Asian Journal of Chemistry Vol. 7, No. 1 (1995) 27–32

Synthesis and Biological Studies of 3,4,5-Trimethoxy benzalanilines

M.R. MANRAO*, CHANDER KANTA, R.C. SHARMA, P.S. KALSI and V.K. KAUL†
Department of Chemistry
Punjab Agricultural University, Ludhiana-141 004, India

Ten Schiff bases of 3,4,5-trimethoxybenzaldehyde have been synthesized by condensing 3,4,5-trimethoxybenzaldehyde with aniline and substituted anilines. The Schiff bases were reduced with sodium borohydride to get secondary amines. All the synthesized compounds were identified and confirmed on the basis of elemental analysis and spectral studies. The synthesized compounds were tested *in vitro* against five fungi *viz., Alternaria tenuis, Ustilago tritici, Sphaeceloma maydis, Puccinia recondita* and *Alternaria triticina* and two nematodes *Caenorhabditis elegaus* and *Ditylenchus myceliophagus*. Some of the compounds have shown promising antifungal and nematicidal activity.

INTRODUCTION

Schiff bases, the condensation products of carbonyl compounds and amines, in general and those containing methoxy group[2], chloro atom[3], hydroxy group[4] and thio group[5] in the phenyl nucleus in particular are reported to possess biological activity. The aim of the present work is to study the effect of presence of three methoxy groups in the C-phenyl nucleus on the course of this reaction and on the antifungal and nematicidal activity of the products. The present article describes the synthesis of ten Schiff bases of 3,4,5-trimethoxybenzaldehyde, their reduction and spectral studies, antifungal activity and nematicidal properties of the products.

EXPERIMENTAL

Synthesis of the Schiff Bases

3,4,5-Trimethoxybenzaldehyde (19.6 g, 0.1 mol) and aniline (9.3 g, 0.1 mol) were taken in benzene (100 mL) in a RB flask (250 mL) fitted with 'Dean and Stark' apparatus. The mixture was refluxed till water (1.8 g, 0.1 mol) was separated out. The mixture was then cooled to obtain the crude product which was recrystallized from ethanol to get white shining crystals of 3,4,5-trimethoxybenzalaniline(Ia).

†Department of Plant Pathology Punjab Agricultural University, Ludhiana-141 004, India.

Reaction, of 3,4,5-trimethoxybenzaldehyde with *p*-toluidine, *o*-anisidine, *p*–anisidine, *p*-phenetidine, *o*-aminophenol, *p*-aminophenol, *m*-chloroaniline, *p*-chloroaniline and *p*-bromoaniline was carried out by following the above procedure to get 3,4,5-trimethoxybenzal-*p*-toluidine (IIa), 3,4,5-trimethoxybenzal-*o*-anisidine (IIIa), 3,4,5-trimethoxybenzal-*p*-anisidine (IVa), 3,4,5-trimethoxybenzal-*p*-phenetidine (Va), 3,4,5-trimethoxybenzal-*o*-hydroxyaniline (VIa), 3,4,5-trimethoxybenzal-*p*-hydroxyaniline (VIIa),3,4,5-trimethoxybenzal-*m*-chloroniline (VIIIa), 3,4,5-trimethoxybenzal-*p*-chloroaniline (IXa) and 3,4,5-trimethoxybenzal-*p*-bromoaniline (Xa). (yield 90–96%).

Sodium Borohydride Reduction of 3,4,5-trimethoxybenzalanilines

3,4,5-Trimethoxybenzalaniline (2.71 g, 0.01 mol) was dissolved in methanol (50 mL) and the solution was warmed to 60°C. To the warm solution added sodium borohydride in small amounts with constant stirring. After the complete addition of sodium borohydride, the solution was filtered, and stirred for 1 h. The reaction mixture was cooled and allowed to stand at room temperature for 24 h. The excess of the solvent was distilled off and water (100 mL) was added to it and then the mixture was placed in an ice bath for 3 h when a solid separated out which was filtered. The crude sample was recrystallised from methanol to get pure crystals of the secondary amine (Ib).

