Turk J Chem
33 (2009) , 257 – 266.

doi:10.3906/kim-0806-3

Synthesis, Spectral Characterization, and Antimicrobial Activity of Arsenic(III) and Bismuth(III) tri[3(2'-hydroxyphenyl)-5-(4-subsituted phenyl)pyrazolinates]

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Received 02.06.2008

Arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates of the type $M(C_{15}H_{12}N_2OX)_3$ [where $C_{15}H_{12}N_2OX$ = 3(2'-hydroxyphenyl)-5-(4-substituted phenyl)pyrazoline] have been synthesized by the reaction of MCl_3 and sodium salt of pyrazolines in 1:3 molar ratio in anhydrous benzene at elevated temperature. These newly synthesized derivatives have been characterized by elemental analysis (C, H, N, As, and Bi), molecular weight measurement, spectral [IR and multinuclear NMR (^1H & ^{13}C)] and x-ray diffraction studies. The bonding mode of pyrazolines and coordination no. of arsenic(III) and bismuth(III) in these derivatives have been discussed. Antibacterial and antifungal potential of free pyrazoline and some arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates have also been discussed.

Key Words: Arsenic , Bismuth , Pyrazolinates, Antimicrobial activity.

Introduction

Pyrazolines are an important class of heterocyclic compounds used in industries as dyes, lubricating oils, antioxidants, and in agriculture as catalysts for decarboxylation as well as inhibitors for plant growth.[1−3]

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Complexation behavior of 3(2'-hydroxyphenyl)-5-phenylpyrazoline with Ni(II), Co(II), and Cu(II) have been investigated in our laboratories.[4] We have also investigated the complexation behavior and antimicrobial potential of 3(2'-hydroxyphenyl)-5-phenylpyrazoline and substituted pyrazolines with tin(IV), organotin(IV), diorganotin(IV), and triorganotin(IV).[5−9]

The antimicrobial activity of organic compounds containing bismuth and antimony is partial in number, while those involving arsenic are more numerous.[10] Bismuth (III) compounds have been used in the treatment of gastrointestinal diseases for about 2 centuries.[11] Bismuth (III) compounds do not require maintenance of a practically neutral stomach pH, which involves the risk of intestinal infections with other bacteria that can no longer be destroyed by the acidic gastric juice.[12] Colloidal bismuth sub-citrate is the most popular bismuth drug in the classical triple therapy.[13−15] Bismuth(III) compounds, which are useful in treatment of peptic ulcers, is now used in the clinic.[16−18]

In continuation to our previous work, it was thought worthwhile to study the complexation behavior of 3(2'-hydroxyphenyl)-5-phenylpyrazoline and substituted pyrazoline with Arsenic (III), Antimony (III), and Bismuth (III). We have studied the synthesis, spectral study, and antimicrobial activity of dichloroantimony(III) pyrazolinates, chloroantimony(III) dipyrazolinates, dichlorobismuth(III) pyrazolinates, and chlorobismuth(III) dipyrazolinates.[19−20] In the present paper we describe the synthesis, spectral characterization, and antimicrobial activity of arsenic(III) tri[3(2'-hydroxyphenyl)-5-(4-substituted phenyl)pyrazolinates] and bismuth(III) tri[3(2'-hydroxyphenyl)-5-(4-substituted phenyl)pyrazolinates].

Experimental

Solvents (benzene, acetone, and alcohol) were rigorously dried and purified by standard methods before use.[21] All the chemicals used were of analytical grade quality. Bismuth trichloride (E.Merck) was used as received and arsenic trichloride was prepared in laboratory by the reaction of arsenic trioxide with thionyl chloride[22]. 2-Hydroxyacetophenone (CDH) and benzaldehydes (E.Merck) were used as received.

