1 mL of absolute EtOH was added. The mixture was stirred at room temperature for 1 h. The yellow precipitate formed after 1 h at room temperature was filtered and dried, yielding 211 mg (63%) of imine **6** as yellow solid: ^{1}H NMR (DMSO-d_6) δ 8.54 (s, 1H, CH=N), 7.48 (d, J = 7.5 Hz, 1H, ArH), 7.34 (t, J = 7.62 Hz, 1H, ArH), 7.30 (d, J = 7.32 Hz, 1H, ArH), 7.18 (t, J = 7.32 Hz, 1H, ArH), 6.92−6.70 (m, 4H, ArH), 4.62 (s, 1H, CH), 3.83 (m, 2H, CH_2), 3.15 (m, 1H, CH_2), 3.02 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6) δ 169.9 (C=O), 167.6 (CH=N), 160.3, 156.2, 136.4, 132.5, 131.8, 128.8, 127.5, 122.4, 118.8, 118.6, 116.6, 116.5 (ArC), 61.02 (CH), 55.61 (CH_2), 46.74 (CH_2); IR (KBr) ν 3430−2934 (broad), 1665 (C=O), 1623, 1452 (C=N), 1384, 834, 760, 700 cm^{-1}; ESI-MS m/z 337 $[M + Na]^+$; HRMS (ESI) calcd for $C_{17}H_{19}N_2O_4$ ($[M + H]^+$), 315.1360; found, 315.1354.

Synthesis of DCHA (*3*). A suspension of imine **6** (250 mg, 0.79 mmol) and 10% catalyst Pd(C) (5% w/w) in 30 mL of MeOH was stirred under H_2 pressure (40 psi) for 6 h at room temperature. The catalyst was removed by filtration on Celite and the solvent by distillation under reduced pressure. Amino acid **3** was obtained as a beige solid (240 mg, 95%): ^{1}H NMR (D_2O) δ 7.20−7.01 (m, 4H, ArH), 6.79−6.66 (m, 4H, ArH), 4.36 (s, 1H, CH), 3.87 (s, 2H, CH_2), 2.84−2.69 (m, 4H, CH_2); ^{13}C NMR (D_2O, CD_3OD) δ 176.9 (C=O), 157.3, 157.1, 132.0, 131.0, 130.3, 129.8, 129.6, 124.9, 121.0, 119.7, 118.0, 117.3 (ArC), 64.8 (CH), 48.9, (CH_2), 45.9 (CH_2), 44.3 (CH_2); IR (KBr) ν 3367−3057 (broad), 1616 (C=O), 1589, 1558, 1460, 1394, 1274, 756 cm^{-1}; ESI-MS m/z 339 $[M + Na]^+$; ESI-MS m/z 317.1 $[M + H]^+$; HRMS (ESI) calcd for $C_{17}H_{21}N_2O_4$ ($[M + H]^+$), 317.1496; found, 317.1495.

Determination of the Purity of the Chelating Agent. The ligand in concentration 1×10^{-4} M was dissolved in NaOH calculated to be 3 times the molar amount of the ligand, and the pH was fixed at 6 by the addition of 2 mM MES buffer (2-(*N*-morpholino)ethanesulfonic acid). Ionic strength was adjusted to 0.1 M by the addition of NaCl. The experimental solution was photometrically titrated at 25.0 ± 0.5 °C during the addition of 4.58×10^{-4} M Fe^{3+} standard solution until the absorbance at 480 nm presented no changes. Finally, the purity of the chelating agent was calculated following the mathematical procedure previously described (*11*).

Determination of Stability Constants by Potentiometric Data. The lowest protonation constants (K_3^H and K_4^H), corresponding to the two amino groups, were determined by potentiometric titration (*16*) of DCHA chelating agent solution (1×10^{-3} M; $\mu = 0.1$ M (NaCl)), previously dissolved in NaOH, with 0.0571 M HCl solution at 25 °C under N_2 atmosphere. Ca^{2+} and Mg^{2+} solutions were added in 1:1 ligand-to-metal ratio before the titration to determine the Ca^{2+} and Mg^{2+} stability constants. Total Ca^{2+} and Mg^{2+} concentrations were measured by atomic absorption spectrophotometry. The stability constants were calculated using the program Hyperquad 2006 (*17*).