Reduction of IIa to Xa was carried out in the similar way to get the respective secondary amines. (yield 80–95%).

***In Vitro* Testing of Antifungal Activity**

Each compound (20 mg) was dissolved initially in ethanol (0.5 mL). The final volume was made upto 10 mL by adding distilled water. The resultant solution of 2000 ppm concentration was diluted serially to 1000, 500, 250, 250, 100, 50 and 25 ppm concentrations. Antifungal activity of the synthesized compounds was tested *in vitro* against *Alternaria tenuis, Ustilago tritici, Sphaeceloma maydis, Puccinia recondita* and *Alternaria triticina* by spore germination inhibition mehtod[6]. In each treatment 100 spores were counted. Cavity slides containing spores of the test fungi in sample solutions were incubated at 25 ± 1°C for 18 h. Percentage spore germination inhibition was calculated by using the formula

$$\frac{\text{Spore germination in control} - \text{Spore germination in treatment}}{\text{Control}} \times 100$$

ED_{50} (Effective dose at which 50% spore germination inhibition was caused) values were calculated by log probability method.

***In Vitro* Screening of the compounds for their Nematicidal Activity**

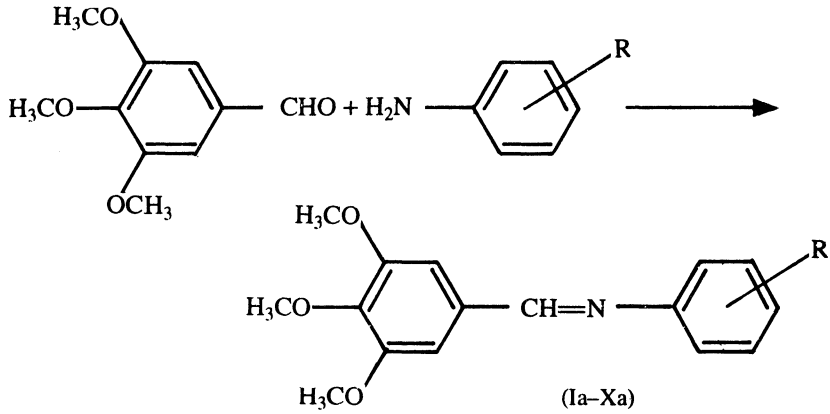
Pure cultures of *Caenorhabditis elegaus* and *Ditylenchus myceliophagus* were raised on *Agaricus bisporus* spawn cultured on wheat media. The cultures were maintained at a temperature 18–24°C and nematodes were extracted from the media following Cobb's seiving and decantation technique. Freshly extracted nematodes of all developmental stages were utilized for the preliminary screening.

The test solutions of the compounds were prepared as mentioned above in the

antifungal testing. About 100 nematodes of each genera were exposed to 2 mL solution of the test compounds and observations for number of living or dead nematodes were recorded after an interval of 48 h. The immobile nematodes with a definite posture were recorded as dead. After 96 h the nematodes were transferred to distilled water and the possible reversal of the immobilized nematodes was recorded.

RESULTS AND DISCUSSION

Ten Schiff bases (Ia to Xa), synthesized by condensing 3,4,5-trimethoxybenzaldehyde with aniline and substituted anilines are recorded in Table 1. These are characterised by elemental analysis and spectral studies.



(Ia–Xa)

TABLE-1
CHARACTERISTICS OF 3,4,5-TRIMETHOXYBENZALANILINES

Compund	R	Colour	m.p.* (°C)	Molecular formula**
Ia	H	Dark yellow	98	$C_{16}H_{17}NO_3$
IIa	*p*-CH_3	Yellow	110	$C_{17}H_{19}NO_3$
IIIa	*o*-OCH_3	Lemon yellow	90	$C_{17}H_{19}NO_4$
IVa	*p*-OCH_3	Light green	136	$C_{17}H_{19}NO_4$
Va	*p*-OC_2H_5	Green	138	$C_{18}H_{21}NO_4$
VIa	*o*-OH	Brown	90	$C_{16}H_{17}NO_4$
VIIa	*p*-OH	Dark Brown	180	$C_{16}H_{17}NO_4$
VIIIa	*m*-Cl	Light grey	70	$C_{16}H_{16}NO_3Cl$
IXa	*p*-Cl	Dirty red	165	$C_{16}H_{16}NO_3Cl$
Xa	*p*-Br	Greyish	125	$C_{16}H_{16}NO_3Br$

*All the melting points are uncorrected.
**All the compounds gave satisfactory elemental analysis.