Ligands were prepared by the reported procedure.[23]

Synthesis of $\mathbf{As(C_{15}H_{12}N_2OX)_3}$

The new arsenic (III) tripyrazolinates of the general formula $As(C_{15}H_{12}N_2OX)_3$ were prepared by the reaction of arsenic(III) trichloride and the sodium salts of pyrazolines in 1:3 molar ratio.

$$AsCl_3 + 3Na(C_{15}H_{12}N_2OX) \xrightarrow[\text{benzene}]{\text{Anhydrous}} As(C_{15}H_{12}N_2OX)_3 + 3NaCl$$

[where X = H , CH_3, OCH_3, and Cl]

Synthesis of $\mathbf{As(C_{15}H_{13}N_2O)_3}$

Freshly cut pieces of sodium (0.194 g; 8.43 mmol) were taken in a flask with excess of isopropanol and refluxed for ∼30 min., till a clear solution of sodium isopropoxide was obtained. The solution of 3(2'-hydroxyphenyl)-5(4-phenyl)pyrazoline (2.00 g; 8.43 mmol) in anhydrous benzene was then added and the reaction mixture

was further refluxed for ∼1 h, giving a yellow colored solution. The reaction mixture was cooled to room temperature and then benzene solution of arsenic trichloride (0.509 g; 2.81 mmol) was added with constant stirring. The reaction mixture was further stirred at reflux temperature for ∼7 h, till the color of the reaction mixture underwent a change. Reaction mixture was filtered to remove the precipitated NaCl. The solvent was removed under reduced pressure from the filtrate. The yellow colored solid thus obtained was reprecipitated in acetone and dried in vacuum to get 1.79 g of $As(C_{15}H_{13}N_2O)_3$. Compounds no. 2, 3, and 4 were prepared by the same method. The analytical details are summarized in Table 1.

Synthesis of $Bi(C_{15}H_{12}N_2OX)_3$

The new bismuth(III) tripyrazolinates of the general formula $Bi(C_{15}H_{12}N_2OX)_3$ were prepared by the reaction of bismuth(III) trichloride and the sodium salts of pyrazolines in 1:3 molar ratio.

$$BiCl_3 + 3Na(C_{15}H_{12}N_2OX) \xrightarrow[\text{benzene}]{\text{Anhydrous}} Bi(C_{15}H_{12}N_2OX)_3 + 3NaCl$$

[where X = H , CH_3, OCH_3, and Cl]

Synthesis of $Bi(C_{15}H_{13}N_2O)_3$

Freshly cut pieces of sodium (0.194 g; 8.43 mmol) were taken in a flask with excess of isopropanol and refluxed for ∼30 min., till a clear solution of sodium isopropoxide was obtained. The solution of 3(2'-hydroxyphenyl)-5(4-phenyl) pyrazoline (2.00 g; 8.43 mmol) in anhydrous benzene was then added and the reaction mixture was further refluxed for ∼1 h, giving a yellow colored solution. The reaction mixture was cooled to room temperature and then benzene solution of bismuth trichloride (0.886 g; 2.81 mmol) was added with constant stirring. The reaction mixture was further stirred at reflux temperature for ∼8 h, till the color of the reaction mixture underwent a change. Reaction mixture was filtered to remove the precipitated NaCl. The solvent was removed under reduced pressure from the filtrate. The reddish brown colored solid thus obtained was reprecipitated in acetone and dried in vacuum to get the 2.30 g of $Bi(C_{15}H_{13}N_2O)_3$. Compounds no. 6, 7, and 8 were prepared. The analytical details are summarized in Table 1.

Physical Measurements

Bismuth was estimated by direct titration with standard EDTA solution using xylonol orange as indicator[24] and arsenic was estimated iodometrically.[24] Infrared spectra were recorded as nujol mulls using CsI cells on a Perkin Elmer Model 557 FT-IR spectrophotometer in the range of 4000-200 cm^{-1}. The ^1H NMR spectra and proton decoupled ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$ on a Bruker DRX-300 spectrometer operated at 300.1 and 75.45 MHz for ^1H & ^{13}C, using TMS (tetramethylsilane) as internal standard. Molecular weights were determined on a Knauer Vapor Pressure Osmometer in $CHCl_3$ at 45 °C. The elemental analysis (C, H, and N) was obtained using a Coleman CHN analyzer. The x-ray diffraction studies were carried out on a Bruker Nonius Kappa CCD diffractometer at room temperature.

Table 1. Physical and Analytical data for arsenic (III) and bismuth (III) tripyrazolinates.