Determination of Stability Constants by Spectrophotometric Data. The first two protonation constants (K_1^H and K_2^H) of the ligand were measured spectrophotometrically (*18*) because the combination of protons with the phenolic groups is accompanied by extensive changes in the absorption spectra at 393.8 and 236.8 nm. Twelve DCHA solutions (1×10^{-4} M; $\mu = 0.1$ M (NaCl)) were prepared, and the pH was adjusted from 10.00 to 13.30 with 0.3−0.5 pH intervals. The spectra were recorded at 200−400 nm in a Shimazdu UV−vis spectrophotometer (Kyoto, Japan) for all solutions. The wavelength on the maximum absorbance and molar absorptivities of L^{4-} and LH_2^{2-} species were initially estimated at pH 12.9 and 9.9, respectively, and used as seeds for the calculations (*11*). For a better result, the constant K_2^H was also refined with the other protonation constants calculated by the potentiometric method using Hyperquad.

Stability constants for the Fe^{3+} and Cu^{2+} chelates were calculated from spectrophotometric data obtained after titration (*11*). Solutions of Fe^{3+} and Cu^{2+} chelates (1:1 metal/ligand ratio, $\mu = 0.1$ M (NaCl)) at 1×10^{-4} and 1×10^{-3} M, respectively, were prepared under N_2 at 25.0 ± 0.5 °C, by slow addition of Fe^{3+} or Cu^{2+} standard solutions. The pH was lowered to 1 by the addition of HCl and titrated with aqueous 0.200 M NaOH titrant to pH 12. The Fe^{3+} chelate solution was back-titrated to pH 2.5, too. The absorbance of the solutions was measured at 430 and 490 nm for the Fe^{3+} chelate and at 650 nm for the Cu^{2+} chelate. Total Fe^{3+} and Cu^{2+} concentrations were measured by atomic absorption spectrophotometry. The protonation constants (K_1^H and K_2^H) and the stability constants (K_{FeL}, K_{FeHL}, K_{FeoHL}, K_{CuL}, K_{CuHL}, and K_{CuH2L}) were calculated from the data by an in-house program using Microsoft Excel Solver (*11*).

Fe^{3+} Chelate Stability versus pH. The behavior of the DCHA/Fe^{3+} chelate was studied in agronomic conditions by modeling. The chelate stability in different conditions was obtained using the equilibrium speciation model VMINTEQ program (*19*). The component database was modified to include the DCHA chelating agent as new component. The thermodynamic database was also modified including every protonation, Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{3+} stability constants for each species formed. Percentage of Fe^{3+} chelated by DCHA was calculated over the pH 4−13 range for two agronomic conditions. The values were also calculated for *rac*-*o*,*o*-EDDHA, *meso*-*o*,*o*-EDDHA, *o*,*p*-EDDHA, and EDTA chelating agents using the two models. The total concentrations of the ligands and Fe^{3+} were 100 μM.

In the first model, Hoagland nutrient solution (*20*) was used as theoretical model to study the behavior of DCHA/Fe^{3+}, using Fe(OH)$_3$amp as possible solid controlling the system solubility. The behavior in soil conditions was calculated in the second model. All soil components that could have some effect on DCHA/Fe^{3+} stability were considered. Two soil types with unlimited and limited Cu^{2+} availability, respectively, were proposed to predict the stability of DCHA/Fe^{3+} with high and low Cu^{2+} levels in soil, respectively. The composition of the theoretical soil is described in **Table 1**.

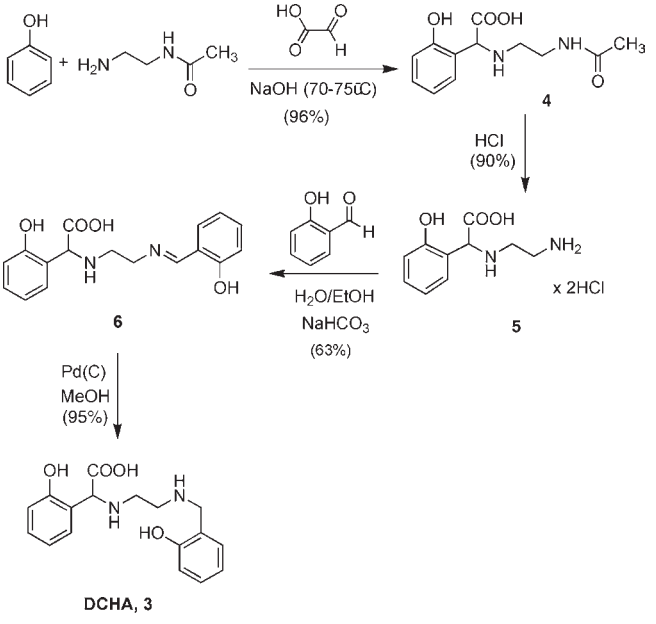
Table 1. Composition of Theoretical Soil Model with Unlimited Cu^{2+} Availability Used To Predict the Stability of DCHA/Fe^{3+} in Soil Conditions

