Mashington,[14] in order to confirm that the analogous imidazole derivatives have only the gauche conformation in between the two polar group X(O,S) and the imidazole ring, N(19), we considered the imaginary plane D [C(7), C(9) and C(16)]. In most cases N(19) are deviated from the imaginary plane D about 2-3 Å, but in the sulconazole nitrate the deviation value is 0.88 Å. While the deviation values of the polar group X(O, S) from the plane D are about 0.18-0.39 Å expected for the miconazole A[2.316 Å]. As shown in Figure 3, in all cases we found that no the molecular conformation have gauche conformation. This result was a contrast to a hypothesis of Mashington *et al.*[14] Thus we examined the total deviations from the imaginary plane D for the two polar group, X(O, S) and N(19). Except for the title compound (about 1.6 Å), these deviation values ranges from about 2.3 to 3.3 Å. As stated above whether these subtle conformational differences, for example, the torsion angles, dihedral angles, the selected distances (*l*) and conformational type of two prlar groups, affect the pharmacological activity of the title compound is difficult to asses, because mainly activity data obtained from *in vitro* experiments are derived from a number of differing techinques and test organisms.

As shown in Figure 2, the unit cell contains independent eight molecule and one-$NO_3$ group are hydrogen bonded to the two neighbored sulconazole molecules [N(19)···O(23)(x, y, z): 2.896(7) Å, 136.9(6)°, N(19)···O(25)(x+0.5, y, z+0.5): 2.974(7) Å, 135.6(5)°]. The molecules are packed by two hydrogen bondings and van der Waals forces.

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# A Structure-Based Activation Model of Phenol-Receptor Protein Interactions

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Data from structure/activity studies in *vir* gene induction system have led to evaluate the working hypothesis of interaction between phenolic inducers and phenol binding proteins. The primary specificity in the association of a phenolic inducer with its receptor in our system is hypothesized to be the hydrogen bonding interactions through the *ortho* methoxy substituents as well as the proton transfer between the inducer and the binding protein. In this paper the proposed working model for phenol-mediating signal transduction was evaluated in several ways. The importance of the general acid-base catalysis was first addressed by the presence of an acidic residue and a basic residue in the phenol binding protein. Series of compounds were tested for *vir* gene expression activity to confirm the generation of a strong nucleophile by an acidic residue and an involvement of a basic residue as a proton acceptor. An attempt was made to correlate the $pK_a$ values of the phenolic compounds with *vir* gene induction activities as inducers to further support the proposed proton transfer mechanism. Finally, it was also observed that the regioselectively attached methoxy group on phenol compounds is required as the proper hydrogen bond accetptor.

## Introduction

*Agrobacterium tumefaciens* causes crown gall tumors, transforming a wide range of gymnosperm and dicotyledonous angiosperms.[1] It initiates the expression of virulence genes in response to host-derived phenolic signals through two component regulatory proteins (VirA and VirG)[2] and putative phenol binding proteins.[3] Several investigations have been made to define structural requirements which confer on a compound inducing *vir* gene expression. Sys-

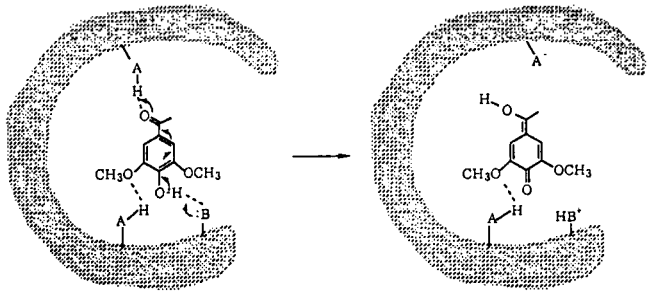

**Figure 1.** A proposed model of interaction of AS in the binding site of the phenol binding receptor of *Agrobacterium*. Hydrogen bond donor (A-H) and an acceptor (-B:) suggested to act as general acid and base are shown in the hydrophobic cleft.

tematic studies on structure/activity relationships were performed in order to confirm the three requirements that phenolic compounds must meet as active inducers.[4,5,6] The critical structural features of the inducers are 1) a phenol hydroxy group, 2) an *ortho*-methoxy or *ortho, ortho*-dimethoxy groups, and 3) carbonyl containing functional groups or cinnamic acid derivatives ($sp^2$ center) oriented *para* to the phenol hydroxy group.