S. No.	Compound	Yield (%)	M. P. (°C)	Analysis (%) : Found (Calcd.)				Mol. Wt. Found (Calcd.)
				C	H	N	M	
1	$As(C_{15}H_{12}N_2OX)_3$	81	202	69.05	4.60	11.57	9.58	782
				(68.70)	(4.96)	(11.68)	(9.53)	(785.92)
2	$As(C_{15}H_{12}N_2OX)_3$	93	218	69.90	5.46	10.19	9.09	824
				(69.57)	(5.43)	(10.14)	(9.04)	(827.92)
3	$As(C_{15}H_{12}N_2OX)_3$	91	222	66.05	5.16	9.63	8.51	872
				(65.75)	(5.13)	(9.58)	(8.55)	(825.92)
4	$As(C_{15}H_{12}N_2OX)_3$	95	206	61.08	4.07	9.50	8.47	884
				(60.80)	(4.05)	(9.46)	(8.43)	(887.92)
5	$Bi(C_{15}H_{12}N_2OX)_3$	89	245	58.88	4.26	9.19	22.86	914
				(59.08)	(4.25)	(9.16)	(22.79)	(916.98)
6	$Bi(C_{15}H_{12}N_2OX)_3$	94	267	60.00	4.68	8.75	21.70	960
				(59.87)	(4.67)	(8.73)	(21.72)	(961.98)
7	$Bi(C_{15}H_{12}N_2OX)_3$	89	248	57.25	4.47	8.30	20.77	1006
				(57.03)	(4.45)	(8.31)	(20.69)	(1009.98)
8	$Bi(C_{15}H_{12}N_2OX)_3$	88	216	53.04	4.42	8.25	20.52	1018
				(52.83)	(4.40)	(8.21)	(20.44)	(1021.98)

Where X = H in **1** & **5**, CH_3 in **2** & **6**, OCH_3 in **3** & **7** and Cl in **4** & **8** compound respectively; and M = As and Bi.

Antimicrobial Studies

Agar disc diffusion technique was used for the screening of in vitro antimicrobial activity.[25] Inoculums of bacteria were prepared in nutrient broth and fungi in potato dextrose agar slant. The cultures were inoculated and incubated for 48 h in case of bacteria and for 5 days in case of fungi. The molten Muller Hinton medium was poured in sterile Petri discs (9 cm in diameter) to get a depth of 5 mm. The medium was left to solidify. There after it was seeded with respective test organisms. For the purpose of seeding, 5 ml sterile water was added to agar slant culture of fungi. The culture was scraped to get suspension of fungi spore. A sterile cotton swab was dipped in the culture/suspension, lightly rubbed over the solidified medium. The plate was left for a few minutes and then used for the test. In 1 ml of acetone solvent, $30\,\mu m$ of each sample to be tested were dissolved. Five millimeter discs of Whatmann filter paper no. 42 were cut and sterilized. The filter paper discs were immersed in the solution of sample; after soaking, the discs were removed and left in sterile Petri discs to permit the solvent to evaporate. After about 10 min the paper discs were transferred to seeded agar plates. Discs were kept on the seeded agar plates. Finally the dishes were incubated at 37 °C for 24 h (for bacteria) and at 30 °C for 72 h (for fungi), where clear or inhibition zones were detected around each disc.

A disc soaked in acetone alone was used as a control under the same conditions and no inhibition zone for acetone was observed. Each distinct inhibition zone was measured as diameter in millimeter, both antibacterial and antifungal activity were calculated as a mean of 3 replicates.

Results and Discussion

Arsenic(III) pyrazolinate is a yellow colored solid, non-hygroscopic, and stable at room temperature. It is insoluble in benzene and hexane but soluble in chloroform, acetone, and coordinating (tetrahydrofuran, dimethylformamide, and dimethylsulphoxide) solvents. Bismuth(III) pyrazolinate isa reddish brown solid, non-hygroscopic, and stable at room temperature. It is insoluble in benzene and hexane but soluble in chloroform, acetone, and coordinating (tetrahydrofuran, dimethylformamide, and dimethylsulphoxide) solvents. The molecular weight measurements in dilute chloroform solution at 45 °C show the monomeric nature of all these compounds. The elemental analysis (C, H, N, Cl, As, and Bi) data are in accordance with the stoichiometry proposed for respective compounds.