UV spectra of the Schiff bases have been recorded in methanol on a manually operated spectrophotometer (Hilger Watts Model-700) using silica cells. Generally four absorption bands at 212, 235, 270 and 340 nm are obtained. Two

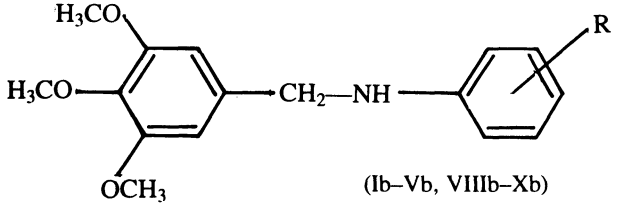
of these bands (235 nm and 340 nm) are sensitive to N-phenyl substitution whereas the other two bands (212 and ~ 270 nm) usually remain unaffected. Those bands which are sensitive to substitution, correspond to electron-transition involving the N-phenyl ring and the bands which remain unaffected correspond to the transition involving the C-phenyl nucleus of the molecule. The band at 270 nm is the band which is used to identify the azomethine (>C=N—) linkage. This band is relatively insensitive to N-phenyl substitution, but substitution by three methoxy groups in the C-phenyl ring gives a small red shift compared to 262 nm value of benzalanilines. This band completely disappears on reduction of the Schiff base with sodium borohydride. Intramolecular hydrogen-bonding which occurs in case of 3,4,5-trimethoxy-(*o*-hydroxyaniline) causes a shift of ~ 10 nm in the value of the band at ~ 235 nm.

Infrared spectra of the Schiff bases have been recorded in nujol. The spectra show bands at 1600–1575 cm^{-1} which are assigned to the azomethine linkage. This band assignment is supported by the chemical evidence as it disappears on reduction of the Schiff bases.

PMR spectra of the Schiff bases have been recorded in $CDCl_3$. PMR spectrum of 3,4,5-trimethoxybenzal-(*p*-methylaniline) indicates six aromatic protons as a multiplet between 2.6 to 2.9 τ, nine methoxy protons at 6.1 τ as a singlet, three methyl protons at 6.7 τ as a siglet and azomethine proton at 1.7 τ also as a singlet. The observed azomethine proton singal at 1.6 τ is in fair agreement with the earlier reported value.

Reduction of the Schiff bases

3,4,5-Trimethoxybenzalanilines (Ia to Xa) are reduced with sodium borohydride in methanol 50–60°C. The use of higher temprature is partially necessitated because of low solubility of the Schiff bases in methanol. The presence of methoxy substituent in the *ortho* position (IIIa) of the aniline moiety of the Schiff base suppresses the reduction because of steric hindrance due to bulky methoxy group in the vicinity of carbon-nitrogen double bond. Further the reduction is not possible when a hydroxy group is present in the N-phenyl ring, only intense colour changes have been observed. The crystalline secondary amines obtained from the reduction of the Schiff bases with sodium borohydride are recorded in Table 2.



(Ib–Vb, VIIIb–Xb)

TABLE-2
CHARACTERISTICS OF DIHYDRO PRODUCTS

Compund	R	Colour	m.p.* (°C)	Molecular formula**
Ib	H	White	60	$C_{16}H_{19}NO_3$
IIb	*p*-CH_3	White	110	$C_{17}H_{21}NO_3$
IIIb	*o*-OCH_3	Chocolate	75	$C_{17}H_{21}NO_4$
IVb	*p*-OCH_3	Light Brown	79	$C_{17}H_{21}NO_4$
Vb	*p*-OC_2H_5	Dusty brown	90	$C_{18}H_{23}NO_4$
VIIIb	*m*-Cl	Creamish	85	$C_{16}H_{18}NO_3Cl$
IXb	*p*-Cl	Dust coloured	120	$C_{16}H_{18}NO_3Cl$
Xb	*p*-Br	Light green	108	$C_{16}H_{18}NO_3Br$

*All the melting points are uncorrected.
**All the compounds gave satisfactory elemental analysis.