Based on the crystallographic information available for proteins which have phenol binding sites, it has been suggested that most of phenol binding proteins contain a largely hydrophobic cleft which is able to accomodate the planar aromatic ring.[7] The mechanism-dependent structural recognition beyond the hydrogen bonding potential of the *para* substituent of dehydroconiferyl alcohol (DCA) has led to develope a model to propose critical changes in the ionization of the two amino acid residues, one proton donor and the other proton acceptor, in the phenol binding site (Figure 1).[8] In this model, a strong nucleophile ($-A^-$), *e.g.* a carboxylate, is predicted to be generated as a result of the initial proton transfer. The developed model allows several predictions that need to be tested for a better and solid one. The predictions are as follows: 1) a nucleophile ($-A^-$) is generated from a proton donor residue (-AH) due to protonating the carbonyl oxygen of the phenolic compound, replacing a leaving group at the α-carbon and causing a covalent modification of the binding protein; 2) a quinone methide is formed as a result of the proton transfer; 3) at least one methoxy group is located at the *ortho* to the hydroxy group providing the hydrogen bond interactions; and 4) a basic residue (-B) in the binding protein acts as a proton accepting site. Upon protonation of the basic residue, the binding protein is expected to undergo a conformational change for signal transduction to occur. In this paper a series of compounds were thoroughly examined in order to evaluate these four predictions.

## Results and Discussion

**Evaluation of an acidic residue as a proton donor.** The phenol binding model proposed in Figure 1 predicts that an acidic residue (-AH) of the binding protein is ionized and becomes a strong nucleophile upon the protonation of the carbonyl oxygen on the phenol compound. Generation of a strong nucleophile in an acidic residue was

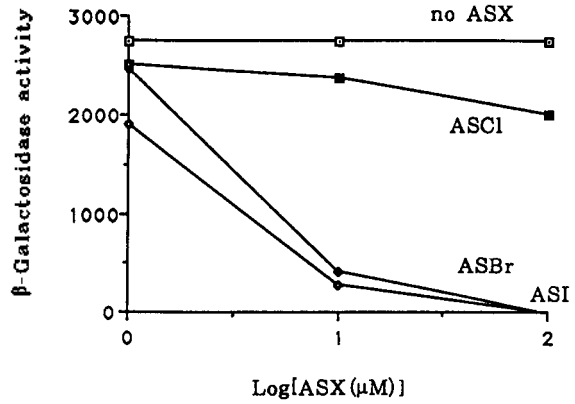


**Figure 2.** Inhibition of *vir* gene expression by halogenated analogs of AS. *A. tumefaciens* strain 358mx was incubated for 8 hr with 100 μM AS in the presence of different concentrations of ASX.

examined by exploiting a mechanism-based inhibitor. Based on the proposed mechanism for both a phenol binding protein[7] and triose phosphate isomerase (TPI),[9] α-bromoacetosyringone (ASBr) was designed and found to act as a specific and irreversible inhibitor in *vir* gene expression.[8] The irreversibility of inhibition by ASBr was demonstrated by both washing experiment[8] and affinity labelling experiment with $^{125}$IBrAv.[3] In order to prove the presence of an acidic residue in a phenol binding site, ASBr was examined in the proposed model. If ASBr occupies the same binding site in Figure 1, a reactive allylic bromide results from the tautomerization. The nucleophile generated in the phenol binding protein attacks and displaces the bromide, producing a covalently modified protein. The importance of a leaving group for displacement reaction at the α-carbon of ASBr was demonstrated by the inhibition assay with several halogenated substrate analogs (Figure 2). The inhibitory effects of these compounds roughly follow the leaving group potential, I≥Br>Cl. In other words a correlation was found between the capacity of halogen substituents as leaving groups and the *vir* inducing activities. The results support that the mechanism-based inhibition occurred *via* the nucleophile generated by deprotonation; for instance, carboxylate anion generation from the deprotonation of carboxylate.