IR spectral data

The infrared spectral data of these compounds are summarized in Table 2. All arsenic(III) pyrazolinates and bismuth(III) pyrazolinates exhibit bands of medium intensity in the region 3320-3312 cm^{-1} due to ν(N-H) stretching vibrations and bands in the region 1626-1594 cm^{-1} due to ν(C=N) stretching vibrations.[5−9,19−20,26] In all arsenic(III) pyrazolinates and bismuth(III) pyrazolinates, the signal ν(C=N) stretching is shifted to lower wave number in comparison to the spectra of free pyrazolines (at ∼1654 cm^{-1}) suggesting the involvement of iminonitrogen in coordination. The signal due to ν(O-H) (originally present at 3080 cm^{-1} in free pyrazolines) is completely absent from the spectra of complexes. The band presents in the region between 1026 and 1032 cm^{-1} in compounds 3 and 7 may be assigned to ν(C-O) stretching indicating the presence of –OCH_3 group.[5−9,19−20,26]

The appearance of 2 new bands in the IR spectra of arsenic(III) pyrazolinates (in comparison to free pyrazolines) in the region 527-515 cm^{-1} and 422-412 cm^{-1} are assigned to ν(As-O) and ν(As-N) stretching vibrations, respectively.[27−31] Similarly, the appearance of 2 new bands in the IR spectra of bismuth(III) pyrazolinates (in comparison to free pyrazolines) in the region 544-536 cm^{-1} and 465-455 cm^{-1} are assigned to ν(Bi-O) and ν(Bi-N) stretching vibrations respectively.[27,31−32] The appearance of these 2 new bands and absence of hydroxyl band suggest that the pyrazoline behaves as monobasic bidentate ligand in these compounds.

Table 2. IR spectral data (cm^{-1}) for arsenic (III) and bismuth (III) tripyrazolinates.

S. No.	Compound	ν(N-H)	ν(C=N)	ν(C-O)	ν(M-O)	ν(M-N)
1	$As(C_{15}H_{12}N_2OX)_3$	3312	1604	-	526	412
2	$As(C_{15}H_{12}N_2OX)_3$	3316	1608	-	524	421
3	$As(C_{15}H_{12}N_2OX)_3$	3320	1594	1026	527	418
4	$As(C_{15}H_{12}N_2OX)_3$	3314	1598	-	515	422
5	$Bi(C_{15}H_{12}N_2OX)_3$	3319	1619	-	536	465
6	$Bi(C_{15}H_{12}N_2OX)_3$	3316	1618	-	540	455
7	$Bi(C_{15}H_{12}N_2OX)_3$	3320	1624	1032	539	463
8	$Bi(C_{15}H_{12}N_2OX)_3$	3316	1626	-	544	458

Where X = H in **1** & **5**, CH_3 in **2** & **6**, OCH_3 in **3** & **7** and Cl in **4** & **8** compound respectively; and M = As and Bi.

Multinuclear NMR spectral studies

The ^{1}H NMR chemical shifts of these compounds are listed in Table 3. In ^{1}H NMR spectra the aromatic protons of arsenic(III) pyrazolinates and bismuth(III) pyrazolinates were observed as a multiplet in the region δ 8.3-6.9 ppm.[5−9,19−20,26] The signals of aromatic protons of different aromatic rings overlap with each other and were observed as a complex pattern; therefore, aromatic signals could not be assigned individually. The peak due to hydroxyl proton originally present at $\delta \sim$11.00 ppm in free pyrazolines is completely absent from the spectra of compounds suggesting the bonding through hydroxyl oxygen atom.[5−9,19−20,26] The appearance of a peak at δ 5.6-5.1 ppm as a broad singlet could be assigned to N-H group originally present at δ 5.4-5.0 ppm in free pyrazolines suggesting the non-involvement of N-H group in bond formation.[5−9,19−20,26] The skeletal protons of 5-membered rings are observed at δ 3.7-3.2 ppm as a triplet and at δ 2.6-2.2 ppm as a doublet could be assigned to CH and CH_2 groups, respectively.[5−9,19−20,26]

The proton decoupled ^{13}C NMR spectra (Table 3) of arsenic(III) pyrazolinates and bismuth(III) pyrazolinates show presence of all important signals with reference to free pyrazolines. The assignments have been made on the basis of available literature along with the spectra of the ligands. The signal observed in the region δ 139.8-122.5 ppm as multiplet could be assigned to aromatic carbon.[5−9,19−20,26] The signal observed at δ 155.5-162.9 ppm, which is due to imino carbon of C=N group, is shifted to downfield in comparison to the spectra of free pyrazolines (at δ 143.5-142.8 ppm) suggesting the involvement of imino nitrogen in coordination.[5−9,19−20,26] All other signals were found at their respective positions as in free pyrazolines.

The complexes have been examined for crystalline/amorphous nature through XRD; all the complexes are amorphous solid.