component	equilibrium	log K^0
$CO_{2(g)}$ (0.0003 atm)	$CO_{2(g)} + H_2O \leftrightarrows 2H^+ + CO_3^{2-}$	−18.15
	possible solids	
soil Ca	soil Ca $\leftrightarrows Ca^{2+}$	−2.50
soil Mg	soil Mg $\leftrightarrows Mg^{2+}$	−3.00
calcite	$CaCO_3 \leftrightarrows Ca^{2+} + CO_3^{2-}$	−8.41
dolomite	$CaMg(CO_3)_2 \leftrightarrows Ca^{2+} + Mg^{2+} + 2CO_3^{2-}$	−3.00
	infinite solids	
soil Cu	soil Cu + $2H^+ \leftrightarrows Cu^{2+}$	2.80
soil Fe	soil Fe + $3H^+ \leftrightarrows Fe^{3+}$	2.70
soil Zn	soild Zn + $2H^+ \leftrightarrows Zn^{2+}$	5.80

RESULTS AND DISCUSSION

The synthesis of (2-(2-((2-hydroxybenzyl)amino)ethylamino)-2-(2-hydroxyphenyl)acetic acid) (**3**, DCHA) started from phenol, *N*-acetylethylenediamine, glyoxylic acid, and NaOH. After an extensive study of the reaction conditions, the acetamide **4** was obtained in nearly quantitative yield as a single ortho isomer. No traces of the corresponding para isomer were observed (^{1}H NMR of the crude reaction mixtures). The acetamide **4** was hydrolyzed with concentrated HCl, and the hydrochloride **5** was obtained after removal of the solvent under reduced pressure. Condensation of hydrochloride **5** with salicylaldehyde was carried out in H_2O/EtOH at pH 7.2 (adjusted with 10% $NaHCO_3$). Under these conditions, imine **6** precipitated in the reaction medium and was obtained by filtration in 65% yield. Subsequent reduction of the imino group with H_2/Pt(C) afforded **2** (95% yield), as a pure compound directly from the reaction medium (**Scheme 1**). The use of $NaBH_4$ or $NaCNBH_3$ also produced DCHA in nearly quantitative yields (spectroscopic). However, in these cases, the product is heavily contaminated with variable amounts of salts and its purification could not be achieved. Although the synthesis of imine **6** has not been reported in the literature, the oxovanadium(IV) complex of **6** (V^{4+}O−EHGS) was described by Pecoraro and co-workers as a product derived from the oxidative decarbonylation of the monooxovanadium complex V^{5+}O-*o*,*o*-EDDHA (*21*−*23*). Transmetalation of V^{4+}O−EHGS with $FeCl_3$ allowed the synthesis and chemical characterization of the ferric chelate of **6**, which was used as a model compound to study the iron binding in proteins (*24*). Structurally related manganese complexes were also reported (*25*).

Scheme 1. DCHA Synthetic Sequence



DCHA/Fe^{3+} was first characterized to predict its capacity to solve iron chlorosis. The used methodology has been previously reported by us (*11*). This methodology includes the calculation of the protonation and stability constants of DCHA with Fe^{3+} and other cations already present in soils such as Ca^{2+}, Mg^{2+}, and Cu^{2+} and the modeling of the behavior of DCHA/Fe^{3+} under agronomic conditions. The titrimetric purity of the chelating agent DCHA determined by photometry (with Fe^{3+}) was

Table 2. Log Protonation and Log Stability Constants[a] for the Chelating Agents (Charges Are Omitted for Simplicity)