**Formation of a quinone methide as an intermediate.** The second prediction for the proposed model is that the tautomerization of a phenolic compound leads to the generation of a quinone methide. In order to understand different *vir* gene induction activities by several phenolics varing the number of methoxy groups *ortho* to the hydroxy group, chemical processes in which the active inducers were transformed by a putative phenolic binding protein (p 10 or p21)[3] were examined. For this purpose it was necessary to compare the thermodynamic aspects of active phenolic inducers with those of their corresponding quinone methide analogs. In this study the enthalpy change of tautomerization ($\Delta H_{taut}$) for each phenolic compound was studied by AM1 calculation (Table 1). Acetosyringone (AS) is more stable than its quinone methide tautomer by 18.8 kcal/mol, which makes the formation of a quinone methide very

**Table 1.** $\Delta H$ for tautomerization for some phenolics

Keto form Enol form

| | $H_{f,ketone}$ | $H_{f,enol}$ | $\Delta H_{taut}$ |
|---|---|---|---|
| R=H R'=H | −59.0 | −40.1 | 18.9 |
| R=H R'=$OCH_3$ | −96.9 | −75.7 | 21.2 |
| R=$OCH_3$ R'=$OCH_3$ | −130.9 | −112.1 | 18.8 |

$H_f$ values are estimated for each tautomer by AM1 calculations and reported with a unit of kcal/mol.

unfavorable. In fact the difference between a value obtained from AM1 calculation and a reported value for toluene is about 4 kcal/mol.[10] This observation indicated that the relative stability of AS with respect to its tautomer, which was calculated by AM1, could be wrong by up to 4 kcal/mol. Nevertheless the heat of tautomerization estimated by the AM1 calculation was very informative because the $\Delta H_{taut}$ values for all the three compounds were very similar within a range of 19-21 kcal/mol, regardless of the number of methoxy groups attached at the *ortho* position of the hydroxy group. Contrary to the different *vir* gene expression activities induced by these phenolics, it was surprising to discover that they have all the similar enthalpy changes upon tautomerization. It suggested that without any catalytic aid from the phenol binding proteins there would be no significant thermodynamic consideration taken into account in order to explain any effect of the number of methoxy groups *ortho* to the hydroxy group.

**Evaluation of a basic residue as a proton acceptor.** From the working model it is also predicted that the efficiency of a proton transfer is related to the capability of a phenolic binding protein to deprotonate the hydroxyl group (Figure 1). Therefore it is envisioned that a phenolic compound with a different $pK_a$ value may have a different activity in *vir* gene induction. The requirement of a proton transfer in *vir* gene induction suggests that any active inducer may have a similar $pK_a$ value to that of AS, which was previously measured as 7.4.[11] In order to obtain a phenolic compound with a dramatically changed $pK_a$ value, a nitro substituent was considered as a candidate because if it were substituted at either ortho or *para* to the hydroxy group, it could lower a $pK_a$ value by 3.1 or 2.8 unit, respectively.[12] Any compounds having a nitro group as a substituent may be too acidic to protonate a basic residue of a phenol binding protein in this model. For this purpose compound **1** (methyl 4-hydroxy-3-methoxy-5-nitrocinnamate) and **2** (4-hydroxy-3-methoxy-5-nitrobenzaldehyde) with a nitro group substituted at the ortho position of the hydroxyl group were prepared[13] (Figure 3a) and assayed for *vir* gene expression (Table 2). These compounds were chosen because their corresponding methoxy derivatives, methy 4-hydroxy-3,5-dimethoxycinnamate (methyl sinapinate) and 4-hydroxy-3,5-dimethoxy benzaldehyde (syringaldehyde), were known to induce the *vir* gene expression with 85%