On the basis of above discussion and available literature, the most plausible geometry around arsenic(III) and bismuth(III) in these complexes are presented in Figure 1.

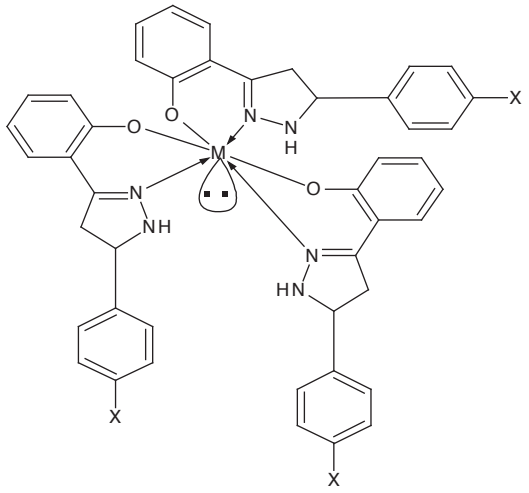

Figure 1. Molecular structure of $M(C_{15}H_{12}N_2OX)_3$ (where M = As and Bi; X = -H, -CH_3, -OCH_3, and –Cl).

Table 3. ^{1}H NMR and ^{13}C NMR data (in δ ppm) for arsenic (III) and bismuth (III) tripyrazolinates.

S. No.	Compound	^{1}H NMR Chemical shift (in ppm)	^{13}C NMR Chemical shift (in ppm)
1	$As(C_{15}H_{12}N_2OX)_3$	8.2-7.0 (27H, m, Ar-H)	139.7-126.5 (Ar-C)
		5.6 (3H, s, NH)	155.5 (C=N)
		3.2 (3H, t, CH)	43.0(CH)
		2.2 (6H, d, CH_2)	26.5 (CH_2)
2	$As(C_{15}H_{12}N_2OX)_3$	8.2-7.2 (24H, m, Ar-H)	139.5-122.5 (Ar-C)
		5.1 (3H, s, NH)	157.5 (C=N)
		3.4 (3H, t, CH)	43.1 (CH)
		2.6 (6H, d, CH_2)	26.5(CH_2)
		0.9 (9H, s, CH_3)	13.8 (CH_3)
3	$As(C_{15}H_{12}N_2OX)_3$	8.3-.7.1 (24H, m, Ar-H)	132.9-126.9 (Ar-C)
		5.5 (3H, s, NH)	157.2 (C=N)
		3.6 (3H, t, CH)	43.3 (CH)
		2.5 (6H, d, CH_2)	26.8 (CH_2)
		3.9 (9H, s, OCH_3)	51.4 (OCH_3)
4	$As(C_{15}H_{12}N_2OX)_3$	8.3-7.3 (24H, m, Ar-H)	138.7-128.7 (Ar-C)
		5.6 (3H, s, NH)	155.5 (C=N)
		3.6 (3H, t, CH)	43.3 (CH)
		2.1(6H, d, CH_2)	26.2 (CH_2)
5	$Bi(C_{15}H_{12}N_2OX)_3$	7.9-7.0 (27H, m, Ar-H)	139.4-126.9 (Ar-C)
		5.4 (3H, s, NH)	161.8 (C=N)
		3.3 (3H, t, CH)	43.5 (CH)
		2.2 (6H, d, CH_2)	26.6 (CH_2)
6	$Bi(C_{15}H_{12}N_2OX)_3$	7.7-6.9 (24H, m, Ar-H)	133.8-122.6 (Ar-C)
		5.1 (3H, s, NH)	162.5 (C=N)
		3.6 (3H, t, CH)	43.2 (CH)
		2.5 (6H, d, CH_2)	26.8 (CH_2)
		0.9 (9H, s, CH_3)	13.7 (CH_3)
7	$Bi(C_{15}H_{12}N_2OX)_3$	7.8-6.7 (24H, m, Ar-H)	138.7-125.5 (Ar-C)
		5.5 (3H, s, NH)	162.6 (C=N)
		3.7 (3H, t, CH)	43.6 (CH)
		2.0 (6H, d, CH_2)	26.3 (CH_2)
		3.8(9H,s,OCH_3)	51.5 (OCH_3)
8	$Bi(C_{15}H_{12}N_2OX)_3$	7.8-6.3 (24H, m, Ar-H)	138.8-123.5 (Ar-C)
		5.4 (3H, s, NH)	162.9 (C=N)
		3.4 (3H, t, CH)	43.3 (CH)
		2.2 (6H, d, CH_2)	26.6 (CH_2)

Where X = H in compounds **1** & **5**, CH_3 in **2** & **6**, OCH_3 in **3** & **7** and Cl in **4** & **8**, respectively; m = multiplet, s = singlet, d = doublet, and t = triplet.