quotient	DCHA (**3**)	*rac*-*o*,*o*-EDDHA (*7*)	*meso*-*o*,*o*-EDDHA (*7*)	*o*,*p*-EDDHA (*16*)
[HL]/[H][L]	11.16	11.88	11.90	11.18
$[H_2L]/[H][HL]$	9.33 ± 0.06	10.80	10.89	10.18 ± 0.04
$[H_3L]/[H][H_2L]$	7.89 ± 0.13	8.67 ± 0.01	8.58 ± 0.04	8.65 ± 0.05
$[H_4L]/[H][H_3L]$	5.98 ± 0.16	6.28 ± 0.11	6.16 ± 0.02	6.19 ± 0.02
[CaL]/[Ca][L]	4.87 ± 0.28	7.99 ± 0.42	7.56 ± 0.49	4.12 ± 0.10
[CaLH]/[Ca][H][L]	14.51 ± 0.26	17.42 ± 0.39	17.10 ± 0.65	14.27 ± 0.16
$[CaLH_2]/[Ca]\ [H]^2[L]$	23.88 ± 0.17	26.87 ± 0.37	26.41 ± 0.64	23.23 ± 0.32
[MgL]/[Mg][L]	5.00 ± 0.22	10.13 ± 0.03	9.44 ± 0.08	5.64 ± 0.16
[MgLOH] [H]/[Mg][L]	-4.31 ± 0.19			
[MgLH]/[Mg] [H][L]			17.51 ± 0.25	15.55 ± 0.03
$[MgLH_2]/[Mg][H]^2[L]$			26.56 ± 0.35	23.83 ± 0.32
[FeL]/[Fe][L]	27.94 ± 0.12	35.86	34.15	28.72 ± 0.05
[FeHL]/[Fe][H][L]	30.16 ± 0.28	35.08	36.56	35.02 ± 0.05
$[FeH_2L]/[Fe][H]^2[L]$				37.35 ± 0.10
[FeOHL] [H]/[Fe][L]	20.18 ± 0.21	23.12	22.81	19.45 ± 0.19
[CuL]/[Cu][L]	22.37 ± 0.18	24.94 ± 0.05	23.68 ± 0.02	21.74 ± 0.38
[CuHL]/[Cu] [H][L]	27.52 ± 0.06	32.87 ± 0.04	32.30 ± 0.00	30.96 ± 0.09
$[CuH_2L]/[Cu][H]^2[L]$	31.88 ± 0.03	37.33 ± 0.07	37.25 ± 0.01	36.17 ± 0.12
$[CuH_3L]/[Cu][H]^3[L]$				38.14 ± 0.07

[a] μ = 0.1 M (NaCl); t = 25 °C.

> 87% in all cases, which is in good agreement with the estimated purity obtained by ^{1}H NMR (considering that the error of this technique in our instrumental conditions is ±4%). The molar absorptivity at pH 6.0 of DCHA/Fe^{3+} (4796 ± 30 L mol^{-1} cm^{-1}) is similar to that of *o*,*o*-EDDHA/Fe^{3+} (4721 ± 16 L mol^{-1} cm^{-1}) as was expected considering their structural similarities.

Stability Constants. The protonation and stability constants of DCHA are shown in **Table 2**. These values are compared with those of *o*,*o*-EDDHA, *rac*-*o*,*o*-EDDHA, *meso*-*o*,*o*-EDDHA, and *o*,*p*-EDDHA obtained in earlier works using the same methodology (*11*, *26*).

The protonation constants of DCHA are in general lower than those of the *o*,*o*-EDDHA meso and racemic isomers and those of *o*,*p*-EDDHA. Because the charge of DCHA (L^{3-}) is lower than those of *o*,*o*-EDDHA and *o*,*p*-EDDHA (L^{4-}), the acidity of phenolate and amine groups is higher in DCHA, giving a lower affinity with protons. The same tendency was observed for the protonation constants of ethylendiaminetetracetic acid (EDTA) (L^{4-}; log K^H = 10.19 (H_1), 6.13 (H_2), 2.69 (H_3), 2.00 (H_4)) versus HEDTA (L^{3-}; log K^H = 9.81 (H_1), 5.37 (H_2), 2.60 (H_3)), where a hydroxymethyl group unable to bond metals replaces one carboxylic group of EDTA (*27*).

DCHA forms square-planar complexes with Ca^{2+} and Mg^{2+}. The magnitudes of DCHA/Mg^{2+} stability constants are higher than those of DCHA/Ca^{2+} (**Table 2**). This tendency is analogous to that observed for *o*,*o*-EDDHA, where the presence of phenolate groups favors the binding to small metals such as Mg^{2+} (*11*). The species MgLOH formed for the DCHA ligand stabilized the chelate at high pH by the deprotonation of one axial molecule of H_2O in the MgL chelate. The low values of the DCHA/Ca^{2+} and DCHA/Mg^{2+} stability constants with respect to those of *o*,*o*- and *o*,*p*-EDDHA indicate the poor affinity of these cations with DCHA. This fact is an advantage for the use of the chelate DCHA/Fe^{3+} in calcareous soils, because a lower competence between Ca^{2+} and Mg^{2+} and Fe^{3+} is expected.