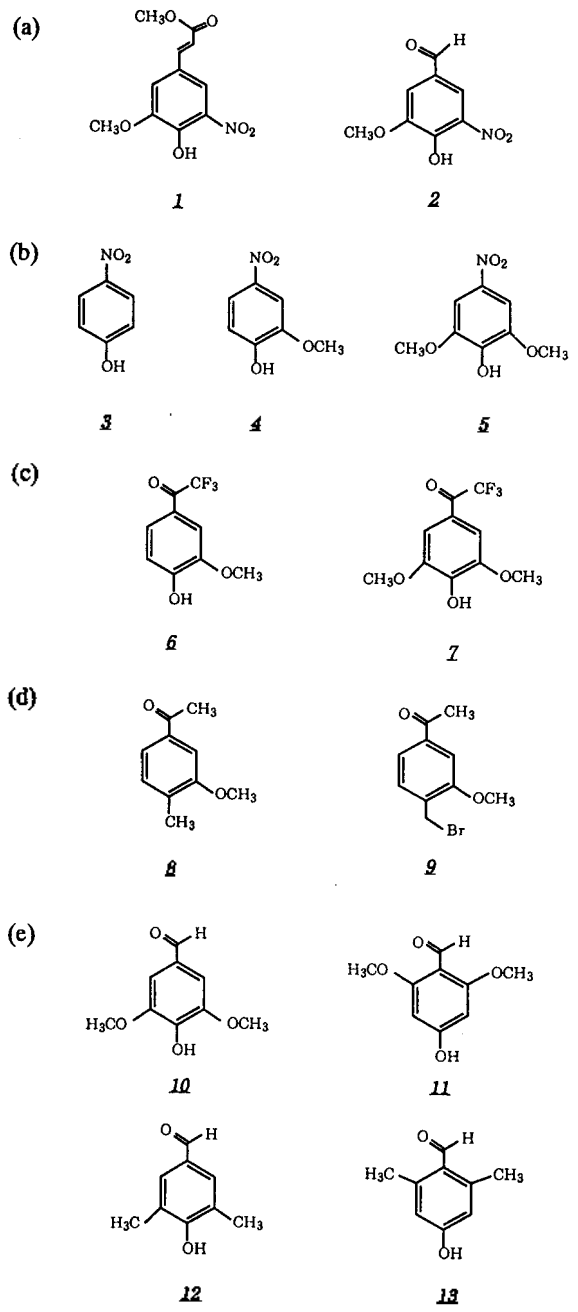


**Figure 3.** Structures of compounds tested for evaluating a working model. (a) The *ortho*-nitro substituted compounds in *vir* gene expression, (b) The *para*-nitro substituted compounds in *vir* gene expression, (c) The trifluoromethyl compounds tested in the $pK_a$ study, (d) The compounds used in inhibition assay to examine a basic residue, and (e) The compounds assayed for the regioselectivity in the recognition.

and 105% activities of AS control, respectively.[14] The difference in their *vir* gene inducing capabilities exerted by the two similar compounds **1** and **2** was, however, remarkable. Compound **1** was surprisingly active up to 81% of AS control even if it's $pK_a$ is expected to be too low as an active inducer. On the other hand, compound **2** was found to be inactive as expected since in the presence of a strong electron withdrawing group such as a nitro group the formyl