Microbial assay

The antibacterial activity of a free ligand and 2 complexes were tested against the bacterial species *Bacillus lichaniformis* and *Vibrio Spp.* and the antifungal activities were tested against *Aspergillus niger* and *Penicillium notatum.* The antimicrobial activity of some antibiotics were also tested and compared with free pyrazoline and its arsenic(III) and bismuth(III) complexes. The results are listed in Table 4.

Antibacterial studies show that the arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates have greater activity towards all tested bacteria than free pyrazoline. The arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates also exhibited greater antifungal activity towards all tested fungi than the free pyrazoline

Comparison of the antimicrobial activities of the free pyrazoline and its arsenic(III) and bismuth(III) complexes with some known antibiotics exhibit the following results :

1. The arsenic(III) tripyrazolinates exhibit comparable and bismuth(III) tripyrazolinates exhibit greater antibacterial effect towards *Bacillus lichaniformis* as compared to free pyrazoline and chloramphenicol.
2. The arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates exhibit comparable antibacterial effect towards *Vibrio spp.* as compared to free pyrazoline and chloramphenicol.
3. The arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates exhibit comparable antifungal effect towards *Penicillium notatum* compared to free pyrazoline and terbinafin.
4. The arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates exhibit comparable antifungal effect towards *Aspergillus niger* compared to free pyrazoline and terbinafin.

It is difficult to make an exact structure and activity relationship between antimicrobial activity and the structure of these complexes. It can possibly be concluded that the complexation of arsenic(III) and bismuth(III) moiety with biologically active pyrazoline ligand results in increased activity of these complexes. Correlation between geometry around central atom in a complex and antimicrobial activity of the same complex is a further area of research.

Table 4. Antimicrobial activity of the free pyrazolines arsenic(III) tripyrazolinate and bismuth (III) tripyrazolinate.

Comp. No.	Fungi		Gram (+ve) bacteria	Gram (–ve) bacteria
	A. niger	*P. notatum*	*B. lichaniformis*	*Vibrio spp.*
1	+	+	+	+
2	++	++	++	++
3	++	++	++++	+++
4	+++	+++	+++	+++

Inhibition values beyond control are + = 6–10 mm, ++ = 11–15 mm, +++ = 16–20 mm, ++++ = 21–25 mm (the values are including disc diameter).

The standards are in the form of sterile Hi-Disc cartridges, each disc containing 30 μm of the drug.

1 = 3(2'-hydroxyphenyl)-5-phenylpyrazoline;

2 = Terbinafin (antifungal agent) and chloramphenicol (antibacterial agent);

3 = compound 1 and **4** = compound 5

Conclusions

The present study describes a series of arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates. Bidentate behavior of the pyrazoline ligands in arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates has been

confirmed by spectral technique (IR ^{1}H NMR and ^{13}CNMR). In arsenic(III) tripyrazolinates and bismuth(III) tripyrazolinates central arsenic(III) and bismuth(III) appear to acquire the coordination number 6 and the most plausible geometry around the arsenic(III) and bismuth(III) is distorted octahedral (Figure 1).[29,31] The bismuth(III) tripyrazolinates exhibit greater antibacterial activity and comparable antifungal activity compared to free pyrazoline and some of the antibiotics, such as chloramphenicol, and the antifungal agent terbinafine, respectively. The arsenic(III) tripyrazolinates exhibit comparable antifungal activity compared to free pyrazoline and some of the antibiotics, such as chloramphenicol, and the antifungal agent terbinafine, respectively.

Acknowledgment

The authors are grateful to SAIF, CDRI, Lucknow (India); Punjab University, Chandigarh (India), IIT, Mumbai (India), and SIF, IISc, Bangalore (India) for providing the necessary spectral and analytical data. One of the authors, Jeeven Singh Solanki is thankful to **Dr. Man Mohan Satnami,** lecturer, Pt. Ravisanker University, Chhatishgarh (India) for his valuable academic suggestions.

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