Visible optical absorption spectra for both DCHA/Fe^{3+} and DCHA/Cu^{2+} are shown in **Figure 2**. Whereas the absorbance maximum of the *o*,*o*-EDDHA/Fe^{3+} is constant at 480 nm throughout the pH range (*26*, *28*), the maximum absorbance of DCHA/Fe^{3+} reveals a shift from 500 nm at pH 4.16 to 430 nm at pH 11.83. This behavior is similar to that of *o*,*p*-EDDHA/Fe^{3+}. An isosbestic point is shown at 450 nm, indicating that there are only two species with different absorptions in the pH range of 7.38−11.83 (in *o*,*p*-EDDHA the isosbestic point appears at 470 nm). The optical absorption spectra obtained for DCHA/Cu^{2+} present the same tendency as for the Cu^{2+} complexes of *o*,*o*-EDDHA and *o*,*p*-EDDHA (*11*, *26*).

DCHA/Cu^{2+} has stability constants between those of *o*,*o*-EDDHA/Cu^{2+} and *o*,*p*-EDDHA/Cu^{2+} chelates (**Table 2**). The *o*,*o*-EDDHA and DCHA ligands are able to complex Cu^{2+} with two phenolate and two amine groups. In the case of *o*,*p*-EDDHA, the phenolate group placed in the para position of one of the aromatic rings cannot bind Cu^{2+} and a carboxylate forms the bond instead. The affinity of phenolate groups for Cu^{2+} is evident because the stability constant of DCHA/Cu^{2+} is higher than that *o*,*p*-EDDHA/Cu^{2+}. The protonated and diprotonated species of DCHA/Cu^{2+} (see CuHL, CuH_2L in **Scheme 2**) are similar to those of *o*,*o*-EDDHA/Cu^{2+} and *o*,*p*-EDDHA/Cu^{2+} (*11*, *26*). However, the diprotonated species CuH_2L produce an important effect on the Cu^{2+} stability constant of DCHA. Whereas in *o*,*o*-EDDHAH_2/Cu^{2+}, Cu is bonded by two amino and two carboxylic groups, in DCHAH_2/Cu^{2+} (CuH_2L in **Scheme 2**) Cu needs a water molecule to occupy one of the coordination positions. Similarly, we observed that in CuLH species, the second carboxylate group should produce a stabilization effect in the *o*,*o*-EDDHA and *o*,*p*-EDDHA chelates, possibly due to the formation of the pentadentated chelates. This effect cannot occur in DCHA/Cu^{2+} because of the lack of the second carboxylate group. Then DCHA/Cu^{2+} species at low pH (protonated and diprotonated) present lower stability than those of *o*,*o*-EDDHA/Cu^{2+} and *o*,*p*-EDDHA/Cu^{2+} chelates (**Table 2**). The main species present in the pH range typical of calcareous soils (over pH 7.5) are $[CuL]^-$ for DCHA and $[CuHL]^-$ for *o*,*o*-EDDHA and *o*,*p*-EDDHA.

The situation with Fe^{3+} is clearly different. The values of stability constants of DCHA/Fe^{3+} (**Table 2**) were considerably lower than those of *o*,*o*-EDDHA/Fe^{3+} and in the range of those measured for *o*,*p*-EDDHA/Fe^{3+}. The stronger binding of Fe^{3+} in *o*,*o*-EDDHA derives from its closed octahedrical structure with six bonds between the ligand and the metallic nucleus. However,