**Table 2.** *vir* gene induction and inhibition

| Compounds | *vir* gene expression (% of 100 μM AS control) |
|---|---|
| 100 μM **1** | 81.0±11.0 |
| 100 μM **2** | 5.4±0.1 |
| 100 μM **3** | 2.0±0.1 |
| 100 μM **4** | 1.9±0.1 |
| 100 μM **5** | 85.0±17.2 |
| 100 μM **6** | 1.3±0.1 |
| 100 μM **7** | 80.5±1.7 |
| 100 μM AS+10 μM **8** | 142.4±13.8 |
| 100 μM AS+10 μM **9** | 82.5±10.5 |
| 100 μM **10** | 106.2±11.0 |
| 100 μM **11** | 1.5±0.1 |
| 100 μM **12** | 6.4±0.1 |
| 100 μM **13** | 16.1±3.5 |

Each compound was assayed for *vir* gene induction at 100 μM concentration and for inhibition at 10 μM in the presence of 100 μM AS. All the β-galactosidase activities were reported as % of the AS control, which was incubated with 100 μM AS at the same condition.

group is easily converted to a diol. The results indicate that the capacity of the $sp^2$ center as an electron withdrawing moiety at the *para* position appears to override the $pK_a$ requirement in this model. In other words it is possible to displace the methoxy group ortho to the hydroxy group with a nitro group to activate *vir* gene expression comparable to AS, depending on the type of functional groups located at the *para* position. This will expand a structural prerequisite of active inducers and inactivators in this system. 2,6-dinitrofluorobenzene (FDNB) was considered as a nitro analog substituted at the ortho position and actually found to inactivate the *vir* gene induction as effectively as ASBr (unpublished data). The other set of phenolic compounds bearing a nitro group at the *para* position of the hydrxyl group, **3** (4-nitrophenol), **4** (2-methoxy-4-nitrophenol), **5** (2, 6-dimethoxy-4-nitrophenol), were also examined for their abilities to induce *vir* gene expression (Figure 3b). Their $pK_a$ values are expected to be the same as 7.15 since the methoxy groups ortho to the hydroxy group do not alter the $pK_a$ values.[12] However, irrespective of the similar $pK_a$ values of these compounds, compound **5** was the only active inducer among them (Table 2). The *vir* inducing activity of compound **5** is attributed to the two methoxy groups which can serve as hydrogen acceptors. These findings suggest that the *para* functional group can be replaced with a nitro group only when there exist two methoxy groups ortho to the hydroxyl group in order to provide a sufficient binding interaction. The *vir* gene activation was examined by trifluoromethyl analogs of acetovanillone and AS to evaluate the effect of trifluoromethyl as a better electron withdrawing group at the *para* position to the hydroxy group. However, the $pK_a$ values of **6** (3-methoxy-4-hydroxy-trifluoroacetophenone) and **7** (3,5-dimethoxy-4-hydroxy-trifluoroaceto phenone) were measured by UV and shown to be similar to that of AS (Figure 4). The reason for the failure in changing the $pK_a$ values of these compounds may be that trifluoromethyl group makes the carbonyl carbon more electrophilic so that it can be attacked by a base (-OH)