A comparative study of the UV and IR spectra of the reduced products with those of the parent Schiff bases reveals that UV spectra of dihydro products lack the absorption maxima at ~ 270 nm. The IR spectra of the dihydro products does not contain absorption bands (~ 1625 cm^{-1}) assigned to azomethine linkage but additional bands appear at 3250 cm^{-1} which are assigned to the N—H group.

Antifungal Activity

Ten Schiff bases and eight reduced products have been tested for their antifungal activity against *A. tenuis, U. tritici, S. maydis, P. recondita* and *A. triticina* by employing spore germination inhibition method at various concentrations. The results have been recorded in terms of ED_{50} values (Table 3). All the Schiff bases except Xa have been found to be effective against one or the other of the test fungi. These Schiff bases have shown promising antifungal activity against *U. tritici*. In general, 3,4,5-trimethoxybenzal-*o*-hydroxyaniline (VIa) has been found to be the most effective compound. This may be attributed to the presence of hydroxy group in the *ortho* position of the N-phenyl nucleus. Only five out of the eight dihydro products tested have shown antifungal activity. These are mostly effective against *P. recondita*. None of the dihydro products has been found to be effective against *Alternaria* sp.

Nematicidal Activity

In all 18 compounds have been tested for their nematicidal activity against *C. elegaus* and *D. myceliophagus* and the results are expressed in terms of EC_{50} values. Schiff bases (Ia-Xa) paralyse most of the nematodes, though not dead. The revival of the paralysed nemathodes is observed in all except three cases, when the chemical solution is replaced by water, thus indicating the reversible action induced by these chemicals. Dihydro products have been found to have no effect on the test nematodes. The three Schiff bases which are found to be effective are IIa against *C. elegaus* (Ec_{50} 1850 ppm) and IIIa and IVa against *D. myceliophagus* (EC_{50} 1850 ppm and 1600 ppm respectively).

TABLE-3
ANTIFUNGAL ACTIVITY OF 3,4,5-TRIMETHOXYBENZALANILINES AND DIHYDRO PRODUCTS (ED_{50} values in ppm)

Compund No.	*A. tenuis*	*U. tritici*	*S. maydis*	*P. recondita*	*A. triticina*
Ia	865	150	600	*	*
IIa	*	270	*	895	*
IIIa	*	210	610	*	*
IVa	*	810	*	*	*
Va	*	*	*	535	*
VIa	560	175	200	355	*
VIIa	*	880	*	220	*
VIIIa	*	610	*	*	*
IXa	790	230	*	245	210
IIIb	*	770	*	*	*
IVb	*	*	*	240	*
Vb	*	690	*	220	*
IXb	*	*	*	375	*
Xb	*	880	910	*	*

*More than 1000 ppm.

REFERENCES

1. D.N. Dhar and C.L. Taploo, *J. Sci. Ind. Res.*, **41**, 501 (1982).
2. M. Rai, Baljit Kaur and B.S. Dhir, *J. Indian Chem. Soc.*, **59**, 416 (1982).
3. H. Pacheco, L. Cronenberger, D. Pillon and J. Thiolliera, *Chem. Abstr.*, **72**, 111001, 111002 (1970).
4. M.R. Manrao and Sunita Kohli, *J. Indian Chem. Soc.*, **63**, 348 (1986).
5. J.R. Sharma, M.R. Manrao, Daljeet Kaur and P.S. Kalsi, *Plant Disease Research*, **6**, 90 (1991).
6. Anonymous, *Phytopathology*, **33**, 627 (1943).

(*Received*: 23 September 1993; *Accepted*: 30 April 1994) AJC-809