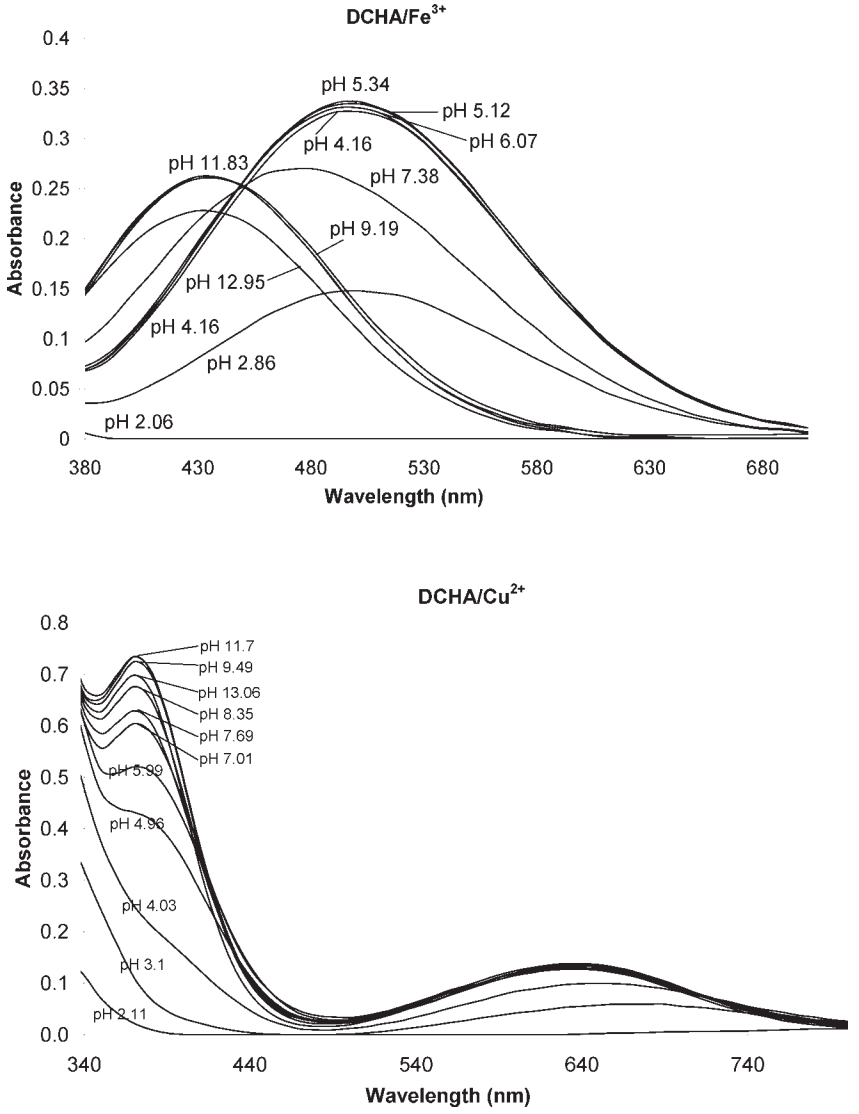
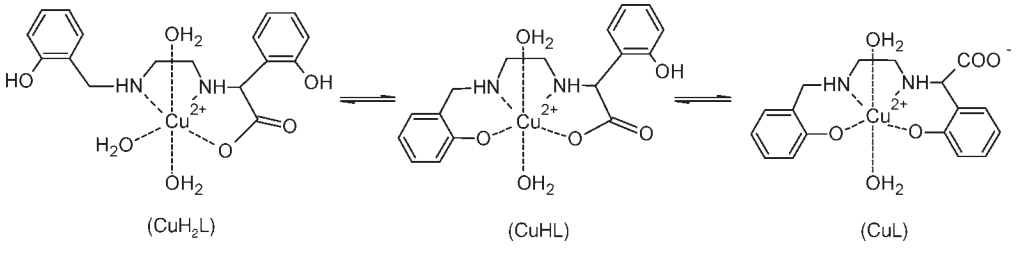


Figure 2. Absorption spectra of DCHA/Fe^{3+} and DCHA/Cu^{2+} as a function of pH. [DCHA/Fe^{3+}] = 1×10^{-4} M, [DCHA/Cu^{2+}] = 1×10^{-3} M, t = 25 °C, μ = 0.100 M (NaCl).

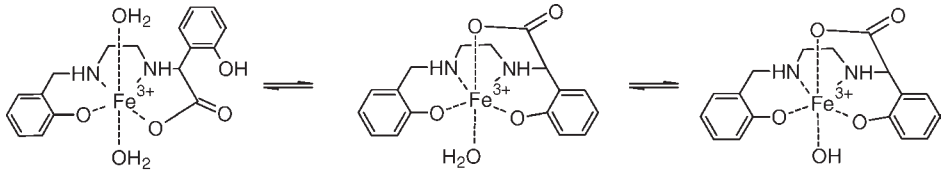
Scheme 2. DCHA/Cu^{2+} Species Formed



the similarity between the values obtained for the stability constants of the DCHA and *o*,*p*-EDDHA ligands is interesting, both having five bonds with the iron nucleus, but two phenolate−Fe bonds in DCHA against one phenolate−Fe and one carboxylate−Fe bond in *o*,*p*-EDDHA. Both DCHA and *o*,*p*-EDDHA complexes should have a water molecule occupying the remaining coordination position in the FeL species (**Scheme 3**). Below pH 2, one of the phenolates of DCHA/Fe^{3+} takes a proton to form FeLH and another water molecule occupies the vacant position. Over pH 7.8, a proton from the coordinated water molecule in FeL is released, forming the FeOHL species (**Scheme 3**). This behavior is analogous to that observed in *o*,*p*-EDDHA/Fe^{3+}.