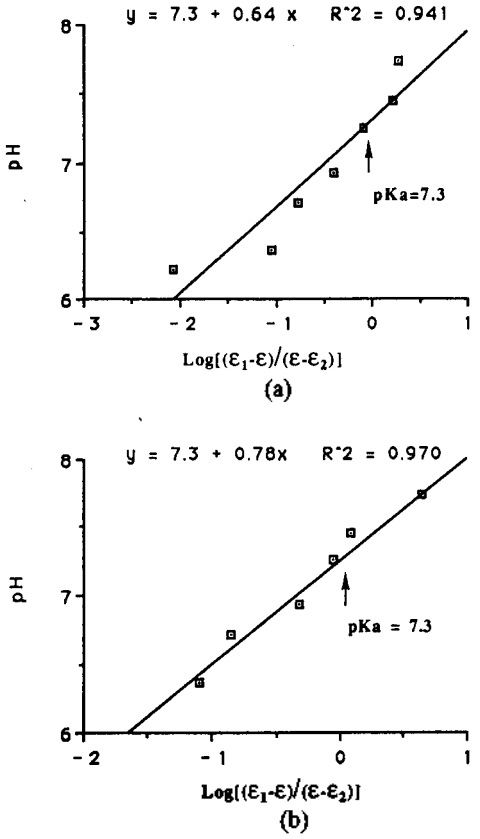


**Figure 4.** The $pK_a$ measurements of trifluoromethyl derivatives, (a) compound **6** and (b) compound **7**. The pH values at which samples were monitored were plotted against $Log[(\varepsilon_1-\varepsilon)/(\varepsilon-\varepsilon_2)]$ to give $pK_a$ values at the intercept with y-axis.

more easily. The resulting hydroxylated compounds lose the electron withdrawing moiety when present in an aqueous solution. Therefore compound 6 was shown to be inactive in *vir* gene expression (Table 2), which was surprising when compared to acetovanillone with 78% of the AS control. On the other hand, two ortho-methoxy groups on the trifluoromethyl ketone derivative did give an active compound **7**, consistent with the result of compound **5** with the *para*-nitro substituent. Based on these data, it is concluded that the presence of two methoxy groups has a priority over the other structural factors. It may be that the tighter binding formed by these substituents overrides the required tautomerization as an activation mechanism. In addition, compounds with other structural moieties at the position of the hydroxy group were also prepared and tested in order to examine the presence of a basic residue in the phenol binding protein. Compound **8** (3-methoxy-4-methylacetophenone) and **9** (4-bromomethyl-3-methoxyacetophenone) were prepared[16] with the different structural variations at the hydroxy position (Figure 3d). The bromine in compound **9** is also expected to be replaced by a basic residue of the phenol binding protein, if any. However, neither of them showed significant inhibition of *vir* gene expression activity at 10 μM. The increased steric hindrance afforded by these substituents around the phenol site on the binding protein may be a crucial factor in the failure for these compounds in controlling *vir* gene expression. Instead, when it was sub-

stituted by fluorine as in FDNB, a basic residue in the binding protein replaced it in a $S_N2$ reaction and was covalently modified by this inhibitor (unpublished data).

**Regioselectivity of methoxy groups.** The importance of the methoxy groups in the active phenolics was confirmed by evaluating the regioselectivity of methoxy groups relative to the phenolic hydroxy groups. Structurally similar compounds **10** (3,5-dimethoxy-4-hydroxybenzaldehyde), **11** (2,6-dimethoxy-4-hydroxybenzaldehyde), **12** (3,5-dimethyl-4-hydroxybenz aldehyde), **13** (2,6-dimethyl-4-hydroxybenzaldehyde) were assayed for *vir* gene expression (Figure 3e). It was found that compound **12** was not an active inducer in *vir* gene expression as much as **10** (Table 2). It was also interesting to note that methyl groups will not substitute for the methoxy groups in *vir* gene induction as shown by **12** and **13** (Table 2). These data suggest that the correct hydrogen bond formation between the methoxy groups and a phenol binding protein is essential to activate *vir* gene expression.

## Experimental

**Materials.** Compounds **1** and **2** were prepared as described in Lee *et al.*[13] Compounds **6** and **7** were synthesized according to Dudley[15] and compounds **8** and **9** were obtained by Zeng.[16] The rest of compounds were obtained from Aldrich.

**Synthesis of 2,6-dimethoxy-4-hydroxybenzaldehyde (11).** Under the Vielsmeyer-Haak reaction condition, 2 mL of phosphorous oxychloride was added dropwise to a mixture of 3,5-dimethoxyphenol (1 g, 6.5 mmol) and 2 mL of dimethylformate, cooled down to 0 °C for 30 min. After 1 hr at room temperature, the reddish orange viscous material was treated with 12 mL $H_2O$. The reddish mixture was extracted with ether three times. The aqueous layer was saturated with sodium acetate and left overnight. An organic solid appeared and was separated by filtration. The solid was stirred with 10 mL ethyl acetate and filtered again. The sparingly soluble solid was recrystallized from methanol to give a reddish brown product (143 mg, 0.8 mmol). $^1$H NMR (500 MHz, $CDCl_3$) δ 10.28 (s, 1H, H-1), δ 6.01 (s, 2H, H-3'), δ 3.84 (s, 6H, -$OCH_3$). EI$^+$, 70 eV, m/z 182 (100, M$^+$), 167 (10, M-$CH_3$), 153 (15, M-CHO), 151 (13, M-$OCH_3$), 122 (5, M-(CHO+$OCH_3$)).

**Bacterial Strains and Medium.** *A. tumrfaciens* strain 358mx (*vir*E:lacZ) ($K_2HPO_4$, 1.72 mM; $NaH_2PO_4\times H_2O$, 7.25 mM; $NH_4Cl$, 18.5 mM; $MgSO_4\times 7H_2O$, 1.22 mM; KCl, 2.00 mM; $CaCl_2\times H_2O$, 0.077 mM; $FeSO_4\times 7H_2O$, 0.021 mM; glucose, 0.05%; pH 7.0) supplemented with 100 g/mL of carbenicillin.[17] Induction medium was prepared as follows: $K_2HPO_4$, 1.72 mM; $NaH_2PO_4\times H_2O$, 0.725 mM; $NH_4Cl$, 18.5 mM; $MgSO_4\times 7H_2O$, 1.22 mM; KCl, 2.00 mM; $CaCl_2\times H_2O$, 0.077 mM; MES hydrate 20 mM and sucrose, 30 g or glucose, 10 g per liter as a carbon source. Induction medium was prepared fresh daily (pH 5.5) and all components were filtered through a 0.45 μm syringe filter.

***vir* gene induction and inhibition assay.** *A. tumefaciens* strain 358mx (*vir*E:lacZ) contains a promoterless *lacZ* gene inserted into *virE* in the Ti plasmid through transposon mutagenesis with Tn3HoHo1.[18] The bacterial cells were grown to an optical density ($OD_{600}$) of 0.2-0.55 on AB minimal medium supplemented with 100 μg/mL of carbenicillin. The cells were pelleted and resuspended at 0.05 $OD_{600}$/mL in induction medium containing various compounds. In general the test compounds were dissolved in 10% DMSO/$H_2O$ mixtures at millimolar concentrations, diluted to appropriate concentrations and filtered through a 0.45 μm syringe filter. These solutions were diluted 10-fold with $H_2O$ to obtain the desired concentrations. Both background and positive control samples contained induction medium only and induction medium supplemented with 100 μM AS, respectively. Samples were placed in a 15 mL sterile centrifuge tube and agitated (200 rpm) at 28 °C for the indicated time. At the end of induction period, samples were either worked up directly for β-galactosidase activity or stored frozen at −76 °C for later analyses. The *vir* gene inhibition assay was carried out as the induction assay with an appropriate concentration of the inhibitor in the presence of 100 μM AS.