It is known that the replacement of two carboxylic groups of EDTA/Fe^{3+} by two phenolate groups (as in the chelate *o*,*o*-EDDHA/Fe^{3+}) produces a noticeable increase in stability (10 units in log *K*) (*29*). Therefore, an increment of around 5 units of log *K* was expected with the substitution of one of the carboxylate groups of the complex *o*,*p*-EDDHA/Fe^{3+} by a phenolate group to form DCHA/Fe^{3+}. However, the Fe^{3+} stability constants of DCHA calculated in this paper are analogous to those obtained for *o*,*p*-EDDHA/Fe^{3+} (*11*).

Scheme 3. DCHA/Fe^{3+} Species Formed



(FeHL) (FeL) (FeOHL)

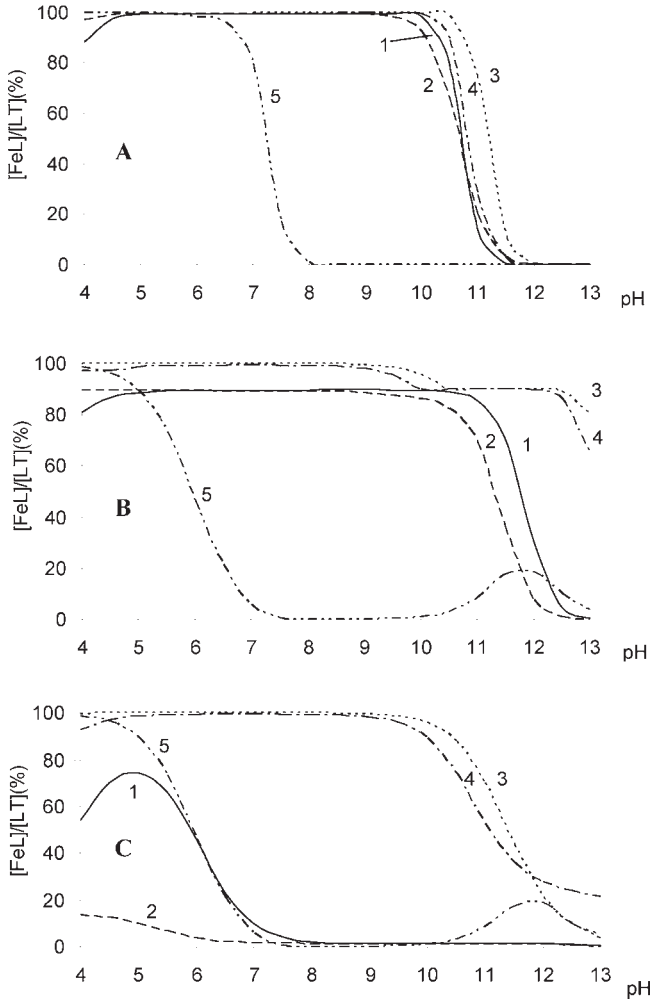


Figure 3. Percentage of chelating agents in solution that are binding Fe^{3+}: comparison of the behavior of (1) DCHA, (2) *o,p*-EDDHA, (3) *rac-o,o*-EDDHA, (4) *meso-o,o*-EDDHA, and (5) EDTA in (**A**) nutrient solution and in soil conditions with (**B**) limited Cu^{2+} and (**C**) unlimited Cu^{2+}.

The results above indicated that the increase in stability is due better to the close geometry of the chelate than to the nature of the coordinating functionalities, namely, phenolate versus carboxylate. The implications of this observation in the mechanism of action of these complexes (their interaction with the enzyme FCR) are being explored by experimental and computational tools in our laboratories.

Fe^{3+} Chelate Stability versus pH. The simple comparison of the stability constants of DCHA and the isomers of EDDHA may not correctly reflect the relative effectiveness of DCHA in agronomic conditions. It is necessary to know the affinities of the ferric and competing ions, mainly Cu^{2+}, for the ligands at a given pH in agronomic conditions. **Figure 3** presents the calculated percentage of iron that remains chelated in nutrient solution (**A**) and in soil conditions with limited (**B**) and unlimited Cu^{2+} (**C**) for DCHA/Fe^{3+} compared to *o,p*-EDDHA/Fe^{3+}, *rac-o,o*-EDDHA/Fe^{3+}, *meso-o,o*-EDDHA/Fe^{3+}, and EDTA/Fe^{3+}. In nutrient solution the polyphenolic chelates behave similarly, maintaining the total chelated iron in solution at agronomic pH values (**Figure 3A**). In the soil model (**Table 1**) the main components that can affect the iron chelate stability are included. Calcium and magnesium equilibria are included in the soil model, because these elements are considered to be the main competitors of weak chelating agents, such as EDTA (*30*) in calcareous conditions. Cu^{2+} equilibrium has been included in the soil model to determine the ability of this metal to replace Fe^{3+} from DCHA/Fe^{3+}. The affinity of *o,p*-EDDHA to Cu^{2+} produces a decrease of the iron chelate (*26*), and the same behavior is observed for DCHA but to a lesser extent (**Figure 3A**,**B**). Normally, calcareous soils to which iron chelates are frequently applied also present low Cu^{2+} availability. The Cu^{2+} concentration in model **C** (near 1×10^{-4} M at pH 8 for DCHA/Fe^{3+}) is high and unusual in soils (*26*). This range of Cu^{2+} concentrations is found only in polluted soils, for example, by the addition of fungicides (*31*). Then, in model **B** the total Cu^{2+} concentration in solution is limited to 1×10^{-5} M. In this case, DCHA/Fe^{3+} is stable until pH 11.5 with a behavior similar to that of *rac-o,o*-EDDHA/Fe^{3+} and *meso-o,o*-EDDHA/Fe^{3+} and slightly higher than *o,p*-EDDHA/Fe^{3+}. In the three soil models EDTA/Fe^{3+} is not effective enough in maintaining Fe^{3+} in solution at agronomic pH.

On the basis of the theoretical modeling of the chelates, it may be concluded that DCHA/Fe^{3+} could be used as a ferric chelate to correct iron chlorosis, and it may be better than EDTA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} for agronomic purposes. This assertion can be made despite the stability constants that show that the affinity of DCHA toward Fe^{3+} is lower than that of *o,p*-EDDHA. However, two additional factors improve the stability of the DCHA/Fe^{3+} in calcareous soils. First, DCHA forms the FeOHL species at lower pH values than *o,p*-EDDHA (7.76 for DCHA and 9.27 for *o,p*-EDDHA) and, second, presents lower charge ([FeOHDCHA]$^{-}$) than *o,p*-EDDHA ([FeOH−*o,p*-EDDHA]$^{2-}$).

Conclusions. The synthesis of (2-(2-((2-hydroxybenzyl)amino)-ethylamino)-2-(2-hydroxyphenyl)acetic) (DCHA/Fe^{3+}), a novel pentacoordinate ferric chelate having two phenol groups and a carboxylate moiety (in addition to the two amino groups) coordinated to Fe^{3+}, has been effected in four steps from phenol, *N*-acetylethylendiamine, glyoxylic acid, and NaOH. The purity of the chelating agent, its protonation, Ca^{2+}, Mg^{2+}, Fe^{3+}, and Cu^{2+} stability constants, together with its ability to maintain iron in solution in different conditions, have been determined. The results obtained in this work indicate that DCHA/Fe^{3+} presents stability constants similar to those of *o,p*-EDDHA/Fe^{3+}, but the theoretical speciation indicates that the species FeOHL favored a higher stability of DCHA/Fe^{3+} at calcareous pH. It is predicted that DCHA/Fe^{3+} combines a good stability in nutrient solution and calcareous soils (due to the presence of two phenolate groups

in its structure) and a fast action to relieve iron chlorosis due to its open nature similar to that of *o*,*p*-EDDHA/Fe^{3+}. Efforts are underway in our laboratories to determine the agronomical efficiency and mode of action of this new iron chelate.

ABBREVIATIONS USED

DCHA, 2-(2-((2-hydroxybenzyl)amino)ethylamino)-2-(2-hydroxyphenyl)acetic acid; *o*,*o*-EDDHA, ethylenediaminedi(2-hydroxyphenylacetic) acid; *o*,*p*-EDDHA, ethylenediamine-*N*-(*o*-hydroxyphenylacetic acid)-*N*′-(*p*-hydroxyphenylacetic acid); *p*,*p*-EDDHA, ethylenediamine-*N*-(*p*-hydroxyphenylacetic acid)-*N*′-(*p*-hydroxyphenylacetic acid); EDTA, ethylenediaminetetraacetic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; NMR, nuclear magnetic resonance; AAS, atomic absorption spectroscopy; IR, infrared spectroscopy; HRMS, high-resolution mass spectrometry; ESI-MS, electrospray ionization mass spectrometry.

Note Added after ASAP Publication

There was an error in the Introduction of the version of this paper published ASAP on June 9, 2010; the correct version published on June 11, 2010.

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