**Measurement of β-galactosidase activity.** The level of *vir* gene expression was monitored by assaying for the amount of the β-galactosidase. The *o*-nitrophenol liberated from *o*-nitrophenol-β-galactoside was used as a monitor of *vir* gene expression and measured by the absorbance of *o*-nitrophenolate at 420 nm. After incubated with each compound, the induced samples were transferred to microfuge tubes (1.5 mL) and microfuged for 5 minutes to collect bacterial cells. The supernatant was removed and the pellets were resuspended in 600 μL of Z buffer ($Na_2HPO_4\times 7H_2O$, 60 mM; $NaH_2PO_4\times 7H_2O$, 40 mM; $MgSO_4\times 7H_2O$, 1 mM; KCl, 10 mM; dithiothreitol, 20 mM, pH 7.0). A 100 μL aliquot was removed, diluted to 1 mL with Z buffer, and the $OD_{600}$ of this solution ($A_{600}$) was read. To the remaining 500 μL of the cells, 20 μL of 0.05% SDS and 20 μL of $CHCl_3$ were added. The cells were vortexed for 10 seconds and then incubated at 28 °C and 200 rpm for 10 minutes. 100 μL of a 4 mg/mL solution of 2-nitrophenyl-β-D-galactosidase (ONPG) in phosphate buffer (0.1 M, pH 7.0) was added to initiate the reaction. After 30 minutes, the reaction was terminated by the addition of 250 μL of 1 M $Na_2CO_3$. Samples were microfuged for 8 minutes and the absorbance was read at 420 nm for 800 μL of the solutions. The amount of β-galactosidase activity was calculated as uints/bacterial cell=$A_{420}\times 1000/T\times A_{600}$ where T is the incubation time.

**Measurement of $pK_a$.** Nine 0.1 M sodium phosphate buffers were adjusted to have a range of pH between 6.0 and 8.0. Each compound was diluted with DMSO/$H_2O$ mixtures to a concentration of 1 mM. 30 μL of these solutions were added to 1 mL of buffers with nine different pHs to have a final concentration of 30 μM. Absorbances were read at $\lambda_{max}$ for all buffer solutions between pH=6.0-8.0. The molar absorptivities, $\varepsilon_1$ and $\varepsilon_2$ at the extremes of the pH range and ε at the different pH buffer solutions were determined using absorbance values at $\lambda_{max}$. A plot of the pH *vs.* $\log[(\varepsilon_1-\varepsilon)/(\varepsilon-\varepsilon_2)]$ gave a straight line and $pK_a$ was determined at the intercept with y-axis.

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# Sharp-Line Electronic Spectroscopy and Ligand Field Analysis of [Cr(*trans*-diammac)](ClO$_4$)$_3$[1]

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The luminescence and excitation spectra of [Cr(*trans*-diammac)](ClO$_4$)$_3$ (*trans*-diammac=*trans*-6,3-dimethyl-1,4,8,11-tetraazacyclotetradecane-6,13-diamine) taken at 77 K are reported. The mid and far-infrared spectra at room-temperature are also measured between 4000 cm$^{-1}$ and 50 cm$^{-1}$. In the excitation spectrum the $^2E_g$ components are splitted by 102 cm$^{-1}$. Using the observed electronic transitions, a ligand field analysis was performed to determine more detailed bonding properties of the coordinated atoms toward chromium(III). According to the results, we can confirm that the six nitrogen atoms have a strong $\sigma$-donor character, and the *trans*-diammac secondary amine has a greater value of $e_\sigma$ than does the primary amine.

## Introduction

The sharp-line spectroscopic study of chromium(III) compound started with six coordinated nitrogen atoms, because the intensities of absorption or excitation spectra at the low temperature of these compound are very strong. In recent years a considerable amount of such spectral data has been accumulated.[2-5] The ligand field optimization of the electronic spectra, including the sharp spin-forbidden transitions promises to provide more detailed information concerning metal-ligand interactions.[6-10] With the use of chromium(III) system, the sharp electronic lines can be found with a precision two orders of magnitude greater than the broad spin-allowed bands. The sharp-line splittings are also quite sensitive to the metal-ligand geometry. Thus, it is possible to extract geometric information from the molecular spectroscopy.[11-13]

There has also been considerable interest in the coordination chemistry of the pendant arm macrocyclic hexaamine ligand, 6,3-dimethyl-1,4,8,11-tetraazacyclotetradecane-6,13-diamine (diammac) with the first row transition metal ions.[14-17] The syntheses and structural characterization of chromium(III) complexes with *trans* and *cis* ligand conformations (Figure 1) have been reported.[16,17] It was found